EVALUATION OF AFRICAN MARIGOLD (*Tagetes erecta* L.) HYBRIDS/VARIETIES FOR YIELD AND RESISTANCE TO BACTERIAL WILT

By

UMESH C.
(2015-12-023)

THESIS

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Dedicated to my beloved parents

And my major Advisor

(Dr. Sreelatha U.)

DECLARATION

I hereby declare that the thesis entitled **"Evaluation of African marigold (*Tagetes erecta* L.) hybrids/varieties for yield and resistance to bacterial wilt"** 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
Date: 26/08/2017

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CERTIFICATE

Certified that the thesis entitled **"Evaluation of African marigold (*Tagetes erecta* L.) hybrids/varieties for yield and resistance to bacterial wilt"** is a record of research work done independently by **Mr. Umesh C. (2015-12-023)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree,diploma,associateship,fellowship to him.

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Introduction

1. INTRODUCTION

Floriculture has been a successful enterprise in generating revenue for small and marginal farmers of the country. The total area under floriculture in India is estimated to be 8.16 lakh ha with the production of 3.2 lakh tonnes of loose flowers during 2014-2015 (NHB, 2015). The Indian subcontinent is gifted with diverse agro-climatic conditions, that provides ample opportunities for the production of all major cut flowers *viz*., rose, carnation, orchids, anthuriums, *etc*. and loose flowers like marigold, jasmine, chrysanthemum, rose, crossandra, tuberose, China aster, *etc*. In India, marigold ranks first among the loose flowers followed by chrysanthemum, jasmine, tuberose and crossandra (Kavitha and Anburani, 2009). This flower crop is one of the most sought after by the small and marginal farmers due to adaptability to wide range of soil and climatic conditions, long duration of flowering and easiness in cultivation.

African marigold (*Tagetes erecta* L.), a member of family Asteraceae, is the most popular loose flower species in the genus *Tagetes*. The wide spectrum of attractive colours, shape, size and good keeping quality of the species has attracted the attention of flower growers. African marigold is also used as a bedding plant and pot plant. The plant extracts are used as cure for boils, ear ache, eye disease and ulcers. Marigolds are sources of essential oils and compounds of unique biological activities for use as agro-chemicals, foods and colourants, nutritional supplements and also find use in cosmetic industry. Chandhoke and Ghatak (1969) reported the broncho-dilatory, tranquilizing and anti-inflammatory properties of marigold essential oil. Of late, many multinational companies are involved in extraction of carotenoid pigments from the flower petals. There are large areas under contract farming of marigold in Karnataka, Andhra Pradesh and Maharashtra and to a limited extent in Tamil Nadu, but most of the extraction units are located in Kerala and Andhra Pradesh. The carotenoids extracted from marigold petal are the major source of pigment for poultry industry as a feed additive to intensify the yellow colour of egg yolks and broiler skin (Narsude *et al*., 2010a). Lutein, which is the major constituent of xanthophyll, is used for colouring the foodstuffs (Singh, 2006). Marigold is also well known to reduce the plant parasitic

nematode (PPN) population in the soil by several means, such as acting as a non-host or a poor host, producing allelopathic compounds that are toxic or inhibit PPN development, creating an environment that favours nematode antagonistic flora or fauna (Wang *et al.*, 2001).

In Kerala, there is demand for marigold flower throughout the year and trade of this flower is maximum during August to January. Truckloads of the flowers are brought from Tamil Nadu and Karnataka during the festival seasons. African marigold can be successfully cultivated in Kerala throughout the year. However, cultivation of this crop for loose flower has been taken up only recently. Growers were using the available local collections of marigold for flower production. But the local collections do not produce flowers conforming to the specifications required for markets, as they are a mix of colours and with poor flower quality. Multi-floreted compact, orange and yellow coloured flowers are in great demand in markets. Recently, many farmers have turned to F_1 hybrids and improved varieties for cultivation. It is also noteworthy that the number of marigold growers are increasing every year in Kerala as they expect a good return from the sale of flowers. Most of the growers purchase the hybrid seeds from some private agencies and procure hybrid seedlings from Bangalore without even knowing the specifications. Though some of the farmers have been successful in raising a good crop, many of them face heavy loss of plants due to bacterial wilt disease. Lot of queries are being raised by the growers regarding suitable high yielding genotypes with wilt resistance.

Bacterial wilt is a devastating disease caused by *Ralstonia solanacearum* and the pathogen causes huge crop loss in economically important crops of Solanaceae family such as tomato, eggplant, potato, and tobacco. In Kerala the disease is a severe menace limiting the cultivation of solanaceous crops. Though many states *viz*., Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu and West Bengal practice commercial cultivation of African marigold, severe incidence of bacterial wilt in marigold has been reported only from West Bengal (Mondal *et al.*, 2014). However, incidence of bacterial wilt in African marigold was observed at Agricultural Research Station, Mannuthy, Kerala, about four years ago and since then the disease was regularly noticed in the observational trial plots at the station. Nimisha (2016) also reported the incidence of bacterial wilt in African marigold under Kerala conditions.

Management of bacterial wilt in the field is very difficult due to the persistent and pervasive nature of the pathogen. Integrated management tactics focus on avoidance, sanitation, crop rotation, cultural practices, fumigation, host resistance, and grafting on disease resistant rootstocks. The best management practices recommended are either to choose wilt resistant cultivars/varieties/hybrids for cultivation or to adopt grafting on wilt resistant compatible rootstocks as all other management practices are of limited practical feasibility. Grafting is widely practiced in solanaceous crops all over the world and this technique is being commercially utilized by vegetable farmers in Kerala who are choosing F_1 hybrids for cultivation especially under precision farming systems. Narayanankutty *et al.* (2015) reported 100 per cent control of bacterial wilt in solanaceous vegetables through grafting.

Observational trials in African marigold at Agricultural Research Station, Mannuthy involving various genotypes including local collections, varieties/hybrids from both public and private sector indicated high incidence of bacterial wilt for majority of the genotypes. Only one local collection (M-1) was found resistant throughout the previous trials (unpublished data). However, a precise scientific study is necessary to evaluate the African marigold genotypes for yield and bacterial wilt resistance. Keeping this background in view, the present study was undertaken with the following objectives:

1. To evaluate African marigold hybrids/varieties for high yield and resistance to bacterial wilt
2. To assess the feasibility of grafting technique as a tool to combat bacterial wilt

Review of Literature

2. REVIEW OF LITERATURE

African marigold (*Tagetes erecta* L.) is an important commercial flower crop cultivated in India. Marigold produces beautiful flowers with a long blooming period and excellent shelf life. Year round cultivation of this crop is possible under prevailing diverse agro-climatic conditions in our country. Wide range of varieties and species are grown in different parts of India. Some of the varieties *viz*., Pusa Narangi Gainda, Pusa Basanti Gainda and Pusa Arpita developed from IARI, New Delhi has found promising under North Indian conditions.

In Kerala also marigold can be successfully cultivated throughout the year. However, cultivation of this crop for loose flower has been taken up only recently. Farmers were using the available local genotypes of marigold for flower production but these collections do not produce flowers conforming to the specifications required in markets. Though some of the farmers have been successfully raising the good crop, many of them facing heavy loss due to bacterial wilt caused by *Ralstonia solanacearum*.

Management of this pathogen is very difficult under field conditions. Tactics that are used to reduce bacterial wilt impact include crop rotation, soil amendments, cultural practices, field equipment disinfection, weed removal, host resistance and grafting with resistant rootstocks. Crop rotation with non-host crops may reduce the *Ralstonia solanacearum* population in the soil and subsequently reduce disease incidence. Grafting with resistant rootstocks has been successful and is currently practiced for bacterial wilt management around the globe (McAvoy *et al.*, 2012). Some of the reviews on which this study is based are given in this chapter.

2.1. Vegetative characters

Major vegetative characters studied in marigold are plant height, plant spread, number of branches, stem girth and leaf area. Studies conducted in different parts of the country like Uttaranchal, Lucknow, Marathwada, Rajasthan, Haryana have recorded

wide variations among the genotypes for growth. Studies on evaluation of genotypes for their vegetative growth and its relation with yield parameters are reviewed here.

An investigation was undertaken by Bharathi and Jawaharlal (2014a) to evaluate twenty eight genotypes of African marigold (*Tagetes erecta*. L.) for growth traits. The genotypes exhibited significant variation for various plant characters. The genotype Dharmapuri local recorded the highest plant height (113.27 cm) and the genotype Bidhan-1 recorded highest number of primary (22.40) and secondary branches per plant (41.47).

Significant variation for all the growth parameters among twenty eight genotypes of African marigold was reported by Ingle *et al.* (2011). The maximum plant height (121.85 cm) was found in the genotype 'AMC-10' and the minimum (58.75 cm) in 'Namdhari Cracker Jack Mix'. The maximum stem girth (18.02 mm) and plant spread (5560.80 cm^2) was found in the genotype 'AMC-7'.

Performance of different African marigold (*Tagetes erecta* L.) genotypes under Marathwada condition was reported by Narsude *et al.* (2010a). They observed significant variations among the genotypes for different plant growth attributes. The genotype 'Pakharsangavi Local' had tallest plants (114.64 cm) and the maximum stem girth (5.37 cm) as compared to other genotypes. The maximum plant spread (64.48 cm) and number of branches per plant (21.46) were recorded in the genotypes 'Tuljapur Local-2' and 'Tuljapur Local-1', respectively whereas, 'Marigold Orange Bunch' and 'Latur Local' were reported with the minimum plant spread (59.58 cm) and number of branches per plant (14.26), respectively.

Singh and Singh (2010) evaluated fourty four genotypes of marigold *viz.*, twenty nine of *Tagetes erecta* (TEG 1 to TEG 29), thirteen of *Tagetes patula* (TPG 1 to TPG 13) and 2 of *Tagetes minuta* (TMG 1 and TMG 2). Maximum number of primary branches per plant (53.67) and stem diameter (1.93 cm) was observed in the genotype 'TMG 1', while the genotype 'TMG 2' performed better in terms of plant height (226.87 cm). The maximum plant spread (79.10 cm) was found in 'TEG 21' followed by 'TEG 22' (70.30 cm).

Choudhary *et al.* (2014) evaluated twenty eight genotypes of African marigold and two genotypes of French marigold for performance under semi-arid conditions of Haryana. Best performance of the crop in terms of plant height was observed in MGH-09-303 (105.97 cm). Plant spread (77.72 cm), number of primary branches per plant (23.47) and number of secondary branches per plant (140.97) were recorded in the genotype Hisar Jaffri-2, whereas maximum stem diameter was observed the MGH-148-8 (1.83 cm).

Nine African marigold genotypes were evaluated for growth, yield and xanthophyll content (Gowda *et al.*, 2016). The genotype 'Local African Tall' performed better for all the plant characters studied. The genotype 'Local African Tall' recorded the maximum plant height (77.26 cm), number of primary and secondary branches per plant (14.08 and 26.00, respectively), number of leaves per plant (285.49).

Raghuvanshi and Sharma (2011) observed highly significant variation among French marigold cultivars for all the traits studied under mid-hill zone of Himachal Pradesh. Cultivar 'Safari Queen' recorded maximum plant height (35.80 cm) which was statistically on par with cv. Harmony Boy (34.90 cm) and lowest plant height (20.07 cm) was observed in cv. 'Cupidon Varie Yellow'. Maximum plant spread (30.37 cm) was recorded in cv. 'Harmony Boy'. The genotype 'Nana Jumbo Bicolor' showed the maximum lateral branches per plant (8.33) and 'Bonanza Bolero' showed minimum (4.13). The cultivar 'Bonanza Bolero' recorded maximum values for leaf area (34.58 cm^2).

Manik and Sharma (2016) evaluated fifteen genotypes of African marigold (*Tagetes erecta* L.) for yield attributes and xanthophyll content. It was found that growth parameters were significantly differing among the genotypes. At 30 and 60 DAT maximum plant height was recorded in the genotype CGSG-1 whereas in genotype CGJS-1 at 90 DAT. Maximum plant spread and primary branches per plant was recorded in the genotype CGSG-1. At 60 and 90 DAT maximum number of secondary branches per plant was recorded in CGRJ-1.

Deepa and Patil (2016) evaluated twelve marigold (*Tagetes* spp.) hybrids under Dharwad condition. The hybrid 'Sarpan-33' exhibited the maximum plant height

(84.87 cm), number of leaves per plant (416.80), maximum secondary branches (45.27) at 90 DAT. Plant spread in North-South was maximum in 'Sarpan-11' and 'Sarpan-33' (56.17) whereas plant spread in East-West was highest in Sarpan-11 (55.90) at 90 DAT. The hybrid 'Sarpan-33' recorded the maximum number of primary branches at 60 and 90 DAT (9.90 and 15.47, respectively).

Sruthi and Anitha (2015) studied the seasonal impact and effect of pinching on growth and flowering on two varieties *viz*., Pusa Narangi Gainda and Pusa Basanti Gainda of African marigold (*Tagetes erecta* L.). The experiment was conducted in three seasons namely, pre monsoon, monsoon and post monsoon with two levels of pinching (P0- no pinching, P1- pinching at 30 days after transplanting). The results revealed the significant influence of seasons and pinching in the two varieties studied. Growth parameters like plant height, number of primary branches, number of secondary branches, leaf area and total biomass varied with pinching during the three seasons. Maximum number of primary branches was observed in January sown crop with pinching whereas the maximum number of secondary branches were recorded in May sown crop with pinching in both the varieties. Leaf area and total biomass was found maximum in May sown crop for both the varieties with pinching.

2.2. Floral characters

Marigold is a herbaceous plant exhibiting rapid vegetative growth during initial stages. It takes about 30-45 days to complete its vegetative growth and at this stage flower bud appears and the plant enters into reproductive phase. Plant growth before flowering is very important for better flower production, and is influenced by various environmental factors. Optimum vegetative growth leads to high production of quality flowers.

Rao *et al.* (2005) conducted a study in 10 cultivars of African marigold for flower yield and carotenoid pigments. The cultivar 'Orange Double' was superior in terms of the days to first flower opening (95.00 days), flowering duration (44.00 days), diameter of flower (13.40 cm) and weight of flower (16.67 g), whereas, in terms of number of flowers per plant (32.30), 'Hyderabad Local Selection-1' was found superior. The highest total carotenoid per gram fresh weight of flower petals was

observed in the cultivar Pusa Narangi Gainda (2.69 mg/g) followed by Orange Double (2.66 mg/g).

Bharathi and Jawaharlal (2014a) evaluated twenty eight genotypes of African marigold (*Tagetes erecta* L.) for flower traits. They observed that not a single genotype was superior for all the traits evaluated. Bud appearance was the earliest in 'Bangalore Local Tall' (29.47 days) but earliest flower bud opening was observed in 'Double Orange' (46.00 days). The highest flower yield per plant was recorded in 'Coimbatore Local Orange' (1.48 kg) followed by 'Coimbatore Local yellow' (1.12 kg).

Gowda *et al.* (2016) conducted studies in 9 African marigold genotypes for growth parameters, yield and xanthophyll content. The study revealed that the genotype 'Local African Tall', had the maximum flowering duration (98.50 days), flower yield per hectare (15.05 t), petal meal yield per hectare (15.15 q) and xanthophyll yield (38.66 kg per hectare). The genotypes 'Indam Yellow' and 'Inca Orange' took the least number of days (17.46 days) for first flower bud appearance and the least number of days taken for 50 per cent flowering was recorded in the genotype 'Indam Yellow' (57 days).

Narsude *et al.* (2010a) reported maximum number of flowers per plant (71.00), flower yield per plant (630.48 g) and yield per hectare (24.67 MT) in the genotype 'Tuljapur Local-1' followed by 'Pakharsangavi Local' and 'Tuljapur Local-2'. Maximum number of days to last picking (109.67) and duration of flowering was longer (56.33 days) in the genotype 'Marigold Orange Bunch', whereas, the genotype 'Mulegaon Local' had shorter duration (42.00 days) of flowering. The minimum days (97.33) for last picking was recorded in genotype 'Tuljapur Local-2'.

Manik and Sharma (2016) evaluated fifteen genotypes of African marigold (*Tagetes erecta* L.) for yield attributes and xanthophyll content. All the genotypes showed variations for yield parameters. Maximum flower diameter (6.58 cm) was observed in the genotype 'CGRJ-1'. The genotype 'CGSG-1' was superior for number of flowers per plant, flower yield per plot and flower yield per hectare. The highest petal meal yield kg^{-1} of flower was recorded in 'Pusa Narangi Gainda'. The genotype

'CGRJ-2' recorded the maximum petal meal yield ha^{-1}. Maximum xanthophylls content kg^{-1} of petal meal and xanthophyll yield ha^{-1} was recorded in the genotype 'CGSG-1'.

Beniwal and Dahiya (2012) evaluated thirty eight genotypes of marigold and observed that five genotypes of African marigold (MGH 133-1, 133-1-1, 160-8-2, 160-8 and 160-9-1) showed promising results with respect to number of flowers per plant (10-64), flower size (5.0-12.27 cm), fresh weight of flower (5.2-22.0 g), days to first flower (72-138) and flowering duration (18-42 days). In French marigold, four genotypes (Hisar Beauty, Hisar Jaffri-2, MGH 17-1 and 8-2) showed better results with respect to number of flowers per plant (20-224), flower size (3.2-6.1 cm), fresh weight of flower (2.0-5.6 g), days to first flower (89-121) and flowering duration (39-55 days).

Raghuvanshi and Sharma (2011) conducted varietal evaluation in French marigold (*Tagetes patula* L.) and observed highly significant variation among cultivars for all the traits studied. Cultivar 'Safari Queen' recorded maximum flower yield per square meter (8.27 kg) while maximum flower diameter (5.26 cm) and 1000-seed weight (2.60 g) was recorded in the cultivar 'Bonanza Bolero'. Longest duration of flowering was recorded in cv. 'Safari Tangerine' (39.67 days) which was at par picking (131.11 g) was observed in cv. 'Honey Comb'. Highest 100-loose flower weight was observed in the cultivar 'Cupid on Varied Orange'. The cv. 'Honey Comb' recorded the maximum carotene content (3747.50 µg/g FW) which was on par with cv. 'Hero Harmony' (3745.83 µg/g FW).

Deepa and Patil (2016) evaluated the twelve marigold (*Tagetes* spp.) hybrids under Dharwad condition. They observed that the hybrid 'Garland Orange' had the maximum flower diameter (8.50 cm), fresh weight of flower (16.89 g) and flower yield per hectare (23.71 t) whereas the dry weight of flower was maximum in the hybrid 'Indam Yellow New' (1.49 g).

Nimisha *et al.* (2016) studied the performance of 8 African marigold (*Tagetes erecta* L.) cultivars with regards to various floral characters, under open field and rain shelter. The parameters, days to 50 per cent flowering, number of flowers per plant, marketable flower yield and shelf life of flowers in all the cultivars were significantly influenced by growing conditions. Flower length, pedicel length and seed yield per

flower of the cultivars were not significant influenced by growing condition. Highest number of flowers per plant (76.83) were recorded in cultivar 'Local Orange' and marketable flower yield (665.02 g per plant) in 'Orange Giant' respectively, grown under rain shelter.

Sruthi and Anitha (2015) studied the seasonal impact and effect of pinching on growth and flowering on two varieties *viz*., Pusa Narangi Gainda and Pusa Basanti Gainda of African marigold (*Tagetes erecta* L.). The experiment was conducted in three seasons *viz*., pre monsoon, monsoon and post monsoon with two levels of pinching (P0-no pinching, P1- pinching at 30 days after transplanting). The results revealed that September sown crop was showing early flowering. There was no influence of season and pinching on flower diameter, length of flower stalk and flower weight. Maximum number of flowers and flower yield was observed in January sown crop in both the varieties with pinching. The overall results showed that among the two varieties, Pusa Basanti Gainda can be recommended for cultivation during pre-monsoon season and during monsoon season, Pusa Narangi Gainda can be recommended for cultivation.

2.3. Bacterial wilt incidence

Bacterial wilt is a devastating disease, caused by the pathogen *Ralstonia solanacearum*. This pathogen is capable of causing the disease to over 200 botanical species from more than 55 plant families in tropical, subtropical and temperate regions of the world. Solanaceous crops like tomato, chilli, brinjal, tobacco and potato are found to be most vulnerable to this pathogen. Apart from solanaceous crops it is also known to infect many ornamentals like *Cosmos spp, Croton hirtus, Dahlia rosea, Euphorbia hirta, Euphorbia prunifolia, Hibiscus cannabinus, Petunia* spp., *Phlox drummondii, Tagetes* spp., *Tectona grandis, Zinnia elegans etc.*

Commercial cultivation of marigold in India is restricted to Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu and West Bengal. Among the different states, severe incidence bacterial wilt in marigold has been reported from West Bengal. The incidence of bacterial wilt disease in African marigold was found to be maximum in the months of July-October under West Bengal condition (Mondal *et al.*, 2014). In African

marigold genotypes the percentage of disease incidence reported to be 2.76-62.23 (Mondal *et al.,* 2011). Bacterial wilt incidence in African marigold genotypes has been observed at Agricultural Research Station, Mannuthy (unpublished data). Nimisha (2016) reported severe incidence of bacterial wilt in African marigold under Kerala conditions.

2.3.1. *Ralstonia solanacearum* biology and epidemiology

Ralstonia solanacearum is a widespread phyto-pathogenic bacterium that causes devastating wilt disease in many economically important crops and ornamentals. It is a gram negative rod-shaped flagellated bacterium that inhabits the soil. It grows well at 28 to 32° C strictly in aerobic conditions (Hayward, 1991; Schaad *et al.,* 2001). It belongs to the β-proteobacteria and is considered a "species complex" (Alvarez *et al.,* 2010). The term "species complex" was first applied to *R. solanacearum* by Gillings and Fahy (1994). *Ralstonia solanacearum* is a highly diverse species complex comprised of four genetically distinct phylotypes that correspond to different geographic origins (Meng, 2013a).

Under wet soil conditions, swimming motility of the bacterium helps in movement from plant to plant. *Ralstonia solanacearum* spreads though many ways such as water flow in the soil, infected plant material, and through contaminated soil or field supplies and equipment (Louws *et al.,* 2010). Wound sites and natural openings on roots of the plants are the major sites for *Ralstonia solanacearum* to enter the plants then invades cortical tissue, multiplies rapidly within the xylem tissue, and effectively clogs the water conduction system, causing the characteristic wilting symptom (Meng, 2013b). Chemotaxis plays an important role in the host-pathogen interaction. Root exudates attract *Ralstonia solanacearum* to host roots, therefore motility is a very important trait for the pathogen (Yao and Allen, 2006). *Ralstonia solanacearum* produces multiple virulence factors to enable invasion such as extracellular polysaccharides, secreted effectors via the type three secretion system, flagella propelled motility via the type four secretion system and cell wall degrading enzymes delivered through the type two section system (Liu *et al.*, 2005).

2.3.2. Infection and symptomatology of bacterial wilt disease

Ralstonia solanacearum enters into the plant through the wounds in the root system (Pradhanang *et al.*, 2005). Natural opening like stomata can be a best point through which the pathogen can enter into the plant (Chupp and Sherf, 1960). Environmental factor like temperature plays a major role that affects multiple plant pathosystems and their interactions with their hosts (Hayward, 1991). The pathogen enters into pith and xylem vessel by crossing intercellular spaces of cortex which leads to vascular plugging and ultimately leading to wilting of plant. Once bacteria are in the xylem, through quorum sensing, they produce extracellular polysaccharides and cell wall degrading enzymes that ultimately cause xylem clogging and tissue maceration (Salie *et al.*, 1997). Lower parts of the stem and root looks normal from outside but vascular bundles inside the stem turns brown along with formation of water soaked appearance in roots (Walker, 1952). Kelman (1953) noticed that the appearance of slimy viscous ooze on transversely sectioned stem at the site corresponding to vascular bundle due to the dehydration of occluded xylem vessels and destruction of surrounding tissues leads to collapse and death of plant.

The visual symptoms in infected plant, first appear in new growth and quickly spread to the rest of the plant causing the whole plant to collapse. Adventitious root formation on the stems of infected plants and yellowing of foliage may be observed in some partially resistant cultivars (Stall, 1991). A milky white exudate can be observed streaming from the cut surface of an infected plant stem when placed in water for few minutes, this distinguishes bacterial wilt from other wilt diseases. The presence of the pathogen can be verified by observation of vascular browning of infected plants and isolation of the pathogen.

2.3.3. Host range

The *Ralstonia solanacearum* has an unusually wide host range about 50 plant families (Hayward, 1991). The host range includes not only herbaceous plants but also several tree and shrub hosts (e.g. mulberry, olive, cassava, rubber, eucalyptus), some leguminous plants (such as groundnut, French bean), and a few monocotyledons (mainly banana, ginger). Deslandes *et al.* (1998) reported that *Arabidopsis thaliana,*

most widely used model plant for genetic engineering is also vulnerable to infection by this pathogen. Over two hundred species, particularly tropical and semi-tropic crops are liable to one or the other races of *Ralstonia*. Worldwide, the most important range of other host crops are *Anthurium* spp., *Capsicum annum* (bell pepper), *Zingiber officinale* (ginger), *Hevea brasiliensis* (rubber), *Manihot esculenta* (cassava), *Ricinus communis* (castor bean) and *Arachis hypogea* (ground nut). Many weeds, *G. cilliata, Galinsoga flora, Solanum nigrum, Polygonum capitata, Portulaca oleracea* and *Solanum cinereum* are also serves as hosts to the bacteria. *Solanum nigrum is* the primary wild host for race 3 (Hayward, 1994).

2.3.4. Survival of the pathogen

Ralstonia solanacearum can exist in the soil for several years without a host by surviving on crop debris and infected plant roots which sustain the pathogen before release back into the soil. Well drained soils with high water retention capacity are favorable for survival of the pathogen (Stall, 1991). Hayward (1991) proposed many explanations, such as the association of the bacteria with plant debris or with several weed hosts, which are symptomless carriers. The race 3, biovar 2, may survive in a latent form due to the weed hosts within the host or in their root areas in the soil. *R. solanacearum* strains are able to survive in water courses in roots of the weed *Solanum dulcamara* (Elphinstone *et al.*, 1998). The ability of pathogen to enter a dormant like 'viable but not culturable' (VBNC) state, like many other soil microbes may also leads for the long-term survival of the bacterium (Grey and Steck, 2001).

2.3.5. Sources of resistance in marigold

Not much work has been carried out on incidence of bacterial wilt in marigold in India and so far no resistant sources has been reported from India. However resistance to bacterial wilt in genotype M-1 which is a local collection was observed in the trials conducted at Agricultural Research Station, Mannuthy (unpublished data). Jones and Benson (1996) reported bacterial wilt resistance in marigold cultivars *viz.*, Cupid, Irish Lace, Cupid Mix, Sparky Mix, Papaya Crush, Naughty Marietta, Pineapple Crush, Rusty Red, Sparky, Pumpkin Crush, Bonanza Yellow, Choice Mix, Copper

Canyou, Fort Knox, Golden Harmony, Goldie, Gypsy Dancer, Orange Lady, Senator Dirksen and Tangerine Gem under North Carolina (USA) condition.

2.4. Evaluation studies against bacterial wilt

Evaluation of eight tomato parental lines and twenty eight F_1 crosses by Sharma *et al.* (2006) revealed that three parental lines *viz.*, CHDT-4, CHDT-5 and CH-180 and three F_1 crosses CHDT-1 x CH-180, CHDT-4 x CHDT-1 and CH-195 x CH-180 were resistant to bacterial wilt. The parental line CHDT-4 recorded the maximum yield followed by CH-180. Among the F_1 crosses, the crosses, CHDT-4 x CHDT-1, CHDT-1 x CH-180 and CH-195 x CH-180 showed resistant reaction. The F_1 cross *viz.*, EC-339074 x EC-386021 was found superior to others with respect to resistance and yield in bacterial sick plot.

Narayanankutty *et al.* (2005) conducted a field evaluation in tomato for bacterial wilt incidence. The study revealed that among the thirty six genotypes used Pusa Ruby showed 100 per cent wilt incidence. Twenty one lines including the resistant check Shakthi were found resistant (< 20 % incidence) and only one genotype (LE 25) recorded 0 per cent wilt incidence. Fourteen lines expressed moderate resistance to wilt and rest were susceptible to wilt.

Investigation to screen tomato genotypes against bacterial wilt (*Ralstonia solanacearum*) under field condition by Tiwari *et al.* (2012) reported high resistance in one genotype 'Cherry Jashpur'.

Bora *et al.* (2011) evaluated fourteen brinjal (*Solanum melongena* L.) genotypes including local ones for resistance to bacterial wilt and yield. The results revealed that the genotype 'Utsav' exhibited lowest bacterial wilt incidence of 2.23 per cent while the genotype Pusa Purple Long (PPL) exhibited the highest wilt incidence (65.8 %) which was considered as susceptible. Consequently the genotype 'Utsav' exhibited the highest yield (168.6 q ha^{-1}) which was 43.4 per cent higher than the best check SM 6-6. The duration of the crop as revealed from flowering and first harvesting was also shorter than the check varieties. 'Utsav' showed highest benefit:cost ratio of 3.64 as against 2.54 in SM 6-6.

Dutta and Rahman (2012) screened varieties and hybrids of tomato against bacterial wilt disease. Observations on per cent plant mortality were recorded starting from 15 days of transplanting till final harvest at an interval of 10 days. The variety 'All Rounder' was recorded 8.98 per cent wilt incidence with lowest VBDI (1.0) towards the disease which was considered as resistant. Four tomato varieties *viz.*, Swarakhsha, Rakshak, Trishul and Arka Alok recorded 10-20 per cent disease incidence which were considered as moderately resistant. F_1 Hybrids Yash, TO 1458, Hybrid 7610 and F_1 Amulya 1744 were recorded 30-70 per cent disease incidence with the VBDI of 2.6, 2.9, 2.7 and 2.8, respectively were moderately susceptible. The genotypes Loknath and Arka Vikash were found highly susceptible with 70-100 per cent and highest VBDI of 4 and 4.5 per cent, respectively.

Ajjappalavara *et al.* (2008) studied the genetics of bacterial wilt in four F_2 population of brinjal which were chosen by 20 F_1 hybrids derived from line x tester crosses of five female x four male parents. The results revealed that none of the parents was good general combiner for all the characters. The parents DWD-1 and Malapur were found to be good combiners with respect to fruit yield per plant. DWD-1, DWD-2, DWD-3 were the best and desirable combiner for the bacterial wilt incidence. Among these DWD-1 is noticed to be good combiner for the fruit stalk length, fruit rind thickness, fruit weight, fruit borer incidence. The hybrid DWD-1 x Malapur was noticed to be a resistant to bacterial wilt with high yielding and purple stripes on fruits.

2.5. Screening against bacterial wilt through artificial inoculation

In addition to the field evaluation, disease establishment through artificial inoculation is also needed for confirmation of the pathogenicity of the causal organism as well as the host reactions. Disease occurrence through artificial inoculation methods depend upon the inoculum concentration, age of the plants, environment where plants are kept and also the reaction of the host.

Artificial inoculation methods frequently used include the naturalized media drench method, stem puncture method, root dip and transplanting into infested soil.

Artal *et al.* (2012) evaluated 3 inoculation methods *viz.*, soil drenching, leaf clipping and axil puncturing to screen tomato, brinjal and chilli for bacterial wilt

resistance. The inoculation through soil drenching recorded significantly highest bacterial wilt incidence in tomato, brinjal and chilli (98.0, 95.0 and 90.0 per cent, respectively) followed by inoculation through axil puncturing which recorded 78.0, 88.0 and 78.0 per cent wilt incidence. However, lowest wilt incidence of 74.0, 48.0 and 40.0 per cent, respectively was recorded in leaf clipping method.

Rahman *et al.* (2011) conducted a study to screen of 8 cultivars *viz.*, Nayantara, Singhnath, Dhundul, Kazla, Marich Begun Luffa, Kata Begun and Uttara eggplant (brinjal) against wilt diseases with high yield in artificially inoculated field. The highest percentage of disease incidence of bacterial wilt (80 %) was recorded in the cultivar 'Luffa' and the lowest wilt incidence was recorded in the cultivar 'Kata Begun' (30 %) at 55 days after transplanting.

Kim *et al.* (2016) evaluated the 285 tomato genetic resources for resistance to *Ralstonia solanacearum* at seedling stage. Disease severity of tomato accessions was investigated from 7 days to 14 days at an interval of 7 days after inoculation of *R. solanacearum* under greenhouse conditions. The results showed that a total of 279 accessions were susceptible with 70 to 90 per cent wilt incidence. Two tomato accessions were moderately resistant, four accessions were highly resistance against *R. solanacearum*. The germplasms were not showing distinct symptom of bacterial wilt for up to 14 days after inoculation.

Onozaki *et al.* (1999a) screened seventy accessions of wild *Dianthus* for resistance against bacterial wilt (*Pseudomonas caryophylli*) by using the cut root soaking method with an inoculum concentration of 10^7 colony forming units (CFU) ml^{-1}. The results showed that two wild species *D. capitatus* ssp. *andrzejouskianus* and *D. hentri* were showing 0 per cent disease incidence (PDI) which were considered as highly resistant. Seven accessions were classified as resistant.

Onozaki *et al.* (1999b) screened two 285 cultivars of carnation for resistance against bacterial wilt by using the cut root soaking method with an inoculums concentration of 10^7 colony forming units (CFU) ml^{-1}. The results revealed that two hundred seven cultivars (74.7 per cent) were found highly susceptible whereas three cultivars 'Wiko', 'Nocto', and 'Sandrosa' possessed adequate resistance.

Thomas *et al.* (2014) screened resistant genotypes of tomato against the *Ralstonia solanacearum.* A pure bacterial inoculum (0.1 OD; 10^8 cfu ml^{-1}) was used for inoculation. Five inoculation methods *viz.*, seed-soaking inoculation, seed-sowing followed by inoculum drenching, 2 week stage through petiole-excision inoculation, soaking of planting medium with inoculum either directly or after imparting seedling root-injury were used. The results revealed that seed-based inoculations or mere inoculum drenching at 2 weeks did not induce much disease in seedlings. Petiole inoculation induced 90-100 per cent mortality in susceptible checks but also 50-60 per cent mortality in normally resistant genotypes within 7-10 days after inoculation.

2.6. Evaluation of bacterial wilt incidence through spot planting technique

Narayanankutty and Peter (1986) conducted a study to confirm host reaction to bacterial wilt in tomato cultivars using 4 inoculation methods *viz.*, root dipping, stem injection, alternate row planting and spot planting with susceptible genotype. The result revealed that among these methods spot planting was more effective in inducing the disease incidence in the resistant cultivar LE 79 (29.54 %). They suggested that spot planting can be recommended for future field screening trials for bacterial wilt resistance in tomato.

2.7. Combating bacterial wilt through grafting on resistant rootstocks

Grafting is an ancient art and science that was adapted to herbaceous vegetables in Japan and Korea in the early 1900's (Munge *et al.,* 2009). Grafting is the creation of a new plant through fusion of two plants, the rootstock (bottom) and scion (top). Grafting combines valued traits from a desirable rootstock variety such as vigor, soil borne disease resistance, increased yield, improved fruit quality, and enhanced environmental stress tolerance with a desired fruiting scion variety that meets specific market demands. Grafting has been widely adapted to greenhouse production systems where plants are intensely cultivated to produce high yields on highly vigorous rootstocks (Munge *et al.*, 2009). Grafting methods for vegetable crops includes the cleft graft, tongue graft, and splice/tube graft method (Lee, 1994). The splice or tube grafting is the most common approach and this method requires decapitation of both scion and

rootstock at a 45° angle. Then the scion top is secured to the rootstock with a silicon tube or clip. Tube grafting is used in the commercial production of grafted seedlings and it is highly effective with small plants. High grafting survival rates of 85-98 per cent can be achieved with the use of humidity chambers for healing grafted plants (Rivard and Louws, 2006).

Rivard and Louws (2008) effectively managed the bacterial wilt in tomato using grafting technique. In naturally infested soil the genotype 'German Johnson' showed 0 per cent bacterial wilt when grafted on the resistant genotypes CRA 66 or Hawaii 7996. Bacterial wilt while incidence for non-grafted 'German Johnson' was 79 per cent and 75 per cent in 2005 and 2006, respectively.

Peregrine and Ahmad (1982) investigated on use of resistant cultivars and grafting technique against bacterial wilt in tomato. It was reported that among the three rootstocks tried, 92.9 per cent survival rate was observed when tomato cultivars were grafted on *Solanum torvum* whereas the other rootstocks *viz.*, *S. melongena* and *Capsicum frutescens* caused losses up to 40 per cent. Among the cultivars tried, 'Roma' was found to be promising.

Bletsos (2003) studied the effect of grafting on growth and yield of eggplant (*Solanum melongena* L.) seedlings (Tsakoniki) grafted on wild species *Solanum torvum* (GST) and *Solanum sisymbrifolium* (GSS). The results showed that grafted plants were more vigorous, as measured by the plant height, main stem diameter and root system weight than the non-grafted 'Tsakoniki'. This resulted in an increased early production (GST, 45.5 per cent; GSS, 18.4 per cent) and late production (GST, 69.3 per cent; GSS, 59.2 per cent) as compared to non-infected controls.

Sakata *et al.* (2007) reported that, watermelon grafted onto bottle gourd causes early formation of female flowers compared with other rootstocks. Yamasaki *et al.* (1994) reported that, flowering is delayed in pumpkin, bottle gourd, wax gourd and watermelon when grafted on watermelon, especially in plants with 'Shintosa' type rootstocks.

Khah (2005) studied effect of grafting on growth, performance and yield of aubergine (*Solanum melongena* L.) in the field and greenhouse. Seedlings of aubergine (*Solanum melongena* L.) 'Rima' were grafted onto two tomato rootstocks, 'Primavera' (*Lycopersicon esculentum* L.) and 'Heman' (*Lycopersicon hirsutum*). Grafted and non-grafted (control) plants were grown in the field and greenhouse. It was found that plants grafted onto 'Heman' (RH) produced 53 per cent more fruit in the greenhouse and 60 per cent in field than the non-grafted (R) plants. The yield of plants grafted onto "Primavera" (RP) was similar to that of the non-grafted plants. Grafted plants (RH and RP) were more vigorous than the non-grafted (R), as indicated by their higher plant height, main stem and leaf weight. This led to earlier harvest. The seed content of fruit from grafted plants was lower than that of the control, indicating better fruit quality.

Alexios *et al.* (2007) studied the fruit yield and quality of watermelon in relation to grafting. Watermelon cv. Crimson Sweet was grafted onto four rootstocks (Long gourd, Early Max, Max-2 and F-14 gourd). The study revealed that grafting increased fruit size, resulting in higher yields than in the non-grafted control. Fruits from grafted plants had a thicker rind and slightly lower total soluble solids content than the fruit from non-grafted plants.

Alan *et al.* (2007) studied the effect of different rootstocks on watermelon plant growth, fruit yield and quality by comparing grafted and non-grafted plants ones under low tunnel for early production and later open field conditions. Watermelon (Matsum and Nakai) cultivar Crispy was grafted onto TZ-148 and RS-841, commercial hybrids of *C. maxima* x *C. moschata* and an experimental rootstock (*L. siceraria*) cv. 64-18. Non-grafted plants were used as control. Grafting significantly affected plant growth. Control plants had short main stem, less number of lateral vines and low root dry weight. Fruit yield was positively influenced by grafting when compared to control for both the growing conditions. There was a difference among grafted plants, cv. 64-18 was significantly poor yielder than other rootstocks. Detrimental effect was not observed with respect to fruits quality for grafted plants. These results showed that grafted plants improved plant growth and yield without any harmful effects on fruit quality.

Cardoso *et al.* (2012) evaluated tomato rootstocks and its use to control bacterial wilt disease. For the evaluation of grafting method for control of bacterial wilt, the Hawaii 7996 (H7996) was used as rootstock, and the cv. Santa Clara, Santa Cruz Kada and Debora Plus were used as the scion plants. The results revealed that grafting on the H7996 rootstock controlled the bacterial wilt disease 100 per cent and did not show any incompatibility with the scion tomato cultivars.

Lin *et al.* (2008) screened the two locally adapted resistant rootstocks *viz.*, eggplant (EG203) and tomato (Hawaii 7996) for management of tomato bacterial wilt. Resistant rootstock EG203 grafted plants exhibited 0 to 2.8 per cent wilt incidence compared with 24.4 to 92.9 per cent wilt in non-grafted plants.

Marsic and Osvald (2004) reported the influence of different grafting methods on the success of grafting and fruit yield of two tomato cultivars. The cultivars 'Monroe' and 'Belle', were used as scion and 'PG 3' and 'Beaufort' as rootstock. The treatments applied in each cultivar were cleft grafted onto 'PG 3' and 'Beaufort', and tube grafted onto 'PG3' and 'Beaufort', and un-grafted control. In both the methods high percentage of (79-100 %) of successful was obtained. A positive effect of grafting was recorded for yield when 'Monroe' was used as scion, and 'Beaufort' as rootstock. When 'Belle' was used as scion, a negative effect of grafting was observed, since the total fruit yield of non-grafted plants was significantly higher than that of plants grafted onto both rootstock cultivars.

2.8. Post-harvest life

Singh and Misra (2008) evaluated the 9 parents and 36 F_1 of marigold. They reported that cross 'Sutton Yellow' x 'Crackerjack Mix' attained the maximum shelf life (8.33 days) under room temperature.

Narsude *et al.* (2010b) studied the 10 genotypes of African marigold (*Tagetes erecta* L.) for shelf life. It was found that maximum vase life (8.87 days) showed by genotype 'African Marigold Double Orange' which was significantly superior over rest of the genotypes and minimum (6.20 days) in genotype 'Marigold Orange Bunch'. Under open condition the genotype 'African Marigold Double Orange' showed the minimum weight loss of flowers (29.80 %) which remained in good condition for two

days and maximum loss in weight (47.06 %) was observed in genotype 'Akolner Local'. Under paper bag storage, the minimum weight loss of flowers (30.94 %) was found in genotype 'Mulegaon Local' and maximum (51.20 %) in genotype 'Marigold Orange Bunch'. Under cold storage condition, minimum weight loss of flowers (11.74 %) was observed in the genotype 'Pakharsangavi Local' and maximum weight loss (21.89 %) in genotype 'Akolner Local'.

Raghuvanshi and Sharma (2011) conducted a varietal evaluation in French marigold (*Tagetes patula* L.) under mid-hill zone of Himachal Pradesh. Maximum storage duration of 8.67 days under cold storage and 4.00 days under ambient conditions was obtained in cv. 'Cupid on Varie Orange'.

Nimisha *et al.* (2016) studied the shelf life of 8 African marigold (*Tagetes erecta* L.) cultivars. The cultivars 'Sonata Orange' and 'Sonata Yellow' grown under rain shelter condition recorded maximum shelf life of flowers (4.79 days).

Materials and Methods

3. MATERIALS AND METHODS

3.1. Experimental site

The present investigation on "Evaluation of African marigold (*Tagetes erecta* L.) hybrids/varieties for yield and resistance to bacterial wilt" was conducted in Agricultural Research Station (ARS), Mannuthy, Thrissur during the year 2016 to 2017. The experimental site was situated at $76^0$10' E longitude and $10^0$32' N latitude at an altitude of 22.5 m above MSL. The selected site was the bacterial wilt sick plot of the station with facilities for drip irrigation and fertigation.

The experimental area is bestowed with tropical humid climate and during the experimental season the area received an average rainfall of 1760 mm. Meteorological observations *viz*., temperature, sunshine, rainfall and relative humidity are furnished in APPENDIX I. Soil analysis of the experimental area revealed that the soil was slightly acidic with a pH of 5.8, organic carbon content 1.07 per cent, available nitrogen 476.6 kg/ha, available phosphorus 29.24 kg/ha and available potassium 536.48 kg/ha.

3.2. Treatments (Genotypes)

Five F_1 hybrids, two varieties and one local collection of African marigold were used for the study. The details of the genotypes used for the study are given in Table 3.1 and Plate 1.

The investigations were carried out in five experiments *viz*.

1. Field evaluation of African marigold genotypes
2. Screening against bacterial wilt through artificial inoculation methods
3. Screening African marigold genotypes through spot planting technique
4. Grafting studies
5. Post-harvest studies

Table 3.1. List of genotypes and their sources

Genotypes	Name of the genotype	Specification	Source
1	Maria 91	F_1 - Hybrid	Sakura seeds Corp. Bengaluru
2	Rupa	F_1 - Hybrid	Sakura seeds Corp. Bengaluru
3	Sakura 031	F_1 - Hybrid	Sakura seeds Corp. Bengaluru
4	Royal Orange	F_1 - Hybrid	Biocarve seeds Pvt. Ltd. Punjab
5	P-4	F_1 - Hybrid	JYK. Seeds. CO., Ltd. China
6	Arka Agni	Variety	IIHR, Bengaluru
7	Arka Bangara 2	Variety	IIHR, Bengaluru
8	M-1	Local collection	Mannuthy, Kerala

3.3. Field evaluation of African marigold genotypes:

The work was carried out during June, 2016 to September, 2016 in the bacterial wilt sick plot of ARS, Mannuthy. Eight available genotypes of African marigold were evaluated following the KAU package of practices (POP) for the crop.

Design - RBD
No. of treatments - 8
No. of replications - 3
Plot size - 2m x 1m
Spacing in a plot - 50 x 50 cm

3.3.1. Nursery practices

Seeds of the marigold genotypes were sown in protrays filled with potting mixture comprising of cocopeat, vermiculite and perlite in the ratio of 3:1:1 and one month old seedlings were transplanted in the main field.

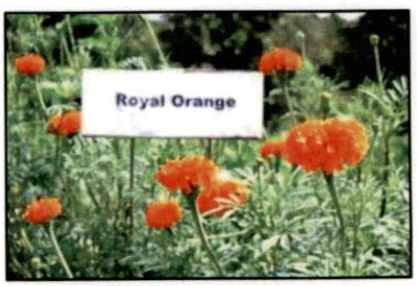
3.3.2. Preparation of main field

The experimental area was cleared and made into beds of size 2m x 1m. Individual beds were separated by 1.0 m x 1.0 m. They were mulched with 30 µ sliver black polythene film. One month old seedlings were transplanted in the well prepared

Maria 91

Rupa

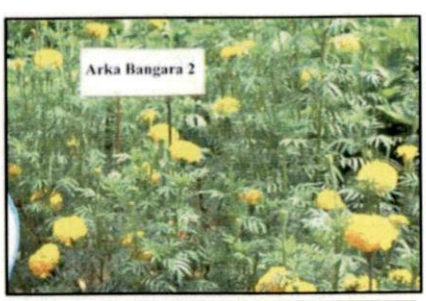
Sakura 031



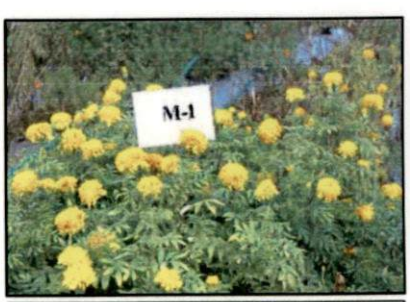
Royal Orange

P-4

Arka Agni



Arka Bangara 2



M-1

Plate 1. African marigold genotypes used for evaluation

main field at a spacing of 50 x 50 cm. The field was laid out in randomized block design (RBD) with three replications. There were eight plants per treatment per replication. A general view of the experimental field is given in Plate 2 and layout of the experimental field is given in Fig 3.1.

3.3.3. Application of manures and fertilizers

Fertilizer application was done as per the KAU package of practices for marigold. For the main field, the land was dug and FYM @ 20 t ha^{-1} was incorporated to the soil. Half dose of N (112.5 kg), full dose of P_2O_5 (60 kg) and half K_2O (30 kg) were applied as basal dose. Fourty five days after planting (DAP) the remaining half dose of N (112.5 kg) and half dose of K_2O (30 kg) were applied through fertigation in 15 equal splits twice in a week. Urea, Factomphos and Muriate of potash were used as source materials for supplying nutrients *viz.*, N, P_2O_5 and K_2O, respectively for the basal dose and Potassium nitrate (13:0.45) and urea were used for fertigation.

3.3.4. Intercultural operations

A single pinching was given at 20 days after planting (DAP) and plants were staked at 45 DAP. Weeding of the interspaces of plots was done at regular intervals. Plant protection measures were undertaken whenever necessary against collar rot, flower eating caterpillars and mites.

3.3.5. Harvesting

Harvesting was done when flowers were fully opened. The harvested flowers were used for recording the observations.

3.3.6. Field evaluation for bacterial wilt incidence

Daily inspection in the field was done to identify the wilt incidence and wilted plants were collected and ooze tested. The bacteria were isolated on TTZ (2, 3, 5 Triphenyl Tetrazolium Chloride) medium (APPENDIX II) and identified as *Ralstonia solanacearum* and further ascertained by conducting Koch's postulates (Plate 3). The severity of the disease incidence in selected genotypes was scored as per the score chart followed by Sinha *et al.* (1988) (Table 3.2).

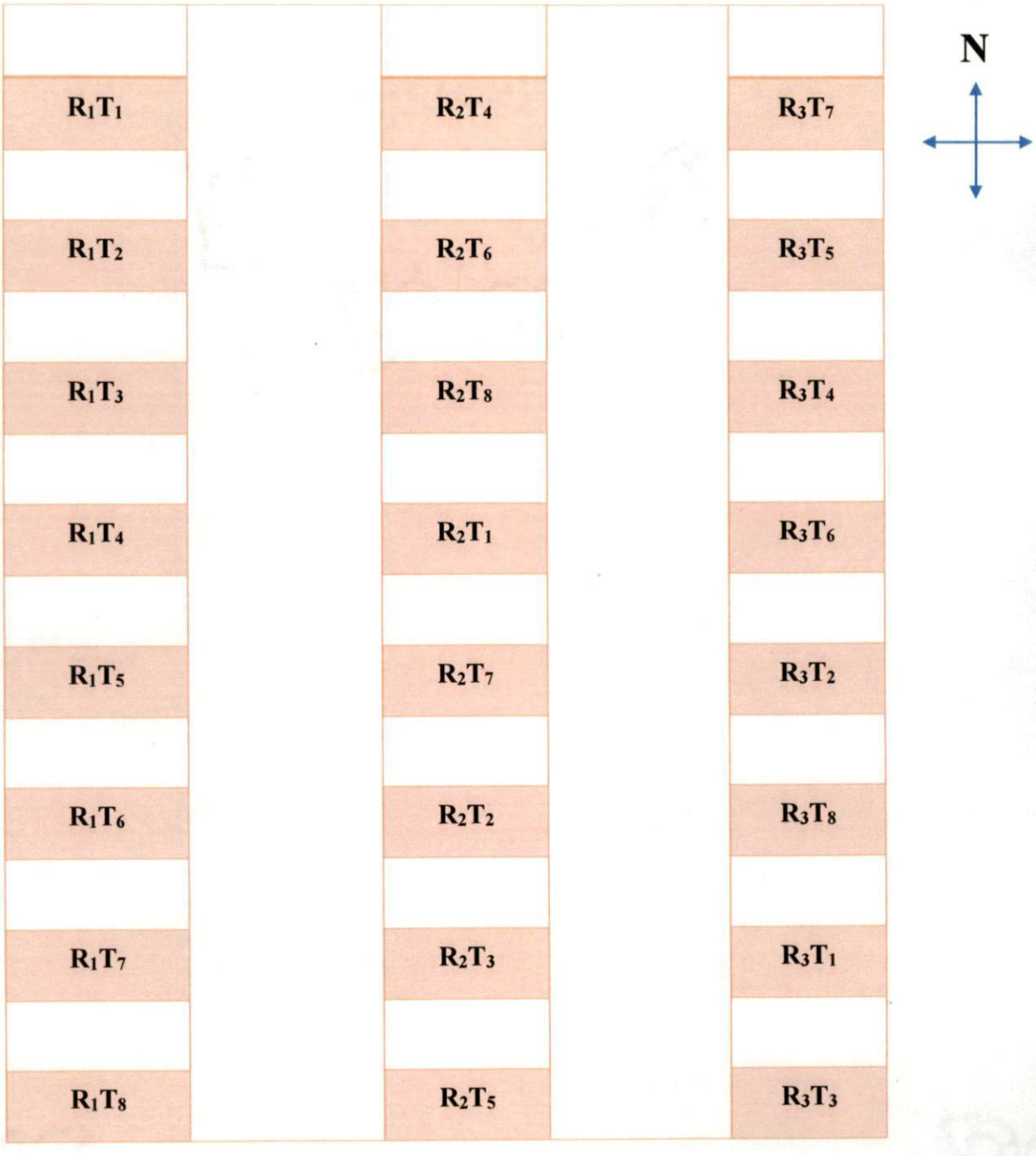
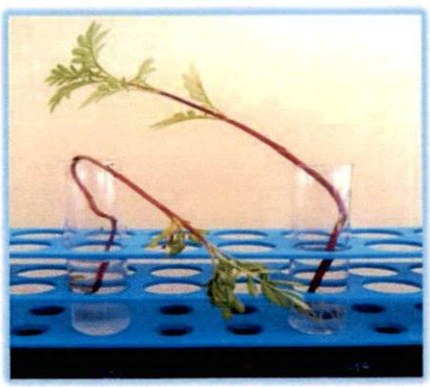


Fig 3.1. Layout of experimental plot for field evaluation

Plate 2. General view of experimental field

Presence of pathogen by ooze test

Isolated bacteria on TTZ medium



Causing disease in healthy seedling

Pathogen re-isolated from diseased seedling

Plate 3. Confirmation of bacterial wilt by Koch's postulates

Table 3.2. Score chart for bacterial wilt incidence

Reaction	Per cent incidence
R (Resistant)	< 10
MR (Moderately resistant)	>10-20
MS (Moderately susceptible)	>20-30
S (Susceptible)	>30-70
HS (Highly susceptible)	>70-100

3.4. Screening against bacterial wilt through artificial inoculation:

The experiment was conducted during September, 2016. Seedlings of the genotypes were raised in soilless medium and three week old seedlings were transplanted to pots filled with sterilized soilless medium comprising of coco peat + vermiculite + perlite in 3:1:1 ratio. The pots were kept in mist chambers (Plate 4). Seedlings of all the genotypes were artificially inoculated with *Ralstonia solanacearum* suspension containing bacterial population at 2.4 x 10^6 cfu ml^{-1}. Three methods of inoculation *viz*., media drenching, root dip and stem injection were tried. For each genotype, five pots were kept as control without inoculation.

Design - CRD

No. of genotypes - 8

No. of inoculation methods - 3 + un-inoculated control

No. of replications - 3

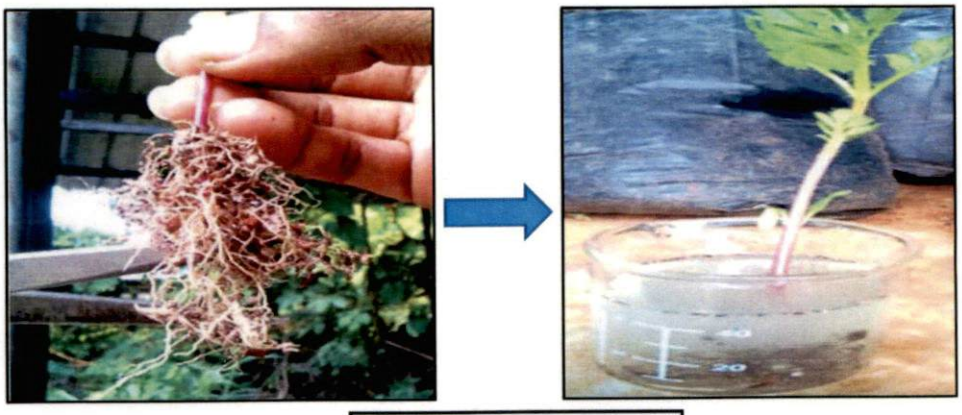
No. of pots/treatment /replication - 5

3.4.1. Inoculation Methods

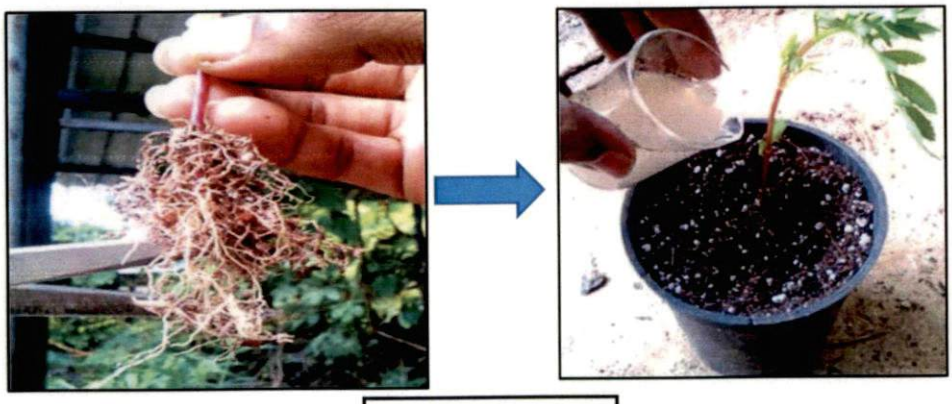
Different methods used for artificial inoculation of the bacterium are illustrated in Plate 5.

1. **Media drenching:** In this method roots of seedlings were trimmed with sterile scissors before planting in pots and 10 ml inoculum was poured into

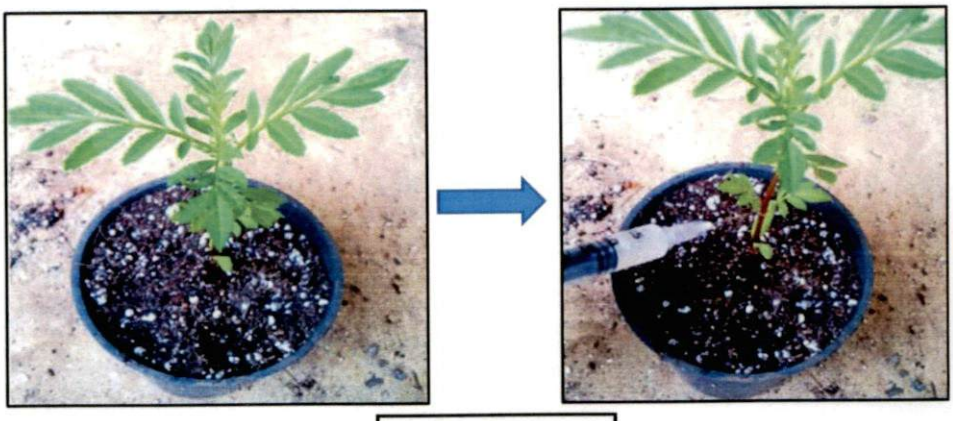
Plate 4. Mist chamber housing of artificially inoculated plants



Root dip method



Drench method



Stem injection

Plate 5. Procedure for artificial inoculation

the root zone (Xian-Gui *et al.*, 2006). One day after planting, the inoculation was repeated by pouring 15 ml of the same inoculum per pot.

2. **Root dip:** Seedlings were uprooted and the root system was washed prior to inoculation. Root tips were trimmed with sterile scissors in order to make a wound and then immediately dipped in 50 ml of bacterial suspension for 2 minutes and planted in pots.
3. **Stem injection:** In this method a pin prick was given on the leaf axils using a syringe and bacterial suspension was injected.

The inoculated plants kept in mist chamber were watered with 20 ml distilled water only when the media was dry. They were not given any nutrients during the study. The plants were monitored daily for a period of 42 days after inoculation (DAI) and the disease incidence in wilted plants of each genotype was confirmed through ooze test and isolation of *R. solanacearum* on TTZ medium (Plate 6).

Severity of the disease incidence in selected genotypes was scored according to the standard score chart (Sinha *et al.*, 1988) as done in field evaluation studies.

3.5. Screening African marigold genotypes through spot planting technique

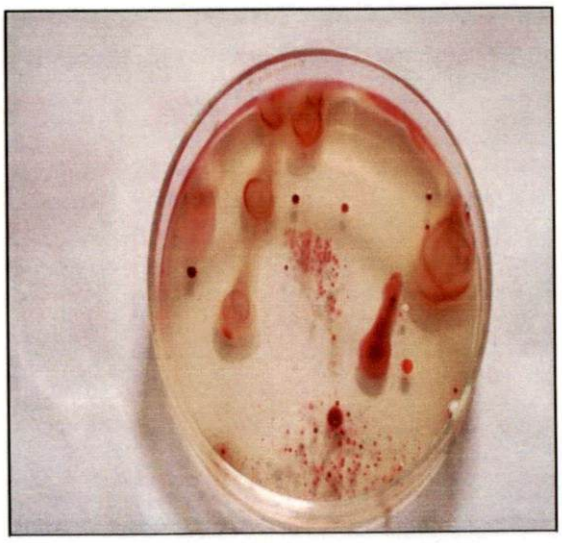
The experiment was conducted during September 2016 to December 2017. For the experiment, seedlings of the seven genotypes *viz.*, Maria 91, Rupa, Royal Orange, P-4, M-1, Arka Agni and Arka Bangara 2 were spot planted with the highly susceptible check genotype Sakura 031. Observations for wilt incidence was recorded as done in field evaluation studies.

3.6. Grafting studies

3.6.1. Field evaluation of grafted and non-grafted plants of African marigold genotypes

Based on results of the experiments on field evaluation of African marigold genotypes and artificial inoculation, studies on evaluation of grafting was undertaken during November 2016 to February 2017. The procedure for grafting is illustrated in Plate 7. For the study, M-1 the local collection which was found 100 per cent wilt free

Ooze test



Isolated bacteria on TTZ medium

Plate 6. Bacterial wilt confirmation after artificial inoculation

in the earlier experiments was selected as rootstock. Genotypes susceptible to bacterial wilt *viz*., Maria 91, Rupa, Sakura 031, Royal Orange, P-4, Arka Agni and Arka Bangara 2 were grafted on the M-1 rootstock through cleft grafting method using grafting clips. Scions from 30 days old seedlings of wilt prone genotypes were grafted onto 45 days old M-1 rootstock. Grafted plants were kept in mist chambers for healing of graft union for 7 days and after that they were taken out and acclimatized in a naturally ventilated green house for 7 days before transplanting to the main field.

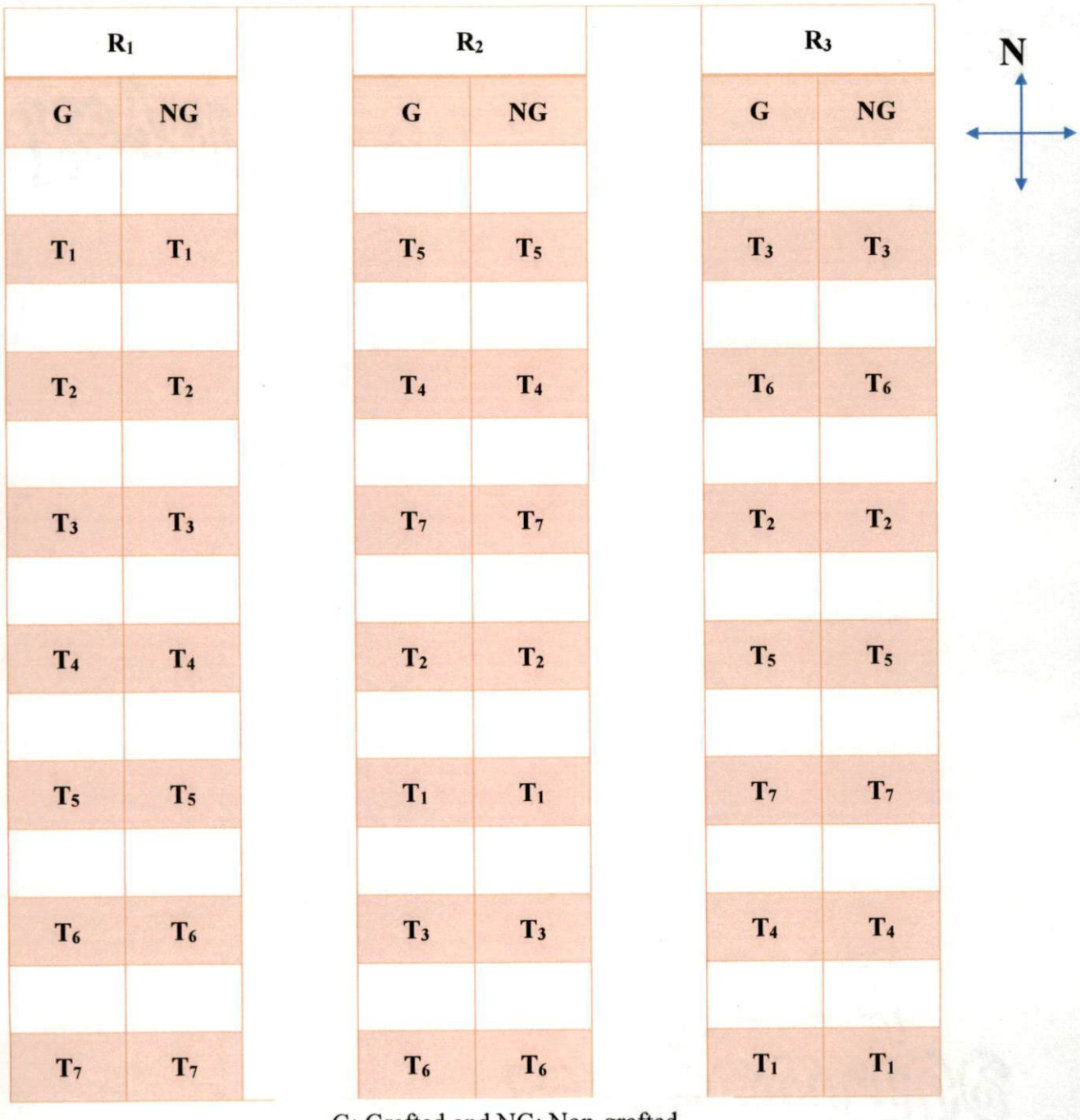
In the main field, grafted and non-grafted seedlings of each genotype was planted in the same bed. Recommended KAU package for the crop was followed in the main field as done during previous experiments. A general view of the experimental field is given in Plate 8 and layout of the experimental field is given in Fig 3.2.

Design	- Split plot (Genotypes as main plot and grafted and non-grafted plants within each genotype as sub plots)
Treatments	- 7
No. of replication	- 3
Plot size	- 3 x 1 m
Spacing	- 50 x 50 cm

3.7. Post-harvest studies

Five flowers with stalk from each genotype were kept in ambient atmospheric conditions during September, 2016. Total initial weight for the five flowers were recorded. Flowers were daily observed for wilting and loss in weight. Shelf life or days to wilt for the genotypes was assessed when 25 per cent of the flowers wilted.

Design	- CRD
No. of genotypes	- 8
No. of replications	- 3



G: Grafted and NG: Non-grafted

Fig 3.2. Layout of experimental plot for grafting studies

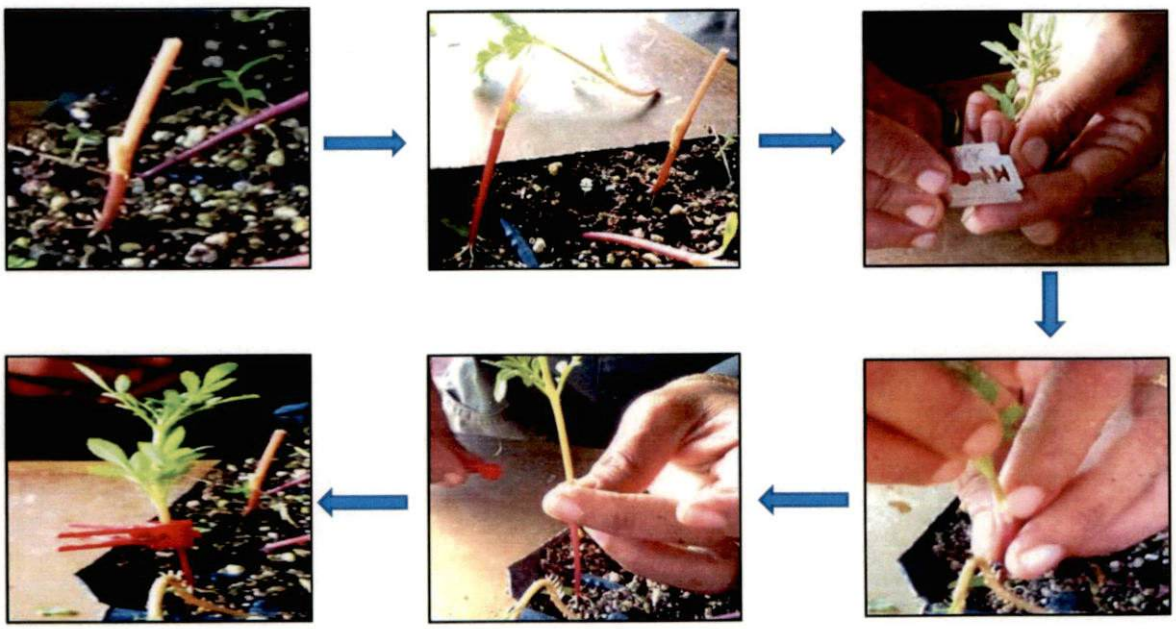


Plate 7. Procedure for grafting technique

Plate 8. General view of the experimental field for grafting studies

3.8. Observations

The following observations were recorded for studies on field evaluation, artificial inoculation, spot planting technique, grafting studies and post-harvest studies.

3.8.1. Plant characters

3.8.1.1. Plant height (cm):

Plant height was measured from base to the tip of the main stem at 30 and 60 DAP in the main field. Observations were recorded for individual plants and the mean height was then worked out in cm.

3.8.1.2. Plant spread (cm):

The plant spread was recorded by measuring the circumference at the top portion at 30 and 60 DAP for individual plants per genotype per replication and the mean spread was calculated.

3.8.1.3. Stem girth (cm):

Stem girth at 2 cm above collar level was measured at 30 and 60 DAP for individual plants per genotype per replication and the average was calculated.

3.8.1.4. Number of primary branches per plant:

The number of branches was counted at 30 and 60 DAP for individual plants and the average was calculated.

3.8.1.5. Petiole colour:

Visual difference in petiole colour were identified with the help of Royal Horticultural Society (RHS) colour chart.

3.8.1.6. Leaf area:

Leaf area was calculated using the leaf area meter instrument (LI-3100C Area meter). True representative four leaves from the middle portion of the stem were selected, area read in the leaf area meter. Observations were recorded for individual plants per genotype per replication and mean leaf area was calculated.

3.8.2. Floral characters

3.8.2.1. Days to bud initiation/formation:

The number of days taken for first flower bud formation was observed for individual plants for each genotype per replication and mean number of days was calculated.

3.8.2.2. Days to flower opening:

The number of days taken to just flower opening from the day of bud initiation was observed for ten flower buds per genotype per replication and mean number of days to flower opening was calculated.

3.8.2.3. Number of flowers per plant:

The number of flowers harvested from individual plants in each genotype per replication was recorded and mean number of flowers was worked out.

3.8.2.4. Flower diameter (cm):

Diameter of ten fully opened flowers from each genotype per replication was measured and the average diameter for each genotype was calculated.

3.8.2.5. Stalk length (cm):

Stalk length of ten randomly selected flowers from each genotype per replication was measured and the mean length was calculated.

3.8.2.6. Flower weight (g):

The weight of ten flowers plucked randomly from each genotype per replication was recorded and average flower weight was worked out and expressed in grams.

3.8.2.7. Petal yield per flower (g):

The weight of ray florets from ten flowers was recorded for each genotype per replication and average weight was worked out and expressed as petal yield per flower.

3.8.2.8. Yield per plant (kg):

The flower yield obtained from individual plants of each genotype per replication was recorded and mean flower yield was worked out.

3.8.2.9. Number of harvests:

Total number of picking of fully opened flowers from the day of first harvest to the last harvest was expressed as number of harvests.

3.8.2.10. Colour of flower:

Visual difference in flower colour were identified with the help of Royal Horticultural Society (RHS) colour chart.

3.8.2.11. Total carotenoids:

Estimation of carotenoids was done according to the method suggested by Arnon (1949). The sample extract was prepared by grinding 200 mg of fresh flower with a pestle and mortar using 10ml of 80 per cent acetone. The homogenate was then filtered in a volumetric flask (25ml) using Whatman Filter paper No. 1. The homogenate was washed out 2-3 times with 5ml of 80 per cent acetone each time and the final volume of filtrate was made up to 25ml of 80 per cent acetone. The filtrate was taken in a cuvette (3/4 volume) and its absorbance was recorded separately at 480 and 510 nm using a spectrometer (using 80 per cent acetone as blank). The carotenoid content was calculated following the formula and expressed in mg g^{-1}.

Total carotenoids: [(7.6 x OD at 480)-(1.49 x OD at 510)] x V/1000 x W

3.8.3. Incidence of bacterial wilt

3.8.3.1. PDI of bacterial wilt:

The number of plants showing the disease incidence was expressed in terms of per cent disease incidence (PDI). The PDI in the plants was calculated by following formula,

$$PDI = \frac{\text{No. of plants affected by wilt disease}}{\text{Total No. of plants under observation}} \times 100$$

3.8.3.2. Stages of incidence

Plants were daily observed for bacterial wilt incidence and date of disease incidence was noted and number of days taken for disease incidence from the day of

transplanting was counted. Number of days to attain the maximum PDI for each genotype per replication was recorded and average number of days was worked out and the genotypes were grouped into the following classes (Table 3.3).

Table 3.3. Stages of bacterial wilt incidence in African marigold

DAP	Stage of incidence
0-30	Early vegetative
31-45	Vegetative
46-60	Early flowering
61-90	Peak flowering

3.8.4. Grafting studies

Observations were recorded on per cent survival of grafts in the field, per cent disease incidence, plant characters and floral characters.

3.8.4.1. Per cent field survival of grafts:

Number of grafted plants survived after transplanting in each genotype per replication was counted and expressed in terms of per cent survival.

3.8.4.2. PDI of bacterial wilt

Both grafted and non-grafted plants in each genotypes were observed for wilting symptoms daily. The plants showing the wilting symptoms were subjected to ooze test and bacteria isolated on TTZ medium for confirmation of bacterial infection. Total number of plants infected with bacterial wilt in each genotype per replication was counted and expressed in terms of per cent of disease incidence (PDI).

3.8.5. Post-harvest studies

3.8.5.1. Days to wilting of flowers (shelf life):

The number of days taken to show more than 25 per cent wilting and discoloration under ambient condition was counted and expressed as number of days to wilt.

3.8.5.2. Loss in weight of flowers:

Five flowers from each genotype were kept in ambient atmospheric conditions. Total initial weight for the five flowers was recorded. The loss in weight of flowers was recorded daily and expressed in terms of physiological loss in weight (PLW) and cumulative physiological loss in weight (CPLW).

3.9. Statistical analysis:

Statistical analysis was done on different parameters using OP-STAT (HAU, Hissar). Analysis of variance studies were conducted to assess the variation among different characters.

Results

4. RESULTS

The present investigation was conducted at Agricultural Research Station, Mannuthy, Thrissur during the year 2016-2017. The work was carried out under five experiments namely 1. Field evaluation 2. Screening against the bacterial wilt through artificial inoculation 3. Screening against the bacterial wilt through spot planting technique 4. Grafting studies and 5. Post-harvest studies. Results of the studies were statistically analyzed and presented in this chapter.

4.1. Field evaluation

Field evaluation of eight genotypes with respect to plant characters, floral characters, yield and incidence of bacterial wilt was conducted during June-September, 2016.

4.1.1. Plant characters

Performance of the genotypes with respect to the plant characters at different stages of growth is presented in Table 4.1(a).

4.1.1.1. Plant height

Significant difference was noticed among genotypes with respect to plant height both at 30 and 60 days after planting (DAP). At 30 DAP, significantly highest plants were recorded in Maria 91 (81.51 cm) followed by P-4 (58.54 cm) and Royal Orange (55.43 cm). The lowest height was observed in M-1 (20.58 cm). However, at 60 DAP, maximum height was recorded in Royal Orange (129.27 cm), followed by P-4 (119.87cm) and Maria 91(113.87 cm) and these genotypes were on par with respect to the parameter. At 60 DAP also the lowest height was observed in M-1 (66.15 cm).

4.1.1.2. Plant spread

Genotypes also showed significant variations with respect to plant spread. At 30 DAP plant spread was maximum for Maria 91 (78.24 cm) followed by Royal Orange

(68.01 cm) and P-4 (66.03 cm). Arka Bangara 2 recorded the lowest spread of 28.66 cm at 30 DAP. Maximum plant spread at 60 DAP was observed in P-4 (107.48 cm) followed by Royal Orange (103.49 cm) and Maria 91 with a spread of 97.74 cm. Lowest plant spread at 60 DAP was recorded in Sakura 031 (57.95 cm).

4.1.1.3. Stem girth

Stem girth recorded on 30 DAP varied significantly among the genotypes. Both Maria 91 and P-4 were on par with respect to the parameter recording a stem girth of 6.55 cm and 6.05 cm, respectively. Both varieties from IIHR *viz*., Arka Agni and Arka Bangara 2 recorded lowest stem girth. However, on 60 DAP stem girth was on par for all the genotypes.

4.1.1.4. Number of primary branches

Significant differences were noticed in the number of primary branches among the genotypes both at 30 and 60 DAP. The genotypes Sakura 031, Royal Orange, Rupa, P-4 and Maria 91 showed higher number of primary branches on 30 DAP and these were on par also. At 60 DAP along with the five above mentioned genotypes, M-1 also showed higher number of primary branches. However, primary branches were the lowest for Arka Agni and Arka Bangara 2 both at 30 and 60 DAP.

4.1.1.5. Leaf area

There was significant differences observed among the genotypes for leaf area. The genotype M-1 recorded maximum leaf area (68.75 cm^2) followed by Maria 91 (40.67cm^2) while minimum leaf area was observed in Arka Bangara 2 (21.07 cm^2).

4.1.1.6. Petiole colour

There was no difference observed among the genotypes for petiole colour. All the genotypes had green coloured petiole.

Table 4.1(a). Field evaluation of African marigold genotypes for plant characters

Genotypes	Plant height (cm)		Plant spread (cm)		Stem girth (cm)		Primary branches		Leaf area (cm^2)	Petiole colour
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days		
Maria 91	81.51	113.87	78.24	97.74	6.55	7.51	11.12	14.20	40.67	Green
Rupa	48.38	97.67	63.24	92.24	5.56	7.28	12.95	15.54	25.74	Green
Sakura 031	41.45	77.12	57.29	57.75	5.43	7.00	13.50	13.84	22.29	Green
Royal Orange	55.43	129.27	68.01	103.49	4.84	7.83	13.01	15.19	34.86	Green
P-4	58.54	119.87	66.03	107.48	6.05	8.52	12.87	15.49	34.85	Green
Arka Agni	33.20	92.37	43.41	82.42	4.10	7.32	6.62	09.45	28.80	Green
Arka Bangara 2	26.20	89.51	28.66	82.46	3.66	7.46	5.87	08.87	21.07	Green
M-1	20.58	66.15	46.13	94.46	4.31	8.35	7.99	13.00	68.75	Green
C.D. (0.01)	05.22	14.06	14.98	14.92	0.87	NS	2.50	03.32	9.12	-
S.E(m±)	01.70	4.59	4.89	4.87	0.27	0.45	0.81	01.08	2.97	-

4.1.2. Floral characters

Floral characters *viz*., days to flower bud initiation/formation, flower opening, flower diameter, stalk length, flower weight and petal yield per flower are given in Table 4.1(b) and characters *viz*., number of flowers per plant, yield per plant, number of harvests, flower colour and total carotenoids content are given in Table 4.1(c).

4.1.2.1. Days to flower bud initiation/formation

Significant differences were observed among the genotypes for the days to initiate flower buds. Except M-1, all the genotypes took almost the same number of days to bud initiation and they were statistically on par with respect to this parameter. However, M-1 took the longest period to initiate bud (50.62 days) and it was significantly late when compared to all other genotypes.

4.1.2.2. Days to flower opening

There was no significance difference observed among the genotypes for days taken to flower opening. However, the genotype Rupa took minimum number of days (10.49) for flower opening which was followed by Sakura 031 (10.70). The genotype Royal Orange took maximum number of days (14.19) for flower opening.

4.1.2.3. Flower diameter

Significant variations were observed for flower diameter among the genotypes which was ranging from 6.45 cm to 9.08 cm (Plate 9). The genotype P-4 recorded the maximum flower diameter measuring 9.08 cm which was on par with Rupa (9.06 cm) while the genotype M-1 recorded the minimum flower diameter (6.45 cm). All other genotypes were on par with respect to the parameter.

4.1.2.4. Stalk length

Stalk length of the genotypes differed significantly among each other (Plate 9). Stalk length was significantly higher for Arka Bangara 2 (11.74 cm), P-4 (11.26 cm), Arka Agni (11.18 cm) and Royal Orange (11.12 cm) and these genotypes were on par with respect to the parameter. These genotypes were followed by Maria 91 and Rupa

with stalk length of 8.67 cm and 7.29 cm, respectively. The stalk length was minimum in the genotype Sakura 031 (5.85 cm) which was on par with the M-1 (6.11 cm).

4.1.2.5. Flower weight

Maximum fresh flower weight was recorded in Rupa (23.36 g) followed by P-4 (22.66 g) and these were significantly higher when compared to all other genotypes. All other genotypes were on par with respect to the parameter with flower weight ranging from 11.36 to 14.46 g.

4.1.2.6. Petal yield per flower

Significant difference was observed in petal yield per flower. Maximum petal yield of 18.16 g was observed in Rupa followed by P-4 (16.56 g) and these genotypes were on par with respect to the parameter. All other genotypes recorded almost similar petal yield per flower ranging from 8.13 g to 10.66 g.

4.1.2.7. Number of flowers per plant

Number of flowers differed significantly among the genotypes and it was highest in P-4 (116.91) followed by Rupa (80.33), Royal Orange (65.83), Arka Bangara 2 (49.04) and Arka Agni (45.29). Number of flowers per plant was the lowest in Sakura 031 (18.15) which was on par with Maria 91 (20.41) and M-1 (36.41).

4.1.2.8. Yield per plant

Genotypes showed significant difference with respect to yield per plant. The maximum flower yield of 1.034 kg was recorded in P-4 and this was followed by Rupa (0.778 kg), Royal Orange (0.466 kg) and Arka Bangara 2 (0.439 kg). Lowest flower yield was recorded in Maria 91 (0.153 kg) which was on par with M-1 (0.234 kg), Sakura 031 (0.348 kg) and Arka Agni (0.359 kg).

4.1.2.9. Number of harvests

Wide variation was observed for the number of harvests. Average number of harvests ranged from 3.83 to 10.00. Except for Maria 91, Sakura 031 and M-1, all other

Table 4.1(b). Field evaluation of African marigold genotypes for floral characters

Genotypes	Days to bud initiation	Days to flower opening	Flower diameter (cm)	Stalk length (cm)	Flower weight (g)	Petal yield/ flower (g)
Maria 91	31.00	10.70	7.75	8.67	13.43	8.96
Rupa	28.12	10.49	9.06	7.29	23.36	18.16
Sakura 031	28.20	10.91	7.37	5.85	14.46	10.66
Royal Orange	30.13	14.19	7.53	11.12	13.40	9.86
P-4	28.70	13.27	9.08	11.26	22.66	16.56
Arka Agni	29.45	12.16	6.75	11.18	11.76	9.03
Arka Bangara 2	28.38	13.15	7.37	11.74	13.90	10.30
M-1	50.62	14.15	6.45	6.11	11.66	8.13
C.D. (0.01)	6.05	NS	0.44	0.96	3.28	3.82
S.E(m±)	1.97	0.92	0.14	0.31	1.07	1.24

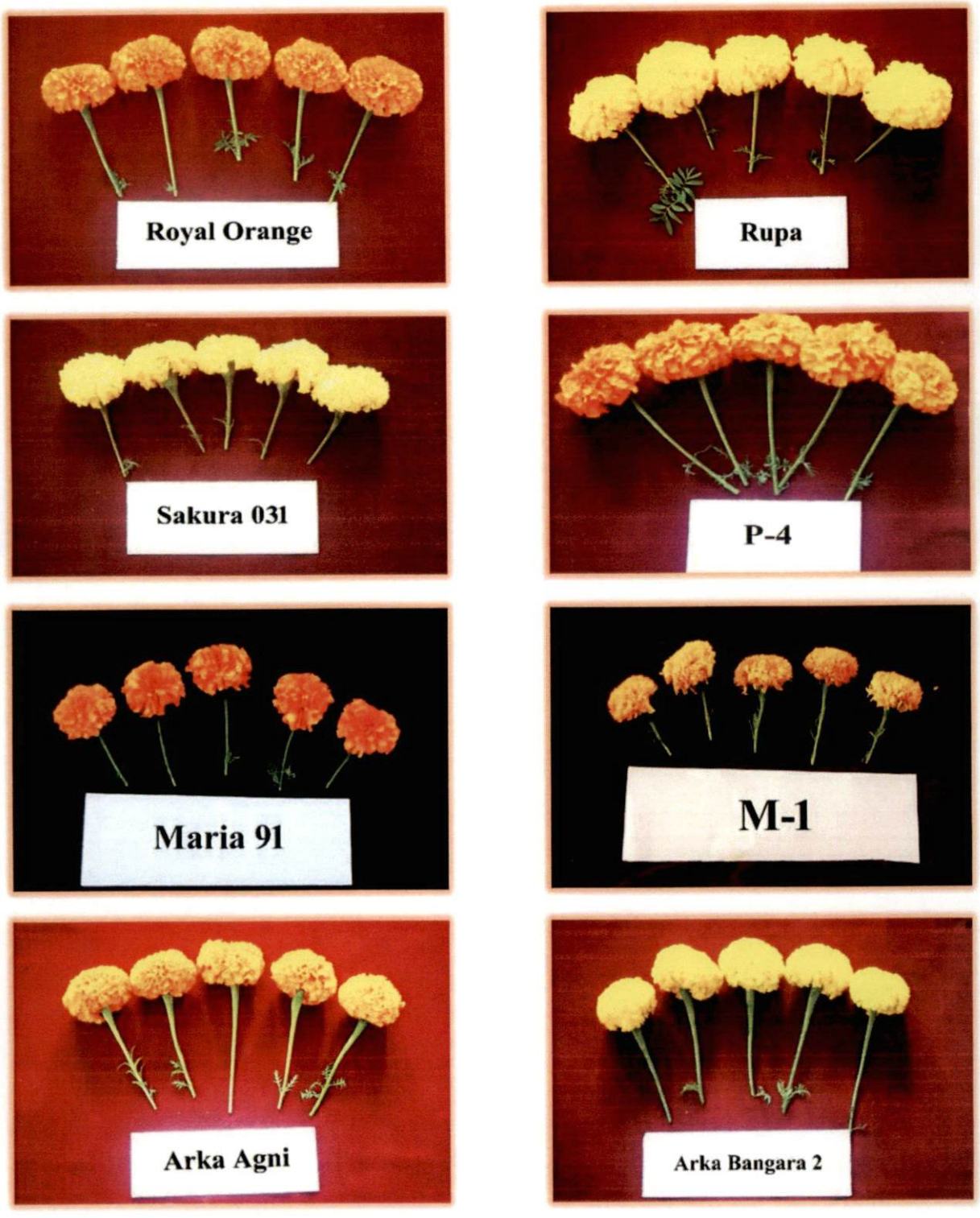


Plate 9. Flower diameter and stalk length in African marigold genotypes

genotypes recorded almost the same number of harvests. Maximum number of harvests was noticed in Arka Bangara 2 (10.00) which was on par with the genotypes P-4 and Arka Agni each with 9.66, Royal Orange and Rupa each with 9.00. Minimum number of harvests was observed in Maria 91 (3.83) which was on par with the variety Sakura 031 (4.00).

4.1.2.10. Flower colour

Flower colour of the genotypes varied from different shades of yellow and orange as given in Table 4.1 (c).

4.1.2.11. Carotenoid content

There was no significance difference with respect to carotenoid content among the genotypes. However, the mean carotenoid content was highest in P-4 (0.074 mg/g FW) followed by Maria 91 (0.062 mg/g FW). Minimum carotenoid content was observed in Arka Bangara 2 (0.033 mg/g FW).

4.1.3. Bacterial wilt incidence

Data on the performance of different genotypes with respect to per cent disease incidence, days to wilting of plants and stages of wilt incidence during field evaluation are presented in the Table 4.1(d) and 4.1(e), respectively.

4.1.3.1. Per cent disease incidence (PDI)

Significant differences were observed with respect to per cent disease incidence among the genotypes (Table 4.1d). The genotype Sakura 031 showed the maximum disease incidence with 100 per cent followed by Rupa (70.83 %) and Maria (45.83 %). Bacterial wilt incidence was nil in the genotype M-1 (Plate 10). Among the other genotypes, Arka Bangara 2 recorded a low PDI of 12.5 followed by P-4 and Royal Orange with the PDI of 25 and Arka Agni with a PDI of 29.16. Based on the PDI, it could be inferred that there are two highly susceptible genotypes (Sakura 031 and Rupa), one susceptible genotype (Maria 91), three moderately susceptible genotypes (Royal Orange, P-4 and Arka Agni), one moderately resistant genotype (Arka Bangara 2) and one resistant genotype (M-1).

Table 4.1(c). Field evaluation of African marigold genotypes for floral characters (contd...)

Genotypes	No. of flowers/ plant	Yield /plant (kg)	No. of harvests	Carotenoid content (mg/g FW)	Colour of flower
Maria 91	20.41 (4.60)	0.153 (1.07)	3.83	0.062 (1.03)	Strong Orangish Yellow
Rupa	80.33 (9.00)	0.772 (1.33)	9.00	0.047 (1.02)	Brilliant Yellow
Sakura 031	18.15 (4.24)	0.348 (1.16)	4.00	0.061 (1.03)	Brilliant Greenish Yellow
Royal Orange	65.83 (8.5)	0.466 (1.21)	9.00	0.052 (1.02)	Vivid Orangish Yellow
P-4	116.91 (10.81)	1.034 (1.42)	9.66	0.074 (1.03)	Vivid Orangish Yellow
Arka Agni	45.29 (6.65)	0.359 (1.16)	9.66	0.044 (1.02)	Strong Orangish Yellow
Arka Bangara 2	49.04 (7.06)	0.439 (1.19)	10.00	0.033 (1.09)	Light Greenish Yellow
M-1	36.41 (6.02)	0.234 (1.11)	4.33	0.054 (1.02)	Light Yellow
C.D. (0.01)	1.82	0.09	1.58	NS	
S.E(m±)	0.59	0.03	0.51	0.00	

Values in the parentheses are transformed data

Table 4.1(d). Field evaluation of African marigold genotypes for bacterial wilt incidence

Genotypes	PDI (%)	Reaction
Maria 91	45.83 (6.82)	S (Susceptible)
Rupa	70.83 (7.92)	HS (Highly susceptible)
Sakura 031	100.00 (10.05)	HS (Highly susceptible)
Royal Orange	25.00 (5.09)	MS (Moderately susceptible)
P-4	25.00 (4.99)	MS (Moderately susceptible)
Arka Agni	29.16 (4.78)	MS (Moderately susceptible)
Arka Bangara 2	12.50 (3.25)	MR(Moderately resistant)
M-1	0.00 (1.00)	R (Resistant)
C.D.(0.01)	3.39	
S.E(m±)	1.10	

Values in the parentheses are transformed data

4.1.3.2. Stages of disease incidence

Wide variations were observed among the genotypes with respect to the stage of bacterial wilt incidence (Table 4.1e). Earliest disease incidence was recorded in the genotypes Maria 91, Rupa and Sakura 031 with a PDI of 8.33, 8.33 and 20.83, respectively. The plants started wilting within 30 DAP. The genotypes Royal Orange and P-4 started wilting within 31-45 DAP whereas, Arka Agni and Arka Bangara 2 started wilting only after 60 DAP. The variety M-1 did not show any wilting symptoms throughout the cropping period (Plate 10). Based on the average number of days taken for wilting to reach the maximum PDI observed in the genotypes, they were classified as "wilting at vegetative stage (Sakura 031) (Plate 10), wilting at early flowering stage (Maria 91) and wilting at peak flowering stage (Rupa, Royal Orange, P-4, Arka Agni and Arka Bangara 2)".

4.2. Screening African marigold genotypes against bacterial wilt through artificial inoculation

Performance of different genotypes against bacterial wilt when artificially inoculated with bacterial suspension is presented in the Tables 4.2(a) and 4. 2(b).

4.2.1. Per cent disease incidence (PDI)

Significant difference was observed with respect to per cent disease incidence among the genotypes, irrespective of the inoculation methods (Table 4.2a). The genotype Sakura 031 recorded 100 per cent bacterial wilt incidence (Plate 11) followed by Arka Agni (93.33 %), P-4 (88.88 %), Arka Bangara 2 (82.22 %) and Maria 91 (71.11 %) which were considered as highly susceptible genotypes. The genotype Royal Orange showed a disease incidence of 44.44 per cent which was considered as susceptible while Rupa with 22.22 PDI was considered as moderately susceptible. The genotype M-1 did not show any wilt incidence and was considered as resistant to bacterial wilt (Plate 11).

Table 4.1(e). Stages of bacterial wilt incidence and days to wilt in African marigold genotypes.

Genotypes	Cumulative PDI at different stages					Remarks
	0-30 DAP	31-45 DAP	46-60 DAP	61-90 DAP	Days to wilt	
Maria 91	8.33	20.83	29.16	45.83	45.33	Early flowering
Rupa	8.33	20.83	37.50	70.83	66.94	Peak flowering
Sakura 031	20.83	58.33	95.83	100.00	43.88	Vegetative
Royal Orange	0.00	4.16	4.16	25.00	68.33	Peak flowering
P-4	0.00	4.16	4.16	25.00	68.50	Peak flowering
Arka Agni	0.00	0.00	0.00	29.16	72.04	Peak flowering
Arka Bangara 2	0.00	0.00	0.00	12.50	67.75	Peak flowering
M-1	0.00	0.00	0.00	0.00	*	No incidence
C.D.(0.01)					13.65	
S.E(m±)					4.38	

Values in the parentheses are transformed data

*Not included for statistical analysis

Highly susceptible genotype (Sakura 031) **Resistant genotype (M-1)**

Plate 10. Bacterial wilt incidence during field evaluation

4.2.2. Days to wilt

When average of all the methods was taken, there was no significant difference observed among the genotypes for the days to wilt to reach maximum disease incidence in each genotype (Table 4.2a). However, the genotype Rupa took maximum number of days to wilt (17.66) followed by Arka Bangara 2 (16.51) and Arka Agni (14.58). Number of days to wilt was the minimum in the genotype Sakura 031 (9.11) followed by Royal Orange (10.24).

4.2.3. Efficacy of inoculation methods

Significant difference was observed with respect to disease incidence among the genotypes but not with respect to the different inoculation methods (Table 4.2b). All the methods showed almost the same efficacy. The root dip method showed 65.83 per cent disease incidence followed by 65.00 per cent incidence in stem injection and 57.55 per cent disease incidence in media drenching. Un-inoculated control in all the genotypes did not show any wilt incidence.

4.3. Screening African marigold genotypes against bacterial wilt through spot planting technique

Genotypes were observed for wilt incidence and the data with respect to PDI and days to wilt observed in spot planting are presented in the Table 4.3.

4.3.1. Per cent disease incidence (PDI)

Hundred per cent wilt incidence was observed for the check genotype Sakura 031 in combination with genotype Maria 91 and M-1. The genotype Maria 91 showed 79.17 per cent disease incidence whereas M-1 was completely wilt free (Plate 12). Sakura 031 spot planted with P-4 and Arka Bangara 2 recorded a wilt incidence of 91.67 per cent. The same PDI was also observed for singly planted Sakura 031. P-4 spot planted with Sakura 031 showed a PDI of 45.83 per cent whereas Arka Bangara 2 planted with Sakura 031 recorded a PDI of 66.67 per cent. The disease incidence in check genotype Sakura 031 ranged from 83.33 to 100 per cent whereas the disease

4.2(a). Bacterial wilt incidence in African marigold genotypes after artificial inoculation

Genotypes	PDI (%)	Days to wilt	Reaction
Maria 91	71.11 (8.61)	11.82	HS (Highly susceptible)
Rupa	22.22 (4.64)	17.76	MS (Moderately susceptible)
Sakura 031	100.00 (10.05)	9.11	HS (Highly susceptible)
Royal Orange	44.44 (6.71)	10.24	S (Susceptible)
P-4	88.88 (9.48)	10.44	HS (Highly susceptible)
Arka Agni	93.33 (9.70)	14.59	HS (Highly susceptible)
Arka Bangara 2	82.22 (9.09)	16.52	HS (Highly susceptible)
M-1	0.00 (1.00)	-	R (Resistant)
C.D.(0.01)	1.72	NS	
S.E(m±)	0.57	2.04	

Values in the parentheses are transformed data

Table 4.2(b). Comparison of inoculation methods for bacterial wilt incidence in African marigold genotypes

Genotypes	Per cent wilt incidence			
	Root dip	Media drenching	Stem injection	Mean A (Genotypes)
Maria 91	88.66 (9.35)	46.66 (6.66)	80.00 (8.95)	**71.11 (8.32)**
Rupa	13.33 (3.38)	26.66 (4.46)	26.66 (4.46)	**22.22 (4.10)**
Sakura 031	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	**100.00 (10.05)**
Royal Orange	53.33 (7.13)	40.00 (6.26)	40.00 (6.26)	**44.44 (6.55)**
P-4	80.00 (8.95)	93.33 (9.70)	93.33 (9.70)	**88.88 (9.45)**
Arka Agni	93.33 (9.7)	93.33 (9.70)	93.33 (9.70)	**93.33 (9.70)**
Arka Bangara 2	100.00 (10.05)	60.00 (7.48)	86.66 (9.35)	**82.22 (8.96)**
M-1	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	**0.00 (1.00)**
Mean B (Methods)	**65.83 (7.45)**	**57.50 (6.91)**	**65.00 (7.43)**	
Factors		**C.D. (0.01)**		
Factor (A)		1.44		
Factor (B)		NS		
Factor (A X B)		NS		

Values in the parentheses are transformed data

Highly susceptible genotype (Sakura 031) **Resistant genotype (M-1)**

Plate 11. Screening against bacterial wilt through artificial inoculation

incidence in genotypes spot planted with Sakura 031 ranged from 0 to 100 per cent (Table 4.3).

4.3.2. Days to wilt

Among the genotype combinations, Arka Agni spot planted with Sakura 031 were the earliest to wilt. Arka Agni took 38.73 days whereas the spot planted Sakura 031 took only 34.84 days to wilt. Sakura 031 planted alone took 39.19 days to wilt. Among the genotypes P-4 took maximum days to wilt (48.81 days) even when spot planted with Sakura 031 and this was followed by Maria 91 (46.72 days) and Arka Bangara 2 (44.67 days). Average number of days to wilt for the check genotype ranged from 34.84 to 50.81 days whereas it ranged from 38.74 to 48.81 days for other susceptible genotypes (Table 4.3).

4.4. Grafting studies

Data on the performance of grafted and non-grafted plants of the genotypes are presented in Table 4.4.1 to 4.4.4.

4.4.1. Field survival of grafts

Highly significant differences were observed among genotypes with respect to per cent survival of grafts in the field (Table 4.4.1). Maximum survival of grafts was observed in P-4 (94.44 %) followed by Arka Agni and Arka Bangara 2 (each with 88.88 %), Maria (83.33 %). These genotypes were on par with respect to the parameter. Minimum field survival of grafts was observed in Sakura 031 (61.11 %) followed by Rupa (66.66 %) and Royal Orange (72.22 %).

4.4.2. Per cent disease incidence (PDI) in grafted and non-grafted plants

Bacterial wilt incidence was not observed in grafted plants of any of the genotypes studied (Plate 13). But wilt incidence ranging from 55.05 to 77.77 per cent was observed in non-grafted plants of all the seven genotypes (Table 4.4.2). However, the genotypes showed no significant difference with respect to per cent disease incidence.

Table 4.3. Bacterial wilt incidence in African marigold genotypes in spot planting

Genotype combination	PDI (%)	Days to wilt
Maria 91 – Sakura 031	79.17 (100.00)	46.73 (43.50)
Rupa - Sakura 031	100.00 (87.50)	43.04 (50.81)
Sakura 031 alone	91.67	39.19
Royal Orange – Sakura 031	75.00 (83.33)	42.45 (44.38)
M-1 – Sakura 031	0.00 (100.00)	- (45.67)
P-4 – Sakura 031	45.83 (91.67)	48.81 (44.17)
Arka Agni- Sakura 031	75.00 (87.50)	38.74 (34.84)
Arka Bangara 2 – Sakura 031	66.67 (91.67)	44.67 (40.67)

Values in parentheses shows PDI and days to wilt for the check genotype

Plate 12. Resistant genotype M-1 with check genotype Sakura 031

Table 4.4.1. Field survival of African marigold grafts

Genotypes	Per cent survival of grafts
Maria 91	83.33 (9.15)
Rupa	66.66 (8.18)
Sakura 031	61.11 (7.86)
Royal Orange	72.22 (8.54)
P-4	94.44 (9.76)
Arka Agni	88.88 (9.44)
Arka Bangara 2	88.88 (9.47)
C.D. (0.01)	1.28
S.E(m±)	0.43

Values in the parentheses are transformed data

Table 4.4.2. Bacterial wilt incidence in non-grafted plants of African marigold genotypes

Genotypes	Per cent disease incidence
Maria 91	61.11 (7.82)
Rupa	72.22 (8.54)
Sakura 031	77.77 (8.86)
Royal Orange	72.22 (8.50)
P-4	55.05 (7.20)
Arka Agni	55.55 (7.39)
Arka Bangara 2	55.55 (7.39)
C.D. (0.01)	NS
S.E(m±)	10.82

Values in the parentheses are transformed data

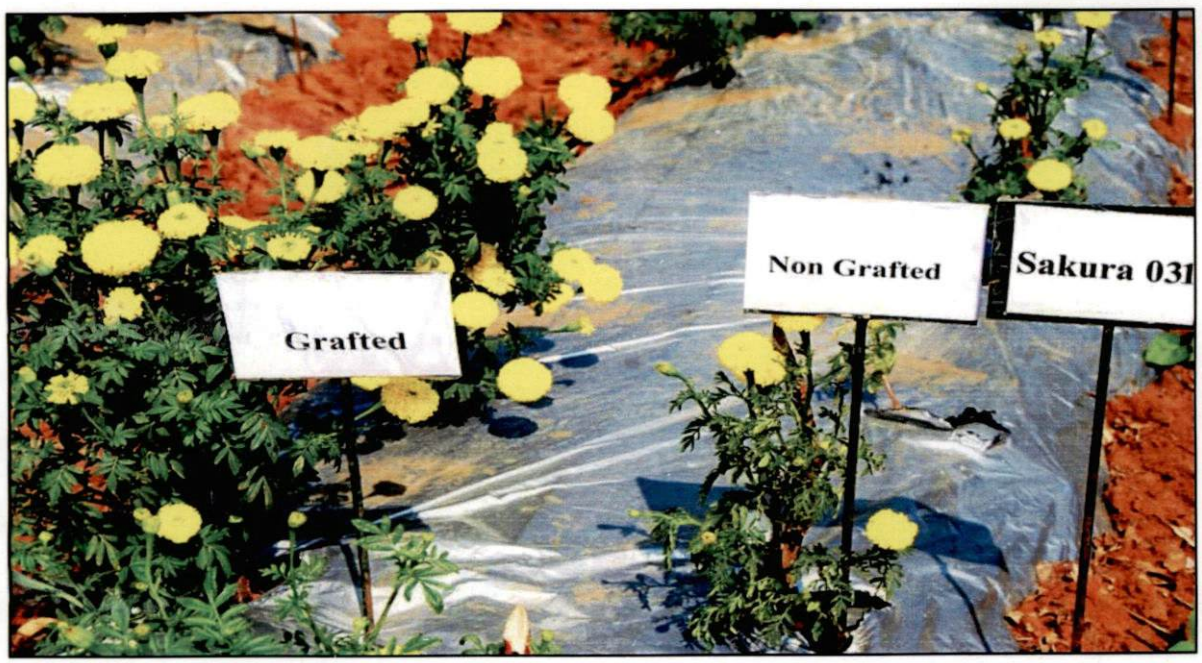


Plate 13. Bacterial wilt incidence during grafting studies

4.4.3. Plant characters

The performance of plant characters, observed in grafted and non-grafted plants of all individual genotypes are presented in Table 4.4.3(a) to 4.4.3(c), respectively.

4.4.3.1 Plant height

At 30 DAP, irrespective whether they are grafted or non-grafted, genotypes showed significant variation in plant height. Height of Royal Orange (47.75 cm) and P-4 (41.56 cm) was significantly higher than all other genotypes. Maria 91 (36.54 cm), Rupa (36.28 cm) and Sakura 031 (34.89 cm) were on par with respect to plant height. Genotypes Arka Bangara 2 and Arka Agni recorded the lowest height of 24.59 cm and 25.91 cm, respectively.

Irrespective of the genotypes, grafted plants were significantly taller than non-grafted plants at 30 DAP. Within a genotype, grafted plants of Royal Orange, P-4, Arka Agni and Arka Bangara 2 were significantly taller than their corresponding non-grafted plants. For all other genotypes there was no difference in plant height between grafted and non-grafted plants (Table 4.4.3a). Within the grafted plants of different genotypes, Royal Orange recorded significantly higher plant height which was also on par with P-4 while in the non-grafted plants, plant height was similar in genotypes *viz*., Royal Orange, Rupa, P-4 and Maria 91.

At 60 DAP irrespective of whether grafted or non-grafted, genotypes showed significant variation for plant height. Royal Orange produced tallest plants followed by P-4, Maria 91 and Rupa. At 60 DAP also, grafted plants were significantly taller than non-grafted plants irrespective of genotypes. No interactions were observed either among genotypes or between grafted and non-grafted plants at 60 DAP.

4.4.3.2. Plant spread

Both at 30 and 60 DAP there were no significant differences observed in plant spread either among genotypes or between grafted and non-grafted plants within genotypes (Table 4.4.3b).

Table 4.4.3(a). Effect of grafting on plant height in African marigold genotypes

Genotypes	Plant height (cm)					
	30 DAP			60 DAP		
	G	NG	**Mean A (Genotypes)**	G	NG	**Mean A (Genotypes)**
Maria 91	35.72	37.37	**36.54**	43.21	45.93	**44.57**
Rupa	33.41	39.15	**36.28**	39.99	44.44	**42.21**
Sakura 031	36.44	33.35	**34.89**	40.52	36.77	**38.65**
Royal Orange	52.81	42.68	**47.75**	66.05	56.57	**61.31**
P-4	44.22	38.90	**41.56**	50.52	46.55	**48.53**
Arka Agni	30.41	21.41	**25.91**	44.58	33.44	**39.01**
Arka Bangara 2	28.19	20.98	**24.59**	45.13	37.94	**41.53**
Mean B (Grafted and Non-grafted)	**37.31**	**33.41**		**47.14**	**43.09**	
Factors	**C.D.(0.01)**			**C.D.(0.01)**		
Factor (A)	7.96			10.06		
Factor (B)	2.46			3.82		
Factor (B) at same level of A	6.60			NS		
Factor (A) at same level of B	9.19			NS		

G - Grafted; NG - Non-grafted

Table 4.4.3(b). Effect of grafting on plant spread in African marigold genotypes

Genotypes	Plant spread (cm)					
	30 DAP			60 DAP		
	G	NG	**Mean A (Genotypes)**	G	NG	**Mean A (Genotypes)**
Maria 91	32.13	37.52	**34.82**	46.19	52.16	**49.18**
Rupa	33.50	42.83	**38.16**	40.02	55.00	**47.51**
Sakura 031	32.02	33.88	**32.95**	41.30	41.75	**41.52**
Royal Orange	43.75	37.40	**40.57**	60.58	51.95	**56.26**
P-4	39.80	38.47	**39.13**	46.08	50.47	**48.27**
Arka Agni	31.15	25.83	**28.49**	53.16	47.61	**50.38**
Arka Bangara 2	29.17	28.28	**28.73**	49.40	56.38	**52.89**
Mean B (Grafted and Non-grafted)	**34.50**	**34.89**		**48.10**	**50.76**	
Factors	**C.D.(0.01)**			**C.D.(0.01)**		
Factor (A)	NS			NS		
Factor (B)	NS			NS		
Factor (B) at same level of A	NS			NS		
Factor (A) at same level of B	NS			NS		

G - Grafted; NG - Non-grafted

4.4.3.3. Primary branches

Significantly high number of primary branches were observed in genotype Royal Orange both at 30 and 60 DAP (Table 4.4.3c). Irrespective of whether grafted or non-grafted, at 30 DAP number of primary branches recorded in Royal Orange (11.79) was on par with P-4 (10.58) whereas at 60 DAP the genotype Royal Orange produced significantly higher number of primary branches (16.30) than all other genotypes. Both at 30 and 60 DAP there was no difference observed in either grafted or non-grafted plants of each genotype and also among the genotypes in grafted or non-grafted condition.

4.4.3.4. Leaf area

Irrespective of the genotypes, leaf area was significantly more in grafted plants (4.22 cm^2) than the non-grafted plants (3.37 cm^2). However, there was no difference in leaf area among the genotypes (Table 4.4.3c).

4.4.3.6. Petiole colour

There was no difference observed in both grafted and non-grafted plants of all genotypes for petiole colour. All the genotypes were showing green coloured petiole.

4.4.4. Floral characters

Floral characters observed in the grafted and non-grafted plants of the genotypes are presented in Table 4.4.4(a) to 4.4.4(d), respectively.

4.4.4.1. Days to bud initiation/formation

There was no significant difference observed for days to bud initiation among the genotypes irrespective of whether they are grafted or non-grafted. However, irrespective of genotypes, grafted plants were significantly earlier to initiate flower buds and on an average they took 28.43 days to initiate flower buds when compared to 29.36 days in non-grafted plants (Table 4.4.4a).

Table 4.4.3(c). Effect of grafting on number of primary branches and leaf area in African marigold genotypes

Genotypes	Number of primary branches						Leaf area (cm^2)		
	30 DAP			60 DAP					
	G	NG	Mean A (Genotypes)	G	NG	Mean A (Genotypes)	G	NG	Mean A (Genotypes)
Maria 91	9.47	9.66	**10.20**	12.21	12.35	**12.28**	5.06	2.49	**3.77**
Rupa	8.41	9.64	**9.41**	10.30	13.92	**12.11**	3.39	3.42	**3.40**
Sakura 031	9.16	8.68	**9.02**	11.25	12.91	**12.08**	4.55	3.64	**4.10**
Royal Orange	14.36	10.8	**11.79**	17.12	15.40	**16.30**	3.88	3.71	**3.80**
P-4	11.47	9.97	**10.58**	14.23	14.36	**14.29**	3.91	4.17	**4.04**
Arka Agni	7.95	7.30	**7.60**	13.38	13.05	**13.22**	4.35	3.27	**3.81**
Arka Bangara 2	7.52	8.13	**7.75**	11.14	13.94	**12.54**	4.36	2.89	**3.62**
Mean B (Grafted and Non-grafted)	**9.79**	**9.17**		**12.82**	**13.70**		**4.22**	**3.37**	
Factors	**C.D.(0.01)**			**C.D.(0.01)**			**C.D.(0.01)**		
Factor (A)	1.41			1.91			NS		
Factor (B)	NS			NS			0.74		
Factor (B) at same level of A	NS			NS			NS		
Factor (A) at same level of B	NS			NS			NS		

G - Grafted; NG - Non-grafted

4.4.4.2. Days to flower opening

Significant difference was observed with respect to days taken for flower opening between grafted and non-grafted plants irrespective of genotypes but not among the genotypes (Table 4.4.4a). Grafted plants took more days (10.37) compared to non-grafted plants (9.19) for flower opening. Within a genotype, significant difference was observed in days to flower opening between grafted and non-grafted plants in Maria 91, Royal Orange and Arka Bangara 2. For all other genotypes grafted and non-grafted plants took similar number of days to flower opening. Within the grafted plants of different genotypes Arka Bangara 2 and Royal orange took significantly more number of days to flower opening when compared to other genotypes. However, in the case of non-grafted plant of different genotypes, all the genotypes except Maria 91 were on par with respect to this parameter.

4.4.4.3. Flower diameter

There was no significance differences observed with respect to flower diameter either among genotypes or grafted and non-grafted plants within each genotype (Table 4.4.4b).

4.4.4.4. Stalk length

Irrespective of whether grafted or non-grafted, significant difference was observed among genotypes with respect to stalk length (Table 4.4.4b). Arka Bangara 2 showed maximum stalk length of 8.8 cm which was on par with Arka Agni with a stalk length of 7.56 cm. Stalk length was significantly less in Rupa (5.49 cm) and Sakura 031 (5.68 cm). Differences were not observed between grafted and non-grafted plants within each genotype.

4.4.4.5. Flower weight

There was no significant difference observed with respect to flower weight for both grafted and non-grafted plants irrespective of genotypes as well as among the genotypes irrespective of grafted or non-grafted condition (Table 4.4.4c). However, maximum flower weight was observed in Arka Bangara 2 for both grafted (7.06 g) and

Table 4.4.4(a). Effect of grafting on days to bud initiation/formation and flower opening in African marigold genotypes

Genotypes	Days to bud initiation/formation			Days to flower opening		
	G	NG	Mean A (Genotypes)	G	NG	Mean A (Genotypes)
Maria 91	28.31	30.05	**29.18**	10.39	9.35	**9.87**
Rupa	29.33	29.19	**29.26**	9.66	9.91	**9.79**
Sakura 031	28.08	29.05	**28.56**	10.08	10.37	**10.23**
Royal Orange	28.47	29.46	**28.96**	10.83	10.00	**10.41**
P-4	29.02	29.22	**29.12**	10.04	10.06	**10.05**
Arka Agni	27.25	29.33	**28.29**	10.72	9.91	**10.32**
Arka Bangara 2	28.54	29.24	**28.89**	10.87	9.80	**10.34**
Mean B (Grafted and non-grafted)	**28.43**	**29.36**		**10.37**	**9.91**	
Factors	**C.D.(0.01)**			**C.D.(0.01)**		
Factor (A)	NS			NS		
Factor (B)	0.38			0.25		
Factor (B) at same level of A	NS			0.66		
Factor (A) at same level of B	NS			0.70		

G - Grafted; NG - Non-grafted

Table 4.4.4(b). Effect of grafting on flower diameter and stalk length in African marigold genotypes

Genotypes	Flower diameter (cm)			Stalk length (cm)		
	G	NG	**Mean A (Genotypes)**	G	NG	**Mean A (Genotypes)**
Maria 91	5.18	5.12	**5.15**	6.69	6.95	**6.82**
Rupa	4.54	5.25	**4.89**	4.96	6.01	**5.49**
Sakura 031	5.38	5.10	**5.24**	6.08	5.29	**5.68**
Royal Orange	5.03	5.28	**5.15**	6.82	6.80	**6.81**
P-4	5.17	4.99	**5.08**	6.28	6.21	**6.25**
Arka Agni	5.52	5.46	**5.49**	7.90	7.23	**7.56**
Arka Bangara 2	5.94	5.74	**5.84**	8.63	8.98	**8.80**
Mean B	**5.25**	**5.28**		**6.77**	**6.78**	
Factors (Grafted and non-grafted)	**C.D. (0.01)**			**C.D.(0.01)**		
Factor(A)	NS			1.56		
Factor(B)	NS			NS		
Factor(B) at same level of A	NS			NS		
Factor(A) at same level of B	NS			NS		

G - Grafted; NG - Non-grafted

non-grafted (7.26 g) followed by Arka Agni for both grafted (6.66 g) and non-grafted (6.06 g) plants. Minimum flower weight was observed in Rupa (4.18 g) for grafted plants and Royal Orange (4.73 g) for non-grafted plants.

4.4.4.6. Petal yield per flower

Petal yield per flower also did not differ either among genotypes or grafted and non-grafted plants within each genotype (Table 4.4.4c). However, maximum petal yield was observed in Arka Bangara 2 for both grafted (4.66 g) and non-grafted (4.93 g) plants followed by Arka Agni for both grafted (4.43 g) and non-grafted (3.86 g) plants. Minimum petal yield was observed in Rupa for grafted plants (2.26 g) and Royal Orange for non-grafted plants (2.73 g).

4.4.4.7. Number of flowers per plant

Irrespective of the genotypes, significant difference was observed for number of flowers per plant between grafted and non-grafted plants. Average number of flowers produced in grafted plants was 57.84 whereas non-grafted plants produced 33.31 flowers per plant (Table 4.4.4d). However, there was no significant difference observed for number of flowers per plant among genotypes. Maximum number of flowers per plant for grafted plant was observed in Arka Agni (108.11) followed by Royal Orange (62.22) whereas minimum in Arka Bangara 2 (34.07). Maximum number of flowers per plant for non-grafts was observed in Rupa (49.88) followed by Arka Agni (46.10) while minimum in Sakura 031 (21.25) which was on par with Arka Bangara 2 (21.28).

4.4.4.8. Yield per plant

Yield per plant was significantly different between grafted and non-grafted plants irrespective of genotypes tested (Table 4.4.4d). Grafted plants showed significantly higher yield (0.155 kg) compared to non-grafted plants (0.111 kg). However, there was no difference with respect to this parameter among genotypes. Though interactive effects were not observed, grafted plants of all genotypes except Maria 91 and Rupa recorded more yield per plant when compared to their corresponding non-grafted plants.

Table 4.4.4(c). Effect of grafting on flower weight and petal yield per flower in African marigold genotypes

Genotypes	Flower weight (g)			Petal yield/flower (g)		
	G	NG	**Mean A (Genotypes)**	G	NG	**Mean A (Genotypes)**
Maria 91	4.78	5.27	**5.02**	2.80	3.27	**3.03**
Rupa	4.18	5.20	**4.69**	2.26	3.20	**2.73**
Sakura 031	5.06	5.80	**5.43**	3.06	3.73	**3.40**
Royal Orange	5.33	4.73	**5.03**	3.13	2.73	**2.93**
P-4	5.46	5.63	**5.55**	3.53	3.73	**3.63**
Arka Agni	6.66	6.06	**6.36**	4.43	3.86	**4.20**
Arka Bangara 2	7.06	7.26	**7.16**	4.66	4.93	**4.80**
Mean B (Grafted and non-grafted)	**5.51**	**5.71**		**3.42**	**3.63**	
Factors	**C.D.(0.01)**			**C.D. (0.01)**		
Factor(A)	NS			NS		
Factor(B)	NS			NS		
Factor(B)at same level of A	NS			NS		
Factor(A)at same level of B	NS			NS		

G - Grafted; NG - Non-grafted

4.4.2.9. Number of harvests

Significant differences were observed for number of harvests among genotypes irrespective of grafted and non-grafted condition and also between grafted and non-grafted plants irrespective of genotypes (Table 4.4.4d). Arka Agni and Arka Bangara 2 recorded significantly higher number of harvests of 10.66 and 9.55 respectively. Average number of harvests for grafted plants (8.81) was also significantly higher than non-grafted plants (6.90). However, no interactive effects were observed either between grafted and non-grafted plants within a genotype or among genotypes within grafted plants or non- grafted plants.

4.5. Post-harvest studies

Results of the studies on post-harvest life of the genotypes are presented in Table 4.5.

4.5.1 Days to wilting of flowers

Significance differences were observed among the genotypes for days to wilting of flowers after harvest. The flowers of the genotypes Sakura 031 and Rupa took maximum days (5.00) followed by P-4 (4.50) to show 25 per cent wilting of flowers. These genotypes were showing significantly better post-harvest life when compared to Maria 91 which took the minimum number of days (3.00) to show 25 per cent wilting of flowers.

4.5.2. Loss in weight of flowers

Flowers of all the genotypes were showing significant difference with respect to physiological loss in weight (PLW) and cumulative physiological loss in weight (CPLW). At day-4 maximum PLW was shown by Maria 91 (43.14 %) followed by M-1 (33.73 %) while lowest PLW shown by Arka Bangara 2 (12.34%). At day-4 maximum CPLW of flowers was shown by M1 (70.99 %) followed by Maria 91 (64.43 %) and Rupa (54.10 %) while minimum CPLW was shown by Royal Orange (43.88 %) which was on par with the Arka Bangara 2 (43.91 %). There is a sudden loss in weight of flowers at day-4 for those genotypes that were showing maximum CPLW.

Table 4.4.4(d). Effect of grafting on number of flowers, yield and number of harvests in African marigold genotypes

Genotypes	Number of flowers per plant			Yield per plant (kg)			Number of harvests		
	G	NG	Mean A (Genotypes)	G	NG	Mean A (Genotypes)	G	NG	Mean A (Genotypes)
Maria 91	45.65	37.58	**41.61**	0.112	0.133	**0.122**	7.66	6.33	**7.00**
Rupa	47.80	49.88	**48.84**	0.104	0.136	**0.120**	8.00	6.00	**7.00**
Sakura 031	54.50	21.25	**37.87**	0.165	0.078	**0.121**	8.33	5.66	**7.00**
Royal Orange	62.22	24.23	**43.23**	0.171	0.073	**0.122**	7.33	5.66	**6.50**
P-4	52.58	32.87	**42.72**	0.131	0.115	**0.123**	7.66	7.00	**7.33**
Arka Agni	108.11	46.10	**77.10**	0.274	0.143	**0.209**	12.00	9.33	**10.66**
Arka Bangara 2	34.07	21.28	**27.68**	0.126	0.093	**0.109**	10.66	8.33	**9.55**
Mean B (Grafted and non-grafted)	**57.84**	**33.31**		**0.155**	**0.111**		**8.81**	**6.90**	
Factors	**C.D.(0.01)**			**C.D.(0.01)**			**C.D. (0.01)**		
Factor (A)	NS			NS			1.385		
Factor (B)	13.67			0.039			0.887		
Factor (B) at same level of A	NS			NS			NS		
Factor (A) at same level of B	NS			NS			NS		

G - Grafted; NG - Non-grafted

Table 4.5. Post-harvest life of African marigold genotypes

Genotypes	Shelf life (days)	Per cent loss in weight of flowers							
		Physiological loss in weight (%)				Cumulative physiological loss in weight (%)			
		D-1	D-2	D-3	D-4	D-1	D-2	D-3	D-4
Maria 91	3.00	14.31	15.09	14.06	43.14	14.31	27.27	37.50	64.43
Rupa	5.00	19.50	15.47	16.13	19.52	19.50	31.96	42.95	54.10
Sakura 031	5.00	11.90	10.51	16.02	19.09	11.90	21.03	33.73	46.42
Royal Orange	4.00	16.44	10.47	13.69	14.00	16.44	25.11	35.21	43.88
P-4	4.50	14.71	13.39	12.20	22.39	14.71	26.13	35.14	49.59
Arka Agni	4.00	20.80	14.95	17.85	16.76	20.80	32.50	44.46	53.30
Arka Bangara 2	4.00	16.02	14.24	11.12	12.34	16.02	27.99	36.04	43.91
M-1	4.00	17.73	25.67	29.00	33.73	17.73	38.73	56.57	70.99
C.D. (0.01)	1.11	3.86	6.91	3.02	8.06	3.86	8.21	7.39	9.94
S.E(m±)	0.36	1.27	2.28	0.99	2.66	1.27	2.71	2.44	3.29

'D' - number of day

Discussion

5. DISCUSSION

African marigold (*Tagetes erecta* L.) is an important annual flower crop which has gained popularity among the growers due to its easy cultivation and wide adaptability. It is used for making garlands, wreaths and religious offerings and is also ideal for garden display both in beds and pots. In Kerala the demand for this crop has increased recently and marigold cultivation has gained popularity in the state. But there are many problems arising due to lack of scientific studies regarding suitable genotypes for cultivation which are having good yield with pest and disease resistance. The most serious and unmanageable disease noticed in marigold in Kerala is bacterial wilt caused by *Ralstonia solanacearum*. Identification of genotypes having desirable plant and floral characters, with good yield along with resistance to bacterial wilt is very essential for successful cultivation of this crop in the state.

Yield in any crop is based on its genetic potential which in turn is influenced by many abiotic and biotic stresses. Causes of major abiotic stresses are environmental and non-biological factors whereas biotic stresses are caused by living organisms. Hence, evaluation of genotypes/cultivars under uniform environment providing similar crop management practices will highlight the best genotype with respect to yield and resistance to biotic stresses. Among the biotic stresses, bacterial wilt is most difficult to control. This devastating disease could be controlled through grafting the susceptible genotypes on wilt resistant rootstocks. This technique is utilized in many Western and Asian countries. Yet, in India possibility of this technique has not been commercially exploited so far. Field trials conducted in grafted solanaceous vegetables have given encouraging results at ARS, Mannuthy. There was 100 per cent control of bacterial wilt and yields were better in grafted plants (Narayanankutty *et al.*, 2015).

The present investigation was undertaken with the objective to evaluate African marigold hybrids/varieties for high yield, with resistance to bacterial wilt and to assess the feasibility of grafting technique as a tool to combat bacterial wilt. The results of the study are briefly discussed hereunder:

5.1. Field evaluation

5.1.1. Plant characters

The genotypes showed significant variations in all plant characters *viz*., height, spread, stem girth, number of primary branches and leaf area. Plant height was maximum for Royal Orange and P-4 whereas it was minimum for M-1 (Fig 5.1). Plant height is attributed to be an important varietal character that depends upon the genetic constitution. Variations among marigold genotypes with respect to plant height have been reported by Khanvilkar *et al*., 2003; Deepa *et al*., 2016; Singh and Mishra, 2008; Bharathi and Jawaharlal, 2014a; Deepa and Patil 2016; Manik and Sharma, 2016.

Wide variations were also observed among the genotypes for plant spread (Fig 5.2). The maximum plant spread was observed in genotype P-4, whereas the minimum plant spread was observed in Sakura 031. The variation in plant spread is attributed to the inherent character of the genotype which is reported by Manik and Sharma (2016). It was also noticed that genotypes with more height recorded greater plant spread and this might be due to direct relationship between these two characters. Raghuvanshi and Sharma (2011) reported a positive relationship between plant height and plant spread.

Significant difference was not observed among the genotypes for stem girth. However, variations in stem girth for African marigold genotypes have been reported by Bharathi and Jawaharlal (2014a) and Choudhari *et al*. (2014). In both these studies more number (28) of genotypes were included for evaluation and some of them were showing significant variation with respect to stem girth. Perhaps the number of genotypes included in the present study was less to show significant variation or the genotypes studied had the same genetic makeup with respect to stem girth.

Another important plant character *viz*., number of primary branches differed significantly among the genotypes (Fig 5.3). Maximum number of primary branches were observed in the genotype Rupa and minimum in genotype Arka Bangara 2. Variations in number of primary branches might be due to genetic makeup and such

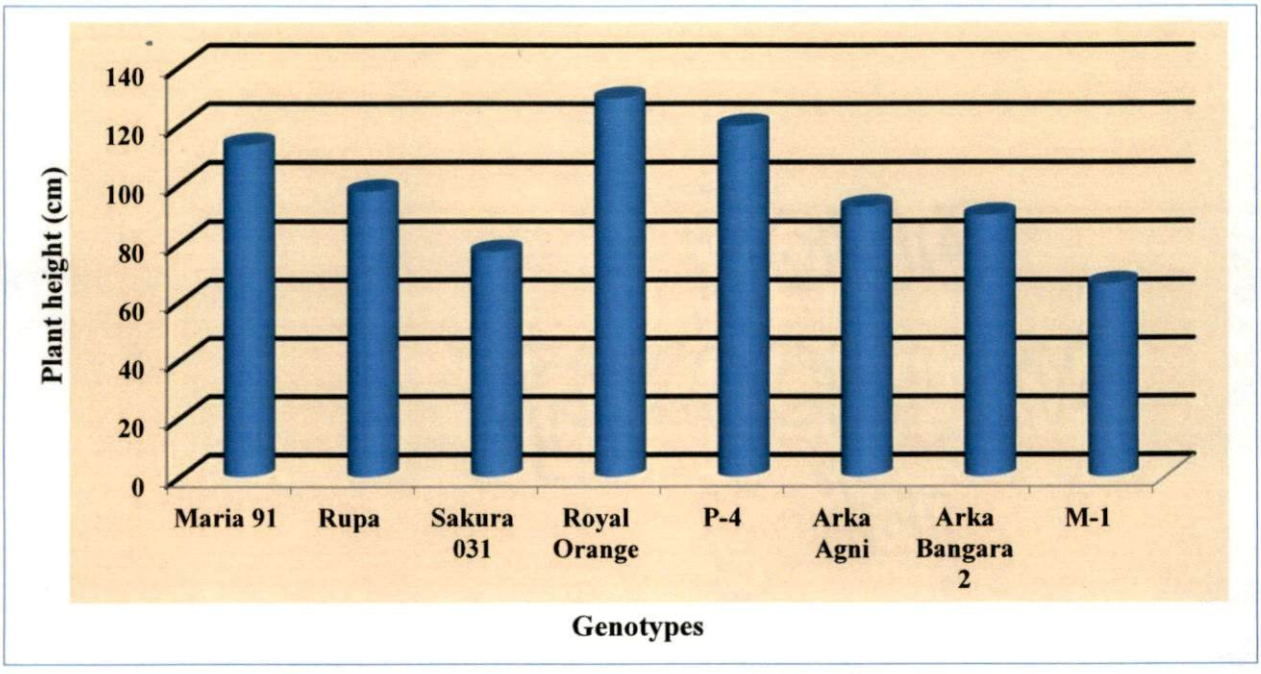


Fig 5.1 Plant height at 60 DAP in African marigold genotypes

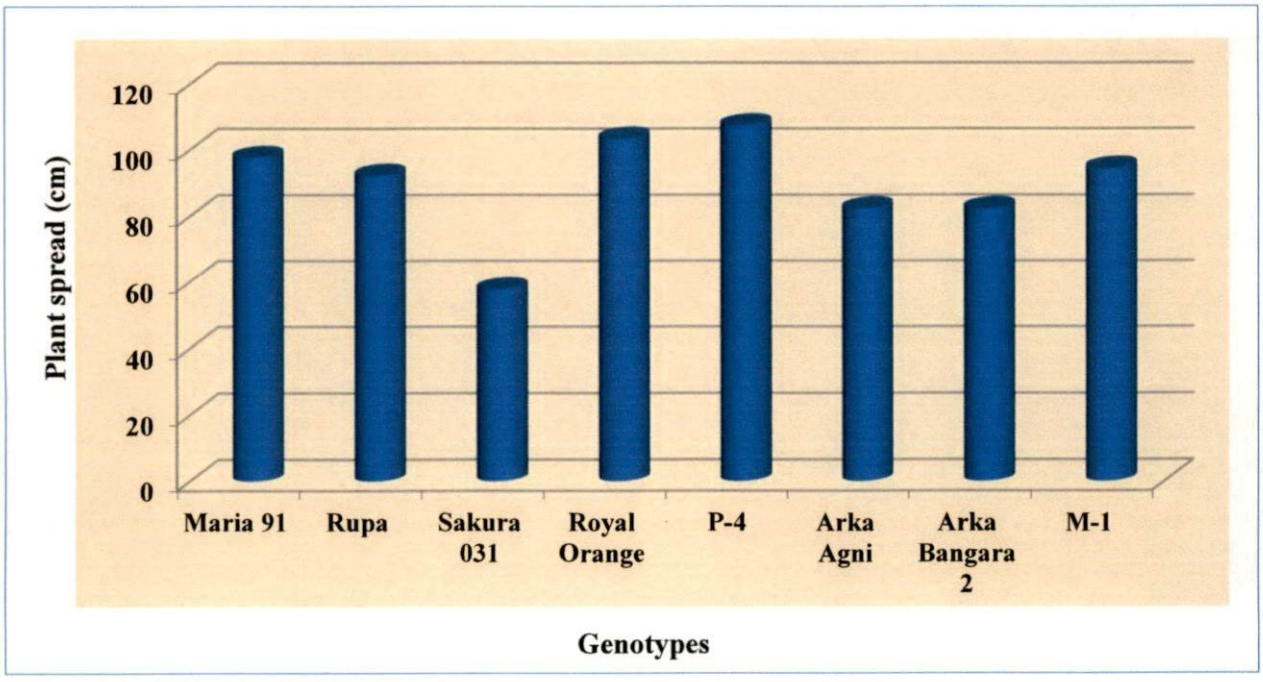


Fig 5.2 Plant spread at 60 DAP in African marigold genotypes

variations in marigold are already reported by Bharathi and Jawaharlal, 2014a; Deepa and Patil, 2016; Manik and Sharma, 2016.

Significant variations among genotypes were also observed in leaf area (Fig 5.4). The genotype M-1 recorded maximum leaf area followed by Maria 91. Minimum leaf area was recorded in Arka Bangara 2. The variation in leaf area among the various genotypes might be attributed to genetic makeup which has been reported by Raghuvanshi and Sharma, 2011; Rao *et al.*, 2005 in marigold.

Varietal evaluation in terms of growth characters have also been observed in African marigold by earlier workers who reported significant variation among cultivars of marigold under different agro-climatic conditions. The maximum plant height, spread, number of primary branches, plant and leaf area might be due to the congenial environment to express the dominant genes in the cultivars.

5.1.2. Floral characters

Results of the evaluation studies on floral characters in terms of days to bud initiation, days to flower opening, flower diameter, stalk length, flower weight, petal yield per flower, number of flowers per plant, flower yield per plant, number of harvests, flower colour and total carotenoids content are discussed here.

Minimum days taken to bud initiation is an indication of earliness of genotype. Minimum days to bud initiation were observed in the genotype Rupa which was on par with all the genotypes except for M-1, which took maximum number of days to initiate the flower buds (Fig 5.5). The marked variation among genotypes for various flowering attributes might be due to their genetic makeup. This could also be due to more dry matter accumulation because of absorption of more nitrogen and other nutrients in addition to prevailing environmental conditions (Rao and Reddy, 2002). These results were in agreement with those of Bharathi and Jawaharlal (2014a) who observed similar trends while evaluating different cultivars of African marigold. Similar findings were also reported by Singh and Singh (2010); Yuvraj and Dhatt (2014) in marigold.

There was no significant difference observed among the genotypes for days to flower bud opening, though many scientists (Bharathi and Jawaharlal, 2014b; Rao and

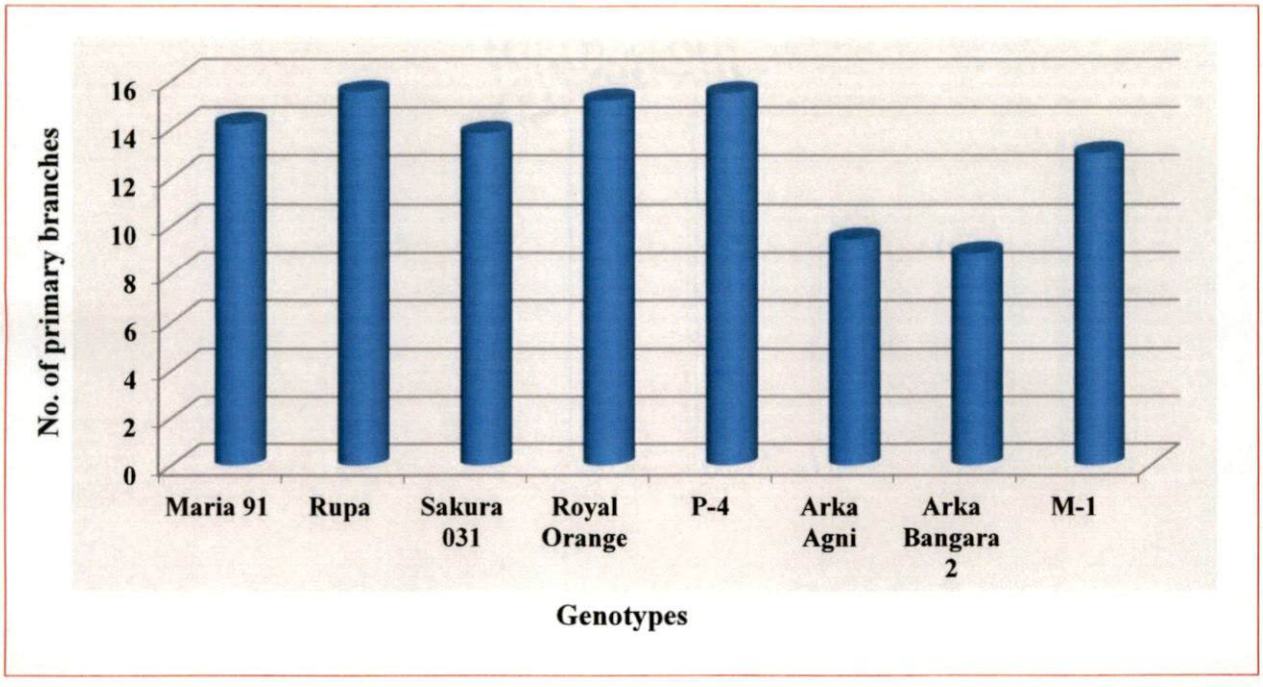


Fig 5.3 No. of primary branches at 60 DAP in African marigold genotypes

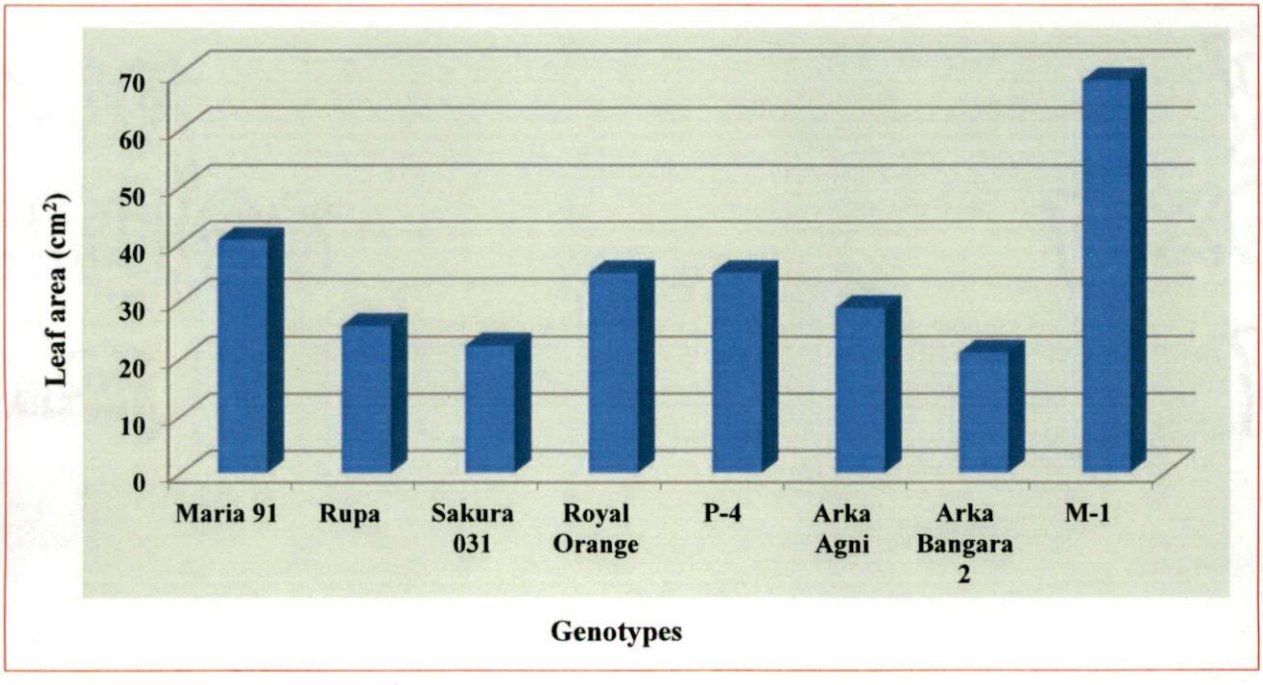


Fig 5.4 Leaf area in African marigold genotypes

Reddy, 2002) have reported marked variation among genotypes for this character. This might also be due to the more number of genotypes included for these studies which resulted in marked variation among the genotypes.

The genotypes differed significantly with respect to flower diameter, which ranged from 6.45 to 9.08 (Fig 5.6). The genotypes P-4 and Rupa recorded the maximum flower diameter and the minimum flower diameter was recorded the genotype M-1. The variation in flower diameter among the genotypes might be due to their genetic makeup that would have influenced higher nutrient uptake (Anuradha *et al.*, 1990). The flower diameter was controlled by genotype-environmental interaction effects. These results were in agreement with Manik and Sharma, 2016; Choudhary *et al.*, 2014; Deepa *et al.*, 2016; Deepa and Patil, 2016.

Stalk length of genotypes differed significantly among themselves. The maximum stalk length was recorded in Arka Bangara 2 which was closely followed by genotype P-4 (Fig 5.7). The stalk length was minimum in the genotype 'Sakura 031' followed by the genotype M-1 and these genotypes were on par with respect to the parameter. Variations in stalk length among African marigold genotypes were reported by Karuppaiah and Kumar (2011).

Significant differences were also observed with respect to weight of individual flower. Maximum fresh flower weight was recorded in the genotype Rupa followed by P-4. Minimum flower weight was observed in M-1 which was found to be on par with Arka Agni (Fig 5.8). Flower weight appeared to be associated with diameter of flower as evident from the results. Therefore, it could be concluded that the variation in fresh flower weight among the genotypes might be due to their genetic makeup. These results were also in accordance with the findings of Rao and Moon, 2005; Narsude *et al.*, 2010a; Deepa *et al.*, 2016.

Petal yield is an important character when industries are concerned. The study also revealed significant differences with respect to petal yield per flower among the genotypes (Fig 5.9). Maximum petal yield per flower was observed in Rupa followed by P-4. The genotype M-1 showed minimum petal yield followed by Maria 91. Genotypes with maximum flower diameter and flower weight yielded high petal

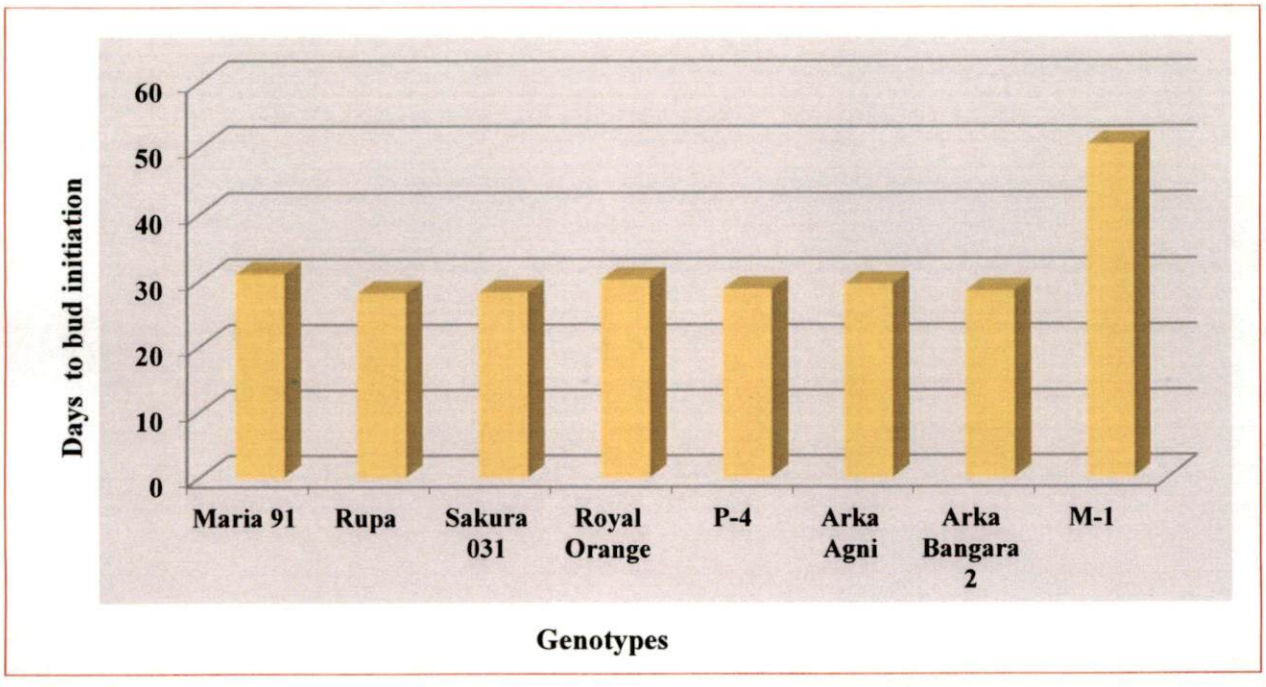


Fig 5.5 Days to bud initiation/formation in African marigold genotypes

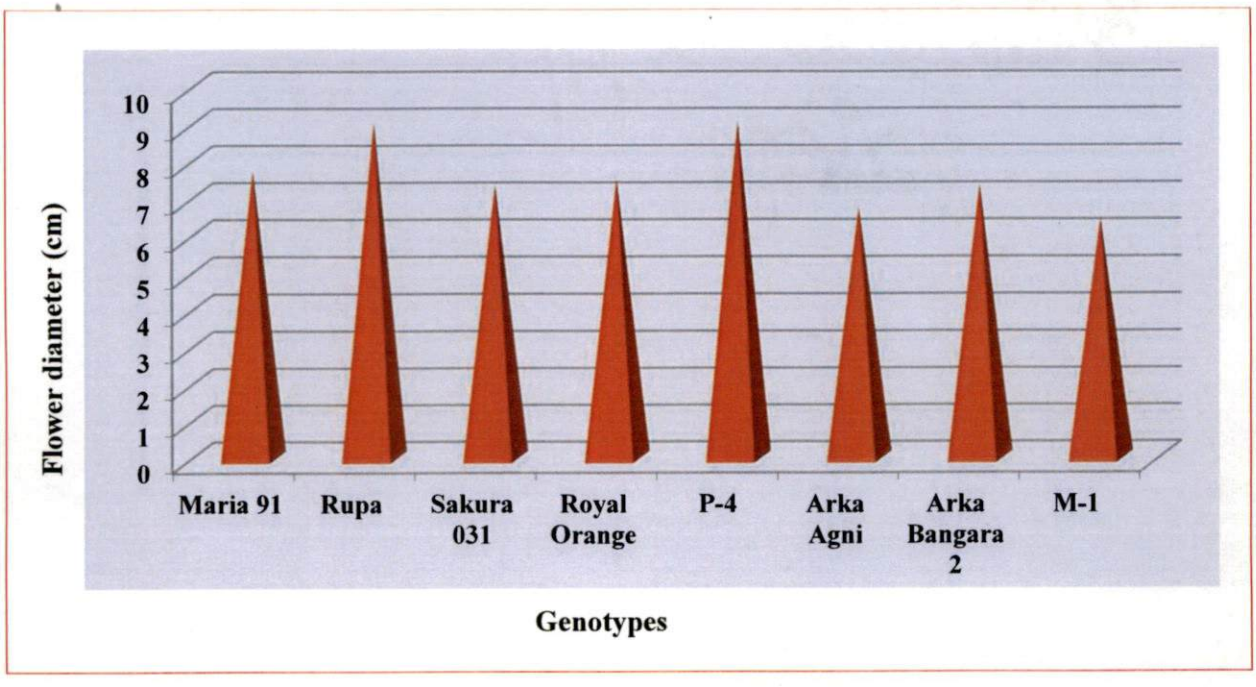


Fig 5.6 Flower diameter in African marigold genotypes

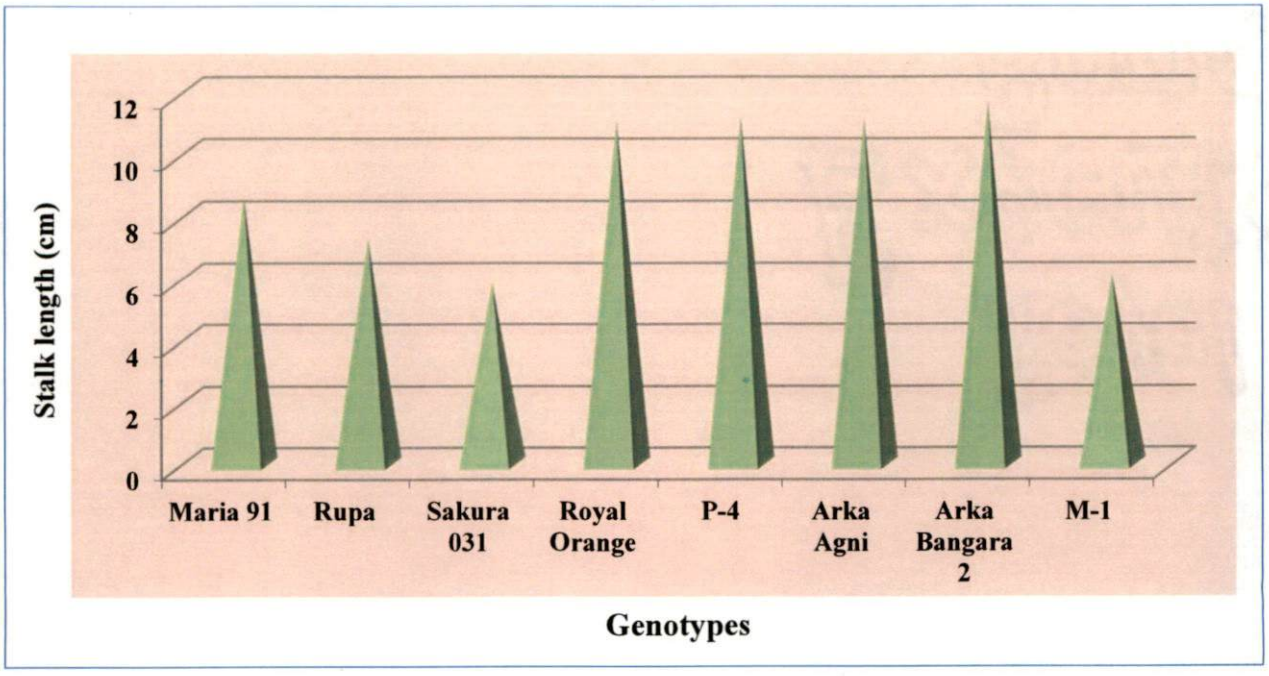


Fig 5.7 Stalk length in African marigold genotypes

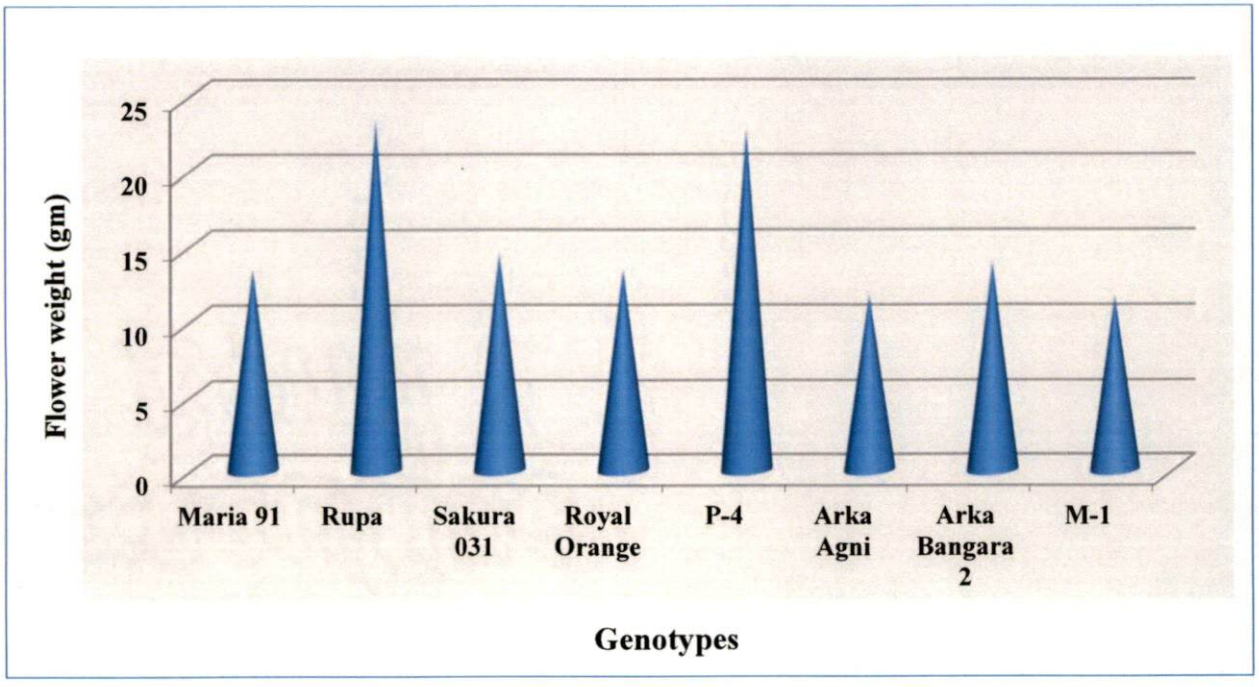


Fig 5.8 Flower weight in African marigold genotypes

weight. The findings of the study are in accordance with those of Manik and Sharma, 2016 and Deepa *et al.*, 2016.

Flower production was maximum in the genotype P-4 which was followed by Rupa. The genotype Sakura 031 recorded the lowest number of flowers per plant followed by the genotype Maria 91 (Fig 5.10). The variation in number of flower per plant might be due to hereditary traits of the genotype. Difference in the photosynthetic efficiency of genotypes might have resulted in enhanced food accumulation in certain genotypes followed by better plant growth and subsequently higher number of flowers per plant (Sunitha *et al.*, 2007). The findings of the study are also in accordance with those of Manik and Sharma (2016), Karuppaiah and Kumar (2011), Naik *et al.* (2005) and Yuvraj and Dhatt (2014).

The maximum flower yield was recorded in P-4 which was very high compared to other genotypes (Fig 5.11) and this was followed by Rupa. The minimum flower yield was recorded in Maria 91 which was followed by M-1. The variation in yield might be due to the inherent capacity of the genotypes. Different photosynthetic efficacy of genotypes might have enhanced food accumulation resulting in better plant growth and subsequently higher number of flowers per plant (Sunitha *et al.*, 2007). This increase in the flower yield could also be due to more number of large sized flowers having higher flower weight. Higher yield might also be contributed by either low incidence of bacterial wilt or extended period of field survival in such genotypes. These results were also in accordance with the findings of Rao and Moon, 2005; Manik and Sharma, 2016; Yuvraj and Dhatt, 2014.

Maximum number of harvests was recorded in the genotype Arka Bangara 2 followed by the genotypes P-4 and Arka Agni (Fig 5.12). Minimum number of harvests were recorded in the genotype Maria 91. This variation among the genotypes could be due to interaction between environment and genetic makeup. These results were also in accordance with the findings of Bharathi and Jawaharlal, 2014 (a) and (b).

The colour of the flowers varied from strong orangish yellow to light greenish yellow colour showing great variations within the genotypes. Variation in the intensity of the flower colour might be attributed to its pigment content (Deineka *et al.*, 2007).

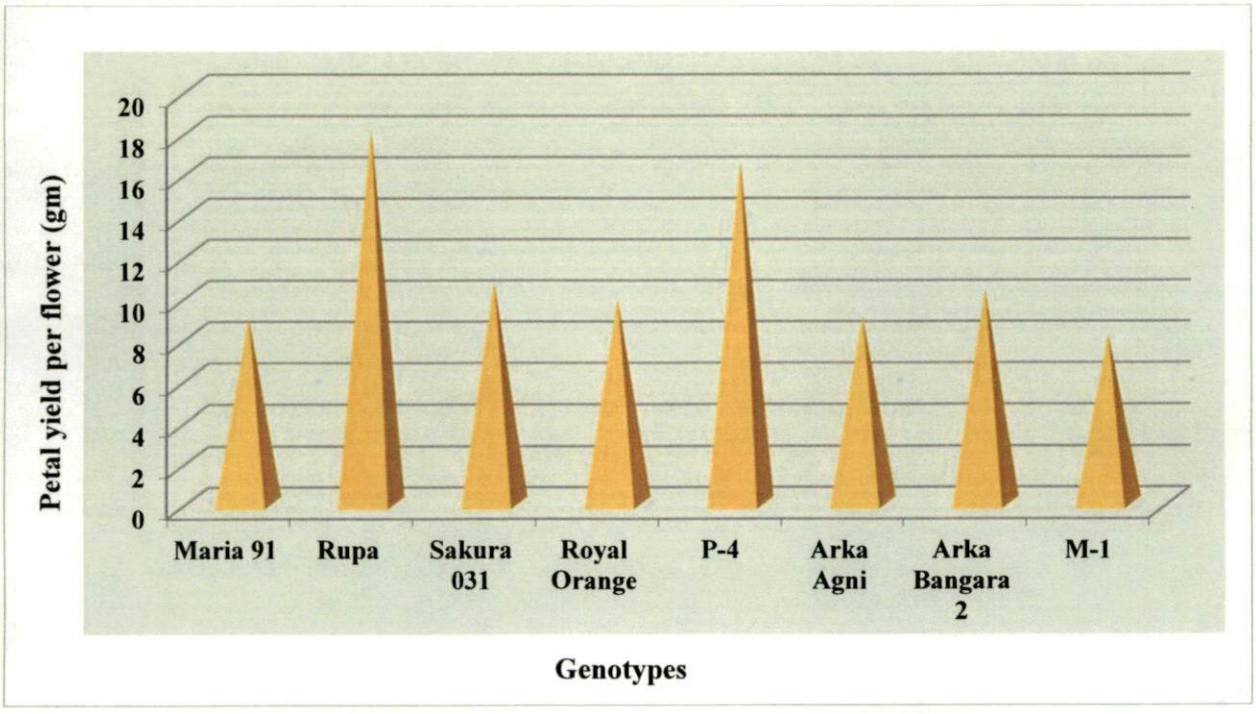


Fig 5.9 Petal yield per flower in African marigold genotypes

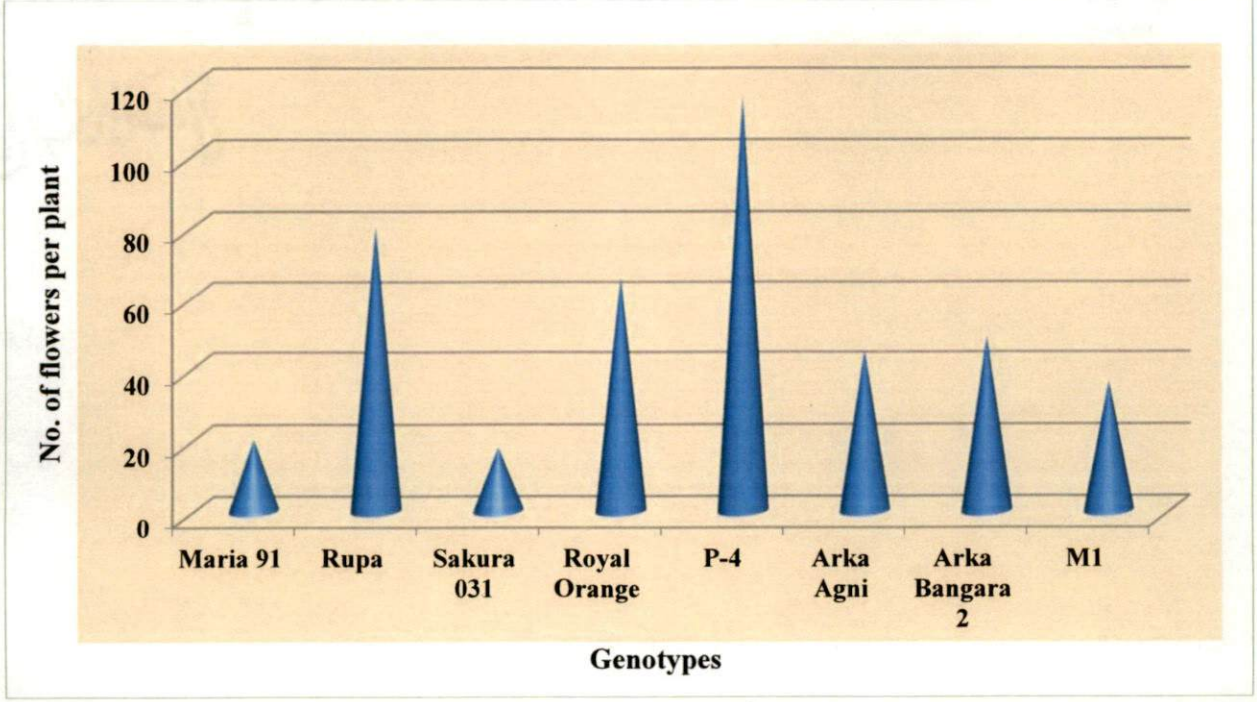


Fig 5.10 Number of flower per plant in African marigold genotypes

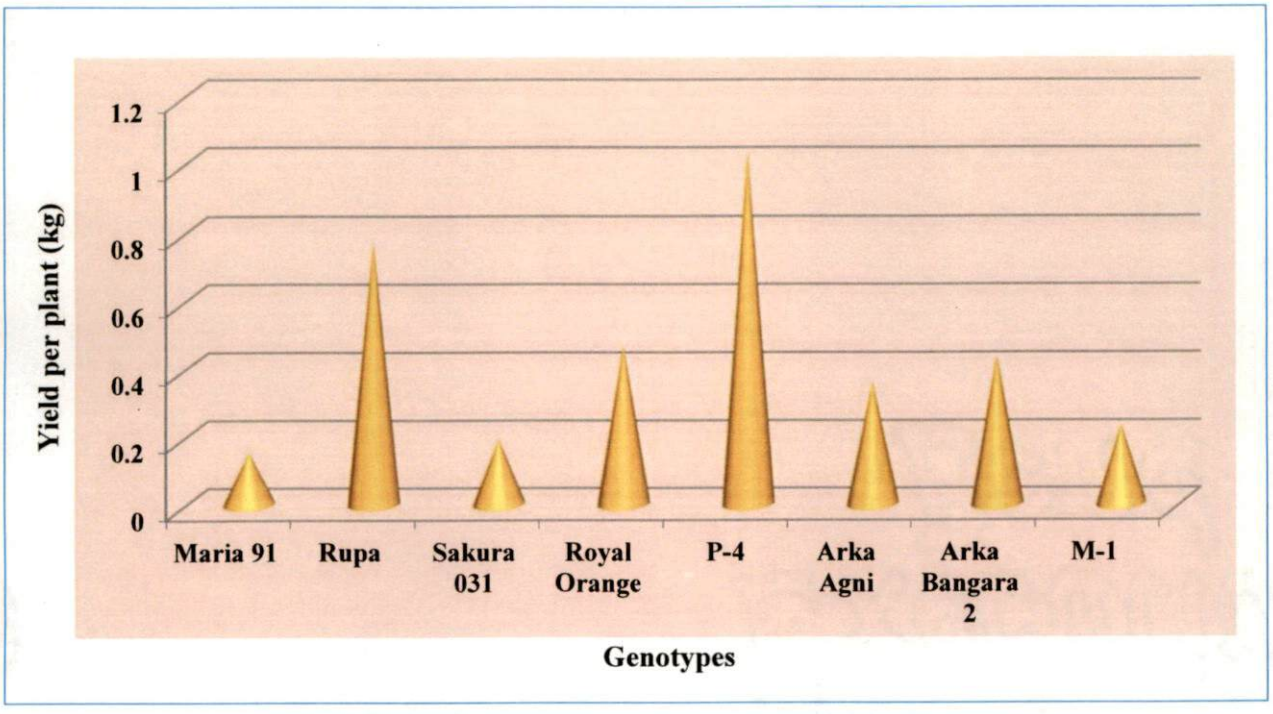
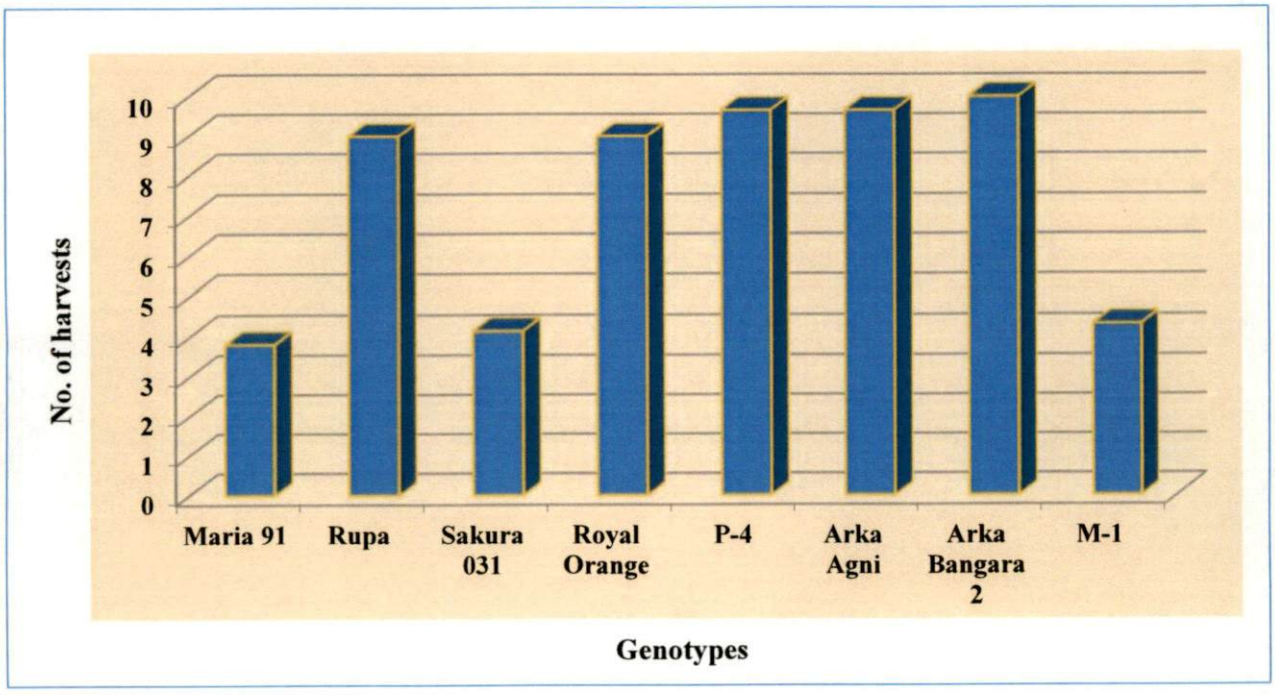


Fig 5.11 Yield per plant in African marigold genotypes



5.12 Number of harvests in African marigold genotypes

Similar findings were reported by Bharathi and Jawaharlal (2014a). However the carotenoid content did not differ significantly among the genotypes studied.

5.1.3. Field evaluation for bacterial wilt resistance

Bacterial wilt is a devastating disease caused by the bacterium *Ralstonia solanacearum.* It is a soil borne pathogen that persists in the field for many years even without host and also get transmitted to adjacent fields through irrigation water, soil, contaminated implements and also due to human intervention.

Breeding for resistance is the most effective strategy to manage bacterial wilt. The easiest and fastest method is to select resistant genotypes for cultivation. Hence screening the genotypes for resistance is very relevant. During field evaluation response of African marigold genotypes for bacterial wilt incidence in terms of per cent disease incidence and stages of disease incidence were observed and discussed here.

The genotype Sakura 031 expressed very high degree of susceptibility (100 %) followed by Rupa (70.83 %) whereas the genotype M-1 was highly resistant and no disease incidence was observed throughout cropping period (Fig 5.13). This variation among the genotypes could be due to interaction between environment and genetic makeup of the host. The variation in disease incidence might also be depended upon the bacterial inoculum concentration in the soil. Baruah *et al.* (2000) and Bora *et al.* (2011) reported similar results while screening the brinjal cultivars.

Performance of a genotype in the field is determined not only by the per cent bacterial wilt incidence but also by the number of days it survived in the field. The genotypes Sakura 031 and Maria 91 were the earliest to wilt (Fig 5.14). These genotypes wilted at vegetative and early flowering stage respectively, which might have resulted in low yield in these genotypes. All other genotypes except M-1 (wilt free) showed wilting at a later stage *viz.*, peak flowering that might have allowed some harvest of flowers. Better yield in Rupa might be attributed to this reason even though the genotype recorded a PDI of 70.83 per cent. Even though the genotypes Arka Bangara 2 and Arka Agni were late (61 to 75 days) to wilt and were with low PDI, the yield was less. This might be due to their genetic makeup. Rahman *et al.* (2011)

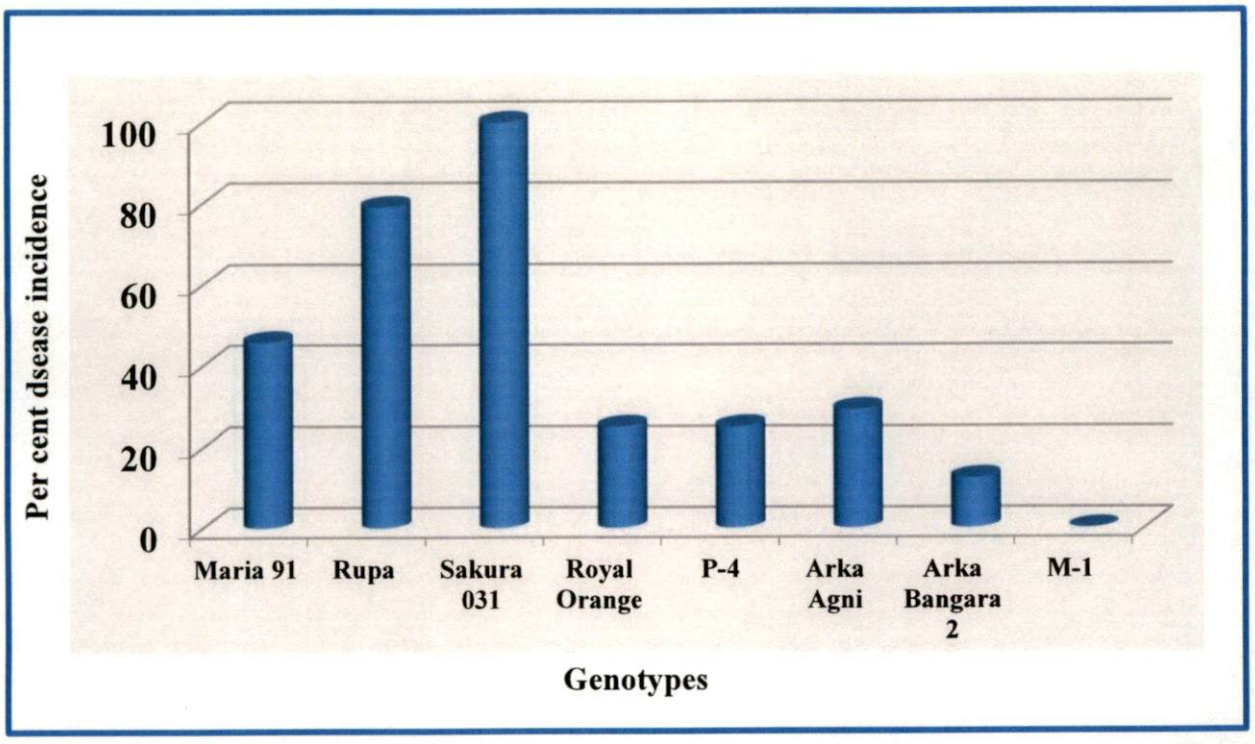


Fig 5.13 Reaction of African marigold genotypes against bacterial wilt incidence in field condition

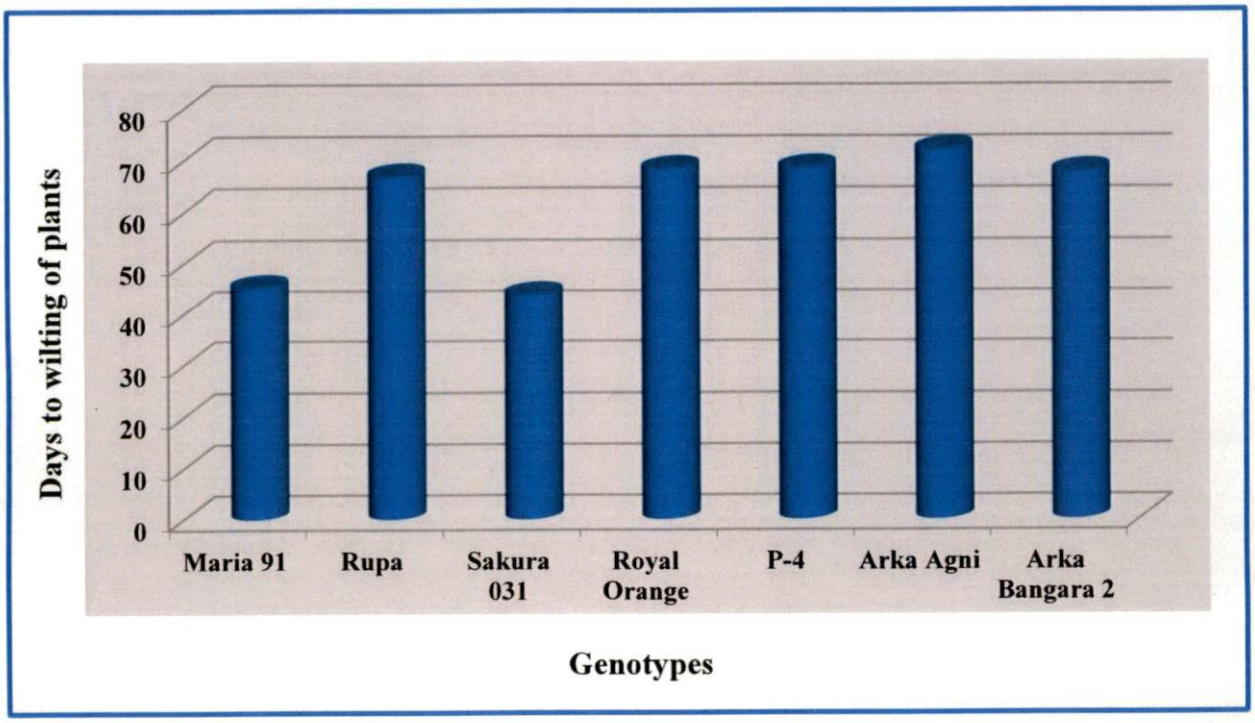


Fig 5.14 Days to wilting of plants in African marigold genotypes

reported wilting of brinjal cultivars at different stages while screening against bacterial wilt disease.

5.2. Screening against bacterial wilt through artificial inoculation

Screening for bacterial wilt resistance in African marigold genotypes is very important for recommending a suitable genotype for cultivation in wilt sick fields. As field evaluation may be influenced by variation in inoculum concentration in soil, screening though artificial inoculation methods was also tried.

Screening through artificial inoculation methods confirmed complete resistance of genotype M-1 and hundred per cent susceptibility of genotype Sakura 031 to bacterial wilt (Fig 5.15). Five genotypes *viz*., Sakura 031, Arka Agni, P-4, Arka Banagra 2 and Maria 91 were scored as highly susceptible whereas Royal Orange was scored as susceptible and genotype Rupa as moderately susceptible. The different reactions of the genotypes against bacterial wilt might be due to their genetic makeup. Such varying reactions of tomato genotypes towards bacterial wilt incidence have been reported by Dutta and Rahman (2012). Moderate resistance may be due to the presence of modifying genes and/or influence of the environment.

There was no significant difference among the three inoculation methods in inducing wilt incidence in the genotypes. However, both root dip and stem injection methods had higher mean per cent disease incidence (65.83 % and 65.00 %, respectively) when compared to media drench method (57.50 %). Fonseca *et al*. (2015) reported stem injection as the most effective inoculation method in *Eucalyptus* spp. against bacterial wilt disease. However, Artal *et al*. (2012) observed media drenching as the most efficient inoculation method in solanaceous crops *viz*., tomato, brinjal and chilli when compared to leaf axil puncturing and leaf clipping methods. Hence it is clear that the efficiency of the inoculation method may also depend upon host. African marigold could be a more sensitive crop and might have equally responded to all the three inoculation methods tried.

5.3. Screening African marigold genotypes through spot planting technique

Screening genotypes by spot planting with susceptible check genotype is an effective screening method in bacterial wilt sick fields. Hence this technique was also tested in the present study. The genotype M-1 did not show wilt incidence even when spot planted with the check genotype Sakura 031 (Fig 5.16). This observation confirmed the immunity of M-1 by precluding any chance for escape by spot planting with Sakura 031. Hundred per cent wilt incidence for the check genotype Sakura 031 was observed in combination with genotype Maria 91 and M-1 for which the PDI were 79.17 and 0, respectively. Narayanankutty and Peter (1986) reported similar results in tomato cultivars by spot planting with Pusa Ruby as the check genotype. Among the other genotypes, Rupa planted with Sakura 031 showed maximum PDI (100 %). Though the genotype Rupa was showing moderate susceptibility during artificial inoculation, when planted with Sakura 031 in the field condition showed higher susceptibility. With regards to days taken to wilt by genotypes and check genotype, the combination of Arka Agni and Sakura 031 were considerably early to wilt than other genotypes (Fig 5.17).

5.4. Grafting studies

Grafting has been extensively used in the greenhouse production as well as open precision farming of horticultural crops where plants are intensely cultivated to produce high yield on vigorous rootstocks. Grafting combines valued traits from a desirable rootstock variety such as enhanced vigor, soil-borne disease resistance, and environmental stress tolerance with a desirable scion variety having high yield with good quality fruits. Grafting enables the deployment of diverse sources of rootstocks to manage site-specific issues while growing market-preferred fruit cultivars as the scion.

Grafting on compatible resistant rootstock is adopted universally in vegetables especially in solanaceous crops to overcome soil borne diseases particularly bacterial wilt. Field trials conducted at ARS, Mannuthy in grafted solanaceous vegetables has given encouraging results with respect to control of bacterial wilt and better yields (Narayanankutty *et al*., 2015).

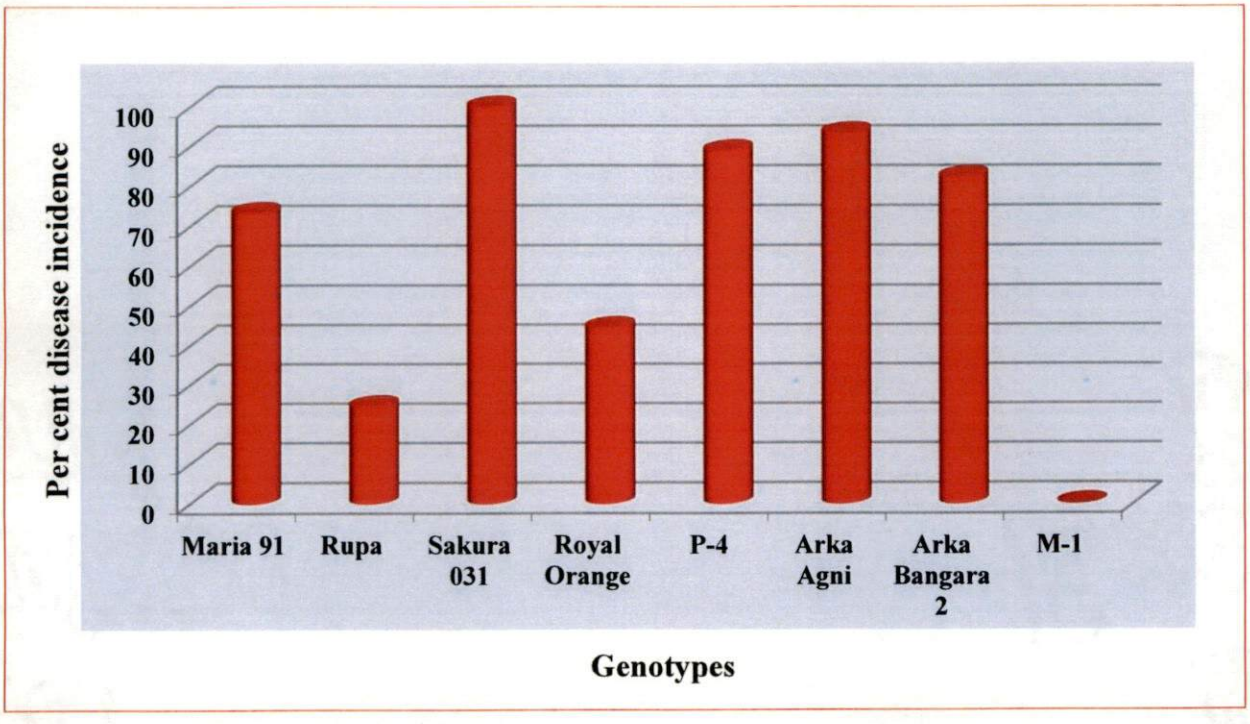


Fig 5.15 Per cent disease incidence (PDI) in genotypes during artificial inoculation

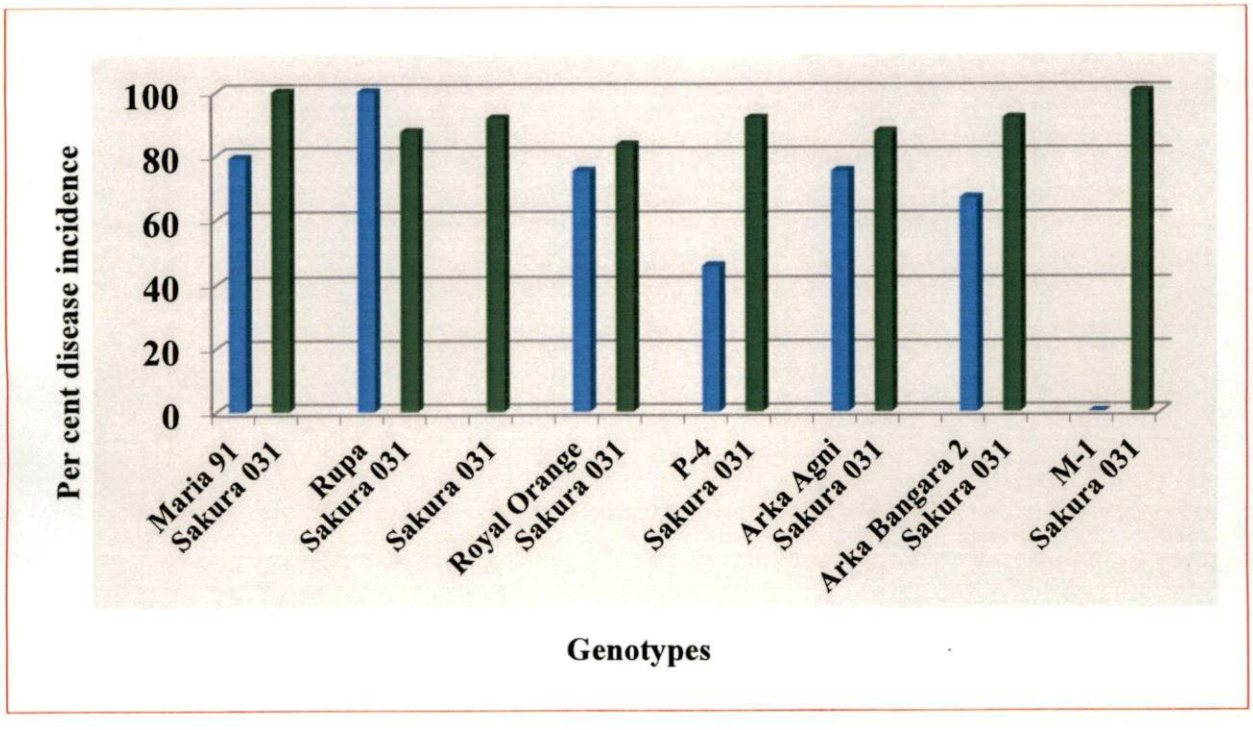


Fig 5.16 Per cent disease incidence (PDI) in genotypes during spot planting

5.4.1. Field survival of African marigold grafts

Grafted plants of almost all the genotypes showed a field survival above 60 per cent (Fig 5.18). However, significant variation was observed among genotypes for field survival of grafts. Maximum survival was recorded in the genotype P-4 while lowest in the genotype Sakura 031. Variation in response of genotypes could be due to their genetic makeup that might have controlled proper graft union and also due to some physiological reasons like lack of cellular recognition, wounding responses, presence of growth regulators, or incompatibility toxins (Andrews and Marquez, 1993; Oda *et al.*, 1993).

5.4.2. Bacterial wilt incidence

There was no disease incidence observed in grafted plants of any genotypes and this might be attributed to the fact that the rootstock M-1 was highly resistant to bacterial wilt. This shows that disease control efficacy depends on the degree of resistance present in rootstock. Control of bacterial wilt in many solanaceous crops through grafting on resistant rootstocks has been reported by Palada and Wu (2007), Rivard and Louws (2008), Rivard *et al.* (2012) and Narayanankutty *et al.* (2015).

Incidence of bacterial wilt was observed in the non-grafted plants of all the seven genotypes indicating the significant impact of grafting on resistant rootstock in control of the disease. Maximum disease incidence was observed in the genotype Sakura 031 while the lowest disease incidence was recorded in Arka Agni and Arka Bangara 2.

5.4.3. Plant characters

There was significance difference observed among the genotypes as well between the grafted and non-grafted plants within genotypes for plant height (Fig 5.19). Irrespective of grafted and non-grafted condition, the genotype Royal Orange produced significantly taller plants than all other genotypes studied. The variation among genotypes could be due to their genetic makeup. Height was in general significantly more for grafted plants irrespective of genotypes. The increase in height may be due to the vigorous root system of the rootstock and also due to resistance to bacterial wilt

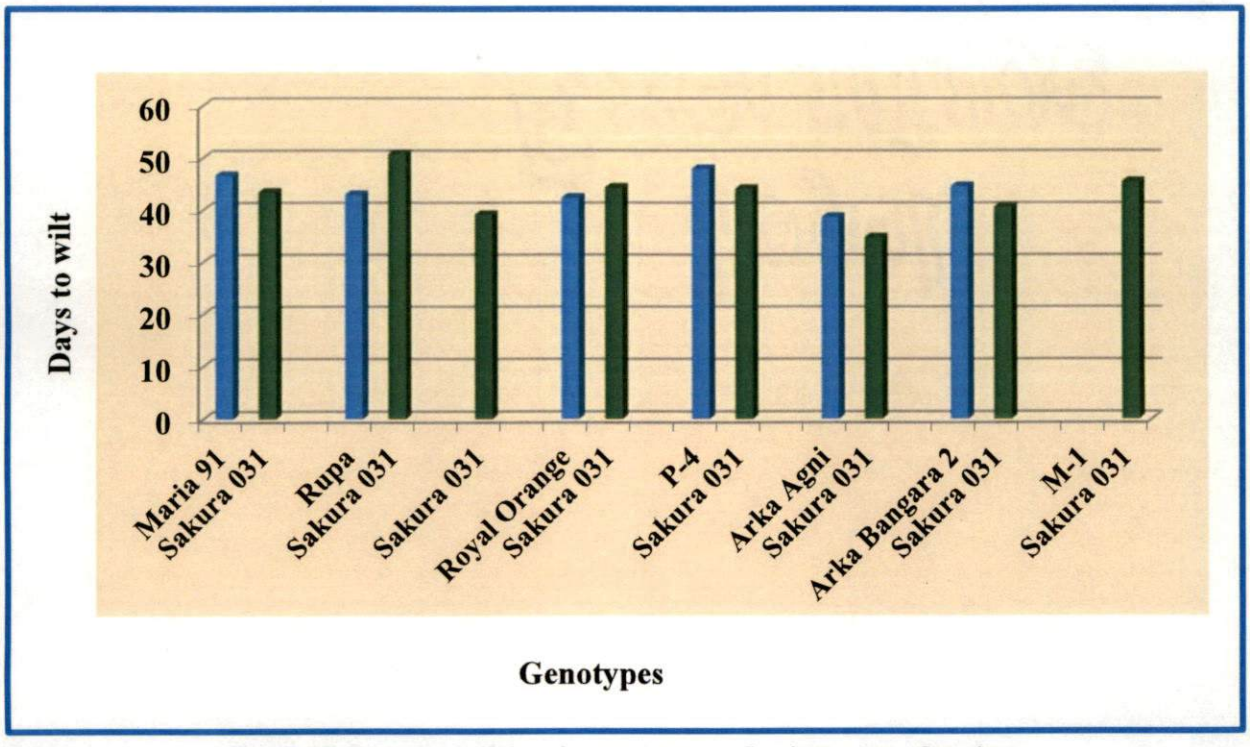


Fig 5.17 Days to wilting in genotypes during spot planting

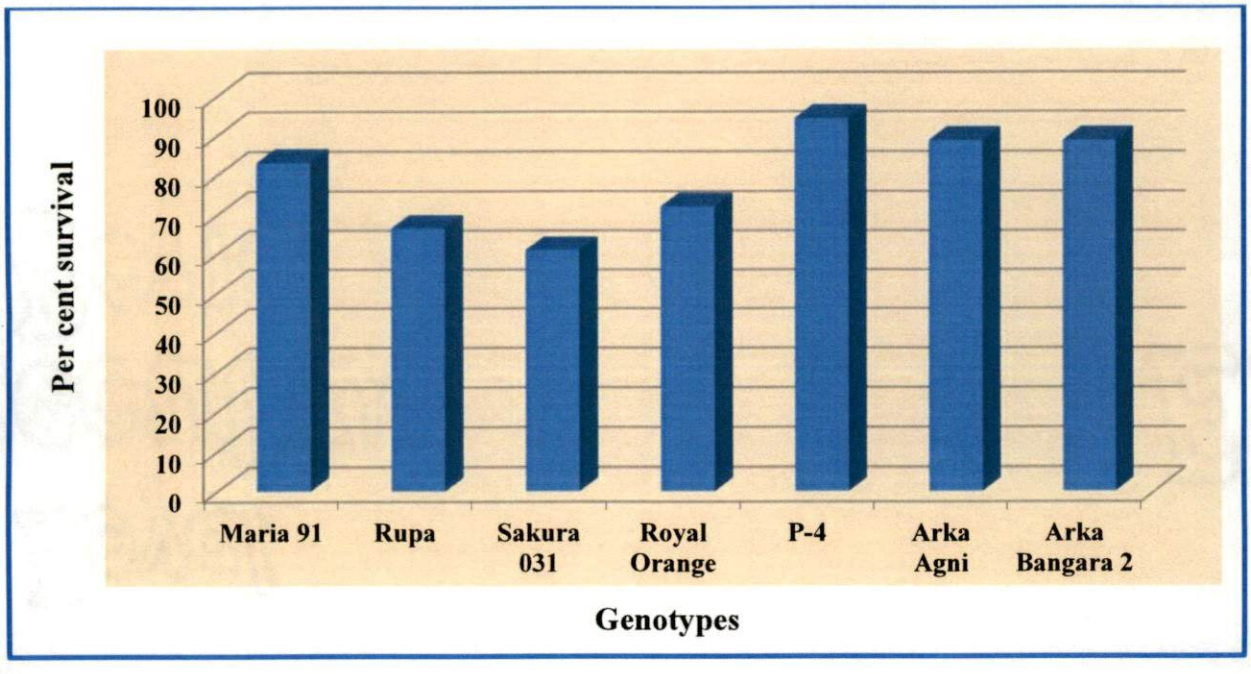


Fig 5.18 Per cent survival of grafts during grafting studies

imparted through grafting. The results of this study are in agreement with the results of Lee (1994), Bletsos (2003) and Khah (2005) who observed increased height and vigor for grafted plants in various vegetables. They have attributed to the increased growth and vigor in grafted plants to healthy root system that supported efficient uptake of water and nutrients.

There was no significance difference observed among the genotypes and as well as between the grafted and non-grafted plants within genotypes with respect to plant spread. Hence it could be inferred that plant spread is not either influenced by genetic makeup or by grafting in the genotypes studied.

Number of primary branches showed significant difference among the genotypes irrespective of whether it is grafted or non-grafted both at 30 and 60 DAP. The genotype Royal Orange recorded the maximum number of primary branches when compared to all other genotypes and this might be attributed to its increased plant height and these two characters are positively correlated as reported by Karuppaiah and Kumar (2011). Grafting did not influence the number of primary branches in different genotypes.

Irrespective of the genotypes, grafted plants showed significantly more leaf area than the non-grafted plants (Fig 5.20). This might be due to the fact that for all the genotypes the rootstock used was M-1 which is a genotype that showed significantly high leaf area during field evaluation. This rootstock might have influenced the leaf area in grafted plants. This is in agreement with the study by Leonardi and Giuffrida (2006) who found that the leaf area in tomato was higher only in plants grafted on a particular stock 'Beaufort' and agree with results on nutrient uptake that was higher in plants grafted on 'Beaufort'. Khah (2005) has also reported that the grafted plants on an average have more leaf area than the non-grafted plants in aubergine.

There was no difference observed in the petiole colour between the grafted and non-grafted plants as well as among the genotypes irrespective of grafted or non-grafted. This might be due to the reason that the petiole colour is a genetically acquired character.

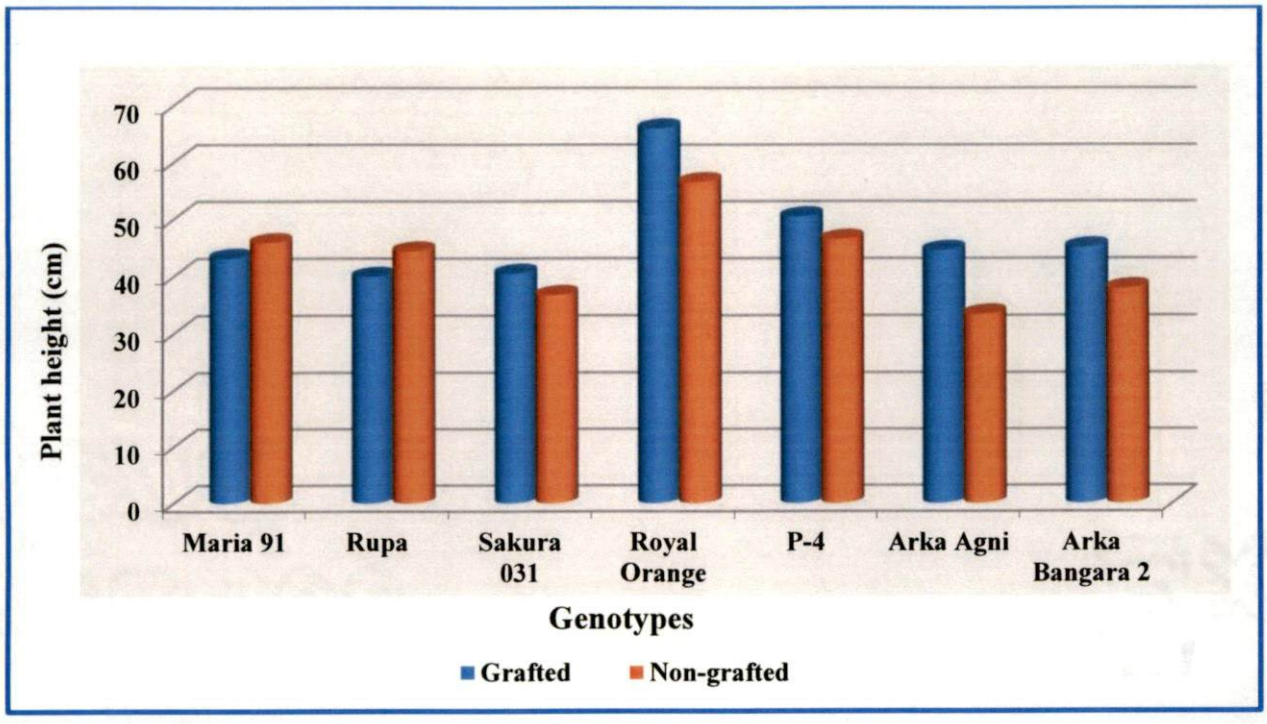


Fig 5.19 Effect of grafting on plant height in African marigold genotypes

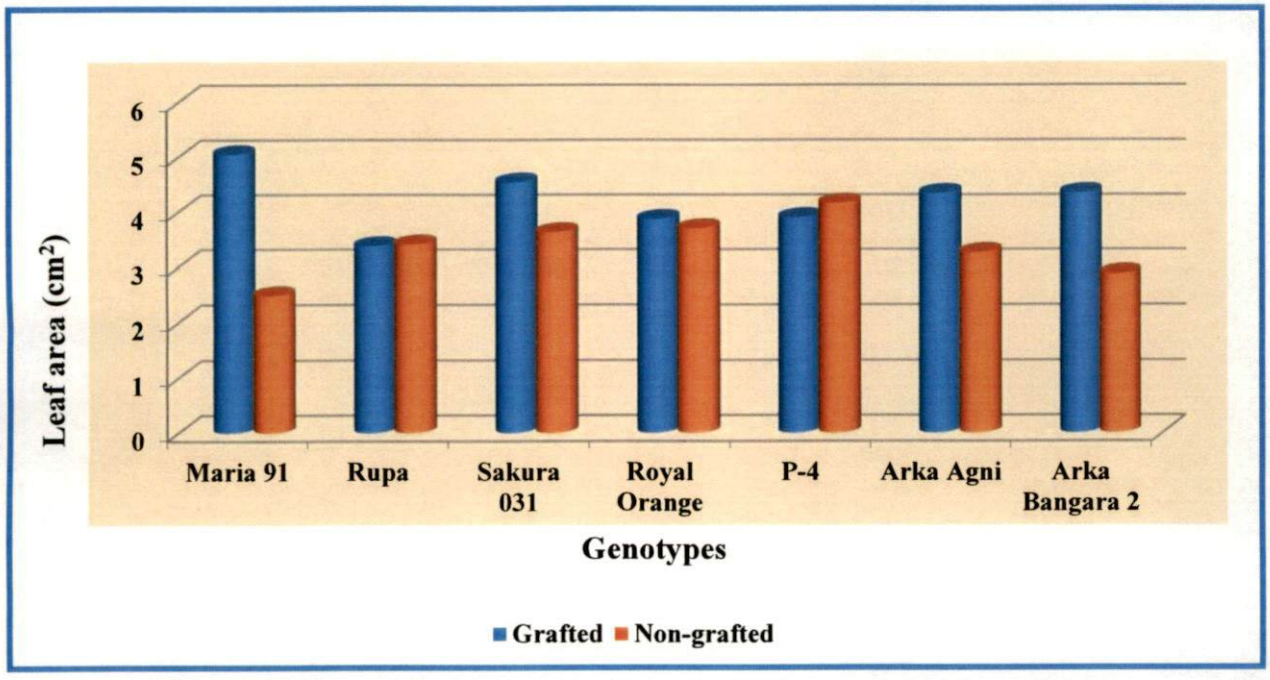


Fig 5.20 Effect of grafting on leaf area in African marigold genotypes

5.4.4. Floral characters

There was no significant difference observed for days to flower bud initiation among the genotypes irrespective of whether it is grafted or non-grafted. This was already revealed during field evaluation that except M-1 all other genotypes initiated flower buds within same period after planting. However significant difference was observed for days to bud initiation between grafted and non-grafted plants irrespective of genotypes. Grafted plants were earlier to show the flower bud initiation compared to non-grafted plants (Fig 5.21). This result is in agreement with the findings of Yetisir and Sari (2003). The earliness in bud initiation may also attributed to the root-scion combination that might have altered amounts of hormones produced which in turn influenced grafted plant organs (Satoh, 1996).

Like the days to bud initiation, significant variation was also observed for days to flower opening between the grafted and non-grafted plants irrespective of genotypes. Grafted plants took more days for flower opening when compared to non-grafted plants (Fig 5.22). This could be due to rootstock-scion combination that might have altered amounts of hormones produced which in turn influenced grafted plant organs (Satoh, 1996).

There was no significant difference observed for flower diameter between the grafted and non-grafted plants irrespective of genotypes and also among the genotypes irrespective of whether they were grafted or non-grafted. Similar results were reported by Farhadi *et al.*, 2016; Mohammad *et al.*, 2009; Quaryoti *et al.*, 2007. This may be due to the environmental factors such as high temperature which prevailed during the flowering period. Clearly, the scion genotype affects final size and quality of flowers in grafted plants, but rootstock effects can drastically alter these characteristics. There are many conflicting reports on changes in quality resulting from grafting. The differences in reported results may be attributed in part to different production environment, type of rootstock/scion combination used, and harvest date.

Significant differences were observed among genotypes for stalk length irrespective of grafted and non-grafted condition. This variation might be due to genetic

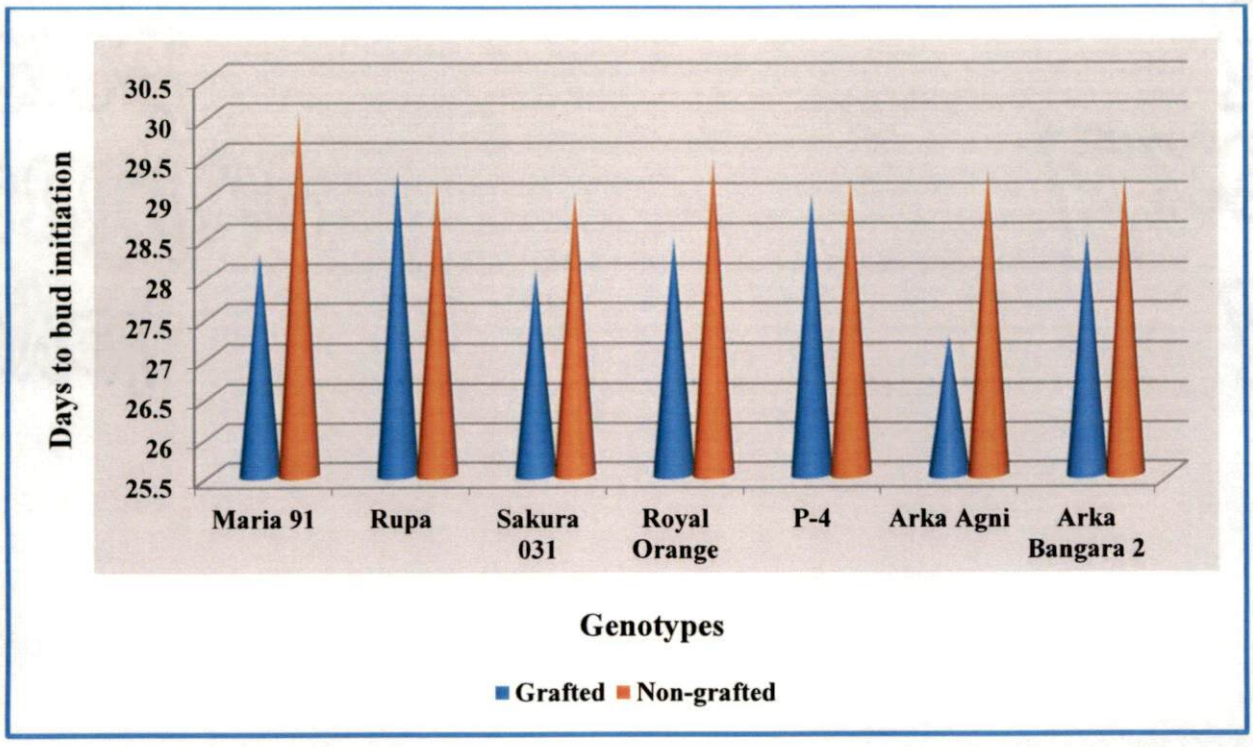


Fig 5.21 Effect of grafting on days to bud initiation/formation in African marigold genotypes

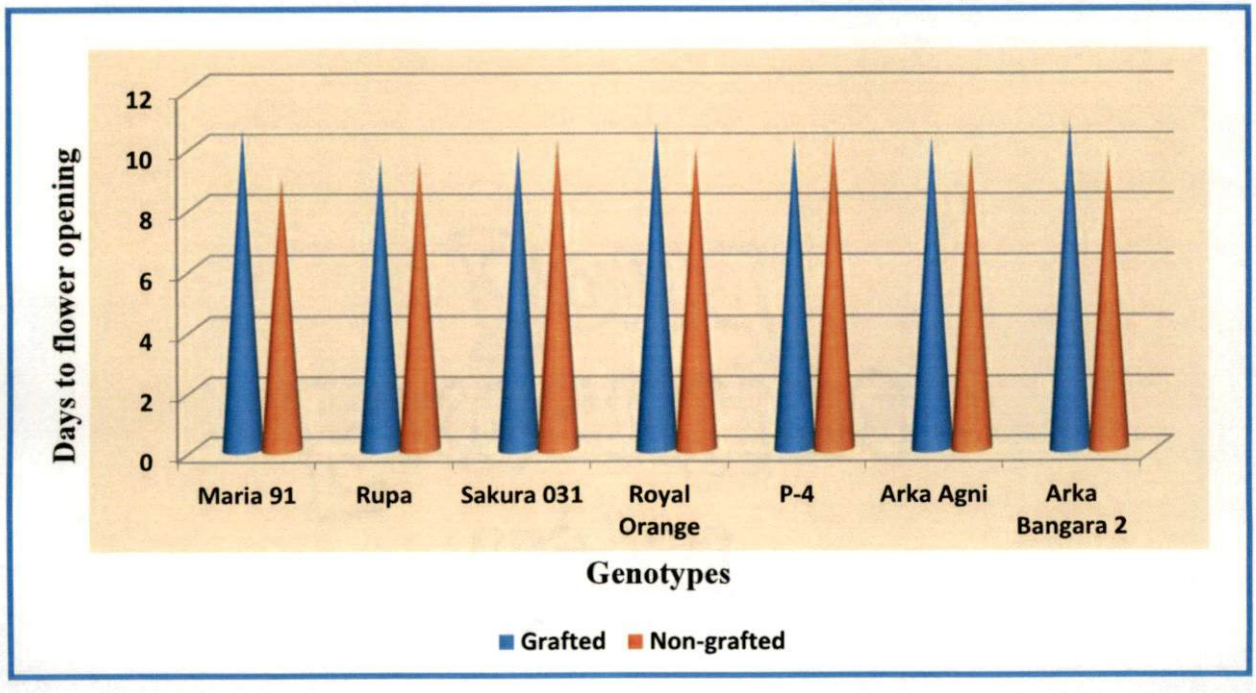


Fig 5.22 Effect of grafting on days to flower opening in African marigold genotypes

character of the genotypes. However stalk length was similar for grafted and non-grafted plants within genotypes.

Flower weight and petal yield per flower did not differ between the grafted and non-grafted plants irrespective of genotypes and also among the genotypes irrespective of whether they were grafted or non-grafted. These findings were similar to the results of Khah (2005) who reported that flower fresh and dry weight in aubergine were not affected by grafting in both the field and greenhouse crops. .

Significant differences was observed for number of flowers per plant between the grafted and non-grafted plants irrespective of genotypes whereas there was no significant difference observed for number of flowers per plant among the genotypes irrespective being grafted or non-grafted. Grafted plants were showing significantly more number of flowers per plant compared to non-grafted plants (Fig 5.23). The findings were similar to the results of Khah (2005) and Ibrahim *et al.* (2001).

Irrespective of the genotypes, grafted plants recorded higher yield than the non-grafted plants (Fig 5.24). The increased yield might be due to the healthy vigorous root system provided by wilt resistant rootstock that might have facilitated better uptake of water and nutrients. This result is supported by similar findings in grafted vegetables by Lee, 1994; Khah, 2005; Khah *et al.*, 2006.

Number of harvests, which is one of the important factor determining yield, showed wide variation among genotypes as well as between the grafted and non-grafted plants within genotypes. More number of harvests in grafted plants might be due to extended crop duration because of grafting (Fig 5.25). The rootstock used might have direct effect on the scion genotypes for extending the crop duration. The variation among the genotypes might be due to the genetic character and relative response to high temperature prevailed during flowering period.

5.5. Post-harvest life

The genotypes showed significant variation for the time taken for 25 per cent wilting of flowers and its loss in weight both in terms of physiological loss in weight (PLW) and cumulative physiological loss in weight (CPLW). Sakura 031 and Rupa

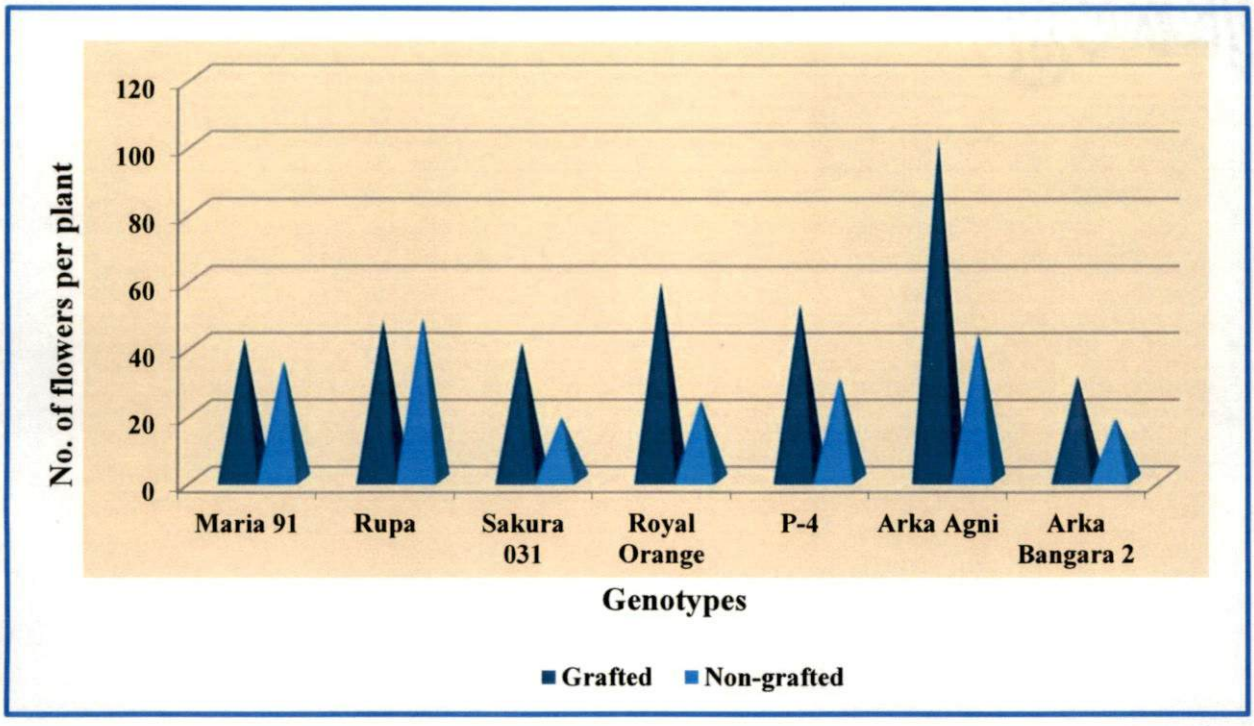


Fig 5.23 Effect of grafting on number of flowers per plant in African marigold genotypes

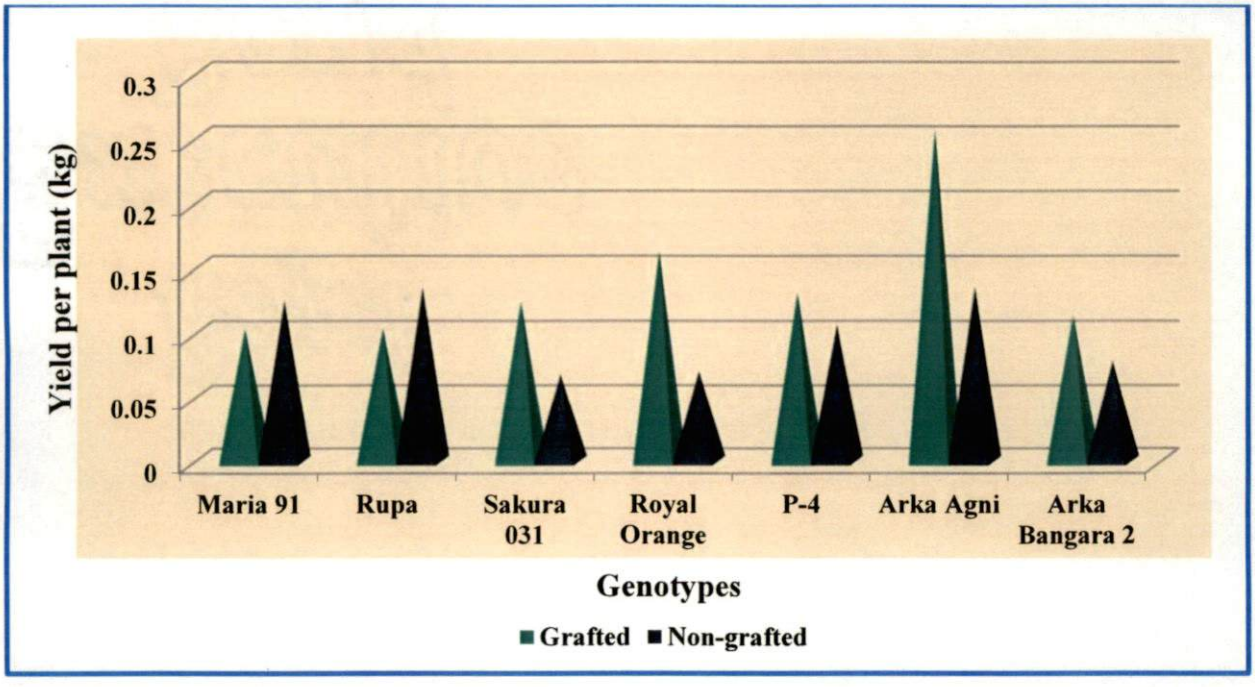


Fig 5.24 Effect of grafting on yield per plant in African marigold genotypes

took maximum number of days to show wilting of flowers followed by P-4. The genotype Maria 91 took minimum number of days to show wilting of flowers (Fig 5.26). The difference in post-harvest life might be attributed to genetic makeup of the genotypes. Raghuvanshi and Sharma, 2011; Singh and Misra, 2008; Kishore (1998) also reported same results in African marigold.

All the genotypes showed significant difference with respect to physiological loss in weight (PLW) and cumulative physiological loss in weight (CPLW) of flowers. All the genotypes showed an increased PLW and subsequently higher CPLW after three days of storage (Fig 5.27 and 5.28). So it seems obvious that flower remain fresh maximum for three days under ambient storage condition. Similar findings were reported by Raghuvanshi and Sharma, 2011; Nimisha *et al.*, 2016.

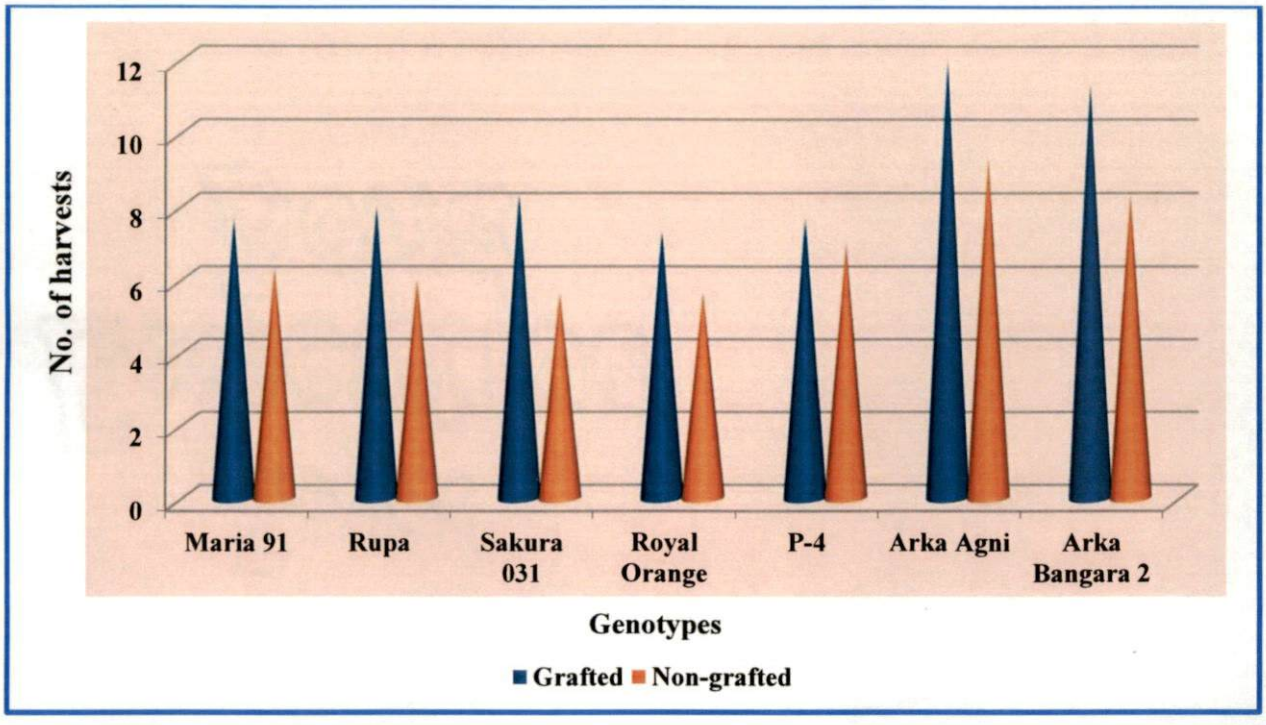


Fig 5.25 Effect of grafting on number of harvests in African marigold genotypes

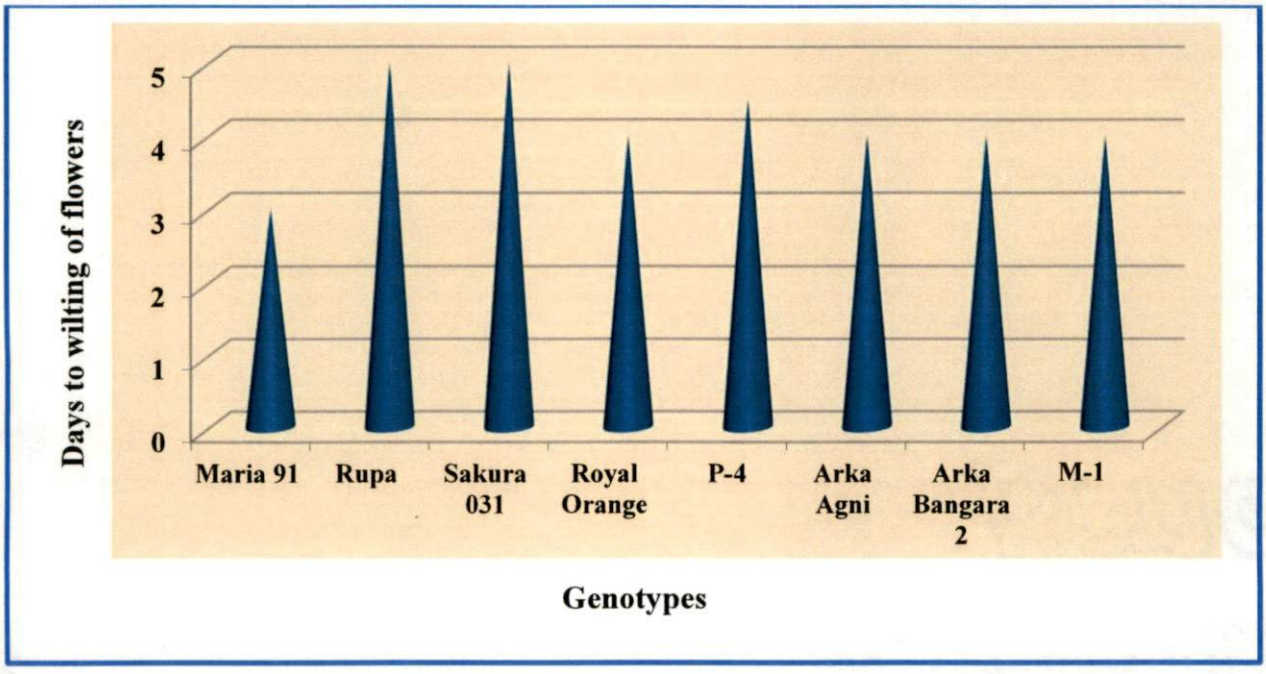


Fig 5.26 Days to wilting of flowers in African marigold genotypes

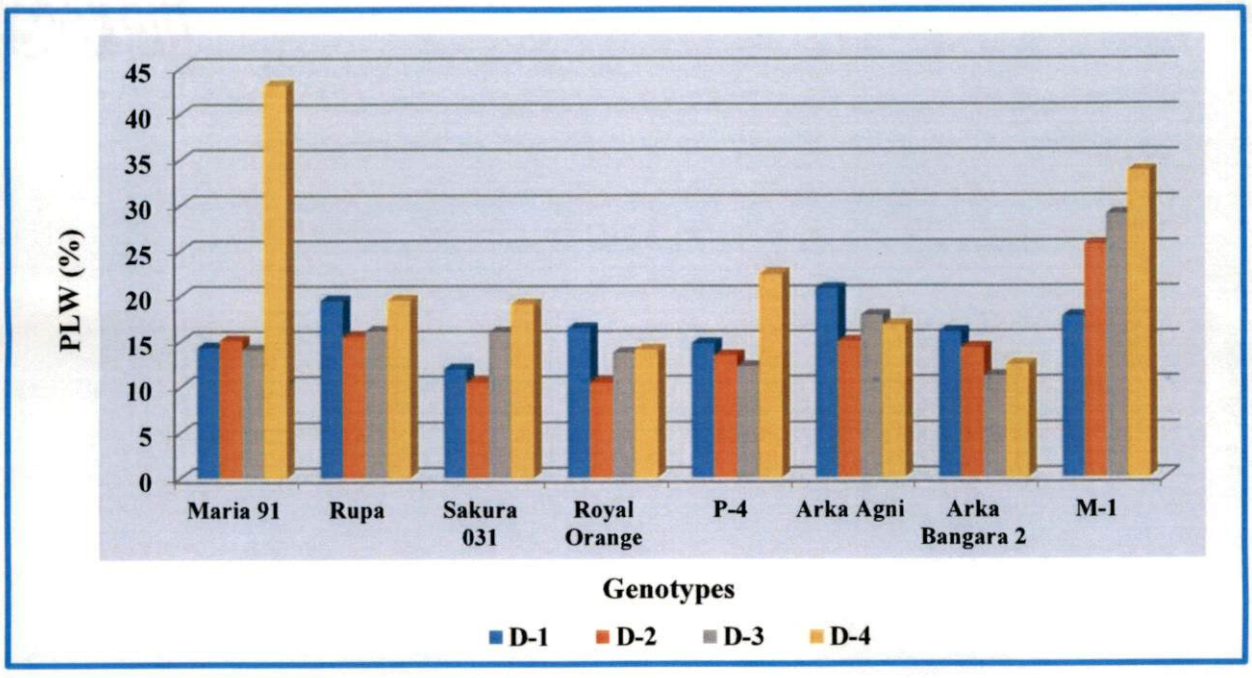


Fig 5.27 Physiological loss in weight (PLW) of flowers in African marigold genotypes

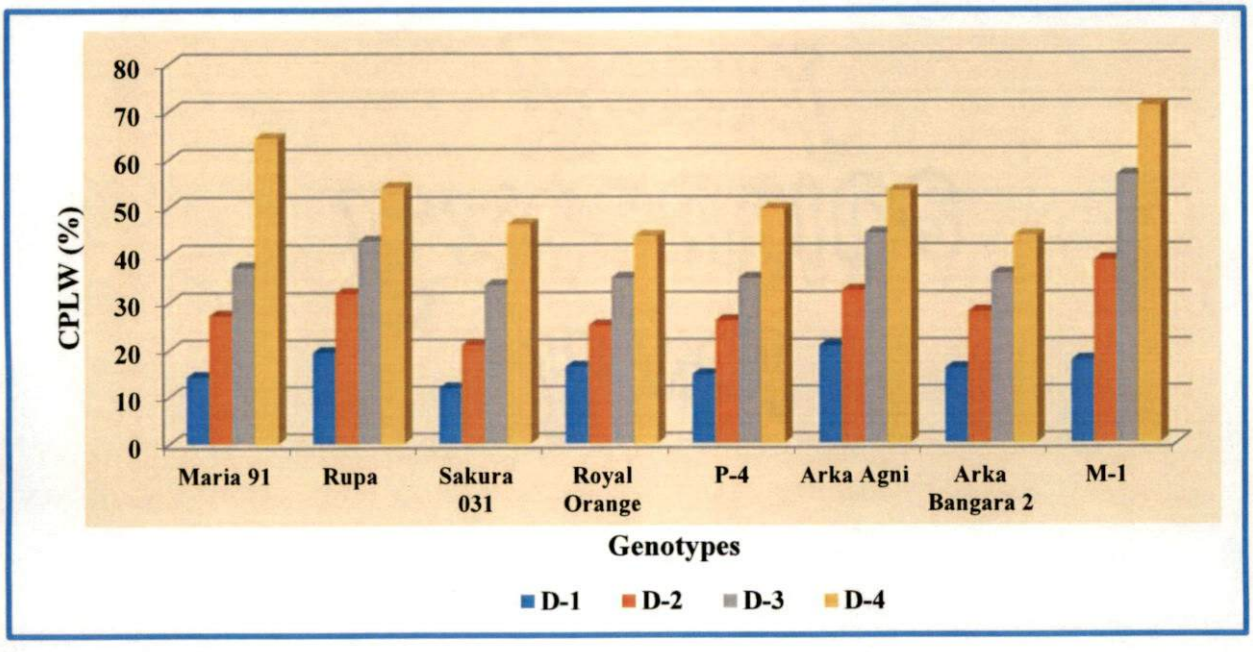


Fig 5.28 Cumulative physiological loss in weight (CPLW) of flowers in African marigold genotypes

Summary

6. SUMMARY

African marigold (*Tagetes erecta* L) is a widely grown annual flower crop. The wide spectrum of attractive colours, shape, size and good keeping quality as well as adaptability to different climatic conditions of this flower crop has attracted the attention of many flower growers. Marigold is predominantly used as a loose flower and in gardening either as bedding plants or as potted plants.

In Kerala also, the demand for this crop is increasing day by day. Particularly during the festivals like Onam the demand for this flower reaches peak in the State. Hence cultivation of this flower has gained momentum in Kerala. However, due to prevalence of the bacterial wilt disease, farmers face losses due to wilting of plants of costly hybrids that are taken up for cultivation. There exist two strategies to tackle such crisis in cultivation of any crop. The first is to identify high yielding genotypes with resistance to bacterial wilt and the second choice is to use grafted plants on wilt resistant rootstocks. Hence identification of suitable African marigold genotypes with high yield, large sized flowers, attractive shape and color, along with resistance to bacterial wilt is very essential. Also important is to screen a wilt resistant rootstock and to study the response of grafted plants on wilt resistant rootstock. Field trials conducted at ARS, Mannuthy in grafted solanaceous vegetables have shown 100 per cent control of bacterial wilt and increased yields (Narayanankutty *et al*, 2015).

Hence considering the growing importance for the flower in the State as well the high occurrence of bacterial wilt disease in the crop, the present investigation entitled as "Evaluation of African marigold (*Tagetes erecta* L.) hybrids/varieties for yield and resistance to bacterial wilt" was undertaken at ARS, Mannuthy with the objective to evaluate eight African marigold genotypes (5 F_1 hybrids, 2 varieties along with 1 local collection) for yield, with resistance to bacterial wilt and to assess the feasibility of grafting as a tool to combat bacterial wilt. Summary of the investigation carried out in five experiments namely field evaluation, screening against the bacterial wilt through artificial inoculation,

screening through spot planting with susceptible genotype, studies on grafting and post-harvest life are given under here.

1. Field evaluation for yield and resistance to bacterial wilt

Genotypes showed wide variation for plant characters such as height, spread, number of primary branches and leaf area. Plant height, plant spread and number of primary branches were maximum in Royal Orange and P-4 which are F_1 hybrids. Leaf area was maximum in genotype M-1. Number of primary branches was almost similar for all the genotypes except the two IIHR varieties *viz.*, Arka Agni and Arka Bangara 2. Stem girth and petiole color were the two characters that did not differ among the genotypes. All the genotypes showed green colored petiole.

Wide variations were also observed among the genotypes for floral characters. Except the genotype M-1, all others took almost the same duration to initiate flower bud. The genotype M-1 was very late to initiate flower bud. However, all the genotypes took almost the same duration for flower opening.

The genotypes Rupa and P-4 produced exceptionally larger flowers having high flower weight and petal yield per flower. Flower size, flower weight and petal yield per flower were low for genotypes M-1 and Arka Agni. The genotypes Royal Orange, P-4, Arka Agni and Arka Bangara 2 produced flowers with longer stalks while stalk length was minimum in Sakura 031. Carotenoid content did not differ among the genotypes. Flower color varied among the genotypes showing different shades of yellow and orange color.

Number of flowers and yield per plant also recorded significant variation among the genotypes. Both these parameters were maximum in P-4 followed by genotype Rupa. Among the varieties, Arka Bangara 2 produced more flowers and higher yield when compared to Arka Agni. All the genotypes except Maria 91, Sakura 031 and M1 recorded almost the same number of harvests.

When overall performance with respect to plant and floral characters were considered, the genotype P-4 was found to be promising.

The genotype M-1 was showing 100 per cent bacterial wilt resistance whereas the genotype Sakura 031 found to be highly susceptible. During field evaluation. Among the hybrids, wilt incidence was low for Royal Orange and P-4 and they were classified as moderately susceptible. Genotypes which were late to wilt (P-4 and Rupa) recorded better yield when compared to the genotypes which were early to wilt (Sakura 031 and Maria 91).

2. Screening of African marigold genotypes against bacterial wilt through artificial inoculation

The genotype M-1 did not show any wilt incidence which was considered as resistant to bacterial wilt. The genotype Sakura 031 recorded 100 per cent bacterial wilt incidence. All the three inoculation methods *viz.*, root dip, media drenching and stem injection showed the same efficacy in testing bacterial wilt.

3. Screening African marigold genotypes under spot planting technique

The genotype M-1 did not show wilt incidence even when spot planted with the check genotype Sakura 031 which showed 100 per cent wilt incidence. This method of field evaluation to test bacterial wilt was very effective avoiding a chance as an escape for the genotypes. The disease incidence in check genotype Sakura 031 ranged from 83.33 to 100 per cent whereas the disease incidence in genotypes spot planted with Sakura 031 ranged from 0 to 100 per cent. Among the genotypes, P-4 recorded the lowest bacterial wilt incidence even when spot planted with highly susceptible Sakura 031.

With respect to the study on bacterial wilt incidence under three modes *viz.*, general field evaluation, artificial inoculation and spot planting technique it could be summarized that consistent response to wilt incidence was shown by two genotypes *viz.*, M-1 and Sakura 031. The genotype M-1 was 100 per cent wilt free whereas Sakura 031 was 100 per cent wilt prone.

4. Grafting studies

Maximum field survival of grafts was observed in P-4 (94.44 %) while minimum in Sakura 031 (61.11 %). Bacterial wilt incidence was not observed in grafted plants of any of the genotypes studied. But per cent wilt incidence ranged from 55.05 per cent to 77.77 per cent in non-grafted plants of the seven genotypes tested.

In general, grafted plants were taller than non-grafted plants. Significant difference were also observed among the genotypes irrespective of whether grafted or non- grafted. There was no significant differences observed in plant spread either among genotypes or between grafted and non-grafted plants irrespective of genotypes. Number of primary branches varied among the genotypes irrespective of grafted and non-grafted condition. The genotypes Royal Orange and P-4 showed more primary branches. Irrespective of the genotypes grafted plants recorded more leaf area than the non-grafted plants.

Irrespective of genotypes, grafted plants were significantly earlier to initiate flower buds and they took average of 28.43 days when compared 29.36 days in non-grafted plants. However, grafted plants on an average took more days (10.37) compared to non-grafted plants (9.91) for flower opening. There was no significance difference with respect to flower diameter, stalk length, flower weight and petal yield per flower in grafted and non-grafted plants. However, stalk length was found to be significantly different among the genotypes. Irrespective of the genotypes grafted plants produced more flowers and high yield per plant than non-grafted plants. Grafting also enhanced the crop duration and facilitated more harvests than non-grafted plants.

5. Post-harvest studies

The shelf life of flowers ranged from 3 to 5 days under ambient condition and this might also depend upon the season of harvest. The flowers of the genotypes Sakura 031 and Rupa took maximum days to wilt (5.00) while Maria 91 took minimum days (3.00).

The study clearly showed that all the genotypes showed considerable variation with respect to yield and resistance to bacterial wilt. The genotype P-4 was found to be promising with high yield, large sized flowers and moderate susceptibility to bacterial wilt. The genotype M-1 was 100 per cent resistant to bacterial wilt while the genotype Sakura 031 was found to be 100 per cent susceptible. The genotype M-1 can be used as rootstock for grafting susceptible genotypes. Grafting the susceptible genotypes on resistant rootstock found to be an effective tool to combat the bacterial wilt. Grafting significantly increased the yield in susceptible genotypes without affecting the flower quality attributes.

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Appendices

APPENDIX - I

Weather data of experimental site (June, 2016-March, 2017)

Month	Mean temperature (°C)		Relative humidity (%)		Bright sunshine (hr)	Rainfall (mm)
	Maximum	Minimum	Morning	Evening		
June-2016	29.80	23.90	95.00	83.00	1.60	654.70
July-2016	29.90	23.50	96.00	75.00	2.30	390.40
August-2016	30.40	23.70	95.00	71.00	4.90	183.50
September-2016	30.30	23.70	95.00	69.00	4.80	86.00
October-2016	31.50	24.10	93.00	68.00	5.50	37.30
November-2016	32.90	23.80	83.00	54.00	5.80	13.80
December-2016	32.70	23.30	85.00	52.00	6.50	52.90
January-2017	34.10	22.90	68.00	37.00	7.60	0.00
February-2017	36.00	23.20	70.00	31.00	8.70	0.00
March-2017	36.10	24.70	85.00	48.00	7.40	13.2

APPENDIX - II

Chemical composition of TTZ medium

Sl. No.	Chemical	Weight in grams
1	Peptone	10.00
2	Casein hydrolysate	1.00
3	Glucose	5.00
4	Agar	20.00
5	Distilled water	1 litre

Autoclave and then add 5 ml of 1% TTZ.

EVALUATION OF AFRICAN MARIGOLD (*Tagetes erecta* L.) HYBRIDS/VARIETIES FOR YIELD AND RESISTANCE TO BACTERIAL WILT

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The present investigation entitled "Evaluation of African marigold (*Tagetes erecta* L.) hybrids/varieties for yield and resistance to bacterial wilt" was undertaken at Agricultural Research Station, Mannuthy during the year 2016-17. Performance of eight African marigold genotypes (5 F_1 hybrids, 2 varieties and 1 local collection) was evaluated under five experiments *viz*., field evaluation for yield and bacterial wilt resistance, screening against bacterial wilt through artificial inoculation, field screening against bacterial wilt through spot planting technique, grafting studies and post-harvest studies.

Field evaluation of genotypes showed wide variation for characters studied. Plant height was significantly more in genotype Royal Orange, P-4 and Maria 91 whereas plant spread was high for P-4, Royal Orange, Maria 91 and M-1. Stem girth did not differ significantly among genotypes. Number of primary branches was more in all genotypes except the IIHR varieties. Maximum leaf area was observed in the genotype M-1 (68.75 cm^2). All the genotypes had green coloured petiole. Except M-1, all the genotypes took period ranging from 28 to 31 days to initiate flower buds whereas M-1 was very late (50.62). However, days to flower opening was almost similar for all the genotypes. The genotypes P-4 and Rupa recorded largest flowers (9.08 cm and 9.06 cm, respectively). Flowers with longer stalk were observed in Royal Orange, P-4, Arka Agni and Arka Bangara 2. Fresh flower weight and petal yield per flower were very high for Rupa (23.36 g and 18.16 g) and P-4 (22.66 g and 16.56 g). The genotype P-4 recorded the maximum number of flowers (116.91) and yield per plant (1.034 kg). Number of harvests was more for genotypes Rupa, Royal Orange, P-4, Arka Agni and Arka Bangara 2. Carotenoid content did not vary among the genotypes. The genotype M-1 was observed as 100 per cent resistant to bacterial wilt whereas Sakura 031 was found to be highly susceptible, expressing disease incidence at vegetative stage itself.

Under artificial inoculation also, the genotype M-1 was found to be resistant to bacterial wilt and Sakura 031 was highly susceptible (100 per cent incidence). All the three inoculation methods *viz*., root dip, media drenching and stem injection recorded

the same efficacy in inducing the disease. Under spot planting technique also, M-1 spot planted with Sakura 031 showed no wilt incidence. Disease incidence in check genotype Sakura 031 ranged from 83.33 to 100 per cent whereas the disease incidence in genotypes spot planted with Sakura 031 ranged from 0 to 100 per cent.

For grafting studies, the genotype M-1 was used as rootstock for the other genotypes. Bacterial wilt incidence was not observed in grafted plants of all the genotypes. However in non-grafts the disease incidence ranged from 55.05 to 77.77 per cent. Maximum field survival of the grafts was recorded in P-4 (94.44 %). Irrespective of the genotypes, grafted plants showed significantly higher plant height and leaf area when compared to non-grafted plants. There were no significant differences observed in plant spread and number of branches between grafted and non-grafted plants. Grafted plants were significantly earlier to initiate flower buds (28.43 days) compared to non-grafted plants (29.36 days). However for flower opening, grafted plants took more days (10.37) compared to non-grafted plants (9.19). There was no significant difference observed between grafted and non-grafted plants with respect to flower diameter, stalk length, flower weight and petal yield per flower. Irrespective of the genotypes, grafted plants showed significantly higher number of flowers, yield per plant and more number of harvests.

Among the genotypes studied, shelf life of flowers ranged from 3 to 5 days under ambient storage condition. All the genotypes showed an accelerated loss in weight of flowers after three days of storage.

The study clearly showed that the genotype P-4 was found to be promising with high yield, large sized flowers and moderate susceptibility to bacterial wilt. The genotype M-1 was 100 per cent wilt resistant whereas Sakura 031 was found to be 100 per cent susceptible to bacterial wilt. The genotype M-1 can be used as rootstock for grafting susceptible genotypes. Grafting the susceptible genotypes on resistant rootstock was found to be an effective tool to combat the bacterial wilt. In general grafted plants of all genotypes produced more yield.