CHARACTERISATION AND EVALUATION OF SOMACLONES IN GINGER (*Zingiber officinale* Rosc.)

by

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(2011-12-109)

DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

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THESIS

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KERALA, INDIA

2013

DECLARATION

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

Vellanikkara
28/12/2013

Aswathy Dev
(2011-12-109)

CERTIFICATE

Certified that the thesis entitled "Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" is a bonafide record of research work done independently by Ms. Aswathy Dev under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of **Ms. Aswathy Dev** (2011-12-109), a candidate for the degree of **Master of Science in Horticulture**, agree that the thesis entitled **"Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)"** may be submitted by Ms. Aswathy Dev, in partial fulfilment of the requirements for the degree.

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Aswathy Dev (2011-12-109)


Affectionately
dedicated
to my
family and friends

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), one of the most renowned spices of the Zingiberaceae family, is widely cultivated for its aromatic and pungent rhizomes and used as spice and as medicine. The essential oil and oleoresin are responsible for the characteristic flavor and pungency. It has gained considerable attention as a botanical dietary supplement in developed countries and especially for its use in treatment of chronic inflammatory conditions (Tjendraputra *et al*., 2001). Population based studies showed that people in South East Asian countries have a much lower risk of colon, gastrointestinal, prostrate, breast and other cancers than their Western counterparts. More than 100 compounds have been reported from ginger, gingerol one of the major pungent components is found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic and cardiotonic effects. Ginger is considered as "generally recognized as safe" (GRAS) by Food and Drug Administration (FDA), USA.

In the global scenario, India continues to be the largest producer, consumer and exporter of ginger and its products, with an area of 1,55,063 ha and 7,55,618 tonnes of dry ginger production (2011-12). The export during 2012-13 was 19,850 tonnes valued at Rs. 16,863.10 lakhs (www.indianspices.com dated 16-07-2013). Kerala, the major ginger growing state occupies an area of 6088 ha with a production and productivity of 33,197 tonnes and 5.45 tonnes ha^{-1} respectively (2010-11). Cochin and Calicut ginger marketed from Kerala are reputed in international market owing to its high instrinsic qualities.

The Indian ginger renowned in the world market is second only to Jamaican ginger, due to high fibre content and yellowish colour, which lower its competitive edge in the world trade. Rhizome rot caused by *Pythium* spp. and bacterial wilt caused by *Ralstonia solanacearum* are the major production constraints in ginger cultivation. Extant ginger cultivars are uniformly susceptible and conventional breeding methods are ineffective, because it is obligatory asexual, propagated exclusively through rhizomes. Conventional breeding techniques like evaluation of germplasm and selection, induction of variability by

mutagenesis and colchiploidy, though found promising, could not tackle these problems to an appreciable extent. Being viable alternatives, biotechnological interventions like *in vitro* pollination, *in vitro* mutagenesis, exploitation of somaclonal variation and genetic transformation were also attempted.

Exploitation of *in vitro* culture induced variation or somaclonal variation as a method to create variability is unique. Somaclonal variation is effectively utilized for producing useful phenotypic variants, improving yield, upgrading quality, imparting tolerance to diseases, drought, chilling and salt. Exploitation of somaclonal variation which has received centre stage attention in spice crops; used for isolating clones with high yield in ginger (Sumsudeen, 1996; Smith and Hamill, 1996; Babu, 1997; Pandey *et al*., 1997; Sharma and Singh, 1997; Rao *et al*., 2000; Freitz *et al.*, 2003; Shylaja *et al*., 2003; Paul, 2006; Kurian, 2010; Shylaja *et al*., 2010), turmeric (Salvi *et al*., 2002; Roopadarsini and Gayatri, 2012), kacholam (Geetha *et al*., 1997; Joseph, 1997), small cardamom (Reghunath, 1989; Lukose *et a.l*,1993; Reghunath and Priyadarshan, 1993; Chandrappa *et al.*,1997; Sudharshan *et al.*, 1997; Kuruvila *et al.*, 2005), large cardamom (Rao *et al*., 2003; Gupta *et al.*, 2005), black pepper (Sanchu, 2000; Sujatha, 2001; Rathy *et al.*, 2005), vanilla (Madhusoodanan *et al.*, 2005), better quality in ginger (Ramachandran and Nair, 1992; Bhagyalakshmi *et al.*, 1994; Rao *et al*., 2000; Paul, 2006; Shylaja *et al*., 2010), and disease tolerance in ginger (Sharma and Singh, 1997; Kavitha *et al*, 2005; Paul, 2006; Shylaja *et al*., 2010), black pepper (Shylaja and Nair, 1996; Sanchu *et al*., 2003) and cardamom (ICRI, 2003). Somaclonal variation in important agronomic traits and composition of aromatic oil was reported and superior somaclonal lines have been screened in palmarosa (Patnaik *et al*., 2000), java citronella (Mathur *et al*, 2006), mint (Kukreja and Dhawan, 2000), geranium (Ravindra *et al*., 2004 and Gauri *et al.*, 2008), jamrosa (Nayak *et al*., 2003) and rauvolfia (Alok, 2009).

Induction of variability through induced polyploidy attempted at Department of Plantation Crops & Spices, College of Horticulture, Vellanikkara, has succeeded in the development of two autotetraploids (Sheeba, 1996) with desirable quality attributes like low fibre content and high aromatic oil and oleoresin but susceptible to the diseases which restricts their commercial utility (Shankar, 2003). In order to increase the spectrum of

variability in these tetraploids, induction of variation *in vitro* through indirect methods of regeneration and mutagenesis was attempted as part of DBT funded project from 2006 to 2010. This has resulted in development of potential variants which on preliminary evaluation revealed wide variability in morphology, yield and reaction to major diseases and pests (Kurian, 2010). Systematic evaluation of these somaclones enables isolation of elite somaclones with desirable agronomic features, quality components and resistance/ tolerance to rhizome rot and bacterial wilt diseases.

The present investigations on "Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" were carried out with the objective of characterization and evaluation of somaclones in ginger for variability in morphology, yield, quality and reaction to rhizome rot and bacterial wilt diseases.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The investigations on "Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" focus on screening of 25 somaclones along with two check varieties and three parent clones for variability in morphological characters, yield and quality attributes and resistance/ tolerance to major pests and diseases. The literature related to these aspects in crop plants with special reference to ginger are dealt in this chapter.

2.1. CLONAL VARIATION IN GINGER

2.1.1. Morphology and Yield

Muralidharan (1973) studied the varietal performance of ginger in Wyanad, Kerala, and concluded that the cultivar Rio-de-Janeiro gave the highest fresh ginger yield, whereas the dry ginger yield was lowest in the cultivar. Dry ginger yield was highest in cultivar Tura. Cultivars Moran, Nadia and Thingui are the other high yielders and were more or less on par with cultivar Rio-de-Janeiro.

Nybe *et al*. (1978) investigated morphological variations in twenty five types of ginger and revealed that all the morphological characters varied among types except for leaf breadth, leaf area index and number of primary fingers. Height of plant, number of leaves per tiller, number of roots per plant was highest in Valluvanad and number of primary fingers per plant and secondary fingers per plant were more in Waynad Local and Bajpai respectively.

Nybe *et al*. (1979 b) reported that number of primary rhizome and internodal length of rhizomes was the most variable characters.

Mohanty *et al.* (1981) studied twenty eight cultivars of ginger for variations in number of tillers, number of leaves, plant height, leaf length, weight of straw, number of adventitious roots, number of root tubers, total number of rhizome fingers, girth of

secondary fingers and rhizome yield. The varietal differences for all the characters studied were significant.

Nybe *et al.* (1982) evaluated twenty eight cultivars for fresh and dry rhizome yield and noted significant differences among them. Fresh rhizome yield was highest in the case of cv. Nadia, followed by cultivars Moran, Bajpai, and Narasapattam. Cultivar Nadia also gave the higest yield of dry ginger. Sreekumar *et al.* (1982) found that cultivar Rio-de-Janeiro and Kuruppumpadi were the best yielders.

Ramachandran *et al.* (1982) and Ramachandran and Nair (1992) reported that successful production of stable tetraploid lines in cvs. Maran and Mananthody. The polyploids were more vigorous than the diploids and flowered during the second year of induction. The stable tetraploid lines had larger, plumpy rhizomes and high yield (198.7 g/ plant). However, the essential oil content was lower (2.3%) than the original diploid cultivar. There was a considerable increase in pollen fertility in tetraploids.

Sheeba (1996) developed two autotetraploids (Z-0-78 and Z-0-86) through colchiploidy from commercial cultivars Rio-de-Janeiro and Himachal Pradesh. The autotetraploids were characterized by slower initial growth which surpassed the diploid control during later stages, reduced number of tillers and leaves with increased leaf area and larger epidermal cells and stomata with reduced number/mm^2 of leaf. The autotetraploids derived from Rio-de-Janeiro showed increased pollen fertility than the diploids. The autotetraploids recorded higher rhizome yield than the corresponding diploids.

Germplasm evaluation carried out at IISR, Kozhikode, resulted in the selection of Varada, one of the important ruling cultivars at present in South and Central India (IISR, 2002).

Sasikumar *et al*. (2003) evaluated 15 bold rhizome accessions selected from ginger germplasm for yield and quality in multilocation trials. Based on the overall superior performance, accessions 35 and 107 were selected, multiplied and released for cultivation under the names IISR Rejatha and IISR Mahima, respectively.

Shankar (2003) evaluated seven colchicine induced variants including two autotetraploids (Z-0-78 and Z-0-86) along with three check varieties to study the extent of variability. The induced variants showed significant variation for all the morphological characters except pseudostem circumference, leaf area and leaf area index. Himachal Pradesh had maximum pseudostem circumference, thickness and leaf thickness while Z-0-86 had maximum leaf breadth. The autotetraploid Z-0-86 derived from Rio-de-Janeiro had maximum number of tillers per plant, number of leaves per plant, root length, leaf area and leaf area index whereas autotetraploid Z-0-78 derived from Himachal Pradesh recorded maximum plant height and pseudostem height. The autotetraploids Z-0-78 and Z-0-86 recorded increased cuticle thickness compared to corresponding diploids [Himachal Pradesh and Rio-de-Janeiro]. All the selected variants recorded lesser stomatal number with increased size compared to check varieties. The autotetraploids recorded maximum epidermal cell area with less number of cells per mm^2. The autotetraploids showed increased pollen fertility compared to diploid parents.

2.1.2. Quality

Ginger

Jogi *et al*. (1978) evaluated 14 cultivars and reported that the fibre content ranged from 4.62 (cultivar Poona) to 6.98 per cent (cultivar Narasapattam). Cultivar Karakkal was lowest in dry recovery followed by cultivars Wynad local and Rio-de-Janeiro. Cultivar Rio-de-Janeiro had the highest oleoresin, whereas cultivar Karakkal had the highest oil. Crude fibre was least in cultivars Nadia and China.

Nybe *et al*. (1982) evaluated 28 cultivars and reported that cultivars Rio-de-Janeiro had the highest oleoresin content of 10.53 followed by Maran (10.05%). Essential oil was highest in Karakkal (2.4%) and crude fibre was highest in Kuruppampadi (6.47%).

Ramachandran *et al.* (1982) and Ramachandran and Nair (1992) reported that successful production of stable tetraploid lines in cvs. Maran and Mananthody. The polyploids were more vigorous than the diploids and had larger, plumpy rhizomes and high yield (198.7 g/ plant).

Sreekumar *et al*. (1982) found that the dry ginger recovery ranged from 17.7 per cent in cultivar China to 28.00 per cent in cultivar Tura. Cultivars having more than 22% dry recovery (cultivars Moran, Jugijan, Ernad Manjeri, Nadia, Poona, Himachal Pradesh, Tura and Arippa) were found to be good for dry ginger production.

Mohanty and Panda (1991) studied induced mutation in ginger. Mutations were artificially induced in 5 ginger cultivars by employing one physical and three chemical mutagens. Twenty selected MV_3 generation mutants, along with the parental material, were compared in a 3-year yield evaluation. The highest yield was given by V_1K_1-3 (22.08 t/ha) followed by Suprabha (16.6 t/ha) and V_2E_5-2 (15.4 t/ha), in contrast to the parental cultivar UP (5.93 t/ha). The performance of 6 highest yielding lines, evaluated in a four year trial, confirmed the superiority of V_1K_1-3 (20.3 t/ha). Based on uniformly high yield, dry recovery, oleoresin and essential oil percentages, V_1K_1-3 mutant was recommended for release under the name Suravi during 1991.

Saika and Shadeque (1992) while evaluated twenty exotic and indigenous cultivars found Moran, Jorhat Hard, Thinladium and Wynad had high fibre contents (7-8%). Although not suitable as raw spices, Moran and Jorhat Hard were suitable for the extraction of oleoresins and volatile oils.

Makasone *et al*. (1999) reported that gingerol contents of the tetraploid strains were much higher than the diploid counterparts and they also showed that differences in pungency intensity between the diploids and the tetraploids, as evaluated by sensory test, were consistent with gingerol contents.

Shankar *et al*. (2003) evaluated seven induced variants along with three check varieties to exploit induced variability in ginger. Among the induced variants, autotetraploids Z-0-78 recorded the maximum driage (22.56%) whereas highest oil content was recorded in Z-0-86 (2.07%). Z-0-86 recorded the lowest fibre content (2.70%) while lowest oil content was recorded in Z-0-97 (1.20%). With respect to oil yield per hectare, Rio-de-Janeiro registered the maximum value of 76.02 kg ha^{-1} followed by autotetraploid Z-0-86 (55.50 kg ha^{-1}). The colour of oil varied from light yellow to dark yellow. Sensory evaluation indicated that Rio-de-Janeiro had good sensory score ("++++") and the least preferred was

Z-0-92 and Z-0-95 ("+"). With respect to oleoresin extracted with acetone and ethyl acetate, Z-0-86 recorded maximum content (9.16% and 7.74% respectively), whereas Rio-de-Janeiro gave the maximum oleoresin yield per hectare (280.15 kg ha^{-1} and 288.66 kg ha^{-1} respectively) followed by Z-0-86 (246.28 kg ha^{-1} and 207.97 kg ha^{-1} respectively). The colour of oleoresin extracted using acetone and ethyl acetate varied from pale brown to dark brown. Sensory evaluation of oleoresin indicated that Rio-de-Janeiro had most pleasing aroma with acetone and ethyl acetate as solvents. When extraction efficiency of solvents was compared, acetone was found to extract more oleoresin content (5.91%) than ethyl acetate (3.86%).

Smith *et al*. (2004) developed a tetraploid line named Buderim Gold, from Queensland a local cultivar. Tetraploid had compared the most favourably with 'Queensland' in terms of the aroma/ flavor profile and fibre content at early harvest, and had consistently good rhizome yield. The tetraploid had large rhizomes sections, resulting in a higher recovery of premium grade confectionery ginger and a more attractive fresh market product.

2.1.3. Screening for pests and disease incidence

2.1.3.1 Rhizome rot

Indrasenan and Paily (1974) reported that cultivar Maran showed field resistance to ginger rot caused by *Pythium aphanidermatum.*

Setty *et al*. (1995) evaluated 18 ginger cultivars against rhizome rot (*Pythium* sp.) and found that cultivars Suprabha and Himachal Pradesh showed less than 3 per cent disease incidence.

Panyanthatta (1997) tested 148 accessions of ginger and 7 related taxa for assessing their reaction to rhizome rot caused by *P. aphanidermatum*. All the accessions were susceptible and the incidence was less in five accessions: namely IISR- 73, 79, 215 and 250.

Nybe and Nair (1979 a) evaluated 25 ginger cultivars for rhizome rot incidence. Among the 25 cultivars, Rio-de-Janeiro showed maximum susceptibility (27.50%) to soft-

rot disease followed by Tafingiya (26.40%), Taiwan (23.40%) and Himachal Pradesh (16.30%). The infection was very mild in the types Maran (3.20%), Vengara (3.40%), Wynad Local (3.50%), Wynad Mananthody (3.60%) and Kuruppampady (3.60%). The incidence was medium in Bajpai (5.32%) and Nadia (7.50%).

Shankar (2003) screened seven variants along with three check varieties and found that Himachal Pradesh showed the least susceptibility to soft rot (20.28%).

2.1.3.2 Bacterial wilt

Indrasenan *et al.* (1982) studied reaction of different ginger types to bacterial wilt caused by *Ralstonia solanacearum* and reported that none of the 30 cultivars tested was resistant to the pathogen and that though they varied in susceptibility all yielded poorly when infected.

Shankar (2003) screened seven variants along with three check varieties and reported that Himachal Pradesh showed the least susceptibility to bacterial wilt (10.14%) which was confirmed by ooze test.

2.1.3.3 *Phyllosticta* leaf spot

Nybe and Nair (1979) evaluated 25 ginger cultivars for leaf spot incidence. The type Taiwan (1.69) was the most susceptible type to leaf spot followed by Narasappatom (1.66), Arippa (1.60), Valluvanad (1.61) and Rio-de-Janeiro (1.56) whereas the type Tafingiva (0.80) recorded the least incidence which was on par with Maran, Bajpai and Nadia.

Setty *et al*. (1995) studied the reaction of 18 cultivars of ginger to *Phyllosticta zingiberi* for 6 months under the coastal climate of Karnataka state. None of the cultivars tested was resistant to the disease. However, the cultivars Narasappattam, Tura, Nadia, Tetraploid and Thingpuri were classified as moderately resistant with a disease index less than 5 percent. Other cultivars, namely, Rio de Janerio, Kunduli Local, Waynad Local, Kuruppampady, Suravi and Karakal, were susceptible with a disease index of more than 10 percent.

Dohroo *et al*. (1987) evaluated Himachal Pradesh lines against Phyllosticta leaf spot and reported that none of the tested materials was resistant to *Phyllosticta zingiberi*; however, eight lines showed moderate resistance.

Singh *et al*. (1999) evaluated ginger germplasm for resistance to Phyllosticta leaf spot disease. Germplasm collections SG 554, VIS 18 and RGS-5 were reported to be resistant to the disease.

Shankar (2003) screened seven variants along with three check varieties to study the field tolerance to leaf spot disease. Z-0-78 and Z-0-95 were found to be the least susceptible cultivars to leaf spot, which is scored as very light (0.68) while Rio-de-Janeiro showed the maximum incidence, which is scored as medium (2.00).

2.1.3.4 Shoot borer

Nybe and Nair (1979 a) studied reaction of various types of ginger to shoot borer and reported that among the 25 cultivars of ginger screened, though not significant, Valluvanad (43.40%) was the most affected one followed by Jorhat (40.20%), Nadia (38.80%), China (37.60%), Assam (36.50%) and Vengara (36.30%). It was also noticed that the types Rio-de-Janeiro (21.30%), Wynad Kunnamangalam (24.30%), Arippa (24.70%) and Thodupuzha (25.80%) were comparatively less susceptible than other types.

Koya *et al*. (1986) conducted studies on yield loss caused by shoot borer (*Conogethes punctiferalis*) in Kerala and results indicated that when 50 percent of the pseudostems in a plant are affected, there was a significant reduction of 38g of yield per plant.

Nybe (2001) reported that when 23 to 24 percent of pseudostems of a plant are infested by the pest, about 25 percent yield loss occur and the pest caused 40 percent yield loss in Kottayam and Idukki districts of Kerala.

Shankar (2003) screened seven variants along with three check varieties and found that Rio-de-Janeiro showed the least percentage of shoot borer attacked tillers (19.99%) whereas Z-0-97 had the least percentage of attacked plants (29.37%).

Devasahayam *et al*. (2010) screened 492 accessions of ginger in the field against the shoot borer for four consecutive years. All the accessions were susceptible to the pest attack. None of the accessions was rated as resistant, whereas, 49, 251, 130 and 62 accessions were rated moderately resistant, moderately susceptible, susceptible and highly susceptible, respectively. Among the popular cultivars, Jorhat, Rio-de-Janerio, Thingpuri and Burdwan were rated as moderately resistant and among the high yielding varieties released by IISR, Calicut, Rejatha was moderately susceptible; Mahima and Varada were susceptible.

2.1.3.5 Rhizome maggot

Premkumar *et al.* (1982) reported that 42% of the diseased rhizomes examined had *Pythium* sp. alone and 58 percent had *Pythium* sp. and maggots (*Mimegralla coeruleifrons*). None of the rhizomes were infested with maggot alone.

Radke and Borle (1982) found that the rotting of rhizomes due to disease occurred first and later the flies preferred such rhizomes for egg laying. Surveys conducted in Kerala indicated that 33.6 per cent of diseased rhizomes contained maggots (*Mimegralla coeruleifrons* and *Eumerus pulcherrimus*); none of the healthy rhizomes contained maggots and also reported that *Mimegralla coeruleifrons* was the most common species occurring in ginger rhizomes (Koya, 1986).

Koya *et al*. (1990) conducted studies under controlled conditions in the green house and in the field involving inoculation with rhizome maggot (*Mimegralla coeruleifrons*) and *Pythium* sp. in various combinations. The studies clearly indicated that the maggots could infest only diseased ginger rhizomes and hence cannot be considered as a primary pest.

2.2. SOMACLONAL VARIATION

2.2.1. Morphology and yield

Ginger

Samsudeen (1996) conducted a study on variability in ginger somaclones. The study indicated that there exist high variability among somaclones in yield and yield attributes,

which resulted in identification of few promising high yielding lines with tolerance to rhizome rot.

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.

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 pseudostem, number of tillers/ plant, number of nodes/ finger and yield of rhizomes. But the CP plants were superior in number of 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. A promising somaclonal variant was isolated from the cultivar Maran with bold rhizomes.

Pandey *et al*. (1997) reported that conventionally propagated plants of ginger cultivar Khin yai prodced 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 in ginger.

Rao *et al*. (2000) observed no significant variation in the morphological characters in adventitious bud regenerants in Jamaican ginger. Yield of somaclones was comparable to that of CP plants. Somaclones took thirteen months for harvest after planting out.

Freitz *et al.* (2003) 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.

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 Maran. Somaclones of cultivar Maran exhibited higher increment in plant height and leaf length compared to clones of cultivar Rio-de-Janeiro.

Paul (2006) studied somaclonal variation in two cultivars of ginger, Maran and Rio-de- Janeiro and found that the somaclones exhibited superiority over control plants in morphological characters such as height of pseudostem (16%), number of tillers (27%) and leaves/clone (38%) and also in growth rate. Clones of Rio-de-Janeiro exhibited superiority in height of pseudostem, tiller number and leaf number. Somaclones of cultivar Maran were better in leaf characters such as length, breadth and area of leaf. The rhizome characters such as number (3% and 12% in primary and secondary fingers respectively), length (39% and 22% in primary and secondary fingers respectively) and girth (26% and 22% in primary and secondary fingers respectively) of fingers that contribute directly to yield were more in the somaclones evaluated. Based on the average yield for three years of field evaluation, nine per cent somaclones were found to be high yielders over conventionally propagated plants. 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.

Kurian (2010) tried induction of somaclonal variation in two polyploids (Z-0-78 and Z-0-86) and a diploid cultivar Himachal Pradesh. Evaluation of 289 somaclones (generated through indirect organogenesis and embryogenesis) indicated that somaclones were less tall with more number of tillers and higher mean yield when compared to control cultivars raised through bud culture. Ten per cent of somaclones produced rhizome yield more than 300g and the percentage yield increase over the control cultivar ranged from 92-148.

Shylaja *et al*. (2010) reported two new ginger varieties; Athira and Karthika, developed at Kerala Agricultural University, from cv. Maran, exploiting somaclonal variation. Athira is a high-yielding, high-quality cultivar suitable for fresh and dry rhizome. Karthika is a high-yielding clone that produces highly pungent rhizomes suitable for the extraction of oleoresin. These varieties are reported to have more tolerance to soft rot and bacterial wilt diseases compared to Maran.

Turmeric

Salvi *et al.* (2002) screened 48 adventitious bud regenerants of turmeric cultivar Elite. Two plants were identified to have variegated leaves. All the micropropagated plants showed 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.

Roopadarsini and Gayatri (2012) isolated five types of somaclonal variants among the 105 bud regenerants, based on the morphological traits at the culture conditions. The variants showing higher values of the metric traits than the regenerants and control with regard to morphological parameters in the first generation were selected for further evaluation in the second generation. The variants isolated based on the morphological traits were subjected to biochemical analysis such as curcumin, oleoresin and volatile oil contents and compared with the normal regenerants and the control plant. Significantly high curcumin, oleoresin and volatile oil contents (%) were observed in somaclonal variants when compared to the normal regenerants and also control plant.

Kacholam

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

A comparative study on growth of regenerants derived through indirect organogenesis, adventitious bud culture and CP plants of kacholam (*Kaempferia galanga*) was conducted

by Joseph (1997), for a period of three months after planting out. Leaves were erect in regenerants derived through indirect organogenesis while horizontal leaves were observed in adventitious bud culture and CP plants. Leaf area was higher in CP plants while tiller number was highest in regenerants derived through indirect organogenesis.

Cardamom

Reghunath (1989) evaluated somaclones 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.

Lukose *et al.* (1993) observed that the number of yielding tillers per plant and leaf area were higher in somaclones compared to sucker derived plants and seedlings of cardamom variety Mudigree 1 and clone 37, when screened for three years. However, it was reported that the corresponding yield improvement was not observed.

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

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 micro-propagated 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%).

Micropropagated cardamom plants were evaluated by Sudharshan *et al.* (1997) for growth and yield. Variation in type of panicle, capsule shape and size were observed in

tissue cultured plants. The overall variability of 4.5 per cent was observed in tissue cultured plants while only three per cent was observed in open pollinated seedling progenies.

Kuruvila *et al.* (2005) conducted a comparative study on field performance of tissue culture derived plants and open pollinated seedlings of cardamom in farmers' fields in Kerala, Karnataka and Tamil Nadu for two seasons. 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 a yield potential of more than 750 kg ha^{-1} under moderate management.

Large cardamom

Rao *et al*. (2003) conducted a comparative study on growth and yield of adventitious bud regenerants and open pollinated seedlings of large cardamom. An increase of 1.5 times in yield contributing characters such as number of total tillers/ clump, productive tillers/ clump, spike/ clump and capsules/ spike and twenty times increment in yield were recorded in the somaclones as compared to open pollinated seedlings.

Gupta *et al.* (2005) also reported that yield contributing characters such as number of productive tillers and spikes/ clump, periodicity in yield and higher yield were recorded in tissue cultured plants when compared to open pollinated plants in large cardamom.

Black pepper

Sanchu (2000) carried out a variability study for morphological, yield and quality parameters in black pepper cultivar Cheriyakaniyakkadan derived through indirect organogenesis. Variability was observed 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 three calliclones 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 parent clones. Among the 61 morphological characters studied, 56 were found homogenous within somaclones of each variety. The somaclones were significantly different from parents 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 on morphological characters such as number of leaves/ plant, length and breadth of leaves. The somaclones produced laterals much earlier than CP plants.

Vanilla

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 and 2004. During the initial years, CP plants recorded higher yield while in later years, somaclones recorded higher yield.

Medicinal and aromatic plants

Kukreja *et al*. (2000) evaluated Japanese mint (*Mentha arvensis*) for agronomically desirable characters. Among 280 plants transferred to the field, a wide range of variation was observed for plant height, leaf-stem weight ratio, herb yield, oil content and oil yield. Variations were also recorded for 4 major constituents of the essential oil, i.e. menthol, menthone, isomenthone and menthyl acetate. A positive correlation was found for oil yield with plant height and herb yield, whereas a negative correlation existed between herb yield and oil content. Based on the initial agronomic assessments on an individual plant basis, 27 somaclones were selected and evaluated with parent plant CIMAP/Hy-77 as standard. Somaclones Sc59 and Sc179, selected on the basis of higher herb yield in the initial screening, recorded 55.8% and 64.3% increase in oil yield over the control, respectively. Somaclones Sc93, Sc114, Sc121 and Sc124 that were selected for their better

oil content exhibited 47.2%, 50.6% 57.5% and 48.2% increase in oil yield over the parent variety, respectively.

Mathur *et al*. (2006) evaluated leaf sheath derived callus regenerants of Java Citronella variety 'Jorhat'. From amongst 230 plants of the first batch, extensive somaclonal variations were observed for seven agronomic traits, vitatics, herbage yield, tiller number, diameter of the bush, length and area of the longest leaf, fresh and dry weight ratio and oil content. In addition, variations were also recorded for six major constituents of the essential oil, i.e., citronellal, citronellol, geraniol, citronellyl acetate, geranyl acetate and elemol. Correlation analysis between agronomic traits revealed a significant negative correlation between oil content and herbage yield.

Gauri *et al.* (2008) evaluated somaclones of rose scented geranium (*Pelargonium graveolens* L'Herit ex Ait.) cv. "Hemanthi" in the field for morphology and yield characters. Two distinct morphotypes were described on the basis of leaf dentation-one resembling the parental cultivar in having highly dentated leaves (HDL) and the other with less dentated leaves (LDL). After repeated field-testing for 3 consecutive years, the HDL clones closely resembled the parental cultivar with respect to the different quantity and quality determining traits, while the LDL group was clearly different. The field established LDL somaclones possessed higher herb yield, number of branches and other oil yield attributing traits as compared to the HDL clones and the parent cultivar. The chemical investigations of the essential oil revealed significant differences between the LDL clones, the HDL clones and the control.

Alok (2009) developed calliclones from two accessions of *Rauvolfia serpentina*, a North Indian accession (RS 1) and another South Indian accession (RS 2). Calliclones of RS 1 and RS 2 varied greatly from control plants in growth performance. In general, calli clones of RS 2 were more vigorous. Calliclones of RS 1 produced exceptionally more number of roots whereas calli clones of RS 2 had more root length. The low chlorophyll content in the calliclones of RS1 indicates the probability for high alkaloid content because of the negative relationship of chlorophyll with alkaloid content. Low stomatal frequency and enlarged size suggest the possibility of polyploids among the calliclones.

2.2.2. Quality

Ginger

Ramachandran and Nair (1992) reported that the tetraploid lines from cultivars Maran and Mananthody gave lower essential oil content (2.3%) than the original diploid cultivar.

Bhagyalakshmi *et al.* (1994) studied mericlones of ginger cultivar Wynad local where the mericlones were comparable to the CP plants in the composition of starch, ash, acetone extract and volatile extract. Also fibre content and rhizome yield were found lower in mericlones.

Rao *et al*. (2000) found that with respect to quality somaclones of Jamaican ginger were superior to the local ginger cultivar Kuruppapady in terms of oil and oleoresin recovery.

Paul (2006) studied somaclonal variation in two cultivars of ginger, Maran and Rio de Janeiro and found that the somaclones exhibited superiority over control plants in quality characters. Somaclones recorded higher dry recovery (19.73%) than conventionally propagated plants (16.02%). Of the two cultivars studied, higher driage was noticed in the clones of cultivar Maran (18.25%) than clones of cultivar Rio-de-Janeiro (15.62- 15.79%). In three clones of cultivar Maran viz. 488M, 110M and 970M, the driage recorded was very high registering driage values of 25, 22.56 and 22.50 per cent respectively. Recovery of essential oil varied between 1.00 to 2.50 per cent. Oil content was found high in somaclones of cultivar Rio-de-Janeiro (1.42 to 2.50%) than clones of cultivar Maran (1.00 to 2.25%). Oleoresin content ranged from 4.31 to 8.93 per cent in the somaclones evaluated. Higher recovery was noticed in clones of Rio-de-Janeiro (4.38 to 8.93%) than the clones of cultivar Maran (4.31 to 8.49%). 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%). Clones of cultivar Maran, M VI (1.96%) and 79 M (2.28%) showed the lowest crude fibre content.

Shylaja *et al*. (2010) reported two new ginger varieties; Athira and and Karthika, developed at Kerala Agricultural University, from cv. Maran, exploiting somaclonal

variation. Athira is a high-quality cultivar suitable for fresh and dry rhizome, has low crude fibre contents and high zingiberene contents. Karthika is a high-yielding clone that produces highly pungent rhizomes rich in gingerol, suitable for the extraction of oleoresin.

Geranium

Ravindra *et al.* (2004) reported that somaclonal variants in *Pelargonium graveolens*, show changes with respect to herb yield, essential oil content and oil components such as linalool and trans-rose oxide.

2.2.3. Screening of somaclones for resistance/ tolerance to pests and diseases

2.2.3.1. Natural screening of somaclones against pests and diseases

Ginger

Sharma and Singh (1997) while evaluating adventitious bud regenerants of cultivar HP found that 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.

Paul *et al*. (2006a) screened somaclones of cultivar Maran and Rio-de-Janeiro for three successive years (2002- 2004) and reported that the incidence of shoot borer was highest in the first year of field evaluation. The incidence ranged from 0-50 per cent in clones of Maran and from 0 - 60 per cent in clones of Rio-de-Janeiro. The infestation was very low in second and third year of evaluation in Maran and Rio-de-Janeiro (0.33% and 0.22% respectively).

Paul *et al*. (2006b) also found that during field evaluation of somaclones of cultivar Maran and Rio-de-Janeiro for a period of three years (2002-2004), rhizome rot incidence was noticed in 14 per cent clones each of Maran and Rio-de-Janeiro. Bacterial wilt disease was noticed in seven per cent clones of Maran and 11 per cent clones of Rio-de-Janeiro.

Shylaja *et al*. (2010) reported of two new ginger varieties; Athira and Karthika, developed at Kerala Agricultural University, from cv. Maran, exploiting somaclonal

variation. Athira and Karthika are more resistant to soft rot [*Pythium* sp.] and bacterial wilt [*Ralstonia solanacearum*] than Maran.

2.2.3.2. Sick plot screening

Ginger

Paul *et al.* (2006) screened somaclones of cultivar Maran and Rio-de-Janeiro in sick field. Fourteen per cent somaclones of cultivar Maran and twelve per cent of Rio-de-Janeiro were not affected by rhizome rot and bacterial wilt diseases in the sick field. When the selected somaclones were screened in soil heavily infected with the pathogen, the somaclones took no infection. The clones selected showed tolerance reaction in artificial inoculation studies also.

2.2.3.3 Screening by artificial inoculation of pathogens

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 sympotms. 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.

Paul *et al*. (2006) screened one hundred and fifty one somaclones of cultivars viz., Maran and Rio-de-Janerio regenerated through bud culture for resistance/ tolerance to bacterial wilt and rhizome rot disease. In artificial inocluation of bacterial wilt pathogen, all clones took infection but subsequent germination of rhizomes was observed in four somaclones vitatics, 970 M, M VI, 364 R, and R XI. In artificial inoculation of rhizome rot pathogen, three somaclones of Maran developed disease symptoms late (M VI, 364 R

and R XI) and subsequent germination of rhizomes were observed. Somaclones of Maran were found more tolerant to diseases as compared to clones of Rio-de-Janeiro.

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 calliclones was assessed using leaf symptom bioassay by artificial inoculating culture disc of *Phytophthora capsici*. Out of the calliclones of four cultivars viz., Kalluvally, Cheriakanyakkadan, Balankotta and Karimunda evaluated, the clones of cultivar Kalluvally exhibited higher variability in disease reaction while the calliclones of Cheriakanyakkadan showed lesser variability and were tolerant to the disease.

Sanchu *et al*. (2003) assessed Phytophthora foot rot disease reaction in calliclones of black pepper cultivar Cheriakanyakkadan using leaf bioassay and could isolate five calliclones of black pepper tolerant to *Phytophthora* foot rot disease.

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 *Pythium vexans* (ICRI, 2003).

Vanilla

Lestari *et al.* (2006) screened vanilla plants for resistance/ tolerance to wilt disease affecting stem base. Radiation treatments to the seed regenerant have produced several somaclones which had morphological variations. Inoculation using *F. oxysporum* strain F117-109-GV-02011 and then planting in the diseased field of Sukamulya, Sukabumi (Indonesia) produced 23 resistant somaclones. *In vitro* selection in MS medium enriched with fusaric acid at 15-75 mg l^{-1} and then repeated with 75mgl^{-1} resulted in resistant clones. Cross selection by MS medium enriched with 50 per cent filtrate also produced resistant

somaclones. The same results were observed in crossing *V. planifolia* and *V. albida* which resulted in resistant plants in the field.

2.2.4 Correlation

Ginger

Mohanty *et al*. (1979) reported that the rhizome yield was positively and significantly correlated with number of tillers, leaves, secondary rhizome fingers, tertiary rhizome fingers, plant height, leaf breadth, girth of secondary rhizome fingers, number and weight of adventitious roots.

Nair *et al*. (1982) and Ratnambal (1982) studied partitioning of phenotypic correlation between yield and morphological characters into direct and indirect effects by the method of path coefficient analysis revealing that the plant height exhibited a high direct effect as well as high indirect effect in the establishment of correlation between yield and other morphological characters.

Nybe *et al.* (1979b) found that morphological characters and economic characters such as length of primary and secondary fingers and girth of primary fingers were found positively correlated with yield.

Sreekumar *et al.* (1981) reported positive correlation of rhizome weight with plant height, number of tillers and leaf number.

Rattan *et al.* (1988) reported that plant height was positively and significantly correlated with number of leaves, leaf length, rhizome length, rhizome breadth and yield per plot. The number of leaves per plant was positively and significantly related to rhizome length, breadth and yield. The rhizome length was also related to rhizome breadth and yield. Leaves per plant had maximum direct contribution to yield per plant, followed by rhizome breadth.

Sasikumar *et al*. (1992a) studied 100 accessions of ginger germplasm for variability, correlation and path analysis. They found that rhizome yield was positively correlated with plant height, tiller and leaf number and leaf length and width. Plant height also had

significant and positive association with leaf and tiller number as well as length and width of leaf. The association of leaf number with tiller number, leaf length and leaf width was also positive and significant. Tiller number had a significant negative association with dry recovery. Leaf width had a positive significance with dry recovery. They concluded that the plant height should be given prime importance in selection program as this character had positive and significant correlation as well as a good direct effect with rhizome yield.

Pandey and Dobhal (1993) observed that the rhizome yield per plant was positively associated with plant height, number of fingers per plant, weight of fingers and primary rhizomes.

Saika and Shadeque (1992) reported that leaves/clump, tillers/clump and shoot height were positively correlated with yield.

Zachariah *et al*. (1993) while classifying 86 ginger accessions into high-, medium- and low-quality types based on the relative contents of the quality components, revealed that the intercharacter association showed a positive correlation with oleoresin, gingerol and shogoal.

Chandra and Govind (1999) reported that the yield was positively and significantly correlated with tillers per clump, internodal distance of rhizome and plant height and was negatively correlated with fibre content in ginger.

Das *et al*. (1999) reported very high positive direct effects of stomatal number, leaf area, leaf number and plant height on rhizome yield; leaf temperature, relative humidity of leaves, stomatal resistance and rate of transpiration showed negligible effects.

Singh *et al*. (1999) grouped 18 cultivars into three clusters under Nagaland conditions based on D^2 analysis. The major forces influencing divergence of cultivars were rhizome yield per plant and oleoresin and fiber contents.

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigations on "Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur, during the period May 2012 and June 2013. Main aspects focused in the study are as follows:

- Evaluation of somaclones for variability in morphological, yield and quality attributes
- Evaluation of somaclones for variability in resistance/ tolerance to major pests and diseases through:
  - Screening of somaclones for natural occurrence of pests and diseases
  - Sick plot screening of somaclones against rhizome rot and bacterial wilt
  - Artificial screening through artificial inoculation technique
- Assessment of genetic variability and identification of promising somaclones

3.1 FIELD EVALUATION OF SOMACLONES

3.1.1 Experimental material

Somaclones developed through indirect methods of regeneration from two induced polyploids of ginger (Z-0-78 from Himachal Pradesh treated with 0.25% colchicine by injection method and Z-0-86 from Rio-de-Janeiro treated with 0.1% colchicine by hole method) and diploid cultivar Himachal Pradesh form the base material for the study.

Twenty five somaclones selected from among 289 somaclones were field planted along with two check varieties (Karthika and Varadha) and three parental cultivars (Z- 0-78, Z-0-86 and HP) and evaluated for variation in morphology, yield, quality attributes and resistance / tolerance to pests and diseases (rhizome rot, bacterial wilt, leaf spot, rhizome maggot and stem borer infestation) (Table 1).

Table 1. Details of somaclones selected for the study

Sl no.	Parent	Somaclones	Mode of regeneration
1	Z-0-78	C 78 129	Indirect organogenesis
2	Z-0-86	C 86 23	
3		C 86 26	
4		C 86 32	
5		C 86 40	
6		C 86 124	
7		C 86 139	
8	Himachal Pradesh	CHP 8	
9		CHP 39	
10		CHP 49	
11		CHP135	
12	Z-0-78	SE 78 26	Indirect embryogenesis
13		SE 78 30	
14	Z-0-86	SE 86 24	
15		SE 86 40	
16		SE 86 42	
17		SE 86 81	
18		SE 86 83	
19		SE 86 131	
20		SE 86 142	
21	Himachal Pradesh	SEHP 8	
22		SEHP 63	
23		SEHP 64	
24		SEHP 73	
25		SEHP 146	

3.1.2 Management of crop

The field was prepared by ploughing and raised beds of size 2m x1m for field evaluation and beds of size ½ m x ½ m for sick plot evaluation were taken with an interchannel of 40cm width. Rhizome bits of 15- 20 g were used as seed material. The crop was managed as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2002).

Planting

Two months after planting

Four months after planting

Six months after planting

Eight months after planting

Plate 1. Experimental field- stages of crop growth

3.1.3 Morphological characters

Morphological characters of field planted somaclones were recorded at five months after planting. Observations from 10 plants were taken and mean was worked out.

3.1.3.1 Growth habit – Growth habit was observed visually and categorized as erect, semi erect and spreading

3.1.3.2 Height of plant – Height of plant was measured from the soil level to the tip of top leaf of main shoot (cm).

3.1.3.3 Number of shoots – Total number of shoots from a single clump was counted.

3.1.3.4 Height of main shoot - Height of main shoot was measured from soil level to the base of top leaf in each clump (cm).

3.1.3.5 Girth of shoot - Girth of shoot was measured from the tallest pseudostem of each clump at 5cm above soil level (cm).

3.1.3.6 Leaf colour- Intensity of leaf colour was visually observed and categorized into light green, green and dark green.

3.1.3.7 Number of leaves on main shoot - Number of leaves on main shoot was counted.

3.1.3.8 Leaves per plant - Leaves per plant were recorded as total number of leaves in all the tillers in a plant.

3.1.3.9 Leaf length -Leaf length was measured on the upper fourth opened leaf of the main shoot (cm).

3.1.3.10 Leaf width - Leaf width was measured on the upper fourth opened leaf of the main shoot (cm).

3.1.3.11 Leaf area– Leaf area was calculated using the equation A = -24 +3.312 x L, where L is the length of the upper fourth opened leaf and 'A' is the leaf area (Sheeba, 1996) (cm^2).

3.1.3.12 Leaf petiole length - Leaf petiole length was measured on the upper fourth opened leaf from the tip of leaf sheath to the base of the blade (cm).

3.1.3.13 Spike length - Spike length was measured from the soil level to the tip of the inflorescence (cm).

3.1.3.14 Bract colour – Colour of the bract tip was visually observed and classified as crimson yellowish white tip.

3.1.3.15 Crop duration - Crop duration was recorded as number of days taken by the crop from the day of planting to attain maturity when aerial portion of plant withered.

3.1.4 Yield attributes

3.1.4.1 Rhizome characters

Number of primary, secondary, tertiary fingers and quaternary fingers, length of primary and secondary fingers, girth of primary and secondary fingers, internodal length and rhizome shape were recorded from 10 clumps at the time of harvest. The rhizomes arising from seed material was taken as primary, those arising from primary fingers were secondary fingers, from secondary tertiary fingers arise and from tertiary arise quaternary fingers. Length, girth and internodal length of primary and secondary fingers and inner core and thickness of primary and secondary fingers were recorded and means were worked out.

3.1.4.2 Fresh rhizome yield

The rhizomes were harvested eight months after planting by uprooting individual clumps. The per plant yield (gm) and per plot yield (kg) were recorded and per hectare yield (tonnes) was computed.

3.1.4.3 Dry yield

The per hectare yield of dry rhizomes (tonnes) was computed from the per hectare fresh yield and driage per cent.

3.1.5 Quality attributes

Rhizomes of the selected 25 somaclones along with two check varieties and their parental clones replicated twice were analyzed for quality attributes like volatile oil, oleoresin and crude fiber content.

3.1.5.1 Dry recovery (%)

One kilogram of fresh rhizome was rough peeled and sun dried till a constant weight was obtained and the dry recovery of rhizome was expressed in percentage.

3.1.5.2 Estimation of volatile oil

Volatile oil was estimated by water cum steam distillation method using Clevenger apparatus as per AOAC (1980) and the recovery of volatile oil was expressed as percentage. Twenty five grams of coarsely ground powder from each somaclone was distilled for three hours for estimation of volatile oil. From the dry rhizome yield per hectare and oil content, oil yield per hectare was computed.

3.1.5.3 Estimation of oleoresin

The content of oleoresin in the sample was estimated using Soxhlet method of extraction as per AOAC (1980). Five grams of powdered sample was reflexed with 125 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 weight of the beaker along with acetone extract was recorded. The recovery of oleoresin was expressed in percentage. From the dry rhizome yield per hectare and oleoresin content, oleoresin yield per hectare was computed.

3.1.5.4 Estimation of crude fibre

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

3.1.5.5 Colour of ginger powder

Colour of rhizome powder was visually observed and classified as light cream, dull cream, dark cream, creamish yellow and yellow.

3.1.6 Characterization of somaclones based on DUS guidelines

The somaclones were characterized based on the following DUS characteristics:

Table 2. Characteristics for DUS characterization

Sl No.	Characteristics	States	Note
1	Plant: Growth habit	Erect Semi-erect Spreading	1 3 5
2	Plant: Height (cm)	Short (<100) Medium(100 – 120) Tall (>120)	3 5 7
3	Plant: Number of shoots	Few (<10) Medium (10 - 15) Many (>15)	3 5 7
4	Plant: Height of shoot (cm)	Short (<75) Medium (75 - 90) Tall (> 90)	3 5 7
5	Shoot: Diameter (cm)	Narrow (<3) Medium (3 - 5) Broad (>5)	3 5 7
6	Shoot: Intensity of green colour	Light green Green Dark green	1 3 5
7	Shoot: Number of leaves on main shoot	Few (<25) Medium (25-35) Many (>35)	3 5 7
8	Leaf: Length (cm)	Short (<25) Medium (25 - 30) Long (>30)	3 5 7

Contd.

Sl No.	**Characteristics**	**States**	**Note**
9	Leaf: Width (cm)	Narrow (<2.5) Medium (2.5 – 3.5) Broad (>3.5)	3 5 7
10.	Leaf: Intensity of green colour	Light green Green Dark green	1 3 5
11	Leaf: Petiole length (cm)	Short (<0.5) Medium (0.5-0.7) Long (>0.7)	3 5 7
12	Spike: Length (cm)	Short (<25) Medium (25 - 35) Long (>35)	3 5 7
13	Colour of the bract tip of fully developed spike	Crimson Yellowish- white tip	3 5
14	Rhizome: Thickness (cm)	Thin (<2) Medium (2-3) Bold (>3)	3 5 7
15	Rhizome: Shape	Straight Curved Zigzagged	1 3 5
16	Crop duration(days)	Short (<200) Medium (200-210) Long (>210)	3 5 7
17	Dry recovery (%)	Low (<16) Medium (16-18) High (>18)	3 5 7

3.2 SCREENING OF SOMACLONES FOR RESISTANCE/ TOLERANCE TO PESTS AND DISEASES

3.2.1 Screening for natural incidence of pests and diseases

The crop was raised and maintained as per Package of Practices Recommendations of KAU and the natural incidence of pests and diseases were recorded. The natural incidence of bacterial wilt and rhizome rot were recorded and the percentage incidence was calculated. Similarly the infestation of shoot borer was recorded as number of infected tillers per plant and leaf spot disease as leaf spots covering the leaf surface.

3.2.1.1 Plant infection

Under natural screening, incidence of rhizome rot and bacterial wilt were observed as a complex along with occurrence of rhizome maggot.

3.2.1.1.1 Incidence of rhizome rot

The incidence of rhizome rot was recorded by observing the presence of fungal pathogen in the infected rhizomes. For that the plants showing yellowing of leaves were uprooted, rhizomes were washed and cut to pieces. The small bits of rhizomes cut from the infected portion were put in a sterile petridish containing sterile water and incubated under low temperature overnight. The presence of *Pythium* was then confirmed by observing these pathogen containing incubated rhizome bits under microscope. The per cent disease incidence (PDI) was calculated using the formula;

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

3.2.1.1.2 Incidence of bacterial wilt

The incidence of bacterial wilt was recorded by conducting ooze test with the infected rhizomes and percent disease incidence was calculated by the formula given in 3.2.1.1.1.

1. *Pythium* culture

2. Soil inoculation of pathogen

3. Plant inoculation of pathogen

4. Mixing of inoculum to plant base

Plate 2. Steps in artificial inoculation of *Pythium aphanidermatum*

1. Bacterial wilt affected plants

2. Pinpricks made on plant base

3. Inoculation of bacterial ooze to pin pricked plant base

Plate 3. Steps in artificial inoculation of somaclones against *Ralstonia solanacearum*

3.2.1.1.3 Incidence of rhizome maggot

The incidence of rhizome maggot was recorded as number of plants infected per bed and percent incidence was calculated by the formula given in 3.2.1.1.1.

3.2.1.2 Incidence of shoot borer

Incidence of shoot borer (*Conogethes punctiferalis*) was recorded as the number of infected tillers per clump and was expressed as percentage of number of tillers infected to total number of plants (Devasahayam *et al.*, 2010).

Scoring of borer incidence was done as per the following scale:

Highly resistant - 0%

Moderately resistant - 0.1 – 12.5%

Moderately susceptible - 12.6 – 17.1%

Susceptible - 17.8 – 21.8 %

Highly susceptible - More than 21.9%

3.2.1.3 Incidence of leaf spot

Incidence of leaf spot disease caused by *Phyllosticta zingiberi* was recorded during the growth period. From the observations recorded, per cent disease incidence (PDI) was calculated by the formula given in 3.2.1.1.1. The percent disease severity (PDS) was calculated using the formula given by Wheeler, 1963.

$$\text{Percentage Disease Severity (PDS)} = \frac{[\text{Sum of all numerical ratings}]}{\text{Total number of leaves x Maximum scale}} \times 100$$

Ten leaves were randomly selected from infected plants and were scored using 0-5 scale as detailed below;

Grade 0 - No infection

Grade 1 - 1- 10% infection

Grade 2 - 10- 25% infection

Grade 3 - 25- 50% infection

Grade 4 - 50- 75% infection

Grade 5 - > 75% infection

3.2.2 Artificial screening

The experiment on artificial screening, for the incidence of rhizome rot and bacterial wilt diseases, was conducted during May to December 2012 in selected twenty five somaclones along with three parental cultivars and two check varieties. Healthy rhizome bits were planted in potting mixture prepared with sand, soil and cowdung in the proportion 1:1:1. Three plants each were maintained for each somaclone and a control. Inoculation of pathogen on healthy plants was done in shaded net house with misting facility.

3.2.2.1 Rhizome rot

For the artificial inoculation, culture of *Pythium aphanidermatum* the pathogen causing rhizome rot in ginger, was from Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur. The culture was multiplied in sterile Petri dishes mediated with potato dextrose agar medium. For that fungal discs were cut from mother culture using cork borer of 5mm size and placed at the center of the mediated Petri dishes and incubated for a week to get full growth of fungal mycelium.

Inoculation of pathogen

The fully grown fungal mycelium in the Petri dishes were taken and mixed with sterilized moistened potting mixture in polythene covers and incubated for a week with adequate humidity. Infected soil was applied to the base of healthy plants by raking to incorporate thoroughly the infected soil to plant base. Plants were observed daily for appearance of symptoms of disease. The symptoms were recorded using a score chart given by Shanmugam, 1996.

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

3.2.2.2 Bacterial wilt

Inoculation of pathogen

The bacterial ooze obtained from the freshly infected rhizomes was used as inoculum for the artificial inoculation of plants. The rhizomes infected with bacterial wilt disease were collected from the farmer's field and washed thoroughly with water to remove dirt. These rhizomes were then cut into small pieces, dipped in sterile water to get maximum inoculum. The concentration of inoculum was adjusted to get a minimum number of 10^6cfu/ml. Pinpricks were made on the base of healthy ginger plants raised in net house and the bacterial inoculum was dropped on to the wound. Observations were taken daily for incidence of bacterial wilt in the inoculated plants. Wilt symptoms were recorded using a 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

3.2.3 Sick plot screening

The selected 25 somaclones along with the check varieties and parent cultivars were planted in sick field where ginger grown in the previous season was severely infected by rhizome rot and bacterial wilt diseases. Beds of size ½ m x ½ m were taken and planting and other management practices were done as per POP recommendation as in the field

evaluation, except that no control measures were taken against diseases. The number of somaclones survived was recorded and expressed in percentage.

3.3 STATISTICAL ANALYSIS

The experiments were conducted in Randomised Block Design with 30 treatments in two replications. Data on characters studied were subjected to statistical analysis, using Spare 1 Package. The data thus obtained were processed for analysis of variance, genotypic and phenotypic coefficient of variation, correlation coefficients and genetic divergence.

3.3.1 Phenotypic and genotypic variance

The variance components were estimated using the formula suggested by Burton and Devane (1953).

Phenotypic variance (Vp) = Vg +Ve

Where

Vg - genotypic variance

Ve - environmental variance

Genotypic variance (Vg) = $(V_T-V_E)/ R$

Where

V_T - mean sum of square due to treatments

V_E – mean sum of square due to error

R - number of replications

Environmental variance (Ve) = V_E

3.3.2 Phenotypic and genotypic coefficient of variation

The variance components were estimated using the formula suggested by Burton and Devane (1953).

Phenotypic coefficient of variation (PCV) = $(Vp^{1/2} \times X)/ 100$

Where

Vp – Phenotypic variance

X – Mean value of characters

Genotypic coefficient of variation (GCV) = $(Vg^{1/2} \times X)/ 100$

Where

Vg – Genotypic variance

X – Mean value of characters

3.3.3 Heritability

Heritability in the broad sense was estimated by the formula suggested by Burton and Devane (1953).

$H2 = (Vg/ Vp) \times 100$

Where

Vp – Phenotypic variance

Vg – Genotypic variance

The range of heritability was categorized as suggested by Robinson *et al*. (1949) as

0 – 30 per cent - Low

30 – 60 per cent – Moderate

60 per cent and above - High

3.3.4 Simple correlation coefficients

Simple correlation coefficients were worked out to study the extent of association between the characters according to the formula suggested by Johnson *et al.* (1955).

3.3.5 Genetic divergence

The genetic divergence among 30 genotypes including 25 somaclones, three parent cultivars and two check varieties was assessed based on 26 quantitative and four

qualitative characters as given by Mahalanobis (1936). Clustering of genotypes using Mahalanobis D^2 value was carried out using computer based iterative method as suggested by Suresh and Unnithan (1996).

RESULTS

RESULTS

The results of the investigations on "Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" are described in this chapter under the following headings:

- Evaluation of somaclones for variability in morphological, yield and quality attributes
- Evaluation of somaclones for variability in resistance/ tolerance to major pests and diseases through:
  - Screening of somaclones for natural occurrence of pests and diseases
  - Sick plot screening of somaclones against rhizome rot and bacterial wilt
  - Artificial screening through artificial inoculation technique
- Assessment of genetic variability and identification of promising somaclones

4.1. FIELD EVALUATION OF SOMACLONES IN GINGER

Somaclones developed through indirect methods of regeneration from two induced polyploids of ginger (Z-0-78 from Himachal Pradesh treated with 0.25% colchicine by injection method and Z-0-86 from Rio-de-Janeiro treated with 0.1% colchicine by hole method) and diploid cultivar Himachal Pradesh form the base material for the study.

Twenty five somaclones selected from among 289 somaclones were field planted along with two check varieties (Karthika and Varadha) and three parental cultivars (Z- 0-78, Z-0-86 and HP) and evaluated for variation in morphology, yield, quality attributes and resistance / tolerance to pests and diseases (rhizome rot, bacterial wilt, leaf spot and stem borer infestation). Among the 25 somaclones, three somaclones were derived from cultivar Z-0-78, thirteen were from cultivar Z-0-86 and nine from cultivar Himachal Pradesh.

4.1.1 Morphological Characters

Morphological characters and yield attributes of 25 somaclones along with two check varieties (Karthika and Varada) and three parent clones (Z-0-78, Z-0-86 and

Himachal Pradesh) were recorded during active plant growth stage at five months after planting. Morphological characters recorded include plant height, number of shoots, height of main shoot, number of leaves on main shoot, total number of leaves, girth of pseudostem, leaf length, leaf width, leaf area, petiole length, spike length and bract colour, and the data are presented in Table 3. Each character of the somaclones was compared with individual parents, individual check varieties and to the mean value of check varieties and parents.

4.1.1.1 Plant height

Plant height recorded among the somaclones ranged between 57.88 and 101.32cm. Somaclone SEHP 63 recorded maximum plant height of 101.32 cm and minimum plant height was noted in somaclone C86 32 (57.88cm).

Compared to individual parents [Z-0-78 (80.10cm), Z-0-86 (79.65cm) and HP (80.85cm)], among the nine somaclones from cultivar Himachal Pradesh, only SEHP 63 was significantly superior and three somaclones (CHP 8, CHP 39 and SEHP 8) were on par with the parent cultivar. Among the three somaclones of cultivar Z-0-78 and among the 13 somaclones of cultivar Z-0-86, none were significantly superior to the respective parents, but SE 86 40 was on par with the parent cultivar Z-0-86. Compared to individual check varieties Karthika (48.43cm) and Varada (56.25cm), 19 and 11 somaclones were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (52.34cm) and that of parent clones (80.20cm), out of the 25 somaclones evaluated, 15 somaclones were found significantly superior to check varieties and somaclone SEHP 63 was significantly superior to parent clones. Nine somaclones were observed on par with check varieties and four were on par with parent types.

4.1.1.2 Number of shoots

Number of shoots recorded among the somaclones ranged between 4.40 and 10.00. Somaclone SE 86 142 recorded maximum number of shoots of 10.00 and minimum number of shoots was noted in somaclone C 86 32 (4.40).

Table 3. Morphological characters of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Somaclones/ parents/ check	Plant height (cm)	No. of shoot	Shoot height (cm)	Girth of pseudostem (cm)	No.of leaves in main shoot	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Petiole length (cm)
1	CHP 8	82.99	8.05	69.89	3.45	21.90	24.57	3.05	57.36	0.30
2	CHP 39	81.99	5.10	69.19	3.38	20.60	26.68	3.06	64.37	0.29
3	CHP 49	76.48	6.05	66.95	2.79	19.70	22.51	2.75	50.53	0.29
4	CHP 135	77.65	5.55	68.03	3.10	21.45	24.70	2.65	57.81	0.25
5	SEHP 8	81.74	9.85	69.89	3.99	18.55	25.01	2.81	58.82	0.34
6	SEHP 63	101.32	7.15	80.68	2.78	22.25	27.41	2.08	66.76	0.26
7	SEHP 64	76.15	5.35	66.33	2.88	21.10	22.64	2.53	50.96	0.29
8	SEHP 73	57.94	6.02	51.16	2.71	15.52	20.99	2.41	45.52	0.24
9	SEHP 146	66.92	6.75	59.75	2.79	18.00	21.55	2.29	47.37	0.28
10	C 78 129	68.68	6.55	56.73	2.94	20.55	22.03	2.26	48.95	0.28
11	SE 78 26	74.10	4.75	61.42	2.78	21.75	20.14	2.21	42.70	0.24
12	SE 78 30	65.72	4.99	56.46	2.81	19.85	20.67	2.41	44.44	0.24
13	C 86 23	75.51	6.50	63.47	2.78	18.60	23.43	2.43	53.58	0.25
14	C 86 26	79.01	9.85	66.55	3.10	18.25	23.98	2.49	55.41	0.31
15	C 86 32	57.88	4.40	48.95	2.64	18.90	21.13	2.21	45.98	0.18
16	C 86 40	66.71	5.15	53.48	2.75	18.55	23.40	2.30	50.17	0.26
17	C 86 124	62.80	5.25	53.21	2.52	19.40	20.52	2.14	43.96	0.30
18	C 86 139	72.20	6.75	63.89	3.04	19.15	22.45	2.08	50.34	0.24
19	SE 86 24	58.91	5.05	52.04	2.71	17.35	21.23	2.20	43.29	0.29
20	SE 86 40	82.23	7.05	69.51	3.28	22.95	21.18	2.40	46.13	0.29
21	SE 86 42	75.55	5.15	63.88	3.08	19.40	22.34	2.43	49.97	0.25
22	SE 86 81	64.94	5.70	58.13	2.71	18.17	22.81	2.42	51.53	0.37
23	SE 86 83	72.27	8.25	64.37	3.07	18.00	23.87	2.47	55.06	0.33
24	SE 86 131	68.53	6.20	58.18	2.93	18.55	26.58	2.72	64.03	0.31
25	SE 86 142	73.61	10.00	63.86	2.76	16.88	22.83	2.36	51.61	0.26
26	Karthika	48.43	5.75	39.56	2.11	16.25	20.03	1.88	42.34	0.26
27	Varadha	56.25	4.40	51.70	2.25	17.80	18.59	1.82	37.57	0.25
28	Z-0-78	80.10	4.95	72.80	2.52	13.15	23.00	2.23	52.18	0.24
29	Z-0-86	79.65	9.15	72.65	3.02	11.90	21.73	2.19	47.95	0.26
30	HP	80.85	4.95	74.50	3.32	13.17	24.20	2.75	56.15	0.28
C D (0.05)		18.06	3.66	19.90	6.37	0.98	3.71	0.52	12.26	0.09
C V %		11.44	26.44	14.68	15.72	15.55	7.50	9.82	11.02	15.00

Compared to individual parents [Z-0-78 (4.95), Z-0-86 (9.15) and HP (4.95)], among the nine somaclones from cultivar Himachal Pradesh, only SEHP 8 was significantly superior and eight were on par with the parent cultivar. Among the three somaclones of cultivar Z-0-78 and among the 13 somaclones from cultivar Z-0-86, none was significantly superior and two (C 78 129 and SE 78 30) were on par to the parent cultivar Z-0-78 and C 86 26 was on par with the parent cultivar Z-0-86. Compared to individual check varieties Karthika (5.75) and Varada (4.40) three and four somaclones each were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (5.10) and that of parent clones (6.35), out of the 25 somaclones evaluated, three somaclones (SEHP 8, C 86 26 and SE 86 142) were found significantly superior to check varieties and none of the somaclones was significantly superior to parent clones. Seventeen somaclones were observed on par with varietal mean and eleven were on par with parental mean.

4.1.1.3 Height of main shoot

Height of main shoot recorded among the somaclones ranged between 48.95cm and 80.68cm. Somaclone SEHP 63 recorded maximum height of main shoot of 80.68cm and minimum height of main shoot was noted in somaclone C 86 32 (48.95cm).

Compared to individual parents [Z-0-78 (72.80cm), Z-0-86 (72.65cm) and HP (74.50cm)], none of the somaclones were found significantly superior to their parents, SEHP 63 was on par with the parent cultivar Himachal Pradesh. Compared to individual check varieties Karthika (39.56cm) and Varada (51.70cm), 16 somaclones were significantly superior to Karthika and SEHP 63 was found significantly superior to Varada. Compared to the mean of check varieties (45.63 cm) and that of parent clones (73.32 cm), out of the 25 somaclones evaluated, nine somaclones were found significantly superior to check varieties. None of the somaclones was superior when the parental means were compared. Sixteen somaclones were observed on par with check varieties and SEHP 63 was on par with parent types.

4.1.1.4 Girth of pseudostem

Girth of pseudostem recorded among the somaclones ranged between 2.52 cm and 3.99 cm. Somaclone SEHP 8 recorded maximum girth of tiller of 3.99 cm and minimum girth of tiller was noted in somaclone C 86 124 (2.52 cm).

Compared to individual parents [Z-0-78 (2.52cm), Z-0-86 (3.02cm) and HP (3.32cm)], among the nine somaclones from cultivar Himachal Pradesh, three somaclones of cultivar Z-0-78 and among the 13 somaclones from cultivar Z-0-86, none of the somaclones were significantly superior but three somaclones each and five were on par with the parent cultivars Himachal Pradesh, Z-0-78 and Z-0-86 respectively. Compared to individual check varieties Karthika (2.11cm) and Varada (2.25cm), six and four somaclones were significantly superior to Karthika and Varada respectively. Compared with the mean of check varieties (2.18cm) and that of parent clones (2.95cm), out of the 25 somaclones evaluated, four somaclones were found significantly superior to check varieties and somaclone SEHP 8 was significantly superior to parent clones. Twenty one somaclones were observed on par with check varieties and eight were on par with parent types.

4.1.1.5 Shoot colour

Different shoot colours recorded were light green and green. Green shoot colour was found dominating (16 somaclones), followed by light green colour (9 somaclones) (Table 7).

4.1.1.6 Leaf colour

Different leaf colours recorded were light green, green and dark green. Green leaf colour was found to be the dominating (14 somaclones), followed by light green colour (10 somaclones). SE 86 40 was observed to have dark green leaf colour (Table 7).

4.1.1.7 Number of leaves in main shoot

Number of leaves in main shoot recorded among the somaclones ranged between 15.52 and 22.95 (Table 3). Somaclone SE 86 40 recorded maximum number of leaves of 22.95 and minimum number of leaves was noted in somaclone SEHP 73 (15.52).

Compared to individual parents [Z-0-78 (13.15), Z-0-86 (11.90) and HP (13.17)], among the nine somaclones from cultivar Himachal Pradesh, six were significantly superior and three were on par with the parent cultivar, among the three somaclones of cultivar Z-0-78, all the three somaclones were significantly superior to the parent cultivar and among the 13 somaclones from cultivar Z-0-86, eight were significantly superior and five were on par with the parent cultivar. Compared to individual check varieties Karthika (16.25) and Varada (17.80), none of the somaclones was significantly superior to Karthika and Varada. Compared to the mean of check varieties (17.02) and that of parent clones (12.74), out of the 25 somaclones evaluated, none of the somaclones was found significantly superior to check varieties and 13 somaclones were significantly superior to parent clones. Twenty three somaclones were observed on par with check varieties and twelve were on par with parent types.

4.1.1.8 Leaf length, width and leaf area

The length and width of the fourth leaf from growing tip was recorded and leaf area was computed. Leaf length recorded among the somaclones ranged between 20.14 cm and 27.41 cm. Somaclone SEHP 63 recorded maximum leaf length of 27.41cm and minimum leaf length was noted in somaclone SE 78 26 (20.14 cm).

Compared to individual parents [Z-0-78 (23.00), Z-0-86 (21.73) and HP (24.20)], among the nine somaclones from cultivar Himachal Pradesh and among the three somaclones of cultivar Z-0-78, none of the somaclones was significantly superior to the parent cultivars, five were on par with the parent cultivar Himachal Pradesh. Among the 13 somaclones from cultivar Z-0-86, SE 86 131 was significantly superior and eight were on par with the parent cultivar. Compared to individual check varieties Karthika (20.03) and Varada (18.59), eight and sixteen somaclones were significantly superior to Karthika

and Varada respectively. Compared to the mean of check varieties (19.31) and that of parent clones (22.90), out of the 25 somaclones evaluated, 10 somaclones were found significantly superior to check varieties and somaclone SEHP 63 was significantly superior to parent clones. Fifteen somaclones were observed on par with check varieties and nine were on par with parent types.

Leaf width recorded among the somaclones ranged between 2.08 cm and 3.06 cm. Somaclone CHP 39 recorded maximum leaf width of 3.06 cm and minimum leaf breadth was noted in somaclone C 86 139 (2.08 cm).

Compared to individual parents [Z-0-78 (2.23 cm), Z-0-86 (2.19 cm) and HP (2.75 cm)], among the nine somaclones from cultivar Himachal Pradesh and among the three somaclones of cultivar Z-0-78, none was significantly superior to the parents, while three and two somaclones were on par with the parent cultivar Himachal Pradesh and Z-0-78 respectively. Among the 13 somaclones from cultivar Z-0-86, SE 86 131 was significantly superior and 10 were on par with the parent cultivar. Compared to individual check varieties Karthika (1.88) and Varada (1.82), 15 and 16 somaclones were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (1.85 cm) and that of parent clones (2.39 cm), out of the 25 somaclones evaluated, 15 somaclones were found significantly superior to check varieties and two somaclones were significantly superior to parent clones. Ten somaclones were observed on par with check varieties and 13 were on par with parent types.

Leaf area recorded among the somaclones ranged between 42.70 cm^2 and 66.76 cm^2. Somaclone SEHP 63 recorded maximum leaf area of 66.76 cm^2 and minimum leaf area was noted in somaclone SE 78 26 (42.70 cm^2).

Compared to individual parents [Z-0-78 (42.17), Z-0-86 (47.95) and HP (56.15)], among the nine somaclones from cultivar Himachal Pradesh and three somaclones of cultivar Z-0-78, none was significantly superior to the parents but five and three somaclones were on par with the parent cultivar HP and Z-0-78 respectively. Among the 13 somaclones from cultivar Z-0-86, SE 86 131 was significantly superior and eight were on par with the parent cultivar. Compared to individual check varieties Karthika (42.34)

and Varada (37.57), eight and 16 somaclones were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (39.95) and that of parent clones (48.76), out of the 25 somaclones evaluated, nine somaclones were found significantly superior to check varieties and four somaclones were significantly superior to parent clones. Fifteen somaclones were observed on par with check varieties and fourteen were on par with parent types.

4.1.1.9 Petiole length

Petiole length recorded among the somaclones ranged between 0.18cm and 0.37cm. Somaclone SE 86 81 recorded maximum petiole length of 0.37cm and minimum petiole length was noted in somaclone C 86 32 (0.18 cm).

Compared to individual parents [Z-0-78 (0.24cm), Z-0-86 (0.26cm) and HP (0.28cm)], among the nine somaclones from cultivar Himachal Pradesh and three somaclones of cultivar Z-0-78, none of the somaclones were significantly superior, but five somaclones and one somaclone (C 78 129) were on par with the parent cultivars HP and Z-0-78 respectively. Among the 13 somaclones from cultivar Z-0-86, SE 86 81 was significantly superior and six were on par with the parent cultivar. Compared to individual check varieties Karthika (0.26cm) and Varada (0.25cm), somaclone SE 86 81 was significantly superior to Karthika and SE 86 81 and SEHP 8 were significantly superior to Varada. Compared to the mean of check varieties (0.26cm) and that of parent clones (0.26cm), out of the 25 somaclones evaluated, somaclone SE 86 81 was found significantly superior to mean of check varieties and parent clones. Thirteen somaclones were observed on par with both check varieties and parent types.

4.1.1.10 Bract colour

Out of the 25 somaclones, 16 showed flowering. The bract colour of spikes were noted, two different colours namely greenish white and crimson. Somaclones C 86 23, SE 86 42 and SE 86 83 showed crimson bract colour and others showed greenish white bract colour (Table 7).

4.1.1.11 Spike length

Spike length was measured as the height of spike from the soil level to the tip of inflorescence. Spike length noted among 16 somaclones ranged from 12.65 to 22. 43 cm. Highest spike length was observed in somaclone SE 86 142 and lowest in SE 86 40 (12.65 cm) (Table 7).

4.1.2 Yield attributes

4.1.2.1 Rhizome characters

Rhizome characters viz. number of primary, secondary, tertiary and quaternary fingers, length, girth, internodal length, thickness of flesh and core of primary and secondary fingers, colour of flesh and core, shape of rhizomes were recorded in somaclones at the time of harvest. Each character was then compared with individual parents, check varieties and to the mean value of check varieties and parents. Rhizome characters of somaclones along with parent cultivars and check varieties are presented in Table 4.

4.1.2.1.1 Number of primary and secondary fingers

Number of primary fingers recorded among the somaclones ranged between 2.40 and 4.20 Somaclone CHP 8 recorded maximum number of primary fingers of 4.20 and minimum number of primary fingers was noted in somaclone C 78 129 (2.40).

Compared to individual parents [Z-0-78 (2.90), Z-0-86 (3.30) and HP (3.73)], five somaclones were on par with the parent cultivar Himachal Pradesh, two were on par with cultivar Z-0-78 and 11 were on par with the parent cultivar Z-0-86. Compared to individual check varieties Karthika (3.80) and Varada (3.00), though none was significantly superior to Karthika, six somaclones were significantly superior to Varada, but eight and twenty three somaclones were on par with Karthika and Varada respectively.

Compared to the mean of check varieties (3.40) and that of parent clones (3.31), out of the 25 somaclones evaluated, though none was significantly superior, fifteen somaclones were observed on par with check varieties and 22 were on par with parent types.

Table 4. Rhizome characters of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Somaclones/ parents/ check	No. of fingers				Length of fingers (cm)		Internodal length of fingers (cm)	
		1^0	2^0	3^0	4^0	1^0	2^0	1^0	2^0
1	CHP 8	4.20	11.30	13.70	4.35	4.81	3.83	0.82	0.66
2	CHP 39	4.10	8.20	8.80	4.92	5.13	4.16	0.72	0.61
3	CHP 49	3.40	10.00	9.90	6.44	4.93	3.86	0.80	0.60
4	CHP 135	4.00	10.50	11.00	5.10	4.82	4.00	0.79	0.68
5	SEHP 8	3.70	10.40	10.40	6.78	4.57	3.36	0.74	0.60
6	SEHP 63	3.40	9.10	12.00	8.85	5.14	4.77	0.89	0.72
7	SEHP 64	4.10	9.80	6.80	2.55	4.33	2.92	0.80	0.54
8	SEHP 73	4.10	8.10	6.80	3.58	4.55	4.03	0.79	0.64
9	SEHP 146	3.50	7.70	9.40	4.35	4.98	3.16	0.74	0.62
10	C 78 129	2.40	5.20	5.60	7.80	4.17	3.30	0.91	0.63
11	SE 78 26	3.90	10.40	9.20	3.38	4.07	3.23	0.85	0.62
12	SE 78 30	4.10	7.90	6.70	2.13	4.17	2.99	0.77	0.67
13	C 86 23	4.00	12.00	12.30	4.70	5.57	4.06	0.94	0.65
14	C 86 26	3.40	8.90	11.70	8.00	4.35	3.94	0.91	0.68
15	C 86 32	3.20	3.70	3.55	1.50	3.92	3.52	0.67	0.58
16	C 86 40	3.40	8.30	11.90	4.93	4.86	3.87	0.79	0.62
17	C 86 124	3.50	7.90	10.40	9.80	3.88	3.33	0.69	0.66
18	C 86 139	3.70	7.80	9.80	5.00	4.48	3.69	0.74	0.69
19	SE 86 24	3.50	6.40	7.45	3.00	3.85	2.66	0.71	0.54
20	SE 86 40	4.10	9.90	13.20	5.18	4.92	4.37	0.91	0.71
21	SE 86 42	3.50	9.30	10.40	5.25	5.08	3.65	0.90	0.70
22	SE 86 81	2.70	7.80	9.00	3.85	5.26	4.38	0.92	0.63
23	SE 86 83	3.40	7.70	9.10	4.60	5.22	3.87	0.92	0.63
24	SE 86 131	3.10	9.40	15.38	6.33	4.73	3.68	0.97	0.69
25	SE 86 142	3.40	11.10	13.10	7.30	4.48	4.09	0.81	0.73
26	Karthika	3.80	4.90	7.40	1.80	3.89	3.71	0.70	0.63
27	Varadha	3.00	6.17	6.84	1.50	3.85	2.99	0.66	0.55
28	Z-0-78	2.90	7.00	6.55	3.20	2.98	3.31	0.42	0.32
29	Z-0-86	3.30	8.08	9.39	4.94	3.24	4.05	0.38	0.39
30	HP	3.73	9.55	10.04	5.11	3.35	3.50	0.58	0.64
C D (0.05)		1.21	4.93	9.54	5.62	1.82	0.98	0.27	0.17
C V %		13.93	24.83	44.49	57.25	12.58	11.46	16.36	12.71

Contd.

Table 4. Rhizome characters of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Variety	Thickness of fingers (cm)		Thickness of core (cm)		Girth of 1^0fingers (cm)	Colour of rhizome
		1^0	2^0	1^0	2^0		
1	CHP 8	3.06	3	1.58	1.56	8.65	CLBR
2	CHP 39	2.9	2.57	1.70	1.33	8.77	C
3	CHP 49	3.21	2.87	1.69	1.59	8.67	CBT
4	CHP 135	3.09	2.56	1.77	1.24	8.97	Y
5	SEHP 8	3.21	2.6	1.73	1.40	8.94	C
6	SEHP 63	2.97	2.79	1.73	1.63	8.08	Y
7	SEHP 64	2.73	2.42	1.49	1.26	8.33	C
8	SEHP 73	2.98	2.76	1.62	1.40	8.81	CLBR
9	SEHP 146	2.45	2.6	1.25	1.28	7.33	CLBR
10	C 78 129	2.61	2.39	1.37	1.27	7.50	CLBR
11	SE 78 26	2.91	2.68	1.63	1.36	7.90	C
12	SE 78 30	2.71	2.5	1.51	1.26	8.18	CLBR
13	C 86 23	2.68	2.63	1.36	1.31	7.89	LY
14	C 86 26	2.55	2.72	1.23	1.44	7.50	LC
15	C 86 32	2.5	2.11	1.42	1.23	7.34	LY
16	C 86 40	2.79	2.79	1.47	1.39	7.85	C
17	C 86 124	2.33	2.16	1.25	1.12	7.40	CLBR
18	C 86 139	2.72	2.67	1.36	1.39	7.96	CLBR
19	SE 86 24	2.54	2.28	1.30	1.16	7.87	C
20	SE 86 40	2.87	2.85	1.43	1.47	7.94	LC
21	SE 86 42	2.8	2.76	1.32	1.52	8.01	CLBR
22	SE 86 81	2.28	2.04	1.20	1.04	7.11	C
23	SE 86 83	2.6	2.55	1.40	1.43	7.53	C
24	SE 86 131	2.95	2.63	1.71	1.35	7.78	LY
25	SE 86 142	2.62	2.39	1.38	1.19	7.72	LY
26	Karthika	2.3	2.39	1.42	1.07	6.80	LY
27	Varadha	2.34	1.87	1.34	1.40	7.08	CLBR
28	Z-0-78	2.56	2.6	1.32	1.22	7.09	Y
29	Z-0-86	2.52	2.38	1.32	1.18	5.97	CBT
30	HP	2.94	2.36	1.54	1.34	7.87	LY
C D (0.05)		0.66	0.77	0.44	0.22	1.33	
C V %		11.23	13.00	10.74	15.57	7.48	

Number of secondary fingers recorded among the somaclones ranged between 3.70 and 12.00. Somaclone C 86 23 recorded maximum number of secondary fingers of 12.00 and minimum number of secondary fingers was noted in somaclone C 86 32 (3.70).

Compared to individual parents [Z-0-78 (7.00), Z-0-86 (8.08) and HP (9.55)], among the nine somaclones from cultivar Himachal Pradesh, five were on par with the parent cultivar, among the three somaclones of cultivar Z-0-78, two were on par with the parent cultivar and among the 13 somaclones from cultivar Z-0-86, seven were on par to the parent cultivar. Compared to individual check varieties Karthika (4.90) and Varada (6.17), nine and four somaclones were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (5.54) and that of parent clones (8.21), out of the 25 somaclones evaluated, six somaclones were found significantly superior to check varieties and none of the somaclones were significantly superior to parent clones. Seventeen somaclones were observed on par with check varieties and 13 were on par with parent types.

4.1.2.1.2 Number of tertiary and quaternary fingers

Number of tertiary fingers recorded among the somaclones ranged between 3.55 and 15.38. Somaclone SE 86 131 recorded maximum number of tertiary fingers of 15.38 and minimum number of tertiary fingers was noted in somaclone C 86 32 (3.55).

Compared to individual parents [Z-0-78 (6.55), Z-0-86 (9.39) and HP (10.04)], among the nine somaclones from cultivar Himachal Pradesh, four were on par with the parent cultivar, among the three somaclones of cultivar Z-0-78, two were on par with the parent cultivar and among the 13 somaclones from cultivar Z-0-86, nine were on par with the parent cultivar. Compared to individual check varieties Karthika (7.40) and Varada (6.84), 19 and 20 somaclones were on par with Karthika and Varada respectively. Compared to the mean of check varieties (7.12) and that of parent clones (8.66), out of the 25 somaclones evaluated, nineteen somaclones were observed on par with check varieties and 18 were on par with parent types.

Number of quaternary fingers recorded among the somaclones ranged between 1.5 and 9.8. Somaclone C 86 124 recorded maximum number of quaternary fingers of 9.8 and minimum number of quaternary fingers was noted in somaclone C 86 32 (1.5).

Compared to individual parents [Z-0-78 (3.20), Z-0-86 (4.94) and HP (5.11)], among the nine somaclones from cultivar Himachal Pradesh, three were on par with the parent cultivar, among the 13 somaclones from cultivar Z-0-86, seven were on par with the parent cultivar and among the three somaclones of cultivar Z-0-78, two somaclones were on par with the parent cultivar. Compared to individual check varieties Karthika (1.80) and Varada (1.50), four and five somaclones were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (1.65) and that of parent clones (4.42), out of the 25 somaclones evaluated, five somaclones were found significantly superior to check varieties and none of the somaclones were superior to parent clones. Twenty four somaclones were observed on par with check varieties and 16 were on par with parent types.

4.1.2.1.3 Length of primary and secondary fingers

Length of primary fingers recorded among the somaclones ranged between 3.85cm and 5.57cm. Somaclone C 86 23 recorded maximum length of primary fingers of 5.57cm and minimum length of primary fingers was noted in somaclone SE 86 24 (3.85 cm).

Compared to individual parents [Z-0-78 (2.98cm), Z-0-86 (3.24cm) and HP (3.35cm)], among the nine somaclones from cultivar Himachal Pradesh, seven was significantly superior and two somaclones were on par with the parent cultivar, among the three somaclones of cultivar Z-0-78, all three somaclones were on par with the parent and among the 13 somaclones from cultivar Z-0-86, nine somaclones were significantly superior and four were on par with the parent. Compared to individual check varieties Karthika (3.89cm) and Varada (3.85cm), five and six of the somaclones were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (4.97cm) and that of parent clones (3.19cm), out of the 25 somaclones evaluated, none of the somaclones were found significantly superior to check varieties and seventeen

somaclones were significantly superior to parent clones. Six somaclones were observed on par with check varieties and eight were on par with parent types.

Length of secondary fingers recorded among the somaclones ranged between 2.66 cm and 4.77 cm. Somaclone SEHP 63 recorded maximum length of secondary fingers of 4.77cm and minimum length of secondary fingers was noted in somaclone SE 86 24 (2.66cm).

Compared to individual parents [Z-0-78 (3.31cm), Z-0-86 (4.05cm) and HP (3.50cm)], among the nine somaclones from cultivar Himachal Pradesh, SEHP 63 was significantly superior and five somaclones were on par with the parent cultivar, among the three somaclones of cultivar Z-0-78, none was significantly superior and among the 13 somaclones from cultivar Z-0-86, though none of the somaclones was significantly superior, four somaclones were on par. Compared to individual check varieties Karthika (3.71cm) and Varada (2.99cm), SEHP 63 was significantly superior to Karthika and nine somaclones were significantly superior to Varada. Compared to the mean of check varieties (2.97cm) and that of parent clones (3.62cm), out of the 25 somaclones evaluated, nine somaclones were found significantly superior to check varieties and somaclone SEHP 63 was significantly superior to parent clones. Fourteen somaclones were observed on par with check varieties and 15 were on par with parent types.

4.1.2.1.4 Internodal length of primary and secondary fingers

Internodal length of primary fingers recorded among the somaclones ranged between 0.67cm and 0.97cm. Somaclone SE 86 131 recorded maximum internodal length of primary fingers of 0.97cm and minimum internodal length of primary fingers was noticed in somaclone C 86 32 (0.67 cm).

Compared to individual parents [Z-0-78 (0.42cm), Z-0-86 (0.38cm) and HP (0.58cm)], among the nine somaclones from cultivar Himachal Pradesh, SEHP 63 was significantly superior and eight somaclones were on par with the parent cultivar, among the three somaclones of cultivar Z-0-78, all the three somaclones were significantly superior and among the 13 somaclones from cultivar Z-0-86, all the somaclones were

significantly superior. Compared to individual check varieties Karthika (0.70cm) and Varada (0.66cm), none of the somaclones were significantly superior to Karthika while C 86 23 and SE 86 131 were significantly superior to Varada and the rest 23 were on par with Varada. Compared with the mean of check varieties (0.68cm) and that of parent clones (0.46cm), out of the 25 somaclones evaluated, somaclone SE 86 131 was found significantly superior to check varieties and 18 somaclones were significantly superior to parent clones. Twenty three somaclones were observed on par with check varieties and seven were on par with parent types.

Internodal length secondary fingers recorded among the somaclones ranged between 0.54 cm and 0.73 cm. Somaclone SE 86 142 recorded maximum internodal length secondary fingers of 0.73 cm and minimum internodal length secondary fingers was observed in somaclones SE 86 24 and SE HP 64 (0.54cm).

Compared to individual parents [Z-0-78 (0.32cm), Z-0-86 (0.39cm) and HP (0.64cm)], among the nine somaclones from cultivar Himachal Pradesh, though none of the somaclones were significantly superior, three somaclones were on par with the parent cultivar, Among the three somaclones of cultivar Z-0-78, all the three somaclones (C 78 129, SE 78 26 and SE 78 30) were significantly superior, among the 13 somaclones from cultivar Z-0-86, twelve somaclones were significantly superior and SE 86 24 was on par with the parent. Compared to individual check varieties Karthika (0.63cm) and Varada (0.55cm), none of the somaclones were significantly superior to Karthika but 13 were on par. Somaclone SE 86 142 was significantly superior to Varada and 23 somaclones were on par with Varada. Compared to the mean of check varieties (0.59cm) and that of parent clones (0.45cm), out of the 25 somaclones evaluated, none of the somaclones was found significantly superior to check varieties and 16 somaclones were significantly superior to parent clones. Twenty two somaclones were observed on par with check varieties and nine somaclones were on par with parent types.

4.1.2.1.5 Rhizome thickness of primary and secondary fingers

Rhizome thickness of primary fingers recorded among the somaclones ranged between 2.28 and 3.21cm. Somaclone SEHP 8 and CHP 49 recorded maximum thickness

of primary fingers of 3.21cm and minimum thickness of primary fingers was noted in somaclone SE 86 81 (2.28cm).

Compared to individual parents [Z-0-78 (2.56cm), Z-0-86 (2.52cm) and HP (2.94cm)], among the nine somaclones from cultivar Himachal Pradesh, three somaclones of cultivar Z-0-78 and 13 somaclones from cultivar Z-0-86, six, three and ten somaclones each were on par with Himachal Pradesh, Z-0-78 and Z-0-86 respectively. Compared to individual check varieties Karthika (2.30cm) and Varada (2.34cm), six and four somaclones were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (2.32cm) and that of parent clones (2.67cm), out of the 25 somaclones evaluated, five somaclones were found significantly superior to check varieties and none of the somaclones were significantly superior to parent clones. Nineteen somaclones were observed on par with check varieties and 14 were on par with parent types.

Rhizome thickness of secondary fingers recorded among the somaclones ranged between 2.04cm and 3.00cm. Somaclone CHP 8 recorded maximum thickness of secondary fingers of 3.00cm and minimum thickness of secondary fingers was recorded in somaclone SE 86 81 (2.04cm).

Compared to individual parents [Z-0-78 (2.60cm), Z-0-86 (2.38cm) and HP (2.36cm)], among the nine somaclones from cultivar Himachal Pradesh, three somaclones of cultivar Z-0-78 and 13 somaclones from cultivar Z-0-86, none of the somaclones was significantly superior while nine somaclones each from cultivar Himachal Pradesh and Z-0-86, were on par with the parent cultivars and somaclone SE 78 26 was on par with the parent cultivar Z-0-78. Compared to individual check varieties Karthika (2.39cm) and Varada (1.87cm), none of the somaclones was significantly superior and 19 somaclones were on par with Karthika, while nine somaclones were significantly superior to Varada and 16 somaclones were on par with Varada. Compared to the mean of check varieties (2.13cm) and that of parent clones (2.45cm), out of the 25 somaclones evaluated, CHP 8 was found significantly superior to check varieties though none was superior to parent

clones. Twenty three somaclones were observed on par with check varieties and 18 were on par with parent types.

4.1.2.1.6 Thickness of core of primary and secondary fingers

Thickness of core in primary fingers recorded among the somaclones ranged between 1.20 cm and 1.77 cm. Somaclone CHP 135 recorded maximum core thickness of 1.77 cm and minimum core thickness was noted in somaclone SE 86 81 (1.20cm).

Compared to individual parents [Z-0-78 (1.32 cm), Z-0-86 (1.32 cm) and HP (1.54 cm)], SE 86 131 alone was found to be significantly superior to Z-0-86, while eleven, three and nine somaclones were on par with the parent cultivars Himachal Pradesh, Z-0-78 and Z-0-86 respectively. Compared to individual check varieties Karthika (1.42 cm) and Varada (1.34 cm), CHP 135 alone was significantly superior and 14 somaclones were on par with Karthika while six somaclones were significantly superior and 19 were on par with Varada. Compared to the mean of check varieties (1.38 cm) and that of parent clones (1.39 cm), out of the 25 somaclones evaluated, three somaclones were found significantly superior and 16 somaclones were on par with check varieties and CHP 135 alone was significantly superior to parent clones but 15 were on par with the parental mean.

Thickness of core in secondary fingers recorded among the somaclones ranged between 1.04 cm and 1.63 cm. Somaclone SEHP 63 recorded maximum core thickness in secondary fingers of 1.63 cm and minimum core thickness was noted in somaclone SE 86 81 (1.04 cm).

Compared to individual parents [Z-0-78 (1.22cm), Z-0-86 (1.18cm) and HP (1.34cm)], among the nine somaclones from cultivar Himachal Pradesh, five somaclones were on par with the parent cultivar; among the 13 somaclones from cultivar Z-0-86, ten were on par with the parent Z-0-86 and all the three somaclones of Z-0-78 were on par with the parent cultivar, though none of the somaclones was superior to the parents. Compared to individual check varieties Karthika (1.07cm) and Varada (1.40cm), three somaclones were significantly superior to Karthika but none of the somaclones was superior to Varada. Compared to the mean of check varieties (1.24cm) and that of parent

cultivars (1.25cm), 19 somaclones each were found on par with both check varieties and parent clones.

4.1.2.1.7 Girth of primary fingers

Girth of primary fingers recorded among the somaclones ranged between 7.11 cm and 8.97cm. Somaclone CHP 135 recorded maximum girth of primary fingers of 8.97cm and minimum girth of primary fingers was noted in somaclone SE 86 81 (7.11 cm).

Compared to individual parents [Z-0-78 (7.09 cm), Z-0-86 (5.97 cm) and HP (7.87 cm)], among the nine somaclones from cultivar Himachal Pradesh and among the three somaclones of cultivar Z-0-78, though none of the somaclones were significantly superior, eight somaclones were on par with the parent cultivar Himachal Pradesh and all the three somaclones were on par with the parent cultivar Z-0-78. Among the 13 somaclones from cultivar Z-0-86, 12 somaclones were significantly superior. Compared to individual check varieties Karthika (6.80 cm) and Varada (7.08 cm), eight and six somaclones were significantly superior to Karthika and Varada respectively. Seventeen and nineteen somaclones were found on par with Karthika and Varada respectively. Compared to the mean of check varieties (6.94 cm) and that of parent clones (6.97 cm), out of the 25 somaclones evaluated, seven somaclones each were found significantly superior to check varieties and parent clones. Eighteen somaclones each were observed on par with check varieties and parent types.

4.1.2.1.8 Colour of rhizome

Different colours of rhizomes noted were cream, creamish yellow, light yellow, yellow with shades of blue and bluish ringed core. Shades of cream, with or without bluish ring were found to be the dominating colour in the rhizomes of somaclones evaluated (Table 4).

4.1.2.1.9 Shape of rhizome

Different rhizome shapes were noticed namely straight, curved and zigzagged. Out of the 25 somaclones evaluated, nine somaclones showed zigzagged rhizome shape, seven

somaclones showed curved shape and in nine somaclones rhizomes were straight (Table 7).

4.1.2.2 Fresh rhizome yield

The data on fresh yield of rhizome per plant, per plot and per hectare are presented in Table 5. Fresh yield of rhizome recorded among the somaclones ranged between 115.01g and 250.94g on per plant basis, 3.68 and 8.03 kg per plot basis and 9.81 to 21.42 tonnes on a hectare basis. Somaclone SE 86 81 recorded the highest per plant yield of 250.54g. Somaclone SE 86 81 recorded maximum fresh yield of rhizome of 8.03 kg per plot and 21.42 tonnes ha^{-1} and minimum fresh yield of rhizome was observed in somaclone SEHP 146 (3.68 kg/ plot and 9.81 t ha^{-1}).

Compared to individual parents [Z-0-78 (10.58 t ha^{-1}), Z-0-86 (11.55 t ha^{-1}) and HP (13.01 t ha^{-1})], among the nine somaclones from cultivar Himachal Pradesh, SEHP 63 was significantly superior and six were on par with the parent cultivar, among the three somaclones of cultivar Z-0-78, all the three somaclones were on par with the parent cultivar and among the 13 somaclones from cultivar Z-0-86, eight somaclones were significantly superior and four were on par with the parent cultivar. Compared to individual check varieties Karthika (11.87 t ha^{-1}) and Varada (13.12 t ha^{-1}), eight and four somaclones (SEHP 63, SE 86 40, SE 86 81 and SE 86 142) were significantly superior to Karthika and Varada respectively. Compared to the mean of check varieties (12.49 t ha^{-1}) and that of parent clones (11.38t ha^{-1}), out of the 25 somaclones evaluated, two somaclones were found significantly superior to check varieties and nine somaclones were significantly superior to parent clones. Nineteen somaclones were observed on par with check varieties and fourteen were on par with parent types.

4.1.2.3 Dry rhizome yield

The dry ginger yield ranged from 1.71 to 3.92 tonnes ha^{-1} in different somaclones evaluated. Highest dry yield was recorded in SE 86 142 (3.92 t ha^{-1}) and the lowest was recorded in SEHP 146 (1.71 t ha^{-1}). The data of dry rhizome yield of somaclones along with three parent cultivars and two check varieties are presented in Table 5.

Table 5. Yield of rhizome of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Somaclones/ parents/ check	Fresh yield per plant (g)	Fresh yield per plot (kg)	Fresh yield (t ha^{-1})	Dry yield (t ha^{-1})
1	CHP 8	203.28	6.51	17.34	2.97
2	CHP 39	184.69	5.91	15.76	2.69
3	CHP 49	183.76	5.88	15.68	2.58
4	CHP 135	199.69	6.39	17.04	2.71
5	SEHP 8	194.69	6.23	16.62	3.11
6	SEHP 63	226.10	7.24	19.31	3.21
7	SEHP 64	137.50	4.40	11.73	2.23
8	SEHP 73	153.56	4.93	13.15	2.19
9	SEHP 146	115.01	3.68	9.81	1.71
10	C 78 129	128.60	4.12	10.99	2.12
11	SE 78 26	171.57	5.49	14.64	3.08
12	SE 78 30	167.04	5.34	14.27	2.95
13	C 86 23	210.63	6.74	17.98	3.09
14	C 86 26	203.44	6.51	17.36	2.89
15	C 86 32	167.97	5.38	14.35	3.07
16	C 86 40	213.13	6.82	18.19	3.50
17	C 86 124	187.66	6.04	16.00	3.28
18	C 86 139	210.16	6.73	17.95	3.59
19	SE 86 24	140.16	4.49	11.97	2.13
20	SE 86 40	232.03	7.43	19.82	3.69
21	SE 86 42	167.19	5.35	14.27	2.79
22	SE 86 81	250.94	8.03	21.42	3.75
23	SE 86 83	222.66	6.63	17.68	3.17
24	SE 86 131	212.50	6.80	18.14	3.22
25	SE 86 142	234.38	7.50	20.00	3.92
26	Karthika	139.1	4.45	11.87	2.40
27	Varadha	153.75	4.92	13.12	2.66
28	Z-0-78	124.1	3.97	10.58	2.34
29	Z-0-86	135.31	4.33	11.55	2.19
30	HP	152.5	4.88	13.01	2.76
C D (0.05)		67.83	2.21	5.90	1.27
C V %		17.19	17.56	17.56	20.18

Compared to individual parents [Z-0-78 (2.34 t ha^{-1}), Z-0-86 (2.19 t ha^{-1}) and HP (2.76 t ha^{-1})], among the three somaclones of Z-0-78, two somaclones (SE 78 26 and SE 78 30) were on par with the parent, among the 13 somaclones of Z-0-86, five clones were superior and seven were on par with the parent and among the nine somaclones of HP, three clones were on par with the parent cultivar. Compared to individual check varieties Karthika (2.6 tha^{-1}) and Varada (2.44 tha^{-1}), three somaclones (SE 86 40, SE 86 81 and SE 86 142) were superior to Karthika while 17 and 19 clones were on par with check varieties Karthika and Varada respectively. Compared to the mean of check varieties (2.53 tha^{-1}) and that of parent clones (2.43 tha^{-1}), out of the 25 somaclones evaluated, SE 86 142 and SE 86 81 were superior to mean parent while SE 86 142 alone was superior to mean check. Nineteen and eighteen clones were on par with mean check and mean parent respectively.

4.1.3 Quality attributes

Quality attributes such as dry recovery, volatile oil content, oleoresin content and crude fibre content were estimated for the twenty five somaclones as well as three parent cultivars and two check varieties. The data are presented in Table 6.

4.1.3.1. Dry recovery

The dry ginger recovery ranged from 15.89 to 21.21 per cent in different somaclones evaluated. Highest dry recovery was recorded in SEHP 8 (21.21%) and the lowest was recorded in CHP 135 (15.89%).

Compared to individual parents [Z-0-78 (22.12%), Z-0-86 (19%) and HP (21.23%)], though none were significantly superior/ on par with HP and Z-0-78, SE 86 142 was found on par with parent cultivar Z-0-86. Compared to individual check varieties Karthika (20.23%) and Varada (20.25%), five somaclones each were on par with check varieties. Compared to the mean of check varieties (20.03%) and that of parent clones (20.78%), out of the 25 somaclones evaluated, five somaclones and SEHP 8 were on par with check varieties and parent clones respectively.

Table 6. Quality attributes of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Somaclones/ parents/ check	Powder colour	Driage (%)	Volatile oil (%)	Oil yield (kg ha^{-1})	Oleoresin (%)	Oleoresin yield (kg ha^{-1})	Crude fibre content (%)
1	CHP 8	DuC	17.12	1.60	47.49	6.17	183.12	3.80
2	CHP 39	LC	17.04	2.20	59.09	8.59	230.71	2.40
3	CHP 49	DC	16.44	1.60	41.25	7.44	191.81	2.88
4	CHP 135	DC	15.89	2.00	54.16	7.24	196.06	2.30
5	SEHP 8	DC	21.21	2.00	62.17	6.05	188.08	3.40
6	SEHP 63	DC	16.61	1.92	61.58	6.13	196.60	4.03
7	SEHP 64	CY	18.98	1.72	38.29	5.47	121.77	2.35
8	SEHP 73	DuC	16.62	1.20	26.22	6.93	151.44	3.37
9	SEHP 146	CY	17.40	2.20	37.55	5.84	99.67	1.85
10	C 78 129	LC	19.29	1.20	25.44	4.78	101.32	1.63
11	SE 78 26	DuC	21.06	1.20	37.00	4.98	153.57	1.25
12	SE 78 30	DuC	20.65	2.20	64.82	5.71	168.24	1.87
13	C 86 23	LC	17.20	2.00	61.80	5.67	175.20	2.33
14	C 86 26	LC	16.64	2.00	57.78	5.07	146.48	2.40
15	C 86 32	LC	20.45	1.60	49.06	5.55	170.18	1.67
16	C 86 40	LC	19.34	1.60	56.02	5.04	176.47	3.35
17	C 86 124	LC	20.52	2.00	65.67	6.13	201.29	1.75
18	C 86 139	LC	19.98	1.50	53.79	4.34	155.65	1.55
19	SE 86 24	LC	17.80	2.32	49.45	5.87	125.12	1.90
20	SE 86 40	CY	18.66	2.32	85.79	6.32	233.69	1.57
21	SE 86 42	CY	19.57	1.80	50.24	5.33	148.76	1.27
22	SE 86 81	LC	17.51	1.92	71.99	5.49	205.87	1.70
23	SE 86 83	CY	17.92	1.60	50.69	5.23	165.72	1.95
24	SE 86 131	LC	17.75	1.50	48.29	5.34	171.90	1.35
25	SE 86 142	CY	19.60	2.32	90.96	4.75	186.22	2.40
26	Karthika	CY	20.23	1.20	28.82	5.70	136.87	3.53
27	Varadha	CY	20.25	1.60	42.51	4.38	116.36	3.36
28	Z-0-78	Y	22.12	1.16	27.15	7.32	171.31	3.48
29	Z-0-86	Y	19.00	2.02	44.33	9.00	197.51	2.74
30	HP	Y	21.23	1.53	42.26	4.82	133.13	4.56
C D (0.05)			0.25	3.06	23.52	0.48	81.26	0.39
C V %			6.60	7.46	20.88	4.10	22.22	7.21

Powder Colour

LC – Light cream DuC – Dull cream DC – Dark cream CY – Creamish yellow Y - Yellow

Recovery of volatile oil varied between 1.20 to 2.32 per cent in the somaclones studied. Highest recovery of volatile oil (2.32%) was registered in the clones SE 86 24, SE 86 40 and SE 86 142. Lowest volatile oil recovery (1.20%) was recorded in somaclones SEHP 73, C 78 129 and SE 78 26.

Compared to individual parents [Z-0-78 (1.16%), Z-0-86 (2.02%) and HP (1.53%)], SE 78 30 was significantly superior to parent cultivar Z-0-78, among the 13 somaclones of HP, six were significantly superior and two were on par and among the nine somaclones of Z-0-86, somaclones SE 86 24, SE 86 40 and SE 86 142 were significantly superior to the parent cultivar. Compared to individual check varieties Karthika (1.2%) and Varada (1.6%), 22 and 13 somaclones were found significantly superior to Karthika and Varada respectively. Somaclones SEHP 64 and SE 86 42 were on par with Varada. Compared to the mean of check varieties (1.40 %) and that of parent clones (1.57 %), out of the 25 somaclones evaluated, 14 and 13 somaclones were found significantly superior to check varieties and parent clones respectively. Eight and seven somaclones were found on par with check varieties and parent clones respectively.

4.1.3.3 Volatile oil yield per hectare

Volatile oil yield per hectare ranged between 25.44 and 90.96 kg ha^{-1}. Highest oil yield was recorded in somaclone SE 86 142 and lowest in C 78 129 (25.44 kg ha^{-1}).

Compared to individual parents [Z-0-78 (27.15 kg ha^{-1}), Z-0-86 (44.33 kg ha^{-1}) and HP (42.26 kg ha^{-1})], among the three somaclones of Z-0-78, SE 78 30 was significantly superior, among the thirteen clones of Z-0-86, three somaclones (SE 86 40, SE 86 81 and SE 86 142) were found significantly superior to the parent cultivar, though none of the somaclones was superior to HP, five were on par. Compared to individual check varieties, Karthika (28.82 kg ha^{-1}) and Varada (42.50 kg ha^{-1}), 13 and three somaclones (SE 86 40, SE 86 81 and SE 86 142) were significantly superior and 10 and 16 somaclones were on par with Karthika and Varada respectively. Compared to mean parent (37.91 kg ha^{-1}) and mean check (35.67 kg ha^{-1}), eight and seven somaclones were superior to mean parent and check respectively. Fourteen and sixteen somaclones were on par with mean parent and check respectively.

4.1.3.4 Oleoresin content

Oleoresin content ranged between 4.34 to 8.59 per cent in the somaclones evaluated. Higher recovery was noticed in the clone CHP 39 (8.59%) and lowest in C 86 139 (4.34%).

Compared to individual parents [Z-0-78 (7.32%), Z-0-86 (9.00%) and HP (4.82%)], though none were significantly superior to Z-0-78 and Z-0-86, all the nine somaclones were found to be significantly superior to parent clone HP. Compared to individual check varieties Karthika (5.7%) and Varada (4.38%), four and 22 somaclones were found to be significantly superior and eight and two (C 78 129 and SE 86 142) were on par with Karthika and Varada respectively. Compared to the mean of check varieties (5.04 %) and that of parent clones (7.05 %), out of the 25 somaclones evaluated, 14 somaclones were found significantly superior to check varieties, seven and three somaclones were found on par with parent cultivars and check varieties respectively.

4.1.3.5 Oleoresin yield per hectare

Oleoresin yield per hectare ranged from 99.67 to 233.69 kg ha^{-1} in the somaclones evaluated. Highest oleoresin yield was noticed in the clone SE 86 40 (233.69 kgha^{-1}) and lowest in SEHP 146 (99.67 kgha^{-1}).

Compared to individual parents [Z-0-78 (136.87 kg ha^{-1}), Z-0-86 (116.36 kg ha^{-1}) and HP (171.31 kg ha^{-1})], though none were significantly superior to Z-0-78 and HP, three somaclones were found to be significantly superior to parent clone Z-0-86. Two, ten and six somaclones were on par with parent cultivars Z-0-78, Z-0-86 and HP respectively. Compared to individual check varieties Karthika (197.51 kg ha^{-1}) and Varada (133.13 kg ha^{-1}), two somaclones (CHP 39 and SE 86 40) were found to be significantly superior to Varada while three and nineteen were on par with Karthika and Varada respectively. Compared to the mean of check varieties (126.67 kg ha^{-1}) and that of parent clones (167.32 kg ha^{-1}), out of the 25 somaclones evaluated, two somaclones (CHP 39 and SE 86 40) were found to be significantly superior to check varieties, 20 and 15 somaclones were found to be on par with mean of check and parent clones.

4.1.3.6 Crude fibre content

Fibre content ranged between 1.25 and 4.03 per cent. Somaclone SEHP 63 showed highest fibre content of (4.03%) and lowest fibre content was recorded in SE 78 26 (1.25%).

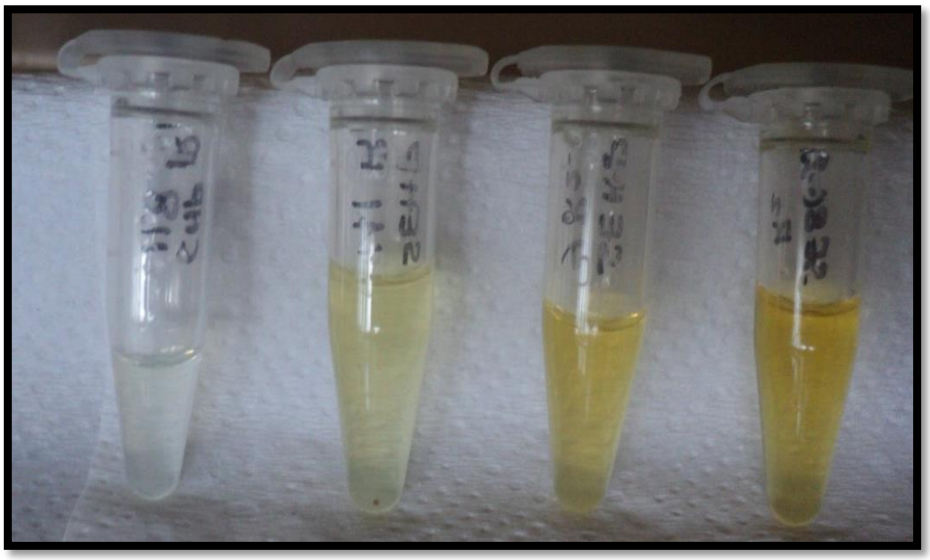
Compared to individual parents [Z-0-78 (3.48%), Z-0-86 (2.74%) and HP (4.56%)], among the nine somaclones of Z-0-86, all the somaclones recorded significantly lower fibre content except somaclone C 86 40 (3.35%) which was on par with the parent cultivar Z-0-86. Compared to individual check varieties Karthika (3.53%) and Varada (3.36%), all the somaclones recorded significantly lower fibre content except SEHP 63 and CHP 8. Somaclone CHP 8 and two somaclones (SEHP 8 and SEHP 73) were on par with Karthika and Varada respectively. Compared to the mean of check varieties (3.45 %) and that of parent clones (3.59 %), out of the 25 somaclones evaluated, SEHP 63 was found to be significantly superior to check varieties and parent clones and CHP 8 was found to be on par with check varieties and parent clones.

4.1.3.7 Colour of ginger powder

Different powder colours recorded include light cream, dull cream, dark cream, creamish yellow and yellow. Among somaclones light cream powder colour was found dominating (11 somaclones) followed by creamish yellow (6 somaclones), dull cream and dark cream (4 somaclones each). The parent cultivars showed yellow powder colour whereas the check varieties were creamish yellow.

4.1.4 DUS characterization of somaclones

Distinctiveness, Uniformity and Stability (DUS) of somaclones were evaluated as per the DUS Guidelines given by PPV & FR Act (2001). Details of characterization of 25 somaclones based on 17 characters are given in the Tables 7 and 8, Plates 5, 6, 7, 8, 9 and 10. The plants were erect or semi erect and majority of somaclones were short statured



Cream Light yellow Yellow Bright yellow

Plate 4. Variability in volatile oil colour

Table 7. DUS characterization of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Somaclones/ parents/ check	Plant: Growth habit	Plant height (cm)	No. of shoot	Shoot height (cm)	Shoot diameter (cm)
1	CHP 8	Erect (1)	Short (3)	Few (3)	Short (3)	Medium (5)
2	CHP 39	Erect (1)	Short (3)	Few (3)	Short (3)	Medium (5)
3	CHP 49	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Narrow (3)
4	CHP 135	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Medium (5)
5	SEHP 8	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Medium (5)
6	SEHP 63	Erect (1)	Medium (5)	Few (3)	Medium (5)	Narrow (3)
7	SEHP 64	Erect (1)	Short (3)	Few (3)	Short (3)	Narrow (3)
8	SEHP 73	Erect (1)	Short (3)	Few (3)	Short (3)	Narrow (3)
9	SEHP 146	Erect (1)	Short (3)	Few (3)	Short (3)	Narrow (3)
10	C 78 129	Erect (1)	Short (3)	Few (3)	Short (3)	Narrow (3)
11	SE 78 26	Erect (1)	Short (3)	Few (3)	Short (3)	Narrow (3)
12	SE 78 30	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Narrow (3)
13	C 86 23	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Narrow (3)
14	C 86 26	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Medium (5)
15	C 86 32	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Narrow (3)
16	C 86 40	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Narrow (3)
17	C 86 124	Erect (1)	Short (3)	Few (3)	Short (3)	Narrow (3)
18	C 86 139	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Medium (5)
19	SE 86 24	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Narrow (3)
20	SE 86 40	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Medium (5)
21	SE 86 42	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Medium (5)
22	SE 86 81	Erect (1)	Short (3)	Few (3)	Short (3)	Narrow (3)
23	SE 86 83	Erect (1)	Short (3)	Few (3)	Short (3)	Medium (5)
24	SE 86 131	Semi- erect (3)	Short (3)	Few (3)	Short (3)	Narrow (3)
25	SE 86 142	Erect (1)	Short (3)	Medium (5)	Short (3)	Narrow (3)
26	Karthika	Semi- erect (3)	Short (3)	Medium (5)	Short (3)	Narrow (3)
27	Varadha	Semi- erect (3)	Short (3)	Medium (5)	Short (3)	Narrow (3)
28	Z-0-78	Erect (1)	Short (3)	Medium (5)	Short (3)	Narrow (3)
29	Z-0-86	Semi- erect (3)	Short (3)	Medium (5)	Short (3)	Medium (5)
30	HP	Semi- erect (3)	Short (3)	Medium (5)	Short (3)	Medium (5)

Contd.

Erect Semi erect

Plate 5. Variability in growth habit

Light green Green Dark green

Plate 6. Variability in shoot colour

Table 7. DUS characterization of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Somaclones/ parents/ check	Shoot: Intensity of green colour	No. of leaves in main shoot	Leaf length (cm)	Leaf width (cm)	Leaf: Intensity of green colour
1	CHP 8	Green (3)	Few (3)	Short (3)	Medium (5)	Green (3)
2	CHP 39	Light green (1)	Few (3)	Medium (5)	Medium (5)	Green (3)
3	CHP 49	Green (3)	Few (3)	Short (3)	Medium (5)	Green (3)
4	CHP 135	Green (3)	Few (3)	Short (3)	Medium (5)	Green (3)
5	SEHP 8	Green (3)	Few (3)	Medium (5)	Medium (5)	Green (3)
6	SEHP 63	Green (3)	Few (3)	Medium (5)	Narrow (3)	Green (3)
7	SEHP 64	Green (3)	Few (3)	Short (3)	Medium (5)	Light green (1)
8	SEHP 73	Light green (1)	Few (3)	Short (3)	Narrow (3)	Light green (1)
9	SEHP 146	Green (3)	Few (3)	Short (3)	Narrow (3)	Light green (1)
10	C 78 129	Green (3)	Few (3)	Short (3)	Narrow (3)	Light green (1)
11	SE 78 26	Light green (1)	Few (3)	Short (3)	Narrow (3)	Light green (1)
12	SE 78 30	Green (3)	Few (3)	Short (3)	Narrow (3)	Light green (1)
13	C 86 23	Light green (1)	Few (3)	Short (3)	Narrow (3)	Green (3)
14	C 86 26	Light green (1)	Few (3)	Short (3)	Narrow (3)	Light green (1)
15	C 86 32	Light green (1)	Few (3)	Short (3)	Narrow (3)	Green (3)
16	C 86 40	Green (3)	Few (3)	Short (3)	Narrow (3)	Light green (1)
17	C 86 124	Green (3)	Few (3)	Short (3)	Narrow (3)	Light green (1)
18	C 86 139	Light green (1)	Few (3)	Short (3)	Narrow (3)	Light green (1)
19	SE 86 24	Green (3)	Few (3)	Short (3)	Narrow (3)	Green (3)
20	SE 86 40	Light green (1)	Few (3)	Short (3)	Narrow (3)	Dark green (5)
21	SE 86 42	Green (3)	Few (3)	Short (3)	Narrow (3)	Green (3)
22	SE 86 81	Green (3)	Few (3)	Short (3)	Narrow (3)	Green (3)
23	SE 86 83	Green (3)	Few (3)	Short (3)	Narrow (3)	Green (3)
24	SE 86 131	Light green (1)	Few (3)	Medium (5)	Medium (5)	Green (3)
25	SE 86 142	Green (3)	Few (3)	Short (3)	Medium (5)	Green (3)
26	Karthika	Light green (1)	Few (3)	Short (3)	Medium (5)	Green (3)
27	Varadha	Green (3)	Few (3)	Short (3)	Medium (5)	Green (3)
28	Z-0-78	Green (3)	Few (3)	Short (3)	Medium (5)	Dark green (5)
29	Z-0-86	Green (3)	Few (3)	Short (3)	Medium (5)	Dark green (5)
30	HP	Green (3)	Few (3)	Short (3)	Medium (5)	Green (3)

Contd.

Light green Green Dark green

Plate 7. Variablity in leaf colour

Yellowish-white bract Pink bract

Plate 8. Variability in bract colour

Table 7. DUS characterization of somaclones in ginger (*Zingiber officinale* Rosc.)

Sl no:	Somaclones/ parents/ check	Petiole length (cm)	Spike length	Colour of bract	Rhizome: Thickness (cm)	Rhizome : Shape	Crop duration (days)	Dry recovery (%)
1	CHP 8	Short (3)	-	-	Bold (7)	Zigzag (5)	Long (7)	Medium (5)
2	CHP 39	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Curved (3)	Short (3)	Medium (5)
3	CHP 49	Short (3)	Short (3)	Yellowish- white tip (5)	Bold (7)	Zigzag (5)	Long (7)	Medium (5)
4	CHP 135	Short (3)	Short (3)	Yellowish- white tip (5)	Bold (7)	Straight (1)	Long (7)	Low (3)
5	SEHP 8	Short (3)	Short (3)	Yellowish- white tip (5)	Bold (7)	Zigzag (5)	Long (7)	Medium (5)
6	SEHP 63	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Curved (3)	Long (7)	Medium (5)
7	SEHP 64	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Curved (3)	Long (7)	High (7)
8	SEHP 73	Short (3)	-	-	Medium (5)	Zigzag (5)	Medium (5)	Medium (5)
9	SEHP 146	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Zigzag (5)	Long (7)	Medium (5)
10	C 78 129	Short (3)	-	-	Medium (5)	Straight (1)	Short (3)	High (7)
11	SE 78 26	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Straight (1)	Short (3)	High (7)
12	SE 78 30	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Straight (1)	Medium (5)	High (7)
13	C 86 23	Short (3)	Short (3)	Crimson (3)	Medium (5)	Zigzag (5)	Long (7)	Medium (5)
14	C 86 26	Short (3)	-	-	Medium (5)	Zigzag (5)	Long (7)	Medium (5)
15	C 86 32	Short (3)	-	-	Medium (5)	Straight (1)	Long (7)	High (7)
16	C 86 40	Short (3)	-	-	Medium (5)	Curved (3)	Long (7)	High (7)
17	C 86 124	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Zigzag (5)	Long (7)	High (7)
18	C 86 139	Short (3)	-	-	Medium (5)	Curved (3)	Medium (5)	High (7)
19	SE 86 24	Short (3)	-	-	Medium (5)	Straight (1)	Short (3)	Medium (5)
20	SE 86 40	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Curved (3)	Long (7)	High (7)
21	SE 86 42	Short (3)	Short (3)	Crimson (3)	Medium (5)	Straight (1)	Long (7)	High (7)
22	SE 86 81	Short (3)	-	-	Medium (5)	Straight (1)	Long (7)	Medium (5)
23	SE 86 83	Short (3)	Short (3)	Crimson (3)	Medium (5)	Curved (3)	Medium (5)	Medium (5)
24	SE 86 131	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Straight (1)	Medium (5)	Medium (5)
25	SE 86 142	Short (3)	Short (3)	Yellowish- white tip (5)	Medium (5)	Zigzag (5)	Long (7)	High (7)
26	Karthika	Short (3)	-	-	Medium (5)	Zigzag (5)	Long (7)	High (7)
27	Varadha	Short (3)	-	-	Medium (5)	Curved (3)	Medium (5)	High (7)
28	Z-0-78	Short (3)	-	-	Medium (5)	Curved (3)	Medium (5)	High (7)
29	Z-0-86	Short (3)	-	-	Medium (5)	Curved (3)	Medium (5)	High (7)
30	HP	Short (3)	-	-	Medium (5)	Curved (3)	Medium (5)	High (7)

Straight

Curved

Zigzagged

Plate 9. Variability in rhizome shape in ginger somaclones

CHP 8

CHP 135

CHP 49

SEHP 8

Plate 10. Somaclones with bold rhizome

Table 8. Grouping of somaclones based on DUS characterization

Sl no.	Characteristics	States	Note	Number of somaclones
1	Plant: Growth habit	Erect	1	12
		Semi erect	3	13
		Spreading	5	0
2	Plant: Height (cm)	Short (< 100)	3	24
		Medium (100-120)	5	1
		Tall (>120)	7	0
3	Plant: Number of shoot	Few (<10)	3	24
		Medium (10-15)	5	1
		Many (>15)	7	0
4	Plant: Height of shoot (cm)	Short (< 75)	3	24
		Medium (75-90)	5	1
		Tall (>90)	7	0
5	Shoot: Diameter (cm)	Narrow (< 3)	3	9
		Medium (3-5)	5	16
		Broad (>5)	7	0
6	Shoot: Intensity of green colour	Light green	1	9
		Green	3	16
		Dark green	5	0
7	Shoot: Number of leaves on main shoot	Few (<25)	3	25
		Medium (25-35)	5	0
		Many (>35)	7	0
8	Leaf: Length (cm)	Short (<25)	3	21
		Medium (25-30)	5	4
		Long (>30)	7	0
9	Leaf: Width (cm)	Narrow (<2.5)	3	17
		Medium (2.5 – 3.5)	5	8
		Broad (> 3.5)	7	0

Contd.

Table 8. Grouping of somaclones based on DUS characterization

Sl no.	Characteristics	States	Note	Number of somaclones
10	Leaf: Intensity of green colour	Light green	1	10
		Green	3	14
		Dark green	5	1
11	Leaf: Petiole length (cm)	Short (<0.5)	3	25
		Medium (0.5 – 0.7)	5	0
		Long (> 0.7)	7	0
12	Spike : Length (cm)	Short (<25)	3	16
		Medium (25 -35)	5	0
		Long (>35)	7	0
13	Colour of the bract tip of fully developed spike	Crimson	3	3
		Yellowish-white tip	5	13
14	Rhizome : Thickness (cm)	Thin (<2)	3	0
		Medium (2-3)	5	21
		Bold (>3)	7	4
15	Rhizome: Shape	Straight	1	9
		Curved	3	7
		Zigzagged	5	9
16	Crop duration (days)	Short (<200)	3	4
		Medium (200-210)	5	5
		Long (>210)	7	16
17	Dry recovery (%)	Low (<16)	3	1
		Medium (16- 18)	5	13
		High (>18)	7	11

with fewer number of shoots and leaves and narrow medium sized pseudostem. Leaves were fewer, short and narrow with short petioles. Rhizome shape observed was straight, curved and zigzagged. Rhizomes were medium in size. Spikes produced in the somaclones had both yellow-white bracts and pink bracts. Somaclones were of long duration with medium to high dry recovery of rhizomes.

4.2. SCREENING OF SOMACLONES FOR RESISTANCE/ TOLERANCE TO PESTS AND DISEASES

Screening of somaclones for resistance or tolerance to pests and diseases were carried out. The methods used include, screening for natural occurrence of pests and diseases, artificial screening against rhizome rot and bacterial wilt and sick plot screening.

4.2.1. Screening for natural occurrence of pests and diseases

Screening of selected somaclones was carried out for the natural incidence of major pests viz; shoot borer, rhizome maggot and diseases viz; rhizome rot, bacterial wilt and *Phyllosticta* leaf spot, during the field evaluation (Table 9).

4.2.1.1 Plant infection

Under natural screening, during the field evaluation, incidence of rhizome rot and bacterial wilt was noted as a complex associated with the incidence of rhizome maggot too. In the infested plants which were uprooted, the presence / absence of *Pythium*, bacteria and rhizome maggot were recorded and are presented as total plant infection. Total plant infection ranged from zero to 36.5 per cent. Pests and disease incidence were not noticed in C 86 139 and the highest infection was noticed in SEHP 73(36.50%). Compared with individual parents [Z-0-78(26.26%), Z-0-86 (39.54%) and HP (17.73)], all the somaclones showed lesser incidence than the respective parents except SEHP 73 (36.50%). Compared to Karthika (38.50%) and Varada (20.00%), all the somaclones except SEHP 73 and C 86 23, recorded lower plant infection. Somaclone SEHP 73 alone recorded highest disease incidence when compared to the mean of check varieties (27.84%) and parent clones (29.25%). Somaclones CHP 135, SEHP 64, SEHP 146, SE 78 26, C 86 23, C 86 40 and C 86 139 were free from rhizome maggot infestation.

Table 9. Natural occurrence of pests and diseases in somaclones

Sl no:	Somaclones / parents/ check	Incidence			Total plant infection (%)	Shoot borer incidence (%)	Leaf spot incidence (%)
		Maggot	Bacterial wilt	Rhizome rot			
1	CHP 8	*		*	11.73	52.11	23.87
2	CHP 39	*	*		15.73	29.99	18.40
3	CHP 49	*	*		17.18	33.76	27.24
4	CHP 135		*		4.68	37.51	16.10
5	SEHP 8	*	*	*	13.46	37.89	12.25
6	SEHP 63	*		*	3.12	41.07	20.51
7	SEHP 64		*		3.45	52.43	19.86
8	SEHP 73	*	*		36.50	51.66	24.85
9	SEHP 146		*		17.00	30.54	12.52
10	C 78 129	*	*		2.76	49.47	14.20
11	SE 78 26		*		3.57	41.64	20.11
12	SE 78 30	*		*	9.39	54.35	16.99
13	C 86 23		*		22.33	41.04	11.44
14	C 86 26	*	*		10.71	29.85	15.72
15	C 86 32	*		*	15.62	63.63	15.14
16	C 86 40		*		3.33	48.44	15.16
17	C 86 124	*	*		4.16	50.94	16.71
18	C 86 139	-	-	-	-	53.31	18.89
19	SE 86 24	*	*		3.52	49.95	10.43
20	SE 86 40	*	*		3.39	29.53	23.10
21	SE 86 42	*	*		8.02	54.44	18.87
22	SE 86 81	*	*		5.28	36.05	9.49
23	SE 86 83	*	*		2.13	42.47	11.58
24	SE 86 131	*		*	3.58	47.69	14.34
25	SE 86 142	*	*		1.08	41.39	17.03
26	Karthika		*		38.50	49.01	22.01
27	Varadha			*	20.00	22.26	28.16
28	Z-0-78	*	*	*	26.26	30.98	15.49
29	Z-0-86	*	*	*	39.54	28.14	21.04
30	HP		*		17.73	34.52	23.14
C D (0.05)					16.62	21.24	10.65
C V (%)					62.45	23.10	27.44

*Incidence present

4.2.1.2 Shoot borer

Shoot borer incidence was highest during the active tiller growth stage. Crop was heavily infected by the pest. Shoot borer incidence ranged between 29.53 and 63.63 per cent. Lowest shoot borer incidence was noted in SE 86 40 (29.53%) and highest in C 86 32 (63.63%). Compared to individual parents [Z-0-78 (30.98%), Z-0-86 (28.14%) and HP (34.52%)], somaclones from HP (CHP 39, CHP 49 and SEHP 146), alone showed significantly lower shoot borer incidence than the parent cultivars. Compared to individual check varieties Karthika (49.01%) and Varada (22.26%), somaclones SE 86 40 (29.53%) and SE 86 42 (54.44%) recorded lowest and highest shoot borer incidence respectively. Compared to mean shoot borer incidence in check varieties (35.63%) and parent clones (31.21%), all the somaclones showed significantly lower incidence of shoot borer.

4.2.1.3 Leaf spot

Per cent disease incidence (PDI) in the somaclones was cent per cent and none of the somaclones were field tolerant to leaf spot disease. Per cent disease severity (PDS) of leaf spot disease in crop ranged between 9.49 and 27.24 per cent. SE 86 81 was the clone least affected by leaf spot disease (9.49 %), where as CHP 49 recorded the highest leaf spot disease incidence (27.24 %). Mean of leaf spot incidence in check varieties was 25.08 % and that in parent clones was 19.89 %. When somaclones were scored for leaf spot incidence, SE 86 81 recorded grade 1(0-10% infection), 23 somaclones recorded grade 2 (10-25% infection) and somaclone CHP 49 recorded grade 3 (25-50% infection). Among the parents Z-0-78, Z-0-86 and HP along with Karthika was classified under grade 2 (10-25% infection) and Varada showed grade 3 infection.

4.2.2. Artificial screening

4.2.2.1 Artificial screening against rhizome rot

Artificial screening against rhizome rot was conducted for the selected 25 somaclones along with two check varieties and three parent clones (Tables 10 and 11, Plate 11). Variation in the disease intensity and time taken for appearance of symptoms was observed among the somaclones. Time taken for appearance of symptoms ranged from a mean of 7

Table 10. Incidence of rhizome rot in artificial screening of somaclones in ginger

Sl no:	Somaclones/ parents/ check	Symptom expression (DAI)	Rhizome rot incidence score							Disease incidence (%)
			1 WAI	2 WAI	3 WAI	4 WAI	5 WAI	6 WAI	7 WAI	
1	CHP 8	10.50	1	4	4	4	4	4	4	100
2	CHP 39	11.66	2	4	4	4	4	4	4	100
3	CHP 49	7.00	1	2	2	3	3	3	3	66.67
4	CHP 135	14.00	0	4	4	4	4	4	4	100
5	SEHP 8	14.00	0	4	4	4	4	4	4	100
6	SEHP 63	18.66	1	3	4	4	4	4	4	100
7	SEHP 64	14.00	0	4	4	4	4	4	4	100
8	SEHP 73	14.00	0	4	4	4	4	4	4	100
9	SEHP 146	14.00	0	3	3	4	4	4	4	100
10	C 78 129	14.00	0	4	4	4	4	4	4	100
11	SE 78 26	14.00	0	4	4	4	4	4	4	100
12	SE 78 30	14.00	1	2	3	3	3	4	4	100
13	C 86 23	16.33	0	1	4	4	4	4	4	100
14	C 86 26	14.00	0	1	1	1	3	3	3	66.67
15	C 86 32	14.00	0	4	4	4	4	4	4	100
16	C 86 40	14.00	0	4	4	4	4	4	4	100
17	C 86 124	18.66	1	3	3	3	3	3	3	66.67
18	C 86 139	10.5	1	4	4	4	4	4	4	100
19	SE 86 24	14.00	0	4	4	4	4	4	4	100
20	SE 86 40	17.50	0	3	4	4	4	4	4	100
21	SE 86 42	17.50	0	4	4	4	4	4	4	100
22	SE 86 81	14.00	1	1	2	2	3	3	3	66.67
23	SE 86 83	14.00	0	4	4	4	4	4	4	100
24	SE 86 131	28.00	0	3	4	4	4	4	4	100
25	SE 86 142	11.66	3	3	3	4	4	4	4	100
26	Karthika	9.33	3	4	4	4	4	4	4	100
27	Varadha	9.33	3	4	4	4	4	4	4	100
28	Z-0-78	14.00	1	4	4	4	4	4	4	100
29	Z-0-86	14.00	0	4	4	4	4	4	4	100
30	HP	14.00	0	1	3	4	4	4	4	100

DAI- Days after inoculation WAI- Weeks after inoculation

Before inoculation

Symptom expression

Re-sprouting stage

Plate 11. Symptom expression and re-sprouting in artificial screening of somaclones against rhizome rot

Table 11. Rhizome rot tolerant accessions in artificial screening of somaclones

Sl no:	Somaclones/ parents/ check	Days taken to re-sprout	Re-sprouting %	Days to re infection	Re infection on resprouts %	No. of tillers retained / plant	Weight of rhizome (g)
1	CHP 39	21.00	66.67	49	33.33	6	18
2	CHP 49*	28.00	33.33	49	100.00	3.5	58
3	CHP 135	35.00	66.67	Nil	Nil	2.5	6
4	SEHP 8	14.00	33.33	70	100.00	0	-
5	SEHP 63	21.00	33.33	42	100.00	0	-
6	SEHP 64	49.00	66.67	63	50.00	1	4
7	SEHP 73	21.00	33.33	Nil	33.33	3	4
8	SEHP 146	35.00	66.67	49	50.00	4	18
9	C 78 129	35.00	33.33	Nil	Nil	4	6
10	SE 78 26	21.00	66.67	42	100.00**	2	14, 6
11	C 86 26*	Nil	Nil	Nil	Nil	8	50
12	C 86 32	28.00	66.67	42	100.00	0	-
13	C 86 124*	21.00	33.33	35	100.00	5	46
14	C 86 139	21.00	33.33	Nil	Nil	4	78
15	SE 86 24	21.00	66.67	Nil	Nil	3.5	24, 106
16	SE 86 40	28.00	33.33	35	100.00	0	-
17	SE 86 42	21.00	66.67	28	50.00	1	-
18	SE 86 81*	Nil	Nil	Nil	Nil	2	24
19	SE 86 83	21.00	33.33	35	100.00	0	-
20	SE 86 131	21.00	33.33	28	100.00	0	-
21	SE 86 142	49.00	33.33	49	100.00	0	-
22	Karthika	21.00	33.33	28	100.00	0	-

*Somaclones which retained 1 plant without any infection till harvest

days in CHP 49 to 28 days in SE 86 131. Days to complete infection and death of plants in somaclones ranged from 14 days to 56 days. Somaclones SEHP 63 took longest period for complete infection and death of plants (56 days). Rhizome rot incidence in the somaclones ranged from 66.67 to 100 per cent, whereas the parents and check varieties showed 100% infection. Out of the 25 somaclones, four somaclones (CHP 49, C 86 26, C 86 124 and SE 86 81) recorded low disease incidence of 66.67% retaining one plant out of three. Nineteen somaclones showed re-sprouting. Days taken to re-sprout ranged between 21 to 49 days. SEHP 8 recorded earliness in sprouting (21days). Re-infection of re-sprouted somaclones was noted from 28 to 70 days. Repeated re-sprouting and re-infection was noted in certain somaclones namely CHP 49, SEHP 63, SEHP 146 and SE 78 26. Among the check varieties, Karthika showed re-sprouting and showed re-infection in 28 days. Mean number of tillers retained per plant 49 days after planting ranged between one and eight tillers. Rhizomes were harvested from 13 somaclones of which four survived infection and nine were re-sprouted ones. Rhizome yield per plant ranged between 4g to 106g (SE 86 24). In the control plants, where no inoculation of pathogen was done the rhizome yield ranged from 4g to 96g. Among the 25 somaclones, twenty one somaclones showed tolerance to rhizome rot disease, but 13 somaclones which yielded rhizomes were relatively more tolerant.

4.2.2.2 Artificial screening against bacterial wilt

Artificial screening against bacterial wilt was conducted for the selected 25 somaclones along with two check varieties and three parent clones (Tables 12 and 13, Plate 12). All the somaclones showed visible disease symptom within a period of four to 14 days. Incidence of the disease ranged between 66.67% and 100%. Days to complete infection and death of plants ranged from 8 to 24 days. Somaclones C 86 23 and SEHP 64 recorded the longest period for complete infection and death of plants. Re-sprouting of rhizomes was noted in six somaclones with a re-sprouting percentage of 33.33 per cent. Number of days taken to re-sprout was 8 days to 36 days, where somaclones SE 78 30 and SE 86 24 showed earliness in re-sprouting and SEHP 63 was found to be late re-sprouting (36 days). Days to re-infection ranged from 16 (SE 86 24) to 32 days in somaclones C 78 129, SE 78

Table 12. Incidence of bacterial wilt in artificial screening of somaclones in ginger

Sl no:	Somaclones/ parents/ check	Symptom expression (DAI)	Incidence of bacterial wilt							Disease incidence (%)
			4 DAI	8 DAI	12 DAI	16 DAI	20 DAI	24 DAI	28 DAI	
1	CHP 8	4.00	1	4	5	5	5	5	5	100.00
2	CHP 39	4.00	5	5	5	5	5	5	5	100.00
3	CHP 49	8.00	2	3	4	5	5	5	5	100.00
4	CHP 135	8.00	3	4	4	4	5	5	5	100.00
5	SEHP 8	8.00	1	5	5	5	5	5	5	100.00
6	SEHP 63	8.00	2	3	4	4	4	4	4	66.67
7	SEHP 64	8.00	1	2	2	2	3	5	5	100.00
8	SEHP 73	8.00	1	2	2	5	5	5	5	100.00
9	SEHP 146	8.00	1	4	5	5	5	5	5	100.00
10	C 78 129	8.00	1	3	3	5	5	5	5	100.00
11	SE 78 26	8.00	1	3	4	5	5	5	5	100.00
12	SE 78 30	8.00	2	5	5	5	5	5	5	100.00
13	C 86 23	8.00	1	4	4	4	4	5	5	100.00
14	C 86 26	8.00	5	5	5	5	5	5	5	100.00
15	C 86 32	8.00	5	5	5	5	5	5	5	100.00
16	C 86 40	8.00	1	5	5	5	5	5	5	100.00
17	C 86 124	8.00	1	5	5	5	5	5	5	100.00
18	C 86 139	8.00	1	4	5	5	5	5	5	100.00
19	SE 86 24	8.00	1	5	5	5	5	5	5	100.00
20	SE 86 40	8.00	1	4	5	5	5	5	5	100.00
21	SE 86 42	14.00	1	5	5	5	5	5	5	100.00
22	SE 86 81	8.00	2	5	5	5	5	5	5	100.00
23	SE 86 83	8.00	1	4	5	5	5	5	5	100.00
24	SE 86 131	8.00	2	5	5	5	5	5	5	100.00
25	SE 86 142	8.00	1	4	4	5	5	5	5	100.00
26	Karthika	8.00	1	2	3	4	5	5	5	100.00
27	Varadha	8.00	2	4	4	4	5	5	5	100.00
28	Z-0-78	8.00	5	5	5	5	5	5	5	100.00
29	Z-0-86	4.00	5	5	5	5	5	5	5	100.00
30	HP	8.00	1	1	5	5	5	5	5	100.00

DAI – Days after inoculation

Table 13. Bacterial wilt tolerant somaclones in artificial screening of somaclones

Sl no:	Somaclones/ parents/ check	Days taken to re-sprout	Re -sprouting (%)	Days to re infection	Re infection (%)
1	CHP 39	16.00	33.33	20	100.00
2	SEHP 63	36.00	33.33	Nil	Nil
3	C 78 129	28.00	33.33	32	100.00
4	SE 78 30	8.00	33.33	32	100.00
5	SE 86 24	8.00	33.33	16	100.00
6	SE 86 142	24.00	33.33	32	100.00

Before inoculation

Symptom expression

Re-sprouting stage

Plate 12. Symptom expression and re-sprouting in screening of somaclones against bacterial wilt

30 and SE 86 142. Out of the six somaclones re-sprouted, SEHP 63 alone was retained without re-infection.

4.2.3. Sick plot screening

The selected 25 somaclones along with the check varieties were planted in sick field where ginger grown in the previous season was severely infected by rhizome rot and bacterial wilt diseases (Table 14). Out of the 25 somaclones evaluated, 19 somaclones survived the adverse conditions in the sick field. The survival rate recorded among the clones ranged from 0.00 to 62.50 per cent in the somaclones. Highest survival was observed in somaclone SE 86 40 (62.50%). Among the clones, the rhizome yield per plot ranged between 2.00g (SEHP 63) and 276.00g (SE 86 40).

4.3. ASSESSMENT OF GENETIC VARIABILITY AND IDENTIFICATION OF PROMISING SOMACLONES

4.3.1 Genetic variability

The coefficient of variation presented in Tables 3, 4, 5 and 6 showed that there is wide variability between somaclones. The number of shoots registered highest coefficient of variation among morphological characters while number of quaternary fingers recorded highest coefficient of variation among rhizome characters. Dry yield was found to have higher coefficient of variation than fresh rhizome yield. Among quality characters, oleoresin yield was observed to have the highest coefficient of variation. Somaclones of ginger showed significant difference between them.

The magnitude of heritable value is the most important aspect of genetic constitution of breeding material, which has close bearing on the response to selection (Panse, 1957). The population mean, range, phenotypic and genotypic coefficients of variation are presented in Table 15. The phenotypic coefficient of variation (PCV) worked out for fifteen characters with significantly positive correlation ranged from 11.75 to 113.23. The genotypic coefficient of variation (GCV) ranged from 1.73 to 112.04, and characters such as plant height, shoot height and leaf area showed lower GCV.

Sick plot

Initial stage

Final stage

Plate 13. Sick plot screening of somaclones in ginger

Table 14. Survival of somaclones in sick plot screening

Sl no:	Somaclones/ parents/ check	Survival (%)	Weight of rhizomes/ plot (g)
1	CHP 8	12.50	36.00
2	CHP 39	37.50	74.00
3	CHP 49	0.00	0.00
4	CHP 135	12.50	25.00
5	SEHP 8	37.50	95.00
6	SEHP 63	12.50	2.00
7	SEHP 64	12.50	26.00
8	SEHP 73	50.00	78.00
9	SEHP 146	25.00	112.00
10	C 78 129	12.50	29.00
11	SE 78 26	25.00	74.00
12	SE 78 30	0.00	0.00
13	C 86 23	0.00	0.00
14	C 86 26	0.00	0.00
15	C 86 32	0.00	0.00
16	C 86 40	12.50	50.00
17	C 86 124	25.00	75.00
18	C 86 139	25.00	64.00
19	SE 86 24	12.50	10.50
20	SE 86 40	62.50	276.00
21	SE 86 42	12.50	41.00
22	SE 86 81	50.00	53.00
23	SE 86 83	0.00	0.00
24	SE 86 131	50.00	166.00
25	SE 86 142	12.50	14.00
26	Karthika	0.00	0.00
27	Varadha	0.00	0.00
28	Z-0-78	0.00	0.00
29	Z-0-86	0.00	0.00
30	HP	0.00	0.00

4.3.2 Heritability

In crop improvement, only genetic component of variation is important since only this component is transmitted to the next generation. The extent of contribution of genotype to the phenotypic variation for the trait in a population is ordinarily expressed as ratio of genetic variance to the total variance, i.e., phenotypic variance, for the trait; this ratio is known as heritability. Thus heritability denotes the proportion of phenotypic variance that is due to genotype, (Singh, 1990). Heritability for different characters is given in Table 15.

High heritability was expressed by all the characters under study- essential oil content (99.23) followed by thickness of primary fingers (98.19), internodal length of primary fingers (97.91), leaf width (97.54), thickness of secondary fingers (95.92), girth of rhizomes (95.24), length of secondary fingers (95.17), number of primary fingers (93.11), girth of pseudostem (93.10), length of primary fingers (93.00), leaf length (87.22) and driage (84.53). Characters such as leaf area (37.97), shoot height (34.08) and plant height (5.18) expressed low heritability.

4.3.3 Correlation

Correlation provides the nature and extent of relationship between yield and yield components. It is essential for simultaneous improvement of yield components and in turn yields. Correlation coefficients of different characters are presented in Table 16. Correlation was found for the characters : shoot height, plant height, leaf length, leaf width and leaf area, girth of pseudostem, number of primary fingers, length of primary and secondary fingers, internodal length of primary and secondary fingers, thickness of primary and secondary fingers, girth of rhizomes, driage and oil content. Highest correlation was recorded among yield and driage followed by rhizome girth, length of primary and secondary fingers, number of quaternary fingers, shoot and plant height, leaf length, width and single leaf area. Number of primary fingers, intermodal length of primary and secondary fingers and thickness of primary and secondary fingers also showed significant correlation with yield.

4.3.4 Genetic divergence

Twenty five somaclones along with three parent cultivars (Z-0-78, Z-0-86 and HP) and two check varieties were grouped into five clusters using Mahalanobis D^2 statistics (Tables 17, 18 and 19, Fig. 1 and 2).

Cluster I had maximum number of genotypes (13 numbers) which included the polyploids CHP 8, CHP 39, CHP 135, C 78 129, C 86 23, C 86 40, SEHP 63, SE 86 24, SE 86 83, SE 86 131 and SE 78 30 along with parent cultivars Z-0-78 and Z-0-86. The check varieties Karthika and Varada were clustered in cluster V with genotypes C 86 26 and SEHP 146 and rest of the three clusters had four genotypes each. The diploid parent Himcahal Pradesh was clustered in cluster II along with SE 86 40, SE 86 42 and SE 86 142. (Cluster III - C 86 139, SEHP 8, SEHP 64 and SE 86 81) and (Cluster IV - CHP 49, C 86 32, C 86 124, SEHP 73 and SE 78 26)].

Cluster I had the maximum intra cluster value (165942.28) and Cluster V had the minimum (6497.99). The intra cluster distance for other clusters was 21526.99 (Cluster II), 19974.31 (Cluster III) and 30931.52 (Cluster IV). The maximum statistical distance was found between the Cluster I and Cluster IV (88929.67) followed by cluster II (84451.61). The distance between the Cluster III and Cluster V displayed the lowest degree of divergence (Table 19).

Somaclones were grouped into thirteen groups based on four qualitative characters (Fig 12). Varada was grouped in Cluster I along with CHP 8, CHP 49, SEHP 63, SEHP 146 and SE 78 26. Parent cultivars HP, Z-0-86 and Z-0-78 were found separately grouped in Clusters II, VIII and XI. Check variety Karthika was grouped in Cluster IX along with C 86 40, SE 86 40 and C 78 129.

Table 15. Estimates of genetic parameters in somaclones of ginger

Sl no:	Characters	Range	Mean	PCV	GCV	Heritability (%)
1	Shoot height (cm)	48.95 - 80.68	62.24	12.68	1.73	34.08
2	Plant height (cm)	57.88- 101.32	72.24	11.75	2.67	5.18
3	Leaf length (cm)	20.14 - 27.41	22.74	20.99	19.60	87.22
4	Leaf width (cm)	2.08 - 3.06	2.40	64.15	63.35	97.54
5	Girth pseudostem (cm)	2.52 - 3.99	2.90	58.72	56.66	93.10
6	Leaf area (cm^2)	42.70 - 66.76	51.09	14.00	8.63	37.97
7	Number of primary fingers	2.40 - 4.20	3.56	53.07	51.21	93.11
8	Length of primary fingers (cm)	3.85 - 5.57	4.45	47.57	45.87	93.00
9	Length of secondary fingers (cm)	2.66 - 4.77	3.68	51.20	50.92	95.17
10	Inter-nodal length of primary fingers (cm)	0.67 - 0.97	0.78	113.23	112.04	97.91
11	Thickness of primary fingers (cm)	2.28 - 3.21	2.72	64.34	68.71	98.19
12	Thickness of secondary fingers (cm)	2.04 – 3.00	2.54	71.98	70.49	95.92
13	Girth of rhizomes (cm)	7.11 - 8.97	7.83	35.74	34.88	95.24
14	Driage (%)	15.89 - 21.21	18.80	23.06	21.82	84.53
15	Volatile oil (%)	1.20 - 2.32	1.77	75.16	74.87	99.23

Table 16. Correlation coefficients between yield and its components

Sl no.	Characters	Correlation coefficient
1	Number of shoots	0.118
2	Shoot height (cm)	0.319*
3	Plant height (cm)	0.351*
4	Number of leaves	0.249
5	Leaf length (cm)	0.306*
6	Leaf width (cm)	0.376**
7	Girth pseudostem (cm)	0.377**
8	Leaf area (cm^2)	0.312*
9	Petiole length	0.083
10	Number of primary fingers	0.279*
11	Number of secondary fingers	0.158
12	Number of tertiary fingers	0.187
13	Number of quaternary fingers	0.532**
14	Length of primary fingers (cm)	0.541**
15	Length of secondary fingers (cm)	0.419**
16	Inter-nodal length of primary fingers (cm)	0.313*
17	Inter-nodal length of secondary fingers (cm)	0.135
18	Thickness of primary fingers (cm)	0.337**
19	Thickness of secondary fingers (cm)	0.279*
20	Girth of rhizomes (cm)	0.614**
21	Driage (%)	0.632**
22	Dry yield ha^{-1}	0.041
23	Volatile oil (%)	0.283*
24	Oleoresin (%)	0.062
25	Crude fibre content (%)	0.004

** Significant at 0.01 level

*Significant at 0.05 level

Table 17. Clustering pattern in 30 genotypess of ginger

Cluster number	Number of accessions in each cluster	Accessions
I	13	CHP 8, CHP 39, CHP 135, C 78 129, C 86 23, C 86 40, SEHP 63, SE 86 24, SE 86 83, SE 86 131, SE 78 30, Z-0-78, Z-0-86
II	4	SE 86 40, SE 86 42, SE 86 142, Himachal Pradesh
III	4	C 86 139, SEHP 8, SEHP 64, SE 86 81
IV	5	CHP 49, C 86 32, C 86 124, SEHP 73, SE 78 26
V	4	C 86 26, SEHP 146, Karthika, Varada

Table 18. Means of variables for five clusters

Clusters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
I	75.39	64.56	2.94	23.79	2.47	54.3	3.52	4.51	3.73	0.77	2.77	2.59	7.9	18.29	1.79
II	78.06	67.94	3.11	22.64	2.49	50.97	3.68	4.46	3.90	0.8	2.81	2.59	7.89	19.77	1.99
III	73.76	64.56	3.16	23.22	2.46	52.91	3.55	4.66	3.59	0.8	2.74	2.43	8.09	19.42	1.79
IV	65.84	56.34	2.69	21.06	2.34	45.34	3.62	4.27	3.59	0.76	2.79	2.52	8.02	19.02	1.52
V	62.65	54.39	2.56	21.04	2.12	45.67	3.43	4.27	3.45	0.75	2.41	2.4	7.18	18.63	1.75

X_1- Shoot height (cm), X_2- Plant height (cm), X_3- Leaf length (cm), X_4- Leaf width (cm), X_5- Girth of pseudostem (cm), X_6- Leaf area (cm^2), X_7- Number of primary fingers, X_8- Length of primary fingers (cm), X_9- Length of secondary fingers (cm), X_{10}- Inter-nodal length of primary fingers (cm), X_{11}- Thickness of primary fingers (cm), X_{12}- Thickness of secondary fingers (cm), X_{13}- Girth of rhizomes (cm), X_{14}- Driage (%), X_{15}- Volatile oil (%)

Table 19. Inter and intra cluster D^2 values among 5 clusters based on quantitative characters

Cluster	I	II	III	IV	V
I	**165942.28**				
II	84451.61	**21526.99**			
III	83918.00	15500.41	**19974.31**		
IV	88929.67	20267.58	19698.48	**30931.52**	
V	80861.88	12172.47	11721.23	17312.57	**6497.99**

The values printed in bold indicate intra cluster distance

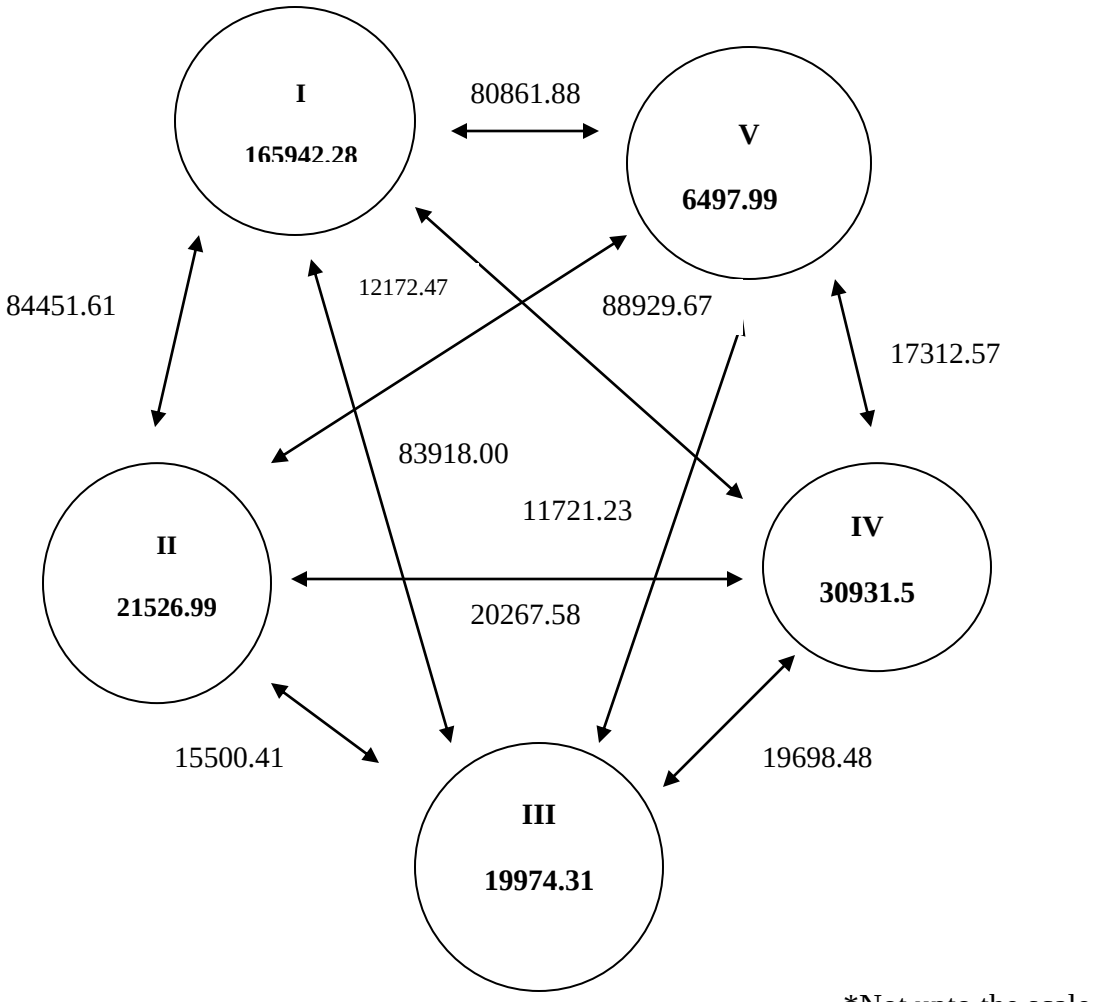


Fig 1. Cluster diagram based on quantitative characters

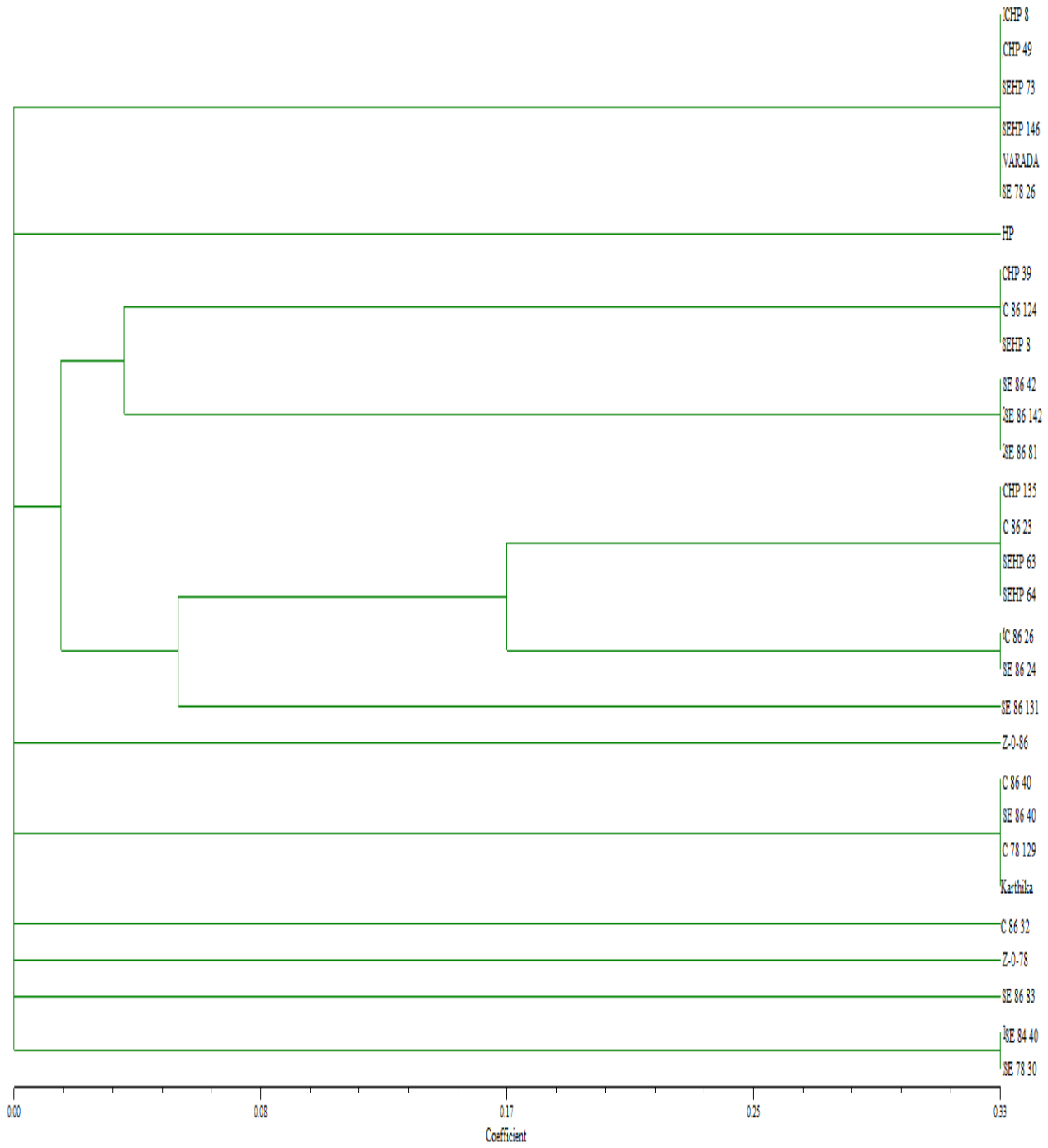


Fig 2. Dendrogram based on qualitative characters

DISCUSSION

DISCUSSION

Ginger is one of the most renowned spices in the world. Ginger being exclusively propagated through vegetative means, natural variability is limited. Somaclonal variation act as a major source of variability for crop improvement and the extent of variation depends on parent cultivar. Somaclones show wide variability which help to identify elite types.

Induction of variability through induced polyploidy attempted at Department of Plantation Crops & Spices, College Of Horticulture, Vellanikkara, has succeeded in the development of two autotetraploids (Sheeba, 1996) with desirable quality attributes like low fibre content and high aromatic oil and oleoresin but susceptible to the diseases which restricts their commercial utility (Shankar, 2003). In order to increase the spectrum of variability in these tetraploids, induction of variation *in vitro* through in direct methods of regeneration and mutagenesis was attempted as part of DBT funded project from 2006 to 2010. This has resulted in development of potential variants which on preliminary evaluation revealed wide variability in morphology, yield and reaction to major diseases and pests (Kurian, 2010).

The present study entitled "Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" was taken up in this background at College of Horticulture, Vellanikkara, during 2011-13 to characterize and evaluate twenty five somaclones derived through indirect methods of regeneration, along with three parent cultivars (Z-0-78, Z-0-86 and Himachal Pradesh) and two check varieties (Karthika and Varada). These twenty five somaclones were selected from a base population of 289 somaclones, which were developed through indirect organogenesis and indirect embryogenesis from three cultivars (two induced polyploids Z-0-78, Z-0-86 and a diploid cultivar Himachal Pradesh), at the Department of Plantation Crops and Spices, College Of Horticulture, Vellanikkara. These somaclones were under evaluation in the department since 2008-09.

4.1 FIELD EVALUATION OF SOMACLONES

The somaclones derived through indirect methods of regeneration from two induced polyploids (Z-0-78 and Z-0-86) and a diploid cultivar Himachal Pradesh were evaluated along with three parent cultivars and two check varieties during 2011-13 for variability in morphology, yield, quality and resistance / tolerance to pests and diseases. Among the twenty five somaclones, three somaclones were derived from Z-0-78, thirteen somaclones from Z-0-86 and nine somaclones were derived from the diploid cultivar Himachal Pradesh.

4.1.1 Morphological characters

Somaclones were found superior to parent cultivars and check varieties in various morphological characters recorded (Table 3, Fig 3, 4, 5 and 6). All the somaclones produced more number of leaves registering maximum percentage increase over the parent cultivars than the check varieties. More number of somaclones also showed increment in leaf area, number of shoots, girth of pseudostem and leaf width. The somaclones were comparatively shorter than the parents cultivars probably because the parents were exceptionally tall when compared to check varieties. Kurian (2010) made similar observation during preliminary evaluation of the base population of 289 somaclones. Somaclones derived from Himachal Pradesh registered maximum increase in morphological characters followed by those from Z-0-86. Cultivar difference in somaclonal variation has been reported by Paul (2006) while evaluating adventitious bud regenerants of Maran and Rio-de-Janeiro who found that somaclones derived from Maran showed wide variation. Somaclones showed superiority over check varieties in plant height, leaf length, leaf width, leaf area and girth of pseudostem and cent per cent of somaclones showed increase in the characters and maximum increase was registered for plant height. High amount of somaclonal variation in morphological characters was reported by Babu *et al*. (2005) and Paul (2006) in ginger, Salvi *et al*. (2002), Roopadarsini and Gayatri (2012) in turmeric, Sudharshan and Bhat (1998) in cardamom, and Sujatha (2001) and Sanchu *et al*. (2002) in black pepper. Superiority of somaclones over control

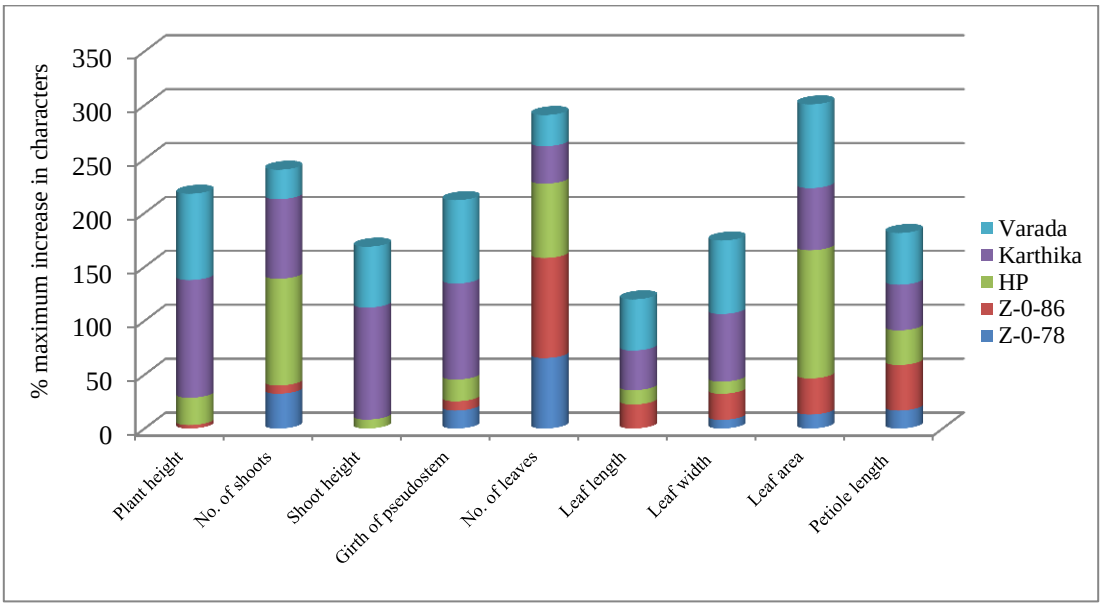


Fig 3. Per cent of maximum increase in morphological characters of somaclones in relation to parents and check varieties

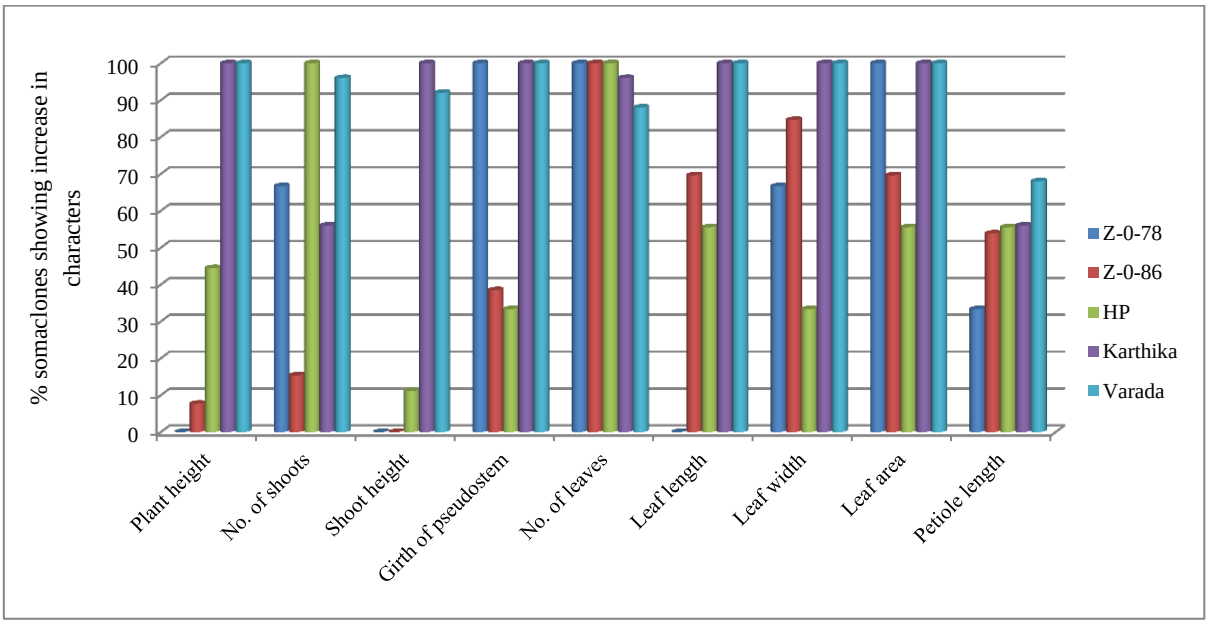


Fig 4. Per cent of somaclones showing increase in morphological characters over parents and check varieties

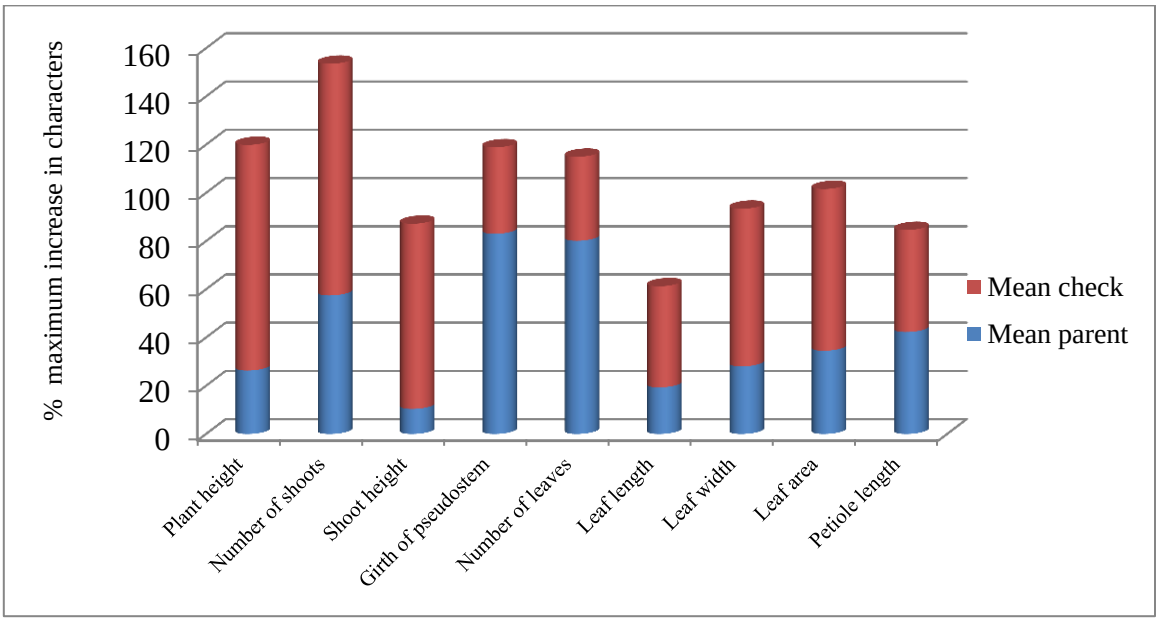


Fig 5. Per cent of maximum increase in morphological characters of somaclones in relation to mean parent and check variety

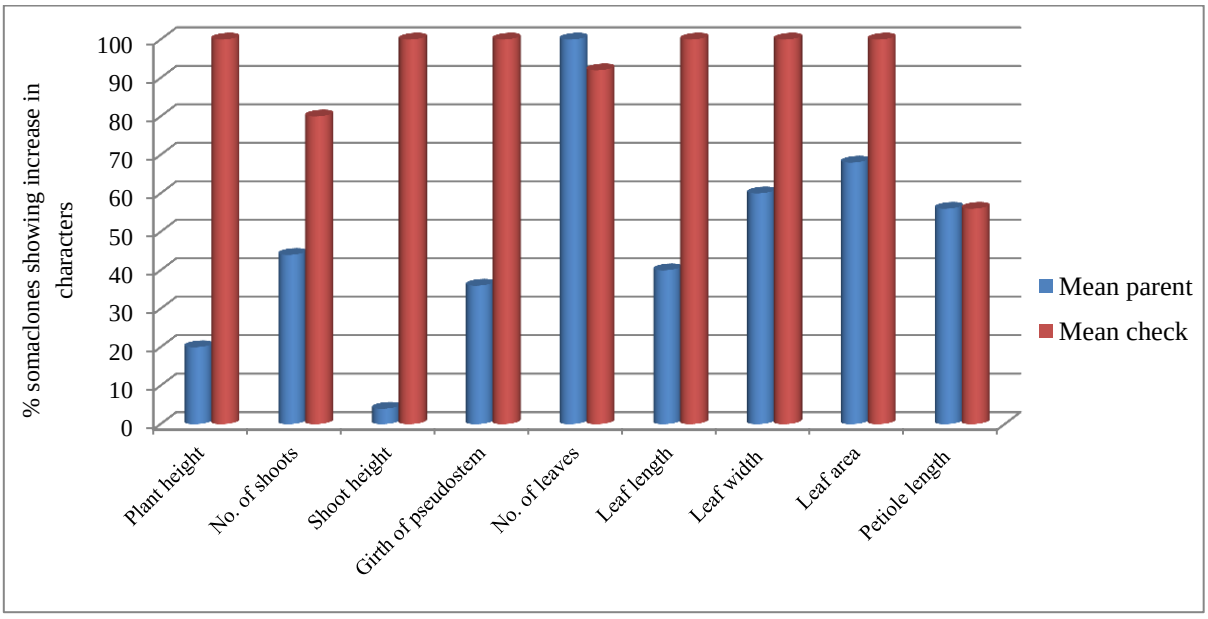


Fig 6. Per cent of somaclones showing increase in morphological characters over mean parent and check variety

plants in various morphological characters was reported in ginger by Babu *et al*. (2005), Freitz *et al*. (2001) and Paul (2006).

4.1.2 Rhizome characters

The somaclones of ginger recorded superiority in rhizome characters compared to parent cultivars and check varieties (Table 4, Fig 7, 8, 9 and 10). Cent per cent of the somaclones showed increase in length of primary and internodal length of primary fingers over parent cultivars and per cent increase was maximum for internodal length of primary followed by number of quaternary and length of primary. Compared to check varieties, cent per cent of somaclones recorded higher rhizome girth but maximum increase was noticed for number of quaternary followed by number of secondary and tertiary fingers. With respect to number of primary, the increase was more compared to parents than check varieties. Babu *et al.* (2005) and Paul (2006), and Salvi *et al.* (2002) also reported similar observations on superiority of somaclones in yield contributing rhizome characters over parent and check varieties in ginger and turmeric somaclones.

Among the different rhizome characters, number of quaternary fingers recorded the highest per cent increase of hundred and five over parent cultivars and 498 per cent over check varieties. Somaclone C 86 124 recorded the highest number of quaternary fingers while C 86 23 had the highest length of primary fingers. Somaclone SE 86 131 recorded the highest internodal length of primary fingers. 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 and Sanchu *et al*. (2000) in black pepper somaclones. Somaclones derived from Z-0-86 recorded maximum increase in rhizome characters followed by those from Z-0-78. Such variations in rhizome characters of somaclones in ginger depending on parent cultivar have been observed by Paul (2006).

4.1.3 Rhizome yield

Somaclones recorded hgher fresh rhizome yield and dry rhizome yield than parents and check varieties (Table 5, Fig 11, 12, 13 and 14). The fresh yield of rhizome ranged

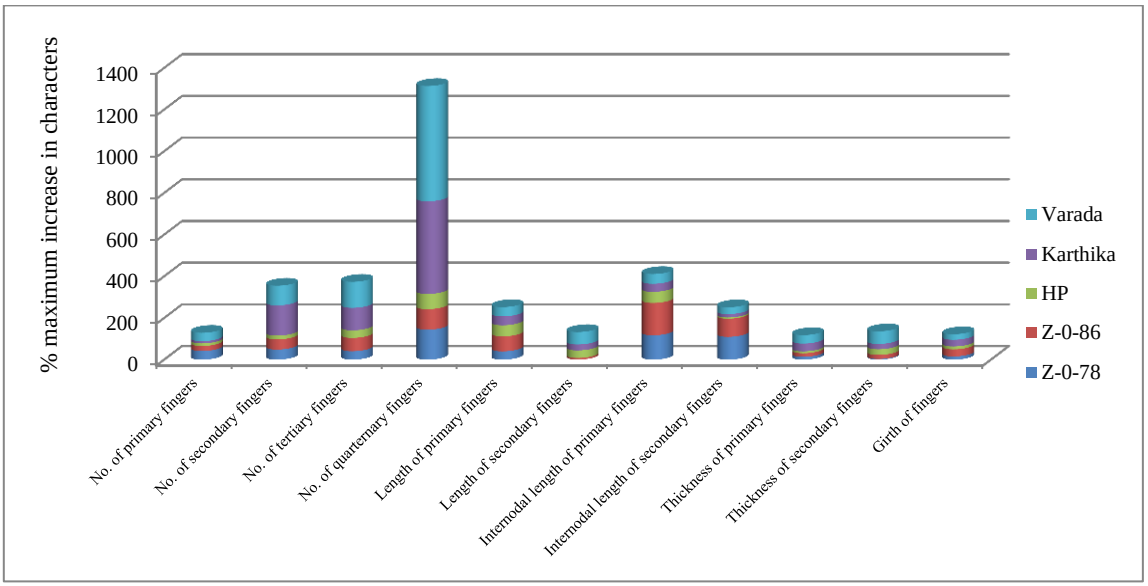


Fig 7. Per cent of maximum increase in rhizome characters of somaclones in relation to parents and check varieties

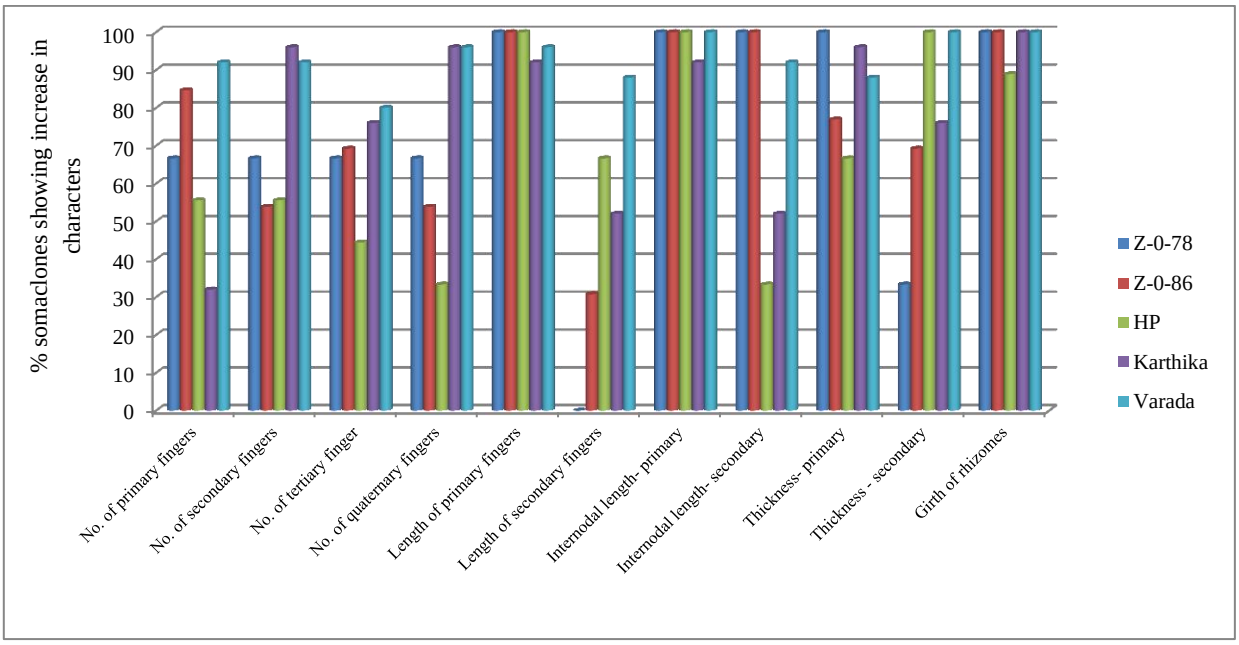


Fig 8. Per cent of somaclones showing increase in rhizome characters over parents and check varieties

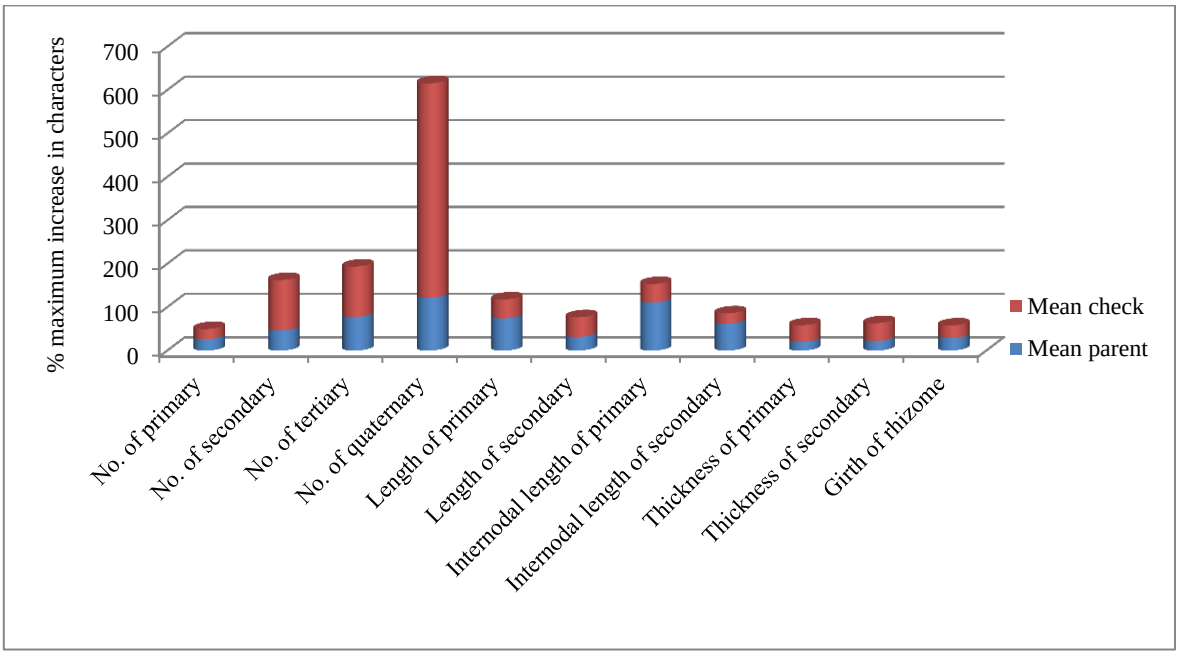


Fig 9. Per cent of maximum increase in rhizome characters of somaclones in relation to mean parent and check variety

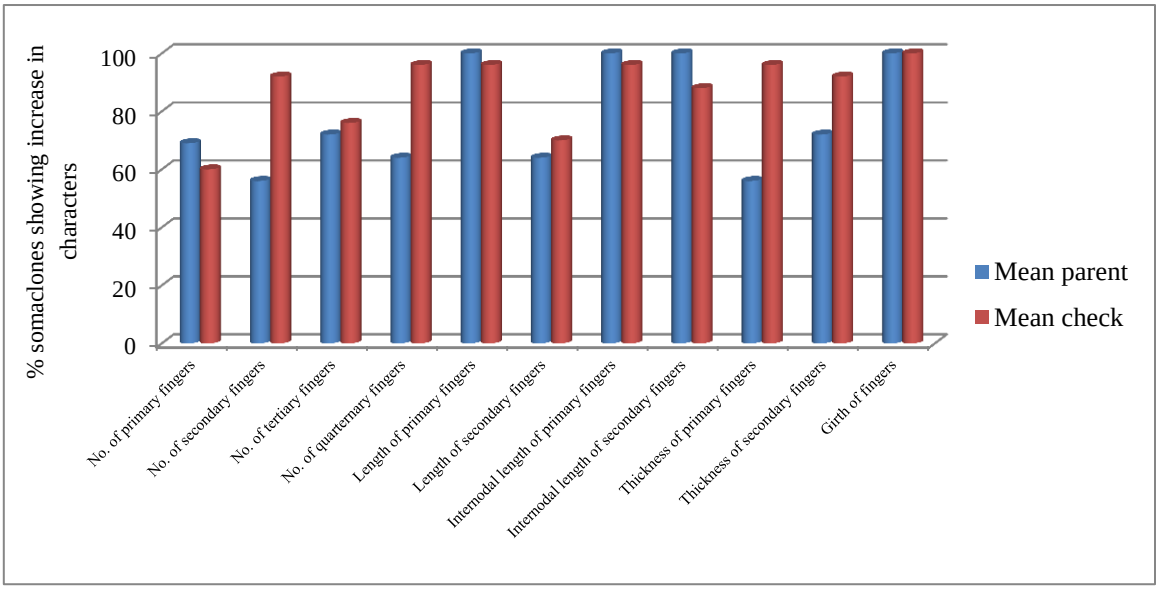


Fig 10. Per cent of somaclones showing increase in rhizome characters over mean parent and check variety

between 9.81 and 21.42 t ha^{-1} and dry yield from 1.71 to 3.92 t ha^{-1} in the somaclones. Yield increment in somaclones was more when compared to parent cultivars than check varieties. Variability in rhizome yield in ginger somaclones were reported by Samsudeen (1996), Shylaja *et al*. (2003), Paul (2006), Sumathi (2007), Kurian (2010) and Shylaja (2010).

Somaclones derived from polyploid parent Z-0-86 recorded higher yield of rhizomes compared to somaclones derived from Z-0-78 and Himachal Pradesh. Fresh rhizome yield per hectare varied from 11.97 to 21. 42 t in somaclones of Z-0-86, 10.99 to 14.64 t in somaclones of Z-0-78 and 9.81 to 19.31 t in somaclones of HP. Somaclones SE 86 81 recorded the highest fresh rhizome yield of 21.42 t ha^{-1} followed by SE 86 142, SE 86 40, SEHP 63, C 86 40 and SE 86 131. Dry rhizome yield per hectare varied from 2.13 to 3.92 t in somaclones of Z-0-86, 2.12 to 3.08 t in somaclones of Z-0-78 and 1.71 to 3.21 t in somaclones of HP. Somaclone SE 86 142 recorded the highest dry rhizome yield of 3.92 t ha^{-1} followed by SE 86 81, SE 86 40, C 86 139 and C 86 40. Paul (2006) reported that the somaclones derived from cultivar Rio-de-Janeiro were high yielding compared to those from cultivar Maran.

4.1.4 Quality attributes

The quality attributes varied significantly in the somaclones compared to parents and check varieties. Somaclones derived from Z-0-86 alone recorded higher dry ginger recovery compared to parents and fourty six per cent of somaclones showed higher dry recovery. Compared to check varieties, twenty per cent of somaclones showed higher dry recovery. Among the somaclones, SEHP 8 recorded the highest dry recovery (21.21%) followed by SE 78 26 (21.06%), SE 78 30 (20.65%) C 86 124 (20.52%) and C 86 32 (20.45%) (Table 6, Fig 11, 12, 13 and 14).

Recovery of volatile oil varied between 1.20 to 2.32 per cent in the somaclones studied. Somaclones derived from polyploid parent Z-0-86 recorded higher volatile oil content. Somaclones SE 86 24, SE 86 40 and SE 86 142 were found to have the highest volatile oil content (2.32 %). Volatile oil yield per hectare ranged from 25.44 to 90.96 kg. The highest oil yield was recorded by SE 86 142 followed by SE 86 40 (85.79 kg ha^{-1}) and

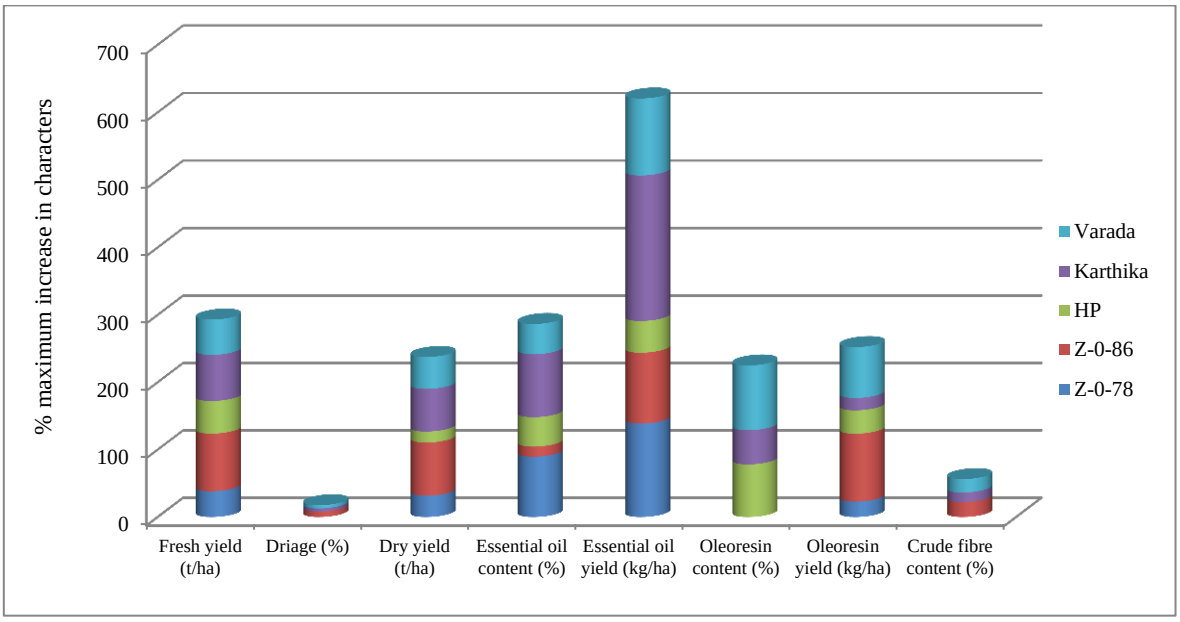


Fig 11. Per cent of maximum increase in yield and quality of somaclones in relation to parents and check varieties

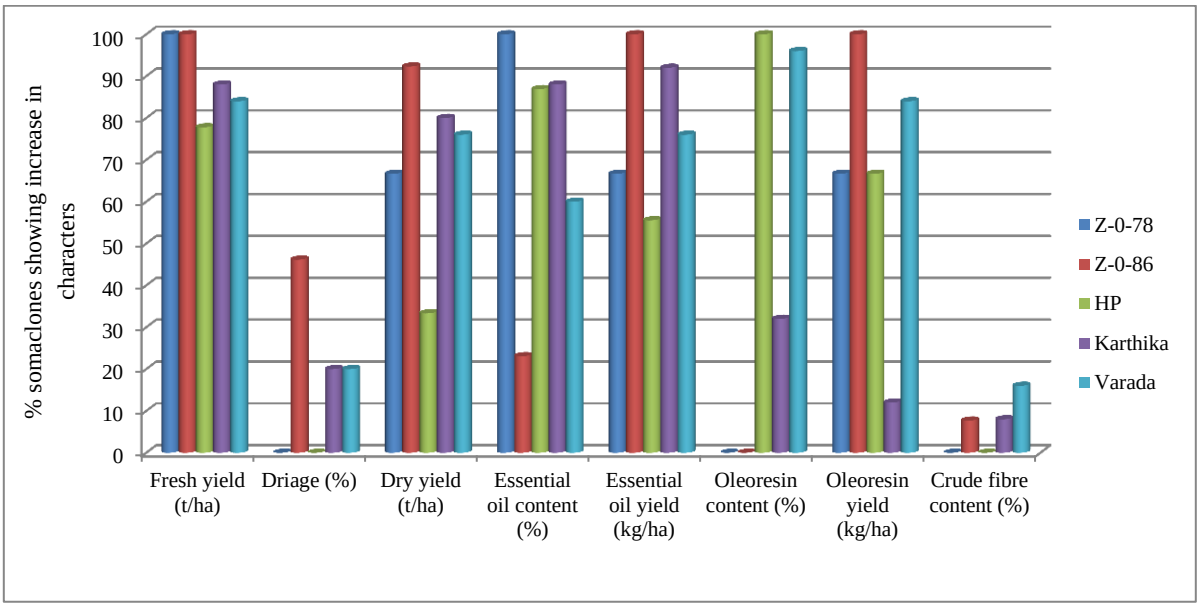


Fig 12. Per cent of somaclones showing increase in yield and quality attributes over parents and check varieties

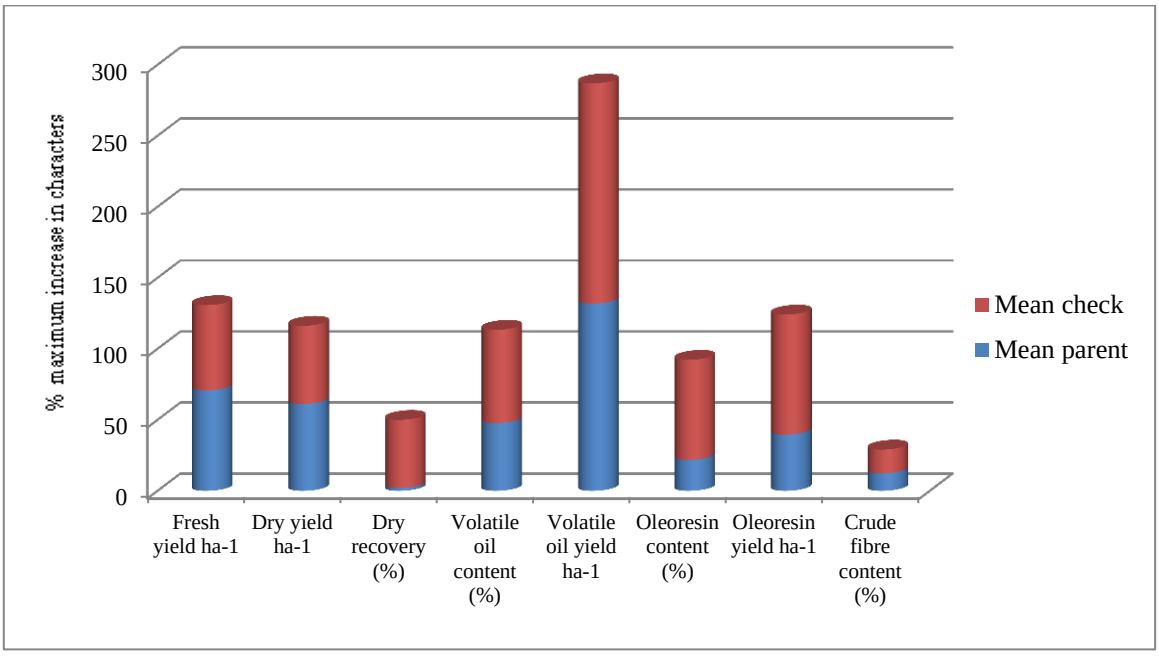


Fig 13. Per cent of maximum increase in yield and quality of somaclones in relation to mean parent and check variety

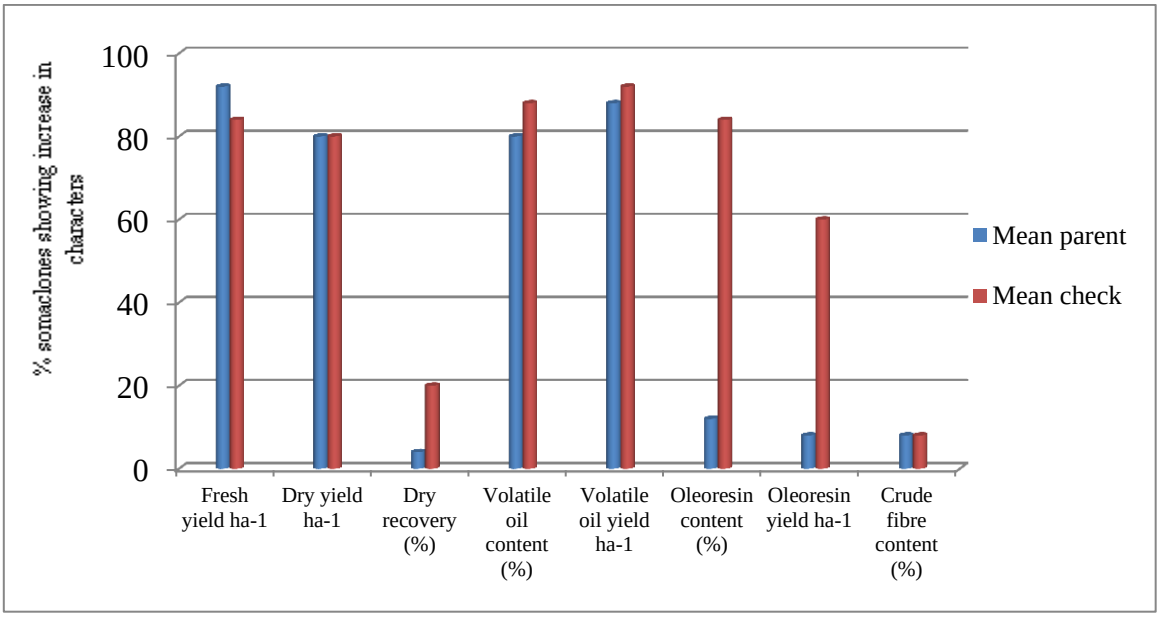


Fig 14. Per cent of somaclones showing increase in yield and quality over mean parent and check variety

SE 86 81(71.99 kg ha^{-1}). The polyploid parent of these somaclones (Z-0-86), derived from Rio-de-Janeiro through colchiploidy, was high in volatile oil content as reported by Sheeba (1996) and Shankar (2003). According to Nybe (1978), Shankar (2003) and Paul (2006), Rio-de-Janeiro excelled all other types in yield of essential oil content when they evaluated different ginger cultivars for quality attributes.

Oleoresin content ranged from 4.34 to 8.59 per cent in the somaclones evaluated. Higher recovery of oleoresin was noticed in cent per cent of somaclones of Himachal Pradesh. Thirty three per cent of somaclones recorded higher oleoresin recovery over parents and sixty four per cent over check varities. Somaclone CHP 39 recorded the highest oleoresin content (8.59 %). Oleoresin yield in somaclones ranged from 99.67 to 233.69 kg ha^{-1} and somaclone SE 86 40 recorded the highest yield followed by CHP 39, SE 86 81, C 86 124, SEHP 63, and CHP 135.

Crude fibre content ranged from 1.25 to 4.03 per cent. Somaclones in general showed lower crude fibre content which is considered as a desirable quality attribute. Ninety seven per cent of somaclones recorded lower crude fibre content over parents and eighty eight per cent over check varieties. Somaclone SE 78 26 recorded low crude fibre content (1.25 %). Somaclones derived from Himachal Pradesh recorded higher crude fibre content compared to those derived from Z-0-78 and Z-0-86.

As observed in the present study, influence of the parental cultivars on somaclonal variation in quality attributes was reported by Paul (2006), wherein somaclones from cultivar Rio-de-Janeiro recorded higher oil and oleoresin content, whereas higher dry recovery and low fibre content were noticed among somaclones of cultivar Maran. Variation in quality attributes in somaclones of ginger were reported by Bhagyalakshmi *et al*. (1994), Rao *et al.* (2000), Paul (2006) and Shylaja *et al*. (2010) and turmeric somaclones by Roopadarsini and Gayatri (2012). Sudharshan and Bhat (1998) observed high oil content of 7.80 per cent in cardamom somaclones compared to open pollinated seedlings. Similar reports were on variation in quality attributes in calliclones of black pepper by Sanchu *et al*. (2002) and Ravindra *et al*. (2004) in geranium.

4.1.5 DUS characterization of somaclones

Distinctiveness, Uniformity and Stability (DUS) of somaclones were evaluated as per the DUS Guidelines given by PPV & FR Act (2001). Twenty five somaclones were characterized and grouped based on 17 characters (Table 8). The plants were erect or semi erect and majority of somaclones were short statured with fewer number of shoots and leaves and narrow medium sized pseudostem. Leaves were fewer, short and narrow with short petioles. Rhizome shapes observed were straight, curved and zigzagged. Rhizomes were medium in size. Spikes produced in the somaclones had both yellow-white bracts and pink bracts. Most of the somaclones were of long duration with medium to high dry recovery of rhizomes. The DUS characterization will be useful in future for plant variety protection.

4.2 SCREENING OF SOMACLONES AGAINST PESTS AND DISEASES

4.2.1 Natural occurrence of pests and diseases

Twenty five somaclones along with three parent cultivars and two check varieties were evaluated in the field for natural occurrence of pests and diseases. In the infested plants which were uprooted, the presence / absence of *Pythium*, bacteria and rhizome maggot were recorded

and presented as total plant infection (Table 9). Rhizome maggot may not be a primary pest and may be infesting diseased rhizomes as observed by Koya *et al*. (1990). Total plant infection ranged from zero to 36.5 per cent. Pests and disease incidence was not noticed in C 86 139. In general, incidence in somaclones was low when compared to parents and check varieties, except SEHP 73 (36.50%).

Shoot borer incidence was high in somaclones, which ranged from 21.53 to 63.63 per cent. This is in agreement with Paul (2006) who also reported high shoot borer incidence in ginger somaclones and that the incidence was influenced by seasons. Somaclone SE 86 40 recorded the lowest shoot borer incidence of 29.53 per cent. The parent cultivar of SE 86 40 was Z-0-86, a polyploid derived from Rio-de-Janeiro. Shankar (2003) reported that the colchicine induced variants from Rio-de-Janeiro showed least incidence of shoot borer

compared to other cultivars. Leaf spot disease severity was found low in somaclones derived from Z-0-86, somaclone SE 86 81 showing least incidence of 9.49 per cent.

4.2.2 Artificial screening of somaclones for resistance / tolerance to rhizome rot and bacterial wilt

4.2.2.1 Artificial screening for resistance / tolerance to rhizome rot disease

Artificial inoculation of *Pythium aphanidermatum* was done in twenty five somaclones along with three parent cultivars and two check varieties, to screen somaclones for reaction to the disease. Somaclones showed higher tolerance to rhizome rot when compared to parents and check varieties which showed cent per cent infection. Four somaclones (CHP 49, C 86 26, C 86 124 and SE 86 81) recorded low disease incidence of 66.67 per cent retaining one plant out of three. Nineteen somaclones showed re-sprouting out of which nine yielded rhizomes. Rhizomes yield from 13 somaclones (four clones which survived infection and nine re-sprouted clones) which showed tolerance to disease ranged between 4g to 106g. Among the check varieties, Karthika showed re-sprouting and showed re-infection in 28 days. Among the 25 somaclones, 21 showed tolerance to rhizome rot disease, but 13 somaclones which yielded rhizomes were relatively more tolerant (Tables 10, 11 and Plate 11).

4.2.2.2 Artificial screening for resistance / tolerance to bacterial wilt disease

Artificial inoculation of *Ralstonia solanacearum* was done in twenty five somaclones along with three parent cultivars and two check varieties, to screen somaclones for reaction to the disease. All somaclones showed visible disease symptom within a period of four to 14 days. Incidence of the disease ranged between 66.67% and 100%. Re-sprouting of rhizomes was noted in six somaclones (SEHP 63, C 78 129, SE 78 30, SE 86 24 and SE 86 142) with a re-sprouting percentage of 33.33 per cent. Number of days taken to re-sprout was 8 to 36 days, where somaclones SE 78 30 and SE 86 24 showed earliness in re-sprouting. Late re-infection (32 days) was noticed in three somaclones, C 78 129, SE 78 30 and SE 86 142. Out of the six somaclones re-sprouted, SEHP 63 alone was retained without re-infection (Tables 12, 13 and Plate 12).

Relative tolerance to both diseases was noticed in four somaclones, CHP 39, C 78 129, SE 86 24 and SE 86 142. Tolerance of somaclones to various diseases was reported by many workers. Paul (2006) reported tolerance of ginger somaclones to rhizome rot and bacterial wilt diseases and two tolerant varieties viz., Athira and Karthika were released (Shylaja *et al*., 2010). Somaclones derived from Maran were reported more tolerant than those from Rio-de-Janeiro (Paul, 2006). Isolation of tolerant calliclones of black pepper to *Phytophthora* foot rot disease through different screening methods was reported by Shylaja *et al*. (1996) and Sanchu *et al*. (2003).

4.2.3 Sick plot screening of somaclones

Somaclones were screened for incidence of rhizome rot and bacterial wilt diseases in a sick field where there was serious incidence of the diseases in the previous year. Nineteen somaclones survived the sick plot conditions giving a rhizome yield in the range of 2 to 276 g per plot (Table 14 and Plate 13).

Nineteen somaclones which survived sick plot include nine somaclones derived from Z-0-86, two somaclones derived from Z-0-78 and eight derived from Himachal Pradesh. Somaclones derived from Z-0-86 recorded highest survival (SE 86 40- 62.50%). As in the present study, Paul (2006) found that somaclones from Rio-de-Janeiro showed higher variation for reaction to diseases. Xie *et al*. (1992) reported two sheath blight resistant lines in rice, LSBR 33 and LSBR 5,by screening 2000 somaclones in field nurseries inoculated with the sheath blight pathogen (*Rhizoctonia solani*). Similar reports have been made by Chuan *et al*. (2000) in banana and Devi *et al.* (2005) in tomato.

4.3 ASSESSMENT OF GENETIC VARIABILITY

4.3.1 Coefficient of variation

An insight into the magnitude of variability present in the somaclones is of importance, as it provides the basis for effective selection. Among the morphological characters, number of shoots showed the highest coefficient of variation, followed by number of leaves and girth of pseudostem. Number of quaternary fingers recorded the highest coefficient of variation among the rhizome characters followed by number of

tertiary and secondary fingers and internodal length of primaries. Coefficient of variation for fresh and dry rhizome yield was 17.56 and 20.78 respectively. Among quality attributes oleoresin yield recorded highest coefficient of variation followed by volatile oil yield whereas the variation was less for the volatile and oleoresin contents. Pests and disease incidence showed fairly high coefficient of variation (Tables 3-6 and 8).

Fifteen characters which showed high correlation with yield in somaclones were studied to estimate genetic parameters. Higher PCV as compared to GCV indicate high environment effect on phenotype for characters such as plant height, shoot height and leaf area. GCV was found very near to PCV for all the rest 12 characters and hence effect of genotype on phenotype expression is also high (Table 15).

4.3.2 Genetic divergence

Somaclones were grouped into five clusters indicating considerable genetic diversity prevailing among them. Polyploid parents (Z-0-78 and Z-0-86) were grouped in Cluster I while the diploid parent cultivar HP was grouped in Cluster II. The check varieties (Karthika and Varada) were grouped in Cluster IV. The maximum genetic divergence among the accessions was noticed in those grouped in Cluster I and least in Cluster IV. Inter cluster distance was highest between Cluster I and Cluster IV indicating that the genotypes grouped in these clusters are more divergent than others (Tables 17, 18, 19 and Fig 11).

Somaclones were grouped into thirteen clusters based on four qualitative characters (Fig 12) suggesting that the somaclones are more divergent in quality attributes. Parent cultivars HP, Z-0-86 and Z-0-78 were separately grouped in Clusters II, VIII and XI. Check variety Varada was grouped in Cluster I while Karthika was grouped in Cluster IX . Singh (1999) grouped 18 cultivars into three clusters based on D^2 analysis and found that rhizome yield per plant, oleoresin and fibre contents were major forces influencing divergence of cultivars.

4.3.3 Aids to selection

4.3.3.1 Correlation

Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for improvement in yield. Correlation of 26 quantitative and four qualitative characters with yield was studied and fifteen characters showed significant and positive correlation (Table 16). Girth of pseudostem was noted to have the highest correlation with yield among morphological characters, followed by leaf width, plant height, shoot height, leaf area and leaf length. Girth of rhizomes had the highest correlation with yield among rhizome characters followed by length of primary and secondary fingers, and thickness and internodal length of primary fingers. Nybe (1979b) reported that length and girth of rhizomes were directly correlated with yield. Chandra and Govind (1999) found that internodal distance of rhizomes was positively correlated with yield. Number, length, internodal length and girth of rhizomes that directly contribute to yield were more in the somaclones evaluated. Among the quality attributes dry recovery recorded highest correlation with yield. Somaclones recorded low crude fibre content compared to parents and check varieties. All the somaclones were taller than the check varieties and the negative correlation of plant height to crude fibre content was reported by Chandra and Govind (1999) is applicable in the present finding too.

4.3.4 Heritability

Heritability above 80 per cent was shown by 12 characters of which volatile oil content recorded highest heritability of 99.23 per cent. Characters such as plant height, shoot height and leaf area showed heritability less than 40 per cent, so selection based on these characters is considerably difficult due to masking effect of environment on genotypic effects. Though significant and positive correlation of plant height with yield, was reported by several workers [Sreekumar *et al.* (1981), Rattan *et al*. (1988), Sasikumar *et al*. (1992), Pandey and Dhobal (1993)], since it is highly influenced by environmental factors it cannot be considered as a selection criteria.

Evaluation of the 25 somaclones based on 26 quantitative characters (morphology, rhizome and yield) and four qualitative characters (volatile oil, oleoresin, crude fibre and driage) revealed wide variability among somaclones, enabling good scope for selection. The somaclones were screened against natural occurrence of pests and diseases and against rhizome rot and bacterial wilt diseases through artificial inoculation and sick plot screening. More number of somaclones showed tolerance to rhizome rot than bacterial wilt disease. Quality analysis revealed that besides having high volatile oil and oleoresin contents, most of the somaclones exhibited low fibre content, a desirable attribute for quality ginger making them suitable as fresh ginger and especially for value added products. Considering yield, quality and tolerance to pests and diseases, six somaclones were selected (SE 86 81, SE 86 142, SE 86 40, SE 86 131, C 86 139 and C 86 124). The selected somaclones registered a yield increase of 28-85% compared to released varieties and 38-85% compared to parent cultivar. The somaclone SE 86 40 is promising for high essential oil and oleoresin yield as well (Table 20).

4.4 FUTURE LINE OF STUDY

The promising somaclones selected needs to be assessed over seasons and locations to study the stability under different environment. Metabolite profiling of the somaclones enables identification of novel chemotypes. Screening of the somaclones for value added products, taking advantage of low fibre profile of the rhizomes should take precedence as this will diversify the end product utilization and safe guard farmers against price crash of the only one primary product, dry rhizomes.

Table 20. Characters of selected superior somaclones, parent cultivar and check varieties

Characters		SE 86 81	SE 86 142	SE 86 40	SE 86 131	C 86 139	C 86 124	Parent cultivar Z-0-86	Check varieties	
									Karthika	Varada
Fresh yield (t ha^{-1})		21.42	20.00	19.80	18.14	17.95	16.00	11.55	11.87	13.12
Driage (%)		17.51	19.60	18.66	17.75	19.98	20.52	19.00	20.23	20.25
Dry yield (t ha^{-1})		3.75	3.92	3.69	3.22	3.59	3.28	2.19	2.40	2.66
Essential oil content (%)		1.92	2.32	2.32	1.50	1.50	2.00	2.02	1.20	1.60
Essential oil yield (kg ha^{-1})		71.99	90.96	85.79	48.29	53.79	65.67	44.33	28.82	42.51
Oleoresin content (%)		5.49	4.75	6.32	5.34	4.34	6.13	9.00	5.70	4.38
Oleoresin yield (kg ha^{-1})		205.87	186.22	233.69	171.90	155.65	201.29	197.51	136.87	116.36
Crude fibre content (%)		1.70	2.40	1.57	1.35	1.55	1.75	2.74	3.53	3.36
Disease incidence (%)		5.28	1.08	3.39	3.58	-	4.16		38.50	20.00
Sick plot survival (%)		✓	✓	✓	✓	✓	✓	-	-	-
Artificial screening	Rhizome rot	✓	✓	✓	✓	✓	-		-	-
	Bacterial wilt	-	✓	-	-	-	-		-	-

C 86 124

C 86 139

SE 86 40

SE 86 81

SE 86 131

SE 86 142

Plate 14. Promising somaclones in ginger

SUMMARY

SUMMARY

Investigations on "Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.), were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur during May 2012 to June 2013. The study was aimed to characterize and evaluate the somaclones for variability in morphology, yield, quality and resistance /tolerance to pests and diseases. The salient findings of the study are listed below.

1. Field evaluation of somaclones.

Evaluation of somaclones based on 26 quantitative characters (related to plant, leaf and yield) and four qualitative characters (dry recovery, volatile oil content, oleoresin content and crude fibre content) revealed wide variability.

- Somaclones exhibited significant variations compared to parent cultivars and check varieties in various characters studied.
- Somaclones exhibited superiority over parent cultivars in morphological characters such as leaf area, number of leaves, number of shoots, girth of pseudostem and leaf width.
- Cent per cent of the somaclones showed increase over check varieties in plant height, leaf length, leaf width, leaf area and girth of pseudostem.
- Somaclones derived from HP registered maximum increase in morphological characters
- Among rhizome characters, number of quaternary fingers recorded maximum increase followed by internodal length of primary fingers and number of tertiary and secondary fingers.
- Somaclones showed superiority over parent cultivars in length and inter-nodal length of primary fingers and quaternary fingers while superiority over check varieties was seen for girth of rhizome, number of quaternary, secondary and tertiary fingers.

- Somaclones derived from polyploid parent Z-0-86 recorded superiority in rhizome characters over other somaclones.
- Ninety two per cent of somaclones showed higher yield over parent cultivars while eighty four per cent showed higher rhizome yield over check varieties.
- Somaclones derived from Z-0-86 alone recorded higher driage and dry yield was also seen to be maximum.
- Somaclones in general recorded low crude fibre content registering its suitability as fresh ginger and for value added products.
- Volatile oil content was found higher in somaclones, seventy per cent over parent cultivars and seventy four per cent over check varieties.
- Higher recovery of volatile oil and oil yield was noticed in somaclones of Z-0-86.
- Oleoresin recovery was high in somaclones derived from HP while oleoresin yield was found high in somaclones derived from Z-0-86.
- DUS characterization of somaclones based on 17 characters as per PPV & FR Act (2001) was done.

2. Screening of somaclones for pests and disease tolerance / resistance

- Somaclones exhibited wide variability in reaction to pests and diseases.
- Screening of somaclones for natural occurrence of pests and diseases revealed that plant infection due to rhizome rot occurring as a complex along with rhizome maggot and bacterial wilt was less compared to parents and check varieties.
- Field tolerance to shoot borer and leaf spot incidence was not observed in somaclones.
- Twenty one somaclones showed tolerance to rhizome rot disease under artificial screening out of which thirteen somaclones (CP 39, CHP 49, CHP 135, SEHP 64, SEHP 73, SEHP 146, C 78 129, SE 78 26, C 86 26, C 86 124, C 86 139, SE 86 24 and SE 86 81) yielded rhizomes, were relatively more tolerant.
- Six somaclones (CHP 39, SEHP 63, C 78 129, SE 78 30, SE 86 24 and SE 86 142) were found tolerant to bacterial wilt disease in artificial screening.
- Four somaclones showed relative tolerance to both rhizome rot and bacterial wilt diseases.

- Nineteen somaclones survived sick field condition.

3. Assessment of variability in somaclones and aids to selection

- Among the different morphological characters studied, number of shoots registered the highest coefficient of variation followed by petiole length, while number of quaternary finger was noticed to have the highest coefficient of variation followed by number of tertiary and secondary fingers among rhizome characters.
- Dry rhizome yield registered higher coefficient of variation than fresh rhizome yield.
- Among quality attributes, oleoresin yield recorded highest coefficient of variation.
- Correlation coefficients worked out for 30 characters indicated that yield is strongly correlated with fifteen characters of which driage showed the highest correlation with yield followed by girth of rhizome, length of primary fingers and secondary fingers.
- GCV was found very near to PCV for 13 characters indicating high effect of genotype on phenotypic expression.
- Visual characters such as leaf width and length, girth of pseudostem, number of primaries, length, thickness, internodal length of primary and secondary rhizomes and girth of rhizomes having high positive correlation with yield and having high heritability appear to be good selection indices in ginger.
- Clustering of 30 genotypes based on 26 qualitative characters and four qualitative characters resulted in 13 and five clusters respectively. The accessions belonging to a quantitative cluster fell in different clusters during qualitative clustering.
- Considering yield, quality and reaction to pests and diseases, six promising somaclones (SE 86 81, SE 86 142, SE 86 40, SE 86 131, C 86 139 and C 86 124) were identified, which registered a yield increase of 28– 85 per cent.
- Somaclone SE 86 40 was found exceptionally suitable for volatile oil and oleoresinextraction.
- The somaclones are especially suitable as fresh ginger and for value added products.

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CHARACTERISATION AND EVALUATION OF SOMACLONES IN GINGER (*Zingiber officinale* Rosc.)

by

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THESIS

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ABSTRACT

The present study entitled " Characterization and evaluation of somaclones in ginger (*Zingiber officinale* Rosc.)" was taken up at College of Horticulture, Vellanikkara, during 2011-13 to characterize and evaluate twenty five somaclones derived through indirect methods of regeneration along with three parent cultivars (Z-0-78, Z-0-86 and Himachal Pradesh) and two check varieties (Karthika and Varada). The experiment was laid out in an RBD design with 30 treatments in two replications.

Wide variability was observed among somaclones for 26 quantitative and four qualitative characters. Extent of variation was more in somaclones when compared to check varieties rather than parent cultivars. Somaclones exhibited superiority over parent cultivars in morphological characters such as leaf area, number of leaves, number of shoots, and girth of pseudostem. Cent per cent of somaclones showed increase over check varieties in plant height, leaf length, leaf width and leaf area. Somaclones derived from Himachal Pradesh registered maximum increase in morphological characters.

Among rhizome characters, number of quaternary fingers recorded maximum increase followed by internodal length of primary fingers and number of tertiary fingers in the somaclones. Cent per cent of somaclones showed increase over parent cultivars in length and inter-nodal length of primary fingers while cent per cent showed increase over check varieties for girth of rhizome. Somaclones derived from Z-0-86 recorded superiority in rhizome characters over those derived from Himachal Pradesh and Z-0-78.

Ninety two per cent of somaclones showed higher yield over parent cultivars while eighty four per cent of somaclones showed higher rhizome yield over check varieties. Somaclones derived from Z-0-86 alone recorded higher driage and dry yield was also seen to be maximum. Somaclones in general recorded low crude fibre content registering its suitability for fresh ginger and value added products. Volatile oil content was found higher in somaclones, seventy per cent over parent cultivars and seventy four per cent over check varieties. Higher recovery of volatile oil and oil yield was noticed in somaclones of Z-0-86. Oleoresin recovery was high in somaclones derived from HP while oleoresin yield was found high in somaclones derived from Z-0-86.

DUS characterization of somaclones based on 17 characters as per PPV & FR Act (2001) was done and this will be useful for plant variety protection.

Assessment of variability in somaclones based on 30 characters indicated that number of quaternary fingers had the highest coefficient of variation followed by number of tertiary and primary fingers, number of shoots and number of leaves. Significant and positive correlation with yield was observed for fifteen characters out of the 30 characters studied. GCV was found very near to PCV for 13 characters indicating high effect of genotype on phenotypic expression. Visual characters such as leaf width and length, girth of pseudostem, number of primaries, length, thickness, internodal length of primary and secondary rhizomes and girth of rhizomes having high positive correlation with yield and having high heritability appear to be good selection indices in ginger. Clustering of 30 genotypes based on 26 qualitative characters and four qualitative characters resulted in 13 and five clusters respectively. The accessions belonging to a quantitative cluster fell in different clusters during qualitative clustering suggesting that the somaclones are more divergent in quality attributes.

Screening of somaclones for reaction to rhizome rot and bacterial wilt through artificial inoculation could locate 13 somaclones tolerant to rhizome rot and six somaclones tolerant to bacterial wilt which was substantiated by sick plot screening and natural screening. Field tolerance to shoot borer and leaf spot incidence was not observed in the somaclones.

Considering yield, quality and reaction to pests and diseases, six promising somaclones were selected (SE 86 81, SE 86 142, SE 86 40, SE 86 131, C 86 139 and C 86 124). The selected somaclones registered a yield increase of 28-85 per cent compared to released varieties and parent cultivars. The somaclone SE 86 40 is promising for high essential oil and oleoresin yield as well. The somaclones are especially suitable as fresh ginger and for value added products.

The promising somaclones selected needs to be assessed over seasons and locations to study the stability under different environment. Metabolite profiling of the somaclones enables identification of novel chemotypes. Screening of the somaclones for value added

products, taking advantage of low fibre profile of the rhizomes should take precedence as this will diversify the end product utilization and safe guard farmers against price crash of the only one primary product, dry rhizomes.