INDUCTION OF VARIATION *IN VITRO* AND FIELD EVALUATION OF SOMACLONES IN GINGER (*Zingiber officinale* Rosc.)

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Induction of variation *in vitro* and field evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

BAP	-	6-benzylaminopurine
NAA	-	α-naphthaleneacetic acid
2,4-D	-	2,4-dichlorophenoxyacetic acid
MS	-	Murashige and Skoog medium (1962)
CP	-	Conventionally propagated
µs	-	micro siemens
µM	-	micro molar
KR	-	kilo rad
Gy	-	Grey
v/v	-	volume in volume
mg l^{-1}	-	milli gram per litre
ppm	-	parts per million
g	-	gram
N	-	normal
i.e.	-	that is
sc	-	subculture
cv.	-	cultivar

Introduction

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), one of the oldest and renowned spices is esteemed for its aroma and pungency. It is much valued as a spice, medicine and vegetable since ancient times. Ginger is used as carminative, stimulant, anti-inflammatory, antiemetic and aphrodisiac in ayurvedic system of medicine. Ginger oil finds use in perfumery and pharmaceuticals. The suitability of ginger for an array of uses has resulted in a hike in demand of this spice worldwide.

In the global scenario, India still continues to be the largest producer, consumer and exporter of ginger. It occupied an area of 85,068 ha with a production of 3,05,930 tonnes. Of the production, 13,000 tonnes of ginger was exported, fetching a foreign exchange earning of Rs.59.50 crores (www. indianspices.com dated 24-2-2006). In Kerala, ginger is cultivated in an area of 8923 ha with a production of 32,412 tonnes. Cochin and Calicut ginger produced in Kerala are famous in international market owing to its high intrinsic qualities.

The production of ginger is handicapped by the occurrence of devastating diseases like rhizome rot caused by *Pythium* spp. and bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al*.. Enormous yield loss was reported from different states of the country due to the above diseases, which account for more than 80 per cent (Bhai *et al*., 2005; Kumar and Hayward, 2005). Attempts to isolate resistant clones using conventional breeding techniques were not successful in ginger as genetic variability available for disease resistance / tolerance is very limited and all the cultivars are susceptible to the diseases. Studies were conducted to manage the diseases using cultural, chemical and biological methods. None of the methods gave absolute control for the two diseases (Kurucheve, 1980; Rani, 1994; Shanmugham, 1996; Vilasini, 1996; Joseph, 1997b; George, 1999).

Breeding through selection and hybridization is not possible in ginger due to lack of variability and absence of natural seed set. The presence of spiny stigmatic surface, low pollen germination and low pollen tube growth prevent fertilization and seed set in ginger (Sathiabhama, 1988). Application of growth regulators for

induction of flowering, effecting pollination and seed set was also not successful in ginger (Usha, 1984). As natural variability stands limited, broadening the genetic base through mutagenesis or tissue culture techniques pave way for exploitation of induced variability for isolating plant types with high yield, quality and resistance/tolerance to diseases.

Earlier crop improvement research on ginger was mainly focusssed on germplasm collection, evaluation and selection of high yielding clones (Nybe, 1978; Rattan, 1994). Induced mutation using γ rays and ethyl methyl sulfonate (EMS) were also attempted in ginger. The mutants isolated were low yielders and the effects of mutagen treatment vanished in subsequent generations (Giridharan, 1984; Dutta and Biswas, 1985; Jayachandran, 1989). Induction of variability through colchiploidy was attempted in ginger and the tetraploids induced were characterized by increased pollen fertility and higher yield of rhizomes than the corresponding diploids (Sheeba, 1996). The field evaluation of induced tetraploids revealed the high susceptibility of tetraploids to rhizome rot and bacterial wilt diseases (Shankar, 2003). Investigations were also made to induce variability in ginger through biotechnological tools. Experiments were conducted to standardise *in vitro* pollination and fertilization techniques in Zingiberaceous spice crops (Valsala, 1994; Bindu, 1997; Renjith, 1999; Vijayasree, 2001; Bhurke, 2002; Shankar, 2003). Through *in vitro* placental pollination, seed set could be achieved in ginger but the germination of seeds was very poor and this technique needs refinement. Under these circumstances, exploitation of somaclonal variation for isolating desirable plant types is of great significance in crop improvement programme of ginger.

Somaclonal variation was extensively used for isolating clones with high yield in sugarcane (Dhumale *et al.*, 1994), mustard (Katiyar, 1997), barley (Li *et al.*, 2001) and banana (Sheela and Nair, 2001; Tang and Tai, 2001; Nwauzoma *et al.*, 2002), better quality in mint (CIMAP, 1992; Kukreja *et al.*, 2000), date palm (Booij *et al.*, 1993), geranium (Gupta *et al.*, 2002; Ravindra *et al.*, 2004) and Jamrosa (Nayak *et al.*, 2003) and disease tolerance in sugarcane (Daub, 1986), tomato (Shahin and

Spivey, 1986; Evans, 1989; Mandal, 1999) and banana (Trujillo and Garcia, 1996; Bhagwat and Duncan, 1998; Chuan *et al.*, 2000; Nwauzoma *et al.*, 2002).

The present investigations on "Induction of variation *in vitro* and field evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" are taken up to induce variability in ginger through *in vitro* techniques, to evaluate somaclones of ginger for morphological, yield, quality parameters and reaction to rhizome rot and bacterial wilt diseases and to characterise the selected superior somaclones using RAPD markers.

Review of Literature

REVIEW OF LITERATURE

Ginger is an important commercial spice crop esteemed for its aroma and pungency. Various diseases of fungal and bacterial origin affect ginger. Of these, rhizome rot (*Pythium* spp.) and bacterial wilt (*Ralstonia solanacearum*) result in considerable crop loss. None of the existing varieties/cultivars are resistant to these devastating diseases. As ginger is exclusively propagated vegetatively, number of clones available is limited. As natural variability is limited in ginger, broadening the genetic base will open up avenues for selection of plant types with high yield and tolerance/resistance to diseases.

Though crop improvement research in ginger started as early as 1950, the earlier efforts were mainly focusssed on evaluation of germplasm and selection of high yielding clones (Nybe, 1978; Rattan, 1994). Mutation breeding studies carried out using physical and chemical mutagens were not successful. Induction of mutation through γ irradiation resulted in reduced yield in ginger (Giridharan, 1984) and mutation induction using ethyl methyl sulfonate (0.05-1.0%) resulted in cytological irregularities (Dutta and Biswas, 1985). Also the effects of mutagen treatments were found vanished in subsequent generations (Jayachandran, 1989). The efforts in polyploidy breeding to obtain flowering and seed set in ginger met with conflicting results. Ramachandran and Nair (1992) found that induced tetraploids were more vigorous and flowered in the second year. Ratnambal and Nair (1982) observed no flowering in the induced tetraploids in ginger. Sheeba (1996) observed increased pollen fertility and higher yield of rhizomes in induced tetraploids than the corresponding diploids. But Shankar (2003) reported that the high yielding capacity of the tetraploids was superseded by the susceptibility of the tetraploids to rhizome rot and bacterial wilt diseases. Thus, lack of variability for resistance to major diseases and extremely rare seed set make conventional breeding programmes ineffective in ginger.

Crop improvement through biotechnological interventions was also attempted in ginger. Valsala (1994) tried *in* vitro pollination and fertilization in ginger.

She could obtain seed set through *in vitro* placental pollination but the seed germination was a problem. Under these circumstances, *in vitro* culture induced variation constitutes an important source of variability for the improvement of this crop. Somaclonal variation has been exploited in many crops for isolation of desirable plant types showing improvement in yield, quality, and resistance/tolerance to biotic and abiotic stresses. Many factors are found to influence the rate of somaclonal variation. They include the mode of regeneration (Skirwin and Janick, 1976; Evans, 1988; Karp, 1989), growth regulators (Evans, 1988; Griesbach *et al.*, 1988; Shoemaker *et al.*, 1991), cultivar (Kurtz and Lineberger, 1983), age of cultivar (Shepard *et al.*, 1980), ploidy level (Heinz and Mee, 1969; Bingham and Mccoy, 1986), explant source (Shepard *et al.*, 1980; Tsai *et al.*, 1992; Gui *et al.*, 1993), duration in culture *in vitro* (Skirvin *et al.*, 1994), proliferation rate of culture (Smith and Drew, 1990), culture conditions (Skirvin *et al.*, 1994) and *in vitro* selection pressure (Bingham and Mccoy,1986).

Somaclonal variation was effectively utilized for producing useful phenotypic variants in carnation (Buiatti *et al.*, 1986), banana (Tan *et al.*, 1993), mulberry (Zaman *et al.*, 1997), rye (Trojanowska, 2002), and pearl millet (Srivastav *et al.*, 2003), for improving yield in sugarcane (Dhumale *et al.*, 1994), mustard (Katiyar, 1997), barley (Li *et al.*, 2001), banana (Sheela and Nair, 2001; Tang and Tai, 2001; Nwauzoma *et al.*, 2002), rice (Rasheed *et al.*, 2003), wheat (Arun *et al.*, 2003) and sunflower (Encheva *et al.*, 2003 and 2004), for upgrading quality in mint (CIMAP, 1992; Kukreja *et al.*, 2000), date palm (Booij *et al.*, 1993), geranium (Gupta *et al.*, 2002; Ravindra *et al.*, 2004), chilli (Anu *et al.*, 2004), and *Cymbopogan* spp. (Nayak *et al.*, 2003), for disease tolerance in sugarcane (Daub, 1986), tomato (Shahin and Spivey, 1986; Evans, 1989; Mandal, 1999), banana (Trujillo and Garcia, 1996; Bhagwat and Duncan,1998; Chuan *et al.*, 2000; Nwauzoma *et al.*, 2002), black pepper (Shylaja, 1996; Sanchu, 2000), barley (Li *et al.*, 2001), wheat (Kinane and Jones, 2001), rice (Araujo *et al.*, 2001a, 2001b and 2002) and sunflower (Encheva *et al.*, 2003), for chilling tolerance in rice (Bertin *et al.*, 1996), for drought tolerance in wheat (Bajji *et al.*, 2004) and for salt tolerance in rice (Zhu *et al.*, 2004).

Several techniques of molecular biology are available now a days for detection of genetic polymorphism in crop plants. Molecular markers have been widely used for estimating genetic diversity, varietal finger printing, linkage mapping, identification of somatic hybrids and for analyzing genetic stability of plants derived through tissue culture. Notable alterations in RAPD profiles have been reported in somaclones of wheat (Brown *et al.*, 1993), rice (Banerjee *et al.*, 1998; Araujo *et al.*, 2001a), sugarcane (Oropeza *et al.*, 1995; Taylor *et al.*, 1995), banana (Damasco *et al.*, 1996; Walther *et al.*, 1997) and pea (Kokaeva *et al.*, 1997).

There are only limited research reports available on the induction of variability in ginger through *in vitro* techniques, field evaluation of somaclones, and their characterization by molecular markers. Hence, similar reports in other crops are included in this review. The review focuses on indirect organogenesis and embryogenesis in Zingiberaceous spice crops, *in vitro* induction of mutation using γ irradiation, field evaluation of somaclones, screening somaclones for resistance/tolerance to diseases and molecular characterization of somaclones using RAPD markers.

2.1 INDUCTION OF VARIATION THROUGH *IN VITRO* TECHNIQUES

2.1.1 Indirect Organogenesis

Ginger

Indirect organogenesis has been reported in ginger from different explants like pseudostem, leaf, shoot tip, shoot meristem and anther in MS medium supplemented with growth regulators like 2,4-D or NAA alone or in combination with BAP.

Choi (1991) initiated calli from base, middle and top portions of pseudostem. Callusing was best observed in base and middle portions of pseudostem in MS medium supplemented with 0.5 ppm NAA. Shoot and root morphogenesis were observed in MS medium supplemented with 0.10 to 1.00 ppm NAA and 1.00 ppm BAP. Instead of NAA - BAP combination, KAU (2001) obtained indirect

organogenesis in ginger from pseudostem explants in medium supplemented with 2,4-D and BAP. Pseudostem base and sheathing leaf base were observed as best explants for induction of calli in the study. Murashige and Skoog medium supplemented with 2,4-D (1-2 ppm) was found good for callusing. Shoot and root morphogenesis were observed in MS medium supplemented with higher levels of BAP and silver nitrate (0.25-2.00 mg l^{-1}).

Calli from shoot tip and shoot meristem were found morphogenic in ginger in studies conducted by Malamug *et al.* (1991), Ishida and Adachi (1997), Rout and Das (1997) and Palai *et al.* (2000). Callusing was highest in MS medium supplemented with 0.5 ppm 2,4-D and 1.0 ppm BAP (Malamug *et al.*, 1991). Shoot and root morphogenesis were achieved in medium incorporated with 1.00 to 3.00 ppm BAP. Shoot proliferation was maximum in medium supplemented with 5.00 ppm BAP and 1.00 ppm NAA. Ishida and Adachi (1997) induced calli from shoot meristem in MS medium supplemented with 2.00 ppm 2,4-D and shoot morphogenesis was observed in medium supplemented with 0.10 ppm NAA and 1.00 ppm BAP. Rout and Das (1997) and Palai *et al.* (2000) initiated calli from shoot meristem of ginger in MS medium supplemented with BAP (1.00-2.00 ppm) and 2,4-D (2.00-5.00 ppm). Organogenesis was observed in medium supplemented with BAP (5.00 ppm), IAA (1.00 ppm) and adenine sulfate (100 ppm). The regenerated plants exhibited 90 – 95 per cent establishment *ex vitro*.

Calli induced from youngest leaf inside the sheathing leaf base of ginger was found morphogenic in studies conducted by Babu *et al.* (1992). Callusing was best observed in MS medium supplemented with 2,4-D at 13.6 µM. Shoot morphogenesis was highest in medium incorporated with 0.9 µM 2,4-D and 22.2 µM BA. Shoot proliferation was maximum in basal medium. Rooting of regenerated shoots was better in liquid MS medium supplemented with 5.4 µM NAA.

Samsudeen *et al.* (2000) and Taesoo *et al.* (2000) reported indirect organogenesis in ginger from anther explants. Samsudeen *et al.* (2000) initiated androgenic calli in MS medium incorporated with 2,4-D (1.00-3.00 ppm). Shoot

morphogenesis was observed in medium supplemented with 2,4-D (0.20 ppm) and BAP (10.00 ppm). Shoot proliferation and rooting were best in medium supplemented with NAA (1.00 ppm). Instead of MS medium, Taesoo *et al.* (2000) used N_6 medium supplemented with NAA (2.00 ppm) for callus induction from anther explants. Shoot morphogenesis was achieved in MS medium supplemented with BAP (1.00-2.00 ppm).

Turmeric

Indirect organogenesis was reported in turmeric from explants like rhizome bud, shoot tips and leaf base. For rhizome bud and shoot tip explants, MS medium supplemented with 2,4-D (2.50-3.00 ppm) was found best for callusing and medium supplemented with NAA (0.10 ppm) and kinetin (1.00 ppm) was best for shoot regeneration (Sunitibala *et al.* 2001).

For callusing of leaf base explants in turmeric, MS medium supplemented with dicamba (2.00 mg l^{-1}) and BAP (0.50 mg l^{-1}) was found best. Shoot morphogenesis was best observed in medium supplemented with BAP (5.00 mg l^{-1}) and TIBA (0.10 mg l^{-1}) or 2,4-D (0.10 ppm). Shoot proliferation was highest in medium supplemented with kinetin (1.00 mg l^{-1}). Root morphogenesis was achieved on basal medium. The regenerated plantlets recorded 90 per cent survival (Salvi *et al.* 2001).

Cardamom

Indirect organogenesis from axenic seedling explants and shoot meristem were reported in cardamom. Rao *et al.* (1982) initiated calli from *in vitro* seedling explants in MS medium supplemented with 2.00 ppm of 2,4-D, IAA or NAA in combination with BAP (2.00 ppm) and 18.00 per cent coconut water. Shoot morphogenesis was observed in medium supplemented with IAA (1.00 ppm) or NAA (1.00 ppm).

Reghunath (1989) initiated calli from shoot meristem explants of cardamom cultivars Malabar, Mysore and Vazhuha. Callusing was best observed in

MS medium supplemented with NAA (4.00 ppm) and BA (1.00 ppm). Shoot morphogenesis was observed in medium supplemented with BAP (2.00-3.00 mg l^{-1}) and kinetin (0.50-1.00 mg l^{-1}). Root morphogenesis was achieved by culturing regenerated shoots in MS medium supplemented with 0.5 per cent activated charcoal for one week and then transferring the cultures to medium supplemented with IBA (1.5 mg l^{-1}) for another week. Cultivar Vazhuka responded better than other cultivars in callusing, shoot and root morphogenesis and subsequent establishment in the soil.

Kacholam

Callus mediated organogenesis was reported in kacholam from explants like rhizome bud and pseudostem in MS medium supplemented with growth regulators like 2,4-D and BAP. Vincent *et al.* (1991) initiated calli from rhizome buds of kacholam in MS medium supplemented with 2,4-D at 1.00 mg l^{-1} and BAP at 0.50 mg l^{-1}. Shoot and root morphogenesis were observed in medium supplemented with 2,4-D at 1.00 mg l^{-1} and BAP at 1.50 mg l^{-1}. The regenerated plants when planted out recorded 85 per cent establishment.

Joseph (1997a) induced profuse callusing on pseudostem explants of kacholam in MS medium supplemented with 2,4-D (1.00 ppm) and BAP (0.25 ppm). Higher concentrations of BAP (1.00 to 10.00 ppm) inhibited callus growth. Shoot and root regeneration were observed in MS medium supplemented with BAP (8.00 ppm). Shoot proliferation was achieved in medium supplemented with adenine sulphate (15.00 ppm), BAP (0.25 ppm) and IAA (2.00 ppm).

Mango ginger

Indirect organogenesis in mango ginger from leaf sheath explants was reported by Prakash *et al.* (2004). Calli were initiated in MS medium supplemented with 9.00 μM 2,4-D. Shoot morphogenesis was achieved in MS medium fortified with 8.8 μM BAP and 2.7 μM NAA.

2.1.2 Indirect Embryogenesis

Somatic embryogenesis has been reported in Zingiberaceous spice crops using explants like leaf, ovary, rhizome bud etc. in MS medium supplemented with dicamba or 2,4-D alone or in combination with BAP.

Ginger

Somatic embryogenesis from young axenic leaves of ginger was reported by Kackar *et al.* (1993) and Tyagi *et al.* (1998). Callusing was best observed in MS medium supplemented with 2.70 µM Dicamba. Embryoid germination was observed in MS medium incorporated with 8.90 µM BAP.

Babu *et al.* (1996a) reported profuse callusing of ovary explants excised from one to two week old ginger flowers in MS medium supplemented with 2,4-D (1.00 ppm) alone or 2,4-D (0.50 ppm) and BAP (1.00 ppm). White globular embryoid like structures were formed in the calli when cultured in medium supplemented with 2,4-D (0.20 ppm) and BAP (10.00 ppm). Embryoid germination was observed in medium incorporated with NAA (1.00 ppm).

Suma and Keshavachandran (2005a and 2005b) induced embryogenic calli on rhizome buds of ginger in MS medium supplemented with 2,4-D (1.00 ppm) and BAP (0.50 ppm). Embryoid germination was observed when calli were transferred to medium supplemented with BAP (3.00 ppm).

Kacholam

Indirect embryogenesis was reported in kacholam from explants like rhizome bud and pseudo stem. Vincent *et al.* (1992) reported that rhizome buds of kacholam developed embryogenic calli in MS medium supplemented with 2,4-D (1.00 ppm) and BAP (0.50 ppm). Calli when transferred to MS medium supplemented with NAA (1.00 ppm) and BAP (0.10 ppm) produced globular embryoids on peripheral layers of the callus. Embryoid germination was observed in hormone free medium.

Joseph (1997a) initiated callus from pseudostem explants of kacholam in MS medium supplemented with 2,4-D (1.00 ppm). Compact, shiny, embryogenic calli were observed in the peripheral region of cultures in medium with BAP (8.00 ppm). Embryoid proliferation was observed in medium incorporated with NAA (3.00 ppm) and BAP (1.00 ppm). Embryo maturation and germination was achieved in basal medium with sucrose at five per cent level.

Lakshmi and Mythili (2003) inducced calli in kacholam using rhizome bud explants. MS medium with 2,4-D (1.00 ppm) and BAP (0.50 ppm) favoured callusing under both dark and light incubation. Formation of embryoids occurred within 23 days in MS medium supplemented with 2,4-D (0.50 ppm), BAP (0.20 ppm) and tryptophan (80.00 μM). Embryo germination was observed in six to eleven days after inoculation in growth regulator free medium.

2.1.3 *In vitro* Mutagenesis

Physical mutagens like γ rays are widely used in plant tissue culture for widening the *in vitro* culture induced variability in many crop plants.

Ginger

KAU (2001) reported the effect of γ irradiation on growth and multiplication of *in vitro* ginger sprouts. The highest doze of γ irradiation that the sprouts could withstand was identified as 20 Gy.

Black pepper

Shylaja (1996) reported *in vitro* induction of mutation using γ irradiation in black pepper cultivars Kalluvally, Karimunda, Balankotta, Cheriakanyakkadan, Panniyur-1 and Panniyur-2. Calli of the cultivar Kalluvally tolerated higher doze of γ irradiation as compared to other cultivars. In all the cultivars, callus growth inhibition was found at higher doses of γ irradiation viz., 40 and 50 Gy. The doze 30 Gy was fixed as maximum dose of γ irradiation that the calli of different cultivars could

withstand. The regeneration capacity of irradiated calli was found very low and the regenerated shoots were weak and chlorotic.

Vanilla

Kuriakose *et al.* (2005) studied the effect of physical and chemical mutagens in vanilla for induction of variability *in vitro*. Vanilla pods and *in vitro* nodal cuttings were subjected to γ irradiation at dozes of 2, 5, 10, 20, 25, 30, 40 and 50 Gy. *In vitro* nodal cuttings were subjected to EMS (0.01 to 0.05 %) for 30 minutes. Good germination in *in vitro* was observed in pods subjected to γ irradiation dozes of 2 to 30 Gy. Pods subjected to 40 and 50 Gy failed to germinate. *In vitro* cultures subjected to γ irradiation dozes of 10 and 20 Gy and 0.5 per cent of EMS exhibited better growth.

Banana

Differential radio sensitivity and post radiation recovery were observed in different *Musa* clones by Novak *et al.* (1990) when they subjected the shoot apices shoots of seven clones of dessert banana, plantain and bluggoe cooking banana to γ irradiation dozes of 15, 30, 45 and 60 Gy at a dose rate of 8 Gy min^{-1}. Decrease in survival rate and shoot number with increasing levels of γ irradiation was reported by Mak *et al.* (1995) and Karmarkar *et al.* (2001).

In banana cultivar Basrai (AAA), lower dozes of irradiation viz., 10 and 20 Gy enhanced multiplication rate of *in vitro* multiple shoot cultures (Karmarkar *et al.*, 2001). Obeidy *et al.* (2002) studied the difference in radio sensitivity of banana cultivars – Grand Nain, Gros Michel and Williams. *In vitro* cultures of these cultivars were subjected to γ irradiation (40 and 60 Gy). At 60 Gy, reduced survival was observed in all the cultivars. The number of shoots was reduced by 65.5, 61.8 and 54.3 per cent in Gros Michel, Williams and Grand Nain respectively when subjected to γ irradiation doze of 60 GY. There was no significant variation in shoot number at 40 Gy in the three cultivars studied. Shoot elongation in Gros Michel decreased with radiation while that of Grand Nain did not significantly vary. Longer shoots were

recorded in Williams at 40 Gy. They also isolated a mutant of Williams which exhibited salt tolerance upto 0.75 per cent NaCl in *in vitro* screening experiments.

Novak *et al.* (1993) reported that differences in sensitivity of shoot tips to γ irrdiation treatment were dependent on ploidy level and hybrid constitution of the A and B genome. They recommended γ irradiation dozes of 25, 35, 40 and 50 Gy for diploids, AAA, AAB and ABB triploids and AAAA tetraploids respectively. From the study, they could isolate mutants superior to Grand Nain with respect to short stature, early flowering, cylindrical fruit bunch, higher fruit quality and higher bunch weight. Tan *et al.* (1993) also isolated short statured early flowering mutants with good bunch characters, high yield and flavour from γ irradiated meristem cultures.

Irradiation of *in vitro* cultures of banana and subsequent field evaluation trials were done by Hui *et al.* (2001 and 2002). They isolated 28 mutants of Grand Nain, which exhibited wide variability in morphological characters such as the colour of pseudostem, leaf-stem ratio and plant shape as compared to control plants. The mutants recorded 10 per cent yield increase over control plants.

Rose

Wilson (1993) reported *in vitro* mutagenesis in rose using γ rays. Bud woods were subjected to γ irradiation doses at 20, 30, 40 and 50 Gy and cultured *in vitro*. Irradiation caused inhibition and reduction in sprouting and survival of the cultures. Two mutants, one with more number of petals and another with reddish yellow petals were isolated from the above study.

Sugarcane

Kumar *et al.* (2001) studied the effect of γ irradiation (10, 20, 30 and 40 Gy) on indirect morphogenesis in six sugarcane cultivars. In all the cultivars, the rate of shoot multiplication decreased with increase in radiation dose. The cultivars KHS 3296, KHS 3347 and Co 62175 were most sensitive to γ irradiation which recorded drastic reduction in shoot number at 10 Gy and complete inhibition at 40 Gy.

Sunflower

Irradiation of *in vitro* cultures and subsequent field evaluation were carried out by Barakat *et al.* (2002) in four cultivars of sunflower. Reduction in callus growth and weight was observed in higher dozes of γ irradiation (300 to 400 Gy). But shoot morphogenesis was found high in irradiated cultures as compared to non irradiated control cultures. The irradiated regenerants recorded reduced plant height, number of nodes, internodal length, stem diameter, leaf area, head diameter and number of ray and disc florets. But the number of branches, leaves and heads increased in the irradiated regenerants as compared to control plants.

Chrysanthemum

Mutation in flower colour and shape of ray florets have been reported in chrysanthemum by resorting to *in vitro* mutagenesis. *In vitro* cultures of chrysanthemum cultivar Lalima were subjected to γ irradiation doses of 0.5 and 1 Gy by Misra *et al.* (2003). From the study, two mutants, one with yellow petal colour and the other with tubular ray florets were isolated, the control plants had red petals and flat spoon shaped ray florets.

2.2 FIELD EVALUATION OF SOMACLONES

Ginger

Field evaluation of ginger somaclones for yield, quality, and tolerance to diseases were attempted by several workers.

Smith and Hamill (1996) observed that adventitious bud regenerants of ginger cultivar Queensland were more vigorous with more number of tillers/plant and lengthy pseudostem than conventionally propagated (CP) plants. But there was no significant difference in yield of rhizomes between the two. Freitz *et al.* (2003) also reported an increase in tiller number, fresh and dry mass of shoots and roots in adventitious bud regenerants as compared to CP plants. But rhizome yield and pseudostem length were more in control plants. Somaclones produced numerous small rhizomes with more number of fleshy roots and tuberous structures at the tips.

Mericlones of ginger cultivar Wynad local were comparable to CP plants in the composition of starch, ash, acetone extract and volatile extract in the studies conducted by Bhagyalakshmi *et al.* (1994). Fibre content and rhizome yield were lower in the mericlones. Rao *et al.* (2000) observed no significant variation in morphological characters in adventitious bud regenerants of Jamaican ginger. Yield of somaclones were comparable with that of CP plants. Somaclones took 13 months to harvest the rhizome after planting out. Quality wise somaclones were superior to the local ginger cultivar Kuruppapady in terms of oil and oleoresin recovery. Pandey *et al.* (1997) reported that conventionally propagated plants of ginger cultivar Khin yai produced higher rhizome yield than adventitious bud regenerants. But rhizomes of adventitious bud regenerants exhibited more branching indicating their high yield potential.

Sharma and Singh (1997) observed no significant difference in height of pseudostem, leaf area, tiller number and yield in adventitious bud regenerants (cultivar Himachal local) and CP plants of ginger. Somaclones were not affected by rotting caused by *Fusarium oxysporum* f. sp. *zingiberi* under field conditions and storage while 54.5 per cent of the rhizomes of CP plants were affected by the fungus during storage.

Babu (1997) compared growth and yield of regenerants derived through adventitious bud regeneration, indirect organogenesis and CP plants. Adventitious bud regenerants exhibited wide variability in height of pseudostem, number of tillers and leaves/plant, girth of rhizome and number of nodes/finger. Regenerants derived through indirect organogenesis recorded wide variability in internodal length of fingers and yield of rhizomes. Somaclones were superior to CP plants in height of pseudostem, number of tillers /plant, number of nodes/finger and yield of rhizomes. But the CP plants were superior in number of and leaves/plant and oleoresin. Somaclones and CP plants were on par with respect to girth of rhizome, internodal length of fingers and dry recovery percentage. He developed a promising somaclonal variant from the cultivar Maran with bold rhizomes.

Variability studies in ginger somaclones conducted by Samsudeen (1996) indicated high variability in somaclones for yield and yield attributes. From the study, a few promising high yielding lines with tolerance to rhizome rot could be identified.

Shylaja *et al.* (2003) studied the response of two ginger cultivars Maran and Rio-de-Janeiro to *in vitro* adventitious bud regeneration. They also evaluated the growth of somaclones for a period of three months. The *in* vitro response was better in cultures of the cultivar Rio-de-Janeiro but the establishment and further growth of regenerants were better in the cultivar Maran. Somaclones of cultivar Maran exhibited higher increment in plant height and leaf length as compared to clones of cultivar Rio-de-Janeiro.

Costus

Pal and Roy (1991) evaluated embryoclones of *Costus speciosus* for morphological, yield and quality parameters. Wide variability in rhizome yield and diosgenin content was observed in embryoclones. Thirty six per cent of embryoclones exhibited higher diosgenin content than CP plants. Embryoclones were morphologically similar to CP plants.

Kacholam

Geetha *et al.* (1997) evaluated field performance of adventitious bud regenerants of *Kaempferia galanga* and *K. rotunda* for three seasons along with conventionally propagated plants. Somaclones were inferior in morphological characters and yield as compared to control plants in the first two seasons but in the third season, both were on par.

Joseph (1997a) compared growth of regenerants derived through indirect organogenesis/embryogenesis, adventitious bud culture and CP plants of kacholam (*K. galanga*) for growth for a period of three months after planting out. Regenerants derived through indirect organogenesis/embryogenesis had erect leaves while adventitious bud regenerants and CP plants had horizontal leaves. Leaf area was

highest in CP plants while tiller number was highest in regenerants derived through indirect organogenesis.

Turmeric

Salvi *et al.* (2002) reported that adventitious bud regenerants of turmeric cultivar Elite showed a significant increase in length of pseudostem, number of tillers, number and length of leaves, number of fingers and fresh rhizome yield per plant as compared to CP plants. Among 48 micropropagated plants, two plants showed variegated leaves.

Cardamom

Lukose *et al.* (1993) observed growth and yield of somaclones, sucker derived plants and seedlings of cardamom variety Mudigere 1 and clone 37 for three years. The number of yielding tillers per plant and leaf area were higher in micropropagated plants but corresponding yield improvement was not observed.

Chandrappa *et al.* (1997) evaluated tissue-cultured plants of promising cardamom selections for their yield for a period of three years. The lines TC 5, TC 6 and TC 7 were found promising and also differed among themselves for yield and yield attributes. Conventionally propagated plants of two ruling varieties viz., Mudigere 1 and Mudigere 2 recorded significantly lower yield as compared to TC 5. Sudharshan and Bhat (1998) also reported that micropropagated plants exhibited 30 per cent yield increase as compared to open pollinated plants. The essential oil content was also higher in micro propagated plants (7.2%) when compared to open pollinated seedlings (6.9%).

Reghunath (1989) analysed growth of regenerants of cardamom cultivar Vazhuka derived through adventitious bud culture and indirect organogenesis along with open pollinated seedlings for a period of nine months. Micropropagated plants exhibited more increment in height of pseudostem, number of leaves and tillers/plant than open pollinated seedlings. Somaclones recorded 50 per cent more number of tillers/plant than seedlings during the growth period observed.

Reghunath and Priyadarshan (1993) noticed variation in plant height and panicle branching character in somaclones of cardamom as compared to clonally propagated plants.

Sudharshan *et al.* (1997) evaluated micropropagated cardamom plants for growth and yield. Tissue culture derived clones showed variations in the type of panicle, capsule shape and size. The overall variability observed in tissue cultured plants was 4.5 per cent as against three per cent in open pollinated seedling progenies.

Kuruvilla *et al.* (2005) carried out on farm evaluation of tissue culture derived plants and open pollinated seedlings of cardamom in farmer's fields in Kerala, Karnataka and Tamil Nadu for two seasons viz., 1993-94 and 1994-95. Somaclones were superior to open pollinated seedlings in growth attributes such as number of tillers, bearing tillers and panicles per clump and also in yield. Irrespective of the seasons and locations, 14 somaclones were identified with yield potential of over 750 kg/ha under moderate management.

Large cardamom

Rao *et al.* (2003) compared growth and yield of adventitious bud regenerants and open pollinated seedlings of large cardamom. The somaclones recorded 1.5 times increase in yield contributing characters such as number of total tillers/clump, productive tillers/clump, spikes/clump and capsules/spike and twenty times increment in yield as compared to open pollinated seedlings. Gupta *et al.* (2005) also reported that tissue cultured plants of large cardamom were superior to open pollinated seedlings in yield contributing characters such as number of productive tillers and spikes/clump, precocity in yield and higher yield.

Black pepper

Sanchu (2000) studied variability in morphological, yield and quality parameters of black pepper cultivar Cheriakaniyakkadan derived through indirect organogenesis. She observed variability in leaf area, number of lateral branches, number of spikes per branch, spike length, number of berries per spike and recovery of

essential oil and piperine. She could isolate five calliclones of black pepper tolerant to *Phytophthora* foot rot disease and a superior somaclone having high yield, quality and tolerance to *Phytophthora* foot rot from the study.

Sujatha (2001) studied variability in axillary bud regenerants of black pepper varieties Panniyur 1, 2, 4 and Subhakara along with parental clones. Out of 61 morphological characters studied, 56 were found homogenous within somaclones of each variety. Significant variation was observed in five traits such as number of branches, angle of insertion of branches and area of young leaf and mature leaf of orthotrope and plageotrope.

Rathy *et al.* (2005) evaluated growth of regenerants of black pepper variety Panniyur-4 derived through axillary bud culture and indirect organogenesis along with CP plants. Somaclones exhibited superiority over CP plants in morphological characters such as number of leaves/plant, length and breadth of leaves. The somaclones produced laterals much earlier than CP plants.

Vanilla

Mary *et al.* (1999) studied variability in somaclones of vanilla derived through *in vitro* seed culture. Tissue cultured plants exhibited variability in leaf morphology and phyllotaxy. Shape of leaves varied from narrow lanceolate to broadly ovate. Somaclones with alternate spiral and alternate distichous phyllotaxy were observed.

Hena (2005) evaluated 360 accessions of vanilla derived through *in vitro* seed culture. Significant variations in length, breadth, area and number of leaves and total growth were observed in the accessions. Twenty accessions were selected which were highly variable from the 360 accessions studied.

Madhusoodanan *et al.* (2005) carried out on farm evaluation of somaclones of vanilla along with CP plants in three states viz., Kerala, Tamil Nadu and Karnataka during 1996 to 2004. During the initial years, CP plants recorded higher yield while in later years, somaclones recorded higher yield.

2.3 SCREENING OF SOMACLONES FOR RESISTANCE / TOLERANCE TO DISEASES

In the present study, screening of somaclones was attempted by several methods like natural screening in sick field, inducing electrolyte leakage from leaves of somaclones using toxic metabolite(s) of pathogens and artificial screening of somaclones using the pathogen. Hence, the reports on production of toxic metabolite(s) by the pathogen, characterization of toxic metabolite(s) and the various methods of screening against diseases are reviewed here under.

2.3.1 Production of Toxic Metabolite(s) by *Pythium* spp and *In Vitro* Screening for the Disease Using the Metabolite(s)

Toxic metabolite(s) produced *in vitro* by *Pythium* spp. produced symptoms similar to that caused by inoculation of pathogen on host plants like *Atropa belladona* (Janardhanan and Husain 1974), maize (Sadik *et al.* 1982) geranium (Desilets and Berlarger 1991; Desilets *et al.* 1991) etc.

Janardhanan and Husain (1974) reported *in vitro* production of a heat stable toxic metabolite and pectolytic enzyme by *Pythium butleri*, which cause root rot of *Atropa belladona.*

Sadik *et al.* (1982) reported isolation and partial characterization of an extracellular phytotoxin produced by *P. aphanidermatum* (Edson) Fitzp., causing stalk rot disease in maize. The metabolite from culture filtrate produced disease symptoms in maize similar to that caused by inoculation of the pathogen.

Desilets and Berlarger (1991) demonstrated that culture filtrate from *P. ultimum* caused infection in geranium. Desilets *et al.* (1991) isolated active phytotoxic compounds from the culture filtrate of *P. ultimum.* The isolated fractions also produced symptoms of root necrosis and root hair breakdown in geranium. The toxic compound was heat stable and water soluble.

In vitro screening for rhizome rot was attempted in ginger by Kulkarni *et al.* (1987). Tolerant plants were isolated by screening calli of ginger cultivars

Mahim and Poonam Local with culture filtrate of *P. aphanidermatum*. Three rhizome samples out of 114 of cultivar Mahim were tolerant to *Pythium* but all the rhizome samples of cultivar Poonam Local were susceptible to the disease. Babu *et al.* (1996 b) also reported *in vitro* selection of resistant plant types in ginger using culture filtrates of *P. aphanidermatum* and *R. solanacearum*.

2.3.2 Production of Toxic Metabolite(s) by *Ralstonia solanacearum* and *In Vitro* Screening for the Disease Using the Metabolite(s)

Maine (1960) reported that the structural integrity and essential physiological processes of host tissues were affected by the extra cellular hydrolytic enzymes and toxins produced by *R. solanacearum*. Paul (1998) reported the methodology for isolation of toxin from *R. solanacearum* causing bacterial wilt in tomato, brinjal and chilli. The toxic metabolite(s) was thermostable and non-host specific. It produced wilt symptoms in detached twigs and seedlings of tomato, brinjal and chilli.

Husain and Kelman (1958) made detailed studies on wilting symptom induced by *R. solanacearum*. They observed that cutlture filtrate of slime forming virulent strains of bacterium contained a heat stable polysaccharide that played the primary role in wilting. The culture filtrate was found to contain heat sensitive cellulase and pectic enzymes. Heating the filtrate and further inoculation slightly reduced their ability to wilt tomato cuttings.

Gowda *et al.* (1977) studied the biological properties of toxic compounds isolated from *R. solanacearum*. They could partially purify the toxin and found that it was nonspecific. Cuttings of host plants kept in 0.2 per cent aqueous toxin solution wilted whereas plants kept in distilled water as control did not wilt. Samuel (1980) obtained a heat stable viscous substance from the culture filtrate of *R. solanacearum* isolated from ginger which could produce wilting symptoms in healthy ginger shoots and had role in the pathogenicity of the bacterium and symptom expression.

Hartriar *et al.* (1985) screened bean calli with culture filtrate of *Pseudomonas syringae* p.v. *phaseolicoli*, causal agent of halo blight. Callus reaction

to the filtrate ranged from totally necrotic with no growth to no necrosis with normal growth. They suggested that callus screening with culture filtrate of *P. syringae* could identify bean cultivars resistant to halo blight.

Hareesh *et al.* (2001) screened ginger calli in modified MS medium supplemented with different concentrations (4, 8 and 16 %) of cell free culture filtrate (CFCF) of *R. solanacearum.* Survival of calli was inversely correlated to concentration of cell free culture filtrate of the pathogen used. Further growth of calli in toxin free medium was observed only from calli from of per cent toxin amended medium.

2.3.3 Natural Screening of Somaclones Against Diseases by Planting in Infected Field

Screening for diseases by planting in field infected by the pathogen is a general practice attempted in many crop plants to assess the disease reaction.

Asparagus

Wacker *et al.* (1990) screened protoclones of *Asparagus officinalis* cultivar Jersey giant along with resistant species, *A. sperengeri* in soil infested with *Fusarium oxysporium* f. sp. *asparagi* and *F. moniliforme*. Variation in disease resistance was observed in protoclones. Resistance was found low in the protoclones as compared to the resistant species *A. sprengeri.*

Rice

Xie *et al.* (1992) identified two sheath blight resistant lines of rice, LSBR 33 and LSBR 5. These lines were selected by field screening 2000 somaclones of rice in field nurseries inoculated with the sheath blight (*Rhizoctonia solani*) pathogen.

Jerusalem artichoke

Somaclones of Jerusalem artichoke (*Helianthus tuberosus* L.) cultivar Nahodka were selected after screening *in vitro* for basal stem and tuber rot pathogen (*Sclerotinia sclerotiarum*). Selected somaclones when screened in soil heavily

infected with *S. sclerotiarum*, showed no field infection. The clones were also found resistant in artificial inoculation studies (Cassells and Walsch, 1995).

Banana

Mericlones of banana cultivar Giant Cavendish were evaluated and ten *Fusarium* wilt resistant clones were identified. From these, a resistant clone 'Tai chiao No.1' was released for commercial planting in Taiwan during 1992. Surveys conducted in orchards planted with Tai Chiao No.1 showed that the percentage of wilt incidence averaged 6.5 per cent during 1994 as compared to 69.0 per cent in Giant Cavendish. During 1995, the wilt incidence was 5.1 per cent in Tai Chiao No.1 while it was 42.6 per cent in Giant Cavendish (Chuan *et al.*, 2000).

Tomato

Mandal (1999) screened regenerants of tomato varieties BWR 1, BWR 6, PKM 1 and culture 340 derived through indirect organogenesis for resistance to bacterial wilt disease by planting in sick field. The regenerants exhibited variability for disease reaction. From the study, few elite clones with bacterial wilt resistance could be isolated.

Devi *et al.* (2005) screened regenerants of tomato variety Shakthi derived through indirect organogenesis along with regenerants of resistant contol, *Lycopersicon pimpinellifolium* for resistance to tomato leaf curl virus. Two calliclones of Shakthi and all the calliclones of *L. pimpinellifolium* were not affected by the virus.

2.3.4 Screening for Diseases by Electrolyte Leakage Method

Assay based on leakage of electrolytes from plant tissues challenged with culture filtrates or toxins has been used in several studies to differentiate susceptible and resistant plants. In several species, loss of electrolytes occurs in tissues infected with the fungus or treated with its toxin (Wheeler and Black, 1963; Samaddar and Scheffer, 1968). Damann *et al.* (1974) showed that an electrolyte leakage assay by

Helminthosporium victoriae toxin is as sensitive as standard seedling root growth assay.

Rice

Selection to brown spot disease in rice using toxin isolated from *Helminthosporium oryzae* was done by Vidyasekharan *et al.* (1990). They screened callus cultures of rice variety IR 8 with toxic metabolite(s) of highly virulent, less virulent and non-pathogenic *H. oryzae* isolates by electrolyte leakage method. Toxic metabolite(s) of highly virulent isolate induced highest leakage of electrolytes. Non-pathogenic isolates did not induce any leakage of electrolytes.

Sugarcane

Leal and Maribona (1991) screened leaves and calli of sugarcane with *Helminthosporium sachari* toxin by electrolyte leakage method and isolated resistant somaclones.

Sundar *et al.* (1999) screened sugarcane varieties Co 671 (susceptible to red rot) and B 091 (resistant to red rot) for resistance to red rot disease. Leaves, internode and calli were screened by electrolyte leakage method using *Colletotrichum falcatum* toxin. The susceptible variety Co 671 recorded a significant increase in loss of electrolytes as compared to that of the resistant variety B O91.

Tomato

Cristinzio and Saccardo (1994) screened different accessions of cultivated and wild species of the genus *Lycopersicon* for resistance to *Phytophthora nicotianae* by electrolyte leakage method. The resistance shown by one accession of *L. esculentum* var. *cerasiforme* was confirmed by artificial inoculation of the pathogen.

Cucumber

Callus response of *Cucumis melo* to toxins produced by *Myrothecium roridum* was studied by Mackay *et al.* (1994). They suggested that electrolyte leakage

studies of calli were not conclusive in establishing membrane as the site of action of toxin. They concluded that electrolyte leakage studies were not useful for *in vitro* screening.

Brinjal

Cristinzio *et al.* (1995) measured eggplant resistance to *Verticilium* wilt (*Verticillium dahliae*) based on electrolyte leakage assay of callus tissue. Loss of electrolytes from the resistant genotypes was significantly lower than that from the susceptible genotypes. *Solanum melongena* lines CCR3 and SM 19/14 and the wild species *S. torvum* exhibited low leakage of electrolytes and were most resistant in artificial screening. The most susceptible cultivars, Lunga violetta and Giant of China exhibited higher leakage of electrolytes.

Black pepper

Shylaja (1996) screened leaves and calli of five black pepper cultivars and *Piper colubrinum*, a species resistant to *Phytophthora* foot rot using concentrated culture filtrate of *Phytophthora* capsici. The highest leakage of electrolytes was shown by Panniyur–1 while the lowest by *P. colubrinum*. The pattern of electrolyte leakage from leaves and calli followed the same trend. She found that tolerance/resistance expressed at the whole plant level was transferred almost to the same extent to induced calli of different cultivars. When regenerants derived from screened and unscreened calli were screened for the disease, she observed low leakage of electrolytes in some regenerants derived from unscreened calli suggesting the possibility of exploiting somaclonal variation as such with out *in vitro* screening for *Phytophtrhora* foot rot resistance in black pepper.

Pineapple

Somaclones of pineapple cultivars Perolera (resistant to *Fusarium subglutinans*) and smooth Cayenne (susceptible to *F. subglutinans*) were screened by electrolyte leakage method using culture filtrate of *F. subglutinans*. Smooth Cayenne

was most sensitive to the culture filtrate while Perolera showed reistance to the culture filtrate (Hidalgo *et al.*, 1998).

2.3.5 Screening by Artificial Inoculation of Pathogen

Rice

Araujo *et al.* (2001a) screened 17 regenerants of rice cultivar IAC 47 derived through indirect organogenesis to rice blast disease along with parent plants. Screening was done in a green house. Plants were inoculated with spore suspension (3×10^5 spores ml^{-1}) of *Pyricularia grisea.* From the study, two somaclones were identified which were resistant to all the isolates of *P. grisea.*

Black pepper

Shylaja and Nair (1996) screened regenerants of black pepper (*Piper nigrum* L.) cultivars derived through indirect organogenesis against *Phytophthora* foot rot disease. The disease reaction of the calliclones was assessed using leaf symptom bioassay by artificially inoculating culture disc of *P. capsici.* Out of the calliclones of four cultivars viz., Kalluvally, Balankotta, Karimunda and Cheriakanyakkadan evaluated, the clones of cultivar Kalluvally exhibited higher variability in disease reaction while the clones of cultivar Cheriakanyakkadan showed lesser variability and were tolerant to the disease. Sanchu *et al.* (2003) also assessed *Phytophthora* foot rot disease reaction in calliclones of black pepper cultivar Cheriakanyakkadan using leaf symptom bioassay. They identified three calliclones, highly tolerant to the disease.

Asparagus

Wacker *et al.* (1990) screened protoclones of Asparagus against *Fusarium moniliforme* and *F. oxysporum* under green house condition and wide variation in disease incidence was noticed in the somaclones.

Banana

Matsumato *et al.* (1999) evaluated mericlones of banana produced from *Fusarium* wilt susceptible var. Maca. Disease resistance was evaluated by artificial

inoculation with the pahogen under green house condition. Regenerants that showed tolerance were transplanted to field and different levels of field resistance to *Fusarium oxysporum* f. sp. *cubense* were observed in the regenerants.

Peach

Hammerschlag *et al.* (1994) reported increased levels of disease resistance to bacterial leaf spot (*Xanthomonas campestris* pv. *pruni*) in toxin screened and unscreened peach regenerants under green house and field conditions.

Apple

Chevreau *et al.* (1998) screened four somaclonal variants of apple var. Greensleeves for fire blight disease (*Erwinia amylovora*) under field and green house condition and the clone R 46/3 was less susceptible to the disease.

Sweet potato

Jin *et al.* (2001) screened 90 sweet potato varieties in green house with *R. solanacearum* culture and crude toxins isolated from the bacteria. They observed significant positive correlation between the disease indices of *R. solanacearum* and toxins isolated from the bacterium. They suggested that crude toxins of the isolates of *R. solanacearum* provide a cheap and exact tool for identifying the resistance to the pathogen. Yong *et al.* (2002) screened sweet potato varieties using crude toxin extracted from *R. solanacearum* in the green house. They found that the variety 97-10 have excellent resistance to *R. solanacearum*. When this variety was screened in diseased areas of Fujian province, it showed good resistance against wilting.

Cardamom

Potted plants of promising cardamom lines were inoculated with spore suspension of *Pythium vexans* to isolate tolerant lines to azhukal disease. Two clones viz., MCC-75 and MHC-10 recorded least incidence of the disease. MHC-10 exhibited tolerance to all the three rhizome rot pathogens viz., *Rhizoctonia solani*, *Fusarium oxysporum* and *P. vexans* (ICRI, 2003).

Ginger

Kavitha *et al.* (2005) studied the resistance/tolerance reaction of five *Zingiber* spp. and other related genera of family Zingiberaceae to rhizome rot disease. Potted plants were inoculated with zoospore suspension (2 x 10^6 spores/shoot) of *Pythium aphanidermatum.* Ginger variety Varada and cultivar Maran were totally susceptible. Wild ginger collected from Wayanad was relatively tolerant. Two accessions of *Z. zerumbet* showed no disease symptoms. Similarly, seven species viz., *Alpinia calcarata, A. galanga, Costus speciosus*, *Curcuma zeodaria*, *Hedichium spicatum*, *Kaempferia galanga* and *K. rotunda* were also not affected by the disease.

Rapeseed

Liu *et al.* (2005) screened double haploid lines of rapeseed (*Brassica napus*) derived from *in vitro* mutagenesis against stem rot disease (*Sclerotinia sclerotiarum*) by five different screening methods viz., *in vitro* screening by toxic metabilite(s), leaf symptom bioassay by artificial inoculation of the pathogen, planting after soaking seedlings in toxin solution, planting seedlings in sick soil under green house and field conditions. Two mutants viz., M083 and M004 were selected with greater resistance to the disease than the donor lines and the resistant control Zhongyou 821.

2.4 MOLECULAR CHARACTERISATION OF SOMACLONES USING RAPD MARKERS

Advances in biotechnology have provided several DNA based molecular markers that open up new vistas in finger printing and documenting genetic variation in plants.

Rice

Polymorphism in banding pattern was detected in regenerants of upland rice cultivar IAC 47 regenerated through indirect organogenesis (Araujo *et al.*, 2001a). The somaclones showed morphological variations and exhibited differences in

reaction to rice blast. In the dendrogram, all the susceptible somaclones clustered in one group and resistant clones in other group.

Lathyrus

Mandal *et al.* (1996) reported polymorphism in RAPD banding pattern in seven somaclones of *Lathyrus sativus*. Wide variability in morphological characters was observed in somaclones and between somaclones and control plant. Similarly, polymorphism in RAPD banding pattern was also detected within somaclones and between somaclones and control plant.

Sugar beet

Munthali *et al.* (1996) employed RAPD analysis for detection of somaclonal variation in sugar beet regenerated through adventitious shoot buds from leaf explants of a single genotype. RAPD analyses were done in parental plant and 120 regenerants using five decanucleotide primers. They observed two polymorphisms in banding pattern and the frequency of polymorphism obtained was similar to the frequencies previously reported in the same clones by isozyme and RFLP techniques.

Garlic

Zahim *et al.* (1999) studied variation in somaclones of five garlic cultivars regenerated through somatic embryogenesis using RAPD markers. RAPD analysis of somaclones revealed a total of 7903 bands, of which 50 were polymorphic. They observed that the frequency of variation was cultivar dependent.

Alfalfa

Piccioni *et al.* (1997) observed no RAPD polymorphism between somaclones of alfalfa regenerated through enhanced release of axillary buds and mother plant. But plantlets regenerated by indirect somatic embryogenesis differed from the donor plant in RAPD profiles.

Tomato

Soniya *et al.* (2001) studied genetic stability of regenerants in tomato variety Shakthi derived through indirect organogenesis using RAPD markers. They observed 90 to 99 per cent genetic similarity between the calliclones and mother plant.

Oil palm

Rival *et al.* (1998) conducted RAPD analysis to investigate the genetic stability of somatic embryo derived clones of oil palm. No intraclonal variability and no difference between mother palm and the regenerants were identified from the analysis.

Tea

Mandal and Chand (2002) characterized 17 somaclones of tea along with control plant using RAPD markers. Four somaclones exhibited polymorphism in banding pattern while 13 somaclones and the control plant showed monomorphism in banding pattern.

Loblolly pine

Tang (2001) characterized 21 regenerants of loblolly pine derived through indirect organogenesis using RAPD markers. Amplification products were monomorphic in all the regenerants tested.

Ginger

Rout *et al.* (1998) studied genetic stability of 14 mericlones of ginger cultivar V_3S_{18} using RAPD markers. Out of 15 decamer primers tried for DNA amplification, three produced amplification. Amplification products were monomorphic for all the clones analysed.

Black pepper

Babu (2000) assessed genetic stability and clonal fidelity of regenerants of black pepper varieties Panniyur-1, 2, 3, 4 and cultivar Karimunda derived through

axillary bud culture using RAPD markers. All the regenerants exhibited uniform banding pattern with their respective source plants. The study confirmed the uniformity and genetic stability of axillary bud regenerants of black pepper. Sujatha (2001) also characterized axillary bud regenerants of black pepper varieties Panniyur-1, 2, 4 and Subhakara using RAPD and isozyme markers. Two clones each of Panniyur-1 and 2 exhibited polymorphism in banding pattern while other clones showed monomorphism.

Turmeric

Salvi *et al.* (2001) reported polymorphism in RAPD banding pattern in regenerants of turmeric cultivar Elite derived through indirect organogenesis. In their further studies with adventitious bud regenerants, they observed no RAPD polymorphism between somaclones and the CP plant (Salvi *et al.* 2002).

Riji (2003) attempted RAPD analysis of 22 regenerants of turmeric derived through indirect organogenesis along with CP plant. Nine variants were detected which exhibited polymorphism in RAPD profiles. None of the calliclones showed similarity towards the CP plant. The clone TSC-1 showed highest polymorphism with the CP plant.

Vanilla

Kuriakose *et al.* (2005) conducted RAPD analysis of embryo derived clones of *Vanilla planifolia* using fifteen primers. Genetic variability to the extent of 58 per cent was observed in embryoclones.

Hena (2005) characterised 20 accessions of vanilla derived through *in vitro* seed culture using RAPD markers. Accessions were grouped into three major clusters with 55 per cent variability.

Mango ginger

Prakash *et al.* (2004) reported polymorphism in RAPD banding pattern in regenerants of mango ginger derived through indirect organogenesis.

Materials and Methods

MATERIALS AND METHODS

The present investigations on "Induction of variation *in vitro* and field evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" were carried out at the Department of Plantation Crops and Spices and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara, Thrissur during April 2002 to October 2005. The study mainly focused on the following aspects.

I. Induction of variation through *in vitro* techniques and production of regenerants (second set somaclones)

II. Field evaluation of already developed somaclones through adventitious bud culture (first set somaclones)

III. Screening of first and second sets of somaclones against rhizome rot and bacterial wilt diseases

IV. Molecular characterization of selected superior somaclones of first set using RAPD markers

The experimental materials used and methodology adopted for conducting various aspects of the study are presented in this chapter.

3.1 INDUCTION OF VARIATION IN GINGER THROUGH *IN VITRO* TECHNIQUES AND PRODUCTION OF REGENERANTS

3.1.1 Induction of Variation Through Indirect Organogenesis / Embryogenesis

3.1.1.1 Source of Explants

The explant sources used for the study included rhizome buds and axenic plants of two ginger cultivars viz., Maran and Rio-de-Janeiro.

3.1.1.1.1 Rhizome Buds

Seed rhizomes of the two cultivars were collected from Regional Agricultural Research Station, Ambalavayal. Rhizome bits after seed treatment with 0.3 per cent Indofil M 45 for 30 minutes were germinated in sterile sand (Plate 1a). Pale yellow sprouts from rhizomes were excised, washed thoroughly, removed the scale leaves and dipped in

teepol solution for 10 minutes and washed with distilled water. The sprouts, after treating with 0.1 per cent Indofil M-45 for 30 minutes were surface sterilized with 0.1 per cent $HgCl_2$ for 10 minutes, washed free off the sterilant, dried and inoculated to modified MS medium supplemented with BAP (3.00 mg l^{-1}).

3.1.1.1.2 Axenic Plants

The protocol reported by Shylaja *et al.* (2003) was followed for producing adventitious bud regenerants in ginger. From the regenerants (Plate 1b), explants like pseudostem (base, middle and top), shoot tip, folded and unfolded leaves were used for inducing organogenic/embryogenic calli.

3.1.1.2 Preparation of Culture Medium

The major and minor elements required for the preparation of various media were of analytical grade and procured from M/s. BDH Laboratories, Sisco Research Laboratories Private Limited (SRL) and Merck Limited. The amino acids, vitamins and plant growth regulators used were of M/s. Merck Limited and Sigma-Aldrich Co.

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was used at half strength in the present investigation. The composition of the medium is given in Appendix-I.

Standard procedures (Gamborg and Shyluk, 1981) were followed for the preparation of MS medium. The pH of the medium was adjusted to 5.6 - 5.8 using 0.1 *N* NaOH or 0.1 *N* HCl. The medium was solidified with 0.7 per cent good quality agar and distributed to test tubes (15 x 2.5 cm) @ 15 ml each. The test tubes were then plugged with non-absorbent cotton. Autoclaving was done at 121°C at 15 psi (1.06 kg/cm^2) for 20 minutes (Dodds and Roberts, 1982). The medium was allowed to cool at room temperature and stored at 26±1°C in the culture room for use within one week.

3.1.1.3 Transfer Area and Aseptic Manipulations

All the aseptic manipulations were carried out under the hood of a clean laminar air flow cabinet (Klenzaids).

1a Sprouting buds from ginger rhizomes

1b *In vitro* adventitious bud cultures of ginger

Plate 1 Explant sources

3.1.1.4 Culture Conditions

The cultures were incubated at 26±1°C in an air-conditioned culture room with 16 h photoperiod (1000 lux) supplied by fluorescent tubes unless otherwise mentioned. Humidity in the culture room varied between 60 to 80 per cent according to the climatic conditions prevailed.

3.1.1.5 Indirect Organogenesis

Indirect organogenesis was attempted in two cultivars viz., Maran and Rio-de-Janeiro using different explants and growth regulator combinations.

3.1.1.5.1 Standardisation of Explants, Growth Regulators and Culture Conditions for Callus Induction

Rhizome buds and different parts of adventitious bud regenerants viz., pseudostem (base, middle and top), shoot tip, folded and unfolded leaves were used as explants for induction of organogenic calli.

Growth regulators like 2,4-D and BAP were incorporated to basal medium at various levels singly and in combinations for inducing calli from different explants. The cultures were incubated in culture room under two conditions viz., dark and light.

Observations were recorded on days taken for callusing, percentage cultures initiating calli and growth score of the calli. Scoring was done based on the spread of the calli and a maximum score of four was given to those that have occupied the whole surface of the medium in the culture tubes. Callus Index (CI) was worked out as follows.

$$CI = P \times G$$

where P is the percentage of cultures initiating calli and G is growth score.

3.1.1.5.2 Standardisation of Growth Regulators and Media Supplements for Shoot Morphogenesis

Growth regulators like 2,4-D, NAA and BAP and media additives like $AgNO_3$ and charcoal were incorporated to the basal medium at different levels singly and in

combinations to standardise the best growth regulator/combination for shoot morphogenesis from induced calli.

Observations were recorded on number of days taken for shoot morphogenesis, percentage regeneration and average number of shoots produced per culture. The regenerated cultures of cultivars Maran and Rio-de-Janeiro were subcultured at one month interval to MS medium supplemented with BAP (3.00 mg l^{-1}) for further shoot proliferation and rooting.

3.1.1.6 Somatic Embryogenesis

Somatic embryogenesis was attempted in two cultivars viz., Maran and Rio-de-Janeiro using rhizome bud explants.

3.1.1.6.1 Standardisation of Growth Regulators and Culture Conditions for Induction of Embryogenic Calli

Growth regulators like 2,4-D and BAP were incorporated to basal medium at different levels singly and in combinations for inducing embryogenic calli from rhizome buds. The cultures were incubated in culture room under two conditions viz., dark and light.

Calli induced were viewed under stereomicroscope and observed for somatic embryogenesis. Percentage of embryogenic calli, number of days taken for induction of somatic embryoids and number of somatic embryoids per culture were recorded.

3.1.1.6.2 Proliferation and Germination of Somatic Embryoids

The somatic embryoids induced were transferred to half MS basal medium and half MS medium supplemented with BAP (3.00 mg l^{-1}) for proliferation and germination of embryoids. The rate of proliferation of somatic embryoids, time taken for germination and percentage germination were recorded.

3.1.2 *In Vitro* Induction of Mutation Using γ Irradiation

The γ irradiation facility available at the Radio Tracer Laboratory, College of Horticulture, Kerala Agricultural University was utilized for the experiment. A ^{60}Co source (Gamma chamber 900 of BARC, Mumbai) with a dose rate of 306.8 Gy / h was used for irradiation.

3.1.2.1 Standardisation of γ Irradiation Dose for In Vitro Mutagenesis

Morphogenic cultures derived from organogenic / embryogenic calli of two cultivars viz., Maran and Rio-de-Janeiro with shoot tips less than 5.00 mm length were subjected to γ irradiation at doses ranging from 10 to 50 Gy at intervals of 10 Gy. After the irradiation, shoot tips were immediately transferred to half strength MS medium supplemented with 3.00 mg l^{-1} BAP. Percentage of cultures putting fourth growth and proliferation rate of shoots at different doses of irradiation were observed. Based on survival of cultures and multiplication rate of shoots, the doses of irradiation that could withstand by different groups of cultures were selected.

3.1.2.2 Irradiation of In Vitro Cultures in the Selected Dose of γ Irradiation

Large-scale irradiation of organogenic / embryogenic callus cultures at the shoot morphogenesis stage were done in the selected dose of γ irradiation. Irradiated cultures were inoculated to regeneration medium. Observations were recorded on the number of shoots regenerated in one month subculture cycle.

3.1.3 Root Characters of Regenerants Derived Through Somatic Organogenesis / Embryogenesis

The plantlets produced through indirect organogenesis, embryogenesis and *in vitro* mutagenesis were compared with respect to number of roots produced and length of roots.

3.1.4 Planting Out, Hardening, Final Survival and Growth of Regenerants

Plantlets with well developed pseudostem and roots were washed free off the medium and planted in bags of size 11 x 8 cm filled with sterile sand, kept under shade

and watered daily. After two weeks, the plantlets were transferred to big poly bags of size 12 x 8" filled with potting mixture in the proportion 1:1:1 sand, soil and cow dung. The polybags were kept in the net house and watered daily.

The plantlets obtained through indirect organogenesis/embryogenesis and *in vitro* mutagenesis were observed for growth parameters for a period of three months and growth observations were recorded at monthly intervals. Observations on number of tillers produced, height of pseudostem and length of the longest leaf were recorded.

3.2 FIELD EVALUATION OF FIRST SET SOMACLONES IN GINGER

3.2.1 Experimental Material

Somaclones of ginger regenerated through *in vitro* adventitious bud culture after passing through 10 to 12 subculture cycles and planted out during 1999-2000 formed the base material for the study.

Eighty three somaclones of cultivar Maran and 87 somaclones of cultivar Rio-de-Janeiro with enough rhizomes for field planting were field planted and evaluated for morphological, yield, quality attributes and resistance/ tolerance to rhizome rot and bacterial wilt diseases along with conventionally propagated (CP) plants for a period of three years (2002-2004).

3.2.2 Management of Crop

The field was prepared by ploughing and mounds of size 50 cm height and 30 cm base diameter were taken at a spacing of 40 cm. Raised beds of size 1 x 1 m were prepared with an interspace of 40 cm between beds. Rhizome bits of 15 to 20 g weight were used as seed material. In first year, planting was done in mounds. In second and third year, planting was done in beds or mounds as per the availability of seed rhizomes. The crop was managed as per the package of practices recommendations of Kerala Agricultural University (KAU, 2002).

3.2.3 Morphological Characters

Morphological characters of the field planted somaclones were recorded at two months interval. Characters like height of pseudostem, number of tillers per plant,

number of leaves per tiller, length, width and area of the last fully opened leaf were recorded. Leaf area was computed as reported by Giridharan (1984).

3.2.4 Incidence of Pests and Diseases

3.2.4.1 Incidence of Shoot Borer

Incidence of shoot borer (*Conogethes punctiferalis*) was recorded as number of infected tillers per clump, and was expressed as percentage infection of total number of tillers. The incidence was recorded from June to October in three years of evaluation.

3.2.4.2 Incidence of Rhizome Rot

Incidence of rhizome rot caused by *P. aphanidermatum* was recorded at fortnightly intervals during the entire growth period of the clones. The percentage infection was worked out noting the number of plants infected out of the total number of plants planted.

3.2.4.3 Incidence of Bacterial Wilt

The incidence of bacterial wilt caused by *R. solanacearum* was recorded at fortnightly intervals during the growth period. The percentage infection was worked out noting the number of plants infected out of the total number of clones planted.

3.2.4.4 Incidence of Leaf Spot

The incidence of leaf spot caused by *Phyllosticta zingiberi* was scored based on the number of spots on the leaf i.e. very light (1 spot), light (2 to 5 spots), medium (6 to 15 spots), severe (16 to 40 spots) and very severe (more than 60 spots) (Nybe, 1978). From each plant, ten leaves were randomly selected for taking observations.

3.2.5 Yield and Quality Attributes of Somaclones

3.2.5.1 Rhizome Characters

Yield contributing characters such as the number of primary, secondary and tertiary fingers, length, girth and internodal length of fingers in a rhizome were recorded at the time of harvest.

The rhizomes originating from the seed material were taken as primary fingers and rhizomes from the primary finger as secondary fingers and rhizomes from from secondary finger as tertiary fingers. Length, girth and internodal length of primary and secondary fingers and thickness of inner core of rhizome were measured and mean worked out. Flesh colour, colour of scale leaves, plumpiness and orientation of the rhizomes were also recorded.

3.2.5.2 Fresh Yield of Rhizome

The rhizomes were harvested eight months after planting by uprooting individual clumps. The fresh yield was expressed in (g) as yield per somaclone.

3.2.5.3 Quality Attributes

The selected 15 high yielding somaclones were evaluated for quality attributes like dry ginger recovery, volatile oil, oleoresin and crude fiber content along with control. Two replications were maintained for each parameter and mean was worked out.

3.2.5.3.1 Dry Ginger Recovery Percentage

The percentage recovery of dry rhizome to fresh rhizome was estimated by sun drying 400 g of fresh rhizome immediately after harvest until a constant weight was obtained.

3.2.5.3.2 Estimation of Essential Oil

Volatile oil was estimated by water cum steam distillation, adopting clevenger method as per AOAC (1980) and the recovery of essential oil was expressed as percentage. Twenty grams of ground ginger powder from each somaclone was used for estimation of volatile oil.

3.2.5.3.3 Estimation of Oleoresin

The content of oleoresin in the sample was estimated using the Soxhlet method of extraction as per AOAC (1980). Five grams of powdered sample was reflexed with 250 ml of acetone. Extraction was continued till the solvent became colourless.

The acetone extract of the sample was transferred to a pre-weighed beaker and the solvent was evaporated and the weight of the beaker was recorded. The recovery of oleoresin was expressed in percentage.

3.2.5.3.4 Estimation of Crude Fibre

The content of crude fibre was estimated as per Sadasivam and Manickam (1992) and expressed as percentage.

3.3 SCREENING OF SOMACLONES AGAINST RHIZOME ROT AND BACTERIAL WILT DISEASES

3.3.1 Production of Toxic Metabolite(s) by *P. aphanidermatum* and Bioassay of the Metabolite(s)

3.3.1.1 Isolation of P. aphanidermatum

The pathogen causing rhizome rot of ginger was isolated from naturally infected rhizomes by following standard isolation procedures (Ricker and Ricker, 1936). The pure culture of the fungus was maintained on potato dextrose agar slants by frequent subculturing (Plate 2). Pathogenicity of the isolated culture was tested by inoculating seven-day-old culture of *P. aphanidermatum* to healthy surface sterilized ginger rhizomes. Inoculated rhizomes were kept in aseptic moist chamber and incubated at room temperature till rotting of rhizomes was observed.

3.3.1.2 Standardisation of Media and Culture Conditions for Production of Toxic Metabolite(s) by P. aphanidermatum

Five mm culture discs of seven day old culture of *P. aphanidermatum* were inoculated to three different liquid media viz., M_1 [Czapek (Dox) agar], M_2 (Richard's solution) and M_3 (Asparagine or synthetic mucor) and incubated for two durations viz., 10 and 15 days in a shaker cum incubator maintained at 27°C with a shaking speed of 100 rpm. Stationary cultures of the pathogen were also maintained in three liquid media for two incubation periods viz., 10 and 15 days. The culture filtrate was collected after filtering successively the liquid cultures through a muslin cloth and Whatman No.1 filter paper. The filtrate was concentrated to one tenth of its volume by keeping the filtrate in a hot plate maintained at 100^0 C to produce concentrated culture filtrate (CCF). Various

Plate 2 Pure culture of *Pythium aphanidermatum*

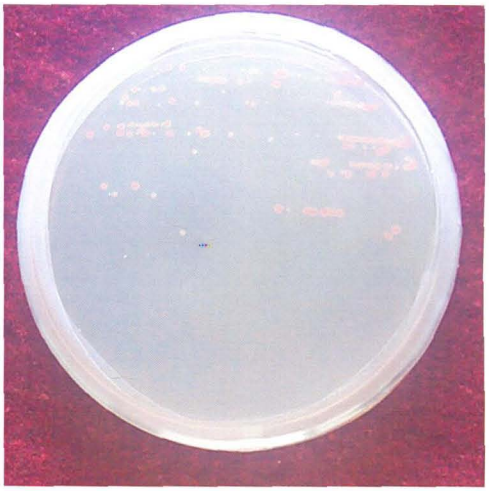


Plate 3 Pure culture of *Ralstonia solanacearum*

media used for the production of toxic metabolite(s) were also concentrated in the same way to get medium control for inoculation/leakage studies.

3.3.1.3 Bioassay of Toxic Metabolite(s) of P. aphanidermatum

Bioassay of toxic metabolite(s) of *P. aphanidermatum* was done by two methods viz., by inoculating CCF to ginger rhizomes and by inducing electrolyte leakage from leaves.

3.3.1.3.1 Bioassay Using Ginger Rhizomes

Mature ginger rhizomes were surface sterilized and five mm deep holes were made using a cork borer. Toxigenicity of the toxic metabolite was tested by inoculating 0.2 ml of CCF in the holes made on the rhizomes. Rhizomes inoculated with concentrated medium without toxic metabolite(s) served as control. Culture discs of *P. aphanidermatum* were also inoculated to rhizomes to compare the symptoms induced by pathogen and toxic metabolite(s) produced by the fungus. Inoculated rhizomes were kept in moist chambers and incubated at room temperature till rotting symptoms were observed. The average diameter of the infected area was calculated.

3.3.1.3.2 Bioassay by Electrolyte Leakage Method

Electrolyte leakage studies were conducted in medium mature leaves of cultivars Maran and Rio-de-Janeiro. Leaves for electrolyte leakage studies were prepared as described by Vidyasekharan *et al.* (1986) and Shylaja (1996). Conductance of ambient solutions was measured in μ Siemens (μs) with a high precision Systronics 20 conductivity meter.

Concentrated culture filtrate extracted from various treatments (three different media, two types of culture and two different incubation periods) were diluted to two dilutions viz., 5 and 10 per cent (v/v). The electrolyte leakage induced from leaves of cultivars Maran and Rio-de-Janeiro by CCF (extracted from 24 treatments) was recorded at 10 minutes interval. The time at which the difference in leakage between successive time intervals appeared maximum was observed and compared for different treatments.

From the 24 treatments, the treatment inducing maximum leakage over medium control was selected for further screening studies.

3.3.2 Production of Toxic Metabolite(s) by *R. solanacearum* and Bioassay of the Metabolite(s)

3.3.2.1 Isolation of R. solanacearum

Ginger plants showing symptoms of bacterial wilt disease were collected. The rhizomes and pseudostem of diseased plants thus collected were subjected to ooze test to confirm the presence of bacterium. Such pieces with profuse bacterial ooze were used for the isolation of the pathogen. They were cut into small bits and surface sterilized with 0.1 per cent mercuric chloride solution for one minute and then washed free off the sterilant. These bits were crushed on a sterilized glass slide with a few drops of sterile distilled water to obtain a bacterial suspension. One loopful of suspension was streaked on Triphenyl Tetrazolium Chloride (TZC) medium to get well-isolated colonies of the bacterium (Kelman, 1954) (Plate 3). The composition of TZC medium is given in Appendix-I.

The cultures were incubated at room temperature for 48 h. Characteristic light pink centered slimy, fluidal bacterial colonies were purified by repeated streaking on TZC medium.

3.3.2.2 Production of Toxic Metabolite(s) by R. solanacearum

The toxin from *R. solanacearum* was isolated as described by Paul (1998). *R. solanacearum* was cultured in peptone casamino acid broth for five days under shaking condition. The bacterial broth was then autoclaved at 15 Ibs pressure for 20 minutes and then filtered. Toxin was precipitated from the extract by adding acetone. The precipitate was allowed to settle overnight and then separated by centrifugation at 5600 rpm for 20 minutes. The precipitate was then washed with acetone and kept for evaporation. The toxic metabilite(s) was then dissolved in distilled water.

3.3.2.3 *Bioassay of Toxic Metabolite(s) of R. solanacearum*

Bioassay of *R. solanacearum* toxin was done by two methods viz., bioassay using ginger shoots and by inducing electrolyte leakage from leaves.

3.3.2.3.1 Bioassay Using Ginger Shoots

The extracted toxin was diluted to different dilutions viz., 1, 5, 10, 25, 50 and 75 per cent v/v with distilled water. Medium mature shoots of ginger were dipped in toxin solutions of varying dilutions and also in toxin solution without dilution for five days. The shoots were then transferred to distilled water and kept for two days. Observations on wilting symptoms were recorded daily. Shoots kept in distilled water served as control.

3.3.2.3.2 Bioassay by Electrolyte Leakage Method

The electrolyte leakage induced from leaves of cultivars Maran and Rio-de-Janeiro, using toxins of different dilutions (2.5, 5.0, 7.5 and 10.0 per cent v/v) was recorded at 10-minutes interval. The time at which the difference in leakage appeared maximum over control (distilled water) was observed. The treatment inducing maximum leakage over control was selected for further screening studies.

3.3.3 Preliminary Screening of Second Set Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

Preliminary screening against rhizome rot and bacterial wilt diseases were carried out in one forty six regenerants of cutivar Maran and 150 regenerants of cultivar Rio-de-Janeiro by electrolyte leakage method. The leaves collected from the clones three months after planting were used for inducing electrolyte leakage with toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum*.

3.3.4 Screening of First Set Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

Detailed screening against diseases was carried out in first set somaclones derived through *in vitro* adventitious bud culture. Three types of screening methods viz.,

natural screening by planting in infected field, screening by electrolyte leakage method and artificial screening using pathogen were attempted in the clones.

3.3.4.1 Natural Screening of Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

Seventy three somaclones of cultivar Maran and 78 somaclones of cultivar Rio-de-Janeiro were screened by planting in a sick field where ginger grown in the previous season was severely infected by rhizome rot and bacterial wilt diseases. Inoculum level in the field was made uniform by ploughing and rhizome bits of somaclones and control plants were planted in mounds. Incidence of the diseases were recorded at fortnightly intervals during the growth period. The percentage infection was worked out noting the number of plants infected, out of the total number planted.

3.3.4.2 Screening by Electrolyte Leakage Method

Seventy seven somaclones of cultivar Maran and 75 somaclones of cultivar Rio-de-Janeiro were screened for resistance/tolerance to rhizome rot and bacterial wilt diseases by electrolyte leakage method.

The disease reaction of somaclones in different methods of screening were also compared.

3.3.4.3 Artificial Screening of Selected Superior Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

Artificial screening experiments were conducted during July to October 2005 in ten somaclones of ginger selected based on yield and disease tolerance. Inoculation was done in a shaded net house with misting facility.

3.3.4.3.1 Artificial Screening Against Rhizome Rot Disease

Artificial screening of somaclones for resistance/tolerance to rhizome rot was done as reported by Shanmugham (1996) with minor modifications. Ginger sprouts emerged from 15 g seed bits, one month after sprouting were used for artificial inoculation. These sprouts were planted in polybags (22 X 15 cm) filled with potting mixture prepared with sand, cow dung and sick soil (soil heavily infected with rhizome

rot disease along with diseased rhizome bits) in the proportion 1:1:1. Three replications were maintained for each clone. The symptoms were recorded daily for a period of six weeks. The symptoms were recorded using a score chart.

Disease score

0 - No disease

1 - 1 - 25 % leaves showing yellowing

2 - 26 - 50 % leaves showing yellowing

3 - 51 - 75 % leaves showing yellowing

4 - More than 75 % leaves showing yellowing and subsequent death

3.3.4.3.2 Artificial Screening Against Bacterial Wilt Disease

One-month-old ginger plants with an average of six leaves/tiller were used for screening studies. Fresh bacterial ooze was collected from wilted ginger plants. Pinpricks were made on second leaf axil and inoculation was done by placing a piece of cotton dipped in the bacterial ooze in the pinpricked area.

Wilt symptoms were recorded daily for a period of six weeks using score chart suggested by Prior and Steva (1990).

Disease score

1 - No symptoms

2 - Inoculated leaf wilted

3 - Two or three leaves wilted

4 - Four or more leaves wilted

5 - Plant dead

3.4 MOLECULAR CHARACTERIZATION OF SELECTED SUPERIOR SOMACLONES

Twelve selected somaclones (eight somaclones of cultivar Maran and four somaclones of cultivar Rio-de-Janeiro), which were found superior in yield and disease reaction in two years field evaluation trials were subjected to RAPD analysis along with CP plants.

3.4.1 Isolation of DNA

Juvenile leaves (last formed leaf) taken from the selected ginger somaclones and CP plants using sterile blade and surface sterilized with 70 per cent alcohol were used for extraction of genomic DNA. DNA isolation methods suggested by Doyle and Doyle (1987) (method I and Ia) and Rogers and Bendich (1994) (method II) were tried with modifications for the extraction of genomic DNA. Reagents required for the preparation of stock and working solutions for isolation of DNA and RAPD analysis are given in Appendix II.

3.4.1.1 Method I

Leaf sample (500 mg) was ground in an autoclaved mortar and pestle with 4.00 ml of 1X extraction buffer and 2.50 ml ß mercaptoethanol. The homogenate was then poured into a centrifuge tube (50 ml) containing three ml of lysis buffer and one ml sarcosin (5%). The contents were mixed by inversion and incubated in water bath at 65°C for 15 minutes. Equal volume of chloroform:isoamyl alcohol (24:1) mixture was added to the sample, mixed gently by inversion and centrifuged at 10,000 rpm for 15 minutes at 4°C. The upper aqueous phase was pipetted out and transferred to a 50 ml centrifuge tube and added 2/3rd volume of chilled isopropanol. The contents were mixed by gentle inversion until the DNA was precipitated and the sample was kept at -20°C for 30 minutes. The DNA was pelleted by centrifuging at 10,000 rpm for 15 minutes at 4°C. The isopropanol was poured off, drained well and the pelleted DNA was washed with 90 per cent alcohol. The pellet was then allowed to air dry and resuspended in 250 µl of TE buffer.

3.4.1.2 Method Ia

Method I was further modified for the quantity of ß mercaptoethanol added and incubation period given for ground leaf in the extraction buffer. The quantity of ß mercaptoethanol was reduced to 20 µl instead of 2.5 ml in the method I. The ground leaf and extraction buffer mixture was incubated for 30 minutes at 65°C instead of 15 minutes in Method I.

3.4.1.3 Method II

One gram leaf sample was ground in liquid nitrogen using a pre-chilled mortar and pestle and transferred to a sterile 50 ml centrifuge tube containing 5.00 ml hot (65°C) 2X extraction buffer. The mixture was then incubated at 65°C for 15 to 20 minutes. Chloroform:isoamyl alcohol treatment was carried out as in Method I. To the aqueous phase obtained after centrifugation, one-tenth volume of 10 per cent CTAB solution was added and mixed gently by inversion. A second treatment with chloroform:isoamyl alcohol mixture was also given. The steps involving DNA pelleting and resuspension were similar to that in Method I.

3.4.2 Estimation of Quality and Quantity of DNA

The quality and quantity of isolated DNA was evaluated by subjecting the DNA to agarose gel electrophoresis.

Agarose was dissolved in TAE buffer and melted by boiling, cooled to 65°C and after adding one µl of ethidium bromide, poured into a horizontal gel casting unit. After 30 minutes, the solidified gel was placed in the horizontal electrophoresis unit filled with 1X TAE buffer (enough to immerse the gel completely). The DNA sample was mixed with gel loading dye in the ratio of 2:1 and loaded in the wells at the cathode side, without overflowing. The electrophoresis was carried out at a constant voltage of 10 V till the dye front reached three fourth distance from the well. The gel was then viewed under UV light in a transilluminator and the image was stored in the Alpha Imager gel documentation system.

3.4.3 Random Amplified Polymorphic DNA (RAPD) Analysis

3.4.3.1 Screening of Random Primers for Amplification of DNA

Genomic DNA of the isolates were amplified using selected random, short oligonucleotide primers.

A total of 27 primers (Operon Technologies, USA) belonging to OPAH, OPE and OPP series available at CPBMB were screened for amplification of genomic DNA.

Those primers, which gave maximum number of reproducible bands, were selected and used for further analysis.

3.4.3.2 RAPD Analysis of Somaclones

Genomic DNA of somaclones was amplified using selected random, short oligonucleotide primers. Reaction mixture consisted of the following.

i)	Template DNA (20.00 ng/µl)	- 5.00 µl/tube
ii)	10X Assay buffer with 15 mM $MgCl_2$ (1X)	- 2.50 µl/tube
iii)	dNTP's (10 mM)	- 1.0 µl/tube
iv)	Taq DNA polymerase (0.6 U)	- 2.0 µl/tube
v)	Primer (5 p moles)	- 1.0 µl/tube
vi)	Sterile milli Q water	- 13.5 µl/tube

The reaction mixture was prepared as a master mix for the required number of reactions. The aliquot of the master mix without template DNA was first prepared. From the master mix, 20.00 µl was pipetted into each PCR tube and 5 µl of template DNA was added. Control samples were also run without template DNA. The reaction mixtures were centrifuged in a microcentrifuge for mixing the components. The contents were overlaid with 25.00 µl mineral oil and PCR tubes were loaded in a thermal cycler (PTC 200) of MJ Research, USA.

Polymerase chain reaction (PCR) was done using short cycling parameters based on Demeke *et al.* (1992). Reactions were carried out in the thermal cycler programmed for one cycle at 94°C for three minutes, 39 cycles repeated running at 92°C for one minute, 37°C for one minute and 72°C for two minutes followed by one cycle at 72°C for five minutes.

Amplified fragments were separated in 1.4 percent agarose gel using 1X TAE buffer, visualized and documented as mentioned in 3.4.2. The RAPD profiles of the somaclones for different primers were scored based on the presence (1) or absence (0) of bands. Percentage polymorphism for individual primer was calculated as

$$\text{Percentage polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

3.5 STATISTICAL ANALYSIS

Statistical analysis of the data recorded was carried out as per the techniques described by Panse and Sukhatme (1985). For RAPD analysis, the data were analysed statistically using NTSYS pc 2.0 software programme. The genetic similarity was estimated by Jaccard's similarity coefficient and dendrogram was constructed employing unweighted pair group method of arithmetic averages (UPGMA).

Results

RESULTS

The results generated from the present investigations on "Induction of variation *in vitro* and field evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" are presented in this chapter.

4.1 INDUCTION OF VARIATION IN GINGER THROUGH *IN VITRO* TECHNIQUES AND PRODUCTION OF REGENERANTS

4.1.1 Indirect Organogenesis

4.1.1.1 Standardisation of Explants, Growth Regulators and Culture Conditions for Callus Induction

The data on the effect of explants, growth regulators and culture conditions on callusing in ginger cultivars Maran and Rio-de-Janeiro are presented in Tables 1a and 1b and the mean performance of the two cultivars in Table 1c.

4.1.1.1.1 Effect of Explants on Callusing

Explants like pseudostem, leaf, rhizome bud and shoot tip were tried for callus induction in ginger. The different explants tried for callus induction showed significant variation with respect to time taken for callusing, percentage callusing and growth of calli in the two cultivars studied.

Irrespective of the cultivars, shoot tip explants responded better than other explants registering early (27.61 days) and higher callusing (77.52%) and higher callus index value (76.41) (Fig. 1a, 1b and 1c and Plate 4). Next to shoot tip, pseudostem base recorded highest callusing and callus growth followed by folded leaf and rhizome bud explants. But rhizome bud explants callused earlier (31 days) than pseudostem base (35 days) and folded leaf (38 days).

Of the different parts of pseudostem (base, middle and top), tried for callusing, base recorded higher callusing percentage (44.86) followed by top (27.8) and middle (25.85). But with respect to callus growth, pseudostem base (37.82)

Table 1a. Effect of explants, growth regulators and culture conditions on callusing in ginger (*Z. officinale* Rosc.) (cultivar Maran)

1/2 MS supplemented with growth regulator (mg l^{-1})	Culture conditions -Dark (D) / Light (L)	**Days taken for callusing								**Percentage callusing								**Callus index							
		1	2	3	4	5	6	Mean		1	2	3	4	5	6	Mean		1	2	3	4	5	6	Mean	
2,4-D (1.00)	D	30.00	30.00	45.00	30.00	44.00	24.67	33.95	32.98	58.33	58.33	23.81	56.25	18.18	100.00	52.48	48.60	58.33	58.33	23.81	56.25	18.18	100.00	52.48	49.56
	L	30.00	30.00	45.00	30.00	30.00	27.00	32.00		66.67	16.67	28.57	33.33	23.08	100.00	44.72		66.67	16.67	28.57	33.33	34.62	100.00	46.64	
2,4-D (2.00)	D	46.00	53.00	53.00	34.28	30.00	30.00	41.05	41.28	37.50	12.50	14.29	38.89	25.00	80.00	34.70	35.83	25.13	6.25	7.15	36.17	25.00	80.00	29.95	30.05
	L	53.00	53.00	53.00	30.00	30.00	30.00	41.50		16.67	25.00	20.00	40.00	20.00	100.00	36.95		8.34	12.50	10.00	30.00	20.00	100.00	30.14	
2,4-D (3.00)	D	30.00	30.00	41.50	37.50	21.00	30.00	31.67	36.73	60.00	40.00	22.22	44.45	38.46	20.00	37.52	37.11	60.00	40.00	22.22	44.45	38.46	20.00	37.52	37.11
	L	53.00	53.00	53.00	31.67	30.00	30.00	41.78		16.67	14.29	16.67	60.00	12.50	100.00	36.69		16.67	14.29	16.67	60.00	12.50	100.00	36.69	
2,4-D (4.00)	D	53.00	53.00	53.00	30.00	30.00	30.00	41.50	42.57	11.11	22.22	11.11	31.58	19.04	22.22	19.55	23.55	11.11	22.22	11.11	31.58	19.04	22.22	19.55	21.73
	L	53.00	53.00	53.00	42.86	30.00	30.00	43.64		12.50	12.50	12.50	77.78	16.67	33.33	27.55		12.50	12.50	12.50	66.89	16.67	22.33	23.90	
2,4-D (3.00) + BAP (0.50)	D	30.00	30.00	30.00	30.00	30.00	21.00	28.50	35.90	100.00	22.22	33.33	77.90	14.29	100.00	57.96	46.81	111.00	22.22	33.33	77.79	14.29	80.00	56.44	45.21
	L	53.00	53.00	53.00	49.80	30.00	21.00	43.30		22.22	25.00	25.00	25.00	16.67	100.00	35.65		22.22	25.00	25.00	15.00	16.67	100.00	33.98	
2,4-D (0.50) + BAP (1.00)	D	30.00	30.00	30.00	30.00	28.50	21.00	28.25	28.67	14.29	12.50	16.67	20.00	64.71	75.00	33.86	34.46	14.29	12.50	16.67	20.00	86.71	75.00	37.53	37.39
	L	30.00	30.00	30.00	30.00	33.50	21.00	29.08		11.11	16.67	16.67	25.00	40.91	100.00	35.06		11.11	16.67	16.67	25.00	54.00	100.00	37.24	
2,4-D (1.00) + BAP (0.50)	D	30.00	30.00	30.00	48.00	30.50	21.00	31.58	30.25	28.57	33.33	16.67	33.33	51.22	80.00	40.52	35.54	28.57	33.33	16.67	33.33	57.37	80.00	41.55	36.06
	L	30.00	30.00	30.00	30.00	32.50	21.00	28.92		16.67	14.29	16.67	33.33	16.67	85.71	30.56		16.67	14.29	16.67	33.33	16.67	85.71	30.56	
Mean	D	35.57	36.57	40.36	34.25	30.57	25.38	33.79*	35.48	44.26	28.73	19.73	43.20	32.99	68.17	39.51*	37.41	44.06	27.84	18.71	42.80	37.01	65.32	39.29*	36.73
	L	43.14	43.14	45.29	34.90	30.86	25.71	37.17*		23.22	17.77	19.44	42.06	20.93	88.43	35.31*		22.03	15.99	18.01	37.65	24.45	86.86	34.16*	
		39.36	39.86	42.82	34.58	30.71	25.55			33.74	23.25	19.58	42.63	26.96	78.30			33.04	21.91	18.36	40.22	30.73	76.09		
CD (0.05)		5.05						2.92*	5.46	13.81						*N.S	14.92	14.55						*N.S	15.72
SE±		1.77						1.02*	1.91	4.84							5.22	5.10							5.50

**Average of two replications

1 - Pseudostem base

2 - Pseudostem middle

3 - pseudostem top

4 - Folded leaf

5 - Rhizome bud

6 - Shoot tip

Table 1b. Effect of explants, growth regulators and culture conditions on callusing in ginger (*Z. officinale* Rosc.) (cultivar Rio-de-Janeiro)

1/2 MS supplemented with growth regulator ($mg\ l^{-1}$)	Culture conditions -Dark (D) / Light (L)	**Days taken for callusing								**Percentage callusing								**Callus index							
		1	2	3	4	5	6	Mean		1	2	3	4	5	6	Mean		1	2	3	4	5	6	Mean	
2,4-D (1.00)	D	30.00	30.00	37.50	30.00	42.00	27.67	32.86	34.49	100.00	83.33	66.67	83.33	15.38	100.00	74.79	64.13	100.00	83.33	66.67	83.33	15.38	100.00	74.79	60.66
	L	30.00	30.00	45.00	54.00	30.00	27.67	36.11		66.67	16.67	83.33	50.00	4.16	100.00	53.47		66.67	16.67	41.67	50.00	4.16	100.00	46.53	
2,4-D (2.00)	D	30.00	30.00	30.00	30.00	25.50	30.00	29.25	29.63	100.00	28.57	25.00	50.00	26.92	62.50	48.83	52.52	50.00	28.57	12.50	25.00	26.92	62.50	34.25	39.83
	L	30.00	30.00	30.00	30.00	30.00	30.00	30.00		100.00	42.86	12.50	50.00	50.00	81.82	56.20		50.00	42.86	6.25	25.00	66.50	81.82	45.41	
2,4-D (3.00)	D	30.00	30.00	38.40	37.00	30.00	30.00	32.57	33.80	37.50	14.29	62.50	57.14	12.50	30.00	35.66	46.60	18.75	7.15	56.25	28.57	12.50	30.00	25.54	32.54
	L	30.00	50.40	32.80	37.00	30.00	30.00	35.03		100.00	62.50	62.50	28.57	16.67	75.00	57.54		50.00	50.00	31.25	14.29	16.67	75.00	39.54	
2,4-D (4.00)	D	30.00	40.00	30.00	42.67	27.42	30.00	33.35	35.76	12.50	37.50	12.50	37.50	40.48	70.00	35.08	29.58	12.50	31.13	12.50	31.13	41.69	70.00	33.16	27.08
	L	49.00	30.00	30.00	60.00	30.00	30.00	38.17		50.00	12.50	12.50	11.11	33.33	25.00	24.07		31.50	12.50	12.50	11.11	33.33	25.00	20.99	
2,4-D (3.00) + BAP (0.50)	D	30.00	30.00	43.00	43.00	30.00	30.00	34.33	35.47	50.00	25.00	50.00	37.50	18.18	100.00	46.78	54.06	50.00	25.00	25.00	37.50	18.18	100.00	42.61	47.29
	L	30.00	30.00	56.60	43.00	30.00	30.00	36.60		87.50	22.22	62.50	62.50	33.33	100.00	61.34		87.50	22.22	31.25	37.50	33.33	100.00	51.97	
2,4-D (0.50) + BAP (1.00)	D	30.00	30.00	30.00	30.00	36.50	30.00	31.08	30.50	28.57	12.50	12.50	25.00	35.00	75.00	31.43	29.59	28.57	12.50	12.50	25.00	35.00	75.00	31.43	29.91
	L	30.00	30.00	30.00	30.00	29.50	30.00	29.92		14.29	12.50	12.50	25.00	22.22	80.00	27.75		14.29	12.50	12.50	25.00	26.00	80.00	28.38	
2,4-D (1.00) + BAP (0.50)	D	30.00	30.00	30.00	52.00	30.50	30.00	33.75	33.71	16.67	16.67	16.67	16.67	52.63	100.00	36.55	31.58	16.67	16.67	16.67	16.67	67.89	100.00	39.10	32.86
	L	30.00	30.00	30.00	52.00	30.00	30.00	33.67		20.00	11.11	12.50	33.33	7.69	75.00	26.61		20.00	11.11	12.50	33.33	7.69	75.00	26.61	
Mean	D	30.00	31.43	34.13	37.81	31.70	29.67	32.46*	33.34	49.32	31.12	35.12	43.88	28.73	76.79	44.16*	44.01	39.50	29.19	28.87	35.31	31.08	76.79	40.13*	38.59
	L	32.71	32.91	36.34	43.71	29.93	29.67	34.21*		62.64	25.77	36.90	37.22	23.91	76.69	43.85*		45.71	23.98	21.13	28.03	26.81	76.69	37.06*	
		31.36	32.17	35.24	40.76	30.82	29.67			55.98	28.44	36.01	40.55	26.32	76.74			42.60	26.59	25.00	31.67	28.95	76.74		
CD (0.05)		3.88						*N.S	4.19	14.11						*N.S	15.24	13.24						*N.S	14.30
SE±		1.36							1.47	4.94							5.34	4.64							5.01

** Average of two replications

1 - Pseudostem base

2 - Pseudostem middle

3 - pseudostem top

4 - Folded leaf

5 - Rhizome bud

6 - Shoot tip

Table 1c. Effect of explants, growth regulators and culture conditions on callusing in ginger (*Z. officinale* Rosc.)*

1/2 MS supplemented with growth regulator (mg l^{-1})	Culture conditions -Dark (D) / Light (L)	Days taken for callusing								Percentage callusing								Callus index							
		1	2	3	4	5	6	Mean		1	2	3	4	5	6	Mean		1	2	3	4	5	6	Mean	
2,4-D (1.00)	D	30.00	30.00	41.25	30.00	43.00	26.17	33.40	33.73	79.17	70.83	45.24	69.79	16.78	100.00	63.63	56.37	79.17	70.83	45.24	69.79	16.78	100.00	63.63	55.11
	L	30.00	30.00	45.00	42.00	30.00	27.34	34.06		66.67	16.67	55.95	41.67	13.62	100.00	49.10		66.67	16.67	35.12	41.67	19.39	100.00	46.59	
2,4-D (2.00)	D	38.00	41.50	41.50	32.14	27.75	30.00	35.15	35.45	68.75	20.54	19.65	44.45	25.96	71.25	41.76	44.17	37.57	17.41	9.83	30.59	25.96	71.25	32.10	34.94
	L	41.50	41.50	41.50	30.00	30.00	30.00	35.75		58.34	33.93	16.25	45.00	35.00	90.91	46.57		29.17	27.68	8.13	27.50	43.25	90.91	37.77	
2,4-D (3.00)	D	30.00	30.00	39.95	37.25	25.50	30.00	32.12	35.27	48.75	27.15	42.36	50.80	25.48	25.00	36.59	41.85	39.38	23.58	39.24	36.51	25.48	25.00	31.53	34.82
	L	41.50	51.70	42.90	34.34	30.00	30.00	38.41		58.34	38.40	39.59	44.29	14.59	87.50	47.11		33.34	32.15	23.96	37.15	14.59	87.50	38.11	
2,4-D (4.00)	D	41.50	46.50	41.50	36.34	28.71	30.00	37.42	39.17	11.81	29.86	11.81	34.54	29.76	46.11	27.31	26.56	11.81	26.68	11.81	31.36	30.37	46.11	26.35	24.40
	L	51.00	41.50	41.50	51.43	30.00	30.00	40.91		31.25	12.50	12.50	44.45	25.00	29.17	25.81		22.00	12.50	12.50	39.00	25.00	23.67	22.44	
2,4-D (3.00) + BAP (0.50)	D	30.00	30.00	36.50	36.50	30.00	25.50	31.42	35.69	75.00	23.61	41.67	57.70	16.24	100.00	52.37	50.44	80.50	23.61	29.17	57.65	16.24	90.00	49.53	46.25
	L	41.50	41.50	54.80	46.40	30.00	25.50	39.95		54.86	23.61	43.75	43.75	25.00	100.00	48.50		54.86	23.61	28.13	26.25	25.00	100.00	42.97	
2,4-D (0.50) + BAP (1.00)	D	30.00	30.00	30.00	30.00	32.50	25.50	29.67	29.59	21.43	12.50	14.59	22.50	49.86	75.00	32.65	32.03	21.43	12.50	14.59	22.50	60.86	75.00	34.48	33.65
	L	30.00	30.00	30.00	30.00	31.50	25.50	29.50		12.70	14.59	14.59	25.00	31.57	90.00	31.41		12.70	14.59	14.59	25.00	40.00	90.00	32.81	
2,4-D (1.00) + BAP (0.50)	D	30.00	30.00	30.00	50.00	30.50	25.50	32.67	31.98	22.62	25.00	16.67	25.00	51.93	90.00	38.54	33.56	22.62	25.00	16.67	25.00	62.63	90.00	40.32	34.45
	L	30.00	30.00	30.00	41.00	31.25	25.50	31.29		18.34	12.70	14.59	33.33	12.18	80.36	28.58		18.34	12.70	14.59	33.33	12.18	80.36	28.58	
Mean	D	32.79	34.00	37.24	36.03	31.14	27.52	33.12	34.41	46.79	29.93	27.42	43.54	30.86	72.48	41.84	40.71	41.78	28.51	23.79	39.06	34.04	71.05	39.71	37.66
	L	37.93	38.03	40.81	39.31	30.39	27.69	35.69		42.93	21.77	28.17	39.64	22.42	82.56	39.58		33.87	19.98	19.57	32.84	25.63	81.78	35.61	
		35.36	36.01	39.03	37.67	30.77	27.61			44.86	25.85	27.80	41.59	26.64	77.52			37.82	24.25	21.68	35.95	29.84	76.41		

1 - Pseudostem base
2 - Pseudostem middle
3 - pseudostem top
4 - Folded leaf
5 - Rhizome bud
6 - Shoot tip

* Derivd from Tables 1a and 1b

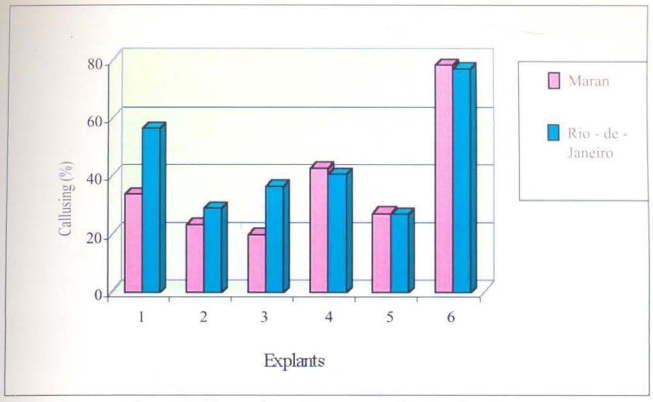


Fig. 1a Effect of explants on callusing in ginger

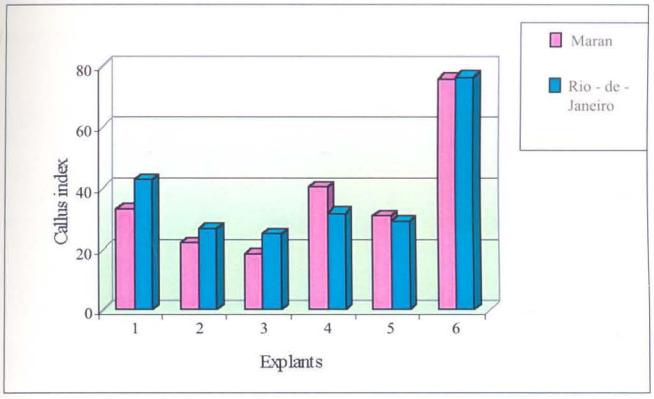


Fig. 1b. Effect of explants on callus growth in ginger

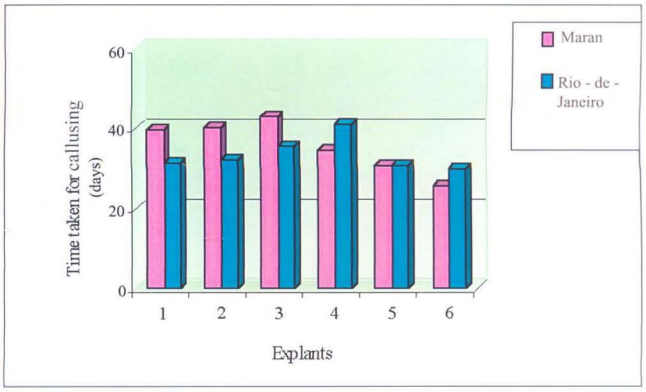
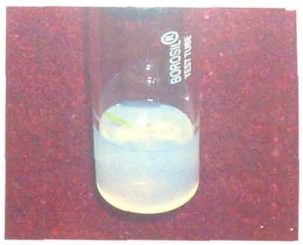


Fig. 1c Effect of explants on time taken for callusing in ginger

1 Pseudostem base
2 Pseudostem middle
3 Pseudostem top
4 Folded leaf
5 Rhizome bud
6 Shoot tip

4a Shoot tip explant



4b Callusing in shoot tip

4c Callusing and morphogenesis

4d Simultaneous shoot and root morphogenesis

4e Shoot elongation

4f Shoot proliferation and rooting

Plate 4 Indirect organogenesis in ginger - various stages

recorded higher growth followed by middle (24.25) and top (21.68) portions. Pseudostem explants registered 35 to 39 days for callus initiation.

Folded leaves were superior to unfolded leaves for callusing and callus growth in ginger. In folded leaves, callusing observed was 41.59 per cent with a callus index value of 35.95. But in unfolded leaves, callusing was not at all observed in the two cultivars studied.

4.1.1.1.2 Effect of Growth Regulators on Callusing

Half MS medium supplemented with various concentrations of 2,4-D (1.0 – 4.0 mg l^{-1}) and combinations of 2,4-D (0.5 – 3.0 mg l^{-1}) and BAP (0.5 – 1.0 mg l^{-1}) were tried for callusing in ginger. Different media combinations differed significantly with respect to callusing and callus growth and time taken for callus initiation. Calli induced at lower concentrations of 2,4-D and combinations of 2,4-D and BAP were hard and compact. At higher levels of 2,4-D (3.00 and 4.00 mg l^{-1}), the induced calli were friable, loose and watery with root hairs.

Half MS medium supplemented with 2,4-D alone (1.00 mg l^{-1}) recorded highest callusing (56.37%) and callus growth (55.11). But for earliness in callusing, combination of 2,4-D with BAP was found best. Medium with 2,4-D – BAP combination (2,4-D at 0.50 mg l^{-1} and BAP at 1.00 mg l^{-1}), took only 29.59 days for callusing as compared to 33.73 days in medium with 2,4-D alone (1.0 mg l^{-1}). Even though early callusing was observed in medium with 2,4-D at 0.50 mg l^{-1} and BAP at 1.00 mg l^{-1}, the percentage callusing was low. Hence, the best medium identified for callusing in 2,4-D – BAP combination was half MS supplemented with 2,4-D at 3.00 mg l^{-1} and BAP at 0.50 mg l^{-1}. The percentage callusing in the medium was 50.44 and callus index was 46.25.

A gradual decline in response to callusing was observed with increasing levels of 2,4-D in the medium. The percentage callusing was 56.37 in medium supplemented with 1.00 mg l^{-1} 2,4-D while it was 26.56 per cent in medium supplemented with 4.00 mg l^{-1} 2,4-D. Similarly, the callus growth was less and the

days taken for callusing were more in medium supplemented with higher levels of 2,4-D.

4.1.1.1.3 Effect of Culture Conditions on Callusing

The two culture conditions tried viz. incubation in dark and light were found on par with respect to callusing and callus growth in the two cultivars studied. But the dark condition favoured earlier callusing in the cultivar Maran. Irrespective of the cultivars, explants and growth regulators, percentage callusing observed in dark was 41.84 per cent while it was 39.58 per cent in light. The callus index ranged from 35.61 to 39.71 in the two culture conditions studied and the cultures took 33 to 36 days for callusing.

4.1.1.1.4 Response of Cultivars to Callusing

The response of two cultivars viz. Maran and Rio-de-Janeiro were compared with respect to callusing, callus growth and time taken for callusing. The explants of cultivar Rio-de-Janeiro responded better than cultivar Maran with respect to the different parameters studied (Table 1a and 1b). The overall callusing percentage observed in cultivar Rio-de-Janeiro was 44.01 while it was 37.41 per cent in cultivar Maran. The callus index values recorded was also higher in cultivar Rio-de-Janeiro (38.59) as compared to cultivar Maran (36.73). The Rio-de-Janeiro explants callused earlier (33.34 days) than Maran (35.48 days).

The response of different explants tried in the two cultivars for callusing was also compared. There was not much variation observed in the response of explants like shoot tip and rhizome bud in the two cultivars. But explants from pseudostem registered higher callusing and callus growth in the cultivar Rio-de-Janeiro. With regard to folded leaf explant, the cultivar Maran recorded higher callusing, callus growth and earliness in callusing than the cultivar Rio-de-Janeiro.

The best medium identified for callusing was half MS supplemented with 1 mg l^{-1} 2,4-D followed by half MS supplemented with 3.00 mg l^{-1} 2,4-D and 0.50 mg l^{-1}

BAP. In the two media, cultivar Rio-de-Janeiro recorded higher callusing and callus growth as compared to cultivar Maran.

Two culture conditions viz. dark and light were also compared in the two cultivars. Incubation under dark and light were found on par with respect to callusing and callus growth. But for earliness in callusing, dark incubation was found favourable in the cultivar Maran.

4.1.1.2 Effect of Growth Regulators and Media Supplements on Shoot Morphogenesis

Growth regulators like 2,4-D, NAA and BAP and supplements like charcoal and $AgNO_3$ were incorporated to basal MS medium at half strength for shoot morphogenesis in ginger. The calli induced from shoot tip explants of the cultivar Maran in half MS medium supplemented with 2,4-D at 1.00 mg l^{-1} were inoculated to shoot regeneration medium to study the response. The best medium identified for shoot morphogenesis in ginger was half MS medium supplemented with BAP at 3.00 mg l^{-1}. Shoot morphogenesis was also observed in medium supplemented with 4 to 5 mg l^{-1} BAP (Table 2). But in medium supplemented with auxin and cytokinin, no shoot morphogenesis was observed. Similarly, in basal half MS medium and medium supplemented with additives like charcoal or $AgNO_3$, no shoot morphogeneses was observed.

Half MS medium supplemented with BAP at 3.00 mg l^{-1} recorded the highest percentage of shoot morphogenesis (38.46) and highest number of shoots per culture (1.63) followed by half MS medium supplemented with BAP at 4.00 mg l^{-1} (15%). Morphogenic potential of the calli decreased with increase in concentration of BAP and the regeneration percentage was 11.11 per cent in half MS medium with higher concentration of BAP i.e. 5.00 mg l^{-1}.

Shoot morphogenesis and proliferation were studied in the two cultivars and the data on the above parameters are presented in Table 3. The calli induced from shoot tip explants in half MS medium supplemented with 2,4-D (1.00 mg l^{-1}) were inoculated to regeneration medium (1/2 MS + BAP 3.00 mg l^{-1}) for shoot

Table 2. Effect of growth regulators and media supplements on shoot morphogenesis in callus cultures of ginger (*Z. officinale* Rosc.) (cultivar Maran)

Sl. No.	Medium	Total no. of calli inoculated	No. of calli with shoot initials	Shoot morpho-genesis (%)	Mean no. of shoots/ culture
1	1/2 MS	28.00	-	-	-
2	1/2 MS + BAP (3.00 mg l^{-1})	78.00	30.00	38.46	1.63
3	1/2 MS + BAP (4.00 mg l^{-1})	20.00	3.00	15.00	1.00
4	1/2 MS + BAP (5.00 mg l^{-1})	18.00	2.00	11.11	1.00
5	1/2 MS + BAP (3.00 mg l^{-1}) + $AgNO_3$ (4.00 mg l^{-1})	17.00	-	-	-
6	1/2 MS + 2,4-D (0.10 mg l^{-1}) + BAP (6.00 mg l^{-1})	10.00	-	-	-
7	1/2 MS + 2,4-D (0.20 mg l^{-1}) + BAP (10.00 mg l^{-1})	8.00	-	-	-
8	1/2 MS + NAA (1.00 mg l^{-1}) + BAP (5.00 mg l^{-1})	11.00	-	-	-
9	1/2 MS + charcoal (0.50%)	8.00	-	-	-

Table 3. Shoot morohogenesis and proliferation in callus cultures of ginger (*Z. officinale* Rosc.)

Cultivar	Mean no. of days taken for shoot morphogenesis	Shoot morphogenesis (%)	Mean no. of shoots in base culture	Mean no. of shoots proliferated in each subculture cycle				Mean total no. of shoots regenerated at the end of IV s.c
				I s.c	II s.c	III s.c	IV s.c	
Maran	29.80	38.46	5.00	+4.25	+16.25	+17.75	+78.00	121.25
Rio-de-Janeiro	26.25	39.39	3.25	+10.00	+30.25	+58.5	+60.00	162.00

Medium - 1/2 MS + BAP (3.00 mg l^{-1})
s.c - subculture

morphogenesis. The cultures of cultivar Rio-de-Janeiro responded better registering early and higher shoot morphogenesis than the cultivar Maran. The callus cultures of cultivar Rio-de-Janeiro took only 26 days for shoot morphogenesis while cultivar Maran took 30 days for shoot regeneration. The percentage of shoot morphogenesis was also slightly higher (39.39) in Rio-de-Janeiro than the cultivar Maran (38.46).

The regenerated shoots of both cultivars were inoculated to half MS medium supplemented with BAP (3.00 mg l^{-1}) for further proliferation. The rate of shoot proliferation was high in cultivar Rio-de-Janeiro recording more number of proliferated shoots in first (10.00), second (30.25) and third (58.50) subculture cycles. Mean number of shoots regenerated at the end of fourth subculture cycle was also high in the cultivar Rio-de-Janeiro recording 162 shoots as compared to 121 shoots in the cultivar Maran. Simultaneous root morphogenesis was also observed in cultures in half MS medium supplemented with BAP (3.00 mg l^{-1}).

4.1.2 Somatic Embryogenesis

4.1.2.1 Standardisation of Growth Regulators and Culture Conditions for Induction of Embryogenic Calli

The response of rhizome bud explants to induction of embryogenic calli and the influence of media and culture conditions on callusing are presented in Tables 4 and 5. Of the seven media tested for induction of embryogenic calli, half MS medium supplemented with 2,4-D (0.5-1.0 mg l^{-1}) and BAP (0.50 - 1.00 mg l^{-1}) produced embryogenic calli in both the cultivars. The two responding media to embryo induction [MS medium supplemented with 2,4-D (0.50 mg l^{-1}) + BAP (1.00 mg l^{-1}) and MS medium with 2,4-D (1.00 mg l^{-1}) + BAP (0.50 mg l^{-1})] were found on par with respect to percentage of embryogenic calli and number of somatic embryoids/ culture. In the two responding media, the embryogenic calli ranged from 82 to 83 per cent and number of embryoids per culture 1.7 – 1.8 and embryo induction occurred 30 days after inoculation (Plate 5). The induced embryoids were found compact.

Embryogenesis occurred both under dark and light conditions. Incubating cultures in dark was significantly superior to incubation in light with respect to

Table 4. Effect of media and culture conditions on induction of embryogenic calli in ginger (*Z. officinale* Rosc.)

1/2 MS supplemented with growth regulator (mg l^{-1})	Culture conditions - Dark (D) / Light (L)	Induction of embryogenic calli		Induction of non embryogenic calli	
		Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro
2,4-D (1.00)	D	-	-	+	+
	L	-	-	+	+
2,4-D (2.00)	D	-	-	+	+
	L	-	-	+	+
2,4-D (3.00)	D	-	-	+	+
	L	-	-	+	+
2,4-D (4.00)	D	-	-	+	+
	L	-	-	+	+
2,4-D (3.00) and BAP (0.50)	D	-	-	+	+
	L	-	-	+	+
2,4-D (0.50) and BAP (1.00)	D	+	+	+	-
	L	+	+	+	+
2,4-D (1.00) and BAP (0.50)	D	+	+	-	+
	L	+	+	-	+

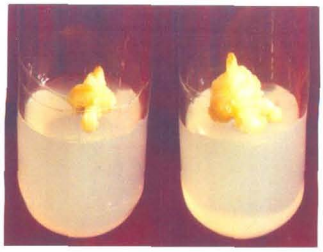
Explant - Rhizome bud

Table 5. Effect of media and culture conditions on induction of somatic embryoids in ginger (*Z. officinale* Rosc.)

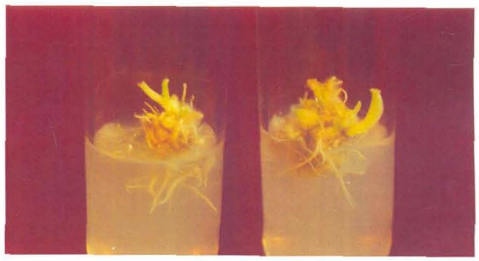
1/2 MS supplemented with growth regulator (mg l^{-1})	Culture conditions - Dark (D)/Light (L)	*Embryogenic calli (%)			*Nonembryogenic calli (%)			*Mean no. of days taken for somatic embryo induction			*Mean no. of somatic embryoids/culture		
		Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean
2,4-D (0.50) and BAP (1.00)	D	83.34	100.00	82.14**	16.66	0.00	17.86	28.50	30.00	30.50**	1.54	1.55	1.84**
	L	78.57	66.67		21.43	33.33		33.00	30.50		1.75	2.50	
2,4-D (1.00) and BAP (0.50)	D	100.00	85.72	83.93**	0.00	14.28	16.07	28.50	29.00	30.00**	1.92	1.67	1.71**
	L	100.00	50.00		0.00	50.00		32.50	30.00		1.75	1.50	
Mean	D	91.67	92.86	92.26***	8.33	7.14	7.74	28.50	29.50	29.00***	1.73	1.61	1.67***
	L	89.29	58.33	73.81***	10.71	41.67	26.19	32.75	30.25	31.50***	1.75	2.00	1.88***
		90.48**	75.6**		9.52	24.40		30.63**	29.88**		1.74**	1.80**	
CD (0.05)		**2.836		**N.S				*N.S		**N.S	**N.S		**N.S
				***2.836						***1.289			***N.S

*Average of two replications

5a Rhizome bud explant



5b Induction of somatic embryoids



5c Germination of somatic embryoids

5d Shoot proliferation from embryogenic callus cultures

Plate 5 Indirect embryogenesis in ginger - various stages

percentage of embryogenic calli. In dark, the percentage of embryogenic calli observed was 92 per cent while in light, it was 74 per cent. The embryogenic calli initiated earlier (29 days) in light than in dark (32 days). The mean number of somatic embryoids/culture did not vary significantly in the two culture conditions.

The two cultivars studied were on par with respect to days taken for embryo induction and mean number of somatic embryoids/culture. In the two cultivars, embryo induction occurred 30 days after inoculation recording 1.7 – 1.8 embryoids per culture. The percentage of embryogenic calli was higher in the cultivar Maran (90) as compared to the cultivar Rio-de-Janeiro (75.60).

4.1.2.2 Proliferation and Maturation of Somatic Embryoids

The induced somatic embryoids of two cultivars were inoculated to half MS basal medium for further proliferation and maturation and the data on proliferation of embryoids are presented in Table 6. The cultivar Rio-de-Janeiro registered higher proliferation of embryoids than the cultivar Maran. The proliferation of embryoids was observed in 45 per cent cultures of cultivar Rio-de-Janeiro as compared to 30 per cent cultures in the cultivar Maran. Mean number of embryoids proliferated after one month of inoculation was also high in cultivar Rio-de-Janeiro registering 2.65 embryoids/culture as compared to 2.11 embryoids/culture in the cultivar Maran. When half MS medium with BAP (3.00 mg l^{-1}) was tried for proliferation of embryoids, rhizogenesis was observed in the two cultivars studied.

4.1.2.3 Germination of Somatic Embryoids

For germination of somatic embryoids in ginger, two media viz. half MS basal and half MS basal with BAP at 3.00 mg l^{-1} were tried. Inclusion of BAP in the basal medium was found to enhance germination of somatic embryoids. In half MS medium supplemented with BAP (3.00 mg l^{-1}), 63.89 per cent embryoids germinated as compared to 24.49 per cent in half MS basal medium without BAP. But, early germination of somatic embryoids was observed in half MS basal medium without BAP registering 21 days for germination while in BAP supplemented medium, the time taken for germination was 26 days.

Table 6. Proliferation of somatic embryoids in ginger (*Z. officinale* Rosc.)

Cultivar	No. of somatic embryoids inoculated	No. of somatic embryoids proliferated after one month	Percentage proliferation	Mean no. of somatic embryoids in base culture	Mean no. of somatic embryoids proliferated in one month	Mean no. of somatic embryoids after one month of culture cycle
Maran	27.00	8.00	29.63	1.70	+0.41	2.11
Rio-de-Janeiro	20.00	9.00	45.00	1.80	+0.85	2.65

Culture condition - Light
Medium - 1/2 MS basal

Table 7. Germination of somatic embryoids in ginger (*Z. officinale* Rosc.)

Medium	No. of somatic embryoids inoculated		No. of somatic embryoids germinated		Mean germination of somatic embryoids (%)			Mean no. of days taken for germination of somatic embryoids		
	Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean
1/2 MS basal	43	35	10	9	23.26	25.71	24.49	20.50	22.00	21.25
1/2 MS + BAP (3.00 mg l^{-1})	18	18	11	12	61.11	66.67	63.89	23.13	28.25	25.69
Mean					42.19	46.19		21.82	25.13	

Culture condition - Light

Table 8. Shoot proliferation in somatic embryo derived cultures in ginger (*Z. officinale* Rosc.)

Cultivar	Mean no. of shoots in base culture	Mean no. of shoots proliferated in each subculture cycle				Mean total no. of shoots proliferated at the end of IV s.c
		I s.c	II s.c	III s.c	IV s.c	
Maran	2.75	+2.75	+4.00	+10.00	+40.25	59.75
Rio-de-Janeiro	5.00	+2.67	+4.00	+14.33	+39.00	65.00

Medium - 1/2 MS + BAP (3.00 mg l^{-1})
s.c - subculture cycle

Of the two cultivars studied, highest germination of somatic embryoids was observed in the cultivar Rio-de-Janeiro, recording 46.19 percent embryoid germination as compared to 42.19 per cent in the cultivar Maran. But, early germination of embryoids was noticed in the cultivar Maran. The time taken for germination of somatic embryoids was 22 days in the cultivar Maran and 25 days in the cultivar Rio-de-Janeiro.

Germination of somatic embryoids was characterised by simultaneous production of root and shoot. The germinated somatic embryoids produced good root and shoot system in half MS medium supplemented with BAP at 3.00 mg l^{-1}. The somatic embryo derived cultures of cultivar Rio-de-Janeiro recorded better shoot proliferation than the cultivar Maran. Mean number of shoots proliferated was high in the cultivar Rio-de-Janeiro registering 65 shoots at the end of fourth subculture as compared to 60 shoots in the cultivar Maran (Table 8).

4.1.3 *In vitro* Induction of Mutation Using γ Irradiation

4.1.3.1 Standardisation of γ Irradiation Dose for In Vitro Mutagenesis

Organogenic / embryogenic callus cultures at the shoot morphogenesis stage were subjected to γ irradiation at five different doses viz. 10, 20, 30, 40 and 50 Gy. The data pertaining to the effect of γ irradiation on culture establishment and proliferation of shoots are presented in Tables 9a, 9b and 9c.

Of the different doses of irradiation tried, highest culture establishment was recorded in cultures irradiated at 10 Gy wherein 95.83 per cent cultures established followed by cultures irradiated at 20 Gy registering 50 per cent establishment (Fig.2). Culture establishment was nil at higher doses of irradiation viz. 40 and 50 Gy. Cent per cent establishment was observed in non-irradiated control cultures.

Total number of shoots proliferated 30 days after irradiation also differed significantly with respect to different irradiation doses. The number of shoots proliferated was highest in cultures irradiated at 10 Gy registering 3.83 shoots/culture, which was also on par with non-irradiated cultures (3.88 shoots/culture). At higher

Table 9a. Effect of γ irradiation on shoot proliferation and establishment of organogenic / embryogenic cultures in ginger (*Z. officinale* Rosc.)

Group	Doze (Gy)	*Mean culture establishment (%)		*Mean no. of shoots in base culture	*Mean shoot proliferation		*Mean no. of total shoots at the end of 30 DAI
		15 DAI	30 DAI		15 DAI	30 DAI	
MC	10.00	100.00	100.00	1.17	+3.17	+0.17	4.51
				(1.284)**	(2.672)	(2.268)	(2.215)
	20.00	83.33	33.33	1.17	+0.50	-0.83	0.84
				(1.284)	(2.109)	(2.034)	(1.112)
	30.00	100.00	0.00	1.83	+0.66	-1.16	1.33
				(1.522)	(2.157)	(1.950)	(1.344)
	40.00	100.00	0.00	1.33	+0.66	-1.67	0.49
				(1.344)	(2.154)	(1.813)	(0.880)
	50.00	83.33	0.00	2.17	-0.66	-0.33	1.18
				(1.601)	(1.783)	(2.157)	(1.246)
	0.00 (control)	100.00	100.00	1.17	+1.17	0.00	2.34
				(1.284)	(2.264)	(2.236)	(1.649)
Mse	10.00	100.00	100.00	1.17	+1.67	+2.50	5.34
				(1.284)	(2.364)	(2.728)	(2.377)
	20.00	100.00	100.00	2.00	+2.00	-0.17	3.83
				(1.559)	(2.435)	(2.167	(2.056)
	30.00	83.33	33.33	1.33	-0.33	-0.33	0.67
				(1.344)	(1.911)	(2.157)	(1.025)
	40.00	83.33	0.00	1.67	-0.17	-0.50	1.00
				(1.451)	(1.955)	(2.109)	(1.144)
	50.00	83.33	0.00	2.00	+0.83	-1.00	1.83
				(1.530)	(2.184)	(1.995)	(1.466)
	0.00 (control)	100.00	100.00	1.17	+1.67	+1.67	4.51
				(1.284)	(2.360)	(2.566)	(2.140)
RC	10.00	83.33	83.33	1.33	+0.83	+0.17	2.33
				(1.344)	(2.180)	(2.272)	(1.604)
	20.00	50.00	33.33	1.33	+0.16	-0.66	0.83
				(1.332)	(2.004)	(1.895)	(1.029)
	30.00	66.66	0.00	1.00	+0.33	-0.83	0.50
				(1.225)	(2.061)	(2.034)	(0.939)
	40.00	100.00	0.00	1.67	+0.33	-0.83	1.17
				(1.434)	(2.063)	(2.020)	(1.202)
	50.00	100.00	0.00	1.33	+1.50	-2.83	0.00
				(1.332)	(2.339)	(1.329)	(0.707)
	0.00 (control)	100.00	100.00	1.00	+2.83	+0.50	4.33
				(1.225)	(2.588)	(2.340)	(2.127)
Rse	10.00	100.00	100.00	1.17	+1.33	+0.66	3.16
				(1.284)	(2.292)	(2.375)	(1.870)
	20.00	100.00	33.33	1.33	+'0.83	-1.67	0.50
				(1.344)	(2.189)	(1.775)	(0.966)
	30.00	50.00	0.00	1.17	-0.33	-0.66	0.18
				(1.284)	(1.911)	(2.073)	(0.707)
	40.00	66.67	0.00	1.00	0.00	+0.17	1.17
				(1.225)	(1.950)	(2.272)	(1.219)
	50.00	33.33	0.00	1.17	-0.66	0.00	0.51
				(1.225)	(1.821)	(2.236)	(0.939)
	0.00 (control)	100.00	100.00	1.33	+0.83	+2.17	4.33
				(1.344)	(2.193)	(2.638)	(2.095)
CD (0.05) for doze means				N.S	0.067	0.195	0.123
CD (0.05) for group means				0.114	N.S	0.174	0.111
CD (0.05) for interaction (doze x group) means				N.S	0.267	0.389	0.247

*Average of six observations

**Values in parantheses indicate transformed values

DAI - Days after irradiation

MC - Plantlets - indirect organogenesis cv. Maran

Mse - Plantlets - indirect embryogenesis cv. Maran

RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro

Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro

Table 9b. Effect of dose of γ irradiation on culture establishment and shoot proliferation in ginger (*Z. officinale* Rosc.)*

Doze (Gy)	Mean culture establishment (%)		Mean no. of shoots in base culture	Mean shoot proliferation		Mean no. of total shoots at the end of 30 DAI
	15 DAI	30 DAI		15 DAI	30 DAI	
10.00	95.83	95.83	+1.21	+1.75	+0.88	3.83
			(1.299)**	(2.377)	(2.411)	(2.016)
20.00	83.33	50.00	+1.46	+0.88	-0.83	1.50
			(1.380)	(2.184)	(1.968)	(1.291)
30.00	75.00	8.33	+1.33	+0.08	-0.75	0.63
			(1.344)	(2.010)	(2.054)	(1.004)
40.00	87.50	0.00	+1.42	+0.17	-0.71	0.92
			(1.363)	(2.030)	(2.054)	(1.111)
50.00	87.50	0.00	+1.63	+0.25	-1.04	0.88
			(1.1.422)	(2.032)	(1.929)	(1.090)
0.00 (control)	100.00	100.00	+1.17	+1.63	+1.08	3.88
			(1.284)	(2.351)	(2.445)	(2.003)
C.D.			N.S	0.153	0.192	0.251
S.E ±				0.055	0.068	0.090

*Derived from Table 9a

**Values in parantheses indicate transformed values

DAI - Days after irradiation

Table 9c. Effect of mode of regeneration on culture establishment and shoot proliferation of γ irradiated cultures in ginger (*Z. officinale* Rosc.)*

Group	Mean culture establishment (%)		Mean no. of shoots in base culture	Mean shoot proliferation		Mean no. of total shoots at the end of 30 DAI
	15 DAI	30 DAI		15 DAI	30 DAI	
MC	92.00	12.00	1.53	+0.87	-0.76	1.64
MC control	100.00	100.00	1.17	+1.17	0.00	2.34
Mse	88.00	36.00	1.64	+0.79	+0.10	2.53
Mse control	100.00	100.00	1.17	+1.67	+1.67	4.51
RC	76.00	8.00	1.34	+0.63	-1.00	0.97
RC control	100.00	100.00	1.00	+2.83	+0.50	4.33
Rse	76.00	12.00	1.14	+0.23	-0.30	1.07
Rse control	100.00	100.00	1.33	+0.83	+2.17	4.33

* Derived from Table 9a

DAI - Days after irradiation

MC - Plantlets - indirect organogenesis cv. Maran

Mse - Plantlets - indirect embryogenesis cv. Maran

RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro

Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro

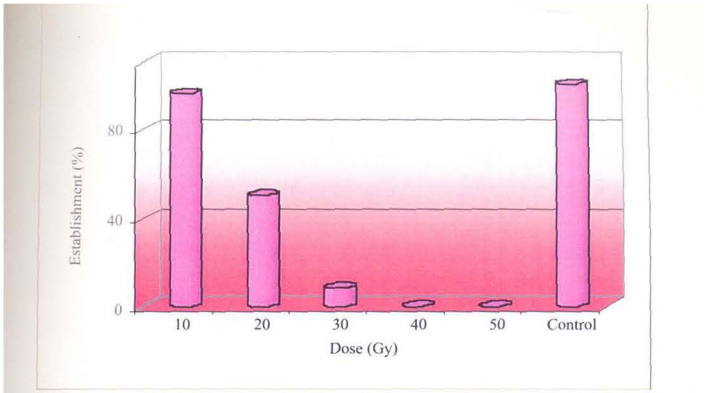


Fig. 2 Effect of dose of γ irradiation on culture establishment in ginger

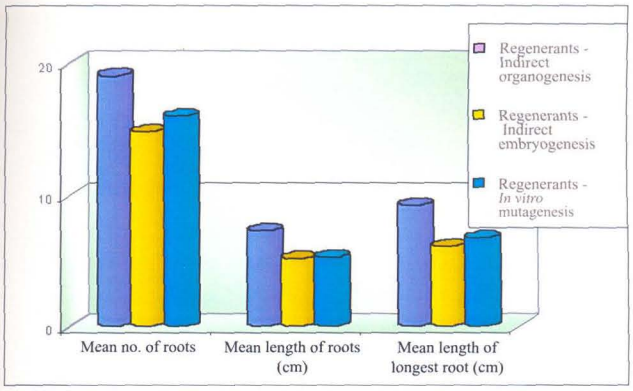


Fig. 3 Root growth in plantlets regenerated through various routes in ginger

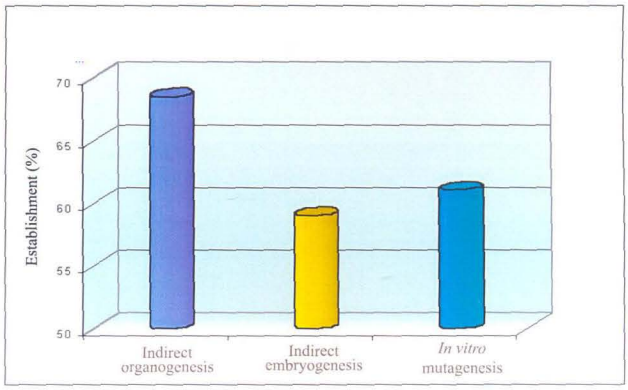


Fig. 4 Establishment of plantlets regenerated through various routes in ginger

doses of irradiation tried, the number of shoots proliferated were less and the shoots formed were weak and stunted. The survival percentage and shoot proliferation were highest in non-irradiated cultures as compared to irradiated cultures (Plate 6).

Out of the four groups of cultures regenerated through various routes, embryogenic cultures of cultivar Maran recorded highest survival (36.00 %) and maximum shoot proliferation (2.53), 30 days after irradiation. The lowest culture establishment (8.00 %) and shoot proliferation (0.97) were recorded in the cultures derived through indirect organogenesis in the cultivar Rio-de-Janeiro.

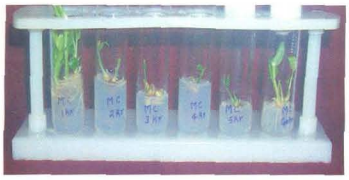
Group x dose interaction was also found significant. The culture establishment and shoot proliferation were found to decrease with increasing levels of γ irradiation. However, the embryogenic cultures of cultivar Maran irradiated at 20 Gy could withstand higher doses of irradiation and recorded cent percent survival 30 days after irradiation.

Evaluating the overall growth performance of the irradiated cultures, the dose of 10 Gy was fixed for large-scale irradiation of organogenic cultures of cultivar Maran and organogenic / embryogenic cultures of cultivar Rio-de-Janeiro. As the embryogenic cultures of cultivar Maran could withstand higher dose of irradiation, the dose 20 Gy was fixed for large-scale irradiation of embryogenic cultures in the cultivar Maran.

4.1.3.2 Irradiation of Cultures in the Selected Dose of γ Irradiation

Irradiation of organogenic / embryogenic callus cultures at the shoot morphogenesis stage was done in the selected dose of γ irradiation. Shoot proliferation was compared among cultures regenerated through various routes in the two cultivars (Table 10).

In general, low dose of γ irradiation (10 Gy) could enhance shoot proliferation in cultures derived through various routes. The shoot proliferation observed in organogenic cultures of cultivar Maran irradiated at 10 Gy was 23.58 while it was 18.58 in non-irradiated control cultures. Similarly, in embryogenic



6a Irradiated cultures of Maran (Indirect organogenesis)

6b Irradiated cultures of Rio-de-Janeiro (Indirect organogenesis)

6c Irradiated cultures of Maran (Indirect embryogenesis)

6d Irradiated cultures of Rio-de-Janeiro (Indirect embryogenesis)

Plate 6 Effect of γ irradiation dose on shoot proliferation in ginger
(showing higher proliferation of shoots in lower doses of irradiation)

Table 10. Shoot proliferation in organogenic / embryogenic cultures of ginger (*Z. officinale* Rosc.) in selected dozes of γ irradiation

Group	Mean no. of shoots in base culture	Mean no. of shoots proliferated in		Mean no. of total shoots at the end of II s.c
		I s.c	II s.c	
MC 10Gy	3.00	+3.50	+17.08	23.58
MC	4.25	+2.33	+12.00	18.58
Mse 20Gy	5.10	+3.30	+6.50	14.90
Mse	3.13	+3.75	+12.75	19.63
RC 10Gy	3.71	+5.14	+17.43	26.28
RC	4.00	+5.00	+17.66	26.66
Rse 10Gy	3.33	+13.67	+9.66	26.67
Rse	3.71	+4.75	+16.50	24.96

Table 11. Root growth in plantlets regenerated through various routes in ginger (*Z. officinale* Rosc.)

Sl. No.	Group	*Mean no. of roots	*Mean length of roots (cm)	*Mean length of longest root (cm)
1	MC	20.70	8.58	11.03
2	RC	17.50	6.04	7.48
3	Mse	15.40	5.74	6.81
4	Rse	14.25	4.65	5.45
5	MC 10 Gy	20.30	6.69	8.73
6	RC 10 Gy	15.90	6.49	8.26
7	Mse 20 Gy	13.60	3.98	5.07
8	Rse 10 Gy	14.20	4.07	4.94
C.D (0.05)		3.658	1.192	1.413
SE±		1.320	0.430	0.510

*Average of 20 observations

MC - Plantlets - indirect organogenesis cv. Maran

RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro

Mse - Plantlets - indirect embryogenesis cv. Maran

Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro

MC 10Gy - Plantlets - indirect organogenesis cv. Maran - irradiated at 10 Gy

RC 10 Gy - Plantlets - indirect organogenesis cv. Rio-de-Janeiro - irradiated at 10 Gy

Mse 20Gy - Plantlets - indirect embryogenesis cv. Maran - irradiated at 20 Gy

Rse 10Gy - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro - irradiated at 10 Gy

cultures of cultivar Rio-de-Janeiro, the shoot proliferation was 26.67 in irradiated cultures and 24.96 in non-irradiated cultures.

Organogenic cultures in general, recorded high shoot proliferation as compared to embryogenic cultures. With regard to cultivar response, the cultivar Rio-de-Janeiro exhibited high shoot proliferation than the cultivar Maran.

4.1.4 Root Characters in Regenerants Produced Through Various Routes

Root characters in regenerants produced through various routes were observed. Regenerants produced through indirect organogenesis exhibited higher root number and root length followed by regenerants produced through *in vitro* mutagenesis and embryogenesis (Table 11 and Fig. 3).

Irrespective of the route of regeneration and doses of irradiation tried, plantlets of cultivar Maran were better in the various root characters as compared to the cultivar Rio-de-Janeiro.

In general, regenerants from non-irradiated source recorded higher root number and root length as compared to regenerants from irradiated calli.

4.1.5 Planting out, Hardening, Final Survival and Growth of Regenerants

The regenerants produced through indirect organogenesis, embryogenesis and *in vitro* mutagenesis were ready for plant out four to five months after culture initiation (Plate 7). Plantlets with well-developed pseudostem and roots were planted in polythene bags filled with sterile sand and hardened for two weeks (Plate 8). After two weeks, the plantlets were transferred to big poly bags filled with potting mixture and kept in the net house for rhizome formation (Plate 9). Growth parameters were recorded in plantlets for a period of three months.

4.1.5.1 Plantlet Establishment

The data relating to establishment and growth of plantlets produced through various routes are presented in Table 12. The regenerants produced through

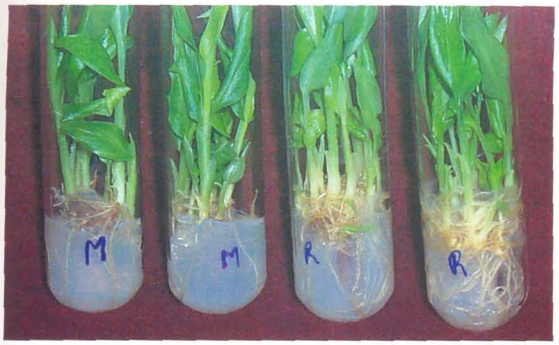


Plate 7. Rooted cultures of ginger ready for plant out

Plate 8. Plantlets of ginger kept for hardening

Plate 9. Hardened plantlets of ginger established in net house for rhizome formation

Table 12. Establishment of plantlets regenerated through various routes in ginger (*Z. officinale* Rosc.)

Sl. No.	Group	No. planted out	No. established - 3 MAP	Establishment (%)*
1	MC	73.00	48.00	65.75
2	RC	66.00	47.00	71.21
3	Mse	72.00	48.00	66.67
4	Rse	74.00	41.00	55.41
5	MC 10Gy	92.00	64.00	69.57
6	RC 10Gy	71.00	40.00	56.34
7	Mse 20Gy	77.00	45.00	58.44
8	Rse 10 Gy	87.00	45.00	51.72

*Establishment three months after planting
MAP - Months after planting
MC - Plantlets - indirect organogenesis cv. Maran
RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro
Mse - Plantlets - indirect embryogenesis cv. Maran
Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro
MC 10Gy - Plantlets - indirect organogenesis cv. Maran - irradiated at 10 Gy
RC 10 Gy - Plantlets - indirect organogenesis cv. Rio-de-Janeiro - irradiated at 10 Gy
Mse 20Gy - Plantlets - indirect embryogenesis cv. Maran irradiated at 20 Gy
Rse 10Gy - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro irradiated at 10 Gy

indirect organogenesis exhibited better establishment (68.48 %) followed by regenerants through *in vitro* mutagenesis (61.04 %) and embryogenesis (59.02 %) (Fig. 4).

Irrespective of the route of regeneration and doses of irradiation tried, the cultivar Maran recorded better survival (65.11 %) as compared to cultivar Rio-de-Janeiro (58.67 %).

In general, regenerants from irradiated calli exhibited low survival (61.04%) as compared to regenerants from non-irradiated source (63.75 %).

4.1.5.2 Growth Analysis in Regenerants Produced Through Various Routes

Morphological characters in regenerants produced through various routes were observed from plant out to three months after planting at monthly intervals and the growth rate of regenerants in various morphological characters are presented in Table 13.

At plant out stage, regenerants produced through indirect organogenesis exhibited more length of pseudostem followed by regenerants of indirect embryogenesis. More tiller number was recorded in regenerants produced through *in vitro* mutagenesis closely followed by regenerants produced through indirect organogenesis. Irrespective of the mode of regeneration, the growth observations recorded gave almost the same values in the two cultivars studied.

Growth observations recorded in regenerants showed that regenerants produced through indirect organogenesis exhibited high growth rate in length of pseudostem during the period of observation. Growth rate in number of tillers and length of leaves were high in regenerants produced through *in vitro* mutagenesis followed by regenerants of indirect embryogenesis. In the two cultivars studied, growth rate recorded for number of tillers and length of leaves were almost the same. But in length of pseudostem, the growth rate was slightly higher in regenerants of cultivar Maran (8.6 cm) as compared to Rio-de-Janeiro (7.71 cm).

Table 13. Growth analysis in plantlets regenerated through various routes in ginger (*Z. officinale* Rosc.)

Sl. No.	Group	At plant out		1 MAP			2 MAP			3 MAP			Increment at 1-3 MAP		
		*Mean tiller no.	*Mean length of pseudostem (cm)	*Mean tiller no.	*Mean length of pseudostem (cm)	*Mean length of longest leaf (cm)	*Mean tiller no.	*Mean length of pseudostem (cm)	*Mean length of longest leaf (cm)	*Mean tiller no.	*Mean length of pseudostem (cm)	*Mean length of longest leaf (cm)	*Mean tiller no.	*Mean length of pseudostem (cm)	*Mean length of longest leaf (cm)
1	MC	4.21	8.24	3.00	12.55	6.93	3.71	16.08	9.13	4.64	33.96	13.89	1.64	21.41	6.97
2	RC	4.50	8.94	4.36	12.65	6.52	4.43	17.25	9.50	5.86	37.47	14.06	1.50	24.81	7.55
3	Mse	4.00	8.41	4.36	11.27	4.90	5.07	26.42	6.06	7.64	43.48	13.88	3.29	32.20	8.98
4	Rse	3.07	5.45	2.86	9.16	5.43	3.36	27.34	13.26	4.29	33.10	13.92	1.43	23.94	8.49
5	MC 10Gy	5.29	8.11	3.29	16.05	7.95	3.86	18.67	9.62	5.86	34.10	14.29	2.57	18.04	6.34
6	RC 10Gy	5.57	8.31	3.36	13.33	7.57	4.64	17.61	11.09	7.00	27.49	15.51	3.64	14.15	7.94
7	Mse 20Gy	2.64	5.61	3.07	8.46	4.91	4.00	13.09	5.69	6.29	40.02	15.65	3.21	31.55	10.74
8	Rse 10 Gy	4.57	6.10	3.29	8.57	4.66	4.07	12.24	5.57	6.29	38.22	15.32	3.00	29.65	10.66
C.D (0.05)		1.628	1.319	N.S	2.414	0.982	N.S	3.368	1.235	1.516	4.266	1.235	1.039	4.294	1.291
SE±		0.580	0.470		0.860	0.350		1.200	0.440	0.540	1.520	0.440	0.370	1.530	0.460

*Average of 14 observations
MC - Plantlets - indirect organogenesis cv. Maran
RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro
MC 10 Gy - Plantlets - indirect organogenesis cv. Maran - irradiated at 10 Gy
RC 10 Gy - Plantlets - indirect organogenesis cv. Rio-de-Janeiro - irradiated at 10 Gy
Mse - Plantlets - indirect embryogenesis cv. Maran
Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro
Mse 20 Gy - Plantlets - indirect embryogenesis cv. Maran irradiated at 20 Gy
Rse 10 Gy - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro irradiated at 10 Gy
MAP - Months After Planting

4.2 FIELD EVALUATION OF FIRST SET SOMACLONES IN GINGER

Somaclones of ginger cultivars Maran and Rio-de-Janeiro regenerated through *in vitro* adventitious bud culture after passing through 10 to 12 *in vitro* subculture cycles were planted out during 1999-2000 for rhizome development. The clones having enough rhizomes for field planting were evaluated for morphological, yield, quality attributes and reaction to rhizome rot and bacterial wilt diseases for three seasons (2002 to 2004) (Plates 10a and 10b). In the first year, 83 somaclones of cultivar Maran and 87 somaclones of cultivar Rio-de-Janeiro were field planted and morphological and yield characters were evaluated in 80 somaclones of Maran and 82 somaclones of Rio-de-Janeiro. Since post emergence rotting was observed in three somaclones of Maran and five somaclones of Rio-de-Janeiro, evaluation for morphological and yield parameters could not be carried out in eight somaclones. Based on yield performance, 72 somaclones of cultivar Maran and 73 somaclones of cultivar Rio-de-Janeiro were selected for evaluation of yield and reaction to diseases in second year. Based on yield and reaction to diseases, 43 somaclones of cultivar Maran and 33 somaclones of cultivar Rio-de-Janeiro were advanced to third year of evaluation. Quality parameters such as dry recovery percentage, contents of essential oil, oleoresin and fibre were assessed in 15 selected superior somaclones. Molecular characterization of selected superior somaclones was attempted using RAPD markers. Conventionally propagated (CP) plants of Maran and Rio-de-Janeiro served as control in the field evaluation and molecular biology experiments.

4.2.1 Morphological Characters

Morphological characterization of field planted somaclones was attempted. The data recorded on morphological parameters, six months after planting in somaclones of two cultivars are presented in Tables 14a and 14b. Frequency distribution of the various morphological parameters recorded is presented in Tables 14a(i) to 14a(vi) for clones of cultivar Maran and 14b(i) to 14b(vi) for clones of cultivar Rio-de-Janeiro. The different characters were evaluated between and within somaclones of two cultivars and between somaclones and CP plants of two cultivars.

4.2.1.1 Height of Pseudostem

Mean height of pseudostem recorded six months after planting ranged from 13.00 to 93.33 cm in the somaclones studied. In general, somaclones of cultivar Rio-

10a. Evaluation in mounds

10b. Evaluation in 1 x 1 m beds

Plate 10 Field evaluation of first set somaclones in ginger

Table 14a. Morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) cultivar Maran

Sl. No.	Somaclone No.	Mean height of pseudostem (cm)	Mean no. of tillers/plant	Mean no. of leaves/tiller	Mean length of longest leaf (cm)	Mean breadth of longest leaf (cm)	Leaf area (cm^2)
1	56 M	61.89	18.67	15.51	19.39	1.42	27.53
2	79 M	65.77	9.56	14.87	21.60	2.29	49.46
3	84 M	65.08	17.75	13.25	23.16	1.71	39.60
4	85 M	63.63	8.67	15.56	21.31	2.01	42.83
5	91 M	45.21	5.40	10.78	18.95	2.16	40.93
6	99 M	45.82	11.29	13.95	17.74	1.21	21.47
7	100 M	73.38	11.40	18.76	21.14	1.29	27.27
8	110 M	58.38	8.38	13.85	18.12	1.72	31.17
9	132M	59.76	16.29	14.93	23.63	2.13	50.33
10	136 M	62.33	9.67	14.33	22.74	1.92	43.66
11	139 M	37.82	5.00	10.11	17.65	1.25	22.06
12	150 M	40.95	6.60	10.00	16.44	1.65	27.13
13	197 M	61.30	16.14	16.21	20.71	1.40	28.99
14	199 M	40.76	12.00	14.00	18.10	1.76	31.86
15	220 M	61.29	14.75	15.54	22.69	2.33	52.87
16	276 M	44.98	16.17	14.66	12.40	0.92	11.41
17	283 M	53.42	15.20	11.93	17.62	1.38	24.32
18	284 M	66.67	18.75	17.40	20.49	1.72	35.24
19	287 M	59.36	21.38	14.85	22.99	2.11	48.51
20	288 M	18.80	3.50	8.00	16.50	1.50	24.75
21	290 M	48.91	8.67	16.00	17.02	1.59	27.06
22	311 M	57.51	12.00	15.66	19.61	1.66	32.55
23	313 M	43.24	9.60	12.06	14.39	1.40	20.15
24	317 M	38.92	8.83	14.66	17.03	1.29	21.97
25	342 M	76.13	23.25	23.04	21.69	1.54	33.40
26	348 M	46.02	14.33	10.44	21.16	1.98	41.90
27	356 M	46.00	16.00	11.66	16.53	1.10	18.18
28	372 M	58.94	15.25	14.50	21.49	1.45	31.16
29	374 M	68.11	12.50	17.67	20.93	1.65	34.53
30	381 M	61.50	20.00	25.00	20.05	1.15	23.06
31	382 M	35.61	9.00	11.17	18.28	1.22	22.30
32	392 M	52.00	10.50	9.83	19.75	1.43	28.24
33	393 M	47.66	14.00	11.00	23.23	1.63	37.86
34	397 M	59.58	12.75	14.26	21.11	1.72	36.31
35	400 M	27.00	12.00	9.33	14.06	1.26	17.72
36	411 M	72.33	23.00	10.00	23.33	1.23	28.70
37	431 M	18.49	3.00	6.50	13.65	0.90	12.29
38	432 M	41.23	17.00	9.00	16.16	0.90	14.54
39	434 M	23.33	3.00	6.00	17.33	1.16	20.10
40	435 M	37.50	3.00	10.50	17.00	1.15	19.55
41	436 M	55.10	15.00	12.33	23.50	1.40	32.90
42	439 M	16.66	12.00	5.00	9.00	1.23	11.07
43	441 M	72.33	19.00	17.66	22.83	1.50	34.25
44	444 M	42.00	10.00	11.66	17.66	1.00	17.66
45	446 M	48.66	19.00	9.33	20.50	1.26	25.83
46	462 M	33.33	9.00	8.00	18.50	1.60	29.60
47	464 M	37.66	8.00	10.00	16.00	1.50	24.00
48	488 M	69.86	24.00	17.66	23.23	1.46	33.92
49	500 M	57.33	18.00	12.33	23.36	1.26	29.43
50	505 M	35.00	8.00	8.66	19.83	1.23	24.39
51	510 M	51.33	12.00	12.00	21.33	0.90	19.20
52	513 M	59.13	20.00	15.33	22.83	1.20	27.40
53	528 M	36.83	7.00	9.50	17.25	1.27	21.91
54	529 M	41.15	12.67	10.78	19.53	1.31	25.58
55	549 M	75.67	15.50	24.67	20.41	1.47	30.00

Contd.

Sl. No.	Somaclone No.	Mean height of pseudostem (cm)	Mean no. of tillers/plant	Mean no. of leaves/tiller	Mean length of longest leaf (cm)	Mean breadth of longest leaf (cm)	Leaf area (cm^2)
56	554 M	60.25	14.33	16.77	21.55	1.42	30.60
57	565 M	45.67	8.00	11.33	18.25	1.08	19.71
58	572 M	70.33	20.00	15.66	25.00	1.66	41.50
59	580 M	61.66	13.00	17.00	20.50	0.96	19.68
60	583 M	60.05	17.50	14.33	22.33	1.65	36.84
61	588 M	61.83	18.00	14.33	23.63	1.57	37.10
62	649 M	18.00	5.00	5.66	12.76	1.10	14.04
63	659 M	63.38	16.00	15.78	22.93	1.61	36.92
64	660 M	55.79	15.67	12.99	20.16	1.35	27.22
65	668 M	26.33	11.50	7.50	17.50	1.41	24.68
66	743 M	57.50	4.00	11.00	22.00	1.06	23.32
67	746 M	57.16	15.00	13.33	20.83	1.77	36.87
68	781 M	26.16	12.00	7.33	15.16	1.00	15.16
69	918 M	64.00	34.00	20.66	17.33	0.93	16.12
70	953 M	36.33	6.00	9.33	17.85	1.52	27.13
71	970 M	46.94	20.00	14.11	16.82	1.24	20.86
72	980 M	41.19	11.00	9.89	19.02	2.09	39.75
73	985 M	40.95	9.00	10.67	15.80	1.00	15.80
74	M I	37.86	9.00	9.44	19.87	1.69	33.58
75	M III	40.40	12.00	11.66	18.43	1.03	18.98
76	M IV	13.00	4.00	5.00	11.16	0.93	10.38
77	M V	33.50	4.00	11.00	12.00	1.25	15.00
78	M VI	60.59	20.13	14.46	22.62	1.83	41.39
79	M VII	59.03	10.00	13.66	23.00	2.36	54.28
80	M VIII	37.27	5.67	10.44	15.77	1.59	25.07
81	Control M	70.07	20.00	15.67	29.00	2.17	62.93
	S.D	15.321	5.869	4.021	3.487	0.366	10.848
	C.V (%)	30.841	45.789	31.297	18.057	25.092	37.631

Mophological observations at six months after planting

Frequency distribution of morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) (cultivar Maran)

Table 14a (i). Height of pseudostem

Group No.	Height of pseudostem (cm)	Frequency (%)
1	12.50 - 20.49	6.25
2	20.50 - 28.49	5.00
3	28.50 - 36.49	6.25
4	36.50 - 44.49	20.00
5	44.50 . 52.49	15.00
6	52.50 - 60.49	20.00
7	60.50 - 68.49	18.75
8	68.50 - 76.49	8.75

Table 14a (ii). Number of tillers/plant

Group No.	No. of tillers/plant	Frequency (%)
1	2.50 - 6.49	15.00
2	6.50 - 10.49	22.50
3	10.50 -14.49	23.75
4	14.50 - 18.49	21.25
5	18.50 - 22.49	12.50
6	22.50 - 26.49	3.75
7	26.50 - 30.49	0.00
8	30.50 - 34.49	1.25

Table 14a (iii). Number of leaves/tiller

Group No.	Number of leaves/tiller	Frequency (%)
1	3.00 - 5.99	3.75
2	6.00 - 8.99	8.75
3	9.00 - 11.99	33.75
4	12.00 - 14.99	28.75
5	15.00 - 17.99	18.75
6	18.00 - 20.99	2.50
7	21.00 - 23.99	1.25
8	24.00 - 26.99	2.50

Table 14a (iv). Length of leaf

Group No.	Length of leaf (cm)	Frequency (%)
1	7.00 - 9.99	1.25
2	10.00 - 12.99	5.00
3	13.00 - 15.99	7.50
4	16.00 - 18.99	31.25
5	19.00 - 21.99	31.25
6	22.00 - 24.99	22.50
7	25.00 - 27.99	1.25

Table 14a (v). Breadth of leaf

Group No.	Breadth of leaf (cm)	Frequency (%)
1	0.90 - 1.10	18.75
2	1.11 - 1.31	23.75
3	1.32 - 1.52	20.00
4	1.53 - 1.73	21.25
5	1.74 - 1.94	5.00
6	1.95 - 2.15	6.25
7	2.16 - 2.36	5.00

Table 14a (vi). Area of leaf

Group No.	Area of leaf (cm^2)	Frequency (%)
1	8.66 - 15.65	10.00
2	15.66 - 22.65	22.50
3	22.66 - 29.65	27.50
4	29.66 - 36.65	17.50
5	36.66 - 43.65	15.00
6	43.66 - 50.65	5.00
7	50.66 - 57.65	2.50

Table 14b. Morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) cultivar Rio-de-Janeiro

Sl. No.	Somaclone No.	Mean height of pseudostem (cm)	Mean no. of tillers/plant	Mean no. of leaves/tiller	Mean length of longest leaf (cm)	Mean breadth of longest leaf (cm)	Leaf area (cm^2)
1	88 R	48.77	15.00	10.00	20.98	1.29	27.06
2	137 R	52.71	5.60	9.80	16.13	1.53	24.68
3	281 R	54.88	12.13	11.08	21.91	1.69	37.03
4	292 R	67.27	30.57	15.38	22.12	1.28	28.31
5	296 R	62.52	26.50	14.25	20.73	1.21	25.08
6	308 R	56.94	15.80	15.33	20.14	1.88	37.86
7	311 R	68.57	10.67	15.22	18.24	1.85	33.74
8	312 R	51.97	17.17	14.08	22.80	1.65	37.62
9	314 R	62.63	16.25	15.17	17.16	1.17	20.08
10	315 R	41.88	12.33	13.00	13.40	1.18	15.81
11	335 R	34.80	10.67	9.67	13.00	1.20	15.60
12	336 R	35.25	10.17	10.61	15.02	1.30	19.53
13	337 R	48.79	9.25	12.50	19.67	1.71	33.64
14	338 R	56.95	14.50	16.17	19.78	1.36	26.90
15	345 R	46.00	9.40	10.96	19.07	1.54	29.37
16	346 R	41.67	11.75	12.83	16.89	1.52	25.67
17	347 R	32.00	5.00	9.00	16.50	1.63	26.90
18	349 R	64.85	17.25	15.08	22.04	1.69	37.25
19	350 R	47.36	13.50	13.58	16.62	1.60	26.59
20	351 R	65.43	19.00	16.50	20.96	1.37	28.72
21	355 R	42.46	10.25	15.55	16.76	1.78	29.83
22	358 R	34.25	9.50	11.00	13.48	1.24	16.72
23	361 R	31.33	8.25	7.16	15.26	1.25	19.08
24	364 R	54.62	17.25	12.33	22.30	1.85	41.26
25	367 R	35.11	8.25	11.66	18.78	1.62	30.42
26	368 R	91.66	19.00	26.00	18.83	1.13	21.28
27	373 R	61.66	14.50	15.83	20.42	1.50	30.63
28	377 R	63.42	16.00	14.66	19.51	1.45	28.29
29	378 R	38.78	12.50	10.00	13.62	1.00	13.62
30	384 R	38.83	18.50	7.17	16.75	1.05	17.59
31	386 R	43.72	14.67	13.67	14.72	1.23	18.11
32	395 R	53.42	12.75	11.92	19.59	2.20	43.10
33	399 R	57.33	20.00	15.00	17.66	1.33	23.49
34	413 R	51.83	12.00	15.83	15.62	1.37	21.40
35	418 R	67.66	17.00	15.66	21.00	1.13	23.73
36	431 R	80.66	21.00	14.00	24.66	1.66	40.94
37	438 R	55.00	6.00	15.00	17.00	0.96	16.32
38	463 R	51.66	18.00	12.00	22.50	1.06	23.85
39	466 R	34.33	5.00	6.00	19.66	1.73	34.01
40	471 R	71.52	14.50	16.33	20.18	1.66	33.50
41	475 R	32.36	12.00	7.33	20.90	1.73	36.16
42	476 R	67.33	12.00	19.66	20.76	1.60	33.22
43	478 R	63.55	25.50	17.67	17.75	1.65	29.29
44	482 R	41.66	13.00	7.50	18.50	1.73	32.01
45	485 R	75.00	23.50	17.83	20.33	1.00	20.33
46	517 R	66.66	25.00	18.33	14.80	1.06	15.69
47	524 R	66.00	20.00	15.00	27.33	1.16	31.70
48	526 R	93.00	31.00	26.66	18.16	1.36	24.70
49	531 R	23.76	5.00	8.50	13.33	1.33	17.73
50	535 R	42.23	9.00	9.16	19.25	1.27	24.45
51	548 R	59.00	9.00	13.00	18.16	1.56	28.33
52	561 R	73.00	11.00	14.66	25.33	1.63	41.29
53	582 R	34.84	6.67	9.55	16.92	1.22	20.64
54	589 R	32.66	10.00	7.66	15.90	1.60	25.44
55	590 R	82.20	18.00	18.00	25.36	1.96	49.71
56	597 R	58.76	19.00	11.00	22.40	1.50	33.60
57	610 R	93.33	21.00	15.67	21.83	1.00	21.83

Contd.

Sl. No.	Somaclone No.	Mean height of pseudostem (cm)	Mean no. of tillers/plant	Mean no. of leaves/tiller	Mean length of longest leaf (cm)	Mean breadth of longest leaf (cm)	Leaf area (cm^2)
58	616 R	45.67	11.00	10.83	19.66	1.23	24.18
59	617 R	81.80	14.00	19.00	23.50	1.43	33.61
60	622 R	61.73	8.00	14.00	19.33	1.36	26.29
61	626 R	49.80	16.00	12.00	19.83	1.20	23.80
62	630 R	86.46	19.00	19.00	24.36	1.26	30.69
63	704 R	45.78	12.60	11.46	19.60	1.68	32.93
64	734 R	59.78	15.00	12.44	20.39	1.35	27.53
65	748 R	39.44	15.33	8.99	18.55	1.56	28.94
66	772 R	39.50	11.50	10.17	19.08	1.22	23.28
67	786 R	65.63	18.00	16.33	21.50	1.52	32.68
68	790 R	43.66	13.33	11.33	17.55	1.87	32.82
69	801 R	54.75	19.33	12.77	19.99	1.78	35.58
70	982 R	35.50	10.00	13.67	11.78	1.01	11.90
71	R I	43.19	4.00	10.75	17.82	1.38	24.59
72	R II	58.55	10.67	15.00	19.26	1.08	20.80
73	R III	55.70	12.00	10.50	22.20	1.65	36.63
74	R IV	40.55	9.67	11.66	15.93	1.42	22.62
75	R V	49.78	10.67	10.33	23.24	1.53	35.56
76	R VI	47.64	9.67	12.44	17.80	1.61	28.66
77	R VII	46.33	13.00	10.66	17.00	1.46	24.82
78	R VIII	79.10	16.00	17.00	25.00	1.73	43.25
79	R IX	51.05	7.00	10.50	23.03	1.67	38.46
80	R X	28.58	4.00	8.50	12.67	1.14	14.44
81	R XI	58.14	15.33	16.00	16.27	1.42	23.10
82	R XII	53.66	20.00	13.33	15.55	1.53	23.79
83	Control R	63.75	14.00	12.00	19.25	2.45	47.16
	S.D	15.739	5.665	3.748	3.248	0.285	8.070
	C.V (%)	29.086	40.632	28.414	17.077	19.590	28.886

Mophological observations at six months after planting

Frequency distribution of morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) (cultivar Rio-de-Janeiro)

Table 14b (i). Height of pseudostem

Group No.	Height of pseudostem (cm)	Frequency (%)
1	22.50 - 31.49	3.66
2	31.50 - 40.49	17.07
3	40.50 - 49.49	20.73
4	49.50 - 58.49	21.95
5	58.50 - 67.49	20.73
6	67.50 - 76.49	6.10
7	76.50 - 85.49	4.88
8	85.50 - 94.49	4.88

Table 14b (ii). Number of tillers/plant

Group No.	No. of tillers/plant	Frequency (%)
1	1.50 - 5.49	6.10
2	5.50 - 9.49	13.41
3	9.50 - 13.49	32.93
4	13.50 - 17.49	23.17
5	17.50 - 21.49	17.07
6	21.50 - 25.49	2.44
7	25.50 - 29.49	2.44
8	29.50 - 33.49	2.44

Table 14b (iii). Number of leaves/tiller

Group No.	Number of leaves/tiller	Frequency (%)
1	4.33 - 7.32	3.66
2	7.33 - 10.32	17.07
3	10.33 - 13.32	31.71
4	13.33 - 16.32	31.71
5	16.33 - 19.32	12.20
6	19.33 - 22.32	1.22
7	22.33 - 25.32	0.00
8	25.33 - 28.32	2.44

Table 14b (iv). Length of leaf

Group No.	Length of leaf (cm)	Frequency (%)
1	11.55 - 13.54	7.32
2	13.55 - 15.54	6.10
3	15.55 - 17.54	18.29
4	17.55 - 19.54	21.95
5	19.55 - 21.54	24.39
6	21.55 - 23.54	14.63
7	23.55 - 25.54	6.10
8	25.55 - 27.54	1.22

Table 14b (v). Breadth of leaf

Group No.	Breadth of leaf (cm)	Frequency (%)
1	0.96 - 1.20	20.73
2	1.21 - 1.45	30.49
3	1.46 - 1.70	32.93
4	1.71 - 1.95	13.41
5	1.96 - 2.20	2.44

Table 14b (vi). Area of leaf

Group No.	Area of leaf (cm^2)	Frequency (%)
1	10.80 - 15.79	6.10
2	15.80 - 20.79	13.41
3	20.80 - 25.79	25.61
4	25.80 - 30.79	21.95
5	30.80 - 35.79	17.07
6	35.80 - 40.79	8.54
7	40.80 - 45.79	6.10
8	45.80 - 50.79	1.22

de-Janeiro (23.76 - 93.33 cm) exhibited more height than clones of cultivar Maran (13.00 – 76.13 cm). Sixteen per cent somaclones of cultivar Rio-de-Janeiro came in the highest classes of frequency table recording height more than 68 cm while only ten per cent clones of cultivar Maran exhibited height more than 68 cm.

Height of pseudostem was found more in 16 per cent somaclones as compared to CP plants. Twenty four per cent somaclones of Rio-de-Janeiro exhibited more height than CP plants while only 7.50 per cent clones of Maran exhibited more height showing the superiority of somaclones of Rio-de-Janeiro over CP plants with respect to height of pseudostem.

Variability for the character studied was almost uniform in the somaclones of two cultivars evaluated, recording a coefficient of variation of 30.841 per cent in clones of cultivar Maran and 29.086 per cent in clones of cultivar Rio-de-Janeiro.

4.2.1.2 Number of Tillers / Plant

The number of tillers ranged from 3 to 34 in the clones evaluated. The number of tillers/plant was found in the range of 3.00 to 34.00 in clones of cultivar Maran and 4.00 to 31.00 in clones of cultivar Rio-de-Janeiro. More somaclones of cultivar Rio-de-Janeiro exhibited higher tiller number than clones of cultivar Maran. Seven per cent clones of Rio-de-Janeiro produced more than 23 tillers/plant while only five per cent clones of Maran came in the range.

When tiller production in somaclones and CP plants was compared, 27 per cent somaclones were found superior to CP plants in tiller production. Forty five per cent somaclones of Rio-de-Janeiro exhibited more tiller production than CP plants while only 7.50 per cent clones of Maran produced more tillers than CP plants. So somaclones of Rio-de-Janeiro were found better in tiller production over CP plants.

In general, wide variability was observed in tiller number in the somaclones evaluated with a coefficient of variation of 40 to 45 per cent. In somaclones of Maran, the variability observed was more than that of Rio-de-Janeiro

clones. The coefficient of variation recorded for the character was 45.789 per cent in clones of Maran as compared to 40.632 per cent in clones of Rio-de-Janeiro.

4.2.1.3 Number of Leaves / Tiller

Mean number of leaves/tiller recorded six months after planting ranged from 5.00 to 26.66 in the somaclones evaluated. The number of leaves was in between 5.00-25.00 in somaclones of Maran and 6.00-26.66 in somaclones of Rio-de-Janeiro. Only 25 per cent somaclones of cultivar Maran recorded high leaf production (15.00-25.00) while 30 per cent clones of cultivar Rio-de-Janeiro produced leaves in the range of 15.00-26.66. Hence, high leaf production was observed in clones of cultivar Rio-de-Janeiro as compared to clones of cultivar Maran.

The leaf production in somaclones was high compared to CP plants. Thirty eight per cent somaclones showed higher leaf production than CP plants. With regard to response of cultivars to leaf production, 57 per cent clones of Rio-de-Janeiro showed higher leaf number over CP plants while only 17.50 per cent clones of Maran recorded higher leaf number over CP plants. So the somaclones of cultivar Rio-de-Janeiro was better in leaf production than clones of cultivar Maran.

High amount of variability was observed in somaclones of Maran in leaf production. The coefficient of variation worked out was 31.297 per cent in clones of Maran as compared to 28.414 per cent in clones of Rio-de-Janeiro.

4.2.1.4 Length, Breadth and Area of Leaf

The length, breadth and area of longest leaf were recorded in different somaclones evaluated. The somaclones of cultivar Maran exhibited more leaf length than clones of cultivar Rio-de-Janeiro. The mean length of longest leaf recorded varied from 9.00 to 29.00 cm in clones of Maran and 11.78 to 27.33 cm in clones of Rio-de-Janeiro. Similarly, breadth of leaf was also high in clones of Maran and ranged from 0.90 to 2.36 cm as compared to 0.96 to 2.20 cm in clones of Rio-de-Janeiro. Hence, the leaf area was also high in Maran somaclones and it ranged from 10.38 to 54.28 cm^2 and 11.90 to 49.71 cm^2 in clones of Rio-de-Janeiro.

The leaf area of somaclones and CP plants when compared, leaf area was found high in CP plants. Only 1.22 per cent clones of Rio-de-Janeiro exhibited higher leaf area than CP plants while none of the clones of Maran recorded leaf area more than that of CP plants.

The somaclones of cultivar Maran exhibited more variability in leaf breadth and leaf area. Coefficients of variation recorded for breadth of leaf and leaf area in somaclones of Maran were 25.092 per cent and 37.631 per cent respectively as compared to 19.590 per cent and 28.886 per cent respectively in clones of Rio-de-Janeiro. The variability for leaf length was almost uniform in the two cultivars.

4.2.2 Growth Rate in Morphological Characters

The growth characters viz. height of pseudostem, number of tillers / plant and number of leaves/tiller were recorded in 80 somaclones of Maran and 82 somaclones of Rio-de-Janeiro during second, fourth and sixth month of planting and the data on morphological characters and growth increment during different periods of observation are presented in Tables 15a and 15b. Frequency distribution for growth characters is presented in Tables 15a(i) to 15a(vi) for clones of Maran and 15b(i) to 15b(vi) for clones of Rio-de-Janeiro.

4.2.2.1 Height of Pseudostem

The height of pseudostem increased at the rate of 7.46 cm / month on an average in the somaclones observed. In CP plants, height of pseudostem increased at the rate of 9.29 cm / month. However, 25 per cent somaclones exhibited superiority in growth rate over CP plants. In majority of somaclones, increase in height of pseudostem was maximum from second to fourth month after planting, the average increase in height being 10 cm per month. In 75 per cent clones of Maran, height of pseudostem increased at the rate of 10.72 cm/month from second to fourth month after planting. Similarly, in 76 per cent clones of Rio-de-Janeiro, the height of pseudostem increased at the rate of 12.21 cm/month.

Table 15a. Growth increment in morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) cultivar Maran

Sl. No.	Somaclone No.	Height of pseudostem (cm)			Growth increment in height of pseudostem (cm)		No. of tillers/plant			Growth increment in no. of tillers/plant		No. of leaves/tiller			Growth increment in no. of leaves/tiller	
		2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP
1	56 M	22.68	48.32	61.89	25.64	13.57	4.00	8.67	18.67	4.67	10.00	5.61	9.11	15.51	3.50	6.40
2	79 M	32.72	49.39	65.77	16.67	16.38	2.88	6.67	9.56	3.79	2.89	7.19	14.17	14.87	6.98	0.70
3	84 M	25.01	64.48	65.08	39.47	0.60	4.00	7.25	17.75	3.25	10.50	10.06	12.25	13.25	2.19	1.00
4	85 M	29.77	50.49	63.63	20.72	13.14	3.17	7.00	8.67	3.83	1.67	5.47	9.93	15.56	4.46	5.63
5	91 M	39.43	41.56	45.21	2.13	3.65	2.30	4.59	5.40	2.29	0.81	6.46	9.57	10.78	3.11	1.21
6	99 M	32.04	43.66	45.82	11.62	2.16	5.43	10.00	11.29	4.57	1.29	9.50	10.20	13.95	0.70	3.75
7	100 M	35.35	69.02	73.38	33.67	4.36	2.80	6.80	11.40	4.00	4.60	8.30	15.53	18.76	7.23	3.23
8	110 M	29.56	52.39	58.38	22.83	5.99	3.25	6.75	8.38	3.50	1.63	6.94	12.17	13.85	5.23	1.68
9	132M	29.38	57.76	59.76	28.38	2.00	3.71	9.29	16.29	5.58	7.00	5.84	10.17	14.93	4.33	4.76
10	136 M	27.83	56.82	62.33	28.99	5.51	3.00	5.00	9.67	2.00	4.67	7.44	11.19	14.33	3.75	3.14
11	139 M	24.67	37.21	37.82	12.54	0.61	1.67	4.00	5.00	2.33	1.00	8.00	9.55	10.11	1.55	0.56
12	150 M	24.30	31.35	40.95	7.05	9.60	1.80	3.80	6.60	2.00	2.80	4.73	9.99	10.00	5.26	0.01
13	197 M	36.63	59.52	61.30	22.89	1.78	6.29	13.00	16.14	6.71	3.14	6.96	13.42	16.21	6.46	2.79
14	199 M	12.13	26.00	40.76	13.87	14.76	8.00	9.00	12.00	1.00	3.00	2.66	8.33	14.00	5.67	5.67
15	220 M	36.17	47.40	61.29	11.23	13.89	2.25	6.50	14.75	4.25	8.25	8.48	10.88	15.54	2.40	4.66
16	276 M	25.39	39.57	44.98	14.18	5.41	3.17	7.00	16.17	3.83	9.17	7.00	11.31	14.66	4.31	3.35
17	283 M	33.84	48.42	53.42	14.58	5.00	4.20	7.40	15.20	3.20	7.80	5.74	11.13	11.93	5.39	0.80
18	284 M	35.77	61.53	66.67	25.76	5.14	3.00	9.00	18.75	6.00	9.75	8.31	13.23	17.40	4.92	4.17
19	287 M	37.53	57.36	59.36	19.83	2.00	4.50	11.88	21.38	7.38	9.50	6.36	12.16	14.85	5.80	2.69
20	288 M	15.00	17.80	18.80	2.80	1.00	1.50	2.50	3.50	1.00	1.00	3.75	5.00	8.00	1.25	3.00
21	290 M	35.25	36.55	48.91	1.30	12.36	1.67	4.67	8.67	3.00	4.00	8.67	8.67	16.00	0.00	7.33
22	311 M	18.49	35.67	57.51	17.18	21.84	3.50	5.17	12.00	1.67	6.83	4.33	10.80	15.66	6.47	4.86
23	313 M	15.38	31.29	43.24	15.91	11.95	5.40	5.40	9.60	0.00	4.20	4.68	10.20	12.06	5.52	1.86
24	317 M	17.79	31.62	38.92	13.83	7.30	3.83	3.83	8.83	0.00	5.00	3.58	8.42	14.66	4.84	6.24
25	342 M	35.46	62.39	76.13	26.93	13.74	3.63	9.63	23.25	6.00	13.62	7.59	15.28	23.04	7.69	7.76
26	348 M	26.39	44.11	46.02	17.72	1.91	3.33	7.00	14.33	3.67	7.33	6.28	10.28	10.44	4.00	0.16
27	356 M	12.38	43.43	46.00	31.05	2.57	4.00	6.00	16.00	2.00	10.00	3.00	10.66	11.66	7.66	1.00
28	372 M	26.69	39.19	58.94	12.50	19.75	2.50	6.00	15.25	3.50	9.25	7.21	10.98	14.50	3.77	3.52
29	374 M	19.04	37.78	68.11	18.74	30.33	2.75	6.33	12.50	3.58	6.17	5.29	11.88	17.67	6.59	5.79
30	381 M	20.09	55.38	61.50	35.29	6.12	5.50	9.00	20.00	3.50	11.00	5.02	12.50	25.00	7.48	12.50
31	382 M	13.61	26.16	35.61	12.55	9.45	4.00	4.00	9.00	0.00	5.00	4.63	6.50	11.17	1.87	4.67
32	392 M	18.50	41.40	52.00	22.90	10.60	3.50	5.00	10.50	1.50	5.50	4.29	8.88	9.83	4.59	0.95

Contd.

Sl. No.	Somaclone No.	Height of pseudostem (cm)			Growth increment in height of pseudostem (cm)		No. of tillers/plant			Growth increment in no. of tillers/plant		No. of leaves/tiller			Growth increment in no. of leaves/tiller	
		2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP
33	393 M	25.50	45.00	47.66	19.50	2.66	2.00	9.00	14.00	7.00	5.00	12.00	10.50	11.00	-1.50	0.50
34	397 M	19.83	42.38	59.58	22.55	17.20	2.00	3.78	12.75	1.78	8.97	4.75	9.90	14.26	5.15	4.36
35	400 M	18.50	23.70	27.00	5.20	3.30	2.00	3.00	12.00	1.00	9.00	4.00	7.33	9.33	3.33	2.00
36	411 M	19.67	59.16	72.33	39.49	13.17	2.00	6.00	23.00	4.00	17.00	4.00	9.25	10.00	5.25	0.75
37	431 M	7.75	11.00	18.49	3.25	7.49	2.00	2.00	3.00	0.00	1.00	2.25	2.50	6.50	0.25	4.00
38	432 M	17.75	40.23	41.23	22.48	1.00	11.00	11.00	17.00	0.00	6.00	4.88	8.00	9.00	3.12	1.00
39	434 M	21.00	22.33	23.33	1.33	1.00	1.00	2.00	3.00	1.00	1.00	6.00	6.00	6.00	0.00	0.00
40	435 M	13.00	17.00	37.50	4.00	20.50	1.00	2.00	3.00	1.00	1.00	3.00	3.00	10.50	0.00	7.50
41	436 M	15.67	43.33	55.10	27.66	11.77	3.00	7.00	15.00	4.00	8.00	4.33	10.00	12.33	5.67	2.33
42	439 M	14.00	15.66	16.66	1.66	1.00	4.00	6.00	12.00	2.00	6.00	4.75	4.75	5.00	0.00	0.25
43	441 M	18.38	49.33	72.33	30.95	23.00	4.00	8.00	19.00	4.00	11.00	8.00	8.00	17.66	0.00	9.66
44	444 M	17.10	25.66	42.00	8.56	16.34	5.00	6.00	10.00	1.00	4.00	3.80	7.00	11.66	3.20	4.66
45	446 M	12.95	47.66	48.66	34.71	1.00	6.00	6.00	19.00	0.00	13.00	4.50	9.33	9.33	4.83	0.00
46	462 M	10.40	21.43	33.33	11.03	11.90	4.00	5.00	9.00	1.00	4.00	3.33	6.75	8.00	3.42	1.25
47	464 M	14.38	36.76	37.66	22.38	0.90	4.00	5.00	8.00	1.00	3.00	5.00	9.66	10.00	4.66	0.34
48	488 M	34.00	53.03	69.86	19.03	16.83	3.00	7.00	24.00	4.00	17.00	7.66	13.25	17.66	5.59	4.41
49	500 M	23.00	57.00	57.33	34.00	0.33	3.00	12.00	18.00	9.00	6.00	9.50	11.75	12.33	2.25	0.58
50	505 M	16.64	24.10	35.00	7.46	10.90	8.00	8.00	8.00	0.00	0.00	4.08	8.00	8.66	3.92	0.66
51	510 M	16.21	50.33	51.33	34.12	1.00	7.00	7.00	12.00	0.00	5.00	4.00	10.25	12.00	6.25	1.75
52	513 M	37.00	55.33	59.13	18.33	3.80	2.00	6.00	20.00	4.00	14.00	8.00	11.50	15.33	3.50	3.83
53	528 M	15.63	31.10	36.83	15.47	5.73	2.00	3.50	7.00	1.50	3.50	4.13	5.75	9.50	1.62	3.75
54	529 M	15.37	31.06	41.15	15.69	10.09	5.33	7.00	12.67	1.67	5.67	3.33	8.72	10.78	5.39	2.06
55	549 M	23.08	50.33	75.67	27.25	25.34	5.00	7.50	15.50	2.50	8.00	4.03	11.13	24.67	7.10	13.54
56	554 M	22.05	59.25	60.25	37.20	1.00	6.00	9.33	14.33	3.33	5.00	5.15	12.66	16.77	7.51	4.11
57	565 M	23.42	33.33	45.67	9.91	12.34	3.50	3.50	8.00	0.00	4.50	4.67	8.00	11.33	3.33	3.33
58	572 M	48.00	54.26	70.33	6.26	16.07	1.00	5.00	20.00	4.00	15.00	13.00	13.10	15.66	0.10	2.56
59	580 M	21.16	55.00	61.66	33.84	6.66	6.00	7.00	13.00	1.00	6.00	6.00	11.75	17.00	5.75	5.25
60	583 M	30.70	58.00	60.05	27.30	2.05	2.00	7.50	17.50	5.50	10.00	4.33	11.25	14.33	6.92	3.08
61	588 M	20.50	40.50	61.83	20.00	21.33	3.00	9.50	18.00	6.50	8.50	8.50	8.70	14.33	0.20	5.63
62	649 M	4.67	15.50	18.00	10.83	2.50	3.00	3.00	5.00	0.00	2.00	2.33	2.50	5.66	0.17	3.16
63	659 M	26.52	62.38	63.38	35.86	1.00	5.67	8.67	16.00	3.00	7.33	6.64	14.67	15.78	8.03	1.11
64	660 M	36.41	50.23	55.79	13.82	5.56	5.00	8.33	15.67	3.33	7.34	9.44	11.22	12.99	1.78	1.77
65	668 M	24.00	25.33	26.33	1.33	1.00	4.00	7.50	11.50	3.50	4.00	5.21	6.50	7.50	1.29	1.00
66	743 M	17.88	42.93	57.50	25.05	14.57	4.00	4.00	4.00	0.00	0.00	5.25	8.75	11.00	3.50	2.25

Contd.

Sl. No.	Somaclone No.	Height of pseudostem (cm)			Growth increment in height of pseudostem (cm)		No. of tillers/plant			Growth increment in no. of tillers/plant		No. of leaves/tiller			Growth increment in no. of leaves/tiller	
		2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP
67	746 M	25.33	52.20	57.16	26.87	4.96	3.50	6.50	15.00	3.00	8.50	5.25	12.29	13.33	7.04	1.04
68	781 M	12.91	25.16	26.16	12.25	1.00	6.00	7.00	12.00	1.00	5.00	4.20	7.00	7.33	2.80	0.33
69	918 M	24.30	60.80	64.00	36.50	3.20	9.00	19.00	34.00	10.00	15.00	7.40	19.33	20.66	11.93	1.33
70	953 M	15.13	28.00	36.33	12.87	8.33	1.50	3.00	6.00	1.50	3.00	6.25	8.33	9.33	2.08	1.00
71	970 M	28.55	36.11	46.94	7.56	10.83	2.67	9.00	20.00	6.33	11.00	9.13	10.00	14.11	0.87	4.11
72	980 M	13.08	29.44	41.19	16.36	11.75	3.50	8.00	11.00	4.50	3.00	5.25	8.89	9.89	3.64	1.00
73	985 M	14.38	32.03	40.95	17.65	8.92	1.50	4.50	9.00	3.00	4.50	6.25	7.13	10.67	0.88	3.54
74	M I	22.21	33.78	37.86	11.57	4.08	4.67	4.67	9.00	0.00	4.33	4.81	7.36	9.44	2.55	2.08
75	M III	11.88	38.70	40.40	26.82	1.70	6.00	6.00	12.00	0.00	6.00	3.83	9.33	11.66	5.50	2.33
76	M IV	3.65	4.75	13.00	1.10	8.25	1.00	2.00	4.00	1.00	2.00	2.00	2.00	5.00	0.00	3.00
77	M V	20.00	32.00	33.50	12.00	1.50	1.00	1.00	4.00	0.00	3.00	5.00	9.00	11.00	4.00	2.00
78	M VI	36.19	58.00	60.59	21.81	2.59	5.13	11.25	20.13	6.12	8.88	7.39	13.65	14.46	6.26	0.81
79	M VII	10.33	28.00	59.03	17.67	31.03	3.00	5.00	10.00	2.00	5.00	3.33	9.33	13.66	6.00	4.33
80	M VIII	14.64	25.94	37.27	11.30	11.33	3.33	5.67	5.67	2.34	0.00	3.35	7.89	10.44	4.54	2.55
81	Control M	39.50	58.67	70.07	19.17	11.40	2.00	8.00	20.00	6.00	12.00	12.00	13.00	15.67	1.00	2.67
	S.D	9.175	14.410	15.321	10.345	7.295	1.907	2.879	5.869	2.275	4.073	2.300	3.023	4.021	2.556	2.616
	C.V (%)	40.424	34.965	30.841	55.872	86.178	51.518	43.848	45.789	79.429	65.150	39.250	30.984	31.297	65.592	84.626

MAP - Months after planting

Frequency distribution of growth increment in morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) (cultivar Maran)

Table 15a (i). Growth increment in height of pseudostem - 2-4 MAP

Group No.	Height of pseudostem (cm)	Frequency (%)
1	0.50 - 4.49	11.25
2	4.50 - 9.49	7.50
3	9.50 - 14.49	21.25
4	14.50 - 19.49	16.25
5	19.50 - 24.49	13.75
6	24.50 - 29.49	13.75
7	29.50 - 34.49	7.50
8	34.50 - 39.49	8.75

Table 15a (ii). Growth increment in height of pseudostem - 4-6 MAP

Group No.	Height of pseudostem (cm)	Frequency (%)
1	< 3.68	36.25
2	3.68 - 7.67	18.75
3	7.68 - 11.67	12.50
4	11.68 - 15.67	16.25
5	15.68 - 19.67	6.25
6	19.68 - 23.67	6.25
7	23.68 - 27.67	1.25
8	27.68 -31.67	2.50

Table15a (iii). Growth increment in number of tillers/plant - 2-4 MAP

Group No.	No. of tillers/plant	Frequency (%)
1	< 1.00	17.50
2	1.00 - 2.99	32.50
3	3.00 - 4.99	35.00
4	5.00 - 6.99	10.00
5	7.00 - 8.99	2.50
6	9.00 - 10.99	2.50

Table 15a (iv). Growth increment in number of tillers/plant - 4-6 MAP

Group No.	No. of tillers/plant	Frequency (%)
1	< 2.50	17.50
2	2.50 - 5.49	32.50
3	5.50 - 8.49	21.25
4	8.50 - 11.49	20.00
5	11.50 - 14.49	3.75
6	14.50 - 17.49	5.00

Table 15a (v). Growth increment in number of leaves/tiller -2-4 MAP

Group No.	Number of leaves/tiller	Frequency (%)
1	-2.78 to -0.79	1.25
2	-0.78 - 1.21	16.25
3	1.22 - 3.21	18.75
4	3.22 - 5.21	27.50
5	5.22 - 7.21	27.50
6	7.22 - 9.21	7.50
7	9.22 - 11.21	0.00
8	11.22 - 13.21	1.25

Table 15a (vi). Growth increment in number of leaves/tiller - 4-6 MAP

Group No.	Number of leaves/tiller	Frequency (%)
1	< 0.77	16.25
2	0.77 - 2.76	35.00
3	2.77 - 4.76	31.25
4	4.77 - 6.76	10.00
5	6.77 - 8.76	3.75
6	8.77 - 10.76	1.25
7	10.77 - 12.76	1.25
8	12.77 - 14.76	1.25

MAP - Months after planting

Table 15b. Growth increment in morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) cultivar Rio-de-Janeiro

Sl. No.	Somaclone No.	Height of pseudostem (cm)			Growth increment in height of pseudostem (cm)		No. of tillers/plant			Growth increment in no. of tillers/plant		No. of leaves/tiller			Growth increment in no. of leaves/tiller	
		2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP
1	88 R	17.85	46.72	48.77	28.87	2.05	3.33	7.00	15.00	3.67	8.00	4.86	9.00	10.00	4.14	1.00
2	137 R	23.17	50.71	52.71	27.54	2.00	2.83	5.40	5.60	2.57	0.20	7.15	9.70	9.80	2.55	0.10
3	281 R	32.82	53.88	54.88	21.06	1.00	2.50	6.75	12.13	4.25	5.38	7.38	10.22	11.08	2.84	0.86
4	292 R	37.46	61.26	67.27	23.80	6.01	4.71	11.29	30.57	6.58	19.28	7.49	12.93	15.38	5.44	2.45
5	296 R	33.75	61.12	62.52	27.37	1.40	6.00	13.25	26.50	7.25	13.25	7.81	13.15	14.25	5.34	1.10
6	308 R	22.03	46.02	56.94	23.99	10.92	3.60	5.20	15.80	1.60	10.60	4.91	12.30	15.33	7.39	3.03
7	311 R	19.24	46.89	68.57	27.65	21.68	4.33	5.33	10.67	1.00	5.34	5.75	12.50	15.22	6.75	2.72
8	312 R	25.49	51.10	51.97	25.61	0.87	4.50	9.50	17.17	5.00	7.67	5.93	11.42	14.08	5.49	2.66
9	314 R	21.73	54.38	62.63	32.65	8.25	4.00	6.33	16.25	2.33	9.92	5.88	10.38	15.17	4.50	4.79
10	315 R	23.89	40.88	41.88	16.99	1.00	3.25	8.33	12.33	5.08	4.00	6.33	12.14	13.00	5.81	0.86
11	335 R	23.69	29.28	34.80	5.59	5.52	3.00	5.33	10.67	2.33	5.34	5.39	8.16	9.67	2.77	1.51
12	336 R	11.98	27.42	35.25	15.44	7.83	4.33	5.00	10.17	0.67	5.17	4.18	8.82	10.61	4.64	1.79
13	337 R	20.33	38.43	48.79	18.10	10.36	2.50	4.50	9.25	2.00	4.75	4.12	7.19	12.50	3.07	5.31
14	338 R	28.88	45.42	56.95	16.54	11.53	2.75	6.50	14.50	3.75	8.00	5.33	10.86	16.17	5.53	5.31
15	345 R	19.71	37.17	46.00	17.46	8.83	2.20	3.80	9.40	1.60	5.60	4.70	7.80	10.96	3.10	3.16
16	346 R	15.97	36.34	41.67	20.37	5.33	5.00	8.00	11.75	3.00	3.75	4.79	7.40	12.83	2.61	5.43
17	347 R	8.00	23.50	32.00	15.50	8.50	2.00	2.00	5.00	0.00	3.00	2.00	7.00	9.00	5.00	2.00
18	349 R	15.67	44.78	64.85	29.11	20.07	5.00	7.00	17.25	2.00	10.25	3.31	10.19	15.08	6.88	4.89
19	350 R	19.29	41.29	47.36	22.00	6.07	5.50	6.50	13.50	1.00	7.00	5.67	7.99	13.58	2.32	5.59
20	351 R	32.69	64.50	65.43	31.81	0.93	2.20	5.00	19.00	2.80	14.00	9.10	14.13	16.50	5.03	2.37
21	355 R	19.21	36.34	42.46	17.13	6.12	5.25	9.00	10.25	3.75	1.25	5.63	9.75	15.55	4.12	5.80
22	358 R	17.04	22.52	34.25	5.48	11.73	3.50	4.83	9.50	1.33	4.67	5.05	7.00	11.00	1.95	4.00
23	361 R	15.75	26.78	31.33	11.03	4.55	2.25	4.75	8.25	2.50	3.50	5.56	6.40	7.16	0.84	0.76
24	364 R	26.30	52.89	54.62	26.59	1.73	4.50	10.75	17.25	6.25	6.50	8.69	11.33	12.33	2.64	1.00
25	367 R	14.99	34.00	35.11	19.01	1.11	3.25	4.33	8.25	1.08	3.92	3.42	8.25	11.66	4.83	3.41
26	368 R	27.50	47.66	91.66	20.16	44.00	2.00	7.00	19.00	5.00	12.00	8.50	9.75	26.00	1.25	16.25
27	373 R	30.50	56.27	61.66	25.77	5.39	1.00	4.00	14.50	3.00	10.50	7.50	12.42	15.83	4.92	3.41
28	377 R	19.26	48.03	63.42	28.77	15.39	3.50	6.75	16.00	3.25	9.25	4.80	10.21	14.66	5.41	4.45
29	378 R	12.28	20.70	38.72	8.42	18.02	6.50	7.00	12.50	0.50	5.50	3.60	7.25	10.00	3.65	2.75
30	384 R	29.17	36.83	38.83	7.66	2.00	3.50	6.50	18.50	3.00	12.00	5.90	6.63	7.17	0.73	0.54
31	386 R	14.19	24.91	43.72	10.72	18.81	3.67	6.67	14.67	3.00	8.00	4.05	6.00	13.67	1.95	7.67
32	395 R	23.03	36.59	53.42	13.56	16.83	2.80	6.60	12.75	3.80	6.15	6.20	9.23	11.92	3.03	2.69

Contd.

Sl. No.	Somaclone No.	Height of pseudostem (cm)			Growth increment in height of pseudostem (cm)		No. of tillers/plant			Growth increment in no. of tillers/plant		No. of leaves/tiller			Growth increment in no. of leaves/tiller	
		2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP
33	399 R	21.25	52.66	57.33	31.41	4.67	4.00	7.00	20.00	3.00	13.00	4.75	12.25	15.00	7.50	2.75
34	413 R	15.00	34.33	51.83	19.33	17.50	7.00	8.50	12.00	1.50	3.50	3.67	9.13	15.83	5.46	6.70
35	418 R	15.86	50.76	67.66	34.90	16.90	7.00	10.00	17.00	3.00	7.00	4.40	11.25	15.66	6.85	4.41
36	431 R	56.00	58.33	80.66	2.33	22.33	1.00	7.00	21.00	6.00	14.00	10.00	11.25	14.00	1.25	2.75
37	438 R	6.00	46.50	55.00	40.50	8.50	1.00	2.00	6.00	1.00	4.00	3.00	8.00	15.00	5.00	7.00
38	463 R	29.25	50.66	51.66	21.41	1.00	4.00	7.00	18.00	3.00	11.00	5.25	11.00	12.00	5.75	1.00
39	466 R	14.00	34.00	34.33	20.00	0.33	2.00	2.00	5.00	0.00	3.00	2.00	6.00	6.00	4.00	0.00
40	471 R	12.89	37.00	71.52	24.11	34.52	6.00	8.00	14.50	2.00	6.50	4.47	9.88	16.33	5.41	6.45
41	475 R	9.90	31.00	32.36	21.10	1.36	2.00	2.00	12.00	0.00	10.00	2.00	3.00	7.33	1.00	4.33
42	476 R	8.67	32.00	67.33	23.33	35.33	6.00	6.00	12.00	0.00	6.00	3.60	8.00	19.66	4.40	11.66
43	478 R	33.17	62.00	63.55	28.83	1.55	3.00	9.00	25.50	6.00	16.50	7.84	15.50	17.67	7.66	2.17
44	482 R	17.00	41.00	41.66	24.00	0.66	5.00	5.00	13.00	0.00	8.00	3.86	6.75	7.50	2.89	0.75
45	485 R	19.40	65.50	75.00	46.10	9.50	5.00	8.00	23.50	3.00	15.50	4.10	13.13	17.83	9.03	4.70
46	517 R	22.33	49.33	66.66	27.00	17.33	3.00	15.00	25.00	12.00	10.00	7.67	15.25	18.33	7.58	3.08
47	524 R	25.50	47.83	66.00	22.33	18.17	4.00	6.00	20.00	2.00	14.00	5.50	10.25	15.00	4.75	4.75
48	526 R	23.67	87.00	93.00	63.33	6.00	4.00	11.00	31.00	7.00	20.00	14.50	19.50	26.66	5.00	7.16
49	531 R	12.50	20.00	23.76	7.50	3.76	3.00	3.00	5.00	0.00	2.00	3.25	5.33	8.50	2.08	3.17
50	535 R	12.67	29.00	42.23	16.33	13.23	3.00	4.50	9.00	1.50	4.50	2.67	5.59	9.16	2.92	3.57
51	548 R	8.33	38.43	59.00	30.10	20.57	3.00	4.00	9.00	1.00	5.00	3.00	7.75	13.00	4.75	5.25
52	561 R	15.25	46.00	73.00	30.75	27.00	4.00	4.00	11.00	0.00	7.00	3.25	9.33	14.66	6.08	5.33
53	582 R	16.89	34.68	34.84	17.79	0.16	5.00	6.00	6.67	1.00	0.67	4.05	9.45	9.55	5.40	0.10
54	589 R	20.00	25.00	32.66	5.00	7.66	2.00	6.00	10.00	4.00	4.00	6.00	6.00	7.66	0.00	1.66
55	590 R	25.50	58.67	82.20	33.17	23.53	2.00	6.00	18.00	4.00	12.00	5.00	13.75	18.00	8.75	4.25
56	597 R	23.00	54.66	58.76	31.66	4.10	2.00	8.00	19.00	6.00	11.00	6.50	10.25	11.00	3.75	0.75
57	610 R	24.67	81.73	93.33	57.06	11.60	3.00	8.00	21.00	5.00	13.00	7.50	15.50	15.67	8.00	0.17
58	616 R	27.00	32.66	45.67	5.66	13.01	2.00	4.50	11.00	2.50	6.50	8.25	8.84	10.83	0.59	1.99
59	617 R	42.00	61.16	81.80	19.16	20.64	1.00	5.00	14.00	4.00	9.00	10.00	13.00	19.00	3.00	6.00
60	622 R	11.00	23.67	61.73	12.67	38.06	3.00	5.00	8.00	2.00	3.00	3.00	6.00	14.00	3.00	8.00
61	626 R	10.50	30.33	49.80	19.83	19.47	4.00	7.00	16.00	3.00	9.00	2.50	10.25	12.00	7.75	1.75
62	630 R	24.33	70.40	86.46	46.07	16.06	3.00	10.00	19.00	7.00	9.00	5.33	13.00	19.00	7.67	6.00
63	704 R	31.87	34.58	45.78	2.71	11.20	2.00	4.83	12.60	2.83	7.77	6.56	9.25	11.46	2.69	2.21
64	734 R	25.06	50.39	59.78	25.33	9.39	4.00	6.33	15.00	2.33	8.67	5.58	10.58	12.44	5.00	1.86
65	748 R	27.98	38.09	39.44	10.11	1.35	2.67	6.67	15.33	4.00	8.66	5.67	8.42	8.99	2.75	0.57
66	772 R	16.59	28.33	39.50	11.74	11.17	3.00	3.00	11.50	0.00	8.50	2.75	7.63	10.17	4.88	2.54

Contd.

Sl. No.	Somaclone No.	Height of pseudostem (cm)			Growth increment in height of pseudostem (cm)		No. of tillers/plant			Growth increment in no. of tillers/plant		No. of leaves/tiller			Growth increment in no. of leaves/tiller	
		2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP	2 MAP	4 MAP	6 MAP	2 - 4 MAP	4 - 6 MAP
67	786 R	33.17	45.11	65.63	11.94	20.52	1.67	6.33	18.00	4.66	11.67	9.83	10.33	16.33	0.50	6.00
68	790 R	22.00	42.33	43.66	20.33	1.33	7.33	10.33	13.33	3.00	3.00	3.66	9.19	11.33	5.53	2.14
69	801 R	20.59	51.29	54.75	30.70	3.46	4.33	6.67	19.33	2.34	12.66	5.18	11.78	12.77	6.60	0.99
70	982 R	11.13	30.00	35.50	18.87	5.50	3.00	5.00	10.00	2.00	5.00	3.38	9.38	13.67	6.00	4.29
71	R I	17.00	33.47	43.19	16.47	9.72	1.60	2.40	4.00	0.80	1.60	5.50	7.93	10.75	2.43	2.82
72	R II	21.83	47.58	58.55	25.75	10.97	3.00	5.00	10.67	2.00	5.67	4.64	10.05	15.00	5.41	4.95
73	R III	36.00	46.70	55.70	10.70	9.00	1.50	4.50	12.00	3.00	7.50	6.00	9.25	10.50	3.25	1.25
74	R IV	19.25	26.55	40.55	7.30	14.00	3.33	4.00	9.67	0.67	5.67	4.44	9.00	11.66	4.56	2.66
75	R V	28.29	47.00	49.78	18.71	2.78	3.33	5.00	10.67	1.67	5.67	7.00	9.50	10.33	2.50	0.83
76	R VI	14.55	41.25	47.64	26.70	6.39	4.00	5.00	9.67	1.00	4.67	3.89	8.39	12.44	4.50	4.05
77	R VII	18.00	40.66	46.33	22.66	5.67	6.00	7.00	13.00	1.00	6.00	3.80	10.25	10.66	6.45	0.41
78	R VIII	48.00	54.67	79.10	6.67	24.43	2.00	10.00	16.00	8.00	6.00	8.00	8.75	17.00	0.75	8.25
79	R IX	14.09	23.92	51.05	9.83	27.13	2.50	4.00	7.00	1.50	3.00	2.34	6.33	10.50	3.99	4.17
80	R X	15.75	19.50	28.58	3.75	9.08	4.00	4.00	4.00	0.00	0.00	3.00	5.17	8.50	2.17	3.33
81	R XI	15.79	35.69	58.14	19.90	22.45	4.00	7.33	15.33	3.33	8.00	5.64	10.44	16.00	4.80	5.56
82	R XII	16.00	42.33	53.66	26.33	11.33	5.00	7.00	20.00	2.00	13.00	3.40	12.50	13.33	9.10	0.83
83	Control R	20.00	31.67	63.75	11.67	32.08	2.00	3.00	14.00	1.00	11.00	7.67	8.67	12.00	1.00	3.33
	S.D	8.883	13.654	15.740	11.293	9.750	1.465	2.485	5.665	2.194	4.192	2.192	2.742	3.748	2.177	2.685
	C.V (%)	41.436	31.995	29.088	53.176	85.249	42.000	39.506	40.632	78.300	54.783	40.809	28.290	28.414	50.381	76.754

MAP - Months after planting

Frequency distribution of growth increment in morphological characters in first set somaclones in ginger (*Z. officinale* Rosc.) (cultivar Rio-de-Janeiro)

Table15b (i). Growth increment in height of pseudostem - 2- 4 MAP

Group No.	Height of pseudostem (cm)	Frequency (%)
1	0.50 - 8.49	14.63
2	8.50 -16.49	15.85
3	16.50 -24.49	34.15
4	24.50 - 32.49	25.61
5	32.50 -40.49	3.66
6	40.50 - 48.49	3.66
7	48.50 - 56.49	0.00
8	56.50 - 64.49	2.44

Table 15b (ii). Growth increment in height of pseudostem - 4-6 MAP

Group No.	Height of pseudostem (cm)	Frequency (%)
1	< 4.08	25.61
2	4.08 - 10.07	29.27
3	10.08 - 16.07	17.07
4	16.08 - 22.07	15.85
5	22.08 - 28.07	7.32
6	28.08 - 34.07	0.00
7	34.08 - 40.07	3.66
8	40.08 - 46.07	1.22

Table 15b (iii). Growth increment in number of tillers/plant - 2-4 MAP

Group No.	No. of tillers/plant	Frequency (%)
1	0.00 - 1.99	34.15
2	2.00 - 3.99	41.46
3	4.00 - 5.99	12.20
4	6.00 - 7.99	9.76
5	8.00 - 9.99	1.22
6	10.00 - 11.99	0.00
7	12.00 - 13.99	1.22

Table 15b (iv). Growth increment in number of tillers/plant - 4-6 MAP

Group No.	No. of tillers/plant	Frequency (%)
1	< 1.00	3.66
2	1.00 - 3.99	14.64
3	4.00 - 6.99	30.49
4	7.00 - 9.99	23.17
5	10.00 - 12.99	14.64
6	13.00 - 15.99	9.76
7	16.00 - 18.99	1.22
8	19.00 - 21.99	2.44

Table 15b (v). Growth increment in number of leaves/tiller - 2-4 MAP

Group No.	Number of leaves/tiller	Frequency (%)
1	< 0.55	2.44
2	0.55 - 2.54	17.07
3	2.55 - 4.54	29.27
4	4.55 - 6.54	34.15
5	6.55 - 8.54	13.41
6	8.55 - 10.54	3.66

Table 15b (vi). Growth increment in number of leaves/tiller - 4-6 MAP

Group No.	Number of leaves/tiller	Frequency (%)
1	< 2.13	32.93
2	2.13 - 5.12	42.68
3	5.13 - 8.12	20.73
4	8.13 - 11.12	1.22
5	11.13 - 14.12	1.22
6	14.13 - 17.12	1.22

MAP - Months after planting

The mean increment in height of pseudostem from second to fourth month after planting was from 1.10 to 39.49 cm in somaclones of Maran and 2.33 to 63.33 cm in clones of Rio-de-Janeiro. The somaclones of Rio-de-Janeiro exhibited higher growth rate than clones of Maran. When clones exhibiting higher increment in height of pseudostem (> 25 cm) were compared in two cultivars, 35 per cent clones of Rio-de-Janeiro and 30 per cent clones of Maran came in the group.

When somaclones and CP plants of two cultivars were compared, 79 per cent somaclones exhibited superiority in growth rate with respect to height of pseudostem over CP plants. Seventy nine per cent somaclones of Rio-de-Janeiro exhibited more growth rate than CP plants as compared to 44 per cent clones of Maran. So somaclones of Rio-de-Janeiro were found superior in growth rate with respect to height of pseudostem over CP plants.

Somaclones of Maran exhibited more variability in growth rate of height of pseudostem than clones of Rio-de-Janeiro. The coefficient of variation recorded for the character was 55.872 per cent in clones of Maran as compared to 53.176 per cent in clones of Rio-de-Janeiro.

4.2.2.2 Number of Tillers / Plant

Number of tillers/plant increased at the rate of 2.44 tillers/month on an average in the somaclones studied. In CP plants, number of tillers/plant increased at the rate of 3.75 tillers/month and 16 per cent somaclones exhibited high growth rate in tiller number than CP plants. The tiller production was found high from fourth to sixth month after planting in different somaclones, the increment during the period being 3.48 tillers/month. In 80 per cent clones of Maran, tiller number increased at the rate of 3.67/month from fourth to sixth month after planting. Similarly, in 89 per cent clones of Rio-de-Janeiro, tiller number increased at the rate of 4.10/month from fourth to sixth month after planting. Clones of Rio-de-Janeiro exhibited higher tiller production than clones of Maran. Increment of more than 13 tillers/plant was noticed from fourth to sixth month in 13 per cent clones of Rio-de-Janeiro as compared to nine per cent in clones of Maran.

When somaclones and CP plants of two cultivars were compared, 20 per cent clones of Rio-de-Janeiro exhibited high tiller production over CP plants as compared to nine per cent clones in cultivar Maran. So somaclones of Rio-de-Janeiro were found better in tiller production over CP plants.

Wide variation in growth rate of tiller production was noticed in clones of Maran. Coefficient of variation for the character studied was 65.150 per cent in clones of Maran as compared to 54.783 per cent in clones of Rio-de-Janeiro.

4.2.2.3 Number of Leaves / Tiller

On an average, number of leaves/tiller increased at the rate of 1.85 leaves/month in the somaclones evaluated. In CP plants, number of leaves/tiller increased at the rate of 0.65 leaves/month. In majority of the somaclones, leaf production was high from second to fourth month after planting, the rate of increase in leaf number being 6.65 leaves/month during the period.

Increment in leaf number from fourth to sixth month after planting varied from -1.50 to 11.93 in clones of Maran and from 0.00 to 9.10 in clones of Rio-de-Janeiro. Twenty four per cent clones of Rio-de-Janeiro exhibited increment of more than 5.25 leaves/tiller while only 18 per cent clones of Maran exhibited increment of more than 4.86 leaves/tiller. So the somaclones of Rio-de-Janeiro exhibited high growth rate in leaf production than clones of Maran.

When leaf production in somaclones and CP plants were compared, 87 per cent somaclones exhibited high leaf production over CP plants. Ninety one per cent somaclones of Rio-de-Janeiro showed superiority in leaf production over CP plants as compared to eighty three per cent clones of Maran. So the somaclones of Rio-de-Janeiro were better in growth rate of leaf number over CP plants.

Variability in increment of leaf number from fourth to sixth month after planting was high in clones of Maran as compared to clones of Rio-de-Janeiro. Coefficient of variation observed for the character studied was 84.626 per cent in clones of Maran as compared to 76.754 per cent in clones of Rio-de-Janeiro.

Overall analysis of growth in somaclones and CP plants of two cultivars with respect to various growth parameters, rate of growth and variability in the characters are summarised as follows.

Evaluation of somaclones for three seasons' revealed the superiority of somaclones over CP plants in various growth parameters and rate of growth (Fig. 5). More somaclones of cultivar Rio-de-Janeiro were found superior to CP plants in respect of growth parameters recorded such as pseudostem height, tiller number and leaf area.

The somaclones of cultivar Rio-de-Janeiro exhibited higher pseudostem height, tiller number and leaf number. Also, more somaclones in the cultivar Rio-de-Janeiro were superior to somaclones of cultivar Maran in the rate of growth of the above morphological characters analysed.

The variability assessment in somaclones of two cultivars showed that clones of cultivar Maran exhibited more variability in morphological characters recorded such as height of pseudostem, number of tillers/plant, number of leaves/tiller and leaf area than clones of Rio-de-Janeiro (Fig. 6). In growth rate of the various morphological characters also, the somaclones of cultivar Maran exhibited more variability showing the higher amount of somaclonal variation in the clones of the cultivar Maran.

4.2.3 Incidence of Pests and Diseases

Incidence of shoot borer, rhizome rot, bacterial wilt and leaf spot were recorded during three seasons of field evaluation.

Shoot borer incidence was highest in the first year of field evaluation. The incidence ranged from zero to fifty per cent in clones of Maran and from zero to sixty per cent in clones of Rio-de-Janeiro (Table 16). In second (2003) and third year (2004) of evaluation, very low infestation (0-33 % and 0-22 % respectively) of shoot borer was observed. The infestation could be controlled by spraying Quinalphos 25 per cent EC as per package of practices recommendations of KAU (KAU, 2002).

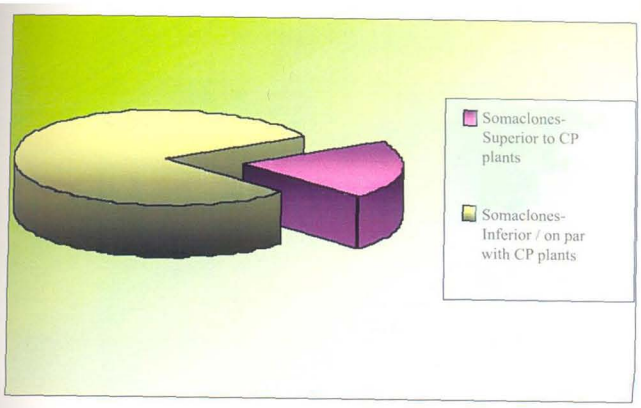


Fig. 5 Comparison of morphological characters in somaclones and conventionally propagated plants in ginger

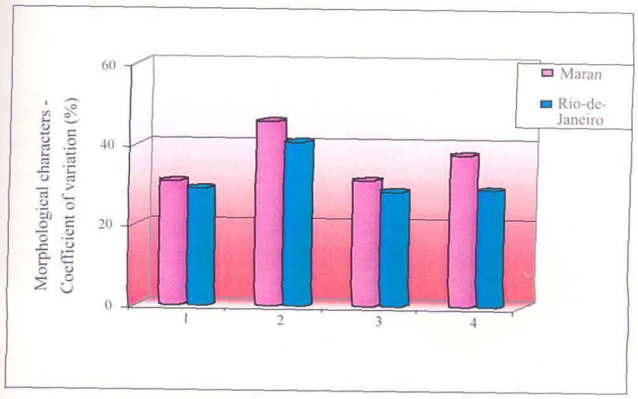


Fig. 6 Somaclonal variation for morphological characters in ginger cultivars

1 Height of pseudostem
2 No of tillers / plant
3 No of leaves / tiller
4 Leaf area

Table 16. Shoot borer incidence in first set somaclones in ginger (*Z. officinale* Rosc.)

Maran					Rio-de-Janeiro			
Sl. No.	Somaclone No.	Shoot borer incidence (%)			Somaclone No.	Shoot borer incidence (%)		
		I Year	II Year	III Year		I Year	II Year	III Year
1	56 M	20.51	-	-	88 R	23.33	-	-
2	79 M	20.92	24.14	-	137 R	35.71	-	-
3	84 M	16.90	-	-	281 R	25.81	14.89	-
4	85 M	34.60		-	292 R	20.09	5.97	22.03
5	91 M	20.37	16.67	-	296 R	12.26	-	-
6	99 M	18.95	10.81	15.15	308 R	11.39	-	19.57
7	100 M	19.30	8.51	16.07	311 R	15.65	-	-
8	110 M	25.42	-	-	312 R	9.73	-	12.86
9	132M	13.14	-	-	314 R	6.15	-	-
10	136 M	27.61	-	-	315 R	8.11	-	-
11	139 M	13.40	-	-	335 R	21.84	-	-
12	150 M	21.21	-	-	336 R	7.37	22.22	-
13	197 M	11.53	10.67	-	337 R	13.51	-	-
14	199 M	25.00	-	-	338 R	18.97	-	-
15	220 M	17.42	-	-	345 R	17.02	-	-
16	276 M	7.24	12.77	-	346 R	19.15	-	-
17	283 M	25.00	-	-	347 R	60.00	-	-
18	284 M	20.00	-	-	349 R	18.84	-	-
19	287 M	14.03	-	-	350 R	18.52	-	-
20	288 M	-	-	-	351 R	23.68	-	-
21	290 M	34.60	-	-	355 R	12.20	-	-
22	311 M	19.42	-	-	358 R	29.79	-	-
23	313 M	16.67	-	-	361 R	27.27	-	-
24	317 M	15.06	8.11	-	364 R	20.29	-	-
25	342 M	16.69	-	-	367 R	42.42	-	-
26	348 M	32.59	-	-	368 R	5.26	-	-
27	356 M	43.75	-	-	373 R	20.69	-	-
28	372 M	26.23	-	-	377 R	20.31	-	-
29	374 M	12.00	-	-	378 R	16.00	-	-
30	381 M	25.00	-	-	384 R	13.51	-	-
31	382 M	5.56	-	-	386 R	34.08	-	-
32	392 M	28.57	-	-	395 R	19.61	-	-
33	393 M	21.43	-	-	399 R	15.00	-	-
34	397 M	27.45	-	-	413 R	25.00	-	-
35	400 M	50.00	-	-	418 R	5.88	-	-
36	411 M	39.13	-	-	431 R	23.81	-	-
37	431 M	-	-	-	438 R	16.67	-	-
38	432 M	41.18	-	-	463 R	33.33	-	-
39	434 M	33.33	-	-	466 R	60.00	20.00	18.33
40	435 M	-	-	-	471 R	13.79	-	-
41	436 M	6.67	-	-	475 R	25.00	-	-

Contd.

Maran					Rio-de-Janeiro			
Sl. No.	Somaclone No.	Shoot borer incidence (%)			Somaclone No.	Shoot borer incidence (%)		
		I Year	II Year	III Year		I Year	II Year	III Year
42	439 M	33.33	-	-	476 R	16.67	-	-
43	441 M	26.32	-	-	478 R	19.61	-	-
44	444 M	-	-	-	482 R	23.08	-	-
45	446 M	26.32	-	-	485 R	17.02	-	-
46	462 M	33.33	-	-	517 R	4.00	-	-
47	464 M	25.00	-	-	524 R	20.00	-	-
48	488 M	25.00	-	-	526 R	-	-	-
49	500 M	22.22	-	-	531 R	60.00	-	-
50	505 M	-	-	-	535 R	16.67	-	-
51	510 M	8.33	-	-	548 R	-	-	-
52	513 M	25.00	-	-	561 R	18.19	-	-
53	528 M	42.86	-	-	582 R	19.19	-	-
54	529 M	31.57	30.77	-	589 R	40.00	-	-
55	549 M	19.35	-	-	590 R	22.22	33.33	-
56	554 M	27.91	-	-	597 R	15.79	13.33	-
57	565 M	25.00	-	-	610 R	14.29	-	-
58	572 M	10.00	-	-	616 R	22.72	-	-
59	580 M	-	-	-	617 R	14.29	-	-
60	583 M	11.43	-	-	622 R	-	-	-
61	588 M	22.22	-	-	626 R	25.00	-	-
62	649 M	-	-	-	630 R	-	-	-
63	659 M	22.96	-	-	704 R	39.68	-	-
64	660 M	31.91	-	-	734 R	24.47	-	-
65	668 M	43.48	-	-	748 R	32.62	-	-
66	743 M	25.00	-	-	772 R	34.78	-	-
67	746 M	23.33	-	-	786 R	11.11	-	-
68	781 M	41.87	-	-	790 R	24.98	-	-
69	918 M	-	-	-	801 R	12.05	-	-
70	953 M	33.33	-	-	982 R	20.00	-	-
71	970 M	6.65	-	-	R I	37.50	-	-
72	980 M	25.00	-	-	R II	25.02	-	-
73	985 M	27.78	-	-	R III	25.00	-	-
74	M I	44.44	21.57	-	R IV	27.61	-	-
75	M III	8.33	-	-	R V	28.12	-	-
76	M IV	0.00	-	-	R VI	-	-	-
77	M V	50.00	-	-	R VII	15.38	-	-
78	M VI	19.87	14.29	16.90	R VIII	12.50	-	-
79	M VII	20.00	-	-	R IX	35.71	-	-
80	M VIII	23.46	-	-	R X	-	-	-
81	Control M	17.64	9.72	21.67	R XI	17.40	-	-
82					R XII	5.00	-	-
83					Control R	7.14	5.71	24.24
	S.D	10.800	7.325	2.904		11.680	9.731	4.310
	C.V (%)	45.100	47.953	16.644		53.794	59.001	22.210

The influence of weather parameters on shoot borer infestation during the three years of evaluation was assessed and correlation coefficients were worked out. Weather data during the period of infestation in three years of evaluation and correlation coefficients are presented in Appendix III and IV. The correlation coefficients worked out revealed that infestation was positively correlated to number of rainy days, minimum temperature and relative humidity and negatively correlated with rainfall and sunshine hours. The low infestation observed during 2003 and 2004 may be due to less number of rainy days, low relative humidity and more sunshine hours recorded during the period.

During field evaluation, rhizome rot incidence was noticed in 14 per cent clones each of Maran and Rio-de-Janeiro (Table 17). Bacterial wilt disease was noticed in 7 per cent clones of Maran and 11 per cent clones of Rio-de-Janeiro. Separate screening experiments were carried out against rhizome rot and bacterial wilt diseases to isolate tolerant/resistant clones to the diseases.

No serious incidence of leaf spot was observed in the somaclones studied during the three seasons of field evaluation.

4.2.4 Yield and Quality Attributes in Somaclones

4.2.4.1 Rhizome Characters

Rhizome characters viz. number of primary, secondary and tertiary fingers, length, girth and internodal length of fingers, thickness of inner core, colour of flesh and scale leaves and orientation of rhizomes were recorded in somaclones in the first year of field evaluation and the data on rhizome characters are presented in Tables 18a and 18b. Frequency distribution for the various rhizome characters is presented in Tables 18a (i-x) and 18b (i-x).

4.2.4.1.1 Number of Primary Fingers

Number of primary fingers varied from one to six in the clones evaluated. The mean number of primary fingers varied from one to six in somaclones of Maran.

Table 17. Incidence of diseases in first set somaclones in ginger (*Z. officinale* Rosc.)

Cultivar	Total no. of somaclones	Somaclones (%) affected by											
		Rhizome rot				Bacterial wilt				Leaf spot			
		I Year	II Year	III Year	Mean	I Year	II Year	III Year	Mean	I Year	II Year	III Year	Mean
Maran	83.00	27.71	11.11	4.55	14.45	14.46	5.55	2.27	7.43	4.82	5.55	6.82	5.73
Rio-de-Janeiro	87.00	24.14	12.32	6.06	14.17	20.69	5.48	6.06	10.74	3.45	6.84	6.06	5.45

Table 18a. Rhizome characters in first set somaclones in ginger (*Z. officinale* Rosc.) cultivar Maran

	Clone No.	No. of fingers			Length of fingers (cm)		Girth of fingers (cm)		Internodal length of fingers (cm)		Thickness of inner core of rhizome (cm)	Colour of		Orientation of rhizome no. of layers
		1^0	2^0	3^0	1^0	2^0	1^0	2^0	1^0	2^0		flesh	scales	
1	56 M	4.00	7.00	9.00	2.60	1.93	6.20	3.93	0.70	0.60	0.60	PY	PY	2
2	79 M	3.00	7.00	8.00	2.60	2.66	7.10	6.00	0.30	0.46	0.30	"	"	"
3	84 M	2.00	6.00	10.00	2.60	1.60	7.00	6.10	0.40	0.50	0.70	"	PYR	1
4	85 M	3.00	7.00	9.00	3.00	2.50	7.50	6.26	0.63	0.40	0.60	"	PY	2
5	91 M	2.00	3.00	5.00	2.70	1.35	4.60	5.20	0.56	0.26	0.70	PYB	"	1
6	99 M	2.00	4.00	8.00	6.80	1.35	4.50	4.55	1.20	0.30	0.60	"	"	"
7	100 M	2.00	4.00	7.00	3.30	2.30	8.00	6.10	0.53	0.56	0.40	"	PYR	4
8	110 M	3.00	9.00	7.00	1.40	2.36	5.50	6.13	0.50	0.40	0.70	"	"	5
9	132M	4.00	12.00	12.00	3.40	2.36	6.00	5.76	0.43	0.50	0.70	PY	"	3
10	136 M	1.00	2.00	4.00	3.00	3.50	6.30	6.00	0.43	0.63	0.70	PYG	"	1
11	139 M	1.00	3.00	7.00	3.10	2.40	5.20	6.10	0.36	0.43	0.60	PYB	PY	"
12	150 M	2.00	6.00	8.00	3.20	2.80	5.00	4.20	0.63	0.56	0.70	PY	PYR	2
13	197 M	2.00	4.00	7.00	5.00	2.55	6.20	5.65	0.80	0.56	0.50	PYB	PY	3
14	199 M	3.00	4.00	7.00	1.40	2.50	2.80	2.75	0.23	0.30	0.30	PY	"	1
15	220 M	2.00	3.00	6.00	2.20	1.85	5.60	4.70	0.43	0.36	0.70	"	"	2
16	276 M	4.00	9.00	10.00	2.60	2.76	6.20	4.43	0.46	0.60	0.50	"	"	"
17	283 M	2.00	4.00	6.00	5.50	2.20	5.20	5.30	0.66	0.80	0.50	"	"	"
18	284 M	3.00	10.00	12.00	3.20	4.30	8.00	5.40	0.53	1.00	0.70	"	"	3
19	287 M	3.00	6.00	9.00	2.60	1.80	5.10	6.50	0.80	0.43	0.70	"	PYBr.	"
20	288 M	2.00	3.00	7.00	1.10	1.85	4.00	5.20	0.20	0.26	0.50	"	PY	2
21	290 M	4.00	6.00	9.00	5.80	1.86	5.20	4.83	0.40	0.36	0.30	"	"	"
22	311 M	3.00	5.00	9.00	3.00	2.30	6.20	6.53	0.70	0.26	0.40	"	PYR	4
23	313 M	4.00	6.00	10.00	6.20	1.53	3.50	4.60	0.60	0.26	0.50	"	PY	2
24	317 M	5.00	7.00	10.00	3.10	2.70	5.10	7.00	0.46	0.46	0.60	"	PYV	3
25	342 M	6.00	11.00	12.00	2.60	2.30	4.50	4.76	0.50	0.43	0.50	"	PY	5
26	348 M	2.00	2.00	5.00	3.10	2.70	2.10	7.00	0.23	0.20	0.50	"	PYR	2
27	356 M	3.00	4.00	8.00	3.10	1.53	6.50	4.73	0.26	0.26	0.50	"	PY	1
28	372 M	2.00	4.00	8.00	4.20	2.15	2.50	6.15	0.60	0.53	0.50	"	PYR	2
29	374 M	3.00	5.00	9.00	4.00	1.86	5.20	5.56	0.56	0.40	0.60	"	PY	"
30	381 M	5.00	6.00	9.00	2.50	2.20	7.80	5.33	0.43	0.43	0.60	"	"	"
31	382 M	3.00	5.00	8.00	2.10	1.50	5.10	4.26	0.33	0.36	0.40	"	"	1
32	392 M	4.00	6.00	9.00	3.00	3.40	6.10	5.10	0.50	0.76	0.50	"	"	2
33	393 M	5.00	7.00	10.00	3.50	2.96	7.30	7.03	0.53	0.53	0.70	PY	PYR	2
34	397 M	3.00	4.00	8.00	3.20	2.50	6.00	4.85	0.40	0.30	1.10	"	LY	3
35	400 M	2.00	2.00	6.00	2.20	3.25	4.50	3.35	0.26	0.50	0.60	"	PY	1
36	411 M	2.00	4.00	8.00	1.80	1.40	4.60	4.65	0.50	0.30	0.60	"	"	"
37	431 M	2.00	2.00	3.00	2.00	2.20	3.00	4.20	0.45	0.46	0.50	"	"	"
38	432 M	3.00	3.00	6.00	4.20	2.20	3.80	2.90	0.50	0.76	0.40	"	"	"
39	434 M	2.00	4.00	3.00	2.20	3.20	3.30	4.00	0.65	0.73	0.40	"	"	2
40	435 M	2.00	2.00	3.00	3.40	1.70	4.70	4.10	0.73	0.40	0.50	"	"	1
41	436 M	2.00	4.00	6.00	2.70	2.40	5.60	5.06	0.60	0.60	0.40	"	PYV	2

Contd.

Sl. No.	Clone No.	No. of fingers			Length of fingers (cm)		Girth of fingers (cm)		Internodal length of fingers (cm)		Thickness of inner core of rhizome (cm)	Colour of		Orientation of rhizome (no. of layers)
		1^0	2^0	3^0	1^0	2^0	1^0	2^0	1^0	2^0		flesh	scales	
42	439 M	2.00	4.00	7.00	2.20	1.70	2.30	1.70	0.20	0.23	0.20	PY	PY	"
43	441 M	3.00	5.00	8.00	4.50	3.03	4.20	4.53	0.70	0.73	0.30	"	"	"
44	444 M	3.00	4.00	8.00	2.80	1.93	4.20	4.00	0.43	0.43	0.50	"	"	"
45	446 M	3.00	5.00	8.00	4.20	2.10	4.20	4.83	0.76	0.46	0.30	"	"	1
46	462 M	2.00	4.00	4.00	2.40	1.40	3.70	3.20	0.23	0.23	0.30	"	"	"
47	464 M	1.00	2.00	6.00	1.00	2.00	3.30	3.20	0.23	0.86	0.40	"	"	2
48	488 M	4.00	9.00	12.00	2.50	2.53	6.20	6.06	0.36	0.40	0.50	"	"	"
49	500 M	2.00	5.00	8.00	2.00	3.55	4.50	4.35	0.26	0.50	0.50	"	"	"
50	505 M	2.00	2.00	4.00	1.70	2.60	3.30	4.60	0.33	0.60	0.50	PYB	"	1
51	510 M	3.00	7.00	10.00	3.00	2.10	5.60	5.36	0.50	0.53	0.60	PYB	"	2
52	513 M	2.00	4.00	8.00	1.60	2.90	4.20	4.50	0.33	0.63	0.60	PY	"	"
53	528 M	2.00	2.00	5.00	3.20	2.50	3.00	3.90	0.56	0.56	0.40	"	"	1
54	529 M	2.00	4.00	5.00	2.00	1.80	2.20	3.20	0.20	0.23	0.30	"	"	"
55	549 M	3.00	2.00	4.00	1.50	1.80	5.20	4.76	0.26	0.30	0.80	"	"	2
56	554 M	5.00	10.00	12.00	2.35	2.23	4.35	6.23	0.46	0.43	0.40	"	PYR	"
57	565 M	2.00	3.00	5.00	3.60	2.20	5.50	5.60	0.53	0.53	0.60	"	PY	"
58	572 M	4.00	8.00	10.00	3.50	3.33	5.20	4.86	0.63	0.60	0.60	"	"	"
59	580 M	4.00	3.00	5.00	3.20	1.43	5.60	6.05	0.46	0.40	0.40	PYB	PYR	"
60	583 M	3.00	8.00	10.00	3.50	2.23	6.50	5.70	0.56	0.56	0.50	PY	"	1
61	588 M	3.00	5.00	7.00	5.50	3.80	5.60	5.10	1.16	0.70	0.50	"	"	2
62	649 M	2.00	3.00	5.00	3.60	4.55	6.50	5.40	0.56	0.53	0.60	"	PY	1
63	659 M	2.00	3.00	5.00	4.70	2.15	5.00	5.55	0.63	0.36	0.50	"	PYV	"
64	660 M	3.00	5.00	8.00	3.00	2.30	4.90	4.96	0.60	0.56	0.50	"	PY	2
65	668 M	3.00	4.00	6.00	5.30	1.86	5.20	6.53	0.56	0.43	0.50	PYB	"	"
66	743 M	2.00	3.00	5.00	4.00	1.65	4.50	4.85	0.53	0.43	0.60	PY	PYR	"
67	746 M	3.00	5.00	7.00	3.80	3.43	5.50	6.50	0.60	0.50	0.50	PY	PYR	3
68	781 M	2.00	2.00	5.00	2.00	1.60	4.10	4.20	0.30	0.43	0.70	"	PY	1
69	918 M	4.00	7.00	10.00	2.70	2.60	5.50	6.06	0.56	0.53	0.70	"	PYV	2
70	953 M	2.00	2.00	5.00	1.00	1.55	3.50	3.70	0.20	0.36	0.60	"	PY	1
71	970 M	3.00	5.00	8.00	3.00	2.53	7.25	5.03	0.46	0.56	0.70	"	"	3
72	980 M	2.00	3.00	5.00	1.55	3.25	4.60	6.95	0.53	0.70	0.50	"	"	"
73	985 M	2.00	4.00	6.00	2.70	1.70	5.75	5.70	0.43	0.36	0.50	"	"	2
74	M I	2.00	3.00	6.00	2.50	2.20	5.85	7.60	0.30	0.30	0.50	"	"	4
75	M III	3.00	5.00	8.00	1.20	2.13	4.65	4.80	0.40	0.33	0.60	"	"	2
76	M IV	2.00	2.00	4.00	2.30	1.00	2.65	1.70	0.20	0.20	0.30	"	"	1
77	M V	3.00	4.00	6.00	2.85	2.25	5.45	5.80	0.90	0.53	0.70	PYB	"	"
78	M VI	3.00	8.00	10.00	3.15	4.43	6.10	5.16	0.50	0.93	0.80	PY	PYR	3
79	M VII	2.00	2.00	5.00	2.15	1.70	5.10	4.60	0.30	0.30	0.70	"	PY	1
80	M VIII	3.00	7.00	10.00	3.10	2.13	5.30	6.30	0.60	0.33	0.50	"	"	1
81	Control M	4.00	8.00	10.00	2.90	3.26	6.70	5.43	0.40	0.46	0.60	"	"	2
	S.D	0.991	2.346	2.305	1.170	0.724	1.366	1.161	0.195	0.169	0.146			
	C.V (%)	35.835	48.353	31.274	39.008	30.834	26.885	22.892	39.806	35.798	27.124			

PY-Pale yellow
PYB- Pale yellow with bluish tinge
PYBr- Pale yellow with brownish tinge
PYG- Pale yellow with greenish tinge
PYR- Pale yellow with reddish tinge
PYV- Pale yellow with violet tinge
1° - Primary
2° - Secondary
3° - Tertiary

Frequency distribution of rhizome characters in first set somaclones in ginger (*Z. officinale* Rosc.) (cultivar Maran)

Table 18a (i). Number of primary fingers

Group No.	No. of primary fingers	Frequency (%)
1	1.00	3.75
2	2.00	43.75
3	3.00	33.75
4	4.00	12.50
5	5.00	5.00
6	6.00	1.25

Table 18a (ii). Number of secondary fingers

Group No.	No. of secondary fingers	Frequency (%)
1	2.00 - 3.00	31.25
2	4.00 - 5.00	37.50
3	6.00 - 7.00	18.75
4	8.00 - 9.00	7.50
5	10.00 - 11.00	3.75
6	12.00 - 13.00	1.25

Table 18a (iii). Number of tertiary fingers

Group No.	Number of tertiary fingers	Frequency (%)
1	3.00 - 4.00	10.00
2	5.00 - 6.00	28.75
3	7.00 - 8.00	31.25
4	9.00 - 10.00	23.75
5	11.00 - 12.00	6.25

Table 18a (iv). Length of primary fingers

Group No.	Length of primary fingers (cm)	Frequency (%)
1	1.00 - 1.72	12.50
2	1.73 - 2.44	17.50
3	2.45 - 3.17	35.00
4	3.18 - 3.89	17.50
5	3.90 - 4.62	7.50
6	4.63 - 5.34	3.75
7	5.35 - 6.07	3.75
8	6.08 - 6.79	2.50

Table 18a (v). Length of secondary fingers

Group No.	Length of secondary fingers (cm)	Frequency (%)
1	0.99 - 1.49	7.50
2	1.50 - 2.00	27.50
3	2.01 - 2.51	33.75
4	2.52 - 3.02	15.00
5	3.03 - 3.53	10.00
6	3.54 - 4.04	2.50
7	4.05 - 4.55	3.75

Table 18a (vi). Girth of primary fingers

Group No.	Girth of primary fingers (cm)	Frequency (%)
1	2.10 - 2.83	7.50
2	2.84 - 3.56	8.75
3	3.57 - 4.30	10.00
4	4.31 - 5.04	17.50
5	5.05 - 5.78	28.75
6	5.79 - 6.51	17.50
7	6.52 - 7.25	3.75
8	7.26 - 8.00	6.25

Table18a (vii). Girth of secondary fingers

Group No.	Girth of secondary fingers (cm)	Frequency (%)
1	1.70 - 2.43	2.50
2	2.44 - 3.17	2.50
3	3.18 - 3.90	7.50
4	3.91 - 4.64	20.00
5	4.65 - 5.38	27.50
6	5.39 - 6.11	22.50
7	6.12 - 6.85	11.25
8	6.86 - 7.59	6.25

Table 18a (viii). Internodal length of primary fingers

Group No.	Internodal length of primary fingers (cm)	Frequency (%)
1	0.20 - 0.32	21.25
2	0.33 - 0.44	18.75
3	0.45 - 0.57	32.50
4	0.58 - 0.69	15.00
5	0.70 - 0.82	8.75
6	0.83 - 0.94	1.25
7	0.95 - 1.07	0.00
8	1.08 - 1.14	2.50

Table18a (ix). Internodal length of secondary fingers

Group No.	Internodal length of secondary fingers (cm)	Frequency (%)
1	0.20 - 0.29	12.50
2	0.30 - 0.39	18.75
3	0.40 - 0.49	23.75
4	0.50 - 0.59	23.75
5	0.60 - 0.69	8.75
6	0.70 - 0.79	7.50
7	0.80 - 0.89	2.50
8	0.90 - 0.99	2.50

Table 18a (x). Thickness of inner core of rhizome

Group No.	Thickness of inner core of rhizome (cm)	Frequency (%)
1	0.20 - 0.30	11.25
2	0.31 - 0.42	12.50
3	0.43 - 0.53	32.50
4	0.54 - 0.64	21.25
5	0.65 - 0.75	18.75
6	0.76 - 0.87	2.50
7	> 0.87	1.25

Table 18b. Rhizome characters in first set somaclones in ginger (*Z. officinale* Rosc.) cultivar Rio-de-Janeiro

Sl. No.	Clone No.	No. of fingers			Length of fingers (cm)		Girth of fingers (cm)		Internodal length of fingers (cm)		Thickness of inner core of rhizome (cm)	Colour of		Orientation of rhizome (no. of layers)
		1^0	2^0	3^0	1^0	2^0	1^0	2^0	1^0	2^0		flesh	scales	
1	88 R	1.00	2.00	4.00	2.50	2.10	5.20	5.50	0.40	0.56	0.40	P.Y	PYB	1
2	137 R	2.00	7.00	9.00	3.00	3.15	6.20	6.60	0.36	0.63	0.50	"	PYV	2
3	281 R	3.00	6.00	9.00	2.80	3.40	5.10	5.70	0.40	0.65	0.70	"	PYR	3
4	292 R	4.00	10.00	15.00	2.60	3.46	6.00	1.48	0.40	0.70	0.70	"	"	"
5	296 R	2.00	3.00	8.00	2.70	2.95	5.00	3.50	0.70	0.76	0.40	"	PY	2
6	308 R	4.00	7.00	10.00	2.80	2.95	7.50	5.30	0.50	0.45	0.60	"	"	3
7	311 R	1.00	2.00	6.00	2.50	2.50	6.00	6.30	0.53	0.56	0.60	"	"	2
8	312 R	4.00	7.00	8.00	2.20	2.30	5.20	4.60	0.50	0.50	0.50	"	"	"
9	314 R	4.00	5.00	7.00	4.20	3.76	4.20	4.63	0.66	0.30	0.60	"	"	"
10	315 R	2.00	3.00	5.00	3.00	2.30	4.50	3.80	0.50	0.43	0.50	"	"	"
11	335 R	3.00	8.00	10.00	4.00	3.26	5.40	5.20	0.50	0.43	0.60	"	"	"
12	336 R	1.00	2.00	6.00	2.50	2.00	4.20	4.20	0.36	0.33	0.30	"	"	"
13	337 R	3.00	6.00	8.00	3.20	1.76	5.50	6.56	0.46	0.36	0.40	"	"	"
14	338 R	3.00	3.00	6.00	3.40	1.90	4.20	3.16	0.36	0.30	0.30	"	"	1
15	345 R	3.00	3.00	5.00	2.70	1.83	5.00	4.93	0.63	0.43	0.40	"	PYV	2
16	346 R	4.00	6.00	8.00	2.30	2.15	5.00	4.00	0.36	0.33	0.70	"	PY	3
17	347 R	1.00	2.00	5.00	2.30	4.20	2.20	4.00	0.43	0.56	0.40	"	"	2
18	349 R	3.00	4.00	6.00	3.00	2.43	5.20	5.63	0.36	0.43	0.60	"	PYV	"
19	350 R	2.00	2.00	5.00	1.80	1.70	4.80	4.50	0.33	0.43	0.40	"	PY	1
20	351 R	2.00	5.00	7.00	4.60	2.60	5.80	5.35	0.43	0.53	0.50	"	PYV	3
21	355 R	2.00	3.00	6.00	3.60	1.70	5.00	5.00	0.66	0.60	0.40	"	PY	1
22	358 R	2.00	2.00	5.00	2.40	2.30	4.60	2.50	0.47	0.63	0.30	"	"	"
23	361 R	2.00	2.00	5.00	1.90	2.25	3.90	4.13	0.45	0.63	0.50	"	"	"
24	364 R	3.00	5.00	6.00	3.50	2.46	7.40	6.73	0.56	0.60	0.60	"	"	2
25	367 R	2.00	4.00	7.00	3.30	3.35	5.30	6.05	0.33	0.56	0.40	"	"	1
26	368 R	4.00	6.00	9.00	5.10	2.10	5.20	4.76	0.70	0.60	0.30	"	"	2
27	373 R	2.00	8.00	12.00	2.20	3.40	5.20	7.15	0.50	0.63	0.50	"	"	"
28	377 R	3.00	7.00	11.00	2.50	2.16	6.20	6.96	0.40	0.36	0.60	"	"	3
29	378 R	2.00	2.00	6.00	1.70	1.30	3.40	2.85	0.26	0.30	0.40	"	"	1
30	384 R	1.00	2.00	5.00	3.50	2.20	1.60	4.30	0.95	0.43	0.50	"	"	"
31	386 R	2.00	2.00	6.00	1.50	1.10	4.00	3.65	0.26	0.30	0.40	"	"	2
32	395 R	3.00	5.00	9.00	4.50	2.65	6.20	5.65	0.53	0.56	1.10	"	"	"
33	399 R	2.00	3.00	7.00	2.60	2.35	5.20	3.95	0.50	0.53	0.30	"	"	"
34	413 R	2.00	3.00	6.00	2.50	1.05	3.50	4.20	0.23	0.30	0.30	"	"	"
35	418 R	3.00	8.00	11.00	1.80	2.10	6.20	5.85	0.40	0.36	0.60	"	"	3
36	431 R	2.00	5.00	9.00	2.80	3.35	6.20	6.35	0.56	0.46	0.60	"	"	"
37	438 R	2.00	3.00	6.00	3.40	2.45	6.00	5.20	0.53	0.66	0.60	"	"	3
38	463 R	2.00	4.00	8.00	3.50	3.35	6.20	6.95	0.53	0.43	0.60	PYB	"	"
39	466 R	2.00	4.00	5.00	3.60	1.20	4.20	3.50	0.56	0.20	0.60	PY	"	2
40	471 R	3.00	5.00	7.00	2.80	1.70	4.50	3.70	0.50	0.40	0.50	PY	PY	
41	475 R	3.00	4.00	8.00	1.30	2.73	5.20	4.13	0.36	0.76	0.60	"	PYV	3
42	476 R	2.00	4.00	6.00	1.30	2.10	3.20	5.20	0.23	0.33	0.60	"	PY	1
43	478 R	4.00	9.00	12.00	2.00	2.53	6.10	5.13	0.43	0.43	0.50	"	"	3
44	482 R	3.00	4.00	8.00	2.20	3.23	4.00	2.90	0.46	0.56	0.40	"	"	2
45	485 R	2.00	3.00	7.00	2.80	2.00	5.20	4.50	0.40	0.33	0.30	PYG	PYR	1
46	517 R	2.00	3.00	6.00	1.10	1.60	3.70	4.05	0.26	0.26	0.40	PY	PY	2
47	524 R	2.00	4.00	9.00	3.80	3.10	4.50	7.15	1.33	1.03	0.50	"	"	"

Contd.

Sl. No.	Clone No.	No. of fingers			Length of fingers (cm)		Girth of fingers (cm)		Internodal length of fingers (cm)		Thickness of inner core of rhizome (cm)	Colour of		Orientation of rhizome (no. of layers)
		1^0	2^0	3^0	1^0	2^0	1^0	2^0	1^0	2^0		flesh	scales	
48	526 R	3.00	6.00	10.00	1.60	2.50	4.50	4.10	0.36	0.66	0.60	"	"	1
49	531 R	2.00	3.00	6.00	2.50	1.40	2.50	2.45	0.53	0.26	0.40	"	"	"
50	535 R	1.00	2.00	7.00	3.30	3.80	2.00	5.20	0.46	0.30	0.60	"	"	3
51	548 R	2.00	3.00	6.00	3.20	2.15	4.50	4.70	0.56	0.53	0.40	"	"	1
52	561 R	3.00	8.00	12.00	1.50	2.70	7.10	6.00	0.30	0.56	0.60	"	"	2
53	582 R	4.00	6.00	8.00	3.20	1.93	5.50	5.90	0.53	0.53	1.00	"	"	"
54	589 R	2.00	4.00	6.00	2.80	1.60	2.50	3.70	0.23	0.26	0.30	"	"	1
55	590 R	1.00	3.00	6.00	2.50	3.20	5.50	6.50	0.45	0.53	0.70	"	PYV	2
56	597 R	2.00	2.00	3.00	3.00	2.50	5.50	4.90	0.70	0.53	0.40	"	PY	"
57	610 R	3.00	8.00	10.00	2.30	2.40	6.00	6.16	0.40	0.36	0.70	"	"	3
58	616 R	2.00	4.00	6.00	2.50	2.90	5.50	5.20	0.56	0.43	0.60	PYB	"	2
59	617 R	2.00	5.00	8.00	4.10	2.45	6.60	6.70	0.43	0.40	0.60	PY	PYV	3
60	622 R	3.00	7.00	9.00	2.00	2.90	1.80	1.25	0.30	0.43	0.20	"	PY	2
61	626 R	2.00	4.00	6.00	2.30	1.80	4.20	4.00	0.50	0.50	0.40	"	"	1
62	630 R	2.00	4.00	8.00	2.80	3.80	7.10	5.03	0.56	0.86	0.70	"	"	3
63	704 R	2.00	4.00	6.00	2.80	2.20	3.50	4.05	0.30	0.33	0.40	"	"	1
64	734 R	2.00	5.00	7.00	2.50	2.35	6.80	7.15	0.43	0.56	0.60	"	PYR	3
65	748 R	4.00	6.00	8.00	5.80	2.10	5.30	4.76	0.50	0.33	0.60	"	PY	2
66	772 R	2.00	2.00	5.00	4.50	2.15	5.60	3.60	0.70	0.40	0.40	"	"	1
67	786 R	3.00	5.00	8.00	2.80	4.73	6.50	5.73	0.60	0.76	0.50	"	"	1
68	790 R	2.00	4.00	8.00	4.70	2.40	5.10	5.35	0.93	0.70	0.70	"	PY	1
69	801 R	2.00	6.00	9.00	2.60	3.13	5.50	6.65	0.53	0.53	0.70	"	"	"
70	982 R	3.00	4.00	7.00	1.90	1.63	4.50	3.10	0.53	0.33	0.30	"	"	1
71	R I	2.00	2.00	6.00	2.00	1.35	6.20	6.00	0.20	0.26	0.80	"	"	"
72	R II	2.00	2.00	5.00	3.50	3.60	5.20	4.35	0.83	1.06	0.50	"	"	"
73	R III	3.00	8.00	11.00	3.00	2.40	7.20	5.75	0.40	0.36	0.50	"	"	2
74	R IV	2.00	2.00	4.00	1.90	1.95	4.00	3.60	0.26	0.33	0.30	"	"	1
75	R V	3.00	6.00	9.00	2.60	2.40	7.00	6.83	0.36	0.46	0.90	"	"	2
76	R VI	2.00	3.00	7.00	1.50	1.80	3.20	4.20	0.23	0.26	0.40	"	"	1
77	R VII	3.00	3.00	6.00	3.60	1.30	2.20	3.10	0.50	0.50	0.30	"	"	2
78	R VIII	2.00	2.00	4.00	5.50	3.00	6.50	5.80	0.83	0.56	0.60	"	"	"
79	R IX	1.00	4.00	6.00	2.80	3.50	5.60	5.20	0.36	0.83	0.60	"	"	"
80	R X	2.00	3.00	5.00	1.80	1.20	3.20	3.20	0.20	0.23	0.40	"	"	1
81	R XI	2.00	2.00	6.00	2.30	2.00	3.60	5.20	0.50	0.36	0.40	PY	PY	2
82	R XII	2.00	3.00	7.00	2.30	2.00	4.50	3.65	0.53	0.40	0.40	"	"	"
83	Control R	4.00	6.00	10.00	3.20	2.60	5.20	6.80	0.45	0.35	0.50	"	"	"
	S.D	0.827	2.012	2.166	0.939	0.750	1.34	1.338	0.181	0.173	0.162			
	C.V (%)	34.321	46.647	29.765	33.235	30.767	27.061	27.537	37.975	35.942	31.638			

PY-Pale yellow
PYB-Pale yellow with bluish tinge
PYG- Pale yellow with greenish tinge
PYR- Pale yellow with reddish tinge
PYV- Pale yellow with violet tinge

1° - Primary
2° - Secondary
3° - Tertiary

Frequency distribution of rhizome characters in first set somaclones in ginger (*Z. officinale* Rosc.) (cultivar Rio-de-Janeiro)

Table 18b (i). Number of primary fingers

Group No.	No. of primary fingers	Frequency (%)
1	1.00	9.76
2	2.00	52.44
3	3.00	26.83
4	4.00	10.98

Table 18b (ii). Number of secondary fingers

Group No.	No. of secondary fingers	Frequency (%)
1	2.00 - 3.00	42.68
2	4.00 - 5.00	30.49
3	6.00 - 7.00	17.07
4	8.00 - 9.00	8.54
5	10.00 - 11.00	1.22

Table 18b (iii). Number of tertiary fingers

Group No.	Number of tertiary fingers	Frequency (%)
1	3.00 - 4.00	4.88
2	5.00 - 6.00	41.46
3	7.00 - 8.00	29.27
4	9.00 - 10.00	15.85
5	11.00 - 12.00	7.32
6	13.00 - 14.00	0.00
7	15.00 - 16.00	1.22

Table 18b (iv). Length of primary fingers

Group No.	Length of primary fingers (cm)	Frequency (%)
1	1.10 - 1.68	8.54
2	1.69 - 2.27	15.85
3	2.28 - 2.85	39.02
4	2.86 - 3.44	14.64
5	3.45 - 4.03	10.98
6	4.04 - 4.62	6.10
7	4.63 - 5.20	2.44
8	5.21 - 5.79	2.44

Table 18b (v). Length of secondary fingers

Group No.	Length of secondary fingers (cm)	Frequency (%)
1	1.05 - 1.50	9.76
2	1.51 - 1.96	15.85
3	1.97 - 2.42	30.49
4	2.43 - 2.88	14.63
5	2.89 - 3.34	13.41
6	3.35 - 3.80	13.41
7	3.81 - 4.26	1.22
8	4.27 - 4.73	1.22

Table18b (vi). Girth of primary fingers

Group No.	Girth of primary fingers (cm)	Frequency (%)
1	1.60 - 2.33	6.10
2	2.34 - 3.07	2.44
3	3.08 - 3.80	9.76
4	3.81 - 4.54	19.51
5	4.55 - 5.28	20.73
6	5.29 - 6.02	19.51
7	6.03 - 6.75	13.41
8	6.76 - 7.50	8.54

Table 18b (vii). Girth of secondary fingers

Group No.	Girth of secondary fingers (cm)	Frequency (%)
1	1.25 - 1.98	2.44
2	1.99 - 2.72	2.44
3	2.73 - 3.45	7.32
4	3.46 - 4.19	21.95
5	4.20 - 4.93	17.07
6	4.94 - 5.67	19.51
7	5.68 - 6.40	14.63
8	6.41 - 7.14	14.63

Table 18b (viii). Internodal length of primary fingers

Group No.	Internodal length of primary fingers (cm)	Frequency (%)
1	0.20 - 0.33	18.29
2	0.34 - 0.47	34.15
3	0.48 - 0.61	32.93
4	0.62 - 0.76	8.54
5	0.77 - 0.90	2.44
6	0.91 - 1.04	2.44
7	1.05 - 1.18	0.00
8	1.19 - 1.33	1.22

Table 18b (ix). Internodal length of secondary fingers

Group No.	Internodal length of secondary fingers (cm)	Frequency (%)
1	0.20 - 0.30	15.85
2	0.31 - 0.40	21.95
3	0.41 - 0.51	19.51
4	0.52 - 0.62	23.17
5	0.63 - 0.73	10.98
6	0.74 - 0.83	4.88
7	0.84 - 0.94	1.22
8	0.95 - 1.06	2.44

Table 18b (x). Thickness of inner core of rhizome

Group No.	Thickness of inner core of rhizome (cm)	Frequency (%)
1	0.16 - 0.25	1.22
2	0.26 - 0.35	13.41
3	0.36 - 0.45	26.83
4	0.46 - 0.55	15.85
5	0.56 -0.65	28.05
6	0.66 - 0.75	9.76
7	0.76 - 0.85	1.22
8	0.86 - 0.95	1.22
9	0.96 - 1.05	1.22
10	1.06 - 1.15	1.22

In majority of the clones of Maran (77.5%), the number recorded was two to three. But in six per cent clones, the number ranged from five to six.

The primary finger production ranged from one to four in somaclones of Rio-de-Janeiro. In majority of the clones (52.44%), the number of primary fingers was two and in 10.98 per cent clones, the number recorded was four.

In the two cultivars studied, clones of Maran produced more primary fingers than clones of Rio-de-Janeiro. Nineteen per cent somaclones of Maran produced primary fingers in the range of 4.00-6.00 while only eleven per cent clones of Rio-de-Janeiro produced more than three primary fingers.

Six per cent somaclones of Maran were superior to CP plants in primary finger production. None of the somaclones of Rio-de-Janeiro produced fingers more than that of CP plants. So the somaclones of Maran were better in finger production over CP plants.

Variability for number of primary fingers was almost uniform in the two cultivars studied recording a coefficient of variation of 35.835 per cent in somaclones of Maran and 34.321 per cent in somaclones of Rio-de-Janeiro.

4.2.4.1.2 Number of Secondary and Tertiary Fingers

Number of secondary fingers recorded ranged from two to twelve in the somaclones evaluated. Similarly, number of tertiary fingers varied from three to fifteen in the somaclones. Secondary and tertiary finger production ranged from 2.00 to 12.00 and 3.00 to 12.00 respectively in somaclones of Maran. Majority of the somaclones of Maran (68.75%) produced secondary fingers in the range of two to five and tertiary fingers in the range of five to eight (60%).

As presented in Table 23b, secondary and tertiary finger production ranged between 2.00 to 10.00 and 3.00 to 15.00 respectively in somaclones of Rio-de-Janeiro. In 73 per cent clones of Rio-de-Janeiro, the number of secondary fingers produced

was two to five and in 70 per cent clones, tertiary fingers produced ranged from five to eight.

The secondary and tertiary finger production was high in clones of Maran than in clones of Rio-de-Janeiro. More than seven secondary fingers were observed in 13 per cent clones of Maran as compared to 10 per cent clones of Rio-de-Janeiro. Similarly, the number of tertiary fingers was more than eight in 30 per cent clones of Maran as compared to 24 per cent in clones of Rio-de-Janeiro.

In 12 per cent somaclones, secondary finger production was found higher than CP plants. Similarly, in seven per cent somaclones, tertiary finger production was higher than CP plants. Sixteen per cent clones of Rio-de-Janeiro recorded higher secondary fingers than CP plants compared to nine per cent clones in Maran. Similarly, nine per cent clones of Rio-de-Janeiro recorded higher tertiary fingers than CP plants compared to six per cent clones in Maran. Thus the clones of Rio-de-Janeiro were superior to CP plants in secondary and tertiary finger production.

Variability was almost uniform in secondary and tertiary finger production in the somaclones of two cultivars studied and the overall variability recorded for the characters was 38.94 per cent.

4.2.4.1.3 Length of Primary and Secondary Fingers

Length of primary fingers ranged from 1.00 to 6.80 cm in the somaclones evaluated. Length of secondary fingers varied from 1.00 to 4.73 cm in the somaclones. Data presented in Table 23a show that mean length of primary fingers varied from 1.00 to 6.80 cm in somaclones of Maran evaluated. Length of secondary fingers ranged from 1.00 to 4.55 cm in the clones of cultivar Maran. Mean length of primary fingers was in between 1.10-5.80 cm and length of secondary fingers was from 1.05 to 4.73 cm in somaclones of Rio-de-Janeiro.

The clones of Maran exhibited superiority in length of primary fingers while clones of Rio-de-Janeiro were superior in length of secondary fingers. When clones exhibiting higher length of secondary fingers (>2.89 cm) were compared, 29

per cent clones of Rio-de-Janeiro came in the range as compared to 19 per cent clones in Maran.

Somaclones exhibited higher primary and secondary finger length over CP plants. Thirty nine per cent of somaclones showed higher length of primary fingers over CP plants. Similarly, 22 per cent of somaclones recorded higher secondary finger length over CP plants. When clones of two cultivars and CP plants were compared, clones of Maran were superior in primary finger length and clones of Rio-de-Janeiro were superior in secondary finger length.

When variability for the characters was looked into, the clones of Maran showed more variability for length primary fingers. Variability was almost uniform in the two clones for length of secondary fingers. The coefficient of variation for length of primary finger was 39.008 per cent in somaclones of Maran as compared to 33.235 per cent in clones of Rio-de-Janeiro.

4.2.4.1.4 Girth of Primary and Secondary Fingers

Girth of primary fingers ranged from 1.60 to 8.00 cm and girth of secondary fingers from 1.25 to 7.60 cm in the clones evaluated. From the results presented in Table 23a, it could be seen that in somaclones of Maran, mean girth of primary fingers varied between 2.10-8.00 cm and girth of secondary fingers between 1.70-7.60 cm. In somaclones of Rio-de-Janeiro, girth of primary of fingers varied from 1.60 to 7.50 cm and secondary fingers from 1.25-7.15 cm.

Girth of primary and secondary fingers were more in somaclones of Maran as compared to Rio-de-Janeiro. Ten per cent somaclones of Maran came in the highest classes of frequency table exhibiting primary finger girth in the range of 7.00-8.00 cm. In clones of Rio-de-Janeiro, 8.54 per cent clones came in the range of 6.80-7.50 cm. Also, 25 per cent clones of Maran recorded secondary finger girth of more than 6.00 cm as compared to 20 per cent clones in Rio-de-Janeiro.

When somaclones and CP plants were compared with respect to finger girth, somaclones were found superior to CP plants. Twenty six per cent somaclones

were found superior in girth of primary fingers and 22 per cent clones in girth of secondary fingers over CP plants. When finger girth in somaclones of two cultivars and CP plants were compared, clones of Rio-de-Janeiro were superior in girth of primary fingers while clones of Maran were superior in girth of secondary fingers.

Variability for girth of primary fingers was almost uniform in somaclones of the two cultivars studied. But for girth of secondary fingers, variability was more in somaclones of Rio-de-Janeiro recording a coefficient of variation of 27.537 per cent. In clones of Maran, the coefficient of variation recorded for the character was 22.892 per cent.

4.2.4.1.5 Internodal Length of Primary and Secondary Fingers

Internodal length of primary fingers ranged from 0.20 to 1.20 cm and secondary fingers from 0.20 to 1.06 cm in the clones evaluated. In somaclones of Maran, internodal length of primary fingers ranged between 0.20-1.20 cm and secondary fingers between 0.20-1.00 cm. Majority of the clones of Maran exhibited internodal length of primary fingers in the range 0.33-0.56 cm and secondary fingers in the range 0.30-0.56 cm.

In somaclones of Rio-de-Janeiro, the length of internodes of primary fingers ranged from 0.20 to 1.33 cm and secondary fingers from 0.20 to 1.06 cm. In majority of the clones of Rio-de-Janeiro, internodal length of primary fingers was in the range of 0.36 to 0.60 cm and length of internodes of secondary fingers in the range of 0.33 to 0.60 cm.

When somaclones and CP plants were compared with respect to internodal length of primary and secondary fingers, 60 per cent somaclones were found superior to CP plants. With regard to cultivar response, clones of Maran were superior in internodal length of primary fingers and clones of Rio-de-Janeiro, in internodal length of secondary fingers.

Variability was almost uniform in internodal length of primary and secondary fingers in the somaclones of two cultivars evaluated.

4.2.4.1.6 Thickness of Inner Core of Rhizome

Thickness of inner core of rhizomes varied from 0.20 to 1.10 cm in the clones studied. The inner core of rhizomes was thicker in clones of Maran than in clones of Rio-de-Janeiro. In 22.50 per cent clones of Maran, the thickness of inner core of rhizomes was more than 0.60 cm as compared to 14.64 per cent clones in Rio-de-Janeiro.

Somaclones and CP plants when compared, 33 per cent somaclones recorded more thickness of inner core of rhizome over CP plants. Clones of Rio-de-Janeiro showed more thickness of inner core of rhizome over CP plants. Forty three per cent clones of Rio-de-Janeiro and 23 per cent clones of Maran were superior to CP plants in thickness of the inner core of rhizome.

The somaclones of Rio-de-Janeiro exhibited more variability for the character studied. The coefficient of variation recorded was 31.638 per cent in clones of Rio-de-Janeiro and 27.124 per cent in clones of Maran.

4.2.4.1.7 Colour of Flesh

Different colours of flesh noticed in ginger clones were pale yellow, pale yellow with tinges of blue and green.

In clones of Maran, eighty five per cent clones had rhizomes with pale yellow flesh colour, 13.75 per cent had rhizomes with pale yellow flesh with a bluish tinge and 1.25 per cent clones had rhizomes with pale yellow flesh with a greenish tinge.

Flesh colours observed in clones of Rio-de-Janeiro were pale yellow in 96.34 per cent of clones, pale yellow with bluish tinge in 2.44 per cent of clones and pale yellow with greenish tinge in 1.22 per cent of clones.

Dominant flesh colour noticed in the clones studied was pale yellow. Only in nine per cent somaclones, the flesh colour varied from pale yellow. Somaclones of

Maran exhibited more variability for the flesh colour. Variable flesh colours were noticed in fifteen per cent clones of Maran and four per cent clones of Rio-de-Janeiro.

4.2.4.1.8 Colour of Scale Leaves

Different scale leaf colours observed in somaclones of ginger were pale yellow, pale yellow with violet, red and brown tinge (Plate 11a). In the cultivar Maran, pale yellow scale leaves were observed in 73 per cent of clones and pale yellow with red scale leaves were observed in 21 per cent of clones.

In somaclones of Rio-de-Janeiro, colours of scales noticed were pale yellow, pale yellow with tinges of blue, red and violet. Pale yellow scale leaves dominated in 84 per cent of clones of Rio-de-Janeiro followed by pale yellow scales with violet tinge in 8.54 per cent of clones.

The dominant scale colour noticed in the clones was pale yellow. As in flesh colour, variation was noticed in colour of scale leaves also. Twenty two per cent somaclones recorded variability in scale colour.

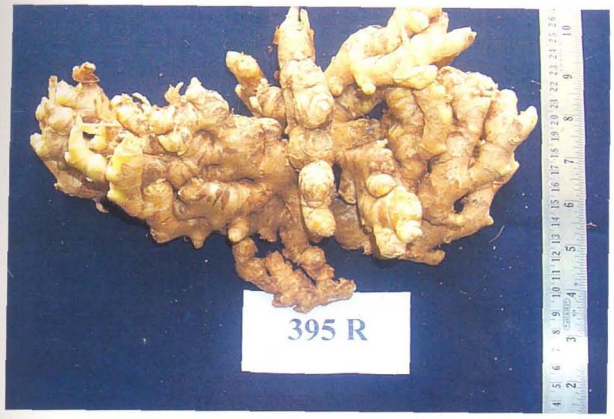
Somaclones of Maran exhibited more variation in scale colour than clones of Rio-de-Janeiro. The colour of scale leaves varied in twenty eight per cent clones of Maran and 16 per cent clones of Rio-de-Janeiro.

4.2.4.1.9 Orientation of Rhizomes

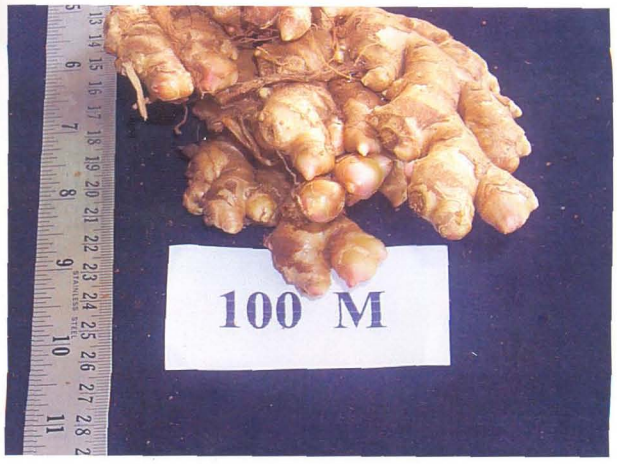
The arrangements of rhizomes were observed in the clones studied. Rhizomes were arranged in layers of one to five (Plate 11b).

In clones of Maran, rhizomes were one layered in 33.75 per cent of clones, two layered in 47.50 per cent of clones, three layered in 12.50 per cent of clones, four layered in 3.75 per cent of clones and five layered in 2.50 per cent of clones.

In somaclones of Rio-de-Janeiro, rhizomes were one layered in 31.71 per cent of clones, two layered in 46.34 per cent of clones and three layered in 22 per cent of clones.



Pale yellow scale leaves



Pale yellow with reddish tinged scale leaves

Plate 11a. Variability in rhizome characters in ginger somaclones
(Colour of scale leaves)

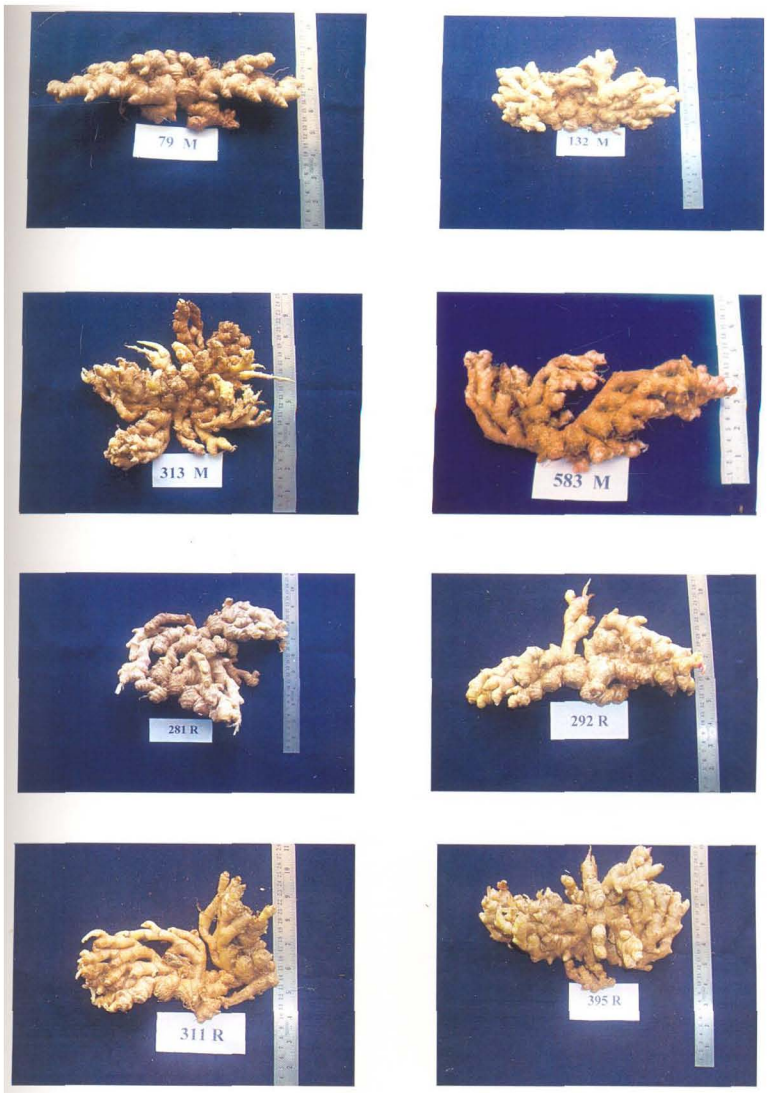


Plate 11b. Variability in rhizome characters in ginger somaclones

Somaclones of Maran recorded more number of layers in rhizomes as compared to clones of Rio-de-Janeiro. Six per cent clones of Maran produced rhizomes with more than three layers while none of the clones produced more than three layers in clones of Rio-de-Janeiro.

Rhizomes of CP plants were two layered in the two cultivars studied. When somaclones and CP plants were compared, 20 per cent somaclones showed superiority in number of layers over CP plants. Somaclones of Rio-de-Janeiro exhibited superiority in number of layers over CP plants. Twenty two per cent clones of Rio-de-Janeiro and 19 per cent clones of Maran recorded more number of layers of rhizomes over CP plants.

Somaclones of Maran exhibited more variability in the character studied recording one to five layered rhizomes as compared to one to three layered rhizomes in clones of Rio-de-Janeiro.

Overall analysis of rhizome characters showed that somaclones were superior to CP plants in the rhizome characters studied such as number, length and girth of fingers and orientation of rhizomes (Fig. 7). More number of somaclones of cultivar Maran were superior to CP plants in number of primary fingers, length of primary fingers and girth of secondary fingers. Clones of Rio-de-Janeiro were better in number of secondary and tertiary fingers, length of secondary fingers, girth of primary fingers and number of layers of rhizomes over CP plants.

Somaclones of cultivar Maran were superior in rhizome characters studied such as number, length and girth of fingers and orientation of rhizomes compared to clones of cultivar Rio-de-Janeiro.

In somaclones of Maran, more variability was observed in length of primary fingers and in somaclones of Rio-de-Janeiro, more variability was observed in girth of secondary fingers (Fig. 8). However, for the other characters studied, variability was almost uniform in the two cultivars studied.

4.2.4.2 Yield

The somaclones were evaluated for three seasons viz. 2002, 2003 and 2004 and the data on per plant yield of rhizome (fresh) for the clones for three years of evaluation are presented in Table 19. The frequency distribution for the parameters is presented in Tables 19a to 19h. Rhizome bits of weight 15 to 20g were used as seed material for planting in somaclones and control plants. Clones yielding less than 100 g fresh rhizomes/plant were considered as low yielders, 100 to 170 g as medium yielders and more than 170 g as high yielders.

In the first year of field evaluation, mean yield of rhizomes ranged between 5.00-330.00 g in somaclones of Maran and 5.00 - 261.66 g in clones of Rio-de-Janeiro. During second year of evaluation, yield recorded ranged from 20.00 to 400.00 g in clones of Maran and 10.00 to 220.00 g in clones of Rio-de-Janeiro. Yield during third year of field evaluation varied between 10.00 - 436.67 g in clones of Maran and 20.00-426.67 g in clones of Rio-de-Janeiro. The yield of somaclones was found to improve in subsequent years of field evaluation.

Based on average yield for three seasons of evaluation, nine per cent somaclones were found superior to CP plants giving an yield increase of 12 per cent over CP plants (Fig. 9 and Plate 12). Eighteen per cent clones of Rio-de-Janeiro were found superior exhibiting an yield increase of 13 per cent over CP plants. Similarly, two per cent clones of Maran were found high yielders than CP plants giving an yield increase of 11 per cent over CP plants.

Yield data in somaclones of two cultivars showed that more somaclones in the cultivar Maran recorded higher yield than the clones of cultivar Rio-de-Janeiro. Thirty per cent clones of Maran came in the highest classes of frequency table recording a mean per plant yield of more than 207 g as compared to 18 per cent clones in cultivar Rio-de-Janeiro. Yield recorded in majority of the clones was also high in somaclones of cultivar Maran as compared to somaclones of cultivar Rio-de-Janeiro. In 65 per cent clones of Maran, yield recorded came in the range 100.00-237.67 g.

Table 19. Fresh yield of rhizomes (per plant) in first set somaclones in ginger (*Z. officinale* Rosc.)

Maran						Rio-de-Janeiro				
Sl. No.	Clone No.	Yield (g)				Clone no.	Yield (g)			
		2002	2003	2004	Mean		2002	2003	2004	Mean
1	56 M	225.00	150.00	230.00	201.67	88 R	83.33	30.00	100.00	71.11
2	79 M	103.33	190.00	145.00	146.11	137 R	85.00	116.67	326.67	176.11
3	84 M	147.50	60.00	203.33	136.94	281 R	175.00	200.00	260.00	216.67
4	85 M	96.66	216.66	245.00	186.11	292 R	261.40	106.67	213.33	193.80
5	91 M	71.00	116.67	293.33	160.33	296 R	172.50	120.00	206.67	166.39
6	99 M	127.14	400.00	390.00	305.71	308 R	182.00	86.67	353.33	207.33
7	100 M	198.00	350.00	220.00	256.00	311 R	83.33	20.00	-	-
8	110 M	113.75	183.33	213.33	170.14	312 R	261.66	183.33	313.33	252.77
9	132M	151.40	200.00	380.00	243.80	314 R	112.00	60.00	-	-
10	136 M	163.33	216.67	133.33	171.11	315 R	90.00	-	-	-
11	139 M	66.66	30.00	110.00	68.88	335 R	130.00	30.00	-	-
12	150 M	72.00	150.00	303.33	175.11	336 R	27.50	75.00	320.00	140.83
13	197 M	210.00	266.67	305.00	260.56	337 R	87.50	-	-	-
14	199 M	50.00	40.00	-	-	338 R	146.25	180.00	390.00	238.75
15	220 M	210.00	320.00	200.00	243.33	345 R	96.25	40.00	250.00	128.75
16	276 M	104.00	60.00	-	-	346 R	100.00	220.00	353.33	224.44
17	283 M	126.00	50.00	290.00	155.33	347 R	5.00	-	-	-
18	284 M	216.25	150.00	270.00	212.08	349 R	120.00	90.00	-	-
19	287 M	166.25	116.67	210.00	164.31	350 R	33.75	-	-	-
20	288 M	20.00	-	-	-	351 R	185.00	60.00	360.00	201.67
21	290 M	153.33	70.00	180.00	134.44	355 R	75.00	-	-	-
22	311 M	98.33	80.00	-	-	358 R	20.00	80.00	-	-
23	313 M	70.00	60.00	-	-	361 R	25.00	20.00	-	-
24	317 M	88.75	30.00	-	-	364 R	148.75	176.67	426.67	250.70
25	342 M	286.25	250.00	386.67	307.64	367 R	60.00	-	-	-
26	348 M	90.00	-	-	-	368 R	140.00	60.00	140.00	113.33
27	356 M	40.00	30.00	-	-	373 R	122.50	60.00	-	-

contd.

Maran						Rio-de-Janeiro				
Sl. No.	Clone No.	Yield (g)				Clone no.	Yield (g)			
		2002	2003	2004	Mean		2002	2003	2004	Mean
28	372 M	116.25	40.00	-	-	377 R	147.50	40.00	-	-
29	374 M	107.50	60.00	30.00	65.83	378 R	5.00	-	-	-
30	381 M	175.00	80.00	40.00	98.33	384 R	50.00	120.00	95.00	88.33
31	382 M	37.50	70.00	-	-	386 R	33.33	200.00	290.00	174.44
32	392 M	85.00	-	-	-	395 R	195.00	50.00	240.00	161.67
33	393 M	240.00	300.00	216.67	252.22	399 R	80.00	-	-	-
34	397 M	88.70	80.00	-	-	413 R	32.50	20.00	-	-
35	400 M	20.00	-	-	-	418 R	35.00	20.00	-	-
36	411 M	140.00	80.00	283.33	167.78	431 R	40.00	50.00	-	-
37	431 M	10.00	-	-	-	438 R	50.00	-	-	-
38	432 M	40.00	92.50	80.00	70.83	463 R	90.00	50.00	190.00	110.00
39	434 M	10.00	20.00	-	-	466 R	45.00	220.00	266.67	177.22
40	435 M	10.00	40.00	-	-	471 R	55.00	30.00	-	-
41	436 M	120.00	110.00	340.00	190.00	475 R	80.00	40.00	-	-
42	439 M	10.00	-	-	-	476 R	40.00	20.00	-	-
43	441 M	120.00	60.00	220.00	133.33	478 R	110.00	50.00	203.33	121.11
44	444 M	40.00	-	-	-	482 R	60.00	-	-	-
45	446 M	20.00	-	-	-	485 R	110.00	60.00	106.67	92.22
46	462 M	10.00	-	-	-	517 R	30.00	-	-	-
47	464 M	20.00	80.00	-	-	524 R	40.00	30.00	-	-
48	488 M	230.00	200.00	200.00	210.00	526 R	90.00	40.00	-	-
49	500 M	100.00	60.00	100.00	86.67	531 R	20.00	10.00	-	-
50	505 M	125.00	140.00	-	-	535 R	100.00	-	-	-
51	510 M	70.00	50.00	-	-	548 R	40.00	-	-	-
52	513 M	150.00	40.00	10.00	66.67	561 R	140.00	70.00	270.00	160.00
53	528 M	30.00	80.00	-	-	582 R	155.00	50.00	40.00	81.67

contd.

Maran						Rio-de-Janeiro				
Sl. No.	Clone No.	Yield (g)				Clone no.	Yield (g)			
		2002	2003	2004	Mean		2002	2003	2004	Mean
54	529 M	60.00	73.33	203.33	112.22	589 R	5.00	-	-	-
55	549 M	140.00	85.00	173.33	132.78	590 R	110.00	50.00	-	-
56	554 M	123.00	360.00	230.00	237.67	597 R	140.00	40.00	-	-
57	565 M	60.00	40.00	200.00	100.00	610 R	80.00	50.00	-	-
58	572 M	260.00	150.00	125.00	178.33	616 R	30.00	40.00	-	-
59	580 M	120.00	-	-	-	617 R	150.00	110.00	65.00	108.33
60	583 M	130.00	60.00	-	-	622 R	10.00	-	-	-
61	588 M	96.66	40.00	-	-	626 R	40.00	50.00	-	-
62	649 M	10.00	-	-	-	630 R	90.00	60.00	-	-
63	659 M	133.33	50.00	220.00	134.44	704 R	80.40	70.00	120.00	90.13
64	660 M	103.33	40.00	200.00	114.44	734 R	145.00	70.00	280.00	165.00
65	668 M	50.00	130.00	320.00	166.67	748 R	100.00	20.00	-	-
66	743 M	60.00	-	-	-	772 R	115.00	60.00	20.00	65.00
67	746 M	93.33	20.00	-	-	786 R	135.00	100.00	120.00	118.33
68	781 M	20.00	60.00	-	-	790 R	98.33	80.00	-	-
69	918 M	330.00	160.00	151.67	213.89	801 R	123.33	50.00	110.00	94.44
70	953 M	40.00	180.00	160.00	126.67	982 R	15.00	-	-	-
71	970 M	290.00	200.00	436.67	308.89	R I	185.00	70.00	60.00	105.00
72	980 M	120.00	200.00	193.33	171.11	R II	93.33	60.00	-	-
73	985 M	100.00	-	-	-	R III	120.00	-	-	-
74	M I	90.00	170.00	320.00	193.33	R IV	40.00	20.00	-	-
75	M III	40.00	-	-	-	R V	183.33	100.00	273.33	185.54
76	M IV	5.00	-	-	-	R VI	35.00	-	-	-
77	M V	30.00	-	-	-	R VII	40.00	-	-	-
78	M VI	306.25	333.33	406.67	348.75	R VIII	225.00	60.00	-	178.33
79	M VII	40.00	40.00	-	-	R IX	57.50	60.00	-	-
80	M VIII	30.00	-	-	-	R X	10.00	-	-	-
81	Control M	200.00	333.33	413.34	315.56	R XI	66.66	140.00	333.33	180.00
82						R XII	40.00	-	-	-
83						Control R	160.00	116.67	336.67	204.45
	S.D	77.165	98.358	103.598	72.396		59.685	52.924	112.655	53.801
	C.V (%)	72.085	76.822	45.667	40.342		64.969	70.389	50.013	34.883

Frequency distribution of fresh rhizome yield in first set somaclones in ginger (*Z. officinale* Rosc.)

Table 19a. Fresh yield (2002) - cultivar Maran

Group No.	Fresh yield (g)	Frequency (%)
1	3.50 - 44.49	27.50
2	44.50 - 85.49	13.75
3	85.50 - 126.49	28.75
4	126.50 - 167.49	13.75
5	167.50 - 208.49	2.50
6	208.50 - 249.49	7.50
7	249.50 - 290.49	3.75
8	290.50 - 331.49	2.50

Table 19b. Fresh yield (2003) - cultivar Maran

Group No.	Fresh yield (g)	Frequency (%)
1	17.50 - 65.49	38.10
2	65.50 - 113.49	19.05
3	113.50 - 161.49	14.29
4	161.50 - 209.49	12.70
5	209.50 - 257.49	4.76
6	257.50 - 305.49	3.17
7	305.50 - 353.49	4.76
8	353.50 - 401.49	3.17

Table 19c. Fresh yield (2004) - cultivar Maran

Group No.	Fresh yield (g)	Frequency (%)
1	6.50 - 60.49	6.82
2	60.50 - 114.49	6.82
3	114.50 - 168.49	11.36
4	168.50 - 222.49	34.09
5	222.50 - 276.49	9.09
6	276.50 - 330.49	15.91
7	330.50 - 384.49	4.55
8	384.50 - 438.49	11.36

Table 19g. Mean fresh yield - cultivar Maran

Group No.	Fresh yield (g)	Frequency (%)
1	63.29 - 99.28	13.95
2	99.29 - 135.28	18.60
3	135.29 - 171.28	23.26
4	171.29 - 207.28	13.95
5	207.29 - 243.28	9.30
6	243.29 - 279.28	11.63
7	279.29 - 315.28	6.98
8	315.29 - 351.28	2.33

Table 19d. Fresh yield (2002) - cultivar Rio-de-Janeiro

Group No.	Fresh yield (g)	Frequency (%)
1	< 33.50	17.07
2	33.50 - 66.49	21.95
3	66.50 - 99.49	20.73
4	99.50 - 132.49	15.85
5	132.50 - 165.49	12.20
6	165.50 - 198.49	8.54
7	198.50 - 231.49	1.22
8	231.50 - 264.49	2.44

Table 19e. Fresh yield (2003) - cultivar Rio-de-Janeiro

Group No.	Fresh yield (g)	Frequency (%)
1	6.50 - 33.49	19.35
2	33.50 - 60.49	41.94
3	60.50 - 87.49	12.90
4	87.50 - 114.49	8.06
5	114.50 - 141.49	6.45
6	141.50 - 168.49	0.00
7	168.50 - 195.49	4.84
8	195.50 - 222.49	6.45

Table 19f. Fresh yield (2004) - cultivar Rio-de-Janeiro

Group No.	Fresh yield (g)	Frequency (%)
1	18.50 - 69.49	12.12
2	69.50 - 120.49	18.18
3	120.50 - 171.49	3.03
4	171.50 - 222.49	12.12
5	222.50 - 273.49	21.21
6	273.50 - 324.49	12.12
7	324.50 - 375.49	15.15
8	375.50 - 426.49	6.06

Table19h. Mean fresh yield - cultivar Rio-de-Janeiro

Group No.	Fresh yield (g)	Frequency (%)
1	62.89 - 86.88	9.09
2	86.89 - 110.88	21.21
3	110.89 - 134.88	12.12
4	134.89 - 158.88	3.03
5	158.89 - 182.88	27.27
6	182.89 - 206.88	9.09
7	206.89 - 230.88	9.09
8	230.89 - 254.88	9.09

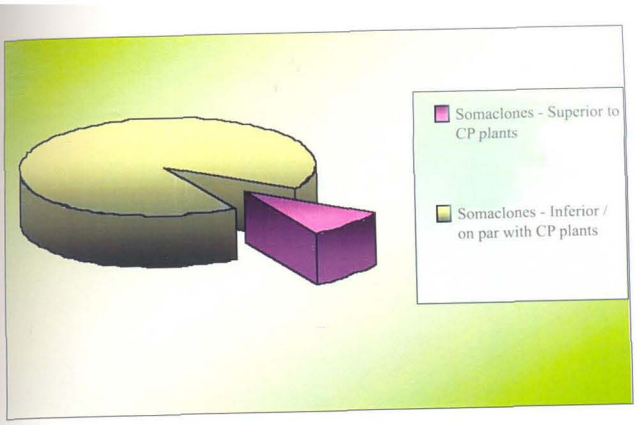


Fig. 9 Comparison of yield in somaclones and conventionally propagated plants in ginger

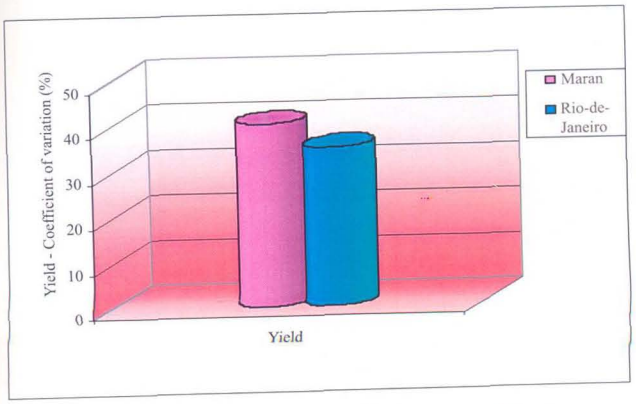


Fig. 10 Somaclonal variation for yield in ginger cultivars

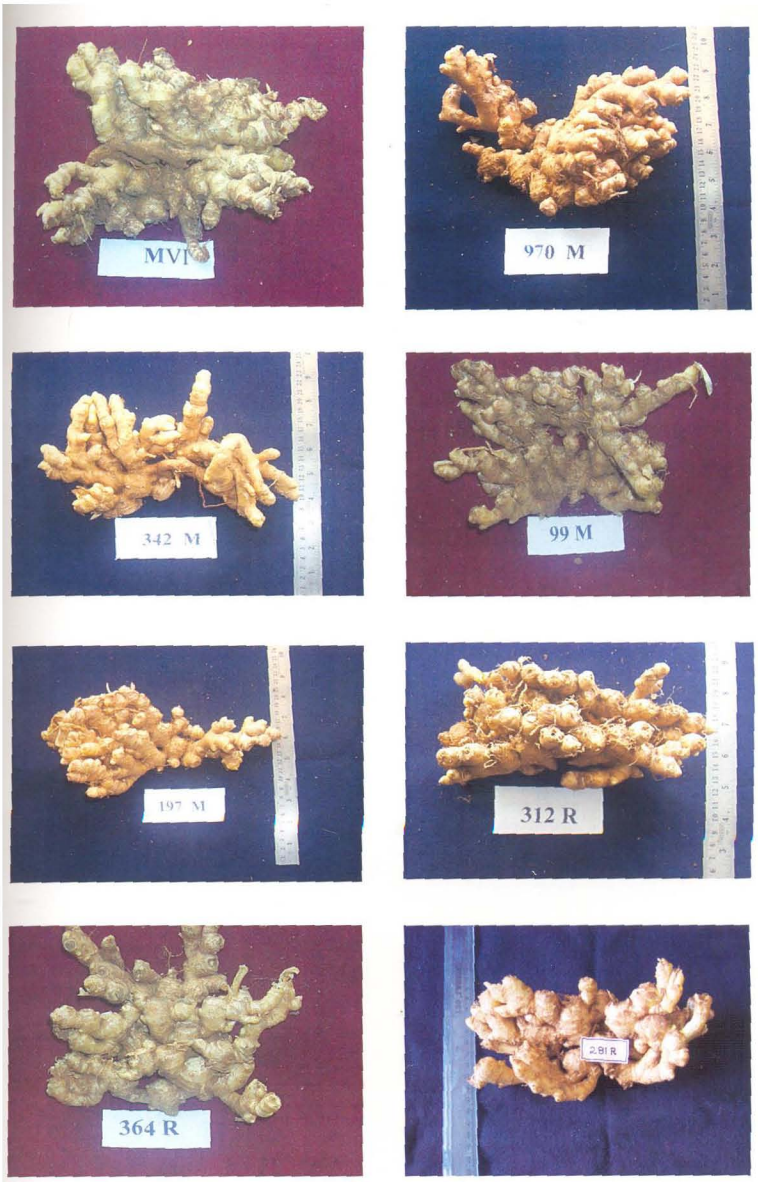


Plate12. High yielding somaclones in ginger

The yield recorded in clones of Rio-de-Janeiro was low and 64 per cent clones came in the range of 83.33-180.00 g.

The somaclones of cultivar Maran exhibited more variability in yield than clones of cultivar Rio-de-Janeiro (Fig. 10). Coefficient of variation recorded for the character was 40.342 per cent in clones of Maran and 34.883 per cent in clones of Rio-de-Janeiro.

Evaluation of somaclones for three seasons' revealed the superiority of somaclones over CP plants in yield. Average per plant yield was found high in nine per cent somaclones. More number of somaclones of cultivar Rio-de-Janeiro were found superior to CP plants in yield. However, somaclones of cultivar Maran recorded higher yield than clones of cultivar Rio-de-Janeiro. The extent of somaclonal variation in yield was also more in clones of cultivar Maran.

The growth parameters and rhizome characters were analysed in the high yielding clones. The high yielding somaclones exhibited superiority in height of pseudostem, number of tillers and leaves and leaf area. The growth rate for the various morphological parameters was also high in the high yielding clones. The rhizome characters like number, length and girth of fingers and layers of rhizomes were more in the high yielding clones.

4.2.4.3 Quality Attributes

Quality parameters like dry ginger recovery, essential oil, oleoresin and fibre content were studied in fifteen somaclones (12 somaclones of Maran and three somaclones of Rio-de-Janeiro) and the results are presented in Table 20.

4.2.4.3.1 Dry Ginger Recovery

The dry ginger recovery ranged from 15.62 to 25 per cent in the different somaclones evaluated. The mean driage recorded in somaclones was 19.73 per cent while it was only 16.02 per cent in CP plants. Thus the somaclones recorded higher recovery of dry ginger as compared to CP plants. Higher dry ginger recovery was

Table 20. Quality attributes in selected somaclones of ginger (*Z. officinale* Rosc.)

Sl.No.	Somaclone No.	*Driage (%)	* Essential oil (%)	*Oleoresin (%)	*Fibre (%)
1	56 M	20.51	1.75	7.21	3.58
2	79 M	18.75	1.50	5.21	2.28
3	85 M	20.51	1.00	6.31	2.33
4	99 M	20.50	2.00	6.27	3.77
5	100 M	21.05	1.75	6.46	3.16
6	110 M	22.56	1.50	5.17	4.25
7	136 M	19.23	1.50	8.48	3.37
8	197 M	19.74	1.50	4.81	5.24
9	342 M	20.50	2.25	7.15	4.16
10	488 M	25.00	1.25	4.31	2.57
11	970 M	22.50	1.75	5.70	3.55
12	MVI	17.95	1.50	5.91	1.96
13	281 R	15.78	1.42	7.37	6.16
14	312 R	15.79	2.50	4.38	4.27
15	364 R	15.62	2.07	8.93	6.86
16	Control M	15.38	1.95	6.65	2.95
17	Control R	16.66	2.25	7.14	3.06

* Average of two replications

noticed in clones of Maran (18-25 %) than in clones of Rio-de-Janeiro (15.62-15.79%).

Of the 12 somaclones of Maran studied, highest percentage of dry ginger recovery was recorded in the clone 488 M (25.00 %) followed by the clones 110 M (22.56 %) and 970 M (22.50 %). Dry ginger recovery in CP plant of the cultivar Maran was 15.38 per cent and all the somaclones studied dominated the CP plant exhibiting 35 per cent increase in dry ginger recovery.

In the somaclones of Rio-de- Janeiro, highest dry ginger recovery was noticed in the clone 312 R (15.79 %) closely followed by the clone 364 R (15.62 %). The CP plant registered almost the same dry ginger recovery as that of the somaclones.

4.2.4.3.2 Essential Oil

Recovery of essential oil varied between 1.00 to 2.50 per cent in the somaclones studied. Essential oil content was found higher in somaclones of Rio-de-Janeiro (1.42 to 2.50 %) than clones of Maran (1.00-2.25 %).

In somaclones of Maran, highest recovery of essential oil was registered in the clone 342 M (2.25 %) followed by 99 M (2.00%). The essential oil content in the CP plant of the cultivar Maran was 1.95 per cent and the clones 342 M and 99 M recorded better recovery of essential oil over the CP plant.

In somaclones of Rio-de–Janeiro, recovery of essential oil was highest in the clone 312 R (2.50 %) followed by the clones 364 R (2.07 %) and 281 R (1.42 %). The essential oil content in the CP plant of cultivar Rio-de–Janeiro was 2.25 per cent and the clone 312 R dominated the CP plant in oil recovery.

4.2.4.3.3 Oleoresin

Oleoresin content ranged between 4.31 to 8.93 per cent in the somaclones evaluated. Higher recovery of oleoresin was noticed in clones of Rio-de-Janeiro (4.38 to 8.93 %) than clones of Maran (4.31 to 8.48 %).

Of the 12 somaclones of Maran, highest recovery of oleoresin was recorded in the clone 136 M (8.48 %) followed by 56 M (7.21 %) and 342 M (7.15 %). The oleoresin recovery in the CP plant of the cultivar Maran was 7.14 per cent and the clones 136 M, 56 M and 342 M dominated the CP plant in oleoresin yield.

In the different somaclones of Rio-de-Janeiro studied, oleoresin content was highest in the clone 364 R (8.93 %) followed by the clones 281 R (8.93 %) and 312 R (4.38 %). Oleoresin content in the CP plant was 7.14 per cent and the somaclone 364 R recorded high recovery of oleoresin over the CP plant

4.2.4.3.4 Fibre Content

Fibre content ranged between 1.96 to 6.86 per cent in the somaclones studied. Somaclones of Maran recorded low fibre content (1.96-5.24 %) as compared to Rio-de-Janeiro (4.27 to 6.86 %). The fibre content in the CP plant of cultivar Maran was 2.95 per cent and the clones M VI, 79 M, 85 M and 488 M recorded low fibre content than the CP plant. Fibre content was lowest in M VI (1.96 %) followed by 79 M (2.28 %) and 85 M (2.33 %). All the somaclones of Rio-de-Janeiro recorded higher fibre content than the CP plant (3.06 %).

4.3 SCREENING OF SOMACLONES FOR RESISTANCE / TOLERANCE TO RHIZOME ROT AND BACTERIAL WILT DISEASES

4.3.1 Production of Toxic Metabolite(s) by *P. aphanidermatum* and Bioassay of the Metabolite(s)

Pythium aphanidermatum was isolated from naturally infected rhizomes. Three different liquid media viz. M_1 [Czapek (Dox) agar], M_2 (Richard's solution) and M_3 (Asparagine or synthetic mucor) and two culture conditions (shaking / stationary) and two incubation periods (10 and 15 days) were tried for production of toxic metabolite(s) of the pathogen. Culture filtrate extracted from each treatment was concentrated to one tenth of its volume by keeping in hot plate maintained at 100^0 C. Toxigenicity of the concentrated culture filtrate (CCF) was tested by bioassay using ginger rhizomes and by inducing electrolyte leakage using the toxic metabolite(s) from leaves of ginger.

4.3.1.1 Bioassay Using Ginger Rhizomes

Mature ginger rhizomes were first cleaned and 0.2 ml CCF was inoculated to the rhizomes. Rotting symptoms were observed in rhizomes when toxic metabolite(s) was inoculated and the symptoms were similar to that of inoculation by culture disc of the pathogen. The CCF extracted from three different liquid media induced rotting symptoms in ginger rhizomes. But when the symptoms produced by CCF and inoculation by pathogen were compared, the rotting area observed was more in direct inoculation of the pathogen (2.27 cm^2) (Table 21 and Plate 13). Among the inoculation treatments using CCF, the area of rotting was more in CCF extracted from medium M_3 (1.96 cm^2) followed by medium M_2 (1.75 cm^2) and medium M_1 (1.66 cm^2).

4.3.1.2 Bioassay by Electrolyte Leakage Method

Electrolyte leakage was induced from medium mature leaves of ginger using toxic metabolite(s) of *P. aphanidermatum* extracted from 24 treatments. Electrolyte leakage was induced immediately after the treatment, which increased with increase in time intervals (Table 22a). The difference in leakage was highest 10 minutes after infiltration with toxic metabolite(s), thereafter the differences showed a decreasing trend. So the leakage of electrolytes induced 10 minutes after infiltration was used for comparison of various treatments.

With regard to three different media tried for production of toxic metabolites(s), maximum toxin accumulation occurred in medium M_3 as evident by the highest leakage of electrolytes in medium M_3 (24.44 µs) followed by medium M_2 (20.31 µs) and medium M_1 (17.49 µs), 10 minutes after infiltration (Table 22b and Fig. 11a).

Of the two incubation periods tried for production of toxic metabolite(s), 15-day incubation accumulated maximum toxic metabolite(s) as evident by the higher leakage values observed in CCF extracted from 15-day incubation treatment (22.05 µs) (Fig. 11b). Similarly, CCF from shake cultures recorded higher leakage of

Table 21. Development of symptoms by toxic metabolite(s) and culture disc of *P. aphanidermatum* in ginger (*Z. officinale* Rosc.)

Treatments	*Area of discolouration / rotting - 3 DAI (cm^2)	Mean area of rotting (cm^2)
M_1T_1 (10 day shaking)	1.73	1.66
M_1T_2 (10 day stationary)	1.70	
M_1T_3 (15 day shaking)	1.69	
M_1T_4 (15 day stationary)	1.53	
M_1 control	0.73	
M_2T_1 (10 day shaking)	1.80	1.75
M_2T_2 (10 day stationary)	1.78	
M_2T_3 (15 day shaking)	1.83	
M_2T_4 (15 day stationary)	1.57	
M_2 control	0.47	
M_3T_1 (10 day shaking)	1.75	1.96
M_3T_2 (10 day stationary)	2.34	
M_3T_3 (15 day shaking)	2.01	
M_3T_4 (15 day stationary)	1.74	
M_3 control	0.76	
Culture disc	2.27	2.27

* Average of three replications

M_1 - Czapek (Dox) agar

M_2 - Richard's solution

M_3 - Asparagine or synthetic mucor

DAI - Days after inoculation

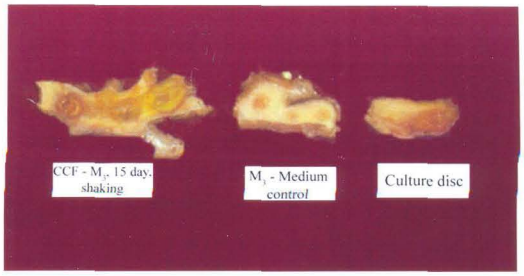


Plate 13. Comparison of symptoms induced by toxic metabolite(s) and culture disc of *Pythium aphanidermatum* in ginger rhizomes

Plate 14. Comparison of symptoms induced by different concentrations of toxic metabolite(s) of *Ralstonia solanacearum* in ginger shoots

Plate 15. Incidence of rhizome rot and bacterial wilt diseases in ginger somaclones in sick field

Table 22a. Electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* from leaves of ginger (*Z. officinale* Rosc.) at different time intervals

Treatments	Concentration of CCF (% v/v)	*Electrolyte leakage over control (µs) at different time intervals (minutes)																							
		M_1								M_2								M_3							
		Maran				Rio-de-Janeiro				Maran				Rio-de-Janeiro				Maran				Rio-de-Janeiro			
		0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30
T_1 10 day shaking	5	8.85	14.30	19.10	18.10	6.65	16.65	19.00	20.50	10.00	18.00	23.00	22.50	8.50	16.50	18.50	19.00	14.85	23.95	23.25	21.70	16.80	23.65	21.00	19.90
T_2 10 day shaking	10	10.95	23.50	28.00	25.50	7.90	15.00	15.00	18.50	13.00	20.00	21.50	25.00	5.50	18.50	22.00	25.50	14.90	24.00	25.05	23.75	18.00	25.60	25.65	29.80
T_3 10 day stationary	5	5.30	12.50	17.60	16.10	2.45	13.20	15.00	16.50	11.00	18.00	16.50	22.50	9.50	16.50	16.00	17.50	14.70	20.80	23.20	21.40	16.35	22.65	23.75	25.30
T_4 10 day stationary	10	5.50	18.00	23.00	21.50	2.70	13.50	14.00	14.00	9.50	28.00	28.00	24.00	10.50	16.50	19.00	26.00	15.85	22.80	24.20	23.25	16.40	24.65	24.95	26.55
T_5 15 day shaking	5	7.35	24.45	28.10	27.60	9.95	19.50	29.50	30.00	14.00	34.00	35.50	25.00	10.50	19.50	24.50	25.50	16.85	28.85	27.90	26.70	17.50	24.10	25.80	24.60
T_6 15 day shaking	10	7.80	26.50	25.50	34.50	11.55	22.00	30.00	31.00	13.00	27.00	28.50	25.00	7.50	23.00	24.50	30.50	17.90	29.60	30.20	29.25	15.85	27.10	27.55	28.25
T_7 15 day stationary	5	8.90	13.85	19.60	18.60	5.55	18.85	20.00	19.00	11.50	26.50	29.50	30.50	5.00	15.50	16.50	22.00	16.60	22.60	28.35	28.60	17.90	23.15	26.75	24.85
T_8 15day stationary	10	8.95	21.00	24.00	23.00	6.50	17.00	16.00	13.50	8.50	10.00	26.50	31.00	4.50	17.50	18.50	20.50	16.95	23.25	21.45	21.25	17.30	24.25	26.15	28.15

*Average of two replications
M_1 - Czapek (Dox) agar
M_2 - Richard's solution
M_3 - Asparagine or synthetic mucor
CCF- Concentrated culture filtrate

Table 22b. Electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* from leaves of ginger (*Z. officinale* Rosc.) 10 minutes after the treatment

Treatments	Concentration of CCF (% v/v)	*Electrolyte leakage over control at 10 minutes after the treatment (µs)						Mean		
		M_1		M_2		M_3				
		Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro	Grand mean
T_1 10 day shaking	5	14.30	16.65	18.00	16.50	23.95	23.65	18.75	18.93	18.84
T_2 10 day shaking	10	23.50	15.00	20.00	18.50	24.00	25.60	22.50	19.70	21.10
T_3 10 day stationary	5	12.50	13.20	18.00	16.50	20.80	22.65	17.10	17.45	17.28
T_4 10 day stationary	10	18.00	13.50	28.00	16.50	22.80	24.65	22.93	18.22	20.57
T_5 15 day shaking	5	24.45	19.50	34.00	19.50	28.85	24.10	29.10	21.03	25.07
T_6 15 day shaking	10	26.50	22.00	27.00	23.00	29.60	27.10	27.70	24.03	25.87
T_7 15 day stationary	5	13.85	8.85	26.50	15.50	22.60	23.15	20.98	15.83	18.41
T_8 15day stationary	10	21.00	17.00	10.00	17.50	23.25	24.25	18.08	19.58	18.83
Mean		19.26	15.71	22.69	17.94	24.48	24.39	22.14	19.35	
		17.49		20.31		24.44				

C.D (0.05) for comparison of cultivars - 0.896

C.D (0.05) for comparison of media - 1.097

C.D (0.05) for comparison of treatments - 1.792

C.D (0.05) for comparison of interaction (medium x treatment) - 3.104

*Average of two replications

** Derived from Table 22a

M_1 - Czapek (Dox) agar

M_2 - Richard's solution

M_3 - Asparagine or synthetic mucor

CCF- Concentrated culture filtrate

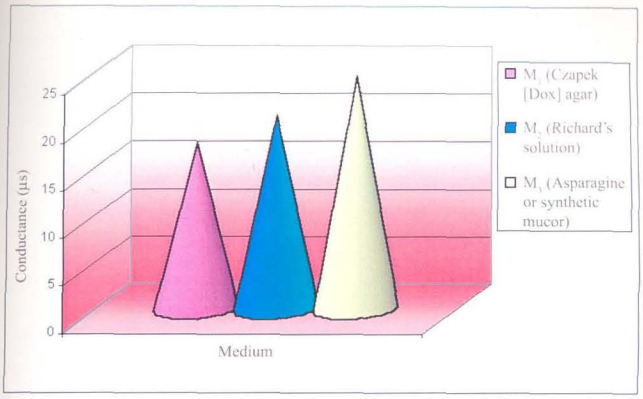


Fig.11a Effect of media on electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum*

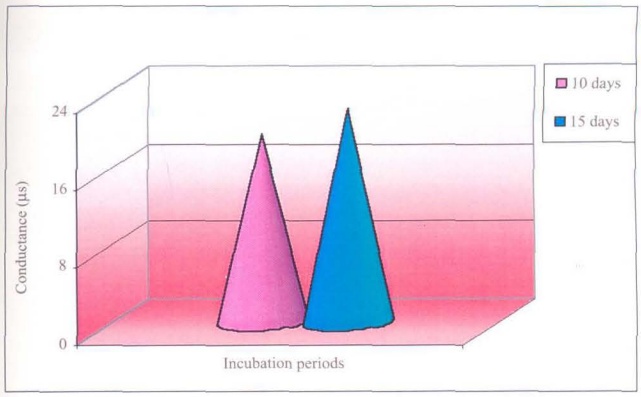


Fig. 11b Effect of incubation periods on electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum*

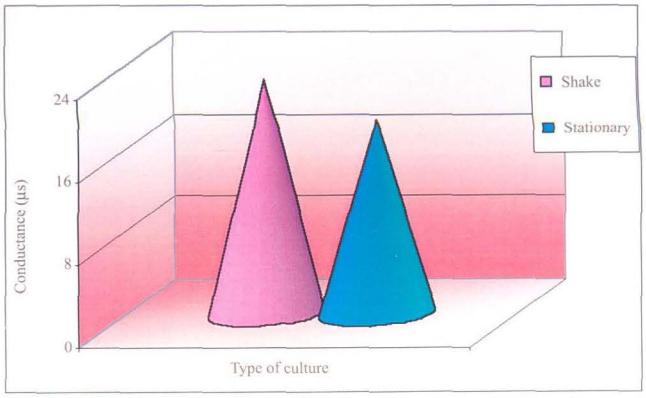


Fig. 11c Effect of type of culture on electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum*

electrolytes (22.72 µs) as compared to CCF from stationary cultures (18.78 µs) (Fig. 11c).

The leakage of electrolytes decreased as the concentration of toxic metabolite(s) decreased. The leakage of electrolytes was 21.60 µs at 10 per cent v/v of toxic metabolite(s) while it was 19.90 µs at 5 per cent v/v of metabolite(s). So the concentration of toxic metabolite(s) giving maximum leakage of electrolytes was selected for further investigations.

After standardising the various parameters responsible for production of toxic metabolite(s) from *P. aphanidermatum*, the treatment T_6 (shake cultures, 15 days incubation period, 10 per cent v/v of CCF) in medium M_3 was selected for production of toxic metabolite(s) for further screening studies and screening of clones against rhizome rot disease.

4.3.2 Production of Toxic Metabolite(s) of *R. solanacearum* and Bioassay of the Metabolite(s)

Ralstonia solanacearum was isolated from bacterial wilt affected ginger plants. The pure culture of the bacterium was maintained by frequent subculturing in Triphenyl Tetrazolium Chloride (TZC) medium. The toxin was extracted as per Paul (1998) by precipitation with acetone.

4.3.2.1 Bioassay Using Ginger Shoots

The toxic metabolite(s) of *R. solanacearum* induced yellowing and wilting symptoms on excised medium mature shoots of ginger (Plate 14). Ginger shoots kept in different concentrations of toxin solution (10, 25, 50 and 75 and 100 per cent) started yellowing from second day of inoculation. However, in lower concentrations of toxin (1 and 5 per cent v/v), the shoots took more time for yellowing. Yellowing was first noticed in lower leaves and gradually spread to upper leaves. On fifth day, shoots in all the toxin concentrations wilted completely. Shoots kept in distilled water did not show any symptoms of wilting. None of the wilted shoots could be recovered

from wilting on transfer to distilled water. Shoots kept in toxin solutions of higher concentrations exhibited more yellowing and wilting.

4.3.2.2 Bioassay by Electrolyte Leakage Method

Different concentrations of *R. solanacearum* toxin (2.5, 5.0, 7.5 and 10.0 per cent v/v) induced electrolyte leakage from leaves of ginger cultivars. The leakage of electrolytes increased with increase in time intervals (Table 23a and Fig. 12). Difference in leakage between successive time intervals was highest 10 minutes after infiltration with toxin and thereafter the differences showed a decreasing trend. So the leakage of electrolytes induced 10 minutes after the infiltration was used in the further studies.

Significant differences in electrolyte leakage values were observed in different concentrations of toxin solutions (2.5, 5.0, 7.5 and 10.0 per cent v/v) tried in the study. The leakage of electrolytes from leaves increased with increase in concentrations of toxin solution. The highest leakage of electrolytes was observed in 10 per cent v/v toxin solution (33.43 µs) and the lowest in 2.5 per cent v/v (26.98 µs) (Table 23b). The concentration of toxic metabolite(s) inducing maximum leakage was hence selected for further studies.

The two cultivars studied were on par with regard to leakage of electrolytes induced by *R. solanacearum* toxin, the leakage values being 28.94 µs in the cultivar Rio-de-Janeiro and 31.11 µs in the cultivar Maran.

4.3.3 Preliminary Screening of Second Set Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

The regenerants produced by indirect organogenesis/embryogenesis and *in vitro* mutagenesis were screened for resistance/tolerance to rhizome rot and bacterial wilt diseases. A total of 296 regenerants (Maran – 146, Rio-de-Janeiro – 150) were subjected to screening. The regenerants produced through various routes were screened against rhizome rot and bacterial wilt diseases by electrolyte leakage method and the data on leakage are presented in Tables 24 and 24a to 24e.

Table 23a. Electrolyte leakage induced by toxic metabolite(s) of *R. solanacearum* from leaves of ginger (*Z. officinale* Rosc.) at different time intervals

Concentration of toxin (v/v) (%)	*Electrolyte leakage over control (µs) at different time intervals (minutes)														
	Maran							Rio-de-Janeiro							
	0	10	20	30	40	50	60	0	10	20	30	40	50	60	Mean
2.50	26.00	28.80	30.90	31.40	32.65	32.85	32.70	23.40	25.15	26.55	27.35	27.45	28.25	28.55	28.71
5.00	27.10	30.10	31.40	32.15	32.40	32.85	32.90	26.65	28.75	30.60	31.40	31.95	32.30	32.85	30.96
7.50	27.95	31.25	32.20	32.75	33.05	33.35	33.75	27.20	29.30	30.90	32.15	32.90	33.60	33.95	31.74
10.00	30.95	34.30	35.95	37.75	38.35	38.85	39.10	29.70	32.55	33.90	34.50	34.70	35.40	35.75	35.13
Mean	28.00	31.11	32.61	33.51	34.11	34.48	34.61	26.74	28.94	30.49	31.35	31.75	32.39	32.78	
	32.63							30.63							

*Average of two replications

Table 23b. Electrolyte leakage induced by toxic metabolite(s) of *R. solanacearum* from leaves of ginger (*Z. officinale* Rosc.) 10 minutes after the treatment*

Concentration of toxin (v/v) (%)	Electrolyte leakage over control (µs) at 10 minutes after the treatment		Mean
	Maran	Rio-de-Janeiro	
2.50	28.80	25.15	26.98
5.00	30.10	28.75	29.43
7.50	31.25	29.30	30.28
10.00	34.30	32.55	33.43
Mean	31.11	28.94	

C.D (0.05) for comparison of cultivars - 2.752

C.D (0.05) for comparison of concentration - 3.892

* Derived from Table 23a

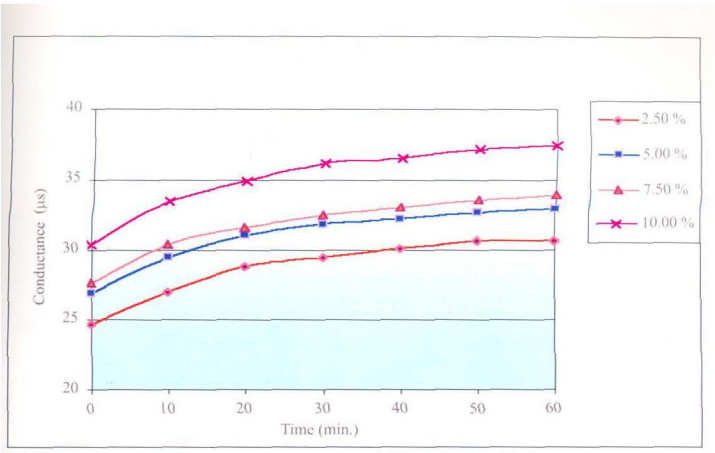


Fig. 12 Effect of different concentrations of toxic metabolite(s) of *R. solanacearum* on electrolyte leakage

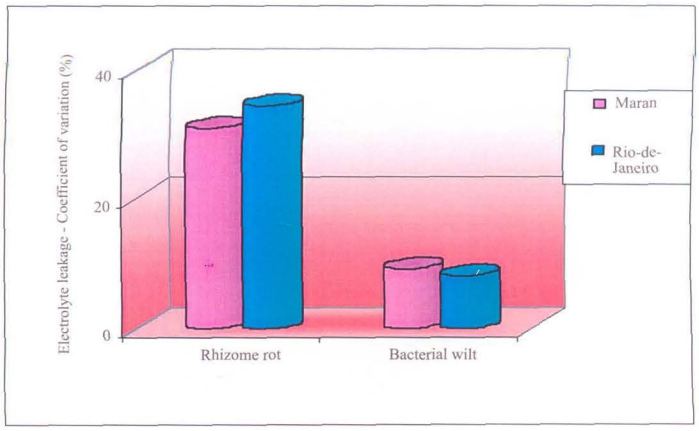


Fig. 13 Variability in electrolyte leakage in second set somaclones of ginger

Table 24. Preliminary screening of second set somaclones in ginger (*Z. officinale* Rosc.) against rhizome rot and bacterial wilt pathogens by electrolyte leakage method

Maran				Rio-de-Janeiro		
Sl. No.	Clone no.	Mean electrolyte leakage (µs)		Clone no.	Mean electrolyte leakage (µs)	
		P. aphani-dermatum	*R.solana-cearum*		*P. aphani-dermatum*	*R.solana-cearum*
1	36 MC	16.00	49.00	30 RC	31.45	47.90
2	120 MC	36.65	33.55	31 RC	20.55	38.00
3	121 MC	17.15	39.90	33 RC	31.00	26.80
4	131 MC	33.25	29.50	34 RC	24.80	43.35
5	213 MC	32.70	41.00	80 RC	19.20	38.75
6	214 MC	28.60	37.90	82 RC	30.20	47.50
7	216 MC	33.00	36.25	83 RC	31.25	29.05
8	218 MC	28.70	46.25	137 RC	21.30	29.90
9	219 MC	35.20	47.05	199 RC	41.00	54.75
10	221 MC	51.15	57.15	201 RC	47.90	38.80
11	223 MC	27.05	34.45	203 RC	42.00	41.45
12	224 MC	38.05	29.85	207 RC	48.25	56.60
13	227 MC	21.20	43.75	286 RC	27.75	38.45
14	231 MC	36.05	40.60	287 RC	22.95	43.55
15	232 MC	22.35	39.25	306 RC	36.40	42.45
16	234 MC	33.75	35.05	425 RC	47.30	41.85
17	235 MC	36.40	37.05	429 RC	24.55	43.25
18	288 MC	36.65	36.90	431 RC	25.90	37.50
19	290 MC	48.50	38.95	432 RC	39.05	55.65
20	292 MC	20.75	41.05	438 RC	46.80	34.80
21	293 MC	22.40	36.60	439 RC	25.90	34.20
22	294 MC	41.15	40.60	440 RC	21.65	36.45
23	295 MC	33.20	35.35	442 RC	28.75	30.50
24	296 MC	47.40	42.05	445 RC	30.60	41.15
25	297 MC	38.90	38.45	452 RC	54.40	37.70
26	298 MC	32.35	36.95	453 RC	23.15	37.80
27	308 MC	28.95	54.95	454 RC	34.30	46.45
28	309 MC	35.80	41.35	456 RC	37.80	40.35
29	314 MC	38.00	40.40	460 RC	37.95	55.95
30	463 MC	41.25	38.80	462 RC	26.15	39.40
31	464 MC	42.90	38.45	597 RC	23.10	50.60
32	466 MC	50.75	47.35	589 RC	41.95	40.75
33	468 MC	26.90	35.40	590 RC	29.10	40.50
34	469 MC	40.10	29.25	598 RC	19.90	50.90
35	471 MC	51.15	36.20	600 RC	35.55	51.40
36	545 MC	36.85	49.05	601 RC	30.95	44.15
37	586 MC	44.95	40.85	602 RC	55.10	63.90
38	616 MC	41.35	41.60	604 RC	56.75	38.25
39	618 MC	34.70	47.35	606 RC	31.15	37.60

Contd.

Maran				Rio-de-Janeiro		
Sl. No.	Clone no.	Mean electrolyte leakage (µs)		Clone no.	Mean electrolyte leakage (µs)	
		P. aphani-dermatum	*R.solana-cearum*		*P. aphani-dermatum*	*R.solana-cearum*
40	640 MC	26.45	40.40	608 RC	60.65	48.25
41	47 MC 10 Gy	24.95	39.00	678 RC	40.80	58.95
42	48 MC 10 Gy	33.70	34.80	692 RC	25.65	37.75
43	49 MC 10 Gy	15.45	43.10	763 RC	46.60	40.65
44	51 MC 10 Gy	25.45	30.00	764 RC	37.35	37.85
45	52 MC 10 Gy	20.85	38.55	53 RC 10 Gy	19.70	40.55
46	95 MC 10 Gy	24.05	45.00	140 RC 10 Gy	31.40	37.25
47	97 MC 10 Gy	35.15	54.95	141 RC 10 Gy	23.35	40.95
48	99 MC 10 Gy	19.70	41.75	145 RC 10 Gy	27.90	45.35
49	100 MC 10 Gy	26.50	45.05	148 RC 10 Gy	16.75	38.95
50	101 MC 10 Gy	27.25	29.10	149 RC 10 Gy	37.40	38.95
51	102 MC 10 Gy	40.30	32.30	151 RC 10 Gy	47.15	38.30
52	103 MC 10 Gy	45.90	30.65	152 RC 10 Gy	20.70	43.40
53	105 MC 10 Gy	21.00	42.65	188 RC 10 Gy	22.15	27.35
54	106 MC 10 Gy	26.00	29.45	189 RC 10 Gy	45.30	57.75
55	108 MC 10 Gy	41.70	30.80	236 RC 10 Gy	28.60	44.60
56	109 MC 10 Gy	30.25	51.35	237 RC 10 Gy	50.50	56.20
57	110 MC 10 Gy	50.00	47.95	238 RC 10 Gy	38.65	40.70
58	156 MC 10 Gy	38.40	38.05	239 RC 10 Gy	41.35	37.70
59	158 MC 10 Gy	29.55	40.75	243 RC 10 Gy	45.40	58.60
60	159 MC 10 Gy	36.95	47.90	245 RC 10 Gy	57.80	45.85
61	163 MC 10 Gy	30.90	32.90	246 RC 10 Gy	29.25	36.25
62	165 MC 10 Gy	36.10	38.00	249 RC 10 Gy	45.40	34.55
63	168 MC 10 Gy	32.70	38.50	271 RC 10 Gy	21.85	37.50
64	169 MC 10 Gy	48.50	43.30	283 RC 10 Gy	44.35	38.95
65	174 MC 10 Gy	55.25	47.75	316 RC 10 Gy	48.40	37.95
66	178 MC 10 Gy	38.35	39.25	349 RC 10 Gy	21.05	46.90
67	182 MC 10 Gy	26.25	40.30	373 RC 10 Gy	25.90	41.50
68	190 MC 10 Gy	19.40	36.40	375 RC 10 Gy	29.90	35.15
69	191 MC 10 Gy	49.65	41.65	376 RC 10 Gy	47.15	58.75
70	192 MC 10 Gy	35.20	38.55	377 RC 10 Gy	30.75	37.80
71	193 MC 10 Gy	43.85	31.00	611 RC 10 Gy	21.35	55.65
72	194 MC 10 Gy	41.40	40.30	612 RC 10 Gy	33.15	26.95
73	196 MC 10 Gy	53.75	44.35	614 RC 10 Gy	43.65	37.70
74	197 MC 10 Gy	52.45	39.85	615 RC 10 Gy	35.30	34.90
75	253 MC 10 Gy	46.95	44.90	660 RC 10 Gy	58.45	37.20
76	254 MC 10 Gy	52.90	41.30	661 RC 10 Gy	35.45	37.90
77	255 MC 10 Gy	15.95	35.20	665 RC10 Gy	45.25	45.15
78	257 MC 10 Gy	55.10	38.60	668 RC 10 Gy	22.10	30.25
79	260 MC 10 Gy	30.35	38.35	666 RC 10 Gy	31.75	39.60
80	265 MC 10 Gy	15.95	42.10	1 RSE	30.60	30.75
81	5 MSE	21.25	31.15	3 RSE	14.85	30.30

Contd.

Maran				Rio-de-Janeiro		
Sl. No.	Clone no.	Mean electrolyte leakage (µs)		Clone no.	Mean electrolyte leakage (µs)	
		P. aphani-dermatum	*R.solana-cearum*		*P. aphani-dermatum*	*R.solana-cearum*
82	6 MSE	25.05	29.70	4 RSE	15.45	30.45
83	8 MSE	21.10	35.95	88 RSE	26.90	31.45
84	9 MSE	32.25	37.90	90 RSE	24.40	26.85
85	11 MSE	20.7	34.05	310 RSE	24.05	29.90
86	12 MSE	26.85	40.05	311 RSE	22.50	34.80
87	13 MSE	14.95	40.60	477 RSE	38.45	37.00
88	14 MSE	18.65	42.95	481 RSE	29.50	38.10
89	16 MSE	24.85	38.15	482 RSE	33.75	38.50
90	19 M SE	31.05	37.70	484 RSE	56.85	34.90
91	20 MSE	26.30	31.20	485 RSE	32.55	41.15
92	21 MSE	18.35	35.10	489 RSE	20.45	37.70
93	23 MSE	17.95	36.70	491 RSE	29.05	34.05
94	24 MSE	25.45	37.85	492 RSE	57.35	35.20
95	25 MSE	22.50	26.45	495 RSE	53.80	45.50
96	26 MSE	21.90	41.00	498 RSE	16.85	29.90
97	27 MSE	14.60	36.35	500 RSE	44.85	38.20
98	28 MSE	17.30	28.05	514 RSE	47.05	29.00
99	44 MSE	49.75	40.80	515 RSE	28.65	38.30
100	75 MSE	36.80	32.35	516 RSE	47.15	40.80
101	76 MSE	39.30	51.10	517 RSE	48.40	33.80
102	78 MSE	37.90	48.85	520 RSE	22.40	54.05
104	122 MSE	29.80	29.65	525 RSE	21.55	38.05
105	123 MSE	30.30	42.05	526 RSE	36.85	39.55
106	124 MSE	20.90	37.80	527 RSE	19.55	39.15
107	125 MSE	19.65	30.15	529 RSE	33.05	37.65
103	126 MSE	19.25	30.20	543 RSE	29.95	42.40
108	546 MSE	30.30	29.00	587 RSE	35.50	37.90
109	643 MSE	44.55	38.70	593 RSE	58.40	34.70
110	644 MSE	31.95	50.25	594 RSE	25.05	40.00
111	656 MSE	26.40	34.65	625 RSE	52.90	40.65
112	657 MSE	23.35	40.65	626 RSE	34.70	47.85
113	722 MSE	35.25	46.85	628 RSE	64.05	30.00
114	761 MSE	51.90	41.00	648 RSE	33.95	29.50
115	54 MSE 20 Gy	21.70	38.35	726 RSE	23.95	37.80
116	55 MSE 20 Gy	14.10	42.85	727 RSE	57.75	33.85
117	56 MSE 20 Gy	30.30	34.35	771 RSE	48.00	38.25
118	57 MSE 20 Gy	18.55	37.60	209 RSE 10 Gy	30.35	38.25
119	58 MSE 20 Gy	18.05	29.80	210 RSE 10 Gy	30.65	35.40
120	59 MSE 20 Gy	27.70	31.05	211 RSE 10 Gy	26.55	33.85
121	60 MSE 20 Gy	28.30	40.75	269 RSE 10 Gy	30.70	38.25
122	61 MSE 20 Gy	32.70	48.90	274 RSE 10 Gy	30.30	36.45
123	164 MSE 20 Gy	19.45	36.65	275 RSE 10 Gy	26.70	39.05

Contd.

Maran				Rio-de-Janeiro		
Sl. No.	Clone no.	Mean electrolyte leakage (µs)		Clone no.	Mean electrolyte leakage (µs)	
		P. aphani-dermatum	*R.solana-cearum*		*P. aphani-dermatum*	*R.solana-cearum*
124	333 MSE 20 Gy	25.00	43.15	384 RSE 10 Gy	37.30	39.05
125	334 MSE 20 Gy	28.45	39.35	389 RSE 10 Gy	43.40	37.75
126	336 MSE 20 Gy	24.45	46.00	395 RSE 10 Gy	31.15	35.05
127	337 MSE 20 Gy	30.35	39.55	397 RSE 10 Gy	18.35	41.20
128	338 MSE 20 Gy	26.20	38.95	401 RSE 10Gy	47.95	30.20
129	570 MSE 20 GY	36.25	50.20	402 RSE 10Gy	18.95	36.05
130	783 MSE 20 Gy	36.25	47.75	418 RSE 10 Gy	45.05	37.20
131	784 MSE 20 Gy	30.95	46.80	419 RSE 10GY	22.10	41.45
132	785 MSE 20 Gy	22.55	49.05	420 RSE 10 Gy	38.00	36.45
133	786 MSE 20 Gy	27.85	46.85	421 RSE 10Gy	63.65	32.55
134	787 MSE 20 Gy	25.10	50.45	422 RSE 10 Gy	30.80	40.45
135	790 MSE 20 Gy	33.85	55.85	423 RSE 10 Gy	24.35	38.10
136	833 MSE 20 Gy	23.05	37.90	535 RSE 10 Gy	16.85	38.00
137	835 MSE 20 Gy	18.70	43.05	536 RSE10 Gy	39.10	36.75
138	894 MSE 20 Gy	26.35	39.25	540 RSE10 Gy	23.55	37.15
139	895 MSE 20 Gy	24.70	46.95	629 RSE10 Gy	33.10	41.00
140	896 MSE 20 Gy	34.30	50.30	650 RSE 10 Gy	48.80	56.20
141	897 MSE 20 Gy	21.50	48.10	651 RSE 10 Gy	34.45	52.05
142	899 MSE 20 Gy	24.60	43.90	652 RSE 10 Gy	22.35	37.85
143	903 MSE 20 Gy	17.35	42.20	730 RSE 10 Gy	34.05	38.60
144	906 MSE 20 Gy	23.75	36.40	731 RSE 10 Gy	46.30	45.75
145	910 MSE 20 Gy	34.60	54.55	732 RSE 10 Gy	49.45	41.15
146	911 MSE 20 Gy	30.95	47.75	733 RSE 10 Gy	29.45	40.60
148				738 RSE10 GY	42.30	51.30
149				739 RSE 10 Gy	28.70	47.75
150				740 RSE 10 Gy	37.00	46.00
151				741 RSE 10 Gy	46.55	44.70
	S.D.	10.205	6.487		11.695	7.364
	C.V. (%)	32.753	16.214		33.679	18.335

MC - Plantlets - indirect organogenesis cv. Maran
MC 10 Gy - Plantlets - indirect organogenesis cv. Maran - irradiated at 10 Gy
Mse - Plantlets - indirect embryogenesis cv. Maran
Mse 20 Gy - Plantlets - indirect embryogenesis cv. Maran irradiated at 20 Gy
RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro
RC 10 Gy - Plantlets - indirect organogenesis cv. Rio-de-Janeiro - irradiated at 10 Gy
Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro
Rse 10 Gy - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro irradiated at 10 Gy

Frequency distribution of electrolyte leakage induced by toxic metabolite(s) of *P.aphanidermatum* and *R. solanacearum* in second set somaclones in ginger (*Z. officinale* Rosc.)

Table 24a. Electrolyte leakage induced by toxic metabolite(s) of *P.aphanidermatum*

Group No.	Electrolyte leakage (µs)	Frequency (%)	
		Maran	Rio-de-Janeiro
1	10.50 - 17.49	6.80	3.31
2	17.50 - 24.49	21.09	19.87
3	24.50 - 31.49	27.21	26.49
4	31.50 - 38.49	24.49	17.22
5	38.50 - 45.49	8.84	11.92
6	45.50 - 52.49	8.84	11.92
7	52.50 - 59.49	2.72	7.28
8	59.50 - 66.49	-	1.99

Table 24b. Electrolyte leakage induced by toxic metabolite(s) of *R. solanacearum*

Group No.	Electrolyte leakage (µs)	Frequency (%)	
		Maran	Rio-de-Janeiro
1	25.20 - 30.10	8.16	7.28
2	30.20 - 35.10	12.93	13.25
3	35.20 - 40.10	31.29	39.07
4	40.20 - 45.10	26.53	19.87
5	45.20 - 50.10	13.61	8.61
6	50.20 - 55.10	6.12	4.64
7	55.20 - 60.10	1.36	6.62
8	60.20 - 65.10	-	0.66

4.3.3.1 *Preliminary Screening of Second Set Somaclones Against Rhizome Rot Disease*

Electrolyte leakage induced by CCF of *P. aphanidermatum* ranged from 14.10 to 64.05 µs in the various regenerants screened. The regenerants of cultivar Rio-de-Janeiro exhibited more leakage of electrolytes (14.85-64.05 µs) than regenerants of cultivar Maran (14.10-55.25 µs).

The regenerants were grouped into three groups based on their leakage values. Regenerants, which came in the first two classes of frequency table (< 24.50 µs) were designated as regenerants of low leakage group, next four classes (24.50 – 52.49 µs) as of medium leakage group and last two classes (> 52.50 µs) as of high leakage group. Of the regenerants screened, 26 per cent came in the low leakage group, 68 per cent in the medium leakage group and six per cent in the high leakage group.

Regenerants of two cultivars when compared, somaclones of cultivar Maran exhibited low leakage of electrolytes than clones of cultivar Rio-de-Janeiro. Leakage of electrolytes was low in 28 per cent regenerants of Maran as compared to 23 per cent regenerants of Rio-de-Janeiro. Hence, the regenerants of Maran exhibited more tolerance to rhizome rot. Majority of the regenerants of both cultivars (68-69%) exhibited leakage of electrolytes in the medium range. More number of regenerants of Rio-de-Janeiro (9%) exhibited high leakage of electrolytes as compared to regenerants of Maran (3%).

Variability for the character studied was almost uniform in the two cultivars recording a coefficient of variation of 32.753 per cent in clones of Maran and 33.679 per cent in clones of Rio-de-Janeiro (Fig.13).

4.3.3.2 *Preliminary Screening of Second Set Somaclones Against Bacterial Wilt Disease*

Leakage of electrolytes induced by toxin of *R. solanacearum* varied between 26.45-63.90 µs in the regenerants of two cultivars studied. The regenerants of Rio-de-Janeiro exhibited more leakage (26.80-63.90 µs) than the regenerants of

cultivar Maran (26.45-57.15 μs). Regenerants, which came in the first two classes of frequency table (< 35.20 μs) were considered as regenerants of low leakage group, next four classes (35.20 - 55.10 μs) as of medium leakage group and last two classes (> 55.10 μs) as of high leakage group. Of the regenerants screened, 21 per cent came in the low leakage group, 75 per cent in the medium leakage group and four per cent in the high leakage group.

In regenerants of two cultivars screened, 21 per cent each of the regenerants of Maran and Rio-de-Janeiro came in the low electrolyte leakage group indicating more tolerance of the regenerants to bacterial wilt disease. Electrolyte leakage was in the medium range in 77 per cent regenerants of cultivar Maran and 72 per cent regenerants of cultivar Rio-de-Janeiro. Only one per cent regenerants of cultivar Maran came in the high leakage group while seven per cent regenerants of cultivar Rio-de-Janeiro came in the group, indicating the high susceptibility of regenerants of cultivar Rio-de-Janeiro to bacterial wilt.

The coefficient of variation was almost uniform for the character in the two cultivars studied. The coefficient of variation was 16.241 per cent in regenerants of Maran and 18.335 per cent in regenerants of Rio-de-Janeiro.

4.3.3.3 Comparison of Electrolyte Leakage in Regenerants Produced Through Various Routes

Electrolyte leakage values induced in regenerants of ginger produced through various routes were compared and the data are presented in Table 24c. The frequency distribution of leakage values in different groups of regenerants is presented in Tables 24d and 24e. Plantlets regenerated through indirect organogenesis / embryogenesis and *in vitro* mutagenesis of two cultivars showed significant variations in electrolyte leakage values induced by toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum*.

When electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* was compared, regenerants derived through indirect embryogenesis exhibited low leakage of electrolytes (31.30 μs) and were more

Table 24c. Electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum* in ginger somaclones regenerated through various routes

Group	No. of plants screened	Mean electrolyte leakage (µs)	
		P.aphanidermatum	*R. solanacearum*
MC	40	34.72	40.15
RC	44	34.43	42.54
Mse	34	27.31	37.20
Rse	38	35.29	36.52
MC 10 Gy	40	34.85	39.64
RC 10 Gy	35	34.99	41.23
Mse 20 Gy	32	26.19	43.27
Rse 10 Gy	33	34.19	40.04
CD (0.05)		4.930	3.033

MC - Plantlets - indirect organogenesis cv. Maran
RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro
Mse - Plantlets - indirect embryogenesis cv. Maran
Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro
MC 10Gy - Plantlets - indirect organogenesis cv. Maran - irradiated at 10 Gy
RC 10 Gy - Plantlets - indirect organogenesis cv. Rio-de-Janeiro - irradiated at 10 Gy
Mse 20Gy - Plantlets - indirect embryogenesis cv. Maran irradiated at 20 Gy
Rse 10Gy - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro irradiated at 10 Gy

Frequency distribution of elecrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum* in second set somaclones in ginger regenerated through various routes

Table 24d. Electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum*

Group No.	Electrolyte leakage (µs)	Frequency (%)							
		MC	RC	Mse	Rse	MC 10 Gy	RC 10 Gy	Mse 20 Gy	Rse 10 Gy
1	10.50 - 17.49	5.00	-	8.82	7.89	7.50	2.86	6.25	3.03
2	17.50 - 24.49	10.00	18.18	35.29	21.05	17.50	22.86	31.25	18.18
3	24.50 - 31.49	15.00	36.36	29.41	18.42	20.00	20.00	43.75	30.30
4	31.50 - 38.49	40.00	13.64	14.71	21.05	20.00	14.29	18.75	18.18
5	38.50 - 45.49	17.50	11.36	5.88	2.63	10.00	22.86	-	12.12
6	45.50 - 52.49	12.50	11.36	5.88	10.53	15.00	11.43	-	15.15
7	52.50 - 59.49	-	6.82	-	15.79	10.00	5.71	-	3.03
8	59.50 - 66.49	-	2.27	-	2.63	-	-	-	-

Table 24e. Electrolyte leakage induced by toxic metabolite(s) of *R. solanacearum*

Group No.	Electrolyte leakage (µs)	Frequency (%)							
		MC	RC	Mse	Rse	MC 10 Gy	RC 10 Gy	Mse 20 Gy	Rse 10 Gy
1	25.20 - 30.10	7.50	6.82	14.71	15.79	7.50	5.71	3.13	3.03
2	30.20 - 35.10	7.50	6.82	23.53	26.32	15.00	8.57	6.25	9.09
3	35.20 - 40.10	37.50	29.55	29.41	39.47	30.00	42.86	28.13	48.48
4	40.20 - 45.10	27.50	27.27	20.59	10.53	35.00	17.14	18.75	21.21
5	45.20 - 50.10	15.00	9.09	5.88	5.26	7.50	11.43	28.13	9.09
6	50.20 - 55.10	2.50	9.09	5.88	2.63	5.00	-	12.50	6.06
7	55.20 - 60.10	2.50	9.09	-	-	-	14.29	3.13	3.03
8	60.20 - 65.10	-	2.27	-	-	-	-	-	-

MC - Plantlets - indirect organogenesis cv. Maran
RC - Plantlets - indirect organogenesis cv. Rio-de-Janeiro
Mse - Plantlets - indirect embryogenesis cv. Maran
Rse - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro
MC 10 Gy - Plantlets - indirect organogenesis cv. Maran - irradiated at 10 Gy
RC 10 Gy - Plantlets - indirect organogenesis cv. Rio-de-Janeiro - irradiated at 10 Gy
Mse 20 Gy - Plantlets - indirect embryogenesis cv. Maran irradiated at 20 Gy
Rse 10 Gy - Plantlets - indirect embryogenesis cv. Rio-de-Janeiro irradiated at 10 Gy

tolerant to the disease (Fig. 14a). In regenerants from irradiated calli, the mean leakage of electrolytes was 32.56 µs and in plantlets regenerated through indirect organogenesis, the mean leakage of electrolytes observed was 34.58 µs.

The cultivar response within each group of regenerants was also analysed. In all the three groups, the regenerants of cultivar Maran exhibited less leakage of electrolytes to toxic metabolite(s) of *P. aphanidermatum* as compared to regenerants of culivar Rio-de-Janeiro. The regenerants of Maran derived through irradiation (20 Gy) of embryogenic calli exhibited low leakage of electrolytes indicating more tolerance of the regenerants to *P. aphanidermatum* as compared to other groups of regenerants.

When electrolyte leakage induced by toxic metabolite(s) of *R. solanacearum* was compared, regenerants derived through somatic embryogenesis exhibited low leakage of electrolytes (36.86 µs) and were more tolerant to the bacterial wilt disease (Fig. 14b). In regenerants produced through indirect organogenesis and *in vitro* mutagenesis, the mean leakage of electrolytes was 41 µs.

4.3.4 Screening of First Set Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

First set of somaclones of Maran and Rio-de-janeiro derived through *in* vitro adventitious bud culture were screened against rhizome rot and bacterial wilt diseases. Detailed screening studies were conducted in the somaclones and three methods of screening (natural screening in sick field, screening by electrolyte leakage method and artificial screening of selected somaclones using the pathogens) were attempted.

4.3.4.1 Natural Screening of Somaclones Against Rhizome Rot and Bacterial Wilt Diseases.

Seventy three somaclones of Maran and 78 somaclones of Rio-de-Janeiro were screened in a sick field where there was serious incidence of rhizome rot and bacterial wilt diseases in the previous year.

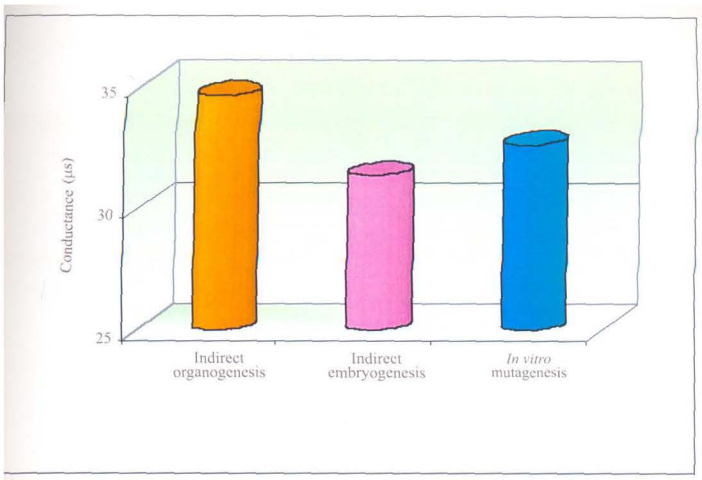


Fig. 14a Electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* in second set somaclones of ginger

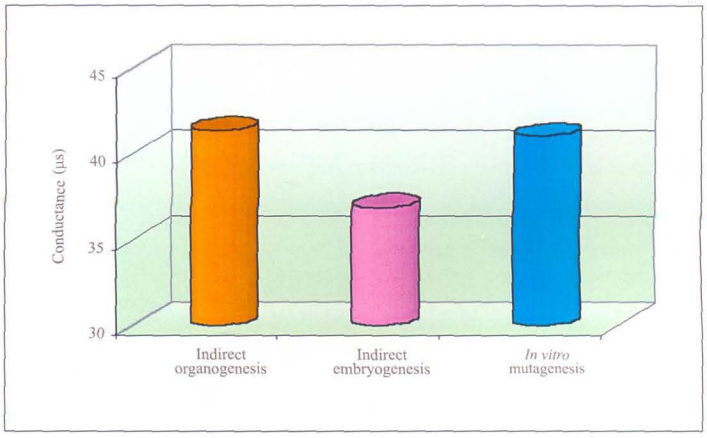


Fig. 14b Electrolyte leakage induced by toxic metabolite(s) of *R. solanacearum* in second set somaclones of ginger

Rhizome rot incidence was noticed in 86 per cent clones of cultivar Maran and 88 per cent clones of cultivar Rio-de-Janeiro (Table 25). Pre emergence rotting was observed in 27.39 per cent clones of cultivar Maran and 25.64 per cent clones of cultivar Rio-de-Janeiro. Post emergence rotting was observed in 58.91 per cent clones of cultivar Maran and 62.82 per cent clones of cultivar Rio-de-Janeiro. Rhizome rot incidence was more in clones of Rio-de-Janeiro than in clones of Maran. Conventionally propagated plants of both cultivars were seriously affected by rhizome rot while the incidence was less in somaclones.

Incidence of bacterial wilt was first noticed five months after planting. Wilt incidence was observed in 29 per cent clones of Maran and 32 per cent clones of Rio-de-Janeiro. Disease incidence was more in clones of Rio-de-Janeiro than in clones of Maran.

Fourteen per cent somaclones of cultivar Maran and 12 per cent somaclones of cultivar Rio-de-Janeiro were not affected by rhizome rot and bacterial wilt diseases in the sick field (Plate 15). Of the nineteen somaclones, which survived in sick field, ten clones were of cultivar Maran and nine were of cultivar Rio-de-Janeiro. The somaclones of cultivar Maran which were not affected by diseases and survived in sick field were 91 M, 99 M, 100 M, 342 M, 393 M, 432 M, 488 M, 668 M, 970 M and M VI. The clones of cultivar Rio-de-Janeiro, which survived in screening field were 281 R, 292 R, 336 R, 364 R, 386 R, 485 R, R I, R V and R XI.

4.3.4.2 Screening by Electrolyte Leakage Method

Electrolyte leakage was induced from leaves of 77 somaclones of cultivar Maran and 75 somaclones of cultivar Rio-de-Janeiro using toxic metabolite(s) of *P.aphanidermatum* and *R. solanacearum* and data are presented in Table 26.

4.3.4.2.1 Sceening Somaclones Against Rhizome Rot Disease

Leakage of electrolytes induced by CCF of *P. aphanidermatum* ranged from 14.75 to 59.85 µs in clones of Maran and from 16.10 to 58.35 µs in clones of Rio-de-Janeiro (Fig. 15a). Somaclones that came in the first two classes of frequency

Table 25. Natural screening of first set somaclones in ginger (*Z. officinale* Rosc.) against rhizome rot and bacterial wilt diseases

Cultivar	Total no. of somaclones	No. of somaclones affected by		% somaclones affected by	
		Rhizome rot	Bacterial wilt	Rhizome rot	Bacterial wilt
Maran	73	63	21	86.30	28.77
Rio-de-Janeiro	78	69	25	88.46	32.05

Table 26. Screening first set somaclones in ginger (*Z. officinale* Rosc.) against rhizome rot and bacterial wilt diseases by electrolyte leakage method

Maran				Rio-de-Janeiro		
Sl. No.	Clone no.	Mean electrolyte leakage (µs)		Clone no.	Mean electrolyte leakage (µs)	
		P. aphani-dermatum	*R.solana-cearum*		*P. aphani-dermatum*	*R.solana-cearum*
1	56 M	22.10	45.30	88 R	19.95	43.75
2	79 M	24.20	41.30	137 R	18.25	50.25
3	84M	45.80	51.20	281 R	31.75	47.30
4	85 M	36.50	42.90	292 R	18.45	49.90
5	91 M	41.45	43.40	296 R	24.85	47.75
6	99 M	37.85	37.45	308 R	20.10	48.95
7	100 M	22.30	45.75	311 R	17.55	37.85
8	110 M	29.55	47.00	312 R	44.55	47.40
9	132 M	24.55	43.95	314 R	49.10	50.15
10	136 M	22.50	45.25	315 R	29.05	50.20
11	139 M	18.70	41.50	335 R	32.55	42.05
12	150 M	24.75	42.85	336 R	18.60	47.50
13	197 M	37.90	49.40	337 R	43.85	48.65
14	199 M	52.45	52.20	338 R	54.85	48.50
15	220 M	28.40	43.50	345 R	16.10	46.15
16	276 M	42.60	49.95	346 R	21.70	50.50
17	283 M	23.85	41.50	349 R	29.65	41.00
18	284 M	41.35	44.40	350 R	45.10	44.70
19	287 M	38.55	45.40	351 R	55.85	45.40
20	288 M	26.25	50.40	355 R	35.80	48.35
21	290 M	44.85	51.90	358 R	34.25	46.60
22	311 M	30.05	45.15	361 R	47.75	49.35
23	313 M	43.40	41.35	364 R	22.30	39.70
24	317 M	39.95	54.70	367 R	24.65	49.25
25	342 M	34.95	45.40	368 R	49.45	49.15
26	348 M	31.00	51.15	373 R	34.05	42.55
27	356 M	32.55	53.40	377 R	37.45	52.35
28	372 M	29.40	50.95	384 R	18.55	45.50
29	374 M	45.70	53.00	386 R	28.75	46.50
30	381 M	19.85	45.00	395 R	50.20	52.00
31	382 M	29.75	47.55	399 R	43.15	53.10
32	392 M	30.90	44.65	413 R	21.95	49.85
33	393 M	24.75	46.30	418 R	27.65	46.75
34	397 M	37.20	40.90	431 R	47.40	51.45
35	400 M	27.95	45.00	438 R	42.00	46.50
36	411M	30.60	48.60	463R	19.00	49.90
37	431 M	17.90	51.45	466 R	23.40	49.45
38	432 M	29.65	41.15	471 R	44.05	54.10
39	434 M	26.25	44.45	475 R	27.35	46.50

Contd.

Maran				Rio-de-Janeiro		
Sl. No.	Clone no.	Mean electrolyte leakage (µs)		Clone no.	Mean electrolyte leakage (µs)	
		P. aphani-dermatum	*R.solana-cearum*		*P. aphani-dermatum*	*R.solana-cearum*
40	435 M	27.30	46.45	476 R	42.15	52.35
41	436 M	33.00	38.40	478 R	44.15	53.80
42	441 M	19.65	44.45	482 R	18.90	43.75
43	444 M	45.45	52.25	485 R	18.00	51.00
44	446 M	30.05	42.20	517 R	33.95	42.75
45	462 M	29.40	43.15	524 R	28.90	47.65
46	464 M	40.80	54.95	526 R	42.05	48.45
47	488 M	19.10	45.65	535 R	45.00	54.10
48	500 M	40.75	42.35	548 R	45.45	52.40
49	505 M	59.85	45.15	561 R	26.45	43.75
50	510 M	29.55	42.65	582 R	16.55	49.20
51	513 M	20.05	47.90	590 R	44.10	52.10
52	528 M	29.25	48.45	597 R	16.90	43.75
53	529 M	19.95	45.85	610 R	44.35	53.80
54	549 M	14.75	42.85	616 R	36.65	41.40
55	554 M	34.60	40.25	617 R	38.55	49.70
56	565 M	18.50	41.20	626 R	43.70	44.80
57	572 M	42.75	46.75	630 R	58.35	42.25
58	580 M	34.40	43.95	704 R	32.80	49.55
59	583 M	38.15	42.15	734 R	25.25	52.15
60	588 M	38.50	44.70	748 R	45.00	52.95
61	659 M	35.70	42.80	772 R	45.35	52.15
62	660 M	16.85	41.70	786 R	23.65	47.00
63	668 M	15.15	48.85	790 R	48.05	52.25
64	743 M	21.90	43.10	801 R	46.35	52.95
65	746 M	41.30	43.40	R I	19.75	46.40
66	918 M	44.45	40.25	R II	45.45	52.00
67	953 M	26.65	46.00	R III	32.90	51.30
68	970 M	18.80	42.50	R IV	28.90	46.60
69	980 M	36.90	48.95	R V	22.15	50.65
70	985 M	37.10	40.40	R VI	42.50	44.45
71	M I	18.55	46.45	R VII	44.05	53.95
72	M III	40.50	49.25	R VIII	23.05	46.00
73	M IV	29.75	49.85	R IX	32.75	48.15
74	M V	40.50	52.90	R XI	21.75	41.65
75	M VI	22.75	42.55	R XII	26.90	52.25
76	M VII	41.05	53.95	Control R	35.00	43.55
77	M VIII	17.60	43.75			
78	Control M	37.95	41.90			
	S.D.	9.623	4.106		11.55	3.841
	C.V. (%)	30.814	9.084		34.333	7.976

Frequency distribution of electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* and *R.solanacearum* in first set somaclones in ginger (*Z. officinale* Rosc.)

Table 26a. Electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum*

Group No.	Mean electrolyte leakage (µs)	Frequency (%)	
		Maran	Rio-de-Janeiro
1	13.00 - 18.99	11.68	13.33
2	19.00 - 24.99	19.48	18.67
3	25.00 - 30.99	22.08	13.33
4	31.00 - 36.99	12.99	13.33
5	37.00 - 42.99	23.38	8.00
6	43.00 - 48.99	7.79	25.33
7	49.00 - 54.99	1.30	5.33
8	55.00 - 60.99	1.30	2.67

Table 26b. Electrolyte leakage induced by toxic metabolite(s) of *R.solanacearum*

Group No.	Mean electrolyte leakage (µs)	Frequency (%)	
		Maran	Rio-de-Janeiro
1	37.00 - 39.99	2.60	2.67
2	40.00 - 42.99	29.87	9.33
3	43.00 - 45.99	29.87	12.00
4	46.00 - 48.99	14.29	28.00
5	49.00 - 51.99	14.29	24.00
6	52.00 - 54.99	9.09	24.00

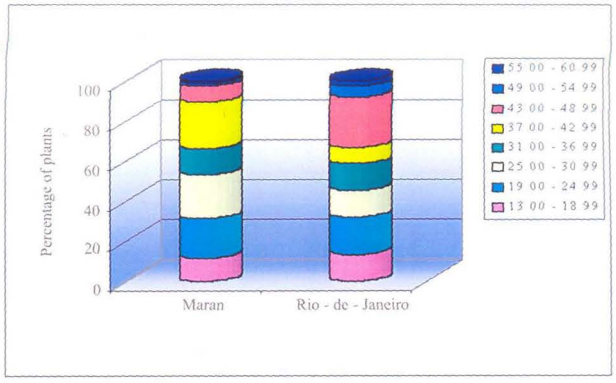


Fig. 15a Variability in electrolyte leakage (µs) induced by toxic metabolite(s) of *P. aphanidermatum* in first set somaclones of ginger

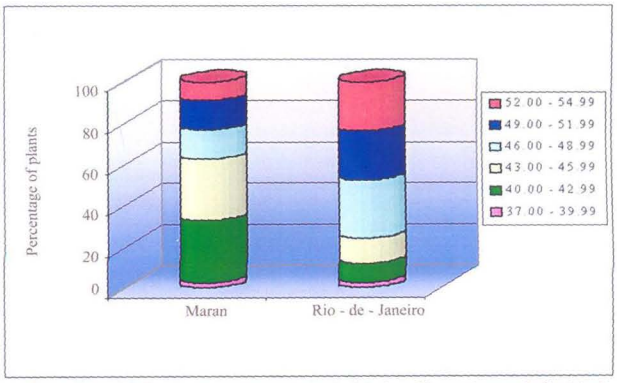


Fig.15b Variability in electrolyte leakage (µs) induced by toxic metabolite(s) of *R. solanacearum* in first set somaclones of ginger

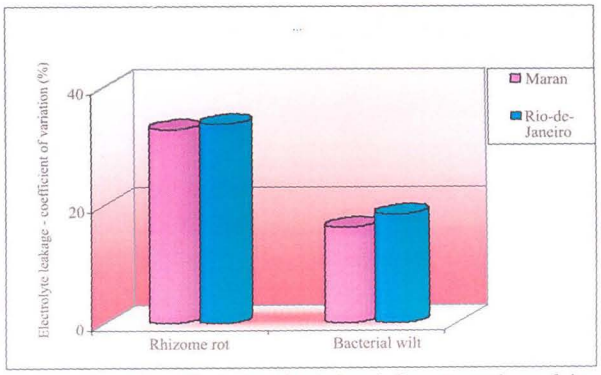


Fig. 16 Variability in electrolyte leakage in first set somaclones of ginger

table, exhibiting leakage less than 25 µs were designated as clones of low leakage group, next four classes as of medium leakage group (25.00 – 48.99 µs) and last two classes (> 49 µs) as of high leakage group. Of the clones screened, 32 per cent somaclones came in the low leakage group, 63 per cent in the medium leakage group and five per cent in the high leakage group.

The leakage of electrolytes was comparatively low in somaclones of Maran indicating tolerance of the clones to rhizome rot disease. The leakage of electrolytes was low in 31 per cent clones of Maran (14.75-24.75 µs) and 32 per cent clones of Rio-de-Janeiro (16.10-24.85 µs). Majority of the clones of Maran (66 %) and Rio-de-Janeiro (60 %) exhibited electrolyte leakage in the medium range. Only three per cent clones of Maran exhibited high leakage values as compared to eight per cent clones of Rio-de-Janeiro..

In general, leakage of electrolytes was found low in somaclones as compared to CP plants. Sixty per cent somaclones recorded lower leakage of electrolytes than CP plants. Clones of Maran exhibited superiority over CP plants in resistance/tolerance to rhizome rot. Seventy one per cent clones of Maran exhibited lower leakage values than CP plants compared to 56 per cent clones in Rio-de-Janeiro.

Variability for disease reaction was more in clones of cultivar Rio-de-Janeiro than clones of cultivar Maran. Coefficient of variation recorded for the character was 34.333 per cent in clones of Rio-de-Janeiro as compared to 30.814 per cent in clones of Maran.

4.3.4.2.2 Screening Somaclones Against Bacterial Wilt Disease

Electrolyte leakage induced by toxin of *R. solanacearum* ranged between 37.45-54.95 µs in clones of Maran and 37.85-54.10 µs in clones of Rio-de-Janeiro. Somaclones that came in the first two classes of frequency table exhibiting leakage less than 43 µs were considered as clones of low leakage group, next two classes as of medium leakage group (43.00 – 48.99 µs) and last two classes (> 49 µs) as of high leakage group. Of the clones evaluated, 21 per cent somaclones recorded low leakage

of electrolytes, 43 per cent exhibited medium leakage and 36 per cent high leakage of electrolytes.

In general, somaclones of cultivar Maran exhibited low leakage values indicating more tolerance of the clones to bacterial wilt disease (Fig. 15b). Thirty two per cent clones of Maran recorded low leakage values as compared to 12 per cent clones of Rio-de-Janeiro. Majority of the clones of Maran exhibited leakage of electrolytes in the medium range while majority of the clones of Rio-de-Janeiro recorded leakage in the high range. Forty eight per cent clones of Rio-de-Janeiro recorded high electrolyte leakage as compared to 23 per cent clones of Maran.

When somaclones and CP plants were compared for electrolyte leakage, 14 per cent somaclones recorded lower leakage values than CP plants. In general, clones of Maran exhibited low leakage values. Seventeen per cent clones of Maran recorded low leakage values than CP plants as compared to 12 per cent clones in cultivar Rio-de-Janeiro.

Variability in electrolyte leakage induced by toxin of *R. solanacearum* was almost uniform in the two cultivars studied (Fig.16).

4.3.4.3 Comparative Evaluation of Natural Screening and Screening by Electrolyte Leakage Method for Assessing the Disease Reaction of Somaclones

Two different screening methods viz. natural screening by planting in sick field and screening by electrolyte leakage method were compared for assessing the disease reaction of the somaclones in the two screening methods.

Fourteen per cent somaclones of cultivar Maran and 12 per cent somaclones of cultivar Rio-de-Janeiro were not affected by rhizome rot and bacterial wilt diseases in the sick field. When somaclones which survived in sick field were screened by electrolyte leakage method for rhizome rot incidence, the clones 100 M, 393 M, 488 M, 668 M, 970 M, M VI, 292 R, 336 R, 364 R, 485 R, R I, R V and R XI came in the low leakage group and the clones 91 M, 99 M, 342 M, 432 M, 281 R and 386 R came in the medium leakage group indicating the tolerance of these clones to

rhizome rot disease. So the clones, which survived in sick field, exhibited the same type of disease reaction in electrolyte leakage studies also.

The clones which survived in sick field when screened for bacterial wilt disease by electrolyte leakage method, the clones 99 M, 432 M, 970 M, M VI, 364 R and R XI exhibited low leakage, 91 M, 100 M, 342 M, 393 M, 488 M, 668 M, 336 R, 386 R and R I exhibited medium leakage and the clones 281 R, 292 R, 485 R and R V exhibited high leakage. So 79 per cent of the clones that survived from infection of bacterial wilt disease in the screening field exhibited the same type of disease reaction in electrolyte leakage studies also.

Comparative evaluation of natural screening and screening by electrolyte leakage method indicated that electrolyte leakage was in the low and medium range in 89 per cent of the somaclones which survived in the sick field.

4.3.4.4 Artificial Screening of Selected Superior Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

Five somaclones each of Maran and Rio-de-Janeiro selected based on yield and tolerance/resistance to rhizome rot and bacterial wilt were subjected to artificial screening for confirmation of the disease tolerance/resistance reaction.

4.3.4.4.1 Artificial Screening Against Rhizome Rot Disease

Artificial screening against rhizome rot disease was done using soil infected with *P. aphanidermatum.* One month old ginger sprouts were planted in potting mixture prepared with sand, cow dung and soil heavily infected with rhizome rot pathogen in the proportion 1:1:1. The symptoms of rhizome rot disease appeared in somaclones nine to 41 days after planting (Table 27). The clone 342 M and the CP plants of Maran and Rio-de-Janeiro (control) were the first to take infection. In two clones viz. 364 R and M VI, there was considerable delay for the appearance of symptoms and it took 34 to 41 days for the appearance of symptoms (Plates 16a - f). Rotting symptoms were noticed in all the somaclones inoculated but the intensity and time taken for appearance of symptoms varied in the different somaclones. In clones (342 M and the CP plants of two cultivars), which took infection first, rotting and

Table 27. Incidence of rhizome rot in selected somaclones of ginger (*Z. officinale* Rosc.) in artificial screening

Clone No.	Days taken for appearance of symptoms	Rhizome rot incidence scores						
		1 WAI	2 WAI	3 WAI	4 WAI	5 WAI	6 WAI	7 WAI
99 M	22.33	0	1	2	3	4	4	4
342 M	9.33	0	2	4	4	4	4	4
393 M	22.67	0	2	3	3	3	4	4
970 M	20.67	0	0	2	3	4	4	4
M VI	40.67	0	0	0	1	1	2	4
Control M	14.00	0	2	4	4	4	4	4
281 R	16.67	0	1	1	3	3	4	4
292 R	21.33	0	1	2	2	3	4	4
364 R	34.00	0	0	0	1	2	3	4
R V	16.33	0	1	2	4	4	4	4
R XI	25.33	0	0	1	3	3	4	4
Control R	12.67	0	3	4	4	4	4	4

* Average of three replications

WAI - Week after inoculation

Table 28. Incidence of bacterial wilt in selected somaclones of ginger (*Z. officinale* Rosc.) in artificial screening

Clone No.	Days taken for appearance of symptoms	Bacterial wilt incidence scores					
		4 DAI	8 DAI	12 DAI	16 DAI	20 DAI	24 DAI
99 M	4.33	2	3	4	5	5	5
342 M	2.33	3	3	4	5	5	5
393 M	3.33	2	3	4	5	5	5
970 M	3.67	2	3	3	3	4	5
M VI	4.33	2	2	3	4	5	5
Control M	3.67	2	2	4	4	5	5
281 R	3.67	2	3	4	4	5	5
292 R	7.67	1	1	3	4	5	5
364 R	3.67	3	3	3	3	4	5
R V	4.33	2	3	4	4	5	5
R XI	3.67	2	3	4	4	5	5
Control R	4.33	2	2	4	5	5	5

* Average of three replications

DAI - Days after inoculation

Plate 16 (a-f) Rhizome rot incidence in selected somaclones of ginger cultivars from third to fifth weeks after inoculation (WAI)

subsequent drying were observed three weeks after inoculation. The somaclones 364 R and M VI, took infection late and rotting and subsequent drying occurred only late after six to seven weeks of inoculation (Fig. 17a and 17b). In five somaclones viz. 99 M, M VI, 364 R, R V and R XI, after rotting of tillers, subsequent germination of rhizome was observed (Plate 18). The clones in which infection of rhizome rot was late and subsequent germination and growth occurred (M VI, 364 R and R XI) were designated as tolerant clones.

4.3.4.4.2 Artificial Screening Against Bacterial Wilt Disease

Artificial inoculation for bacterial wilt disease was done using fresh bacterial ooze collected from wilted ginger plants. Time taken for the appearance of bacterial wilt symptoms ranged from two to eight days (Table 28). The clone 342 M was the first to take infection and wilt (2.33 days). Wilting symptoms appeared eight days after inoculation in the clone 292 R (Plates 17a - f). The clones 99 M, 342 M and 393 M were the first to wilt completely. Wilting symptoms were noticed in these clones 16 days after inoculation. In the clones 970 M and 364 R, wilting was noticed 24 days after inoculation (Fig. 18a and 18b). Gradual development of wilting symptoms was noticed in all the somaclones inoculated with bacterial ooze. In four somaclones viz., 970 M, M VI, 364 R, and R XI, subsequent germination of rhizomes after wilting of the tillers was noticed (Plate 19). The clones that took infection late and in which subsequent germination and growth occurred (970 M, M VI and 364 R) were designated as tolerant clones.

Three different screening methods viz. natural screening in sick field, screening by electrolyte leakage method and artificial screening using the pathogen were compared for assessing the disease reaction of somaclones by the three methods of screening. Four somaclones viz. 970 M, M VI, 364 R and R XI were found tolerant in artificial screening experiment. These clones survived in natural screening also. Moreover, the above four clones came in the low electrolyte leakage group when screened with toxic metabolite(s) *P. aphanidermatum* and *R. solanacearum*. So the clones that were found tolerant in natural screening and electrolyte leakage studies exhibited the same type of disease reaction in artificial screening also. The clones

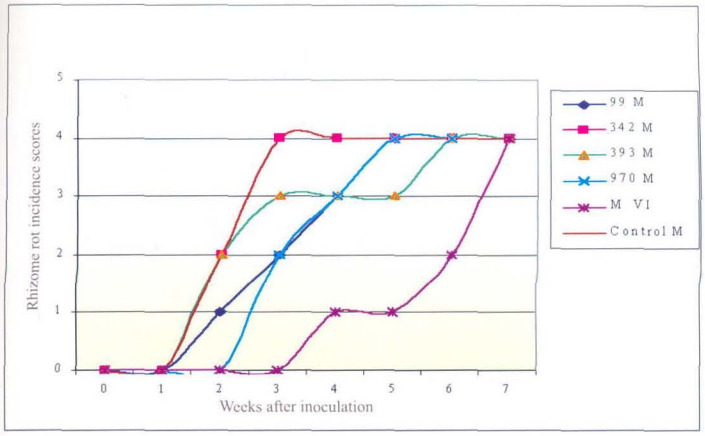


Fig. 17a Incidence of rhizome rot in selected somaclones of ginger in artificial screening (cultivar Maran)

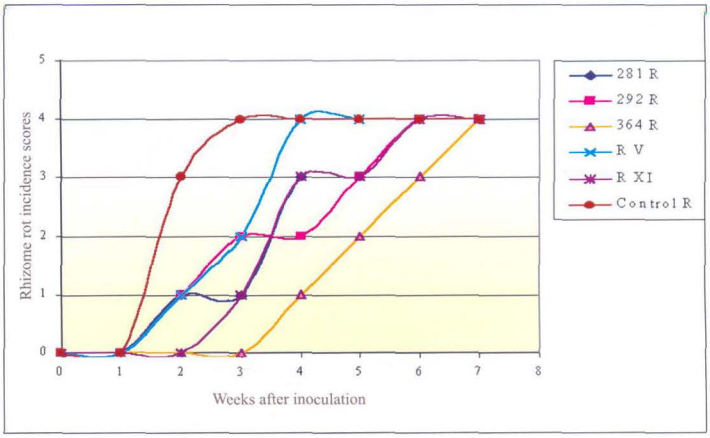


Fig. 17b Incidence of rhizome rot in selected somaclones of ginger in artificial screening (cultivar Rio-de-Janeiro)

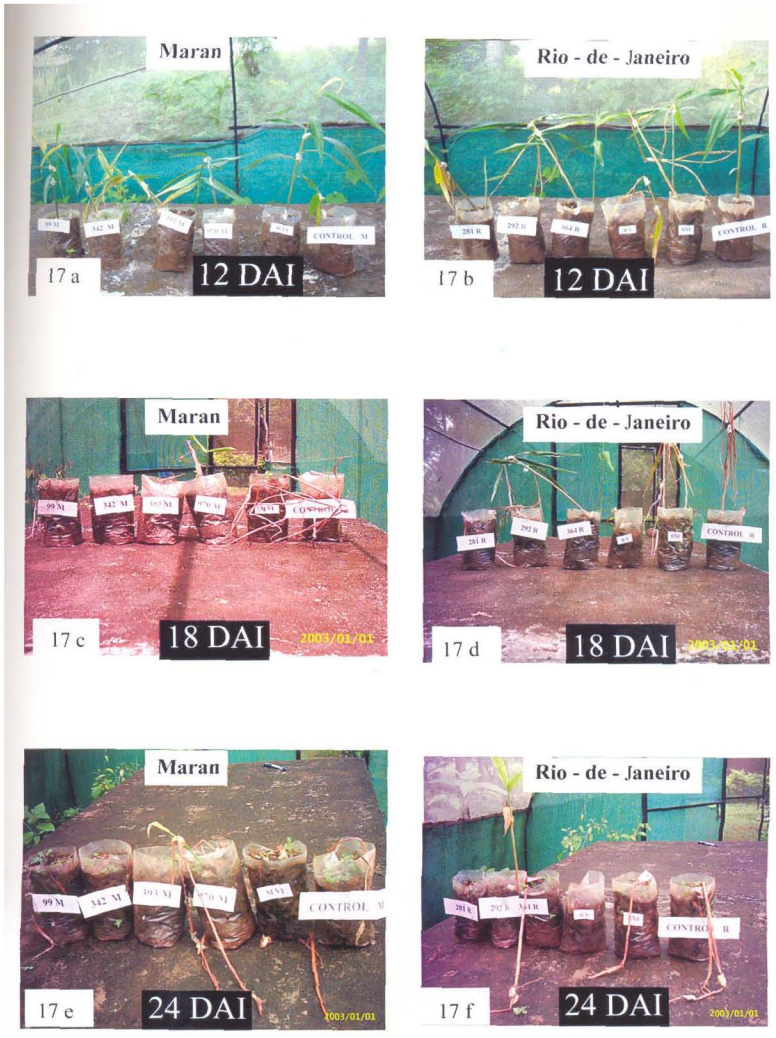


Plate 17 (a-f) Bacterial wilt incidence in selected somaclones of ginger cultivars from 12 to 24 days after inoculation (DAI)

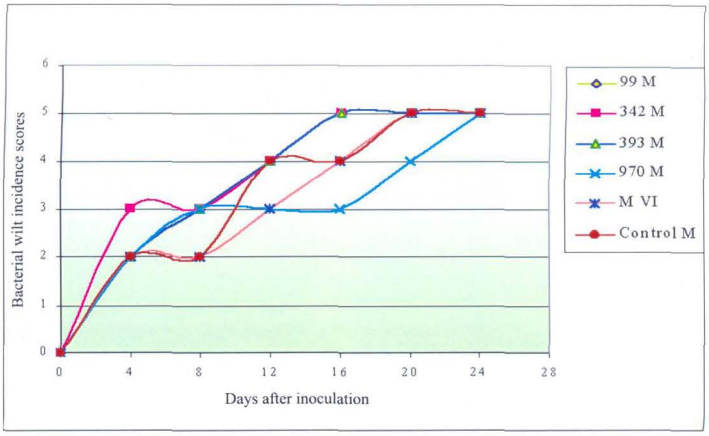


Fig. 18a Incidence of bacterial wilt in selected somaclones of ginger in artificial screening (cultivar Maran)

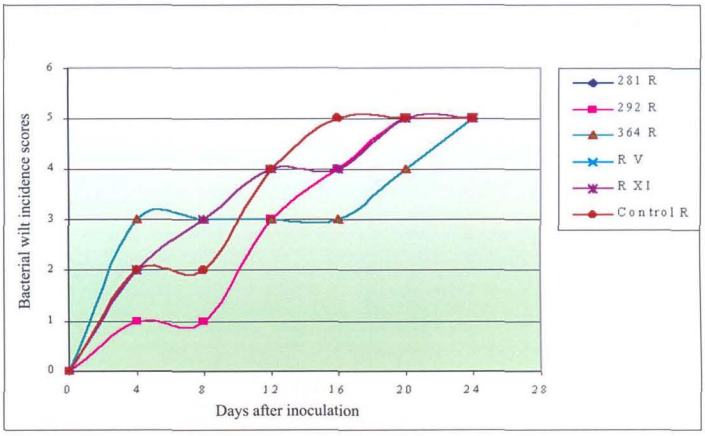


Fig. 18b Incidence of bacterial wilt in selected somaclones of ginger in artificial screening (cultivar Rio-de-Janeiro)

Plate 18 Subsequent germination of rhizomes
(after inoculation with *P. aphanidermatum*)

Plate 19 Subsequent germination of rhizomes
(after inoculation with *R. solanacearum*)

M VI and 364 R showed tolerance to both rhizome rot and bacterial wilt diseases while the clone 970 M showed tolerance to rhizome rot and R XI to bacterial wilt disease in artificial screening experiment.

4.4 MOLECULAR CHARACTERISATION OF SELECTED SUPERIOR SOMACLONES

Molecular characterisation of 12 selected superior somaclones (first set) along with CP plants of two cultivars was done using RAPD technique.

4.4.1 Isolation of Genomic DNA in Ginger

Genomic DNA was isolated from 12 superior somaclones along with CP plants of two cultivars selected for the study. DNA isolation was done by three methods viz. Doyle and Doyle (1987) with modifications (method I and Ia) and Rogers and Bendich (1994) (method II). Quality of DNA isolated was assessed by agarose gel electrophoresis. Of the three methods tried for DNA isolation, good quality DNA in sufficient quantity was obtained in method Ia (Plate 20 and 21). In method I and II, quantity of DNA was low and degradation of DNA was observed (Table 29).

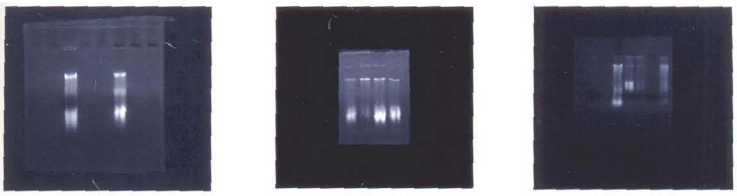
4.4.2 Random Amplified Polymorphic DNA (RAPD) Analysis

4.4.2.1 Screening of Random Primers for DNA Amplification

Twenty seven random primers were screened for amplification of DNA with the selected reaction mixture using template DNA of cultivar Maran. Five primers (OPP 16, OPP 17, OPAH 1, OPAH 3 and OPAH 5), that gave good amplification with clear and distinct bands were selected for further analysis. The results of primer screening are presented in Table 30.

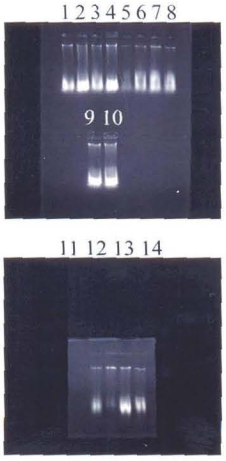
OPE series

Out of eight primers of OPE series screened, none of the primers of the series gave good amplification. The number of bands ranged from zero to two. So primers of OPE series were not selected for further investigation.



Method I Method Ia Method II

Plate 20 Genomic DNA of ginger isolated by different methods



1. M VI
2. 342 M
3. 100 M
4. 393 M
5. 918 M
6. 970 M
7. 99 M
8. 488 M
9. 281 R
10. 386 R
11. 364 R
12. 292 R
13. M (CP)
14. R (CP)

Plate 21 Genomic DNA of selected somaclones in ginger isolated by method Ia

Table 29. Quality of genomic DNA of ginger (*Z. officinale* Rosc.) isolated by different methods

Method of DNA isolation	Quality of DNA	Nature of bands
Method I	Fair	Partially smeared
Method Ia	Good	Clear, narrow
Method II	Poor	Totally smeared

Table 30. Primer screening for DNA amplification in ginger (*Zingiber officinale* Rosc.)

Sl. No.	Primer code	Primer sequence	No. of bands	Quality of bands	Remarks
1	OPE 5	GCAGGGAGGT	Zero	No amplification	Rejected
2	OPE 6	AAGACCCCTC	Zero	No amplification	Rejected
3	OPE 7	AGATGCAGCC	Two	Clear, less no. of bands	Rejected
4	OPE 8	TCACCACGGT	Zero	No amplification	Rejected
5	OPE 9	CTTCACCCGA	Zero	No amplification	Rejected
6	OPE 10	CACCAGGTGA	Zero	No amplification	Rejected
7	OPE 11	GAGTCTCAGG	Zero	No amplification	Rejected
8	OPE 12	TTATCGCCCC	Zero	No amplification	Rejected
9	OPP 1	GTAGCACTCC	Zero	No amplification	Rejected
10	OPP 2	TCGGCACGCA	Zero	No amplification	Rejected
11	OPP 3	CTGATACGCC	Zero	No amplification	Rejected
12	OPP 4	GTGTCTCAGG	Zero	No amplification	Rejected
13	OPP 5	CCCCGGTAAC	Zero	No amplification	Rejected
14	OPP 6	GTGGGCTGAC	One	Smeared	Rejected
15	OPP 7	GTCCATGCCA	Zero	No amplification	Rejected
16	OPP 8	ACATCGCCCA	Zero	No amplification	Rejected
17	OPP 13	GGAGTGCCTC	Zero	No amplification	Rejected
18	OPP 14	CCAGCCGAAC	Zero	No amplification	Rejected
19	OPP 15	GGAAGCCAAC	Zero	No amplification	Rejected
20	OPP 16	CCAAGCTGCC	Four	Clear, distinct	Selected
21	OPP 17	TGACCCGCCT	Four	Clear, distinct	Selected
22	OPAH 1	TCCGCAACCA	Four	Clear, distinct	Selected
23	OPAH 2	CACTTCCGCT	Zero	No amplification	Rejected
24	OPAH 3	GGTTACTGCC	Eight	Clear, distinct	Selected
25	OPAH 4	CTCCCCAGAC	Zero	No amplification	Rejected
26	OPAH 5	TTGCAGGCAG	Eight	Clear, distinct	Selected
27	OPAH 6	GGCTTGGCCT	Zero	No amplification	Rejected

OPP series

From the OPP series, 13 primers were screened and two primers viz. OPP 16 and OPP 17 gave good amplification with four clear and distinct bands. Hence, the two primers that gave good amplification were selected for further analyses.

OPAH series

Six primers from OPAH series were screened for DNA amplification, of which, three (OPAH 1, 3 and 5) gave good amplification with four to eight clear and distinct bands. Repeated tests also gave the same results and hence the three primers of OPAH series were selected for further analyses.

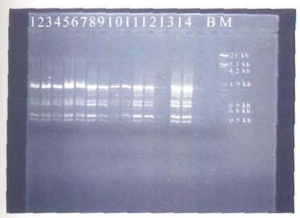
4.4.2.2 RAPD Analysis of Somaclones with Selected Primers

RAPD analysis in selected somaclones was done using the primers selected (Plate 22). Genetic similarity within and between somaclones of two cultivars and CP plants were assessed. The coefficient of genetic similarity was calculated by computing Jaccard's similarity coefficient. Primer OPAH 3 showed the highest polymorphism (23.75%) while the primer OPP 16 showed the least polymorphism (0.00) (Table 31).

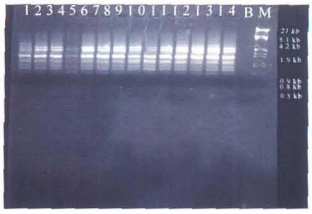
4.4.2.2.1 Genetic Variability in Somaclones

The matrix of genetic dissimilarity in somaclones of cultivar Maran pertaining to the pooled data obtained from five selected primers is presented in Table 32a. Analyses of RAPD profiles of the five selected primers indicated that genetic variability existed within and between somaclones of two cultivars and CP plants.

The percentage of genetic variability varied from zero to 31.6 per cent in somaclones of cultivar Maran with an average variability of 16.8 per cent. Highest genetic dissimilarity of 31.6 per cent was observed between two somaclones viz. M VI and 488 M followed by M VI and 342 M and 488 M and 342 M with 24.4 per cent variability. The highest genetic similarity of 100 per cent was observed between somaclones 393 M and 918 M. The genetic dissimilarity between the CP plant and the



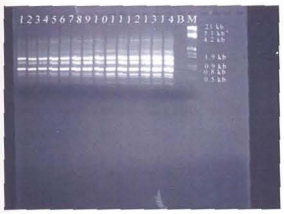
22a OPAH 1



22b OPAH 3

22c OPAH 5

22d OPP 16



22e OPP 17

1. 281R
2. 386 R
3. 364 R
4. 292 R
5. M VI
6. 342 M
7. 100 M
8. 393 M
9. 918 M
10. 970 M
11. 99 M
12. 488 M
13. R (CP)
14. M (CP)

B - Control
M - Molecular weight marker

Plate 22. RAPD profiles of selected somaclones in ginger

Table 31. RAPD polymorphism with selected primers in ginger (*Z. officinale* Rosc.)

Sl. No.	Primer	Total no. of bands	No. of polymorphic bands	% polymorphism
1	OPP 16	112.00	0.00	0.00
2	OPP 17	103.00	19.00	18.45
3	OPAH 1	96.00	22.00	22.92
4	OPAH 3	80.00	27.00	33.75
5	OPAH 5	103.00	18.00	17.48

Table 32a. Matrix of dissimilarity index in selected somaclones of ginger (*Z. officinale* Rosc.) (cultivar Maran)

	M VI	342 M	100 M	393 M	918 M	970 M	99 M	488 M	Control M
M VI	0.000								
342 M	0.244	0.000							
100 M	0.220	0.024	0.000						
393 M	0.243	0.175	0.195	0.000					
918 M	0.243	0.175	0.195	0.000	0.000				
970 M	0.184	0.075	0.098	0.158	0.158	0.000			
99 M	0.158	0.098	0.073	0.179	0.179	0.026	0.000		
488 M	0.316	0.244	0.220	0.194	0.194	0.231	0.205	0.000	
Control M	0.225	0.025	0.049	0.154	0.154	0.051	0.075	0.225	0.000

Table 32b. Matrix of dissimilarity index in selected somaclones of ginger (*Z. officinale* Rosc.) (cultivar Rio-de-Janeiro)

	281 R	386 R	364 R	292 R	Control R
281 R	0.000				
386 R	0.083	0.000			
364 R	0.108	0.028	0.000		
292 R	0.200	0.128	0.103	0.000	
Control R	0.128	0.103	0.077	0.122	0.000

somaclones ranged between 2.5 per cent (342 M) to 22.5 per cent (M VI and 488 M). Evidently, M VI had accumulated maximum genetic changes and had the highest genetic variability (0.877) with the CP plant and rest of the somaclones. Followed by M VI, the clone 488 M exhibited high genetic variability with the CP plant and rest of the somaclones.

The genetic variability in somaclones of Rio-de-Janeiro varied between 2.8 to 20 per cent with an average variability of 10.8 per cent (Table 32b). Highest variability of 20 per cent was observed between 292 R and 281 R and lowest variability of 2.8 per cent was observed between 364 R and 386 R. The clone 364 R shared lowest variability (7.7 %) from the CP plant while the clone 281 R showed highest dissimilarity with the CP plant (12.8 %). The somaclone 292 R was totally distinct from other somaclones and the CP plant.

The somaclones of ginger exhibited 15.7 per cent genetic variability. The extent of genetic variation was more in somaclones of cultivar Maran (0 – 31.6 %) with an average variability of 16.8 per cent. Somaclones of cultivar Rio-de-Janeiro exhibited 2.8 to 20 per cent variability with an average variability of 10.8 per cent. The extent of genetic variation from the CP plant was also more in somaclones of cultivar Maran with an average variability of 12 per cent. The somaclones of cultivar Rio-de-Janeiro exhibited 10.8 per cent variability with the CP plant.

4.4.2.2.2 Dendrogram of Ginger Somaclones Using RAPD Data

The dendrogram constructed using the pooled data of the RAPD analysis with five different primers expressed the total genetic make up of the somaclones and the CP plants.

The somaclones of Maran had two clusters based on the dendrogram (Fig. 19a). The first cluster consisted of M VI alone showing its distinctness from rest of the somaclones and the CP plant. Within the second cluster, 488 M differed from rest of the somaclones. The clones 393 M and 918 M shared maximum similarity with each other followed by the clones 342 M and 100 M and 970 M and 99 M. The clones 342 M and 100 M shared maximum similarity with the CP plant.

Fig. 19a Dendrogram of RAPD profiles of somaclones in ginger (*Z. officinale* Rosc.) cultivar Maran

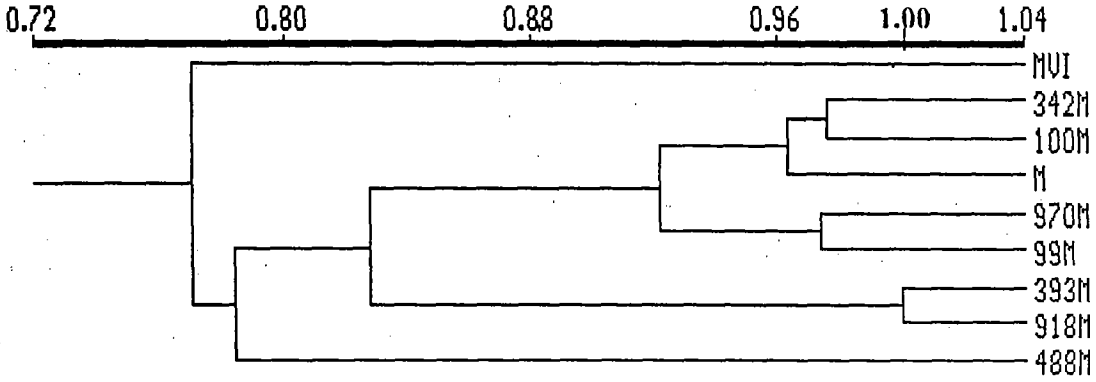
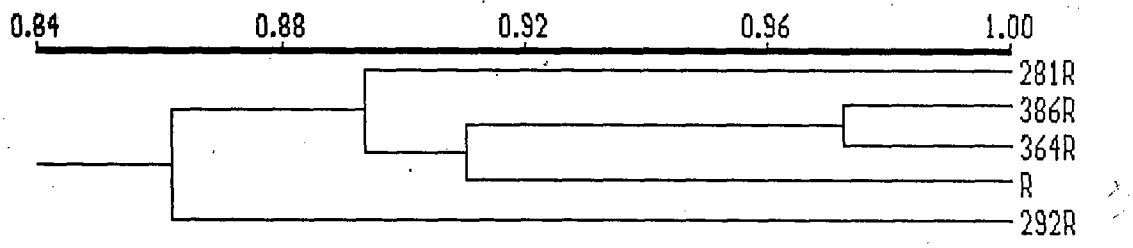


Fig. 19b Dendrogram of RAPD profiles of somaclones in ginger (*Z. officinale* Rosc.) cultivar Rio-de-Janeiro



The dendrogram constructed for the somaclones of cultivar Rio-de-Janeiro formed two clusters (Fig. 19b). The first cluster consisted of 292 R alone showing its distinctness from rest of the somaclones and the CP plant. In the second cluster, 281 R differed from rest of the somaclones. The clones 386 R and 364 R shared maximum similarity with each other and the CP plant.

RAPD analysis revealed the genetic variability within and between somaclones of two cultivars and somaclones and CP plants. In the somaclones of cultivar Maran analysed, M VI exhibited highest polymorphism from rest of the somaclones of the cultivar and CP plant. In somaclones of cultivar Rio-de-Janeiro, the clone 292 R differed from rest of the somaclones of the cultivar and the CP plant.

Discussion

DISCUSSION

Ginger is propagated exclusively by vegetative means. So the natural variability in ginger is limited. However, the existing population shows some amount of variability for morphological and yield attributes. Available variability in ginger is not fully exploited for crop improvement due to poor flowering and lack of seed set. Therefore, somaclonal variation serves as an important source of variability for crop improvement in ginger.

The present investigations on "Induction of variation *in vitro* and field evaluation of somaclones in ginger" were taken up to induce variation through *in vitro* techniques, to evaluate somaclones for yield, quality and tolerance / resistance to diseases and to characterise the selected superior somaclones using RAPD markers.

5.1 INDUCTION OF VARIATION IN GINGER THROUGH *IN VITRO* TECHNIQUES AND PRODUCTION OF REGENERANTS

5.1.1 Standardisation of Indirect Organogenesis

Pseudostem, leaf, bud and shoot tip explants were tried for callus induction in ginger. The different explants tried for callusing showed significant variation with respect to callusing and callus growth in the two cultivars studied. Of the seven explants tried for callusing, shoot tip explants responded better than other explants registering early (27.61 days) and higher callusing (72.52%) and higher callus index value (76.41) (Fig. 1a, 1b and 1c and Plate 4). Next to shoot tip, pseudostem base recorded highest callusing and callus growth followed by folded leaf and rhizome bud explants (Tables 1a, 1b and 1c). Callusing was not observed in unfolded leaves of both cultivars.

The better response of shoot tip explants to callusing may be due to its juvenile nature and the presence of more metabolically active cells in the explant. Moreover, shoot tip being the seat of active synthesis of auxins, could be induced to multiply at an accelerated rate and hence the response was better. Similar response of shoot tip explants to callusing and callus growth was reported by several workers in

ginger (Malamug *et al.*, 1991; Rout and Das, 1997; Palai *et al.*, 2000) and turmeric (Salvi *et al.*, 2001). Poor response of unfolded leaves to callusing was reported in ginger by Babu (1997) and Suma and Keshavachandran (2005a and b) and in kacholam by Joseph (1997a).

Half MS medium supplemented with various concentrations of 2,4-D (1.00 – 4.00 mg l^{-1}) and combinations of 2,4-D (0.50 – 3.00 mg l^{-1}) and BAP (0.50 – 1.00 mg l^{-1}) were tried for callusing in the present investigation. Different media combinations differed significantly with respect to callusing and callus growth and time taken for callusing. The best medium identified for callusing was half MS supplemented with 2,4-D at 1.00 mg l^{-1} followed by half MS supplemented with 3.00 mg l^{-1} 2,4-D and 0.50 mg l^{-1} BAP.

The favourable effect of 2,4-D in callusing may be due to stimulated cell division in 2,4-D incorporated medium or increased DNA content in cells as observed in tobacco cell suspension cultures by Miyazawa *et al.* (2002) or due to accumulation of large amounts of endogenous IAA in cells as reported in carrot callus cultures by Michalczuk *et al.* (1992). It is also possible that 2,4-D acts indirectly in cell cultures by disturbing the endogenous auxin metabolism of cells. The favourable effect of 2,4-D on callusing was reported in Zingiberaceous spice crops like ginger (Ramachandran and Nair, 1992; Babu, 1997; Babu *et al.*, 1992 and 1996a; Rout and Das, 1997; Samsudeen *et al.*, 2000) and mango ginger (Prakash *et al.*, 2004). The beneficial effect of 2,4-D - BAP combination in callusing was reported in ginger by Malamug *et al.* (1991), Rout and Das (1997), Babu *et al.* (1996), Palai *et al.* (2000), Samsudeen *et al.* (2000) and Suma and Keshavachandran (2005a and b) and in Kacholam by Vincent *et al.* (1991 and 1992)

In the present investigation, a gradual decline in response to callusing was observed with increasing levels of 2,4-D in the medium. This observation correlates with the study conducted by Eapen and George (1993), Baker and Wetztein (1994) and Loiseau *et al.* (1995) in peanut where deleterious effects of higher concentrations of 2,4-D in callusing are discussed.

Calli induced at lower concentrations of 2,4-D and combinations of 2,4-D and BAP at various levels were hard and compact. But at higher levels of 2,4-D (3.00 and 4.00 mg l^{-1}), the induced calli were friable, loose and watery with root hairs. The friability of induced calli in 2,4-D incorporated medium may be due to the presence of highly vacuolated and elongated cells in the calli as reported by Davis and Keathley (1992). The friable texture of calli grown in medium supplemented with 2,4-D was reported in ginger by Rout and Das (1997) and Babu (1997), in mango ginger by Prakash *et al.* (2004) and in Kacholam by Joseph (1997a). The hairy nature of calli grown in medium supplemented with 2,4-D was reported by Malamug *et al.* (1991) in ginger.

The two culture conditions tried viz. incubation under dark and light were found on par with respect to callusing and callus growth in the two cultivars studied. But for earliness in callusing, dark incubation was found favourable in the cultivar Maran. The favourable effect of dark incubation may be due to less degradation of auxins in dark as reported by Arzate *et al.* (1997). The favourable influence of dark incubation was also reported by Joseph (1997a) in kacholam when indirect organogenesis was attempted in this crop.

The explants of cultivar Rio-de-Janeiro responded better to callusing and callus growth than the cultivar Maran. In the cultivar Rio-de-Janeiro, callusing was observed in 44.01 per cent of cultures, 33.34 days after incubation expressing a callus index value of 38.59. In the cultivar Maran, callusing was observed in 37.41 per cent cultures with a callus index of 36.73 after 35.48 days of incubation. Similar genotype dependent response of *in vitro* cultures in callusing and callus growth and various stages of morphogenesis has been observed by several workers in spice crops like kacholam (Reghunath, 1989), ginger (Palai *et al.*, 2000; Shylaja *et al.*, 2003), black pepper (Shylaja, 1996) and garlic (Barandiaran *et al.*, 1999). The difference in performance of the two cultivars to callusing and callus growth may be due to difference in endogenous auxin-cytokinin balance of genotypes (Looney *et al.*, 1988) as well as difference in *in vitro* uptake of exogenous cytokinins (Marino, 1988).

Growth regulators like 2,4-D, NAA and BAP and supplements like charcoal and silver nitrate were incorporated to basal MS medium at half strength for shoot morphogenesis in ginger. The highest shoot morphogenesis was observed in half MS medium supplemented with BAP at 3.00 mg l^{-1} followed by BAP at 4.00 mg l^{-1} (Table 2). Morphogenic potential of the calli decreased with increase in concentration of BAP and the regeneration percentage was lowest (11.11) in medium with higher concentration of BAP (5.00 mg l^{-1}) tried in the experiment. Similar observations were also reported by Shirin *et al.* (2000) in kacholam. Many workers have highlighted the favourable effects of BAP to promote callus mediated shoot regeneration in Zingiberaceous crops like ginger (Malamug *et al.*, 1991; Samsudeen *et al.*, 2000), mango ginger (Prakash *et al.*, 2000) and Kacholam (Vincent *et al.*, 1991).

Palai *et al.* (2000) made detailed studies on biochemical changes during indirect organogenesis in ginger. The study revealed that contents of carbohydrate, protein and activity of acid phosphatase were maximum during organogenesis as compared to that of callusing and rooting.

Shoot morphogenesis was observed in 39 per cent of shoot tip derived callus cultures, 28 days after inoculation in half MS medium supplemented with 3.00 mg l^{-1} BAP. Ishida and Adachi (1997) obtained only 27.7 per cent shoot morphogenesis in callus cultures of ginger cultivar Oshoga. Babu *et al.*, (1996b) got shoot morphogenesis 120 to 162 days after inoculation of explant. But in the present study, higher and early shoot morphogenesis were observed.

The callus cultures of cultivar Rio-de-Janeiro registered early and higher shoot morphogenesis and proliferation than the cultures of cultivar Maran (Table 3). The rate of shoot proliferation was also high in Rio-de-Janeiro. The superiority of cultivar Rio-de-Janeiro in shoot proliferation was reported by Shylaja *et al.* (2003), when they regenerated ginger through adventitious bud culture.

The shoot and root morphogenesis were simultaneous in *in vitro* cultures of ginger in half MS medium supplemented with BAP (3.00 mg l^{-1}). The simultaneous

emergence of shoot and root primordia in the same medium was also reported in Zingiberaceous spice crops like ginger (Hosoki and Sagawa, 1977; Ramachandran and Nair, 1992), mango ginger (Prakash *et al.*, 2004) kacholam (Vincent *et al.*, 1991; Geetha *et al.*, 1997; Shirin *et al.*, 2000), small cardamom (Rao *et al.*, 1982) and large cardamom (Sajina *et al.*, 1997).

5.1.2 Standardisation of Somatic Embryogenesis

For the induction of embryogenic calli, rhizome bud explants were tried in the present study (Tables 4 and 5). Of the seven media tested, embryogenesis occurred in two media viz. half MS medium supplemented with 2,4-D (0.50 mg l^{-1}) and BAP (1.00 mg l^{-1}) and 2,4-D (1.00 mg l^{-1}) and BAP (0.50 mg l^{-1}). The induction of embryogenic calli and somatic embryos were on par in the two responding media. The embryogenic calli ranged from 82 to 83 per cent and number of embryoids 1.7 to 1.8 per culture and embryo induction occurred 30 days after inoculation (Plate 5).

Somatic embryogenesis was reported from rhizome bud explants in ginger by Suma and Keshavachandran (2005a and b) and in kacholam by Vincent *et al.* (1992) and Lakshmi and Mythili (2003). Similarly, the favorable effect of BAP and 2,4-D on somatic embryogenesis was reported in ginger by Babu (1997), Babu *et al.* (1996a) and Suma and Keshavachandran (2005a and 2005b). The requirement of both an auxin and a cytokinin in the culture medium for somatic embryogenesis in sorghum was reported by Bhaskaran and Smith (1989).

Embryogenic and non-embryogenic calli were observed when rhizome buds were cultured in half MS medium supplemented with 2,4-D and BAP each at 0.50 and 1.00 mg l^{-1}. The production of embryogenic and organogenic calli in the same medium was also reported by Kackar *et al.* (1993), Suma and Keshavachandran (2005a and b) and Babu *et al.* (2005) in ginger and by Joseph, (1997a) in kacholam.

Incubating cultures under dark was significantly superior to incubation under light with respect to percentage embryogenic calli. Percentage of embryogenic calli observed in dark was 92 per cent while it was only 74 per cent in light. But for early induction of embryogenic calli, incubation in light was superior. Calli were

initiated 29 days after inoculation in light and it took 32 days in dark. The mean number of somatic embryoids/culture did not vary significantly in both the culture conditions. The number of embryoids produced/culture ranged from 1.67 to 1.88 in the two incubations tried. The favourable influence of dark incubation for embryo induction was reported in kacholam by Joseph (1997a). In contrast, Babu *et al.* (1996a) and Lakshmi and Mythili (2003) observed light as an important signal for somatic embryogenesis in ginger and kacholam respectively.

The two cultivars studied were on par with respect to days taken for embryogenesis and mean number of somatic embryoids/culture. But significantly higher percentage of embryogenic calli was recorded in the cultivar Maran. This may be due to the differences in endogenous level of plant growth regulators in the target cells.

The proliferation of somatic embryoids in two cultivars were compared by inoculating the induced embryoids to half MS basal medium (Table 6). In medium with BAP (3.00 mg l^{-1}) tried for proliferation of somatic embryoids, rhizogenesis was observed. The transfer of induced embryoids to hormone free medium will induce stress that triggers embryo maturation and proliferation process. Similar observations on requirement of an auxin for induction of embryoids and a medium devoid of growth regulators for maturation was reported by Babu *et al.* (1996a) in ginger.

For the germination of somatic embryoids in ginger, two media viz. half MS basal and half MS basal with BAP at 3.00 mg l^{-1} were tried. Inclusion of BAP in the basal medium was found to enhance germination of somatic embryoids (Table 7). Kackar *et al.* (1993) and Suma and Keshavachandran (2005a and b) also reported the enhanced germination of somatic embryoids in ginger when BAP was incorporated to MS medium.

The cultivar Rio-de-Janeiro registered higher proliferation of embryoids (45 %) as compared to Maran (30 %). Mean number of embryoids proliferated after one month of inoculation was also high in Rio-de-Janeiro registering 2.65 embryoids/culture as compared to 2.11 embryoids/culture in Maran. Highest

germination of somatic embryoids was observed in the cultivar Rio-de-Janeiro. But early germination of embryoids was noticed in the cultivar Maran. The germinated somatic embryos produced good root and shoot system in half MS medium supplemented with BAP at 3.00 mg l^{-1}. Further shoot proliferation in embryogenic cultures was also highest in the cultivar Rio-de-Janeiro. The mean number of shoots proliferated was also high in cultures of Rio-de-Janeiro registering 65 shoots at the end of fourth subculture as compared to 60 shoots in Maran (Table 8). Better response of Rio-de-Janeiro cultures to *in vitro* shoot proliferation was reported by Shylaja *et al.* (2003).

5.1.3 *In vitro* Induction of Mutation Using γ Irradiation

Organogenic / embryogenic callus cultures at the shoot morphogenesis stage were subjected to γ irradiation at five different doses viz. 10, 20, 30, 40 and 50 Gy (Tables 9a, 9b and 9c). Of the different doses of irradiation tried, highest culture establishment was recorded in cultures irradiated at lower dose of γ irradiation (10 Gy) wherein 95.83 per cent cultures established 30 days after irradiation followed by 20 Gy registering 50 per cent establishment (Fig. 2 and Plate 6). Culture establishment was nil at higher doses of irradiation tried viz. 40 and 50 Gy. In non-irradiated cultures, cent percent establishment was observed.

The number of shoots proliferated 30 days after irradiation was highest in the cultures irradiated at 10 Gy registering 3.83 shoots/culture, which was on par with non-irradiated cultures (3.88 shoots/culture). At higher doses of irradiation tried viz. 30, 40 and 50 Gy, the number of shoots proliferated were less and the shoots formed were weak and stunted. The survival percentage and shoot proliferation were highest in non-irradiated cultures.

The deleterious effects of higher doses of γ irradiation may be because γ irradiation in general caused inhibition of tissue growth along with failure of RNA synthesis and subsequently protein (Bajaj *et al.*, 1970). Also, radiations are known to hamper auxin synthesis leading to even its complete destruction (Sparrow *et al.*, 1952 and Gordon, 1957). Hence, cell division may be inhibited after exposure to ionizing

radiation because of chromosome damage. As observed in the present study, the retarding effects of higher doses of γ irradiation on culture proliferation and establishment were reported in crops such as maize (Moustafa *et al.*, 1989), rose (Wilson, 1993), banana (Mak *et al.*, 1995; Karmarkar *et al.*, 2001), orchid (Sobhana, 2000) and sugarcane (Kumar *et al.*, 2001).

Embryogenic cultures subjected to γ irradiation recorded higher culture establishment (24 %) and shoot proliferation (1.8) than organogenic cultures. Similarly, cultures of cultivar Maran recorded higher establishment (24 %) and shoot proliferation (2.09) than cultures of cultivar Rio-de-Janeiro. Embryogenic cultures of Maran could withstand higher doses of irradiation (20 Gy) and recorded cent percent survival of cultures 30 days after irradiation. This might be due to the inherent ability of cultivar Maran to withstand the stress conditions. The effect of genotype in the response to γ irradiation was reported by Pine *et al.* (1992) in pear, Novak *et al.* (1993) in banana and Shylaja (1996) in black pepper.

Organogenic cultures in general recorded high shoot proliferation as compared to embryogenic cultures. With regard to cultivar response, Rio-de-Janeiro exhibited high shoot proliferation than Maran. Low dose of irradiation (10 Gy) was found to enhance shoot proliferation in cultures derived through indirect organogenesis and embryogenesis (Table 10). This is in agreement with the report made by Karmarkar *et al.* (2001) in banana wherein lower doses of γ irradiation viz. 10 and 20 Gy enhanced multiplication rate of *in vitro* multiple shoot cultures. Charbaji and Nabulsi (1999) in grape and Barakat *et al.* (2002) in sunflower also observed high shoot morphogenesis and more shoot length in irradiated cultures when compared to non-irradiated cultures.

5.1.4 Root Characters, Plantlet Establishment and Growth Analysis in Regenerants Derived Through Various Routes

Regenerants were produced in the present study through indirect organogenesis/embryogenesis and *in vitro* mutagenesis. Regenerants produced through indirect organogenesis exhibited better root characters and good *ex vitro* establishment followed by regenerants from irradiated calli (Tables 11 and 12 and

Fig. 3 and 4). Plantlets produced through indirect organogenesis exhibited more length of pseudostem (8.59 cm) at plant out stage (Table 13). Regenerants produced through *in vitro* mutagenesis recorded more tiller number (4.51) at plant out stage closely followed by regenerants produced through indirect organogenesis. Plantlets produced through indirect organogenesis recorded high growth rate in length of pseudostem (9.36). Growth rate in number of tillers and length of leaves were high in regenerants produced through *in vitro* mutagenesis followed by regenerants produced through indirect embryogenesis. Similar difference in growth parameters in regenerants produced through various routes was reported in kacholam by Joseph (1997a). The regenerants of kacholam, derived through indirect organogenesis/ embryogenesis had erect leaves while adventitious bud regenerants and CP plants had horizontal leaves. Leaf area was highest in CP plants while tiller number was highest in regenerants derived through indirect organogenesis.

In the two cultivars studied, irrespective of the route of regeneration, the regenerants of cultivar Maran recorded better root characters and hence *ex vitro* establishment was better in regenerants of the cultivar. At plant out stage and three months after planting, the growth parameters recorded in regenerants of both cultivars gave almost the same values. But growth rate in length of pseudostem was slightly higher in regenerants of Maran (8.6 cm) as compared to Rio-de-Janeiro (7.71 cm).

As observed in the present study, Nybe (1978) and Shankar (2003) also reported better root characters in the cultivar Maran. The better establishment observed by regenerants of cultivar Maran may be due to its better root characters.

5.2 FIELD EVALUATION OF FIRST SET SOMACLONES IN GINGER

The somaclones of two ginger cultivars derived through *in vitro* adventitious bud culture after passing through 10 to 12 *in vitro* subculture cycles and planted out during 1999-2000 for rhizome development were field planted and evaluated for three seasons (2002-2004) for morphological, yield, quality attributes and reaction to rhizome rot and bacterial wilt diseases. Conventionally propagated

(CP) plants of cultivars Maran and Rio-de-Janeiro served as control in the field evaluation experiment.

5.2.1 Morphological Characters

Somaclones were found superior to CP plants in various morphological characters recorded (Fig. 5) and growth rate of different parameters observed. The height of pseudostem recorded was more in 16 per cent of somaclones than CP plants. Twenty seven per cent of somaclones were found superior to CP plants in tiller production. The leaf production was also found high in the somaclones evaluated as compared to CP plants. Thirty eight per cent somaclones showed higher leaf production over CP plants (Tables 14a, 14b, 15a and 15b). Similar observations were also reported by many workers in ginger when they compared tissue culture derived plants with CP plants. As in the present study, Smith and Hamill (1996) observed that adventitious bud regenerants of ginger cultivar Queensland were more vigorous than CP plants. Freitz *et al.* (2003) and Babu *et al.* (2005) also reported that somaclones of ginger were better in morphological characters than CP plants. The superiority of somaclones over CP plants in the various morphological characters were also reported in spice crops like turmeric (Salvi *et al.* 2002), small cardamom (Reghunath, 1989; Reghunath and Priyadarshan, 1993; Sudharshan and Bhat, 1998), large cardamom (Rao *et al.*, 2003) and black pepper (Sujatha, 2001; Rathy *et al.*, 2005).

Wide variability was observed in morphological characters (Fig. 6) and in growth rate of various parameters recorded in the somaclones evaluated. Somaclones exhibited wide variability in number of tillers/clone, leaf area and also in growth rate of different parameters recorded. The high amount of somaclonal variation in morphological characters were also reported by Babu *et al.* (2005) in ginger somaclones, Salvi *et al.* (2002) in turmeric somaclones, Sudharshan and Bhat (1998) in cardamom somaclones, Sanchu *et al.* (2002) and Sujatha (2001) in black pepper somaclones and Hena (2005) in vanilla somaclones.

Morphological characters were also compared in somaclones of two cultivars. Clones of Rio-de-Janeiro exhibited superiority in height of pseudostem,

tiller number and leaf number. The high tillering capacity of the cultivar Rio-de-Janeiro has been reported by Nair (1975), Nybe (1978), Sheeba (1996) and Shankar (2003). Somaclones of cultivar Maran were better in leaf characters such as length, breadth and area of leaf. This is in agreement with Nair (1975) and Nybe (1978), they reported that cultivar Maran exhibited more leaf area than the cultivar Rio-de-Janeiro.

5.2.2 Yield and Quality Attributes of Somaclones

5.2.2.1 Rhizome Characters

The somaclones of ginger recorded superiority in rhizome characters when compared to CP plants (Tables 18a and 18b) (Fig. 7). Number of primary fingers was found high in three per cent somaclones and secondary fingers in 12 per cent somaclones evaluated. Thirty nine per cent somaclones showed higher length of primary fingers and 22 per cent somaclones showed higher length of secondary fingers than CP plants. Twenty six per cent somaclones were found superior in girth of primary fingers and 22 per cent clones in girth of secondary fingers. With respect to internodal length of primary and secondary fingers, 60 per cent somaclones were found superior to CP plants. So the rhizome characters like number, length and girth of fingers that contribute directly to yield were more in the somaclones evaluated. Pandey *et al.*, (1997), Babu *et al.* (2005) and Salvi *et al.* (2002) also reported similar observations on superiority of somaclones in yield contributing characters over CP plants in ginger and turmeric somaclones.

Somaclones exhibited wide variability in rhizome characters like number of primary and secondary fingers but the variability observed was almost uniform in somaclones of two cultivars.

Variability in yield contributing characters in somaclones of spice crops was reported by Chandrappa *et al.* (1997), Sudharshan *et al.* (1997) and Sudharshan and Bhat (1998) in cardamom somaclones and Sanchu *et al.* (2002) in black pepper somaclones.

When yield-contributing characters were analysed in somaclones of the two cultivars, somaclones of cultivar Maran exhibited superiority in most of the rhizome characters such as number, length and girth of fingers and orientation of rhizomes. Nybe (1978) and Sheeba (1996) reported that the cultivar Maran registered more number of secondary fingers and higher length of secondary fingers than the cultivar Rio-de–Janeiro when they evaluated different cultivars for yield and quality parameters.

5.2.2.2 Yield

The yield of somaclones was evaluated for three seasons and the yield of clones was found to improve in subsequent years of field evaluation (Table 19). Similar observations on yield improvement in somaclones in subsequent generations was reported in kacholam by Geetha *et al.* (1997).

Based on average yield for three years' of field evaluation, nine per cent somaclones were found high yielders over CP plants (Fig. 9). Rao *et al.* (2000) got comparable yield for adventitious bud regenerants in ginger with CP plants. But Salvi *et al.* (2002) reported superiority in yield of adventitious bud regenerants in turmeric over CP plants. Similar observations on yield improvement in somaclones over CP plants were reported in spice crops like small cardamom (Kuruvilla *et al.*, 2005) and large cardamom (Rao *et al.*, 2003; Gupta *et al.*, 2005).

Variability in rhizome yield observed was high in somaclones of cultivar Maran with a coefficient of variation of 40.342 per cent as compared to 34.883 per cent in clones of cultivar Rio-de-Janeiro (Fig. 10). Similar variability in rhizome yield in somaclones of ginger has been reported by Samsudeen (1996) and Babu *et al.* (2005) and in cardamom by Chandrappa *et al.* (1997). Sanchu *et al.* (2002) also observed wide variability for yield in thirty calliclones of black pepper evaluated.

Even though somaclones of cultivar Rio-de-Janeiro exhibited superiority in morphological characters such as length of pseudostem and tiller number, somaclones of cultivar Maran were found to give high rhizome yield as compared to clones of cultivar Rio-de-Janeiro. Mean yield per plant varied between 65.83-348.75 g in clones

of Maran and 65.00-252.77 g in clones of Rio-de-Janeiro. The high yielding capacity of the cultivar Maran was reported by Nybe (1978) and Sheeba (1996). This may be due to better root and leaf characters exhibited by clones of cultivar Maran and also due to better rhizome characters. Several workers have reported the high positive and direct effect of number of leaves, length, breadth and area of leaves on yield in ginger (Ratnambal *et al.*, 1982; Rattan *et al.*, 1988; Sasikumar *et al.*, 1992; Das *et al.*, 1999).

5.2.2.3 Quality Attributes

Somaclones recorded higher dry ginger recovery (19.73 %) than CP plants (16.02 %) (Table 20). Of the two cultivars studied, higher driage was noticed in clones of cultivar Maran (18-25 %) than clones of cultivar Rio-de-Janeiro (15.62-15.79 %). In three clones of cultivar Maran viz. 488 M, 110 M and 970 M, the driage recorded was very high registering driage values of 25, 22.56 and 22.50 per cent respectively. Higher recovery of dry ginger in cultivar Maran has been reported by Nair (1969), Nair (1975), Nybe (1978) and Shankar (2003). Poor drying percentage of Rio-de-Janeiro has also been reported by Kannan and Nair (1965), Muralidharan (1973) and Nair (1975).

Recovery of essential oil varied between 1.00 to 2.50 per cent in the somaclones studied. Oil content was found high in somaclones of cultivar Rio-de-Janeiro (1.42 to 2.50 %) than clones of cultivar Maran (1.00-2.25 %). According to Mathew *et al.* (1972), Nybe (1978) and Shankar (2003), Rio-de-Janeiro excelled all other types in yield of essential oil when they evaluated different cultivars for quality attributes.

Oleoresin content ranged from 4.31 to 8.93 per cent in the somaclones evaluated. Higher recovery of oleoresin was noticed in clones of Rio-de-Janeiro (4.38 to 8.93 %) than the clones of cultivar Maran (4.31 to 8.48 %). Higher oleoresin conent in the cultivar Rio-de-Janeiro has been reported by Lewis *et al.* (1972), Mathai (1972), Muralidharan (1973), Nair (1975), Nybe (1978) and Shankar (2003).

Fibre content ranged from 1.96 to 6.86 per cent in the somaclones studied. Somaclones of cultivar Maran recorded low fibre content (1.96 to 5.24 %) as

compared to cultivar Rio-de-Janeiro (4.27 to 6.86 %). Another significant observation in the present study was that in some of the clones of cultivar Maran, the fibre content recorded was as low as 1.96 percent in M VI and 2.28 per cent in 79 M. Nair (1975) and Shankar (2003) also observed low crude fibre content in cultivar Maran as compared to cultivar Rio-de-Janeiro.

The somaclones evaluated recorded 34 percentage increase in dry ginger recovery, 13 per cent in essential oil, 17 per cent in oleoresin and 25 per cent decrease in fibre content over CP plants.

Variations in quality attributes in somaclones of ginger were reported by Babu *et al.* (2005). Sudharshan and Bhat (1998) observed high oil content of 7.8 per cent in cardamom somaclone SKP 14 TC while the content in open pollinated seedlings were 7.40 per cent. Similar report on variation in quality attributes in calliclones of black pepper was reported by Sanchu *et al.* (2002).

5.3 SCREENING SOMACLONES AGAINST RHIZOME ROT AND BACTERIAL WILT DISEASES

5.3.1 Production of Toxic Metabolite(s) of *P. aphanidermatum* and Bioassay of the Metabolite(s)

Toxic metabolite(s) was found to accumulate in culture filtrate of *P. aphanidermatum*. The production of toxic metabolite(s) *in vitro* by *P. aphanidermatum* was confirmed by bioassay using ginger rhizomes and by electrolyte leakage assay (Tables 21, 22a and 22b). Toxic metabolite(s) of *P. aphanidermatum* produced rotting symptoms in ginger rhizomes similar to that of inoculation by culture disc of the pathogen thereby fulfilling the criteria prescribed for a phytotoxin by Graniti (1972). The toxic metabolite(s) induced electrolyte leakage from leaves. *In vitro* production of toxic metabolite(s) by *Pythium* spp. and production of symptoms by the metabolite(s) similar to that caused by infection with the pathogen has been reported by Janardhanan and Husain in *Atropa belladonna* (1974), Sadik *et al.* in maize (1982), Desilets and Berlarger (1991) and Desilets *et al.* (1991) in geranium and Kulkarni *et al.* (1987) in ginger.

Crude culture filtrate from three media viz. M_1 [(Czapex (Dox) agar) Onion *et al.* (1981)], M_2 (Richard's solution) and M_3 [(Asparagine or synthetic mucor) Hesseltine, 1954] produced rotting symptoms in ginger rhizomes. The area of rotting was more in CCF extracted from medium M_3 (1.96 cm^2) followed by M_2 (1.75 cm^2) and M_1 (1.66 cm^2). In electrolyte leakage assay also, highest leakage of electrolytes was observed in medium M_3 (24.44 µs) followed by medium M_2 (20.31 µs) and medium M_1 (17.49 µs), 10 minutes after treatment (Fig. 11a). Of the two incubation periods tried for production of toxic metabolite(s), 15-day incubation was found to accumulate maximum toxic metabolite(s) as evident by higher leakage values for CCF extracted after 15-day incubation period (22.05 µs) (Fig. 11b). According to Shaw (1981), production of toxin by different micro organisms varied widely according to the culture conditions, nutrients, nutrient sources, P^H of the medium, temperature and light conditions.

Concentrated culture filtrate from shaking cultures recorded higher leakage of electrolytes (22.72 µs) as compared to CCF from stationary cultures (18.78 µs) (Fig. 11c). Production of phytotoxin *in vitro* in shake cultures of *Phytophthora nicotianae* var. *parasitica* and *P. capsicii* was reported by Ballio *et al.* (1972) and Shylaja *et al.* (1997) respectively. The principal advantage of giving agitation is that fermentation will proceed at a faster rate, probably because it allows more rapid diffusion of oxygen through culture medium and media constituents (Shaw, 1981). The leakage of electrolytes decreased as the concentration of toxic metabolite(s) decreased. The leakage of electrolytes recorded was 21.60 µs in 10 per cent v/v concentration of toxic metabolite(s) while it was 19.90 µs in 5 per cent v/v concentration of metabolite(s).

The toxic metabolite(s) accumulated in culture filtrates of *P. aphanidermatum* was found to withstand heating, suggesting the thermostable nature of the metabolite(s). Hence in the present study, culture filtrate was concentrated at 100^0 C in a hot plate. Heat stability of the toxic metabolite(s) produced by *Pythium* spp. was reported by Janardhanan and Husain (1974), Sadik *et al.* (1982) and Desilets *et al.* (1991).

5.3.2 Production of Toxic Metabolite(s) of *R. solanacearum* and Bioassay

In the present study, *R. solanacearum* produced toxic metabolite(s) *in vitro* as confirmed by bioassay of toxic metabolite(s) using ginger shoots and by inducing electrolyte leakage from leaves (Tables 23a and 23b). The toxic metabolite(s) of *R. solanacearum* induced wilting symptoms on excised ginger shoots (Plate 14). None of the wilted shoots recovered from wilting when transferred to distilled water. Different concentrations of toxins viz. 2.5, 5.0, 7.5 and 10.0 per cent (v/v) extracted from *R. solanacearum* induced electrolyte leakage from leaves of ginger cultivars. Electrolyte leakage from leaves increased with increasing concentration of toxin. The highest leakage of electrolytes was observed in 10 per cent (v/v) toxin solution (33.43 µs) and the lowest in 2.5 per cent (v/v) toxin (26.98 µs) (Fig. 12). The production of toxic metabolite(s) *in vitro* by *R. solanacearum* was also reported by several workers (Gowda *et al.*, 1977; Samuel 1980; Hartriar *et al.*, 1985; Paul 1998; Jin *et al.*, 2001; Hareesh *et al.*, 2001; Yong *et al.*, 2002). Jin *et al.* (2001) observed significant positive correlation between the disease indices of *R. solanacearum* and toxins isolated from the bacterium. They suggested that crude toxins of the isolates of *R. solanacearum* provide a cheap and exact tool for identifying resistance to the pathogen.

5.3.3 Natural Screening of Somaclones in Sick Field Against Rhizome Rot and Bacterial Wilt Diseases

Seventy three somaclones of Maran and 78 somaclones of Rio-de-Janeiro were screened against rhizome rot and bacterial wilt diseases in a sick field in which there was serious incidence of rhizome rot and bacterial wilt diseases in the previous year. In the present investigation, it was observed that the incidence of rhizome rot was more in clones of cultivar Rio-de-Janeiro than in clones of cultivar Maran (Table 25). Nair (1969), Nair (1975) and Nybe (1978) reported that cultivar Maran exhibited tolerance reaction to rhizome rot disease under field conditions. Wilt incidence was more in clones of Rio-de-Janeiro than in clones of Maran. This is in agreement with Shankar (2003) who carried out ooze test in freshly harvested rhizomes of cultivars Maran, Rio-de-Janeiro, Himachal Pradesh and seven variants derived through

colchicine treatment. The cultivar Rio-de-Janeiro showed maximum ooze of the bacterium while the intensity of bacterial ooze was moderate in cultivar Maran.

In the present study, fourteen percent somaclones of cultivar Maran and 12 per cent somaclones of cultivar Rio-de-Janeiro were not affected by rhizome rot and bacterial wilt diseases in the sick field (Plate 15). As in the present study, two sheath blight resistant lines were isolated (LSBR 33 and LSBR 5) in rice by screening 2000 somaclones in field nurseries inoculated with the sheath blight (*Rhizoctonia solani*) pathogen (Xie *et al.*, 1992). Similarly, for selection of somaclones of Jerusalem artichoke (*Helianthus tuberosus* L.) resistant to basal stem and tuber rot pathogen (*Sclerotinia sclerotiarum*), *in vitro* and field-screening methods were employed by Cassells and Walsch (1995). When the selected somaclones were screened in soil heavily infected with the pathogen, the somaclones took no field infection. The clones selected showed tolerance reaction in artificial inoculation studies also. Similar studies on isolation of tolerant/resistant somaclones in natural screening in sick field have been reported in banana by Chuan *et al.* (2000) and in tomato by Mandal (1999) and Devi *et al.* (2005). In the present study also, field evaluation and natural screening of somaclones were done simultaneously. The clones that survived in the sick field showed no incidence of the disease in the evaluation plot showing the effectiveness of natural screening method in the selection of plant types tolerant to diseases.

5.3.4 Screening of Somaclones Against Rhizome Rot and Bacterial Wilt Diseases by Electrolyte Leakage Method

In the present investigation, CCF induced quick electrolyte leakage from leaves suggesting the possibility of plasmalemma as the site of action of the toxic metabolite(s) (Tables 22a and 23a). Plasmalemma has been suggested as the primary site of action for some pathotoxins, although there is no conclusive evidence for this in any case (Rudolph, 1976; Scheffer, 1976 and Yoder, 1980). Changes in plasmalemma characteristics occur quickly after exposure to toxin. In several species, loss of electrolytes occurs in tissues infected with the fungus or treated with its toxin (Wheeler and Black, 1963; Samaddar and Scheffer, 1968) or with its culture filtrates

(Collins and Scheffer, 1958). Damann *et al.* (1974) showed that an electrolyte leakage assay by *Helminthosporium victoriae* toxin is as sensitive as standard seedling root growth assay. Hence, the increase in conductivity of lechates of host tissues when treated with pathogenic toxins has been used as a sensitive assay for many *in vitro* studies of pathogenicity.

Seventy seven somaclones of cultivar Maran and 75 somaclones of cultivar Rio-de-Janeiro (first set) were screened in the present study against rhizome rot and bacterial wilt diseases using toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum* (Table 26). In general, somaclones exhibited low leakage of electrolytes as compared to CP plants. According to Cristinzio *et al.* (1995) and Sundar *et al.* (1999), loss of electrolytes from the resistant genotypes is significantly lower than that from the susceptible genotypes. Sixty per cent somaclones recorded lower leakage of electrolytes than CP plants when screened with toxic metabolite(s) of *P. aphanidermatum*. Similarly, 14 per cent somaclones recorded lower leakage values than CP plants when screened with toxic metabolite(s) of *R. solanacearum*. Similar studies on selection of disease resistant somaclones by electrolyte leakage method was reported by Leal and Maribona (1991) in sugarcane against brown eye spot disease. They screened leaves and calli of sugarcane varieties with *Helminthosporium sachari* toxin by electrolyte leakage method and isolated resistant somaclones.

The second set somaclones produced by indirect organogenesis/ embryogenesis and *in vitro* mutagenesis were also screened against rhizome rot and bacterial wilt diseases. A total of 296 regenerants (Maran – 146, Rio-de-Janeiro – 150) were subjected to screening for diseases. Regenerants derived through somatic embryogenesis exhibited less leakage of electrolytes when screened with toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum* indicating more tolerance of the regenerants to the two diseases (Tables 24 and 24a to 24e) (Fig. 14a and 14b)). Liu *et al.* (2005) reported that doubled haploid lines of rapeseed derived through somatic embryogenesis exhibited greater resistance to stem rot disease (*Sclerotinia sclerotiarum*) than the donor lines and the resistant control Zhongyou 821.

The response of two cultivars to leakage of electrolytes indicated that the somaclones of cultivar Maran recorded low leakage of electrolytes and were tolerant to rhizome rot and bacterial wilt diseases. The leakage of electrolytes was low in somaclones of first and second sets in the cultivar Maran. The tolerance/reaction of cultivar Maran to rhizome rot disease and susceptibility of cultivar Rio-de-Janeiro to the disease has been reported by Nair (1969), Nair (1975) and Nybe (1978). According to Shankar (2003), cultivar Rio-de-Janeiro showed maximum intensity of bacterial ooze while the intensity was moderate in cultivar Maran when ooze test was done in freshly harvested rhizomes.

5.3.5 Artificial Screening of Selected Superior Somaclones Against Rhizome Rot and Bacterial Wilt Diseases

Artificial screening was done in five somaclones each of cultivars Maran and Rio-de-Janeiro, selected based on yield and tolerance reaction to diseases. In three somaclones, the symptoms of rhizome rot disease appeared late (M VI, 364 R and R XI) and subsequent germination of rhizomes and growth were noticed in these clones (Table 27, Fig. 17a and 17 b, Plates 16 and 18). Similarly, the incidence of bacterial wilt was noticed late in three somaclones (970 M, M VI and 364 R) (Fig. 18a and 18b, Plates 17 and 19) and subsequent germination and growth were noticed in the clones (Table 28). Araujo *et al.* (2001a) have highlighted the tolerance / resistance reaction exhibited by somaclones of rice in artificial screening experiments. They screened 17 somaclones of rice cultivar IAC 47 against rice blast disease by inoculating spore suspension (3×10^5 spores ml^{-1}) of *Pyricularia grisea* to the plant. From the study, two somaclones were identified which were resistant to all the isolates of *P. grisea.*

Three different screening methods viz. natural screening in sick field, screening by electrolyte leakage and screening by artificial inoculation of the pathogen were carried out for assessing the disease reaction of somaclones. The clones that were found tolerant in natural screening and electrolyte leakage studies exhibited the same type of disease reaction in artificial screening also. The clones M VI and 364 R showed tolerance to both rhizome rot and bacterial wilt diseases in three different

screening methods. In asexually propagated species, transmission of the variant traits through at least two successive clonal propagation cycles provides reasonable assurety of a true genetic base. Multiple screening methods were employed in several screening experiments to isolate tolerant / resistant plant types to diseases. Isolation of tolerant calliclones of black pepper to *Phytophthora* foot rot disease by conducting different screening methods were reported by Shylaja *et al.* (1996) and Sanchu *et al.* (2003). Similarly, the tolerance of sweet potato var. 97-10 to wilt disease was reported by Yong *et al.* (2002) by screening sweet potato varieties with crude toxin of *R. solanacearum* in green house and further screening of selected types in disease affected areas.

5.4 MOLECULAR CHARACTERISATION OF SELECTED SUPERIOR SOMACLONES

Genomic DNA was isolated from 12 superior somaclones of cultivars Maran and Rio-de-Janeiro, selected based on yield and tolerance to diseases along with CP plants of two cultivars by modified Doyle and Doyle (1987) method (method Ia) (Plate 21). The quality of DNA isolated was assessed by agarose gel electrophoresis. Twenty seven random primers were screened for amplification of DNA using template DNA of cultivar Maran. Five primers (OPP 16, OPP 17, OPAH 1, OPAH 3 and OPAH 5) that gave good amplification with clear and distinct bands were selected for characterization of the somaclones. Percent polymorphism exhibited by primers ranged from zero (OPP 16) to 33.75 per cent (OPAH3) (Table 31 and Plate 22). The primers OPAH 3 and OPAH 1 gave highest polymorphism indicating the presence of heterologous sequences in the genomic DNA. Amplification by the primer OPP 16 has given no polymorphism indicating the presence of homologous sequences.

DNA amplification with different primers revealed polymorphism in banding pattern in the somaclones of Maran and Rio-de-Janeiro. Of the different somaclones of cultivar Maran, M VI exhibited highest polymorphism with CP plant and rest of the somaclones. With regard to somaclones of cultivar Rio-de-Janeiro, the clone 292 R expressed highest polymorphism in banding pattern suggesting that

in vitro conditions have induced varied amount of genetic changes in different somaclones.

Somaclones of ginger exhibited 15.7 per cent genetic variability. The extent of genetic variation was more in somaclones of cultivar Maran (0 – 31.6 %) with an average variability of 16.8 per cent. Somaclones of cultivar Rio-de-Janciro exhibited 2.8 to 20 per cent variability with an average variability of 10.8 per cent. The extent of genetic variation from the CP plant was more in somaclones of cultivar Maran (12 %) than clones of cultivar Rio-de-Janeiro (10.8 %). Analysis of genetic variability in different somaclones indicated that all the somaclones had varied degree of genetic difference within clones and between clones and CP plants. The dendrogram constructed from the pooled data of the RAPD scores with five different primers expressed the genetic make up of the somaclones and the CP plants (Fig. 19a and 19b). Soniya *et al.* (2001) suggested that *in vitro* regeneration process is mainly responsible for RAPD banding difference in the somaclones. Riji (2003) reported polymorphism in RAPD profiles of nine turmeric somaclones regenerated through indirect organogenesis. The clone TSC-1 isolated in the study showed highest polymorphism with the CP plant. Results obtained in the present investigation indicated induction of random changes during the *in vitro* regeneration process of the somaclones.

In the present investigation, protocols for indirect organogenesis / embryogenesis and dose of γ irradiation for *in vitro* mutagenesis in ginger were standardised. The regenerants produced through various routes (second set somaclones) were subjected to growth analysis. Plantlets derived through indirect organogenesis exhibited more pseudostem height and plantlets produced through *in vitro* mutagenesis exhibited higher tiller number. Regenerants of indirect organogenesis exhibited higher root number and root length and higher plantlet establishment followed by regenerants of *in vitro* mutagenesis and indirect embryogenesis. Variability exhibited for morphological characters in the regenerants produced through various routes was almost uniform.

The first set somaclones regenerated through *in vitro* adventitious bud culture were evaluated for morphological parameters, yield, quality and reaction to diseases. Based on the study, chances of getting somaclones with high yield, quality and tolerance to rhizome rot and bacterial wilt diseases were confirmed. The somaclones were found superior to CP plants for various growth parameters, yield, quality and tolerance to diseases. Twenty one per cent somaclones were found superior to CP plants in growth characters like height of pseudostem, number of tillers / plant, number of leaves / tiller and leaf area and 36 per cent in growth rate of the characters. For rhizome characters, 25 per cent clones were found superior and for yield of fresh rhizome / plant, nine per cent clones were superior. The high yielding somaclones were found superior in various morphological characters and growth rate. In the present study, even though large number of somaclones (76 clones in third year) was evaluated in field, only nine per cent somaclones exhibited superiority in yield over CP plants giving an yield increase of 12 per cent.

Quality analysis done in first set somaclones indicated the superiority of somaclones in quality attributes like dry ginger recovery, percentage of essential oil, oleoresin and fibre content. The clones 488 M, 110 M and 970 M exhibited dry ginger recovery in the range of 22.50 to 25 per cent exhibiting 19 to 56 per cent increase over CP plants. The clones 342 M and 312 R recorded essential oil content in the range of 2.25 to 2.50 percent giving 7.14 to 19.05 per cent increase over CP plants. The somaclones 342 M, 281 R, 136 M and 364 R recorded oleoresin content in the range of 1.96 to 2.57 per cent giving an increase of 3.62 to 29.42 per cent over CP plants. Fibre content was found low (1.96 to 2.57 %) in the clones 488 M, 85 M, 79 M and M VI exhibiting 14.62 to 34.88 per cent decrease over CP plants.

In vitro production of toxic metabolites of *P. aphanidermatum* and *R. solanacearum* and their bioassay were standardized in the present study. Screening procedures were also developed for rhizome rot and bacterial wilt diseases in ginger. Preliminary screening done against diseases in second set somaclones by electrolyte leakage method revealed that 24 per cent of the clones exhibited low leakage of electrolytes showing their tolerance to diseases. Regenerants derived through indirect

embryogenesis showed higher tolerance to diseases in the electrolyte leakage assay, which needs further confirmation in field evaluation and detailed screening trials. Detailed screening of first set somaclones against diseases was done by three methods viz. screening in sick field, by electrolyte leakage method and by artificial inoculation of the pathogens. In natural screening of somaclones in sick field, 13 per cent somaclones were not affected by rhizome rot and bacterial wilt diseases. Screening by electrolyte leakage method indicated that 32 per cent somaclones were tolerant to rhizome rot disease and 21 per cent to bacterial wilt disease exhibiting low leakage of electrolytes. Artificial screening of ten selected somaclones against rhizome rot and bacterial wilt diseases revealed that the clones M VI, 364 R and R XI were tolerant to rhizome rot disease and the clones 970 M, M VI and 364 R were tolerant to bacterial wilt disease. Based on the three screening methods, the clones M VI and 364 R were found tolerant to both rhizome rot and bacterial wilt diseases (Plate 23). The morphological, yield, quality parameters and disease reaction of the selected clones are presented in Table 33.

The assessment of variability done in somaclones of ginger by different methods revealed the occurrence of high amount of somaclonal variation in ginger. Somaclones exhibited wide variability in characters like number of tillers / plant, number of secondary fingers, internodal length of primary fingers and yield of fresh rhizomes / plant. The somaclones also exhibited wide variations for growth rate in morphological characters. The extent of somaclonal variation in ginger was cultivar dependent and the cultivar Maran exhibited higher variability suggesting the possibility of exploiting somaclonal variation for selection of elite plant types. However, the variability observed for reaction to diseases was low in the somaclones studied as compared to morphological and yield parameters. The variability observed was high for rhizome rot disease as compared to bacterial wilt disease. Somaclones of cultivar Rio-de-Janeiro exhibited higher variation for reaction to diseases. The mode of regeneration tried also determined the extent of somaclonal variation in ginger. The regenerants derived through indirect embryogenesis exhibited high amount of somaclonal variation for reaction to diseases. Molecular characterisation done using RAPD markers revealed the occurrence of genetic variation in somaclones. The

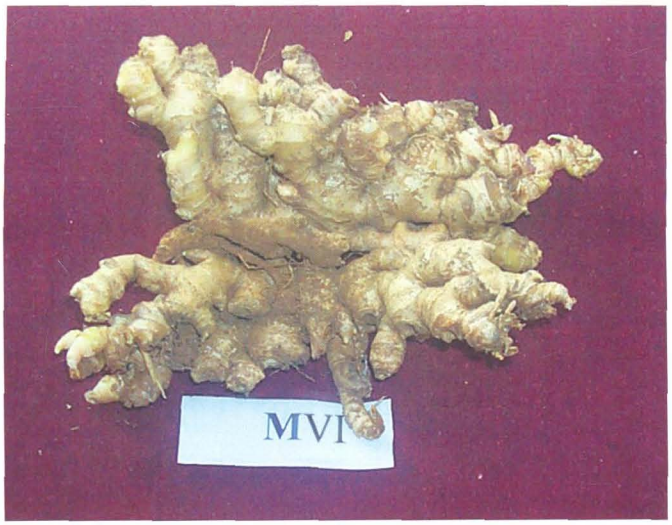


Plate 23 Selected superior somaclones in ginger

genetic variability observed was high in the cultivar Maran. The genetic variability from CP plant was also high in the cultivar Maran.

Table 33. Characters of selected superior somaclones and conventionally propagated plants in ginger (*Z. officinale* Rosc.)

Character		Clone No.		Conventionally propagated	
		M VI	364 R	Maran	Rio-de-Janeiro
Fresh yield of rhizomes / plant (g)		348.75	250.70	315.56	204.45
Dry ginger recovery (%)		17.95	15.62	15.38	16.66
Essential oil (%)		1.50	2.07	1.95	2.25
Oleoresin (%)		5.91	8.93	6.65	7.14
Fibre (%)		1.96	6.86	2.95	3.06
Reaction to diseases					
Survival in sick field		Survived	Survived	Not survived	Not survived
Electrolyte leakage (µs) induced by	*P. aphanidermatum*	22.75	22.30	37.95	41.90
	R. solanacearum	42.55	39.70	35.00	43.55
Artificial screening		Tolerant	Tolerant	Susceptible	Susceptible

The high amount of somaclonal variation observed in cultivar Maran make it an ideal source material for further exploitation. Among the somaclones produced through various routes, further investigation should be focused on somatic embryo derived regenerants because of the high amount of somaclonal variation and high degree of tolerance observed in regenerants against diseases which however needs further confirmation in field evaluation trials. The regeneration and screening protocols developed in the present study could be utilised in further investigations. The superior somaclones identified in the study need to be evaluated in the farmers' fields at multilocations to assess their performance in the actual farming situation.

Summary

SUMMARY

Investigations on "Induction of variation *in* vitro and field evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" were carried out at the Department of Plantation Crops and Spices and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara, Thrissur during April 2002 to October 2005. The study was aimed to induce variability in ginger (cultivars Maran and Rio-de-Janeiro) through in *vitro* techniques, to evaluate somaclones of ginger for morphological, yield, quality and reaction to rhizome rot and bacterial wilt diseases and to characterise the selected superior somaclones using RAPD markers. The salient findings of the study are listed below.

1. The technique of indirect organogenesis in ginger was standardised.

- Shoot tip explants were identified as the best explant for callusing, registering early (27.61 days) and higher callusing (72.52%) and higher callus growth.
- Half MS medium supplemented with 2,4-D at 1.00 mg l^{-1} was most effective for callusing and callus growth.
- Incubation of cultures in dark and light were found on par with respect to callusing and callus growth in the two cultivars.
- Half MS medium supplemented with BAP at 3.00 mg l^{-1} recorded highest percentage of shoot morphogenesis (38.46).
- Shoot and root morphogenesis were simultaneous in callus cultures and root morphogenesis occurred in half MS medium supplemented with BAP (3.00 mg l^{-1}).
- The cultivar Rio-de-Janeiro recorded higher callusing and callus growth, early and high shoot morphogenesis and high rate of shoot proliferation than the cultivar Maran.

2. The technique of indirect embryogenesis in ginger was standardised.

- Half MS medium supplemented with 2,4-D and BAP each at 0.50 and 1.00 mg l^{-1} induced embryogenic calli from rhizome bud explants.

- Incubating cultures under dark was significantly superior to incubation under light with respect to production of embryogenic calli.
- Half MS basal medium was found good for proliferation and maturation of somatic embryoids.
- Inclusion of BAP (3.00 mg l^{-1}) in the basal half MS medium was found to enhance germination of somatic embryoids.
- The cultivar Maran recorded higher percentage of embryogenic calli (90) than the cultivar Rio-de-Janeiro (76). The cultivar Rio-de-Janeiro registered higher proliferation and germination of somatic embryoids.

3. The dose of γ irradiation for *in vitro* mutagenesis in ginger was standardized.
   - The organogenic cultures of two cultivars and embryogenic cultures of cultivar Rio-de-Janeiro could withstand γ irradiation dose of 10 Gy.
   - The embryogenic cultures of cultivar Maran could withstand higher dose of γ irradiation (20 Gy).
4. Root characters and plantlet establishment in regenerants produced through various routes were studied.
   - Regenerants produced through indirect organogenesis exhibited better root characters and plantlet establishment.
   - Regenerants of cultivar Maran were better in various root characters and plantlet establishment as compared to cultivar Rio-de-Janeiro.
   - Regenerants derived through *in vitro* mutagenesis recorded more tillering at plant out and high growth rate in number of tillers.
5. Field evaluation of first set somaclones for three seasons (2002, 2003 and 2004) revealed the superiority of somaclones over CP (conventionally propagated) plants in various morphological characters yield, quality and tolerance to diseases.
   - Somaclones exhibited superiority over CP plants in morphological characters such as height of pseudostem, number of tillers and leaves/clone and also in growth rate.

- The somaclones of cultivar Rio-de-Janeiro exhibited higher length of pseudostem and number of tillers and leaves/clone and high growth rate in height of pseudostem, number of tillers and leaves/clone.
- Somaclones of cultivar Maran were better in leaf characters such as leaf length, breadth and area of leaf.
- The cultivar Maran exhibited higher somaclonal variation for morphological characters like number of tillers and leaves/clone and also in growth rate.
- Somaclones of cultivar Maran exhibited superiority in rhizome characters.
- Nine percent somaclones exhibited superiority in yield over CP plants.
- Somaclones of cultivar Maran exhibited higher yield than the cultivar Rio-de-Janeiro.
- The extent of somaclonal variation for yield was more in somaclones of cultivar Maran.
- Higher driage and low fibre content were noticed in somaclones of cultivar Maran.
- Higher recovery of essential oil and oleoresin were noticed in somaclones of cultivar Rio-de-Janeiro.

7. Standardised the production of *in vitro* toxic metabolite(s) and screening procedures for rhizome rot and bacterial wilt diseases in ginger.

- Toxic metabolite(s) was found to accumulate *in vitro* in culture filtrate of *P. aphanidermatum.*
- Toxic metabolite(s) of *P. aphanidermatum* accumulated maximum in shake, liquid cultures of medium M_3 [(Asparagine or synthetic mucor) Hesseltine, 1954].
- Bioassay of toxic metabolite(s) of *P. aphanidermatum* produced rotting symptoms in ginger rhizomes similar to that of inoculation by culture disc of the pathogen.
- The toxic metabolite(s) of *R. solanacearum* induced wilting symptoms on excised ginger shoots similar to that of inoculation of bacterial ooze.
- The toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum* induced quick electrolyte leakage from leaves of ginger.

- Preliminary screening of 296 somaclones (second set) against rhizome rot and bacterial wilt diseases by electrolyte leakage method revealed that 24 per cent of the clones exhibited low leakage of electrolytes showing their tolerance to diseases.
- Detailed screening of first set somaclones against diseases was done by three methods viz. screening in sick field, by electrolyte leakage method and by artificial inoculation of the pathogens.
    - In natural screening of somaclones in sick field, 13 per cent somaclones were not affected by rhizome rot and bacterial wilt diseases.
    - Screening by electrolyte leakage method indicated that 32 per cent somaclones were tolerant to rhizome rot disease and 21 per cent to bacterial wilt disease exhibiting low leakage of electrolytes.
    - Artificial screening of ten selected somaclones against rhizome rot and bacterial wilt diseases revealed that the clones M VI, 364 R and R XI were tolerant to rhizome rot disease and the clones 970 M, M VI and 364 R were tolerant to bacterial wilt disease.
    - Based on the three screening methods, the clones M VI and 364 R were found tolerant to both rhizome rot and bacterial wilt diseases.

8. Twelve selected superior somaclones were characterised using RAPD markers.
    - DNA isolation by modified Doyle and Doyle (1987) method (method Ia) gave good quality DNA in sufficient quantity.
    - Five primers of the series OPAH and OPP gave good DNA amplification.
    - The primers OPAH 3 and OPAH 1 exhibited highest polymorphism in banding pattern
    - The somaclones of ginger exhibited genetic variability.
    - The extent of genetic variation was more in somaclones of cultivar Maran (0 – 31.6 %) with an average variability of 16.8 per cent.
    - Somaclones of cultivar Rio-de-Janeiro exhibited 2.8 to 20 per cent variability with an average variability of 10.8 per cent.
    - The extent of genetic variation from the CP plant was more in somaclones of cultivar Maran (12 %) than in clones of cultivar Rio-de-Janeiro (10.8 %).

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* Originals not seen

Appendices

APPENDIX I

Chemical composition of different media used in the study

Constituents	Quantity (mg/l)
1) Murashige and Skoog (1962) medium	
Major elements	
$CaCl_2.2H_2O$	440.00
$FeSO_4.H_2O$	27.800
KNO_3	1900.00
KH_2PO_4	170.00
$MgSO_4.7H_2O$	370.00
NH_4NO_3	1650.00
Na_2EDTA	37.30
Minor elements	
$CaCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
H_3BO_3	6.200
KI	0.830
$MnSO_4$	22.300
$Na_2MoO_4.2H_2O$	0.250
$ZnSO_4$	8.600
Organic constituents	
Glycene	2.000
Myoinositol	100.000
Nicotinic acid	0.500
Pyridoxine HCl	0.500
Thiamine HCl	0.100
Sucrose	30.000 g
Agar	7.500 g
2) Potato Dextrose Agar (PDA) medium	
Potato	200 g
Dextrose	20 g
Agar	20 g
3) Czapek (Dox) agar medium (Onion *et al.*, 1981)	
$NaNO_3$	2000.00
KH_2PO_4	1000.00
$MgSO_4.7H_2O$	500.00
KCl	500.00
$FeSO_4.7H_2O$	10.00
Sucrose	30 g
4) Richard's solution	
KNO_3	10 g
KH_2PO_4	5 g
$MgSO_4.7H_2O$	2500.00

Constituents	Quantity (mg/l)
$FeCl_3$	20.00
Sucrose	50 g
5) Asparagine or synthetic mucor medium (Hesseltine, 1954)	
Dextrose	40 g
Asparagine	2 g
KH_2PO_4	500
Thiamine chloride	5.0
6) Triphenyl Tetrazolium Chloride medium (TZC)	
Peptone	10 g
Casamino acid	1000.00
Glucose	5000.00
Agar	20 g
pH	6.8
7) Peptone casamino acid broth	
Peptone	10 g
Casamino acid	1000.00
Glucose	5000.00
pH	6.8

Appendix II

Reagents used for molecular biology works

I. Extraction buffer (4X)

Sorbitol	- 256.00 g
Tris HCl	- 48.00 g
EDTA disodium salt	- 7.40 g
sodium metabisulfate	- 3.80 g
Milli Q water	- 1000 ml

II. Lysis buffer (CTAB 2X buffer)

CTAB	- 2 %
Tris HCl (pH 8)	- 100 mM
Na_2 EDTA (pH 8)	- 20 mM
NaCl	- 1.4 M

III. CTAB 10 %

CTAB	- 10 g
Milli Q water	- 100 ml

IV. TE buffer

Tris (pH 7.4)	- 10 mM
EDTA (pH 8)	- 1 mM

V. Isopropanol

VI. Chloroform: Isoamyl alcohol mixture (24:1, v/v)

VII. 5% sarcosin

VIII 90% Ethanol

IX TAE buffer (50X)

Tris base	- 242 g
Glacial acetic acid	- 57.10 ml
EDTA 0.5 M (pH 8)	- 100.00 ml
MilliQ water	- 1000 ml

X Gel loading dye

Glycerol	- 60 %
TAE buffer	- 30 %
1% Bromophenol blue	- 10 %

XI Ethidium bromide 1.00 mg in 1.00 ml (stock)

Appendix III

Monthly weather data during crop period at Vellanikkara

2002

Month	Max. temp. (^{0}C)	Min. temp. (^{0}C)	Mean RH (%)	Rain fall (mm)	Rainy days	Sunshine hours
January	32.80	22.70	62.00	0.00	0	8.10
February	34.30	22.40	50.00	0.00	0	8.30
March	36.20	24.10	63.00	16.20	2	8.20
April	35.00	24.80	71.00	50.80	4	7.80
May	32.60	24.50	87.00	308.40	12	5.70
June	30.00	23.30	86.00	533.50	22	2.70
July	29.80	23.10	77.00	354.20	21	3.40
August	28.90	22.90	83.00	506.60	19	3.10
September	31.10	23.00	71.00	124.00	8	7.80
October	30.80	23.20	45.00	387.70	19	4.40
November	31.80	23.40	50.00	22.10	3	6.30
December	32.30	22.10	62.00	0.00	0	8.70

2003

Month	Max. temp. (^{0}C)	Min. temp. (^{0}C)	Mean RH (%)	Rain fall (mm)	Rainy days	Sunshine hours
January	33.20	22.90	50.00	0.00	0	9.40
February	34.70	23.60	63.00	162.10	5	9.20
March	34.60	24.10	67.00	94.80	4	8.50
April	34.60	25.00	72.00	23.80	3	7.50
May	34.00	25.00	72.00	403.00	3	6.30
June	30.90	23.80	80.00	570.60	19	4.00
July	29.50	22.20	84.00	492.60	22	2.50
August	30.00	23.40	83.00	490.10	19	4.20
September	31.00	22.70	79.00	53.70	7	7.30
October	30.80	23.10	81.00	276.60	14	5.60
November	31.50	23.90	66.00	18.20	1	7.10
December	32.20	21.90	61.00	0.00	0	9.10

2004

Month	Max. temp. (^{0}C)	Min. temp. (^{0}C)	Mean RH (%)	Rain fall (mm)	Rainy days	Sunshine hours
January	33.40	22.30	58.00	0.00	0	9.60
February	35.20	22.50	50.00	0.00	0	9.60
March	36.50	24.20	61.00	8.60	1	8.60
April	34.80	25.20	69.00	60.20	6	7.40
May	30.40	23.60	84.00	578.30	21	3.40
June	29.60	22.30	85.00	786.00	24	3.30
July	29.30	22.90	85.00	369.60	24	3.3
August	28.50	23.10	83.00	386.90	14	4.40
September	30.80	23.60	80.00	208.80	10	5.10
October	30.80	23.40	73.00	493.20	11	6.00
November	31.40	23.70	65.00	71.70	3	7.10
December	30.10	22.60	55.00	0.00	0	8.90

Appendix IV

Correlation coefficients worked out with weather parameters and shoot borer incidence in somaclones of ginger

Weather parameters	June	July	August	September	October
Rain fall (mm)	-0.495**	-0.476**	+0.489**	-0.041	+0.015
Rainy days	+0.096	-0.611**	+0.403**	-0.150	+0.752**
Minimum temperature (^{0}C)	+0.150	+0.545**	-0.651**	-0.150	-0.150
Maximum temperature (^{0}C)	-0.178	0.743**	-0.213	+0.611**	-0.403
Relative humidity (%)	+0.510**	-0.403**	+0.810**	-0.765**	+0.529**
Sunshine hours	-0.683**	+0.470**	-0.802**	-0.520**	-0.786**

INDUCTION OF VARIATION *IN VITRO* AND FIELD EVALUATION OF SOMACLONES IN GINGER (*Zingiber officinale* Rosc.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Investigations on "Induction of variation *in vitro* and field evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" were carried out at the Department of Plantation Crops and Spices and Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara, Thrissur during April 2002 to October 2005. The objectives of the study were to induce variability in ginger through *in vitro* techniques, to evaluate somaclones of ginger for morphological, yield and quality parameters and for reaction to rhizome rot and bacterial wilt diseases and to characterise the selected superior somaclones using RAPD markers. The studies were carried out in two cultivars of ginger viz., Maran and Rio-de-Janeiro.

Protocol for indirect organogenesis was developed in ginger using shoot tip explants. Half MS medium supplemented with 2,4-D at 1.00 mg l^{-1} exhibited higher callusing and callus growth. For shoot morphogenesis and rooting, half MS medium supplemented with BAP at 3.00 mg l^{-1} was found ideal.

The protocol for indirect embryogenesis was developed in ginger using rhizome bud explants. The explants produced embryogenic calli in half MS medium supplemented with 2,4-D (0.50-1.00 mg l^{-1}) and BAP (0.50-1.00 mg l^{-1}). Dark incubation of cultures favoured production of embryogenic calli. Basal half MS medium was found good for proliferation and maturation of somatic embryoids. Inclusion of BAP (3.00 mg l^{-1}) in the basal half MS medium was found to enhance germination of somatic embryoids.

Dose of γ irradiation for *in vitro* mutagenesis of organogenic / embryogenic cultures of ginger was standardized. Plantlets were produced from irradiated calli of the two cultivars.

Growth analysis in regenerants produced through various routes showed that regenerants produced through indirect organogenesis exhibited better root characters and plantlet establishment. Regenerants produced through *in vitro* mutagenesis exhibited better tillering characters.

Evaluation of somaclones (first set) for three seasons viz. 2002, 2003 and 2004 revealed the superiority of somaclones over conventionally propagated (CP) plants in yield and quality attributes. Of the somaclones of two cultivars studied, somaclones of cultivar Maran recorded higher yield, recovery of dry ginger and low fibre content.

Screening procedures were standardised for rhizome rot and bacterial wilt diseases in ginger. The toxic metabolites of pathogens were found to accumulate *in vitro*. The symptoms produced by the toxic metabolites were similar to that of inoculation by the pathogens. The toxic metabolites induced quick electrolyte leakage from leaves of ginger. Detailed screening done in first set somaclones could locate two somaclones (M VI and 364 R) showing tolerance to rhizome rot and bacterial wilt diseases. Preliminary screening done in second set somaclones by electrolyte leakage method revealed that 24 per cent clones exhibited low leakage of electrolytes showing their tolerance to diseases.

Analyses of various morphological and yield parameters and reaction to diseases revealed the occurrence of high amount of somaclonal variation in ginger. The extent of somaclonal variation observed was high in somaclones of cultivar Maran for morphological, yield and quality attributes. Somaclones of cultivar Rio-de-Janeiro exhibited higher variation for reaction to diseases.

Documentation of selected superior somaclones of first set was done using RAPD markers. The RAPD analysis done in selected clones revealed the occurrence of genetic variation in ginger somaclones. The genetic variability was high in somaclones of cultivar Maran (16.8 %) as compared to cultivar Rio-de-Janeiro (10.8 %).

The regeneration and screening protocols developed in the present study could be utilised in further investigations. The superior somaclones identified in the study could be advanced to further evaluation programmes.