ASSESSMENT OF VARIABILITY AND COMPATIBILITY IN *Tagetes* spp.

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

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COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2007

DECLARATION

I hereby declare that the thesis entitled **'Assessment of variability and compatibility in *Tagetes* spp.'** 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.

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CERTIFICATE

Certified that this thesis entitled **'Assessment of variability and compatibility in *Tagetes* spp.'** is a bonafide record of research work done independently by **Mr. Kishore Boddu** under my guidance and supervision and formed the that it has not been previously formed the basis for the award of any degree, diploma, fellowship or associateship to him .

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ACKNOWLEDGEMENT

It is with great respect and devotion, I place on record my deep sense of gratitude and indebtedness to my major advisor ***Dr. Sujatha. R,*** *Assistant Professor, Department of Plant Breeding and Genetics and chairperson of my Advisory Committee for her sustained and valuable guidance, constructive suggestions, unfailing patience, friendly approach, constant support and encouragement during the conduct of this research work and preparation of the thesis. I gratefully remember her knowledge and wisdom which nurtured this research project in right direction without which fulfillment of this endeavor would not have been possible.*

I place a deep sense of obligation to ***Dr. V. V Radhakrishnan****, Associate Professor and Head, Department of Plant Breeding and Genetics, College of Horticulture and member of my Advisory Committee for the help and co-operation received from her during the entire programme. He in spite of a busy schedule has offered constructive suggestions for the betterment of this manuscript.*

I am deeply indebted to ***Dr. Jyothi Bhaskar****, Assistant Professor, Department pomology and floriculture, College of Horticulture, and member of my Advisory Committee for her unstinted support, critical comments and valuable suggestions during the preparation of this manuscript.*

I am very thankful to ***Dr. D. Girija****, Assistant Professor, CPBMB, Vellanikkara for kindly providing facilities and timely help at various stages of my work.*

I am extremely delighted to place on record my profound sense of gratitude to Dr. Presanna Kumari, Associate Professor, Department of Plant Breeding and

Genetics, for her unbounded support, valuable suggestions and whole- hearted co-operation towards the satisfactory fulfillment of this endeavor

I am very thankful to Dr. C.R. Elsy, Assistant Professor, Department of Plant Breeding and Genetics for the guidance she extended, the encouragement he showered is of great value to me. Her uncompromising attitude towards work and working culture enabled me to learn great strides in my professional career.

I am very thankful to Dr. Dijie Bastian Assistant professor, Department of Plant Breeding and Genetics for her kindly support through out my M. Sc course.

I extend my profound thanks to Dr. P.K. Rajeevan, Associate Professor and Associate Dean, College of Horticulture for his unclenching support during the fledging stages of my M. Sc course.

I am extremely grateful to all the staff members of Department of Plant Breeding and Genetics, for their valuable help during the course of study

I am genuinely indebted to Sri. S. Krishnan, Assistant Professor, Department of Agricultural Statistics for his valuable assistance and guidance during the statistical analysis of the data.

I take this opportunity to thank my seniors Vishnu vardhan reddy Banda and Bhaskar reddy. D, for their support and encouragement.

Words cannot really express the true friendship that I relished from Shareesh Nalpadi and all my M.Sc. Classmates, for the heartfelt help, timely suggestions and back-up which gave me enough mental strength to get through all mind-numbing circumstances.

Ineffable thanks to my dearest Mamatha Reddy Eruva, Pradeep kumarBemberi, sharma tulasi, Madhu kumar korla, Ravi boddu, Ranjit boddu for their eternal support and honest guidance.

I express my sincere thanks to Valsala chechi and the Research Assistants in the Department of Plant Breeding and Genetics and CPBMB, and the AINP on M&AP workers for the timely and wholehearted co-operation and assistance they always accorded me during the course of investigation.

I am happy to place on record my sincere thanks to my dear juniors Devi prasad, Madhu, Rahul, Jagadish, Alok, Dinesh, Thyagarajan, Manikandan, and Satish raj for their immeasurable help and support during the course of this study and I thankfully acknowledge Arun babu for his valuable help in computer work.

I deeply indebted to my family members without whose moral support, blessings and affection this would not have been a success.

*Finally, I bow my head before **THE ALMIGHTY** whose blessings enabled me to undertake this venture successfully.*

21/11/07

Kishore Boddu

To my beloved Friends and Parents

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Introduction

1. INTRODUCTION

Marigold, botanically known as *Tagetes* belonging to the family Asteraceae is a native of Mexico. In India the area under marigold is around 17,600 hectares with a production of more than 2,00,000 metric tones and ranks first among the loose flowers (Naik *et al.*, 2004). Karnataka, Haryana, Maharashtra, New Delhi, Punjab, Tamil Nadu and West Bengal have sizeable area under the crop where as in Kerala the cultivation is limited to isolated traits in Palghat and Ernakulam districts.

Marigolds are premier annuals, which perform well in dry, hot, sunny locations and produces dark green, fine textured foliage and bright coloured flowers through out the year. This flower has gained considerable popularity due to its excellent keeping quality, attractive colours and wider adaptability to different agroclimatic conditions. Apart from the floristic use the demand for various products such as marigold meal, olcoresins, meal pellet and natural pigments extracted from the flowers is increasing in the world market. It is reported that India's share is 25 per cent in the Rs. 300 crore world market for these products (Venkataraman, 2005). It is gaining commercial significance with the diversity of genotypes and uses which it can be put to. The extraction of food colourant in the form of luetin has emerged as a dietary supplement in poultry industry. Purified extract from marigold petals mainly containing xanthophyll dipalmitate (lutein dipalmitate) is marketed as an ophthalmologic agent under the name Adaptional (Bioquimix Reka, Mexico).

In India Marigolds are commonly used for making garlands for religious and social functions and in gardens for beautification in beds and borders. In addition, marigolds are highly effective in keeping the nematode population in soil under control (Bose, 1989). Over past, five different species of *Tagetes* have been introduced in Indian gardens (Darokar *et al.*, 2000). Two different species of *Tagetes* commonly cultivated are:

1. African marigold (*Tagetes erecta*) (2n= 2x= 28)

2. French marigold (*Tagetes patula*) (2n= 4x= 56)

African marigolds, some times referred to as American marigolds, are larger plants than the French type, often with fewer and larger double flowers. In the double-flowered cultivars, there are crested doubles, whose flowers are mounded and full, and anemone doubles whose flowers are flat and wide with a recessed center. This species is of great horticultural importance and is grown commercially for its exquisite blooms. It is popular with landscape designers due to its varied height and colours for use in shrubberies and as herbaceous border in gardens. French marigold plants are usually smaller than African types. Most of them are only six to eight inches in height but some cultivars may reach up to 12 inches. Though double flower petal arrangements are available, single and semi double are common. The single flowered cultivars stand up to heavy rain and humidity in the South India better than double flowered cultivars.

Marigolds had a strong fragrance that some people found objectionable. Newer cultivars are somewhat less pungent. Presently most of the cultivated types are open pollinated progenies which are less vigourous, uneven in height and with low yield. Keeping in view of the importance of marigolds and for successful exploitation in flower industry, it requires proper selection of cultivars and development of superior varieties and hybrids belonging to different species. The choice of appropriate parents is an important criterion in any hybridization programme. Information on nature of gene action governing the yield and yield components as well as various physiological and biochemical traits in marigold are essential for identifying potentially useful parents. The success of breeding programme depends upon the quantum of genetic variability available for exploitation together with the heritability estimates, association of different traits and compatibility.

Hence the present study was carried out with the following objectives.

1. Assessing the spectrum of variability and heritability for various attributes.
2. Understanding the association of flower yield and yield attributes at genotypic and phenotypic level.
3. Grouping of accessions based on genetic divergence and identification of superior genotypes on the basis of selection index.
4. Studying the reproductive biology for testing the self and cross compatibility between different genotypes.
5. Assessing the superiority of the hybrids from the parents based on heterosis studies.
6. Characterization of superior hybrids based on biochemical and RAPD analysis.

Review of Literature

2. REVIEW OF LITERATURE

Tagetes, commonly known as marigold (F: Asteraceae), is a native of Mexico. It is an annual shrub growing wild and cultivated for its ornamental value and fragrance (Darokar *et al.*, 2000). Among different types, African marigold (*Tagetes erecta L.*) is popular with landscape designers, for use in shrubberies and as herbaceous border in gardens due to its varied height and colours. French marigold (*Tagetes patula L.*) is ideal for hanging baskets and window boxes.

The relevant literature pertaining to crop improvement in marigold is reviewed under the following headings. In those aspects in which sufficient literature was lacking, reports on related crops like sunflower, gerbera, dhalia belonging to the same family are also cited.

2.1. Variability

2.2. Reproductive biology

2.3. Self and cross compatibility

2.4. Heterosis

2.5. Biochemical analysis

2.6. Molecular characterization

2.1. Variability studies

Several authors reported wide range of variation in plant and flower characters among the marigold genotypes.

2.1.1. Extent of variation for yield and related traits

Singh *et al.* (1998) in a comparative study involving four varieties each of African and French marigold observed that average yield in each type was 164.889 and 130.278 q/ha respectively. Kennedy (1997) in a study on twenty diverse genotypes of African marigold with respect to five seed characters

observed highly significant differences for vigour index, germination percentage and seedling dry weight.

Variability studies by Singh *et al.* (2003) to assess the performance of 12 marigold cultivars of both African and French marigold revealed that in French marigold maximum number of branches after 60 days of transplanting was 12.31 per plant, maximum duration of flowering was 64.33 days and minimum was 44.33 days. The maximum number of flowers per plant was 59.6 and minimum recorded was 20.3. For African marigold, the duration of flowering ranged from 65.33 days to 45.43 days. The earliness of flowering recorded was 26.53 days after transplanting (DAT) and longest time to flower recorded was 36.33 days after transplanting (DAT). The maximum duration of flowering recorded was 65.3 days. The maximum number of flowers per plant and maximum fresh weight of flower recorded were 44.30 and 15 g respectively.

A study conducted by Verma *et al.* (2004) using 12 genotypes of African marigold and 20 genotypes of French marigold observed that the plants reached a height of 208.01 cm and leaf length of 18.86 cm. The highest value for peduncle length was 6.8 cm and that of number of branches for plant was 25.80. The highest stem diameter was 1.81 cm, the highest plant canopy spread was 6855.11 cm^2 and the highest flower diameter recorded was 7.67 cm. but Singh *et al.* (2003) observed that the maximum diameter of flower at full open stage was 7.87 cm.

Singh and Singh (2005) studied the performance of 29 genotypes of African marigold and observed significant variation for all the growth and flowering parameters. The highest values reported for these characters were as follows: number of primary branches per plant (18), number of flowers per plant (85.67), dry weight of leaf (0.34 g per plant),dry weight of flower (6.68 g), flower longevity (66 days), duration of flowering (135.33 days) and days taken for bud initiation (30.67 days). The evaluation of 13 genotypes of French marigold also revealed wide variation. Flower diameter recorded a maximum of 4.03 cm, yield of flower 309.40 g per plant, peduncle length 1.73 cm and

fresh weight of flower 2.736 g. Maximum number of seeds per peduncle was 95, where as minimum seed yield per plant was 23.44 g.

2.1.2. Biometrical analysis of quantitative traits

Several workers in marigold have attempted the biometrical analysis of yield components and quality aspects. The relevant literature pertaining to these studies are reviewed under the following sub heads.

a) Genetic variability

b) Heritability, genetic advance and genetic gain

c) Correlation studies

d) Path coefficient analysis

e) Genetic divergence

f) Selection index

a) Genetic variability

The success of any breeding programme for evolving superior cultivars with improved characters depends upon the nature and magnitude of genetic variability. Many reports revealed the extent of variability in this crop by working out the genotypic and phenotypic coefficients of variation.

Kshirsagar *et al.* (1995) in an evaluation study of 14 cultivars of sunflower reported that variation was greatest for seed yield per plant, followed by plant height and 100 seed weight.

A study conducted by Janakiram and Rao (1995) in African marigold showed that PCV was high in closer spacing (30 X 20 cm) than in wider (30 X 30 cm) spacing except for the traits, number of lateral branches and flower size. The GCV was higher at closer spacing for characters like days to flower, plant height, number of flowers per plant and total yield per plant.

Patil *et al.* (1996) in genetic variability studies in sunflower observed that the range of variation was maximum for number of seeds per head followed by

weight of head and seed yield. Mostly, higher mean value was associated with greater range. The magnitude of variation was maximum for weight of head followed by seed yield.

Sirohi and Behera (2000) observed that PCV was higher than GCV for all the characters studied in gerbera. Highest GCV and PCV were recorded for number of flowers per plant followed by number of branches per plant.

Singh and Sen (2000) observed lowest coefficient of variation for duration of flowering and highest for flower yield (kg/ plot) in French marigold, while in African marigold it was lowest for plant height 60 days after transplanting and highest for flower dry weight.

Kishore and Raghava (2001) in a study conducted on 18 genotypes of African marigold with respect to 16 different characters reported a high range of variation for seed yield per hectare, flower weight per plant and days to flowering. In general, the PCV were higher than the GCV. Both PCV and GCV were found high for flower weight per plant, followed by flower yield per hectare and seed yield per flower.

A study by Patnaik and Mohanty (2002) on the genetic variability in 13 African marigold cultivars revealed that the PCV and GCV were highest for seed yield. Tabulated data were presented on the mean performance of the 13 African marigold cultivars in terms of plant height, number of primary branches, plant spread, leaf area, number of days to flowering, stalk length, flower size, flower weight, depth of flower, duration of flowering, number of flowers per plant and flower yield per plant.

Singh *et al.* (2002) discussed the genetic variability of African marigold based on qualitative and quantitative characters.

In an investigation to understand the variation in carotenoid content (total carotenoids, total xanthophyll, xanthophyll esters, free xanthophyll and xanthophyll) and yield components (plant height, plant spread, number of branches, days to flower, number of flowers per plant, flower weight, flower

diameter, flowering duration and flower yield) among 10 African marigold genotypes, all the characters showed higher phenotypic variance than genotypic except for flower diameter. Among the yield components, flower diameter showed the greatest variation. Total carotenoids and all its fractions showed higher phenotypic than genotypic variance (Sreekala *et al.*, 2002).

A study conducted by Sujatha and Shiva (2002) on 51 inbreds and 3 control genotypes of sunflower observed that the range of variation was highest for per cent autogamy followed by plant height and oil yield, where as it was lowest for number of days to 50 per cent flowering and stem girth. The magnitude of variation was highest for oil yield, 100 seed weight, head diameter, per cent autogamy, husk per cent, seed density, plant height, seed test weight and oil content.

In marigold, Verma *et al.* (2002) reported genotypic and phenotypic coefficient variations in 33 germplasms. The differences among genotypes were significant for all the characters observed (leaf length, leaf width, pedicel length, number of branches per plant and stem diameter). The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) in all the characters. Sreekala *et al.* (2002) also observed that all the characters (plant height, plant spread, number of branches, days to flower, number of flowers per plant, flower weight, flowering duration and flower yield) showed higher PCV than GCV except for flower diameter. In their study, flower weight and plant canopy showed high PCV and GCV, but Mathew *et al.* (2005) observed that the GCV and PCV was maximum for seed yield along with dry weight of flowers, flower yield, seed vigour and fresh weight of flowers.

Nair and Shiva (2003) observed that range of variation was maximum in cut flower yield per m^2 followed by leaf area in 25 genotypes of gerbera.

In dahlia, Singh (2003) observed significant difference among different germplasms for all the characters except days to fifty per cent flowering. High PCV and GCV were found for number of flowers per plant followed by dry

flower weight whereas narrow difference between PCV and GCV were noticed for days to 50 per cent flowering and fresh flower weight.

Rao *et al.* (2003) studied the genetic variability character association for 11 characters with 82 genotypes of sunflower and observed that the highest PCV and GCV of variation were recorded for number of filled seeds per head (48.127 and 47.776 respectively).

Talukdar *et al.* (2003) in a study on eleven cultivars of spray chrysanthemum reported that the estimates of GCV and PCV clearly revealed maximum genetic variation for leaf number followed by number of primary branches and floral characters, the maximum genetic variation for number of flowers per plant followed by number of ray florets.

b) Heritability, genetic advance and genetic gain

Kshirsagar *et al.* (1995) evaluated 14 genotypes of sunflower and estimated that heritability was highest for plant height and 100 seed weight, while that for yield was moderate.

Kennedy (1997) in a study involving 20 diverse genotypes of African marigold reported that there was high heritability along with high genetic advance for vigour index and germination percentage, indicating that these traits will be amenable to selection. High heritability coupled with low genetic advance was noted for 1000-seed weight and seedling length.

In a study conducted on 12 cultivars each of African marigold and French marigold, Singh and Sen (2000) observed high heritability with high genetic advance for flower yield per plot and per hectare in both the species.

Sirohi and Behera (2000) in gerbera observed high heritability and high genetic advance for number branches per plant, disc diameter, number of petals per flower and yield of flower.

Kishore and Raghava (2001) in a study conducted on 18 genotypes of African marigold reported high estimates of heritability for all the characters tested. The highest heritability values were recorded for days to flowering,

flower diameter and shelf life of flowers, followed by plant spread, number of primary branches and seed yield. High heritability and genetic gain was observed for seed yield and flower yield per plant. High heritability and low genetic gain were observed for days to flowering, flower diameter and shelf life of flowers. Medium heritability and low genetic gain were observed for plant spread, number of primary branches and seed yield per hectare.

A study by Patnaik and Mohanty (2002) revealed that the genetic advance was highest for seed yield whereas heritability in the broad sense was highest for flower weight.

In a study conducted by Sujatha and Shiva (2002) on 51 inbreds and 3 control genotypes of sunflower high heritability coupled with high genetic advance was recorded for plant height, per cent autogamy, per cent seed set and yield per plant. High heritability along with genetic advance was observed for the number of leaves per plant, head diameter, stems girth, days to 50 per cent flowering, 100 seed weight and oil yield.

In a study, Mishra and Roy (2005) estimated that narrow sense heritability in sunflower was low for head diameter followed by leaf length, leaf width and oil content; moderate for days to maturity, flowering time and plant height and high for days to first flowering, days to 50 per cent flowering and seed yield per plant

Nair and Shiva (2003) observed that heritability estimates and genetic advance were highest for cut flower yield and leaf area in gerbera.

In an experiment by Mathew *et al.* (2005) to study the variability and heritability of 24 characters in 15 genotypes of African marigold, high heritability along with high genetic advance were recorded for characters such as number of buds per plant, number of flowers per plant, flower yield and seed vigour, is a criteria for selection. However, Sreekala *et al.* (2002) observed high values of heritability for flower diameter (92.20%) among yield components, indicating that selection can be a good method for the genetic

improvement of this character. High values of heritability in the broad sense were recorded for plant height, plant spread, number of branches, and flower yield per plant.

Mathew *et al.* (2005) reported high heritability along with high genetic advance for characters such as number of buds per plant, number of flowers per plant, flower yield and seed vigour.

c) Correlation studies

Studies conducted by Pratap *et al.* (1999) on 10 divergent genotypes of African marigold, revealed that plant height had a positive and significant relationship at the genotypic and phenotypic levels with spread of plant and number of lateral branches. Spread of plant was also positively and significantly correlated with size and yield of flowers per plant. In French marigold, plant height showed positive and significant correlations with number of lateral branches, days to bud visibility and first and last picking of flowers. Spread of plant showed a positive correlation with number of flowers per plant. Days to bud visibility were positively and significantly correlated with first and last picking of flowers. First and last picking of flowers were also positively and significantly correlated.

Sirohi and Behera (2000) reported that in chrysanthemum there was positive and significant phenotypic association of yield with number of flowers per plant, plant spread and number of branches per plant. They suggested that selection based on these characters would be more effective for chrysanthemum improvement.

Chikkadevaiah *et al.* (2002) in sunflower revealed that seed yield was positively and significantly associated with seed volume, weight, hull percentage, percentage autogamy, days to 50 per cent flowering and oil yield. Oil content was positively associated with plant height, number of flowers per plant, seed volume, hull percentage, head diameter, percentage autogamy, days to 50 per cent flowering, 100 seed weight and seed yield.

Mohanty *et al.* (2002b) carried out a correlation analysis on 13 diverse genotypes of African marigold for 13 characters related to growth and flowering. They reported that yield of flowers per plant showed a significant and positive correlation with plant height and plant spread (East-West and North-South direction) and phenotypic and genotypic level, whereas number of primary branches, leaf area, flower size, individual flower weight and number of flowers per plant were positively and significantly correlated to flower yield at genotypic level. A significant and negative correlation of days to flower with duration of flowering was observed both at the genotypic and phenotypic level.

Studies conducted by Sujatha and Shiva (2003) on 25 genotypes of gerbera revealed that number of ray florets had high direct correlation with flower yield but the correlation is negligible due to undesirable indirect effect. Hence they suggested that restricted selection had to be imposed in order to exploit direct effect.

Studies conducted by Nehru and Manjunath (2003) in sunflower revealed that genotypic correlation coefficients were higher than phenotypic correlation coefficients. In all the characters studied, seed yield was positively associated with growth and yield components. Among the yield components, correlation of number of seeds per head was maximum followed by head diameter. Stem girth, 100 seed weight and plant height showed greater magnitude of correlation. The within and between correlation of growth and yield components were positive. Days to 50 per cent flowering showed non-significant association with seed yield and other four characters except for oil content and volume weight had negative significant and non-significant association. The correlations among yield components were positive, encouraging rapid improvement of seed yield.

Rao *et al.* (2003) in sunflower observed the presence of significant positive association of seed yield with the number of filled seeds per head, test weight, head diameter, stem girth, number of leaves per plant and plant height. In contrast, seed yield was negatively associated with oil content.

A study conducted by Kumar *et al.* (1989) in *T. minuta* revealed positive associations among plant height, plant spread, number of primary branches, number of leaves, dry matter production, number of inflorescence, herbage, oil and flower yield *etc.*, indicating that improvement in one component character led to the concurrent improvement of another. Plant height, plant spread, number of primary branches, number of leaves, dry matter production, oil content, herbage and flower yield had a significant and positive association with essential oil yield. Flower yield exhibited a significant and positive correlation with herbage yield, the number of inflorescence showed positive but non-significant association with oil and flower yields. Correlation between any two growth components except plant height was positive and significant.

A study on African marigold conducted by Naik *et al.* (2004) in two different seasons (*Kharif* and *Rabi*) analysed the association between 13 component characters which were related to growth and flowering and their relative contribution to xanthophyll yield. It was reported that the xanthophyll yield per hectare was positively and significantly correlated with petal meal yield per hectare, flower yield per plant, number of secondary branches, total dry matter production, leaf area, flower size, flower yield per hectare, xanthophyll content per kilogram of petal meal, plant height and number of petals per flower both at phenotypic and genotypic level during *Kharif* season. In *Rabi* season also majority of them showed significant and positive association with xanthophyll yield except days to 50 per cent flowering and fresh flower weight at both the levels.

Mathew *et al.* (2005) assessed the relative contribution of different yield related characters on flower and seed yield in African marigold. Characters such as seed yield per plant and number of flowers per plant showed highly significant correlation with flower yield, while the number of flowers, number of buds and flower yield per plant showed highly significant correlation to seed yield.

d) Path coefficient analysis

Patil *et al.* (1996) showed that the direct effect was maximum for number of seeds per head followed by 100-seed weight and weight of head in respect of both seed yield and oil yield. The maximum indirect effect for any character was through seeds per head.

Bandyopadhyay *et al.* (1997) in a study to assess the relative contribution of different yield attributing characters to the seed yield of African marigold observed that, the number of seeds per flower and zinc uptake by the flower were found to have the greatest positive effects on seed yield. Leaf area constant and average flower weight had the greatest negative effects on seed yield.

In a study by Sirohi and Behera (1999) on direct and indirect effects of various morphological parameters, number of flowers per plant was reported to have high direct effect on yield through number of branches.

Ashok *et al.* (2000) in sunflower observed that days to maturity, plant height, diameter of stem and diameter of capitulam showed the highest direct effects on yield. Percentage of oil content and 100-seed weight was positively correlated with yield, but showed negative direct effects on yield. Days to 50 per cent flowering was negatively correlated and showed negative direct effects on yield. Harvest index showed positive direct effects on yield, but was negatively correlated with yield. The narrow differences between genotypic and phenotypic values indicate that the environment does not play a major role in the relationship between traits.

Nirmala *et al.* (2000) in sunflower reported that head diameter, days to first flowering and number of leaves influenced the yield directly and improvement of this crop could be achieved through the component traits is significant.

Study by Chikkadevaiah *et al.* (2002) revealed that the direct effect on seed yield was highest for oil yield followed by 100-seed weight. The highest

indirect effect for seed yield and oil was minimum through oil yield and yield per plant respectively.

In a study by Sreekala *et al.* (2002) on the direct and indirect effects of various morphological parameters on the total carotenoid yield of marigold lines and cultivars observed that, plant height had low direct effect, but very high indirect negative effect through flower yield on the carotenoid yield. It also had high positive effect through the number of flowers. Plant spread had low direct effect, very high positive indirect effects through flower diameter and flower yield per plant and high indirect negative effect through flower weight. Branch number had high positive indirect effect through flower diameter. Days to flowering had negligible direct effects, but high negative effect through flower weight. The direct effect of flower number was high and negative, but the indirect effect through flower weight was significant and positive. Flower weight had high negative direct effect but high positive indirect effects through flower diameter and flower yield. Flower diameter had very high positive direct effect on carotenoid content, negative indirect effect through flower weight and very high positive effect through yield per plant. The total flowering duration had high positive indirect effect on carotenoid yield through flower weight but very high negative effect through flower yield per plant. Flower yield had high direct positive effect, very high negative indirect effect through flower weight and very high positive indirect effect through flower diameter.

A study conducted by Nehru and Manjunath (2003) showed that the direct effect was maximum for number of seeds per head followed by test weight on seed yield per plant, while seed yield had maximum direct effect on oil yield. The maximum indirect effect for any character was through seeds per head which itself was a major direct contributor.

Rao *et al.* (2003) in sunflower observed that number of filled seeds per head, test weight and head diameter had maximum direct effects on seed yield

indicating that selection based on these characters will increase seed yield per plant in sunflower.

Mathew *et al.* (2005) in an experiment with African marigold observed that, maximum direct effect on flower yield was exhibited by number of flowers per plant followed by fresh weight of flower. Positive and highly significant correlations with flower yield was obtained for number of buds per plant, number of flowers per plant, fresh weight of flower and dry weight of flower.

e) Genetic divergence

Sankarapandian *et al.* (1996) studied fifty-four genotypes of sunflower for their genetic divergence by D^2 analysis and grouped them into seven clusters based on six characters. Based on the intercluster distance and cluster mean for various characters, potential parents were identified from different clusters for hybridization programme.

The Genetic divergence analysis using D^2 method conducted by Manjula *et al.* (2001) on 46 non-oil seed sunflower genotypes based on 14 characters grouped them into 11 clusters. The inter-cluster D^2 values ranged from 288.17 to 3972.34.

Reddy and Devasenamma (2004) studied 58 inbreds and three control cultivars of sunflower for their genetic divergence using D^2 analysis and the 61 genotypes were grouped into 19 clusters. Cluster I had the maximum number of genotypes (29), whereas cluster II had the least number of genotypes (7). The highest inter-cluster distance was recorded between cluster XIV and XIX (322.26) whereas intracluster distance was highest in cluster XV (36.44).

Genetic divergence studies conducted by Rao *et al.* (2003) in inbreds and elite lines of sunflower revealed that the genotypes differed significantly for all characters studied. Multivariate analysis grouped the 94 genotypes into 10 clusters based on D^2 values. Mean values of clusters for seed yield and yield components indicated the existence of considerable distance between the various genotypes. The genotypes exhibited random pattern of distribution into

various clusters, indicating that genetic diversity and geographical diversity are not related. Among the characters, number of filled seeds had the maximum contribution to genetic divergence followed by plant height, oil content and test weight.

f) Selection index

In a study conducted by Patil (1997) in 225 accessions of sunflower from 24 countries, selection indices were computed from four yield components *viz.*, (plant height, head diameter, weight of head and number of filled seeds) were involved in formulating selection index.

Ojha and Roy (1998) observed that the selection index based on plant height, head diameter, seed filling percentage, yield per plant and plot yield showed maximum expected progress in biparental populations of sunflower. Plot yield was reported to be the most important trait in the construction of selection index, in that its absence caused maximum reduction in the gain.

2.2. Reproductive biology

In a study of pollen morphology by acetolysis method, Srivastava (1976) found two morphological types in pollen (spine with low basal cushion and spine with high basal cushion). He observed that there was a change in pollen morphology in relation to the genomic constitution of various cytotypes (2x, 4x, 3x, 6x) of marigold. In 2x (African marigold) the basal cushion is less and the spinal column is conspicuously elongated, in 4x (French marigold) the basal cushion is higher and the length of the spinal column is reduced than that in African marigold. In the 3x hybrid and the 6x amphidipolid there is remarkable increase in the height of the basal cushion and consequent reduction in the length of spinal column.

Srivastava (1994) studied the SEM micrographs of the pollen grains of the hybrids of the cross African marigold x French marigold and reported micropuncta as the dominant feature of female parent. He found that pollen

micropuncta were dense in both the pollen grains of hybrid and African marigold but sparse in that of French marigold.

Study conducted by Mohanty *et al.* (2002a) on floral biology of African marigold in different cultivars revealed that flower bud initiation varied from 17 to 24 days, while bud maturity after initiation ranged from 13 to 24 days. The color break occurred 2-5 days after bud maturity, opening of florets took 3-6 days after bud maturity, the complete florets opened within 10 to 17 days. Time of anthesis ranged between 05.00 and11.00 am in different cultivars with the maximum percentage of floret opening (43-62 per cent). Anther dehiscence started at the time of floret opening or a bit longer after floret opening. When the florets were pollinated on the day of opening, very little or no seed set was observed in various cultivars. The percentage of seed germination ranged from nine to 20 when hand pollination was conducted on the third day of opening. Stigma receptivity was maximum up to 70 per cent when the florets were hand pollinated on the fifth day of anthesis.

2. 3 Compatibility studies

The interspecific hybrids (3n) between African marigold (2n= 24) and French marigold (2n= 48) have been produced in USA, which were of intermediate characters (Bose, 1989). These hybrids were dwarfs like French marigold but had bigger flowers like that of African marigold and were early flowering and medium in height (60 cm).

In an experiment on hybridization between *H.* annuus (2n=34) and different hexaploid *Helianthus spp.* Georgieva (1992) reported that the hybrids with *H. tuberosus* (2n=102) showed high degree of sterility and their fertility was improved by three to four backcrosses. The high cross incompatibility with *H. rigidus* (2n=102) was partly overcome by using *H. rigidus* (2n=102) as the seed parent. There was a greater degree of gametic compatibility between *H. annuus* (2n=34) and *H. resinosus* (2n=102) than was the case with the other two crosses and there was no reciprocal effect, but the F1 hybrids had abnormal meiosis leading to reduced fertility.

Skaloud and Kovacik (1995) studied the response to different types of self-fertilization (geitonogamy and autogamy) in six lines of sunflower. The incidence of lines with a high degree of self-fertility in the case of geitonogamy (self-pollination within a single head) was high, but self-fertile lines were rare in the case of autogamy (self-pollination of single florets). Crosses of geitonogamously self-fertile lines showed lower autogamous self-fertility than their parents.

Doddamani *et al.* (1997) in sunflower reported that all genotypes recorded higher seed yield, seed set, autogamy and self-compatibility during summer compared to the monsoon. Inbred lines were comparatively more self-compatible than hybrids and varietal population.

Raghava (1999) reported the development of two new varieties, *viz.*, Pusa Narangi Gainda and Pusa Basanti Gainda through pedigree method of breeding. F_1 hybrids were produced by using the apetalous type of male sterility.

A study conducted in safflower (F: Asteraceae) to assess self incompatibility and seed set under different kinds of bagging such as fine muslin cloth, craft paper and butter paper by Patil (2003) revealed that bagging of individual bud adversely affected seed setting, which ranged from 1.67 to 2.41 seeds per capsule. Different genotypes showed different seed setting in different types of bagging. Butter paper bagging was superior to the other bagging treatments for seed setting, while significant reduction in seed setting was reported in muslin cloth.

Self and cross compatibility studies conducted in dhalia (F: Asteraceae) showed that seed set occurred under both open pollinated (94 %) and bagged condition (87.5%), producing viable seeds (Behr and Debner, 2004). The seedlings of bagged seeds were subjected to molecular finger printing using RAPD and were reported to be produced by self-pollination proving the absence of any self-incompatibility, whereas the high seed set under open pollination was an indication of out crossing.

Fukai *et al.*, (2004) in *Dendranthema* (F: Asteraceae) reported that crosses between tetrapoloid X tetrapoloid combinations were compatible, but no seedlings were obtained from octaploid X octaploid combinations. The tetrapoloid X octaploid combination was partially compatible. The ploidy level was almost intermediate between that of the parents. Some progenies had a larger chromosome number than expected. Male sterile traits appeared in progenies derived from specific cross combinations between tetraploid and octaploid and found that the reciprocal crosses between chrysanthemum (*D. grandiflorium*) and other *Dendranthema* species were possible when the appropriate genotype was carefully chosen. Hybrid progenies showed a wide range of variation in the flowers. The hybrids were reported to require shorter days to flower than the parents do.

Nikolova (2004) reported successful seed set on the cross compatibility in a cross between wild perennial diploid species and the cultivated species of sunflower. Germination and viability of seeds were taken as an indication of successful interspecific hybridization and also the compatibility was reported to be significantly higher when the wild species was used as the male F_1 hybrids showed annual growth habit, heterosis and high level of variation for plant height, total number of disk florets and inseminated disk florets.

2.4. Heterosis

A study conducted by Kumar *et al.* (1989) on hybrid vigour in marigold for economic characters revealed that out of 27 hybrids, 10 expressed significant and positive heterobeltiosis in respect of plant height and duration of flowering. The maximum heterobeltiosis recorded for plant height was 32.50 per cent. The highest percentage of heterobeltiosis for number of branches recored was 34.41 per cent and the range was –42.04 to 34.41 per cent. Thirteen hybrids registered negative heterobeltiosis for days to first flowering and the range was from –9.95 to 12.21 per cent. The heterobeltiosis for duration of flowering ranged from –17.17 to 13.76 per cent. The heterobeltiosis for flower diameter ranged from –74.76 to 63.97 per cent. For flower weight, eight hybrids exhibited positive and

significant heterobeltiosis and the maximum for this character recorded was 96.76 per cent. The range for number of flowers was from 0.93 to 57.67 per cent. Positive heterobeltiosis was recoded in 17 hybrids and the maximum was 59.75 per cent. In respect of yield of flowers per plant, the maximum heterobeltiosis was 748.55 per cent.

A diallel analysis carried out by Reddy *et al.* (1989) including reciprocals observed that heterosis over better parent ranged from –8.91 to 75.30 per cent in metric traits like plant height, number of branches per plant, days to 50 per cent flowering, flower diameter, flower weight, number of flowers per plant, and flower yield per plant. Reciprocal differences were also significant in crosses for all the characters except number of branches per plant and days to 50 per cent flowering.

Gupta *et al.* (2001) in a line x tester programme observed heterosis in all traits except for stalk length and number of seeds. The highest heterosis was observed in flower yield (510.12 %).

A study conducted by Mohanty *et al.* (2003) on both the African marigold and French marigold showed highly significant difference in variance among parents and among the crosses for all characters, except flower stalk length, flower diameter and flower weight. The mean sum of squares due to parents vs. crosses was highly significant for all characters indicating the presence of significant heterosis.

Mohanty *et al.* (2003) in African marigold reported that the negative heterosis was preferred for days to first flower. Heterosis ranged from –26 to 15 per cent and –2 to 19 per cent over mid parent (MP) and better parent (BP) respectively. The range of heterosis for flower diameter varied from –19 to 34 per cent and –9 to 27 per cent over MP and BP respectively. The range of heterosis for flower size recorded was –19 to 34 per cent over MP and –9 to 27 per cent over BP. Among the 24 crosses, 4 crosses over MP and 20 crosses over BP manifested heterosis for flowering duration. Number of flowers was identified as the main component of yield, which ultimately resulted in increased flower yield

and the heterosis ranged from –8 to 98 per cent and –25 to 43 per cent over MP and BP respectively. Relative heterosis in flower ranged from -5 per cent to 260 per cent, while heterobeltiosis ranged from –16 to 211 per cent. Heterosis for flower yield in many crosses was attributed to significant positive heterosis in component traits like flower number, flower size and flower weight. It was reported that the hybrids which had high mean performance for different yield related characters did not exhibit high heterobeltiosis except, some crosses because better or the best parent involved in these hybrids and high mean expression.

Sreekala and Raghava (2003) reported that heterosis could be exploited for total carotenoid and its commercially important fractions in marigold. Specific combining ability variance was predominant for total content of carotenoids, lutein content of xanthophyll esters. General and specific combining abilities and heterosis were highly significant. Heterobeltiosis was also positive. General combining ability (GCA) variances were not significantly correlated to performance *per se*. There was also no correlation between performance *per se* of normal petalled pollen parents and the performance of crosses made between male-sterile (female) and male-fertile (pollen) parents. These findings suggest that carotenoid content should not be the only criterion considered in the selection of parental lines.

A study was conducted by Velmurugan *et al.* (2003) on heterosis and combining ability effects for xanthophyll content in African marigold by using 11 parents (3 lines and 8 testers). Two lines recorded higher values than the overall mean of lines. Four tester parents recorded higher values than the overall mean of tester parents. Among 24 crosses, three had positive and eight had negative heterosis towards relative heterosis. Relative heterosis, heterobeltiosis and standard heterosis ranged between -18.13 and 12.96 per cent, -10.83 and 24.12 per cent, and between -22.81 and 8.77 per cent, respectively.

2.5. Biochemical analysis

Carotenoids are one of the most abundant groups of pigments present in all green tissues as a component of chloroplast and are responsible for yellow to red colouration of many flowers and fruits where they are conferred to the chromoplasts (Goodwin and Briton, 1988).

The marigold (*Tagetes spp.*) is a well-recognized source of carotenoid and xanthophylls pigments due to which it is used for salad dressing, for making ice creams, dairy products, soft drinks, bakery products, jam and confectionary.

Alam *et al.* (1968) suggested that, xanthophyll is the major carotenoid in marigold flower petal of which, luetin accounts for 80-90 per cent.

Kasemsap *et al.* (1990) evaluated 22 genotypes of *T. erecta* and *T. patula* for xanthophyll extraction and recorded that, in general *T. erecta* (15249.5 ppm) showed higher xanthophyll content than *T. patula* genotypes (maximum 12918.7 ppm).

Sreekala *et al.* (2002) in a variability study on 10 selected genotypes of African marigold assessed high variability for total carotenoids, compared to morphological characters or yield components. Heritability estimates were also high for different carotenoids. Path analysis for total carotenoid yield revealed that there was a very high genotypic and phenotypic correlation between total carotenoid yield, morphological and yield characters. The direct influence on total carotenoid yield was maximum for flower yield per plant, followed by flower diameter and flower weight.

Naik *et al.* (2004) reported that pinching has positive effect on xanthophyll yield which might be due to variation in petal meal per hectare and flower yield per hectare. Also, spraying chemicals especially DAP at (2 %) spray recorded maximum xanthophyll yield (15.51 Kg/ha).

2.6. Molecular characterization

Random Amplified Polymorphic DNA analysis was carried out by Darokar *et al.* (2000) to assess the extent of genetic diversity in six accessions each of *T. minuta* and *T. patula* collected from different geographical parts of India. Out of 20 decamer random primers used to amplify the genomic DNA, 15 responded positively to yield amplification products as discrete bands in both the cases. In case of *T. minuta* only seven per cent polymorphism was observed and all the six accessions showed 95-100 per cent similarity. In the case of *T. patula*, more than 90 bands were produced out of which 70 per cent were polymorphic. Relatively, much higher variation was observed among the accessions of *T. patula* collection.

Faure *et al.* (2002) crossed the annual diploid sunflower with the perennial diploid species *H. mollis* and *H. orgyalis*. Hybridization success was low but in cytological examination, all plants appeared to be diploid. However, the phenotypes of these diploids were not intermediate between the parents and despite great variation, they resembled the female parent-type predominantly. Thirty five per cent of plants obtained from sunflower pollinated with perennial *Helianthus* had a phenotype resembling the female sunflower parent. The hybrids and parents were subjected to RAPD and RFLP analysis. It was reported that only five per cent of the minimum number of expected RAPD and RFLP bands from male parents were recovered in plants produced from mature seeds after pollination of sunflower by *H. mollis*. More hybrids were found among plants obtained from embryo rescue, with an average of 25 per cent of the male parent bands recovered per plant. Analysis of individual plants indicated the occurrence of various levels of hybridization. There was a significant positive correlation between the number of phenotype traits related to hybrid status and the number of bands derived from the male parent.

Sherawat *et al.* (2003) examined the genetic variation in 13 commercial chrysanthemum cultivars using RAPD. Genetic variation was studied using 60 random decamer primers, of these, 31 primers amplified the genomic DNA.

For the cultivars tested, between two to 21 bands were obtained for each primer and out of a total 257 clear and reproducible bands, 239 were polymorphic. The amplified DNA fragments normally ranged from 0.55 to 2.00 kb. For 11 out of the 13 genotypes, the primers screened revealed RAPD fragment(s) unique to a particular cultivar. RAPD data of different cultivars were used to calculate squared Euclidean distance matrix and based on this, cluster analysis was done using unweighted pair group cluster analysis by arithmetic mean. Genetic variation amongst cultivars was high enough to divide them into two major groups. These groupings were in consistent with their morphological differences and geographical distribution. The results indicated that RAPDs were efficient for the identification of chrysanthemum cultivars and for the determination of genetic relationships.

Kumari *et al.* (2006) assessed the molecular diversity in *T. erecta* by using RAPD and ISSR markers. The genomic DNA extracted from callus cultures were used to generate PCR profiles. RAPD amplifications were generated using four random decamer primers viz., OPE-7, OPE-14, OPI-1, and OPI-6 selected after screening 40 primers. Total 25 bands were generated from RAPD reaction of which 13 were found to be polymorphic. The dendrogram constructed showed four major clusters. The principal component analysis showed maximum of 52 per cent variability distributed on the 1st dimension and 12 per cent on the 2nd dimension. Though the callus was induced from a single explant source, it showed considerable amount of variability existing at molecular level.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation on 'Assessment of variability and compatibility in Tagetes spp.' was carried out at the College of Horticulture, Kerala Agricultural University, Vellanikkara during the period from 2006 to 2007. The crop was raised in the field attached to the All India Network Project on Medicinal and Aromatic plants, in the Department of Plant Breeding and Genetics and the laboratory works were conducted at the Department of Plant Breeding and Genetics as well as Center for Plant Biotechnology and Molecular Biology (Plate 1).

Materials

The experimental material consisted of a heterogeneous population of African and French marigold collected from different places in Andhra Pradesh, Karnataka and Kerala (Table 1). There were 14 different accessions of African type and 7 accessions in French type. African types were laid out in the field in randomized block design with two replications and the standard cultivation practices as per the Package of Practice Recommendations KAU (2002) were adopted. The French types were raised in pots and as the number of plants available in each variety was different, proper statistical analysis could not be carried out.

Methods

The project consisted of the following experiments:

3.1 Evaluation of variability in African marigold (*T. erecta*) and French marigold (*T. patula*) based on morphological traits

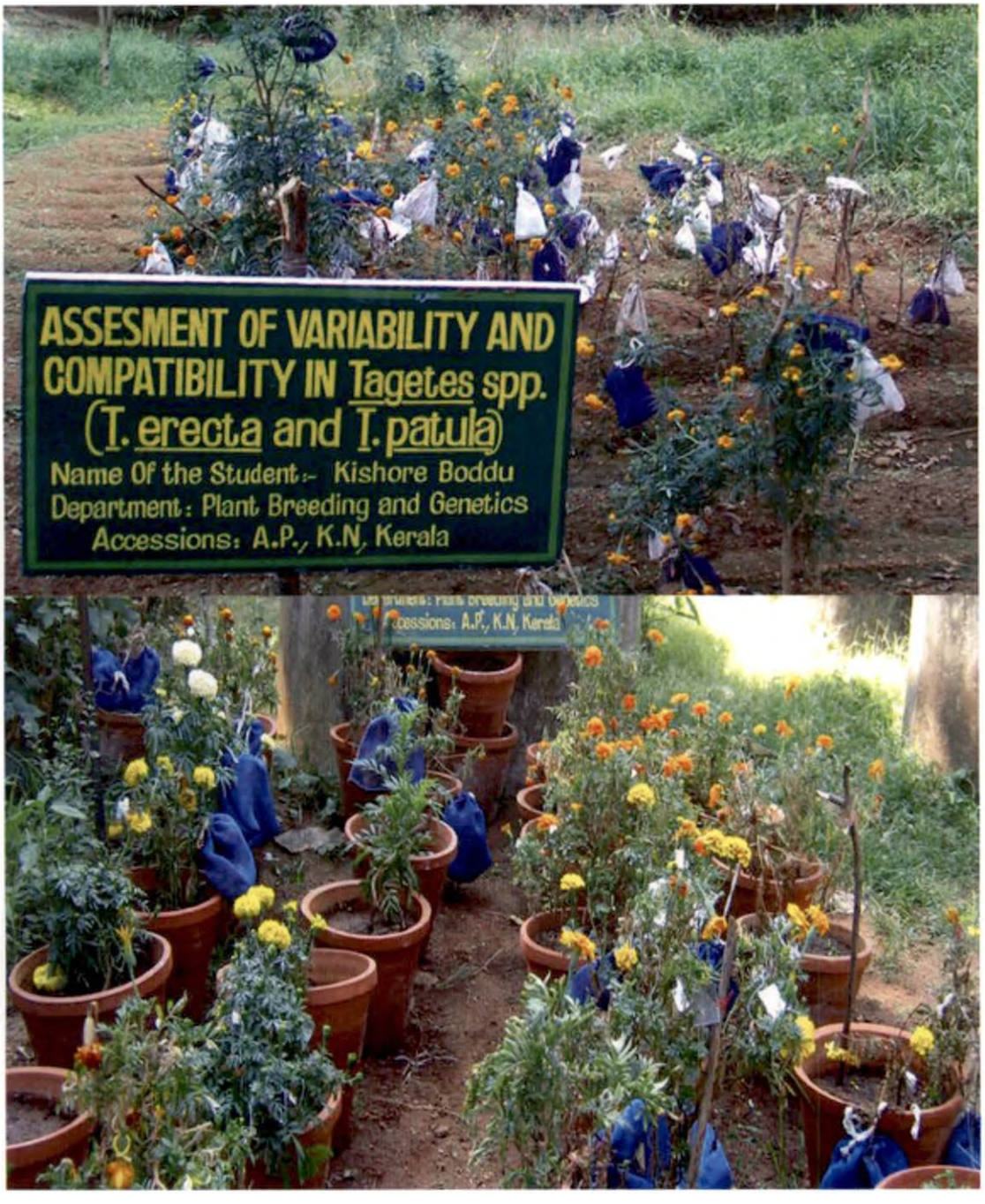
3.2 Study of reproductive biology and compatibility

3.3 Estimation of heterosis and biochemical as well as molecular characterization of F_1 hybrids

Table 1. Experimental material in African and French marigolds

Sl. No.	Accession	Place of collection	Number of plants
African marigold			
1	Thiruvambadi (Th)	Thiruvambadi, Thrissur, Kerala	5
2	Peringavu (P)	Peringavu, Thrissur, Kerala	5
3	Kapugal (Ka)	Kapugal, Andhra Pradesh	5
4	Chembukavu (C)	Chembukavu, Thrissur, Kerala	6
5	Thammara (T)	Thammara, Andhra Pradesh	5
6	Pilathara Yellow (Pil.Y)	Pilathara, Kannur, Kerala	6
7	Hessargatta (Kah)	Hessargatta, Karnataka	6
8	Trikaripur (Tr)	Trikaripur, Kerala	6
9	Kodad (Ko)	Kodad, Andhra Pradesh	6
10	Sreemannarayana nagar (S)	Sreemannarayana nagar, Andhra pradesh	6
11	Pilathara orange (Pil.O)	Pilathara, Kerala	6
12	Vellanikkara (VK 1)	Vellanikkara, Thrissur, Kerala	6
13	Ananthagiri (A)	Ananthagiri, Andhra Pradesh	6
14	Vellanikkara (VK 2)	Vellanikkara, Thrissur, Kerala	6
French marigold			
1	Boy O Boy (BOB)	Banglore	9
2	Safari Bolor (S.B)	Banglore	4
3	Safari Tangerine (S.T)	Banglore	4
4	Little Hero Fire (L.H.F)	Banglore	3
5	Boy Spry (B.S)	Banglore	6
6	Trichur Local (T.L)	Trichur	2
7	French Vanilla (F.V)	Banglore	1

Plate1. General view of the field



3.1 Evaluation of variability in African marigold (*T. erecta*) and French marigold (*T. patula*) based on morphological traits

Observations were recorded from all the plants and the mean was worked out for each accession. To record the leaf and some of floral characters five plants were selected from each accession to keep the sample size uniform. Pinching was carried out in all the plants 30 days after transplanting.

3.1.1 Extent of Variation for quantitative characters

a) Plant height

Height of each individual plant was recorded in centimeters.

b) Stem girth

Stem girth at collar region of each plant was measured in centimeters using a twine and scale.

c) Number of branches

Number of branches was counted from each plant at full blooming stage.

d) Leaf length

Four functional leaves from the top 3rd to 6th nodes were selected from each of the five plants per accession. The length was measured in centimeters and mean worked out.

e) Leaf width

Width of the selected leaves were measured in centimeters and mean worked out.

f) Duration of the crop

Number of days from date of sowing to death of plants was recorded for counting total duration of crop in each selected plant.

g) Days to flower

The numbers of days were counted from date of sowing to the formation of first bud and the mean was worked out for each accession.

h) Pedicel length

Pedicel length in centimeters was measured from four flowers per plant from the selected five plants in each accession and average length worked out.

i) Number of flowers

Number of flowers from each selected plant was counted throughout the flowering season and mean number of flowers per plant was worked out.

j) Fresh weight of flowers

After recording number of flowers, four flowers from each plant were randomly selected, their total weight was taken and average weight was worked out.

k) Flower diameter

Four fully opened flowers were selected randomly from each of the five plants per accession to record the diameter (cm) and average worked out.

l) No. of whorls of ray florets

From the selected four flowers per plant, whorls of ray florets were counted and average was calculated.

m) Flower yield

Flower yield was calculated by multiplying the number of flowers per plant and fresh weight of flowers.

n) Seed set percentage

Four dried flowers from each plant were selected randomly and the number of mature, black and healthy seeds was counted separately for calculating the mean.

3.1.2 Statistical analysis of data and estimation of genetic parameters based on morphological traits

The phenotypic data obtained from African marigold were subjected to analysis of variance (Singh and Choudhary, 1985). The following genetic parameters were estimated to compare the different genotypes to elucidate the correlation between various characters and to develop suitable selection criteria for the crop.

a) Variance and coefficient of variation

The phenotypic and genotypic coefficients of variations were calculated by the formula suggested by Burton and Devane (1953). The estimates of PCV and GCV were classified as

Less than 25 per cent = low

25-50 per cent = moderate

> 50 per cent = high

b) Heritability

Heritability in the broad sense (h^2) was estimated using the formula suggested by Burton and Devane (1953) and expressed in per cent. The range of heritability was categorized as follows (Robinson *et al.*, 1949).

0-30 per cent = low

31-60 per cent = moderate

60 per cent and above = high

c) Genetic advance and genetic gain

The expected genetic advance (GA) of the genotypes was measured by the formula suggested by Lush (1949) and Johnson *et al.* (1955) at five per cent selection intensity using constant K as 2.06 given by Allard (1960). The GA calculated was used for estimation of genetic gain (GG) as percentage of the mean value of the character under study.

The genetic gain was classified according to Johnson *et al.* (1955) as follows

1-10 per cent	= low
11-20 per cent	= moderate
21 per cent and above	= high

d) Correlation coefficients

The phenotypic and genotypic covariances were worked out in the same way as the variances were calculated. Mean product expectations of the covariance analysis are analogous to the mean square expectation of the analyses of variance. The different covariance estimates were calculated by the method suggested by Fisher (1937).

e) Path coefficient analysis

In path coefficient analysis the correlation among cause and effect were partitioned into direct and indirect effects of casual factors on effect factor. The principles and techniques suggested by Wright (1921) and Li (1955) were used for the analysis using the formula given by Dewey and Lu (1959).

f) Genetic divergence

The genetic divergence among 14 accessions was assessed based on different characters as given by Mahalanobis (1936). Clustering of genotypes using Mahalanobis D^2 value was carried out using the computer oriented iterative algorithm method as suggested by Suresh and Unnithan (1996).

g) Selection index

Smith (1937) model was used for formulating the selection index. This is desired to select plants, the merit (H) of which is linearly expressed as:

$$H = a_1G_1 + a_2G_2 + \ldots\ldots\ldots + a_n G_n$$

Where, G_1, G_2.....G_n represents the genotypic values of characters and a_1, a_2...a_n denote the weights to be assigned to each of the character.

3.2 Study of Reproductive Biology and Compatibility

3.2.1 Floral features

Eighteen unopened buds were collected at six different stages of development *viz.*, buds at initiation stage, sepal breaking stage, composite bud stage (where unfurling of floret was expected the following day), first ray floret opening stage, first whorl of ray floret opening stage and full bloom stage (Plate 3a). Time of anthesis, anther dehiscence and stigma receptivity were recorded. To keep the buds fresh and growing, growth solutions of different compositions were tried and the best was selected. The selected buds were kept in this solution for one week and the following observations were recorded. For studying the pollen stigma characters slides were prepared using saffranin/ acetocarmine dye and photographs were taken at 4X and 10X magnifications in a trinocular research microscope (Labomed)

a) Flower colour:

The flower colour was observed from each plant and classified as orange orange yellow, lemon yellow and yellow.

b) Insects visiting:

The possible pollinators visiting the flowers were observed throughout the flowering season.

c) Time of anthesis:

The time of breaking of sepals and of exertion of first floret and the outermost row of florets were recorded.

d) Time of anther dehiscence:

Buds of different maturity i.e. very immature, one day prior to sepal breaking, fully opened floret and fully opened flowers were observed.

e) Pollen fertility:

Pollen fertility was checked by counting normal fertile pollen using acetocarmine method and fertility was calculated as percentage of viable pollen.

f) Time of stigma receptivity:

Time of stigma receptivity was observed in the selected buds at different stages as mentioned above.

3.2.2 Compatibility studies

Self and cross compatibility were tested using selected plants.

a) Selfing

To study the self-compatibility in African marigold two different kinds of bags were used.

1. Nylon net bags (500 microns)
2. Butter paper bags

Individual buds in each plant were bagged at bud initiation stage until seed set. In each plant half of the bagged flowers left undisturbed and in the remaining ones, assisted self pollination was done by brushing two flowers of the same plant. Germination test for selfed seeds was conducted by sowing the seeds in plastic bowls and germination percentages were recorded.

b) Crossing

Parents were selected based on plant vigour and floral characters *viz.*, plant height, number of branches, number of flowers, flower colour, number of whorls of ray florets *etc.* The crossing was conducted by brushing the flowers from first ray floret opening stage to second whorl of ray floret opening stage between flowers from the selected parents. The brushing of flowers was continued for three days in the morning hours and pollinated flowers were covered immediately with bags. And only the first two whorls of seeds were collected for heteosis studies.

3.3 Estimation of heterosis and biochemical as well as molecular characterization of selected F_1 hybrids

3.3.1 Heterosis

From among all the hybrids superior ones showing high heterotic potential were identified. Relative heterosis and heterobeltiosis were estimated for all the hybrids as per the procedure of Hayes *et al.* (1995).

3.3.2 Biochemical characterization

Carried out in superior F_1 progenies of selected crosses along with their parents. Total carotenoid and xanthophyll contents were calculated by taking 500 mg of fresh flower sample. The sample was ground in 20 ml of acetone in a mortar and pestle in presence of few crystals of anhydrous sodium sulphate. The homogenate was kept undisturbed for 30 minutes and the supernatant was decanted into a beaker. Repeated the process twice and the pooled supernatant was transferred into a separating funnel. Equal quantity of petroleum ether was added and mixed thoroughly until the two layers separated. The lower layer was discarded and the upper layer was collected into a beaker and absorbance was recorded at 452 nm.

Standard curve preparation

One mg of beta-carotene was dissolved in 25 ml chloroform and made up the volume to 250 ml with petroleum ether (1ml= 0.1mg or 100). 10 ml of this

solution was diluted to 100 ml again with petroleum ether (1 ml= 10 µg). Pipetted out 5,10,20,25 and 30 ml of this solution to separate 100 ml volumetric flasks, added 3 ml of acetone to each sample and made up the volume to 100 ml with petroleum ether. The concentration was 0.5, 1.0, 2.0, 2.5 and 3.0 µg /ml and measured the absorbance at 452 nm using 3 per cent acetone in petroleum ether as blank. The absorbance was plotted against concentration (Fig.1).

Based on these values total carotenoid and xanthophyll content was calculated using the following formula .

$$\mu g \text{ of carotene/sample} = \frac{\text{Concentration of carotene solution as read from standard curve (µg/ ml)} \times \text{Final volume} \times \text{Dilution}}{\text{Weight of sample}}$$

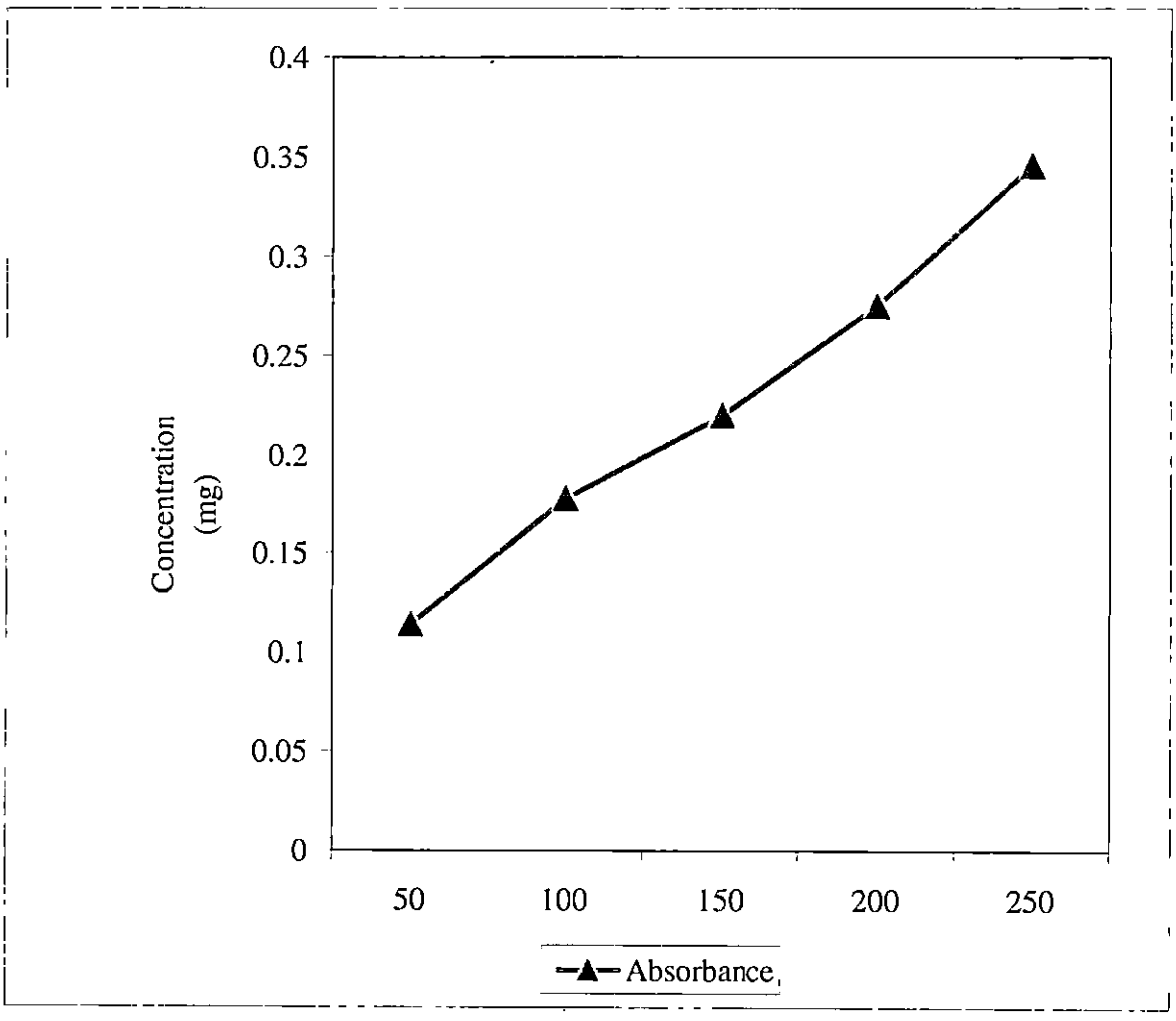
3.3.2 RAPD analysis

To characterize the F_1 hybrids in relation to the parents RAPD assay was carried out. DNA was isolated following modified Doyle and Doyle (1987) method using selected parents and their progenies.

Procedure:

One g of fresh tender leaves were collected from the selected plants, wiped with 70 per cent alcohol and transferred to ice cold mortor along with 50 µl beta mercaptoetanol and a pinch of sodium meta bisulphate, The sample was ground into fine powder using liquid nitrogen and the powder was transferred to a 50ml centrifuge tube containing 4 ml extraction buffer, 3ml lysis buffer and 1 ml sarcosine. The homogenate was incubated for 45min at 65 ^{O}C added 8 ml of (24:1) chloroform: isomylalchohol and centrifuged at 10,000 rpm for 10 min. at 4^{O}C. Supernatant was collected, 1/3 volume of chilled isopropanol was added and incubated at -20 ^{O}C for 30 min.

Fig.1 Standard curve of ß carotene



The precipitated DNA was pelleted by centrifugation at 10,000 rpm for 10 min. at 4 ^{O}C. The pellet was collected and washed first with 70 per cent alcohol and 100 per cent alcohol, air-dried and dissolved in 100 µl of TE buffer

The quality was checked by electrophoresis on 0.7 per cent agarose gel using 1X TAE buffer. The gel was then viewed using a transilluminater and the image was stored in the Alpha Imager Gel Documentation System (Alpha Innotech Ltd). The DNA was quantified using a NanoDrop (NanoDrop Technologies, Inc. USA) by measuring the absorbance at 260 and 280 nm and the samples were used for RAPD assay.

Reaction mixture

Reaction mixture consisted of 50 ng of DNA, 0.3 unit of Taq DNA polymerase, 100 µM each of dNTPs, 1.5 mM of $MgCl_2$ and 10 p mol. of decamer primers for each reaction tube. A mater mix for the required number of reactions was prepared first and aliquots were dispended into 0.5 ml or 0.2 ml PCR tubes. The PCR was carried out in a Thermal Cycler (Master cycler personal) from Eppendorf, Germany.

Thermal cycle

Initial denaturation	94 ^{O}C (3min)	
Denaturation	92 ^{O}C (1min)	40cycles
Annealing	37 ^{O}C (1min)	
Extension	72 ^{O}C (2min)	
Final elongation	72 ^{O}C (5min)	

Screening of random primer

A total of 20 decamer primers of OPA, OPS and OPY series (Operon Technology, USA) were screened for amplification of genomic DNA extracted from a selected cross with the parents and three F_1 samples. Details of the primers are provided in Appendix 2. From these, three primers *viz.*, OPA-2, OPY-9 and OPY-10 that gave good amplification and showing

polymorphism were selected for screening the 30 samples belonging to six selected crosses. The amplified products were separated through electrophoresis on 1.2 per cent agarose gel. The gel was viewed under UV light and image documented.

Results

4. RESULTS

The success of any crop improvement programme depends upon the precise information available on the magnitude of variability, heritability, nature and extent of character association with the yield and its related characters.

4.1 Evaluation of variability

Observations were recorded from all the 109 genotypes (80 plants belonging to 14 accessions of African type and 29 plants belonging to seven varieties of French type) with respect to 14 characters. Since the crop is heterozygous, individual plants belonging to the same accession in African type showed variation for the different characters. Hence, plants under each accession were given serial number one to six and the observations from the 80 genotypes were used to calculate the range and mean values (Fig 2 and 3). Statistical analysis was confined to the data from African type accessions only as the sample size in French type were varying and too less to obtain meaningful estimates in some of the varieties.

4.1.1 Extent of variation for quantitative characters

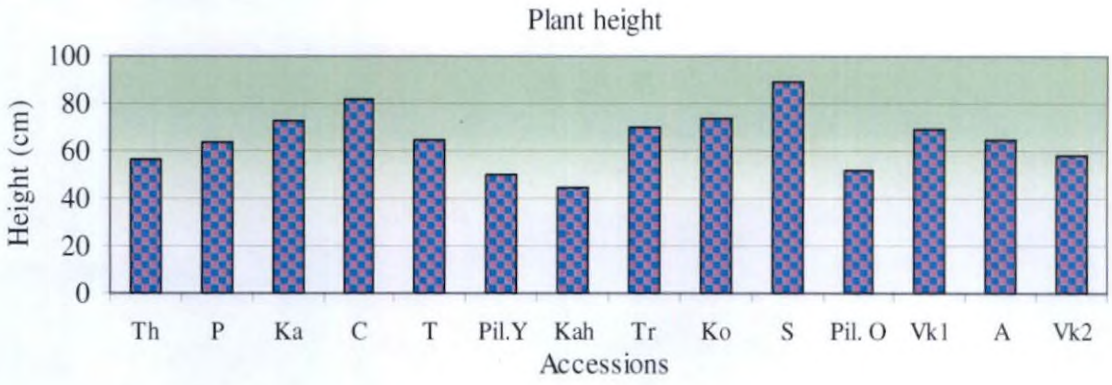
The mean values for the 14 different characters were calculated for each of the accession in African marigold (Table 2) as well as French marigold (Table 3). The genotypic and phenotypic coefficients of variation (GCV and PCV) of the 14 characters were also calculated based on the population mean.

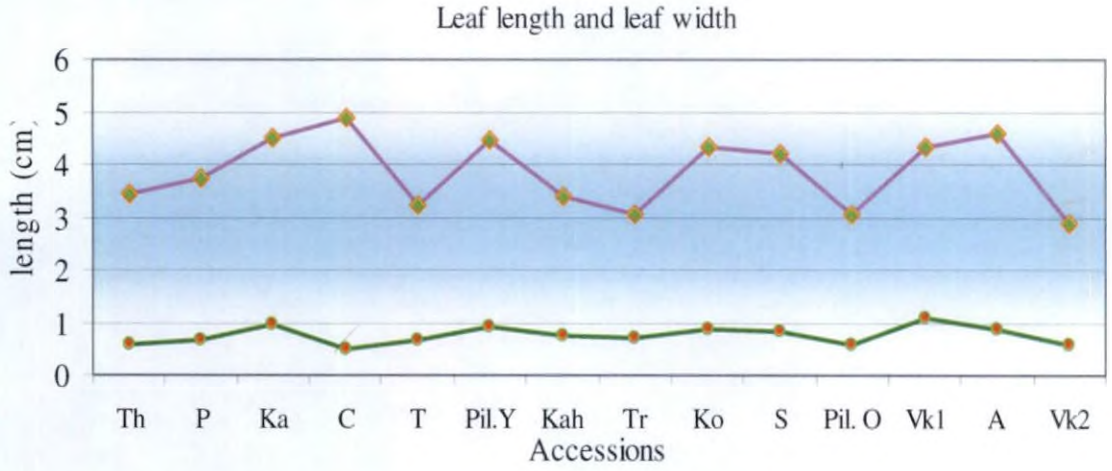
The data from African marigold was subjected to the analysis of variance to check whether extent of variability shown for each character is significant or not (Table 4). The analysis showed significant difference between the accessions for all the characters studied except stem girth, duration of the crop, days to flower, number of flowers and flower diameter.

a) Plant height

In African types, the height ranged from 30 cm to 156 cm. S-6 was the tallest genotype and Th-1 was the shortest. The GCV and PCV for plant height

Fig. 2 Variability for different vegetative characters in African marigold





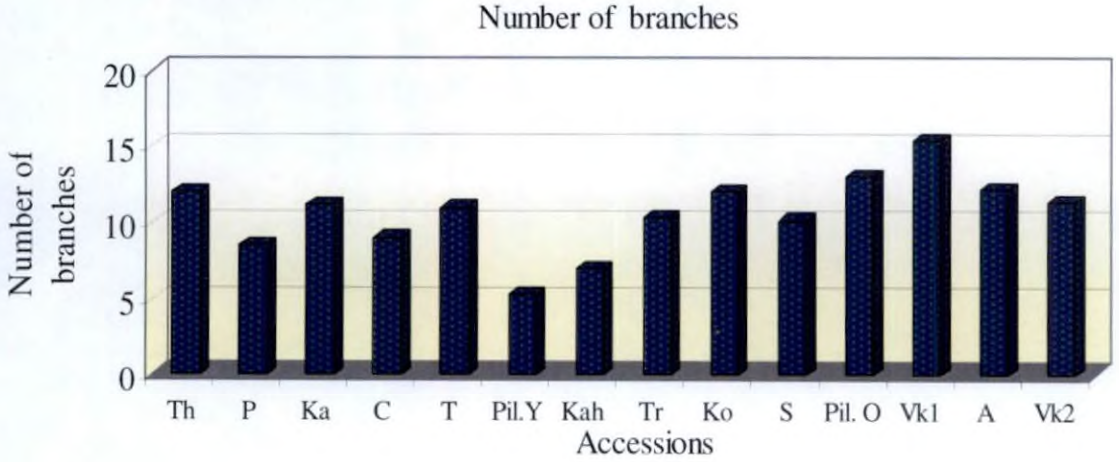
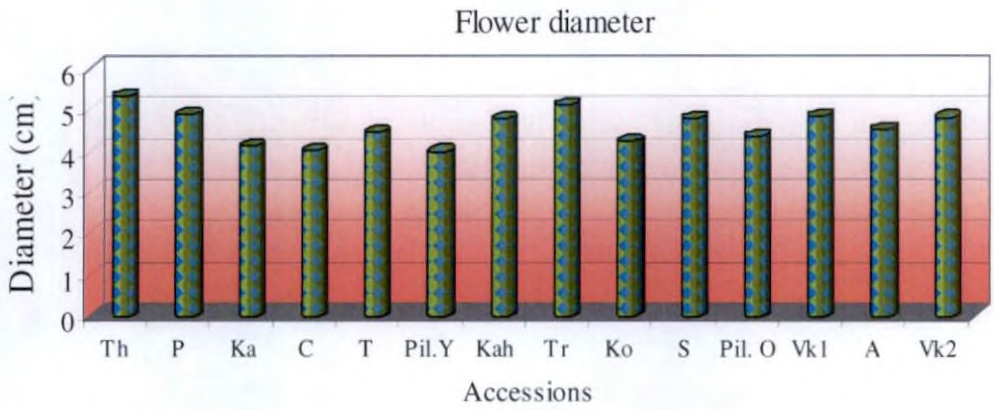
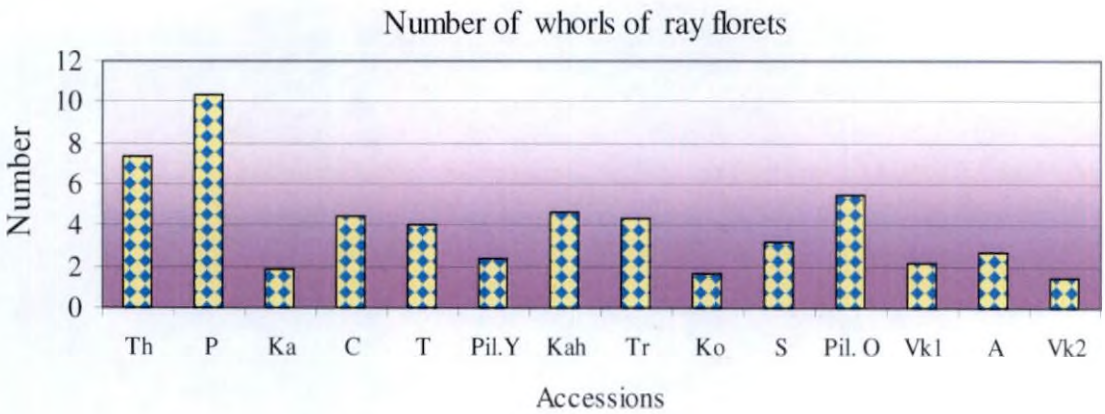


Fig.3 Variability for different floral characters in African marigold





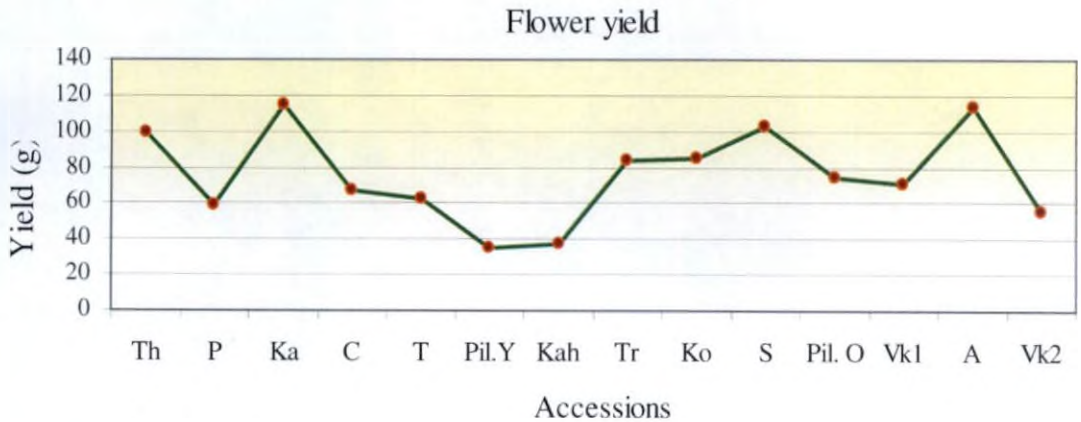


Table 2. Mean values for different characters in different accessions of African marigold

Accessions	Plant height (cm)	Stem girth (cm)	Leaf length (cm)	Leaf width (cm)	Duration of the crop (no. of days)	No. of branches	Days to flower	No. of flowers	Pedicel length (cm)	Flower diameter (cm)	No. of whorls of ray florets	Fresh weight of flowers (g)	Seed set (%)	Flower yield (g)
Th	55.91	3.83	3.43	0.58	103.16	12.00	55.00	32.16	5.85	5.33	7.33	3.09	83.66	100.12
P	64.00	3.63	3.75	0.70	105.50	8.50	73.00	19.83	6.68	4.86	10.3	2.98	81.65	59.07
Ka	73.16	5.05	4.50	0.98	101.66	11.16	56.66	24.66	6.18	4.11	1.83	4.64	81.67	114.69
C	81.83	4.58	4.88	0.50	102.00	9.00	60.00	24.50	3.53	4.00	4.50	2.77	79.83	67.91
T	64.16	5.43	3.23	0.68	94.500	11.00	58.00	28.33	4.28	4.43	4.00	2.22	78.33	62.68
Pil.Y	50.00	2.68	4.45	0.95	108.00	5.33	60.00	14.16	4.35	3.98	2.33	2.48	77.83	35.09
Kah	44.16	3.10	3.40	0.75	103.16	7.00	65.00	18.67	5.68	4.75	4.66	2.09	81.66	38.02
Tr	70.00	4.10	3.05	0.71	104.00	10.33	65.00	29.66	6.18	5.12	4.33	2.83	81.66	83.90
Ko	73.67	3.90	4.33	0.90	98.00	12.00	65.00	25.67	5.38	4.21	1.66	3.34	81.83	85.02
S	89.00	4.20	4.21	0.85	103.16	10.16	61.83	25.83	5.71	4.78	3.16	4.04	83.66	103.75
Pil. O	51.83	3.75	3.06	0.58	95.33	13.00	55.83	31.83	4.66	4.35	5.50	2.30	84.33	74.66
Vk1	69.17	3.62	4.35	1.12	96.50	15.33	55.00	24.50	5.85	4.83	2.16	2.89	88.16	70.75
A	64.16	4.41	4.61	0.88	106.00	12.16	65.00	31.16	4.20	4.51	2.66	3.64	81.16	113.54
Vk2	58.33	3.51	2.91	0.61	109.00	11.33	62.50	22.33	6.11	4.80	1.50	2.55	84.66	55.97
Population mean	64.95	3.98	3.87	0.79	102.14	10.58	61.27	25.23	5.33	4.57	4.00	2.99	82.15	76.08
GCV	17.04	11.90	14.46	17.61	3.33	19.18	6.12	15.68	15.47	5.07	57.87	22.16	2.63	30.62
PCV	20.99	22.84	20.02	23.17	5.33	27.85	10.05	25.02	19.86	11.79	65.36	26.00	3.76	36.26

Table 3. Mean values for morphological characters in French marigold

Accessions	Plant height (cm)	Stem girth (cm)	Leaf length (cm)	Leaf width (cm)	Duration of the crop (no.of days)	No. of branches	Days to flower	No. of flowers	Pedicel length (cm)	Flower diameter (cm)	No. of whorls of ray florets	Fresh weight of flowers (g)	Seed set %	Flower yield (g)
Boy O Boy	37.56	2.78	1.71	0.64	74.34	3.23	26.23	14.67	4.46	2.78	8.00	1.85	18.00	27.13
Safari Bolor	33.75	3.45	2.17	0.55	73.75	10.75	26.00	19.75	4.77	4.45	4.00	0.57	31.75	11.35
Safari Tangerine	30.25	2.95	2.62	0.5	74.5	8.00	26.5	15.75	4.60	2.95	4.00	0.71	44.5	11.18
Little Hero Fire	26.34	3.00	2.03	0.63	75.34	8.34	29.34	24.34	4.90	3.40	3.00	0.57	37.00	13.94
Boy Spry	34.50	2.50	2.68	0.81	75.00	8.34	26.17	22.5	5.46	3.84	4.00	0.76	21.67	15.88
Trichur Local	59.00	1.75	2.05	0.45	70.50	3.13	25.00	4.00	3.55	3.55	4.00	0.51	17.50	2.04
French Vanilla	30.00	4.00	5.50	1.10	72.00	6.01	32.00	12.00	4.80	4.80	9.00	2.48	2.00	29.76

Table 4. Mean sum of squares form ANOVA for morphological characters in African marigold

Source of variation	D.f	Plant height (cm)	Stem girth (cm)	Leaf length (cm)	Leaf width (cm)	Duration of the crop	No. of branches	Days to flower	No. of flowers	Pedicel length (cm)	Flower diameter (cm)	No. of whorls of ray florets	Fresh weight of flowers (g)	Seed set %	Flower yield (g)
Replication	1	0.72	0.09	1.38	1.16	32.12	6.33	154.02	9.89	1.87	0.06	2.68	0.14	0.70	451.41
Treatment	13	**308.53	1.05	*0.91	*0.05	41.24	*12.84	52.05	55.56	**1.80	0.34	**12.19	**1.04	*14.22	**1304.18
Error	13	63.44	0.60	0.28	0.01	18.04	4.57	23.91	24.24	0.44	0.23	1.47	0.16	4.85	218.50
CD (0.05)		17.20	1.68	1.15	0.25	9.177	4.62	10.56	10.63	1.43	1.05	2.62	0.87	4.76	31.93

*p= 0.05 **p= 0.01

were 17.04 and 20.99 respectively. In French types mean plant height varied from 26.34 cm (L.H.F) to 59 cm (Trichur local).

b) Stem girth

Stem girth ranged from 1.5 cm in Ka-3 to 7.2 cm in P-4 and T-1. The GCV and PCV were 11.90 and 22.84 respectively. In French types mean value for stem girth ranged from 1.75 cm (Trichur local) to 4 cm (F.V).

c) Number of branches

In different accessions of African marigold Vk1 had maximum number of branches and Pil.Y had the minimum. The GCV and PCV were 19.18 and 27.85 respectively. In French types the mean values for number of branches in different accessions ranged from three (Trichur local) to 10.75 (S.B).

d) Leaf length

Leaf length varied from 7.6 cm (Vk2-2) to 1.9 cm (Vk-2-5) in African marigold. The GCV was 14.46 and PCV was 20.02. In French types mean leaf length ranged from 1.71 cm (B.O.B) to 5.5 cm (F.V).

e) Leaf width

Leaf width ranged from 0.4 cm to 1.8 cm in different accessions of African marigold. The GCV and PCV were 17.61 and 23.10 respectively. Ko-2 had maximum leaf width and Ka-3 and C-1 had the minimum. In French types the mean value for leaf width ranged from 0.45 cm (Trichur local) to 1.1 cm (F.V).

f) Duration of the crop

Duration of the crop varied from 89 days (T) to 110 days (Vk2) in different accessions of African marigold. The GCV was 3.33 and PCV was 5.33. In French types, the mean value for duration of crop in different accessions ranged from 70.5 days (Trichur local) to 75.34 days (L.H.F).

g) Days to flower

In different accessions of African marigold Th was the earliest to flower (55 days) and P (73 days) was the latest. The GCV and PCV were 6.12 and 10.05 respectively. The range of days to flower was from 50 to 78 days. In French types the accession Trichur local took minimum number of days to flower (25) and L.H.F took maximum number of days (29.34).

h) Pedicel length

Pedicel length varied from three cm (T-2) to 8.6 cm (Ka-1) in different accessions. The GCV was 15.47 and PCV was 19.86. Accession P (6.68 cm) has got highest mean pedicel length and accession Pil.Y (3.53 cm) has got the minimum value for mean pedicel length. In French types, the accession Trichur local recorded minimum mean value for pedicel length (3.55 cm) and B.S recorded maximum mean value for pedicel length (5.46 cm).

i) Number of flowers

The number of flowers per plant ranged from eight (Pil.Y-4) to 48 (Th-4). The GCV and PCV were 15.68 and 25.02 respectively. In French types the number of flowers in different accessions varied from four (Trichur local) to 24.34 (L.H.F).

j) Fresh weight of flowers

Average fresh weight of flowers in the 80 genotypes ranged from 1.08 g (Kah-6) to 5.9 g (A-4). Mean value for fresh weight of flowers was highest in Ka (4.64 g) and lowest in Kah (2.09 g) whereas GCV, and PCV were 22.16 and 26 respectively. In French types, the mean value for fresh weight of flowers recorded maximum in F.V (2.48 g) and minimum in Trichur local (0.51 g).

k) Flower diameter

Flower diameter ranged from 2.5 cm (Pil.Y-3) to 7 cm (Ka-2) in different accessions The GCV and PCV were 5.07 and 11.79 respectively. Th (5.33 cm) has got highest mean value for flower diameter and Pil.Y (3.98 cm) has got the

lowest. In French types flower diameter in different accessions ranged from 2.78 cm (BOB) to 5.1 cm (F.V).

l) The number of whorls of ray florets

The number of whorls of ray florets per flower ranged from one to 12 in the 80 genotypes. The GCV and PCV were 57.87 and 65.36. Accession P showed maximum mean value for the number of whorls of ray florets (10.33) and Vk-2 Showed the minimum mean value (1.5). In French types the mean value for number of whorls of ray florets ranged from three (L.H.F) to nine (F.V).

m) Flower yield

Mean value for yield ranged from 114.69 g (Ka) to 35.09 g (Pil.Y) in different accessions. The GCV and PCV were 30.62 and 36.26 respectively. In French types, the mean value for yield recorded maximum in BOB (27.13 g) and minimum (2.04 g) in Trichur local.

n) Seed set percentage

Seed set percentage in different genotypes ranged from 72 (T-2) to 92 (A-4) and the character recorded highest mean value in P (88.16) and lowest in Pil.Y (77.83). GCV was 2.63 and PCV 3.76 respectively. In French types seed set percentage ranged from two (F.V) to 44.5 (S.T).

4.1.2 Statistical analysis of data and estimation of genetic parameters

a) Variance and coefficient of variation

For all the characters studied, PCV showed higher values than GCV, indicating the predominant role of environment in the expression of these traits.

b) Heritability (in broad sense)

Heritability (in broad sense), genetic advance and genetic gain in African marigold are presented in Table 5. Highest heritability was observed for number of whorls of ray florets (78.39) followed by fresh weight of flowers (72.67), flower yield (71.30) and plant height (65.89).

Table 5. Heritability, genetic advance and genetic gain for different characters in African marigold

Characters	Heritability (%)	Genetic advance	Genetic gain (%)
Plant height	65.89	18.50	28.49
Stem girth	27.13	0.51	12.76
Leaf length	52.18	0.83	21.52
Leaf width	57.80	0.21	27.58
Duration of the crop	39.14	4.38	4.29
Number of branches	47.47	2.88	27.23
Days to flower	37.05	4.70	7.67
Number of flowers	39.25	5.10	20.23
Pedicel length	60.68	1.32	24.82
Flower diameter	18.53	0.20	4.50
Number of whorls of ray florets	78.39	4.22	105.55
Fresh weight of flowers	72.67	1.16	38.92
Seed set percentage	49.10	3.12	3.80
Flower yield	71.30	40.52	53.26

The other characters exhibited moderate range of heritability such as pedicel length (60.68) leaf width (57.60), leaf length (52.18), seed set percentage (49.10), number of branches (47.47), number of flowers (39.25) and days to flower (37.05). Lowest values were obtained for Stem girth (27.13) and flower diameter (18.53).

c) Genetic advance and genetic gain

Genetic advance was highest for yield (40.52) and lowest for flower diameter (0.20). Genetic gain was highest for number of whorls of ray florets (105.55) followed by yield (53.26) and fresh weight of flowers (38.92). Low genetic gain was observed for characters days to flower (7.67), flower diameter (4.50), duration of the crop (4.29) and seed set percentage (3.80). Moderate genetic gain was observed for characters like number of flowers (20.23) and stem girth (12.76).

d) Correlation studies

The genotypic and phenotypic correlations of the various characters with yield were worked out (Table 6 and 7).

It was observed that yield was significantly and positively correlated with number of flowers (rg= 0.7460; rp= 0.9626) and fresh weight of flowers (rg= 0.8887; rp= 0.7862) both at genotypic and phenotypic level. Significant and positive genotypic as well as phenotypic correlations were also observed between leaf length and leaf width (rg= 0.8292 and rp= 0.6933) as well as number of branches and number of flowers (rg= 0.6190 and rp= 0.7567) (Fig. 4 and Fig. 5).

Correlation between flower diameter and number of whorls of ray florets (rg= 0.7402), plant height and fresh weight of flowers (rg= 0.8383), stem girth and fresh weight of flowers (rg= 0.6696), leaf length and fresh weight of flowers (rg= 0.6359) were significant at only genotypic level. For the vegetative characters *viz.* plant height (rg= 0.6168), stem girth (rg= 0.7729) and number of branches (rg= 0.6081) only genotypic correlation was significantly correlated with flower yield. Significant and positive genotypic correlation was observed between

Fig.4 Genotypic correlation between different characters in African marigold

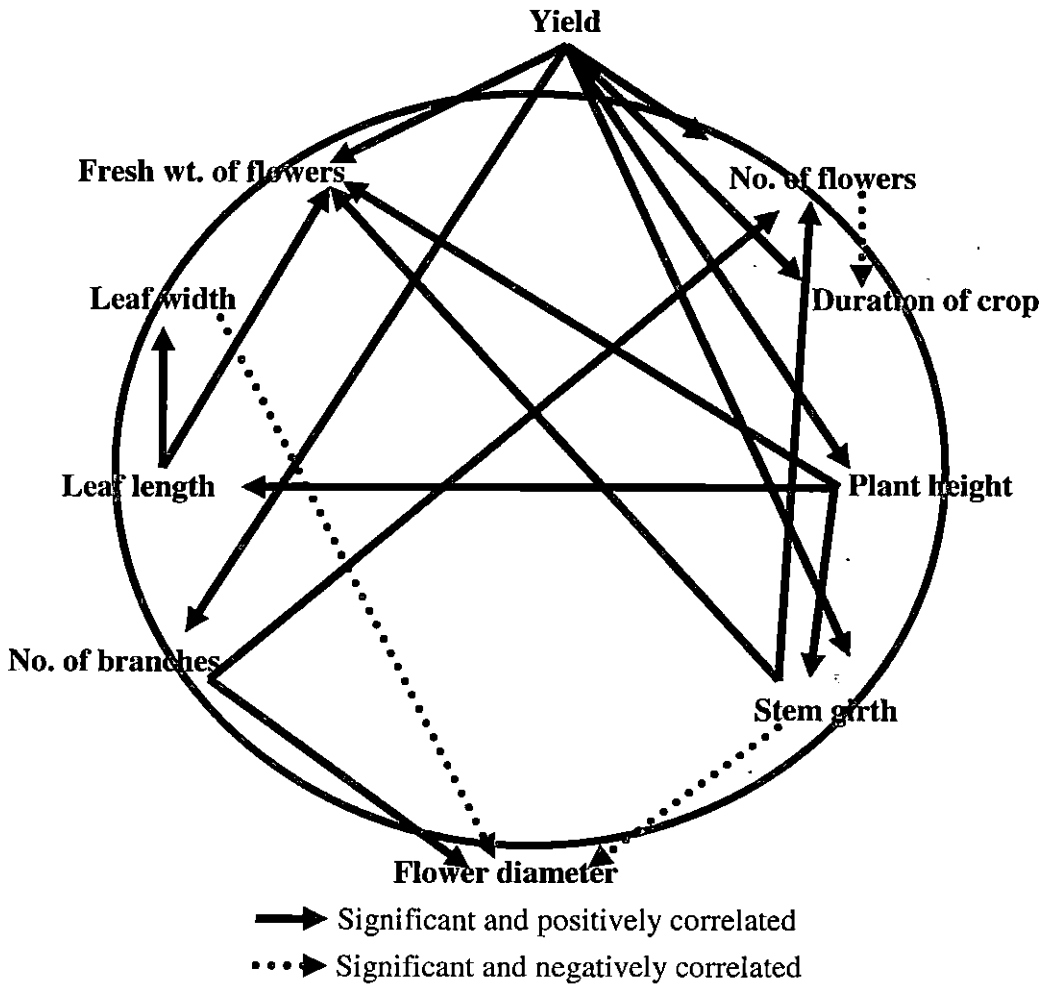


Fig. 5 Phenotypic correlation between different characters in African marigold

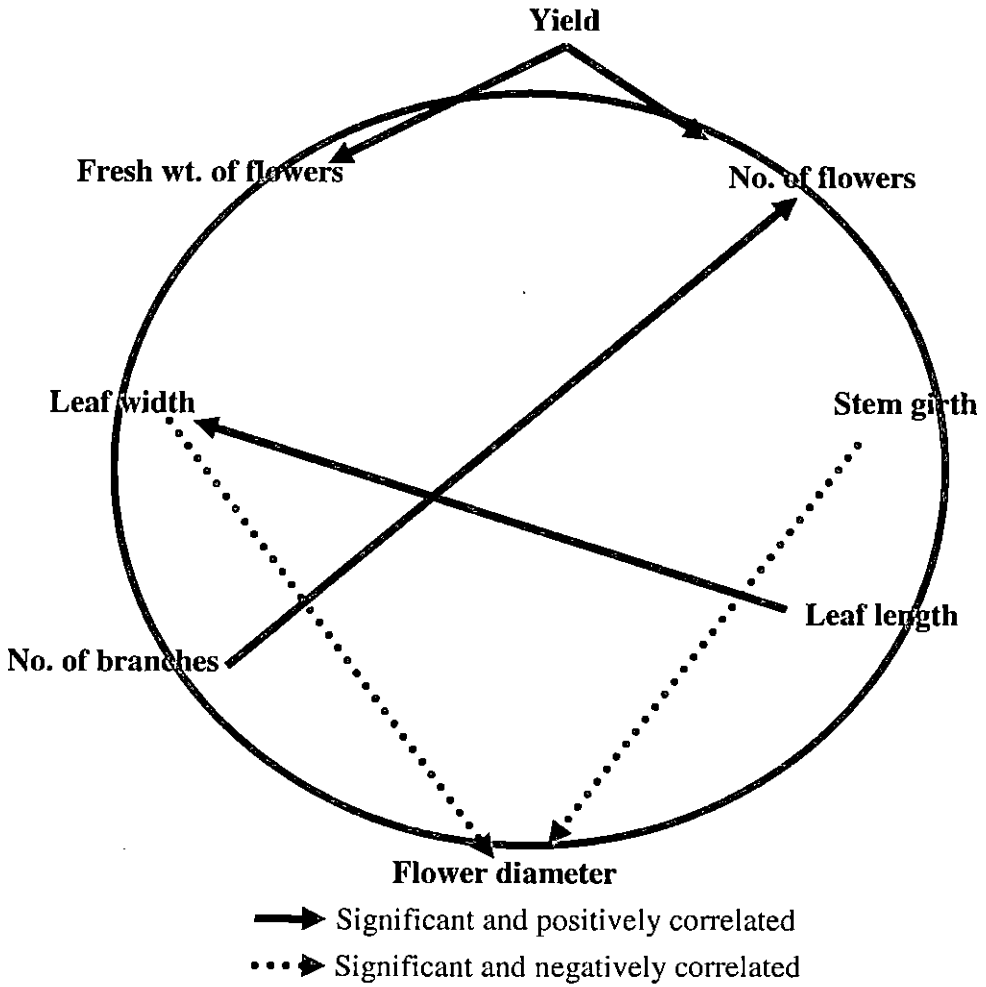


Table 6. Estimates of genotypic correlation coefficients for yield and its components in African marigold

Characters	Stem girth	Leaf length	Leaf width	Duration of the crop	No. of branches	Days to flower	No. of flowers	Pedicel length	Flower diameter	Number of whorls of ray florets	Fresh weight of flowers	Seed set %	Flower yield
Plant height	0.6336*	0.5946*	0.4153	0.0208	0.1048	0.2199	0.0984	0.0554	-0.1537	-0.2191	0.8383**	-0.0503	0.6168**
Stem girth		-0.0319	0.0249	-0.3013	0.3242	-0.0244	0.6861*	-0.4905	-0.6584*	-0.0989	0.6696*	-0.7210*	0.7729*
Leaf length			0.8292**	0.4286	-0.0930	0.2280	-0.2353	-0.5642	-0.1299	-0.4879	0.6359*	-0.3021	0.3790
Leaf width				-0.1203	0.04616	-0.1888	-0.5645	-0.1231	-0.5981*	-0.7574*	0.5696	0.2304	0.1735
Duration of the crop					-0.8139*	0.4093	-0.7868*	0.3235	0.5640	0.0451	0.1764	-0.1981	-0.1859
No. of branches						-0.5645	0.6190*	0.1787	0.7150*	-0.2051	0.4586	0.9655**	0.6081*
Days to flower							-0.4134	0.3735	0.3755	0.6307*	0.0377	-0.2146	-0.1548
No. of flowers								-0.1935	0.9940*	0.1646	0.3640	0.3002	0.7460**
Pedicel length									0.9302*	0.2387	0.2656	0.6553*	0.0887
Flower diameter										0.7402*	-0.3043	0.6700*	0.2409
Number of whorls of ray florets											-0.3477	-0.1210	-0.1785
Fresh weight of flowers												0.0146	0.8894**
Seed set %													0.1277

** Significant at 1% level, * Significant at 5% level

Table 7. Estimates of Phenotypic correlation coefficients among yield and its components in African marigold

Characters	Stem girth	Leaf length	Leaf width	Duration of the crop	No. of branches	Days to flower	No. of flowers	Pedicel length	Flower diameter	Number of whorls of ray florets	Fresh weight of flowers	Seed set %	Flower yield
Plant height	0.5506	0.4416	0.2761	-0.2258	0.3389	-0.0610	0.2931	-0.0420	-0.1129	-0.2001	0.4952	0.0938	0.5062
Stem girth		0.2012	-0.0420	-0.4824	0.3742	-0.2821	0.5151	-0.1078	-0.0468	-0.0945	0.3037	-0.0187	0.5072
Leaf length			0.6933*	-0.1130	-0.0681	-0.1539	-0.2221	-0.2572	-0.3279	-0.1920	0.4904	-0.1220	0.2217
Leaf width				-0.0479	0.1346	-0.0787	-0.2158	0.0587	-0.2902	-0.4355	0.4001	0.0236	0.1450
Duration of the crop					0.4259	0.5064	-0.2920	0.1569	0.0408	0.0158	0.0984	-0.1826	-0.0789
No. of branches						-0.3856	0.7567*	0.1030	0.1153	-0.1989	0.1089	0.5827*	0.5238
Days to flower							-0.3065	0.2356	0.0709	0.1729	-0.0459	-0.2825	-0.2190
No. of flowers								-0.0708	0.1155	0.0403	0.1097	0.2592	0.9626*
Pedicel length									0.5547	0.2156	0.2770	0.4313	0.1301
Flower diameter										0.3277	0.0898	0.4767	0.1018
Number of whorls of ray florets											-0.1569	-0.0122	-0.0910
Fresh weight of flowers												0.1949	0.7862*
Seed set %													0.2950

* significant at 5% level

plant height and stem girth (rg= 0.6336), plant height and leaf length (rg= 0.5946), stem girth and number of flowers (rg= 0.6861), number of branches and flower diameter (rg= 0.7150) *etc.*

Some of the characters showed significant negative correlation at genotypic level such as correlation between stem girth and flower diameter (rg= -0.6584), Correlation between leaf width and flower diameter (rg= -0.5981) and duration of the crop and number of flowers (rg= -0.7868) were also negative and significant at genotypic level.

e) Path coefficient analysis

The correlations between yield and component characters were portioned into direct and indirect effects and presented in Table 8.

Fresh weight of flowers exhibited the highest positive direct effect and significant correlation with yield (0.8013), followed by number of flowers per plant (0.5955), leaf width (0.2205), flower diameter (0.0982), days to flower (0.0520), duration of the crop (0.0170), leaf length (0.0036) and number of whorls of ray florets(0.0020). Days to flower had positive direct effect (0.0520) on yield but it was negatively correlated (-0.1855) to yield. Direct effect of plant height on yield was negative (-0.1792), but have high positive indirect effects through traits like fresh weight of flowers (0.6870) and leaf width (0.0937) and number of flowers (0.0500). Its correlation with yield was found to be positive. Stem girth also showed positive indirect effects through fresh weight of flowers (0.5686) and number of flowers (0.4203).

Number of branches had negative direct on yield (-0.1078) but it was positively correlated to yield, due to high positive indirect effect through fresh weight of flowers (0.3859) and number of flowers (0.3615). Negative correlation (-0.1785) was recorded between number of whorls of ray florets due to negative indirect effect of fresh weight of flowers (-0.2863) but it had positive direct effect on yield (0.00207). Residual effect due to other factors was 0.0372.

Table 8. Path coefficients showing direct (Diagonal) and indirect effects (non diagonal) on flower yield in African marigold

Characters	Plant height	Stem girth	Leaf length	Leaf width	Duration of the crop	No. of branches	Days to flower	No. of flowers	Pedicel length	Flower diameter	Number of whorls of ray florets	Fresh weight of flowers	Seed set %	Correlation with yield
Plant height	**-0.1792**	-0.0130	0.0020	0.0937	0.0003	-0.0096	0.0126	0.0500	-0.0031	-0.0157	-0.0004	0.6870	-0.0017	0.6168
Stem girth	-0.1158	**-0.0202**	-0.0002	0.0082	-0.0048	-0.0345	0.0002	0.4203	0.0270	-0.0735	-0.0002	0.5686	0.0290	0.7729
Leaf length	-0.1082	0.0011	**0.0036**	0.1847	0.0081	0.0101	0.0136	-0.1043	0.0297	-0.1406	-0.0010	0.5176	0.0114	0.3790
Leaf width	-0.0761	-0.0007	0.0030	**0.2205**	-0.0022	-0.0049	-0.0101	-0.3475	0.0069	-0.0639	-0.0016	0.4583	-0.0086	0.1735
Duration of the crop	-0.0034	0.0057	0.0017	-0.0293	**0.0170**	0.0916	0.0208	-0.4972	-0.0172	0.0622	0.0001	0.1462	0.0073	-0.1850
No. of branches	-0.0159	-0.0064	-0.0003	0.0100	-0.0145	**-0.1078**	-0.0302	0.3615	-0.0094	0.0783	-0.0004	0.3859	-0.0363	0.6081
Days to flower	-0.0434	-0.0001	0.0009	-0.0431	0.0068	0.0627	**0.0520**	-0.2524	-0.0196	0.0410	0.0013	0.0345	0.0076	-0.1548
No. of flowers	-0.0150	-0.0142	-0.0008	-0.1286	-0.0142	-0.0654	-0.0220	**0.5955**	0.0104	0.1105	0.0003	0.3068	-0.0111	0.7460
Pedicel length	-0.0110	0.0106	-0.0021	-0.0297	0.0057	-0.0198	0.0199	-0.1212	**-0.0511**	0.0966	0.0005	0.2126	-0.0245	0.0887
Flower diameter	0.0287	0.0151	-0.0052	-0.1434	0.0108	-0.0859	0.0217	0.6698	-0.0503	**0.0982**	0.0016	-0.2769	-0.0254	0.2409
Number of whorls of ray florets	0.0394	0.0020	-0.0018	-0.1702	0.0008	0.0221	0.0346	0.1035	-0.0122	0.0782	**0.0020**	-0.2863	0.0046	-0.1785
Fresh weight of flowers	-0.1537	-0.0143	0.0023	0.1261	0.0031	-0.0519	0.0023	0.2280	-0.0135	-0.0339	-0.0007	**0.8013**	-0.0001	0.8894
Seed set %	-0.0086	0.0160	-0.0011	0.0519	-0.0034	-0.1073	-0.0108	0.1809	-0.0343	0.0683	-0.0002	0.0030	**-0.0365**	0.1277

Residual value: 0.0372

f) Genetic divergence

Using Mahalanobis D^2 statistics the 14 accessions of African marigold were grouped into five clusters. The different clusters and their variable means are presented in Table 9.

Among the five clusters, cluster number I had maximum number of accessions (5), cluster II had three accessions where as the rest of the clusters consisted of two accessions each.

Accessions included in cluster I were Th, P, Ka, T and A and they had highest mean value for number of whorls of ray florets (5.2331), stem girth (4.4733 cm) and fresh weight of flowers (3.3184 g). Cluster II consisted of accessions C, Tr, S and recorded highest mean values for plant height (80.2782 cm), leaf length (4.0485 cm) and flower yield (85.1882 g).

Cluster III included Vk1 and Vk2 accessions and had the highest mean value for leaf width (0.86 cm), number of branches (13.3332), pedicel length (5.9823 cm), flower diameter (4.8154 cm) and seed set percentage (86.4159). Cluster IV consisted of two accessions Pil.Y and Kah and recorded highest mean value for days to flower (62.5023 days) and duration of the crop (105.5823 days). Cluster V consisted of Ko and Pil.O and had highest mean value for number of flowers (28.7522).

Inter and Intra cluster D^2 values among the 5 clusters (Table 10) revealed that cluster IV had maximum intra cluster distance (16911.9701) where as II had minimum (331.6223). Maximum inter cluster distance was between cluster I and cluster IV (382457.4102) and minimum inter cluster distance was between cluster II and cluster V (19897.3923) (Fig 6).

g) Selection index

Characters to be considered were selected based on their phenotypic correlations, direct and indirect effects on yield, variability, heritability and genetic gain. Accordingly selection based on plant height, stem girth, number of flowers, number of whorls of ray florets, fresh weight of flowers, flower yield

Table 9. Mean performance of accessions in different clusters of African marigold

Cluster No.	Plant height (cm)	Stem girth (cm)	Leaf length (cm)	Leaf width (cm)	Duration of the crop	No. of branches	Days to flower	No. of flowers	Pedicel length (cm)	Flower diameter (cm)	No. of whorls of ray florets	Fresh weight of flowers (g)	Seed set %	Flower yield (g)
I	64.2822	4.4733	3.9062	0.7672	102.1651	10.9667	61.5331	27.2332	5.4392	4.6532	5.2331	3.3184	81.2962	70.0585
II	80.2782	4.2935	4.0485	0.6881	103.0554	9.8339	62.2782	26.6661	5.1452	4.6336	4.1239	3.2172	81.7216	85.1882
III	63.7501	3.5671	3.6327	0.8670	102.7506	13.3332	58.7507	23.4104	5.9823	4.8154	1.8332	2.7254	86.4159	63.3626
IV	47.0823	2.8920	3.9259	0.8501	105.5823	6.1671	62.5023	16.4176	5.0175	4.3655	3.5365	2.2891	79.7477	36.5656
V	62.7526	3.8254	3.7567	0.7401	96.6655	12.5067	60.4174	28.7522	5.0226	4.2829	3.5831	2.8221	83.0894	79.8454

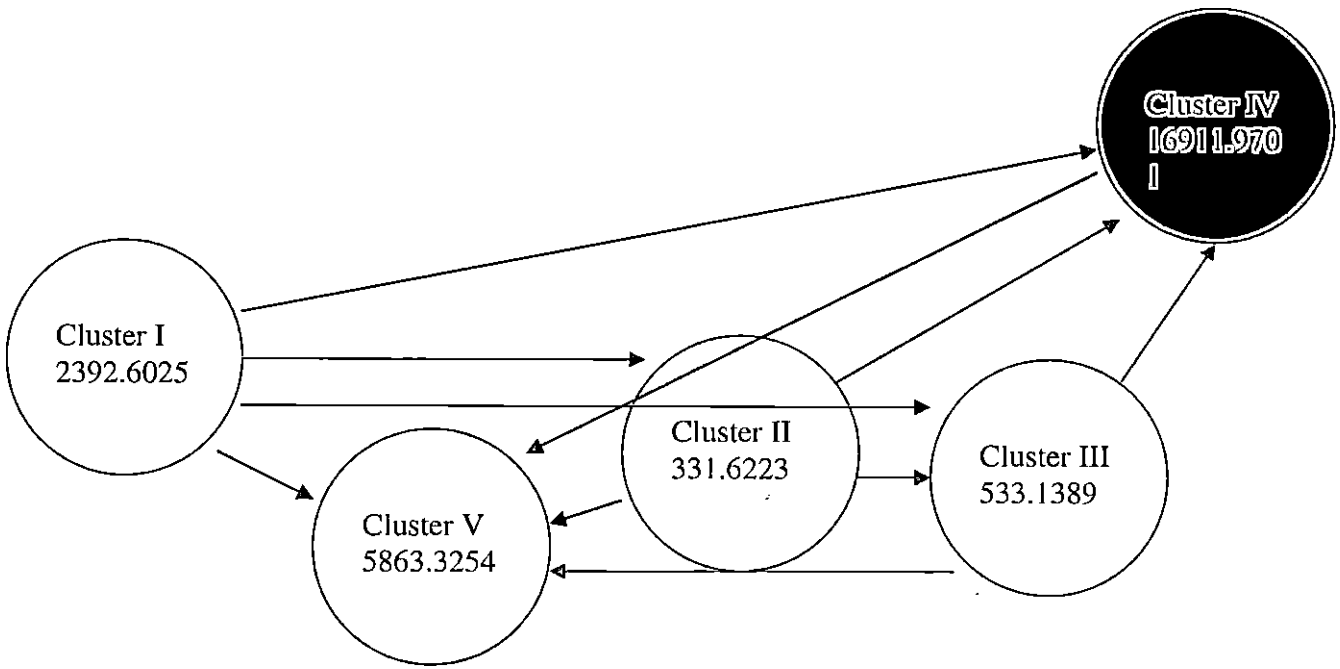
Table 10. Inter and intra cluster D^2 value among different clusters of African marigold

Cluster	I	II	III	IV	V
I	**2392.6025**				
II	87426.1587	**331.6223**			
III	204681.0000	25259.4612	**533.1389**		
IV	382457.4102	107036.5387	31391.7249	**16911.9701**	
V	27668.3189	19897.3923	87278.3881	213163.6423	**5863.3254**

The values printed in bold indicates intra cluster D^2 values

Fig.6 Inter and intra cluster distances

Inter cluster distances between I & II 87426.1587, I & III 204681.0000, I & IV 382457.4102, V 27668.3189 & II & III 25259.4612, II & IV 107036.5387, II & V 19897.3923, III & IV 31391.7249, III & V 87278.3881, IV & V 213163.6423.



were found effective and the selection indices were formulated based on efficiency over direct selection and number of characters involved (Table 11).

4.2 Reproductive biology and compatibility studies

For taking up any crop improvement programme, the knowledge of floral biology of the crop is a prerequisite. In the present study 18 flower buds at six different stages were collected to study the floral biology. A preliminary study was taken up to identify the best growth solution for keeping the buds fresh and growing for one week. Among the different growth solutions tried (Table 12) Al_2SO_4 (3 g l^{-1}) + Kinetin (25 mg l^{-1}) was found best suited for keeping the buds fresh.

4.2.1. Floral features

a) Flower type

Flowers in marigold are typical Asteracea flowers, consisting of two different whorls of flowers outer whorls of ray florets (♀) and inner disc florets and the inflorescence is heterogamous head.

b) Flower colour

Ranged from lemon yellow to dark orange (plate 2a and 2b) in different accessions. Even within the same accession individual plants varied with respect to the colour.

c) Insects visiting

Ants, Dragonflies, Grasshoppers and bees are the insects that were commonly observed during the period of pollination.

d) Time of anthesis

Ranged from 6 am to 10 am in different accessions, with anther dehiscence occurring between 9 am-12 noon and completed by 2 pm.

Plate2a. Variability for floral characters in African marigold

Plate 2b. Variability for floral characters in French marigold

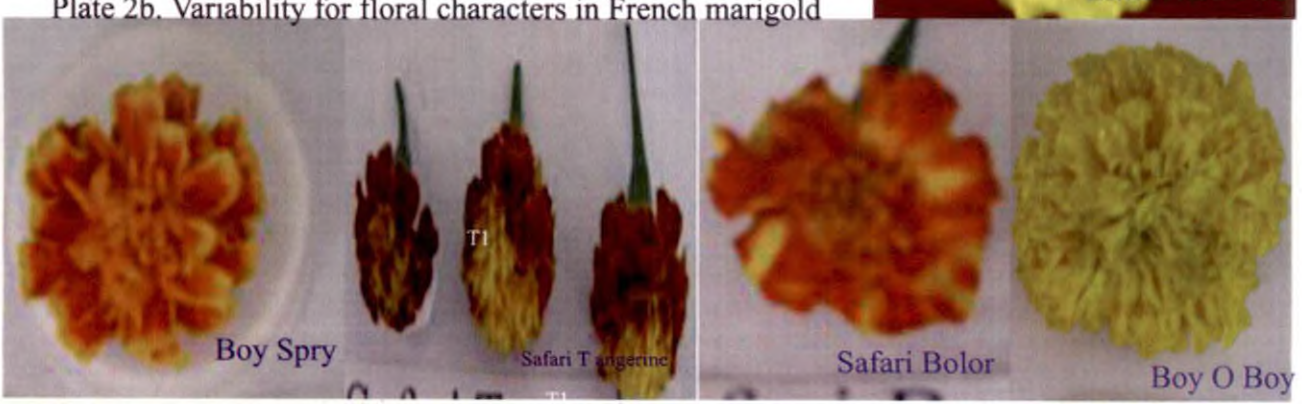


Table 11. Ranking of accessions of African marigold

Sl .No.	Accessions	Selection index	Rank based on selection index	Rank based on flower yield
1	Th	104.51	4	4
2	P	81.16	10	11
3	Ka	126.72	1	1
4	C	85.16	9	9
5	T	80.40	12	10
6	Pil.Y	67.75	13	14
7	Kah	65.33	14	13
8	Tr	95.62	6	6
9	Ko	101.58	5	5
10	S	114.55	3	3
11	Pil. O	88.62	8	7
12	Vk1	90.49	7	8
13	A	116.48	2	2
14	Vk2	81.12	11	12

Table 12. Composition of the growth solutions used for keeping the buds fresh

Sl. No	Composition
1	$Al_2 SO_4$ 3 g l^{-1}
2	Kinetin 25 mg l^{-1}
3	$Al_2 SO_4$ 3 g l^{-1} + Kinetin 25 mg l^{-1}
4	$Al_2 SO_4$ 3 g l^{-1} + Sucrose 5%
5	Kinetin 10 g l^{-1}
6	Kinetin 25 mg l^{-1} + Sucrose 5%
7	HQ 10 g l^{-1}
8	$AgNO_3$ 1 g l^{-1}
9	$Al_2 SO_4$ 40 g l^{-1}

e) Time of anther dehiscence

It was observed that the day of anther dehiscence coincides with the opening of the first ray floret (Plate 3b). Stigma remained receptive for four to six days but papillae remained succulent only for 2-3 days in different accessions (Plate 3c).

f) Pollen fertility

Ranged from 98-99 per cent in different accessions (Plate 4).

4.2.2. Self and cross compatibility studies

a) Selfing

Self compatibility was tested by bagging individual flowers of selected plants using butter paper bags and nylon bags (Plate 5). Selfing by just bagging individual flowers and artificial selfing by rubbing two flowers of the same plant and then bagging were carried out and the seed set percentage was worked out. Also the selfed seeds were subjected to germination test (Plate 6).

The average seed set percentage in nylon net bags was slightly higher than butter paper bags in both types of selfing. In flowers which were just bagged, nylon net bagging gave average seed set of 12.41 per cent where as in butter paper bagged flowers it was 6.3 per cent only. The average seed set percentage in artificial selfing in nylon net-bagged flowers was 12.68 per cent, where as in butter paper bagged flowers it was 8.4 per cent.

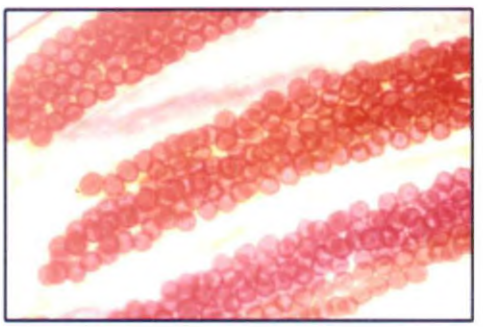
b) Crossing

To study the cross compatibility, 18 crosses were conducted between plants of African marigold (including four reciprocal hybrids also) and seven direct crosses, 10 inter specific crosses between plants of African and French type (Table 13). In all the intra specific crosses, seed set and germination of seeds were normal. For the inter specific crosses normal looking black seeds were obtained but germination was recorded only in those crosses where African types were used as the female parent. There was no difference in compatibility status due to reciprocal hybrids.

Plate 3a. Different stages of flower development ()

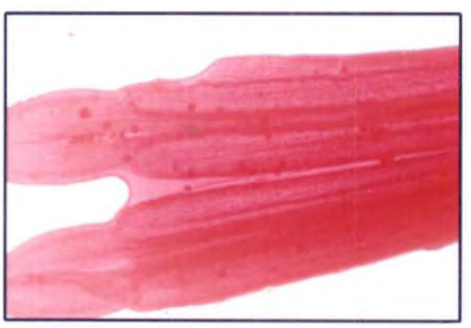
Plate 3b. Anther at different stages of flower development

Immature anther
at 1st, 2nd, 3rd stages (20X)



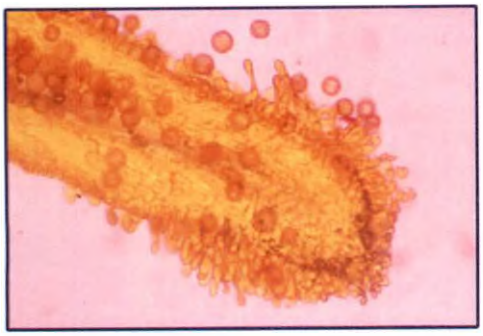
Anther just before
dehscence 4 th stage (25X)

Anther during dehiscence
at stage 5 th stage (25X)

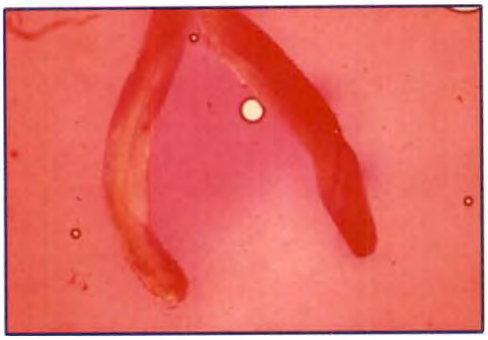


Anther during dehiscence
at 6th stage (25X)

Plate 3c. Stigma at different stages of flower development



Receptive stigma showingthe papillae and pollen grains at 3 rd stage (25X)

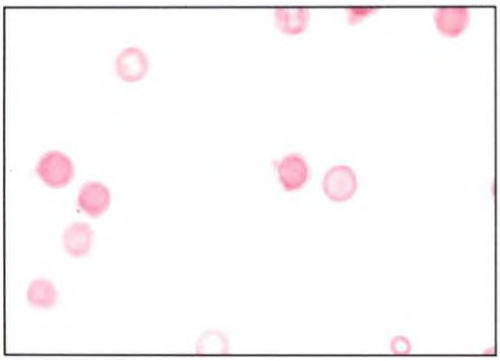


Stigma after pollination at 4th stage (25X)

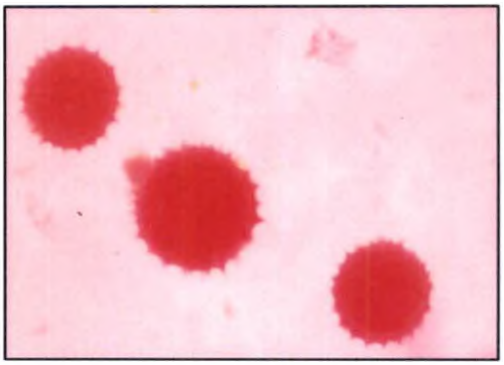
Dried stigma at 5th stage (25X)

Dried stigma at 6th stage (25X)

Plate 4. Pollen fertilty



Fertile and sterile pollen grains (10X)



Fertile pollen with spiny exime (25X)

Table 13. Intra and inter specific hybridization of different genotypes of African and French marigold

Sl. No.	Parents (Accession name with plant number)	Seed set percentage	No. of seeds germinated
1	Kah-2 X Th-6	60	15
2	Tr-5 X Pil.O-3	55	10
3	Pil.O-3 X Tr-5	90	57
4	Th-1 X C-6	76	27
5	C-6 X Th-1	53	12
6	Vk2-3 X Tr-3	56	13
7	Tr-3 X Vk2-3	80	30
8	Th-1 X A-3	30	2
9	Vk2-3 X T-5	48	8
10	Th-6 X Ka-3	57	13
11	Ko-2 X A-5	36	5
12	Vk1-5 X Pil.O-4	44	7
13	Pil.O-5 X Th-1	32	2
14	Ka-3 X Th-6	44	8
15	T-3 X Vk1- 1	46	7
16	Ka-3 X T-3	30	0
17	Ko-2X A-5	28	0
18	Th-1X A-4	24	0
19	Vk1-1 X L.H.F	25	5
20	L. H. F X Vk1-1	10	0
21	Th-6 X S.B	30	4
22	S. B X Th-6	12	0
23	Tr-3 X S.T	34	6
24	S. T X Tr-3	16	0
25	Ka-2 X F. V	13	0
26	F. V X Ka-2	15	0
27	Pil.O-4 X B. S	14	0
28	B. S X Pil.O-4	10	0

4.3 Estimation of heterosis

A total of 28 crosses were carried out of which 18 crosses produced F1 seedlings (Table 13). In the remaining 10 crosses, there was no seed germination. There were 15 intra specific crosses using different genotypes of African marigold out of which, four were reciprocal hybrids and three inter specific crosses (African x French type). The reciprocals were included for heterosis study to identify the differences, if any, due to maternal effect. Morphologically the F_1s of inter specific crosses resembled the African marigold (♀) more than the French type (♂). In each cross, the superior F_1 hybrid was selected based on the yield-correlated characters and heterobeltiosis (over the better parent-BP) was worked out for the intraspecific hybrids (Table 14), intraspecific hybrids from direct and reciprocal hybrids (Table 15) and interspecific hybrids (Table 16). Similarly relative heterosis (over the mid parental value-MP) was also worked out for the intraspecific hybrids (Table 17), intraspecific hybrids from direct and reciprocal hybrids (Table 18) and interspecific hybrids (Table 19). In general, the F_1 s showed wide variation for heterosis with respect to each of the selected trait.

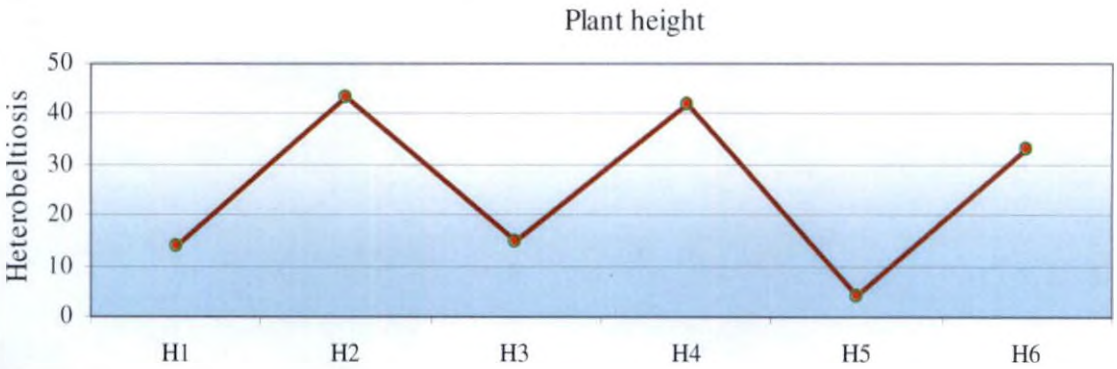
a) Plant height

Among the 18 successful hybrids, 14 expressed positive heterobeltiosis for this character. The maximum heterobeltiosis (51.42) and relative heterosis (86.67) for this character was recorded in the hybrid Pil.O-3 X Tr-5. The hybrid Kah-2 X Th-6 showed lowest heterobeltiosis (-80.00) as well as lowest relative heterosis Vkl-5 X Pil.O-4 (-40.29) for this character. In the hybrids of reciprocal hybrids much difference were not recorded except for the hybrid (Th-6 X Ka-3) in which, the direct cross had low heterobeltiosis than that from the corresponding reciprocal cross. (Fig 7 a and 8 a).

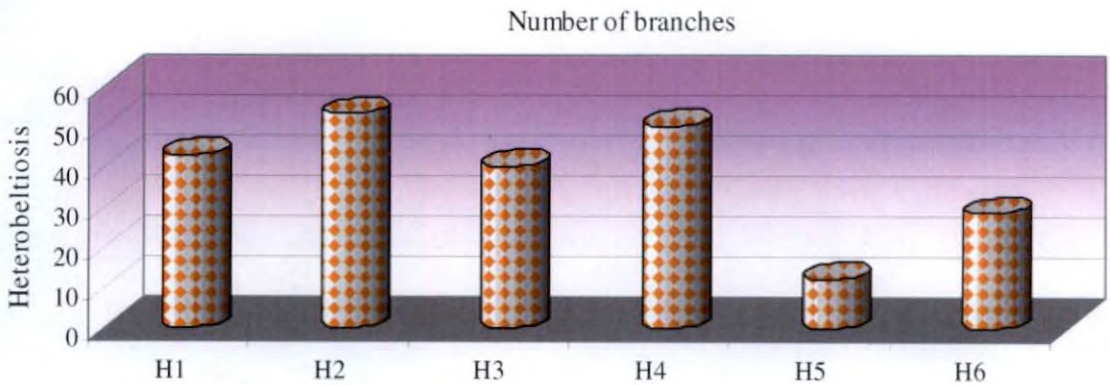
Among the three inter specific hybrids only one showed positive heterobeltiosis (Vkl-1 X L.H.F) and the range was 3.75 (Vkl-1 X L.H.F) to -20.00 (Tr-3 X S.T) whereas all the three showed positive relative heterosis with a range of 49.53 (Vkl-1 X L.H.F) to15.83 (Tr-3 X S.T).

Fig. 7 Heterobeltiosis for significant characters in hybrids of African marigold

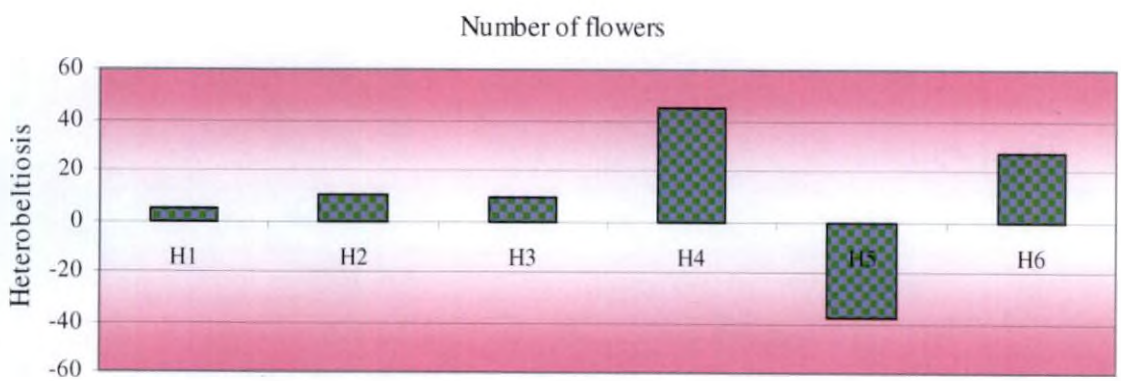
a)



b)

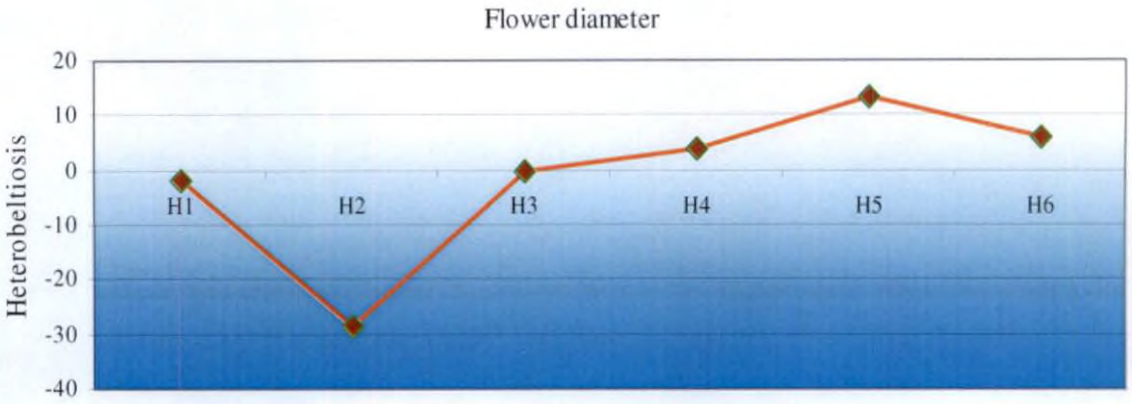


c)

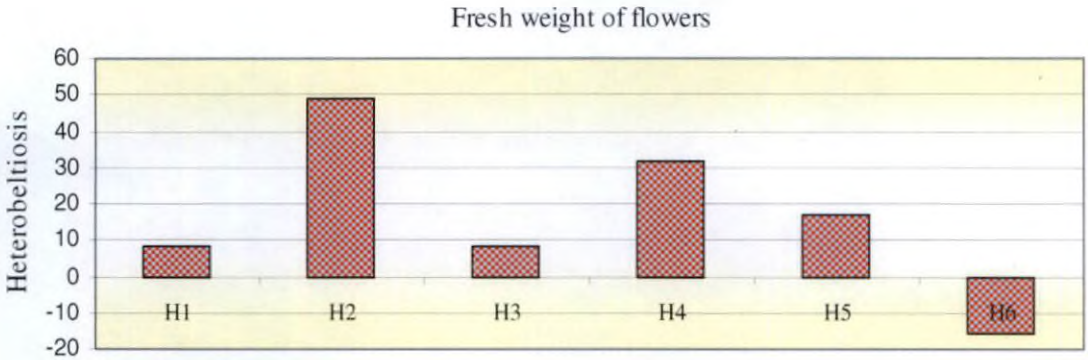


H1: Th-1 X C-6, H2: Tr-5 X Pil.O-3, H3: Th-1 X A-3,
H4: Vk1-5 X Pil.O-4, H5: Th-6 X Ka-3, H6: Tr-3 X Vk2-3

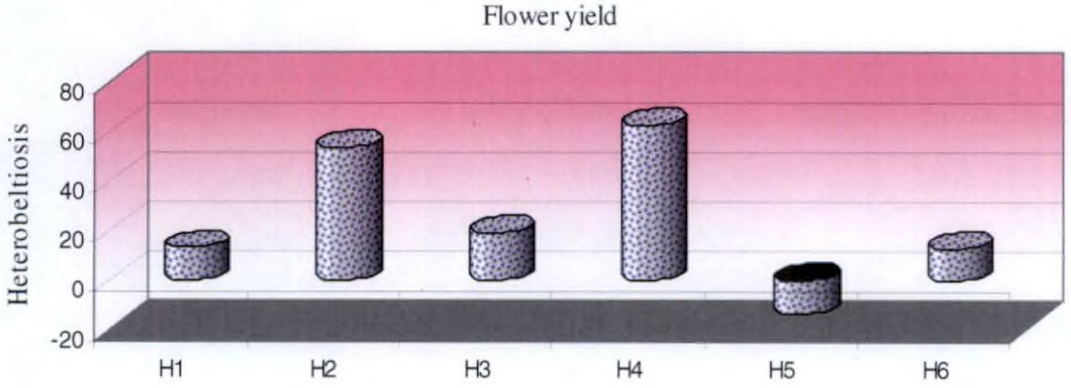
d)



e)



f)



H1: Th-1 X C-6, H2: Tr -5 X Pil.O-3, H3: Th-1 X A-3,

H4: Vk1-5 X Pil.O-4, H5: Th-6 X Ka-3, H6: Tr-3 X Vk2-3

Table 14. Heterobeltiosis for the superior hybrids in direct crosses of African marigold

Character	Kah-2 X Th-6	Vk1-5 X Pil.O-4	Th-1 X A-3	Vk2-3 X T-5	Pil.O-5 X Th-1	T-3 X Vk1-1	Ko-2 X A-5
Plant height	-80.00	41.86	15.11	28.57	2.66	3.75	-8.64
Stem girth	-128.57	7.31	7.89	-29.03	12.50	17.94	-5.40
Leaf length	13.15	5.71	-19.04	-13.34	-7.69	-180.76	-42.85
Leaf width	66.67	41.66	55.56	30.00	0.00	-133.34	18.18
No. of branches	12.50	50.00	40.00	26.31	0.00	11.12	-6.67
Duration of the crop	6.36	0.00	1.81	17.97	-22.72	3.57	-2.85
Days to flower	9.09	7.69	7.69	24.24	9.09	10.44	7.69
No. of flowers	-25.71	45.00	9.52	-25.00	-36.84	13.34	-40.00
Pedicel length	8.00	-11.12	0.00	0.00	-25.00	-27.45	-16.67
Flower diameter	12.24	4.00	0.00	2.04	-20.00	-18.42	-28.26
Flower color	Orange yellow	Yellow	Orange yellow	Orange yellow	Orange	Lemon yellow	Yellow
Number of whorls of ray florets	-124.00	0.00	0.00	-400.00	-140.00	83.34	-300
Fresh weight of flowers	-1.80	31.93	8.53	27.50	-121.89	-34.78	-3.92
Seed set %	6.89	4.76	3.34	-7.86	-11.53	-10.97	9.52
Flower yield	-28.00	62.56	19.21	3.34	-21.33	-16.81	-45.51

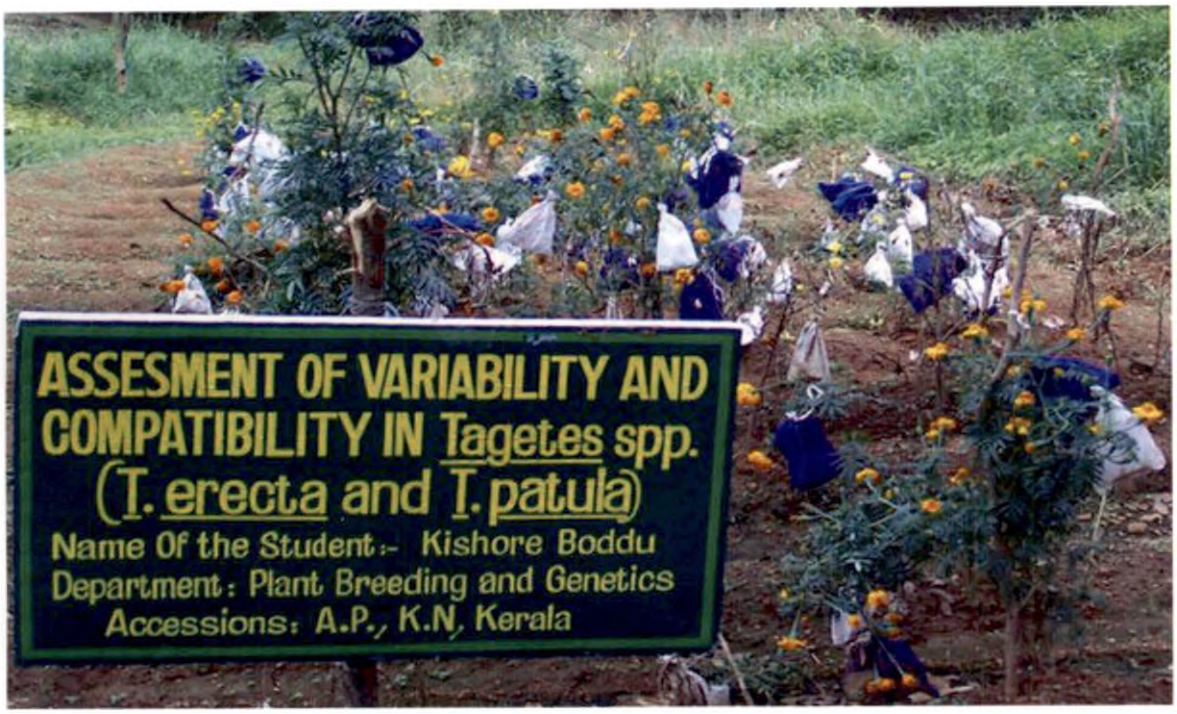
Table 15. Heterobeltiosis for the superior hybrids in direct and reciprocal crosses of African marigold

Character	Pil.O-3 X Tr-5	Tr -5 X Pil.O-3	Th-1 X C-6	C-6 X Th-1	Th-6 X Ka-3	Ka-3 X Th-6	Tr-3 X Vk2-3	Vk2-3 X Tr-3
Plant height	51.42	43.34	14.11	13.09	4.00	10.76	33.34	30.56
Stem girth	12.50	-2.43	12.50	16.67	-68.42	2.23	-5.56	5.00
Leaf length	-10.04	-13.04	0.00	-5.00	-43.17	-14.28	-17.85	21.42
Leaf width	45.45	3.34	27.27	0.00	63.63	50.00	12.50	41.67
No. of branches	50.00	52.94	42.85	57.14	12.50	0.00	28.57	37.50
Duration of the crop	12.72	12.72	3.57	-2.85	6.36	8.03	-4.76	-4.76
days to flower	4.76	6.25	0.00	3.22	10.71	37.5	7.69	9.09
No. of flowers	15.62	10.00	5.26	5.00	-37.50	-41.17	26.67	31.25
Pedicel length	6.25	-17.64	1.96	-47.05	11.53	2.50	13.04	1.63
Flower diameter	0.00	-28.57	-1.69	-22.44	13.46	-9.43	5.88	0.00
Flower color	Orange	Orange	Orange yellow	Lemon yellow	Orange yellow	Lemon yellow	Yellow	Orange yellow
Number of whorls of ray florets	0.00	87.50	0.00	-140.00	0.00	-1100	0.00	0.00
Fresh weight of flowers	40.59	48.70	8.53	4.33	17.03	11.55	-15.93	19.15
Seed set %	10.52	10.58	1.13	2.24	7.95	-4.81	-7.50	-6.97
Flower yield	49.87	53.83	13.10	9.11	-14.07	-24.86	12.82	43.00

Table16. Heterobeltiosis for the superior hybrids in Inter specific crosses (African x French)

Character	Vk1-1 X L.H.F	Th-6 X S.B	Tr-3 X S.T
Plant height	3.75	-4.28	-20.00
Stem girth	-3.20	7.89	11.90
Leaf length	-14.06	-75.00	16.00
Leaf width	-27.27	0.00	0.00
No. of branches	-60:00	25.00	-33.34
Duration of the crop	7.69	3.57	1.81
days to flower	3.22	7.69	-13.34
No. of flowers	-18.18	-26.67	-6.67
Pedicel length	4.41	-6.38	-16.00
Flower diameter	-2.17	-15.38	9.25
Flower color	Orange	Yellow	Lemon yellow
No. of ray florets	0.00	-1100	70.00
Fresh weight of flowers	-6.89	-17.18	19.04
Seed set %	-3.40	-8.75	-7.69
Flower yield	-16.67	-48.43	-34.92

Plate 5. Selfing of selected plants using butter paper and net bags



a) African marigold

b) French marigold

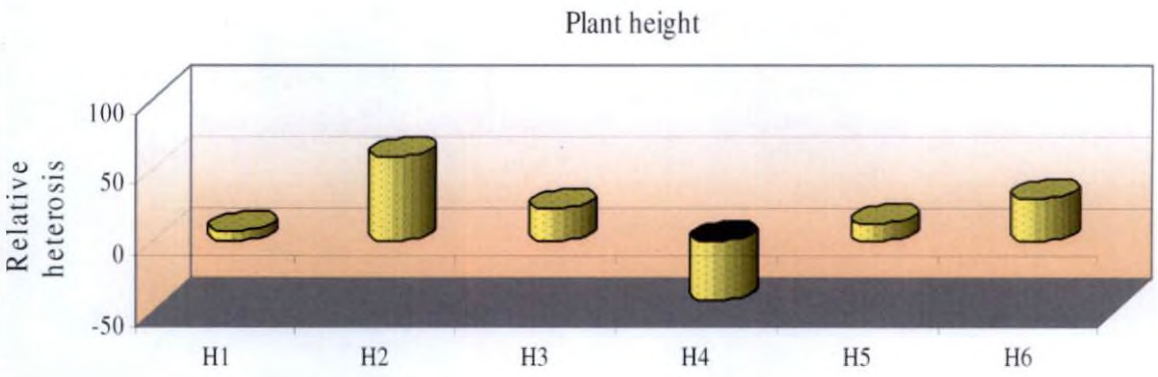
Plate 6. Germination test for selfed seeds of African marigold

a) Three days after germination

b) 25 days after germination

Fig. 8 Relative heterosis for significant characters in hybrids of African marigold

a)



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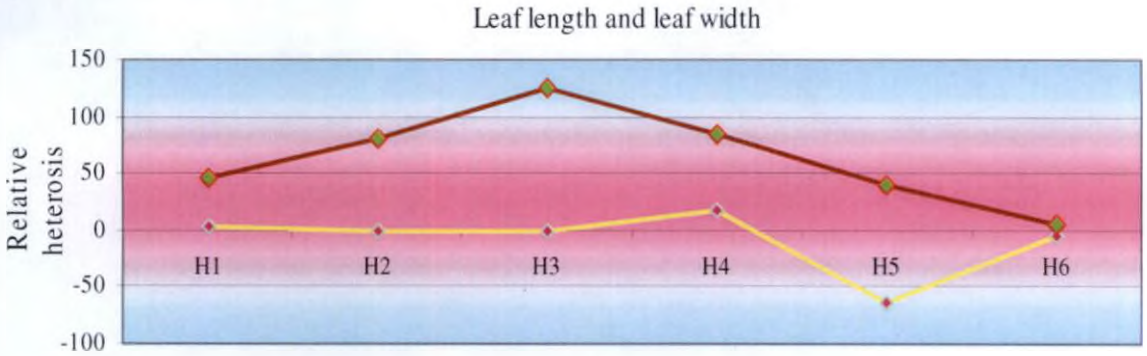
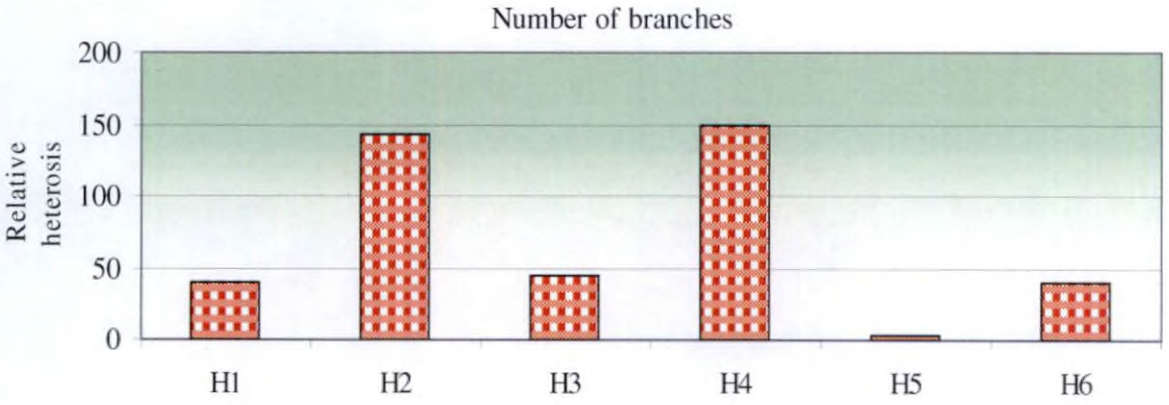
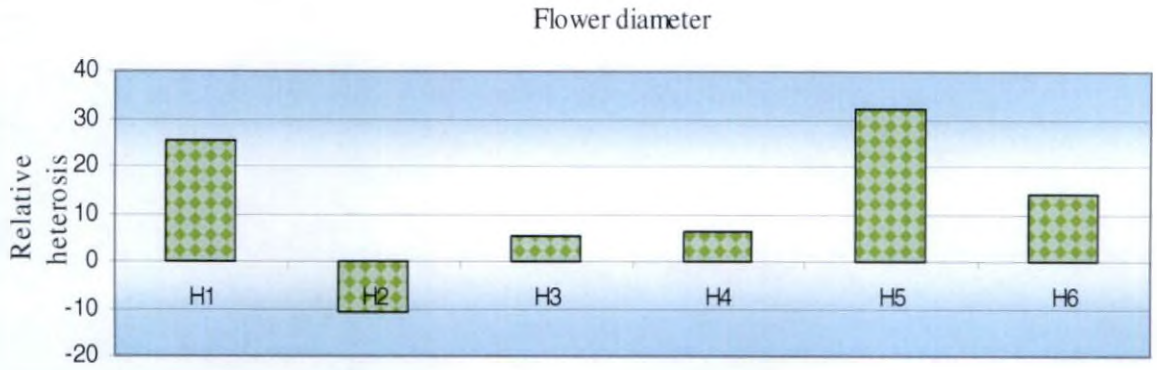


c)

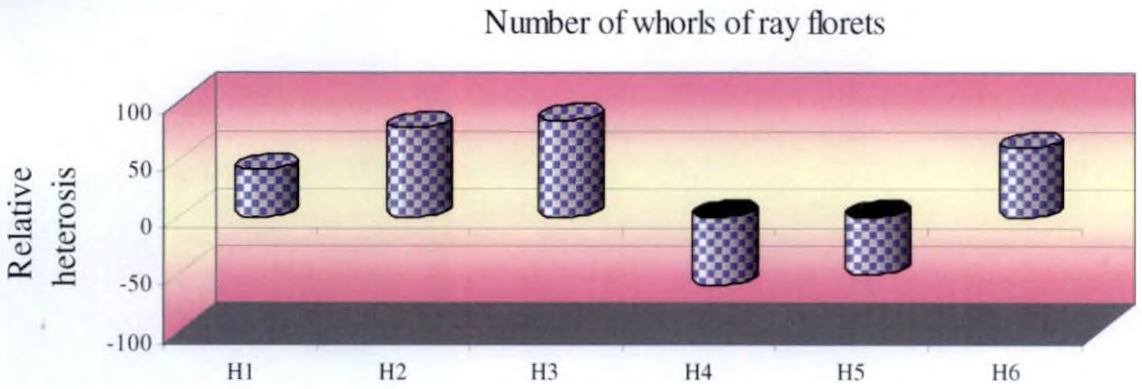


H1: Th-1 X C-6, H2: Tr-5 X Pil.O-3, H3: Th-1 X A-3,
H4: Vk1-5 X Pil.O-4, H5: Th-6 X Ka-3, H6: Tr-3 X Vk2-3

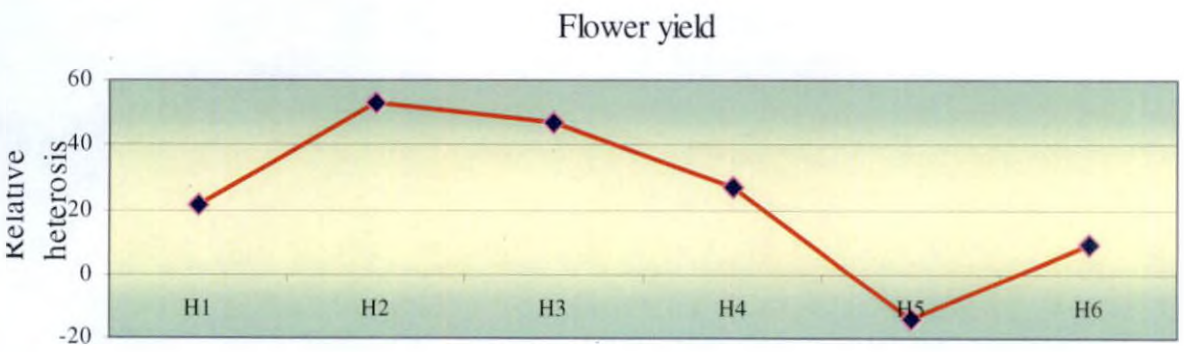
d)



e)



f)



H1: Th-1 X C-6, H2: Tr-5 X Pil.O-3, H3: Th-1 X A-3,

H4: Vk1-5 X Pil.O-4, H5: Th-6 X Ka-3, H6: Tr-3 X Vk2-3

Fig. 9 Total carotenoid content in parents and hybrids of African marigold

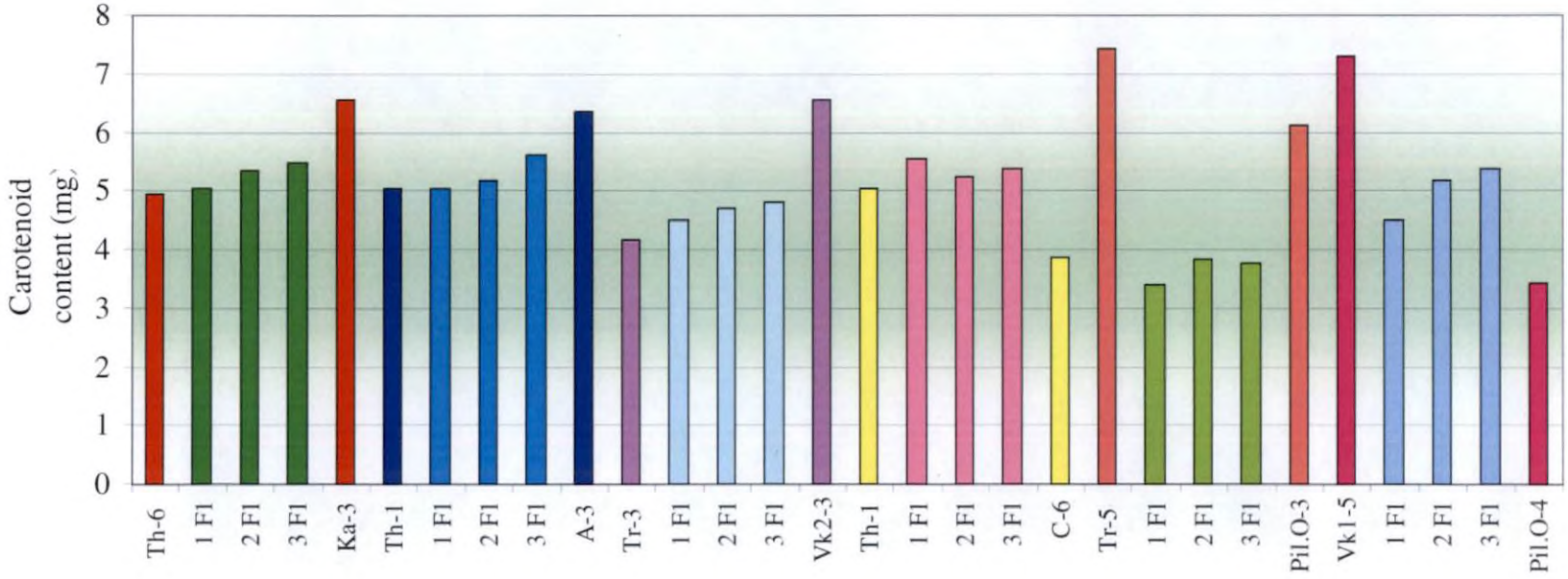


Table 17. Relative heterosis for the superior hybrids in direct crossesof African marigold

Character	Vk1-5 X Pil.O-4	Vk2-3 X T-5	Th-1 X A-3	Kah-2 X Th-6	Pil.O-5 X Th-1	T-3 X Vk1-1	Ko-2 X A-5
Plant height	-40.29	10.22	23.18	7.85	18.00	8.12	-8.02
Stem girth	-47.66	12.32	8.57	-45.16	6.25	-28.20	-13.51
Leaf length	18.42	27.27	0.00	-3.34	2.56	-109.61	-57.14
Leaf width	84.61	118.18	125.00	30.00	6.25	-83.34	18.18
No. of branches	150.00	14.28	45	21.05	-8.34	11.12	20.00
Duration of the crop	4.26	0.91	1.85	15.23	-11.93	12.05	2.85
days to flower	-15.38	18.18	0.00	32.00	16.67	17.91	0.00
No. of flowers	116.21	20.68	15.06	-2.78	-54.16	-20.00	0.00
Pedicel length	-8.25	0.00	12.72	-1.67	-35.00	-16.67	-96.15
Flower diameter	6.52	12.35	5.45	4.08	9.00	-21.05	-2.17
Flower color	Orange yellow	Yellow	Orange yellow	Thick orange	orange	Light yellow	yellow
No. of ray florets	-60.00	60.00	84.61	-2.00	-130.00	50.00	-250.00
Fresh weight of flowers	5.26	69.39	64.38	31.87	-95.50	-17.60	-18.39
Seed set %	7.40	13.51	13.04	-7.92	-12.82	-3.60	4.76
Flower yield	27.04	72.70	47.23	29.98	-20.03	-40.86	-17.85

Table 18. Relative heterosis for the superior hybrids in direct and reciprocal crosses of African marigold

Character	Pil.O-3 X Tr-5	Tr -5 X Pil.O-3	Th-1 X C-6	C-6 X Th-1	Th-6 X Ka-3	Ka-3 X Th-6	Tr-3 X Vk2-3	Vk2-3 X Tr
Plant height	86.67	60.00	6.91	5.66	12.67	-33.84	30.43	25.21
Stem girth	33.34	13.88	0.00	5.00	-63.15	-28.89	-1.36	9.58
Leaf length	0.00	0.00	3.70	-1.23	-63.04	-21.42	-5.08	42.37
Leaf width	120.00	80.00	46.67	33.34	40.09	31.25	6.67	33.34
No. of branches	128.57	142.85	40.00	86.67	3.125	10.00	40.00	60.00
Duration of the crop	7.84	7.84	6.16	-0.47	8.18	11.16	-3.66	-3.66
days to flower	0.00	1.58	0.00	3.34	10.71	31.25	1.53	12.28
No. of flowers	72.97	62.16	2.56	2.56	12.50	-41.17	0.00	5.26
Pedicel length	28.00	2.00	27.50	-15.00	15.38	32.50	1.63	15.00
Flower diameter	14.89	-10.63	25.33	4.25	32.00	-1.88	14.28	-2.04
Flower color	Orange	Orange	Orange yellow	Light yellow	Orange yellow	lemon yellow	Yellow	Orange yellow
No. of ray florets	-77.78	77.28	41.17	-41.17	-50.00	-700	60.00	60.00
Fresh weight of flowers	14.70	32.84	25.00	19.51	-30.37	22.52	4.89	50.43
Seed set %	10.52	11.84	4.14	5.32	6.25	0.00	0.00	10.00
Flower yield	49.59	53.23	22.00	18.41	-14.00	-9.23	9.43	40.79

Table 19. Relative heterosis for the superior hybrids in inter specific crosses (African x French)

Character	Vk1-1 X L.H.F	Th-6 X S.B	Tr-3 X S.T
Plant height	49.53	22.14	15.83
Stem girth	-1.58	6.57	21.42
Leaf length	42.23	-35.41	4.00
Leaf width	10.00	18.75	14.28
No. of branches	-23.07	25.00	-33.24
Duration of the crop	26.48	18.75	16.36
days to flower	39.32	33.07	20.00
No. of flowers	-12.00	13.34	-40.00
Pedicel length	18.26	-4.25	8.00
Flower diameter	12.19	-0.96	9.25
Flower color	Orange	yellow	lemon yellow
No. of ray florets	-50.00	-700	65.00
Fresh weight of flowers	70.65	32.34	50.95
Seed set %	8.00	-8.04	9.23
Flower yield	33.41	41.36	24.12

b) Stem girth

There were seven F_1 plants which showed negative heterosis for stem girth. Pil.O-3 X Tr-5 recorded maximum relative heterosis (33.34) and T-3 X Vk1-1 recorded maximum heterobeltiosis for this character. Only for one hybrid (Pil.O-3 X Tr-5) greater values of heterobeltiosis and relative heterosis was recorded in direct crosses than the corresponding reciprocal hybrids. The hybrid (Kah-2 X Th-6) showed lowest heterobeltiosis (-128.57) and hybrid (Th-6 X Ka-3) recorded the lowest relative heterosis (-63.15).

Out of three, inter specific hybrids only one showed positive heterosis than BP and MP. The range of heterobeltiosis was 11.90 (Tr-3 X S.T) to -3.20 (Vk1-1 X L.H.F) and the relative heterosis ranged from21.42 (Tr-3 X S.T) to -1.58 (Vk1-1 X L.H.F).

c) Number of branches

For number of branches, out of 18 hybrids 16 hybrids showed positive heterobeltiosis The range for relative heterosis for this character was 150 (Vk2-3 X T-5) to -33.24 (Tr-3 X S.T), and heterobeltiosis ranged from 57.14 (C-6 X Th-1) to -60.00 (Vk1-1 X L.H.F). All the hybrids from direct crosses except (Th-6 X Ka-3, Pil.O-3 X Tr-5) recorded greater values of heterobeltiosis and relative heterosis than their corresponding reciprocal hybrids. Only two (Ko-2 X A-5 and Tr-3 X S.T) showed negative heterobeltiosis. Relative heterosis was found negative in Tr-3 X S.T and Vk1-1 X L.H.F. (Fig 7 b and 8 c).

Among three inter specific hybrids one recorded positive heterobeltiosis (Th-6 X S.B) and one recorded negative relative heterosis (Tr-3 X S.T), the range of heterobeltiosis was 25 (Th-6 X S.B) to -60 (Vk1-1 X L.H.F) and the range of relative heterosis was 25 (Th-6 X S.B) to -33.24 (Tr-3 X S.T).

d) Leaf length

The range of heterosis for leaf length was 21.42 (Vk2-3 X Tr-3) to -180.76 (T-3 X Vk1-1) over BP and 42.37 (Vk2-3 X Tr-3) to -109.61 (T-3 X Vk1-1) over MP. Among direct and reciprocal hybrids only two (Th-6 X Ka-3, Tr-3 X Vk2-3)

recorded higher values of heterobeltiosis and relative heterosis in direct cross compared to reciprocal cross. Five hybrids registered positive heterobeltiosis and ten hybrids showed positive relative heterosis for this character. (Fig 8 b).

Among three inter specific hybrids one recorded positive heterobeltiosis (Tr-3 X S.T), one recorded negative relative heterosis (Th-6 X S.B) and the range of heterobeltiosis was 16 (Tr-3 X S.T) to -75.00 (Th-6 X S.B) and range of relative heterosis was 42.23 (Vk1-1 X L.H.F) to -35.41 (Th-6 X S.B).

e) Leaf width

The range of heterobeltiosis for leaf width was 66.67 (Kah-2 X T-6) to -133.34 (T-3 X Vk1-1) to and relative heterosis ranged from 125 (Th-1 X A-3) to -83.34 (T-3 X Vk1-1). All direct crosses recorded greater values of heteroeltiosis and relative heterosis than reciprocal hybrids except Tr-3 X Vk2-3. Out of 18 hybrids 11 showed positive heterosis for leaf width.

Out of the three, inter specific hybrids only one recorded negative heterobeltiosis (Vk1-1 X L.H.F) and the other two were on par with the BP.

f) Duration of the crop

Duration of the crop showed positive heterosis in 12 hybrids and varied from 17.97 (Vk2-3 X T-5) to -22.27 (Pil.O-5 X Th-1) over BP and ranged from 26.48 (Vk1-1 X L.H.F) to -11.93 (Pil.O-5 X Th-1) over MP. Among direct and reciprocal hybrids all direct hybrids recorded higher value than reciprocal hybrids.

All the three inter specific crosses recorded positive heterosis than MP and BP. The range of heterobeltiosis was 7.69 (Vk1-1 X L.H.F) to 1.81 (Tr-3 X S.T) and the range of relative heterosis was 26.48 (Vk1-1 X L.H.F) to 16.36 (Tr-3 X S.T).

g) Days to flower

Negative heterosis was preferred for days to flower and it ranged from -13.34 (Tr-3 X S.T) to 37.5 (Ka-3 X Th-6) and -15.38 (Vk1-5 X Pil.O-4) to 39.32 (Vk1-1 X L.H.F) over BP and MP respectively. Only the hybrid Vk1-5 X Pil.O-4

showed negative relative heterosis for days to flower. All the crosses reported lesser values for heterobeltiosis and relative heterosis compared to reciprocal hybrids.

Among the three inter specific hybrid only one (Tr-3 X S.T) recorded negative heterosis than BP and all the three recorded positive heterosis than MP. The range of heterobeltiosis was from 20 (Tr-3 X S.T) to 39.32 (Vk1-1 X L.H.F) for the character.

h) Pedicel length

Heterobeltiosis in pedicel length ranged from 13.04 (Vk2-3 X Tr-3) to -47.05 (C-6 X Th-1) and relative heterosis ranged from 32.50 (Ka-3 X Th-6) to -96.15 (Ko-2 X A-5). All hybrids from direct crosses recorded greater values than respective reciprocal hybrids for heterobeltiosis and relative heterosis .

Among the interspecific hybrid only one hybrid recorded positive heterosis (Vk1-1 X L.H.F) than better parental value and only one recorded negative heterosis than better parental value (Th-6 X S.B). The range of heterobeltiosis 4.41 (Vk1-1 X L.H.F) to -16.00 (Tr-3 X S.T) and the range of heterobeltiosis was 18.26 (Vk1-1 X L.H.F) to -4.25 (Th-6 X S.B).

h) Number of flowers

For number of flowers per plant, which is highly correlated to flower yield, the heterosis ranged from the 116.21 (Vk2-3 X T-5) to -54.16 (Pil.O-5 X Th-1) to over MP. The heterobeltiosis ranged from 45.00 (Vk1-5 X Pil.O-4) to -41.17 (Ka-3 X Th-6). Out of 18 successful hybrids, 9 showed positive heterobeltiosis and 12 showed positive heterosis. Among direct and reciprocal hybrids all hybrids from direct crosses except Tr-3 X Vk2-3 recorded greater values of heterobeltiosis and relative heterosis than those from reciprocal hybrids. (Fig 7 c and 8 e).

Among the three successful inter specific hybrids all the three recorded negative heterosis than BP and only one recorded positive heterosis than MP. The range of heterobeltiosis was -6.67 (Tr-3 X S.T) to -26.67 (Th-6 X S.B) and the range of relative heterosis was -13.34 (Th-6 X S.B) to -40.00 (Tr-3 X S.T).

j) Fresh weight of flowers

Out of 18 hybrids 11 hybrids showed positive heterobeltiosis and 14 hybrids Showed positive relative hetrosis. The highest heterobeltiosis recorded was 48.70 (Tr-5 X Pil.O-3) and lowest was recorded in (Pil.O-5 X Th-1), the highest relative heterosis was recorded in Vk2-3 X T-5 (69.39) and lowest was in Pil.O-5 X Th-1 (-95.50). In case of all the reciprocal hybrids positive heterobeltiosis was recorded. Only one interspecific hybrid showed positive heterobeltiosis for fresh weight of flowers and the range was from 19.04 (Tr-3 X S.T) to -17.18 (Th-6 X S.B) and all the three interspecific crosses showed positive heterosis over mid parental value, the range was from 70.65 (Vk1-1 X L.H.F) to 32.34 (Th-6 X S.B). (Fig 7 e)

k) Flower diameter

Out of 18 hybrids, 11 crosses showed positive heterobeltiosis and relative heterosis for the character flower diameter and three (Pil. O-3 X Tr-5 , Tr-3 X Vk2-3, Th-1 X A-3) hybrids were on par with the BP. The heterobeltiosis ranged from13.46 (Th-6 X Ka-3) to -28.26 (Ko-2 X A-5) and relative heterosis ranged from 32.00 (Th-6 X Ka-3) to -21.05 (T-3 X Vk1-1). All the hybrids from direct crosses recorded greater values of heterobeltiosis and relative heterosis than their respective reciprocal hybrids except Tr-3 X Vk2-3. (Fig 7 d and 8d)

Among three inter specific hybrids only one recorded positive heterobeltiosis (Tr-3 X S.T) and only one recorded negative relative heterosis (Th-6 X S.B). The range of heterobeltiosis was from 9.25 (Tr-3 X S.T) to -15.38 (Th-6 X S.B) and the range of relative heterosis was 12.19 (Vk1-1 X L.H.F) to -0.96 (Th-6 X S.B).

l) Flower colour and number of whorls of ray florets

Flower colour ranged from light yellow to orange, for number of whorls of ray florets seven hybrids showed negative heterobeltiosis and 10 hybrids showed negative relative heterosis value.

m) Yield of flowers

Out of 18 hybrids 10 hybrids showed positive heterosis over best parent and the range of heterosis was 62.56 (Vk1-5 X Pil.O-4) to -48.43 (Th-6 X S.B) and four hybrids recorded negative heterosis over mid parental value and the range of heterosis over mid parental value was 72.70 (Vk2-3 X T-5) to -20.03 (Pil.O-5 X Th-1). In case of three reciprocal hybrids positive heterobeltiosis was recorded and in only one hybrid it was negative. All three interspecific hybrids showed negative heterobeltiosis for flower yields and the range was from -34.92 (Tr-3 X S.T) to -16.67 (Vk1-1 X L.H.F) and all the three interspecific hybrids showed positive heterosis over mid parental value, the range was from 41.36 (Th-6 X S.B) to 24.12 (Tr-3 X S.T). (Fig 7 f and 8 f).

n) Seed set percentage

Seed set percentage registered maximum value of heterobeltiosis in the hybrid Tr-5 X Pil.O-3. The range of heterosis for this character was 10.58 (Tr-5 X Pil.O-3) to -11.53 (Pil.O-5 X Th-1) over BP and 13.51 (Vk2-3 X T-5) to -12.82 (Pil.O-5 X Th-1) over MP. Out of 18 hybrids nine showed positive heterobeltiosis and 4 showed negative relative heterosis. Among the direct and reciprocal hybrids seed set percentage recorded greater values of hetrosis in corresponding reciprocal hybrids than direct crosses.

All the three inter specific crosses recorded negative heterosis with respect to BP and one (Th-6 X S.B) recorded negative heterosis with respect to MP. The range of heterobeltiosis was -3.40 (Vk1-1 X L.H.F) to -8.75 (Th-6 X S.B) and the range of relative heterosis was 28.00 (Vk1-1 X L.H.F) to -8.04 (Th-6 X S.B).

4.4. Biochemical and molecular characterization

Six crosses which produced the superior hybrids were selected based on the heterosis analysis (Table 20). Two more superior hybrids produced by each of these crosses were also included for the biochemical analysis and molecular characterization, plate 7.

4.4.1. Total carotenoid content

In general, the carotenoid content for parents ranged from 3.44 mg (Vk1-5) to 7.42g (Tr-5) and in hybrids it ranged from 4.52 mg to 5.6 mg. The over all mean of parents was 5.56 mg and for the hybrids it was 5.54 mg respectively.

The carotenoid content in (Th-1) was 5.04 mg and in (C-6) it was 3.86 mg. The hybrids between these two parents had 5.54 mg, 5.26 mg, 5.38 mg respectively.

In the cross (Tr-5 X Pil.O-3) the carotenoid content of parents was 7.42 mg and 6.12 mg respectively and it was 3.39 mg, 3.82 mg, 3.76 mg for F1 plants.

The carotenoid content in (Th-1) was 5.04 mg and in (A-3) 6.34 mg. Hybrids between these two parents showed 5.04 mg, 5.16 mg, 5.6 mg respectively.

The carotenoid content in (Vk1-5) was 7.30 mg and in (Pil.O-4) was 3.44 mg and that of the hybrids was 4.52 mg, 5.16 mg and 5.38 mg. Mean value for only one hybrid (5.6 mg) recorded higher values than over all mean of parents (Fig 9).

In the cross. (Th-6 X Ka-3) the carotenoid content of parents was 4.94 mg and 6.56 mg per gram of fully opened flowers and in three F_1 hybrids the quantity was 5.04 mg, 5.34 mg and 5.48 mg.

In the cross (Tr-3 X Vk2-3) the carotenoid content of parents was 4.18 mg and 6.56 mg respectively whereas it was 4.52 mg, 4.70 mg and 4.80 mg in the three F_1 plants.

Table 20. Best crosses selected for biochemical and molecular characterization

Sl. No.	Parents	Genotypes	No. of superior F_1s
1	Th-1 X C-6	R1P1 X R8P6	3
2	Tr-5 X Pil.O-3	R10P2 X R6P3	3
3	Th-6 X Ka-3	R7P6 X R9P5	3
4	Th-1 X A-3	R1P1 X R8P3	3
5	Vk1-5 X Pil.O-4	R13P3 X R2P3	3
6	Tr-3 X Vk2-3	R4P6 X R7P6	3

Plate 7. Hybrids of African marigold

a) Th-1 X C-6

Th-1

X

C-6

b) Tr-5 X Pil.O-3

Tr-5

X

Pil.O-3

c) Th-1 X A-3

X

Th-1 A-3

F1

d) Vk1-5 X Pil.O-4

X

Vk1-5 Pil.O-4

F1

e) Th-6 X Ka-3

X

Th-6 Ka-3

f) Tr-3 X Vk2-3

X

Tr-3 Vk2-3

4.4.2 Molecular characterization of selected parents and hybrids

RAPD analysis:

Based on the heterotic potential of the F1s six crosses and three hybrids from each cross were selected for further characterization using biochemical and RAPD analysis.

As the reproducibility of analysis depends greatly on the various factors involved in DNA isolation, the quality and quantity of the reaction mixture for PCR and thermal profile applied, the standardization of the technique in a specific crop is an essential prerequisite. Hence, in the present investigation also a preliminary experiment was carried out to standardize the various steps and to select suitable primers showing polymorphism.

Very tender, fresh leaves (1 g) yielded good quality DNA in sufficient quantity. There was no browning of the extract if suitable antioxidants were added.

From the various protocols reviewed, CTAB method (Rogers and Bendich, 1988) and the protocol suggested by Doyle and Doyle (1987) were tried. The latter was found to be a quick method and yielded good quality DNA in sufficient quantity (Plate 8).

Based on the review of literature, initially 20 (OPA 1-10, OPS 1-5 and OPY 6-10) primers were screened and those primers which produced four -six bands were selected for PCR analysis of the 12 parents and 18 hybrids (Plate 9).

The RAPD products generated by each of the three selected primers gave characteristic bands in both parents and hybrids (Table 21). Altogether the three primers produced a maximum of 20 bands the size of which ranged from approximately 500 bp to 5148 bp. The average number of bands per primer for each cross is 5.8 (Plates 10, 11 & 12).

In the cross Th-1 X C-6, the comparison of the banding pattern of the hybrids with that of the parents revealed four monomorphic and 16 polymorphic bands produced by the three primers. Nine bands were common to hybrids and the

Plate 8.Genomic DNA of African marigold

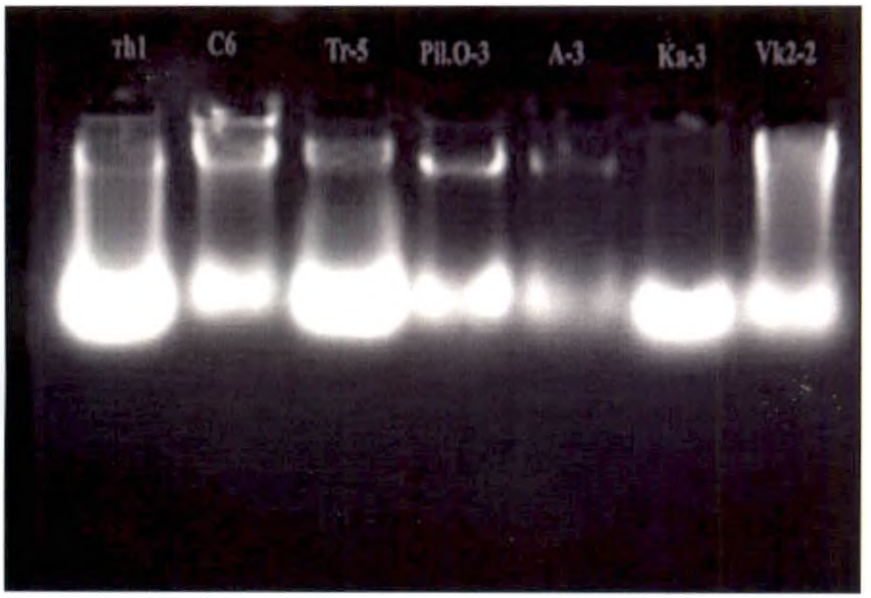
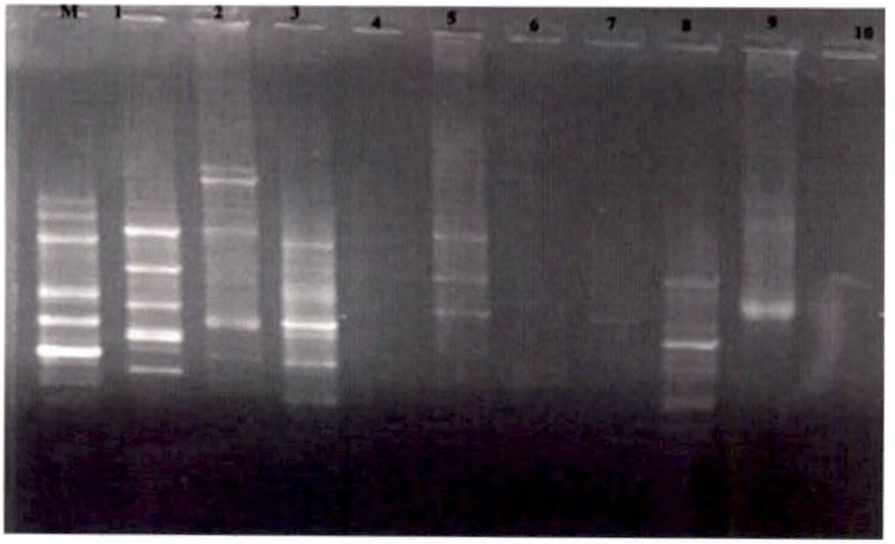


Plate 9. Primer screening using DNA from Tr-5

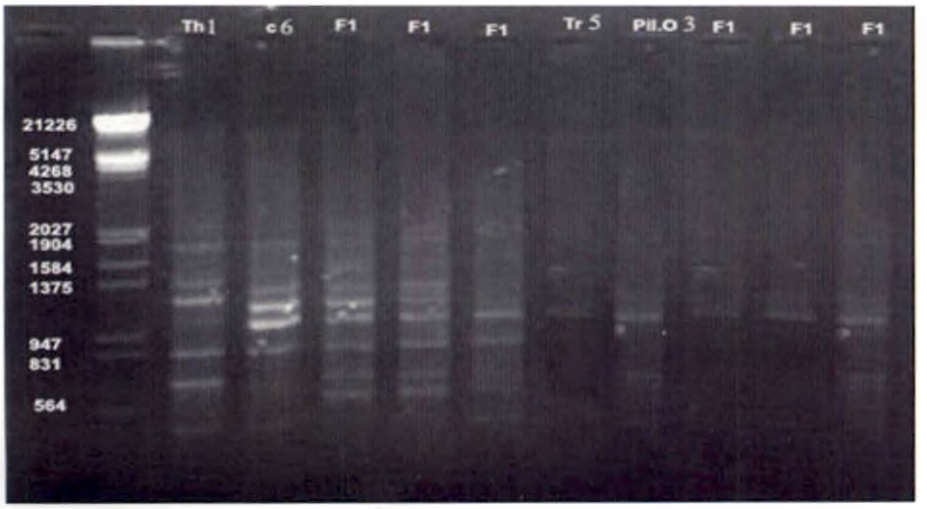


Decamer primer OPA 1-10

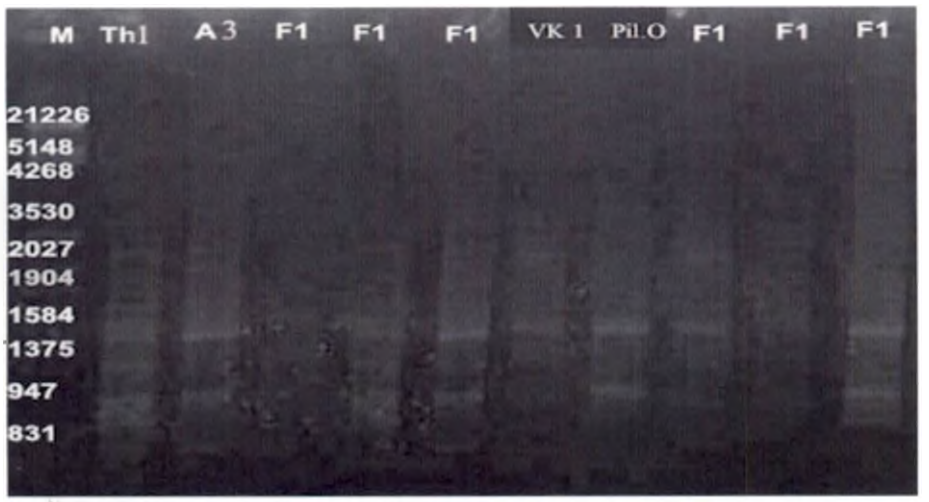
Table 21. RAPD Polymorphism between the parents and hybrids of different crosses of African marigold

Sl. No	Cross	OPY 9		OPY 10		OPA 2		Total		Grand total
		Mono morphic	Poly morphic	Mono morphic	Poly morphic	Mono morphic	Poly morphic	Mono morphic	Poly morphic	
1	Th-1 X C-6	3	4	0	8	1	4	4	16	20
2	Tr-5 X Pil.O-3	3	4	2	4	1	4	6	12	18
3	Th-1 X A-3	1	6	1	4	0	5	2	15	17
4	Vk-1-5 X Pil.O-4	1	5	2	3	2	4	5	12	17
5	Th-6 X Ka-3	4	3	1	6	1	2	6	11	17
6	Tr-3 X Vk2-3	2	3	1	5	1	3	4	11	15

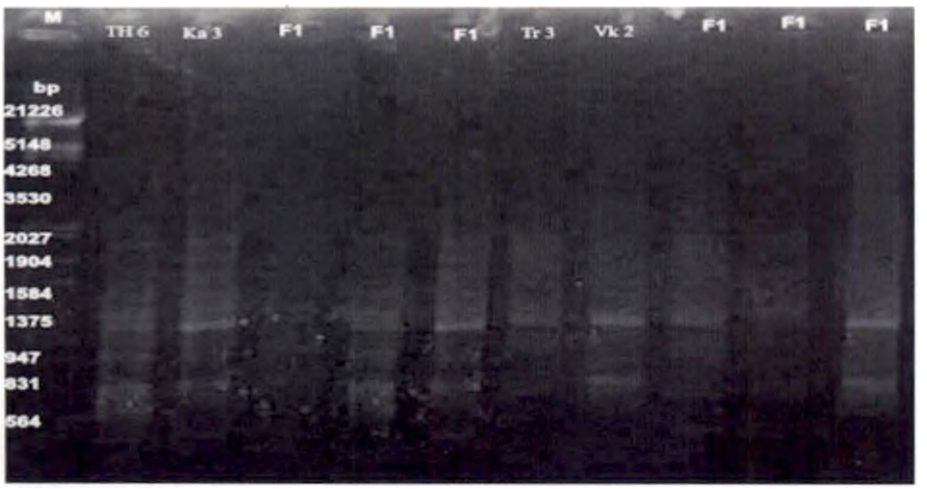
Plate 10. RAPD profile of parents and hybrids with primer OPY 9



1- Marker, 2-6 Th-1 X C-6, 7-11 Tr-5 X Pil.O-3

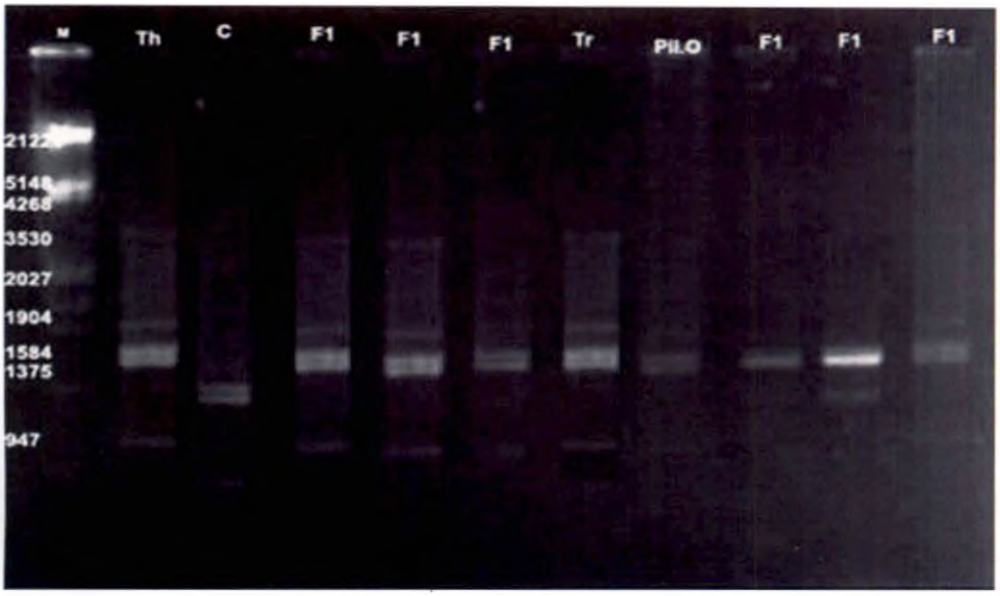


1-Marker, 2-6 Th-1 X A-3, 7-11 Vk1-5 X Pil.O-4



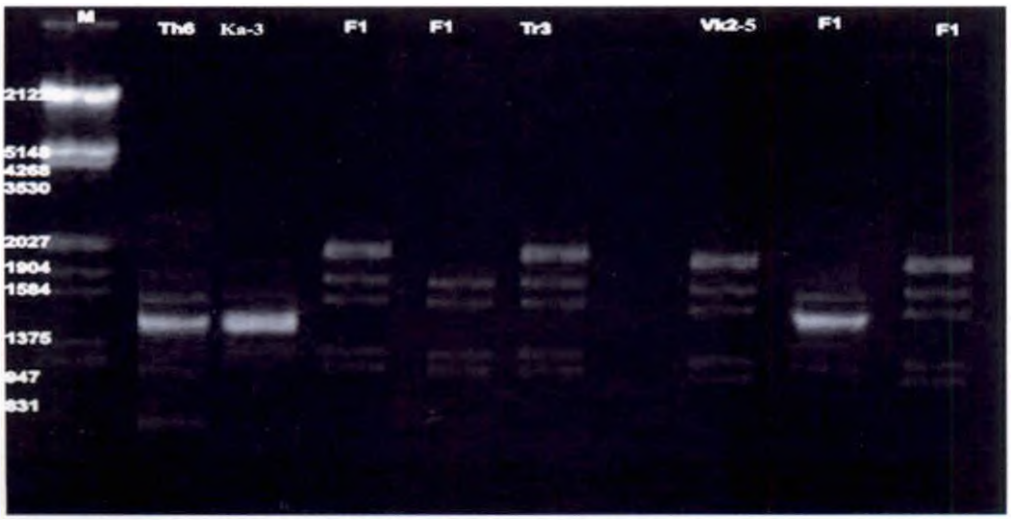
1-Marker, 2-6 Th-6 X Ka-3, 7-11 Tr-3 X Vk2-5

Plate 11. RAPD profile of parents and hybrids with primer OPY 10



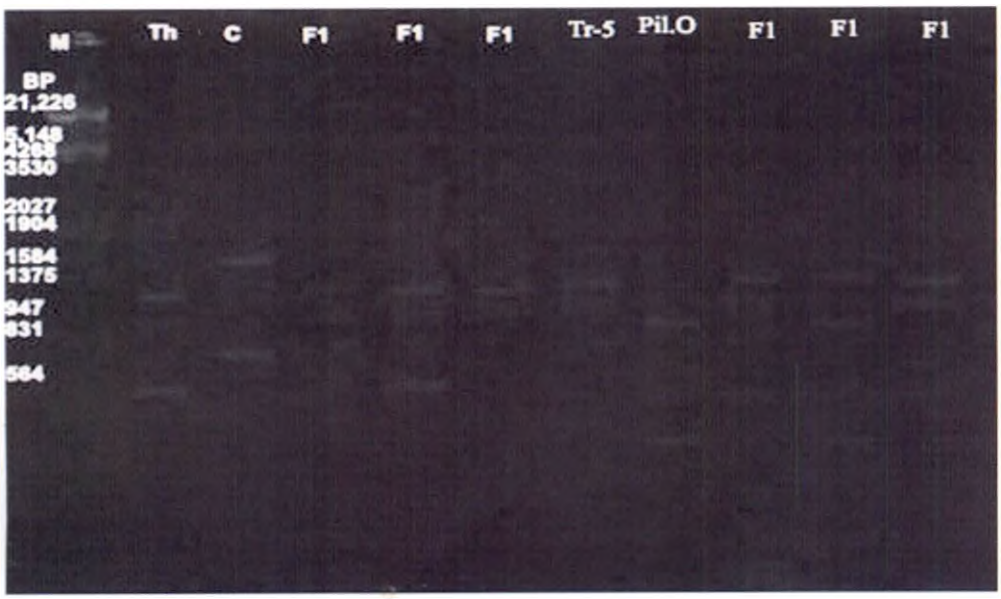
1- Marker, 2-6 Th-1 X C-6, 7-11 Tr-5 X Pil.O-3

1-Marker, 2-6 Th-1 X A-3, 7-11 Vk1-5 X Pil.O-4

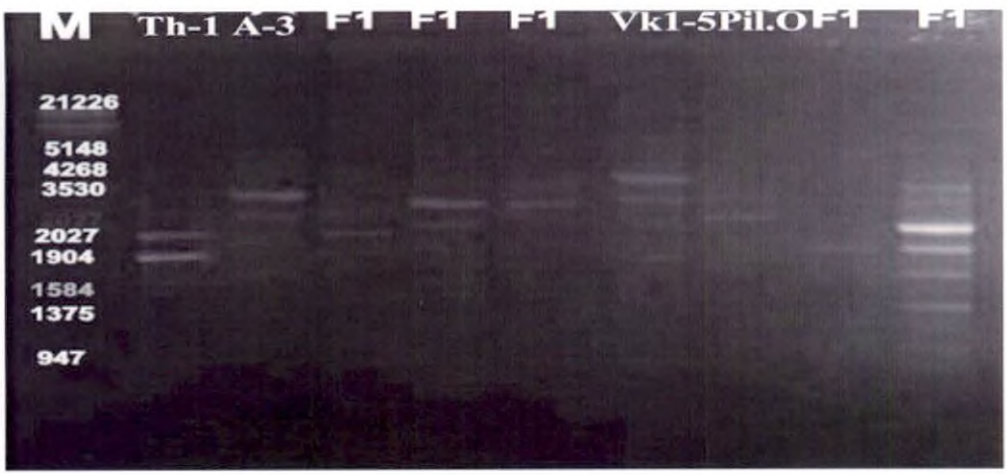


1-Marker, 2-6 Th-6 X Ka-3, 7-11 Tr-3 X Vk2-5

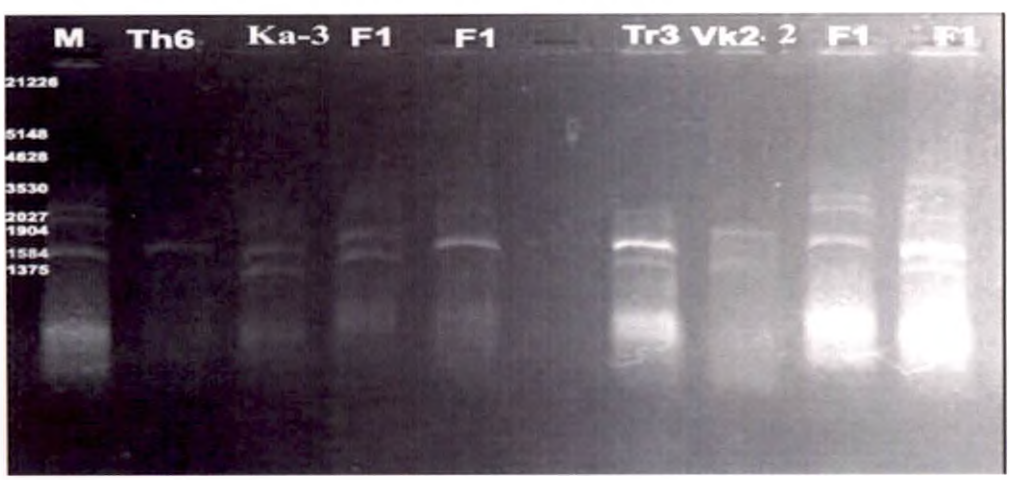
Plate 12. RAPD profile of parents and hybrids with primer OPA 2



1- Marker, 2-6 Th-1 X C-6, 7-11 Tr-5 X Pil.O-3



1-Marker, 2-6 Th-1 X A-3, 7-11 Vk1-5 X Pil.O-4



1-Marker, 2-5 Th-6 X Ka-3, 7-10 Tr-3 X Vk2-5

female parent (Th-1) and 11 were polymorphic whereas only four bands were common to the male parent (C-6) and the hybrids and 16 bands were polymorphic. The three hybrids differed with respect to six bands

When the banding pattern of the hybrids were compared to that of both parents in the cross Tr-5 X Pil.O-3, six monomorphic and 12 polymorphic bands were observed. The comparison of hybrids with female parent revealed six monomorphic and 12 polymorphic bands. Only five bands were common to the male parent and the hybrids, the rest 13 bands were polymorphic. Among the hybrids 13 bands were polymorphic.

In the cross Th-1 X A-3, hybrids and parents differed with respect to 15 bands out of 17. Two bands were monomorphic and 15 bands were polymorphic in female parent and hybrids where as five bands were monomorphic and 12 bands were polymorphic in male parent and the hybrids. The three hybrids showed polymorphism with respect to 13 bands.

Banding pattern of the hybrids and parents in the cross Vk-1-5 X Pil.O-4, showed five monomorphic bands and 12 polymorphic bands. Five bands were common to female parent (Th-6) and hybrids where as male parent and hybrids shared eight bands. Only 10 bands were polymorphic between the three hybrids.

In the cross Th-6 X Ka-3 six monomorphic bands and 11 polymorphic bands were produced by the three primers. When the hybrids were compared with each parent separately, eight bands were monomorphic in the female parent and hybrids whereas only seven bands were common to the male parent and the hybrids. The three hybrids differed with respect to only three bands.

When the banding pattern of the hybrids were compared to that of both parents in the cross Tr-3 X Vk2-3, four monomorphic bands and 11 polymorphic bands were observed. The hybrids showed monomorhism with respect to five bands when compared to the female parent (Tr-3) as well as male parent (Vk2-2). Only seven bands were polymorphic between the three hybrids.

Pair wise comparison of amplicons between the female and male parents of each cross showed that in the first cross the parents (Th-1 and C-6) differed with respect to 13 bands, in second cross (Tr-5 and Pil.O-3) with respect to six bands, in third cross (Th-1 and A-3) with respect to eight bands, in fourth cross (Vk1-5 and Pil.O-4) and fifth cross (Th-6 and Ka-3) with respect to 10 bands and sixth cross (Tr-3 and Vk2-3) with respect to four bands.

The bands of approximate size 1375 bp and 1584 bp were present in all the parents whereas 1904 bp band was found in all except Th-6 and A-3. Similarly the bands of approximate size 1375 bp and 1584 bp were shared by all the hybrids except two and 1904 bp was present in all the hybrids except three.

Discussion

5. DISCUSSION

African marigold (*T. erecta*), also referred to as American marigolds, are larger plants than French types, often with fewer but larger double flowers. This crop is popular with landscape designers due to its varied height and colours in shrubberies and as herbaceous border in gardens. French marigold (*T. patula*) is a versatile commercial flower crop in India with excellent growth, good flower yield and ideal for hanging baskets and window boxes.

All the germplasm exhibited significant variation in terms of vegetative characters and floral characters.

In any breeding programme, assessment of variability existing in the germplasm of the species under investigation is a prerequisite. In the present investigation, 14 accessions of African marigold collected from different eco-geographical locations of Kerala, Andhra Pradesh and Karnataka and seven varieties of French marigold collected from local nurseries were assessed for the genetic variability. The variability observed for 14 morphological characters have been analysed statistically to identify important yield related traits and to formulate a selection index to aid in selection programmes. The reproductive biology of both the species was studied and self and cross compatibility was assessed. Out of the selected hybrids, successful ones were subjected to heterosis analysis. The biochemical as well as molecular characterization of the parents and F_1 hybrids was carried out in selected superior hybrids and their parents. The results obtained are discussed in detail.

5.1 Variability studies of African marigold

The variability observed in 14 accessions African marigold is summarized below. Even though the accessions were collected from different places with different agro climatic conditions, in the present study flower initiation and total crop duration was found to be more or less uniform in the Vellanikkara conditions.

5.1.1 Extent of variation for quantitative characters

The accessions of African marigold differed significantly with respect to several vegetative and floral characters. The analysis of variance showed significant differences among the genotypes for the characters like plant height, leaf length, leaf width, number of branches, pedicel length, number of whorls of ray florets, fresh weight of flowers, seed set percentage and yield. Kishore and Raghava (2001) also observed the significant variation for these characters. The analysis of variance does not showed significant difference for number of flowers, which was in agreement with the results obtained by Gupta *et al.* (2001). Though the variation observed for number of flowers and flower diameter were non-significant, the total yield differed significantly between the accessions. This is because the fresh weight of flowers showed highly significant variation ($p= 0.01$) between the accessions and the total yield is a function of number of flowers and mean fresh weight of flowers.

Yield of flowers and flower weight showed maximum variance followed by number of whorls of ray florets. Kennedy (1997) and Singh and Sen (2000) reported maximum variance for yield of flowers and flower weight.

Though the flower size was more or less uniform as there was no significant variation for flower diameter, the acceptability varied because of the highly significant variation showed by number of whorls of ray florets.

In French marigold as the number was not uniform in all the accessions statistical analysis could not be carried out. Trichur Local was the tallest (59 cm) among the seven genotypes and Little Hero Fire was a dwarf. French vanilla had a thick stem (4cm) also compared to Trichur Local (1.75 cm). The French Vanilla in general had thick stem, larger leaves as well as heavy (2.48 g) and large flowers (5.1 cm). The duration of crop was more or less same in the different accessions (70.5 to 75.34 days) and flowering started with in 25-29 days in all. Trichur Local recorded minimum mean value for pedicel length (3.55 cm) and Boy Spry recorded maximum mean value for pedicel length (5.46 cm).The plant spread as indicated by the number of branches was high in

Safari Bolor. Little Hero Fire was found to be a heavy yielder (24.34 flowers) where as in Trichur Local there were only four flowers and the weight of flowers also minimum (0.51 g). The mean value for yield was recorded maximum in Boy O Boy (27.13 g) and minimum (2.04 g) in Trichur Local. Flower colour in different accessions ranged from single (Yellow, creamy white) to multiple colours (Yellow mixed with red, Dark red ray florets with yellow center disc). These results are in agreement with results obtained by Singh *et al.* (2003) and Singh and Singh (2005).

5.1.2. Coefficient of variation, Heritability, Genetic advance and Genetic gain

All the characters showed higher phenotypic variance compared to genotypic variance. Consequently, genotypic coefficient of variation for all the characters under study were low compared to respective phenotypic coefficient variation, which indicated greater G X E interactions. The results obtained by Kishore and Raghava (2001) and Sreekala *et al.* (2002) were also in agreement with this.

The heritability estimates, which indicate the accuracy with which genotypes could be identified by its phenotypic performance, was also worked out considering the influence of environment as indicated by the difference between genotypic and phenotypic coefficient of variation. Magnitude of heritable variability is the most important aspect of genetic constitution of all the breeding material, which has close bearing on the response to selection (Panse, 1957). In the present study, heritability values were high for yield of flowers, number of whorls of ray florets and plant height, which is in agreement with the results reported by Singh and Sen (2000). These findings suggest that there is a scope for improvement of these characters through direct selection. Sreekala *et al.* (2002) reported high heritability for plant height, flower weight and flower yield. Patnaik and Mohanty (2002) also recorded high heritability for fresh weight of flowers.

Low heritability values were recorded for stem girth and flower diameter. This, combined with wide variation in genotypic and phenotypic coefficient of

variation, suggest that improvement of these characters through selection is not viable. All other characters showed moderate heritability.

Heritability values along with genetic gain are more useful criterion in predicting the reluctant effects for the best individual (Johnson *et al.*, 1955). High heritability along with high genetic gain was observed for yield, fresh weight of flowers, number of whorls of ray florets and plant height. This indicates that the scope for improvement of these characters through direct selection is high. Singh and Sen (2000) as well as Kishore and Raghava (2001) also reported high heritability and genetic advance for yield of flowers and fresh weight of flowers in marigold. Talukdar *et al.* (2003) reported high heritability and genetic advance for both ray and disc florets in chrysanthemum.

Medium heritability and medium genetic gain was observed for leaf length, number of branches, number of flowers and pedicel length. This indicates that the heritability is due to additive gene effects and selection may be effective. Low heritability and medium genetic gain was obtained for stem girth, indicating that the character is governed by additive gene effects, but there is environmental effect as indicated by low heritability and selection may be effective to some extent

Flower diameter exhibited low heritability along with low genetic gain, indicating that this character is highly influenced by environmental effects and selection could be ineffective.

5.1.3. Correlation and Path coefficient analysis

The correlation among various characters revealed the inter relationship between important characters. The significant and positive genotypic correlation between the characters such as plant height with stem girth and leaf length indicate that the inter relationship between the characters determine the overall vigor of the plant. Improvement of these characters may have a significant effect on increasing the fresh weight of flowers, ultimately contributing to the total yield.

Genotypic correlation coefficients in general were found to be higher than the corresponding phenotypic correlation coefficients for most of the characters indicating a strong association between the various characters studied. But the phenotypic expression of these strong correlations for different quantitative traits under study might have lessened by the influence of environment, as indicated by the higher level of significance in phenotype than genotype. Naik *et al.* (2004) also reported such effects. The direction of significance of both phenotypic and genotypic correlations was same in all the characters.

A perusal of data of present study revealed that plant height had significant and positive correlation with stem girth and leaf length at genotypic level. This shows that taller plants will have stronger stem with longer leaf. Similarly, plant height had significant and positive genotypic correlation on fresh weight of flowers and total flower yield, which indicates the influence of vigour of the plant in producing larger flowers in more number. The result is in agreement with Mohanty *et al.* (2003).

Stem girth showed significant and positive correlation with number of flowers and it had negative effect on flower diameter at genotypic level. As the number of flowers increases, the size may get reduced to some extent. Sirohi and Behera (2000) also reported that in chrysanthemum, plant spread had negative correlation with flower diameter at phenotypic level.

Leaf length showed positive and significant correlation with leaf width and fresh weight of flowers at genotypic level and only at phenotypic level on leaf width. The increase in total photosynthetic area might be contributing to the yield. Sreekala *et al.* (2002) reported similar result that plants with more plant spread (cm^2) had positive and significant effect on flower weight and flower yield. Senthil kumar *et al.* (2004) reported flower yield exhibited a significant and positive correlation with herbage yield. However, leaf width had negative and significant correlation on flower diameter at genotypic level. Pratap *et al.* (1999) also recorded a negative correlation between leaf area (cm^2) and size of the flower at phenotypic level.

Number of branches showed significant and positive correlation with number of flowers, flower diameter, seed set percentage and yield at phenotypic level. Therefore, improvement of this character will also improve these correlated characters in a positive direction. Sirohi and Behera (2000) in chrysanthemum and Singh (2003) in dahlia reported similar results. Number of branches had negative direct effect on yield, which was in agreement with the results obtained by Sreekala *et al.* (2002).

The significant and negative correlation of total duration of crop to the total number of flowers indicates that the effective flowering period is more important. If the vegetative growth is more extended, the total duration may extend but it may not contribute to the total number of flowers produced by that plant. Sreekala *et al.* (2002) also reported that flowering duration was significantly and negatively correlated with yield.

The significant and negative correlation of total duration of crop to yield may be due to high negative indirect effect through number of flowers (-0.4972) and leaf width (-0.0293).

Number of flowers was significantly and negatively correlated with flower diameter which is in agreement to the general observation that when flower number increases flower size decreases. Similar results were recorded by Sirohi and Behera (2000) in chrysanthemum.

Negative correlation was observed between number of whorls of ray florets and yield due to negative indirect effect through fresh weight of flowers. Similar results were reported by Sirohi and Behera (2000) in chrysanthemum that number of petals per flower was negatively correlated with yield.

Fresh weight of flowers exhibited the highest positive direct effect and significant correlation on yield followed by number of flowers per plant. Sirohi and Behera (2000) in chrysanthemum also recorded positive and significant correlation between yield and number of flowers per plant. Also in agreement with the results obtained by Sreekala *et al.* (2002). Mathew *et al.* (2005)

recorded that fresh weight of flowers and flowers per plant had maximum direct effect on flower yield.

5.1.4. Genetic divergence and Selection index

Fourteen accessions of African marigold were grouped into five clusters. Among the five clusters, cluster I has maximum number of accessions (5), cluster II had three accessions where as the rest of the clusters consisted of two accessions each.

Accessions included in cluster I had highest mean value for number of whorls of ray florets as well as highest mean value for stem girth and fresh weight of flowers. Cluster II recorded highest mean values for plant height, leaf length and flower yield. Cluster III had the highest mean value for leaf width, number of branches, pedicel length, flower diameter and seed set percentage. Cluster IV recorded highest mean value for days to flower and duration of the crop. Cluster V had the highest mean value for number of flowers.

From inter and intra cluster distances it was recorded that the intra cluster distances were smaller than inter cluster distances. Cluster IV had maximum intra cluster distance where as II had minimum. Maximum inter cluster distance was between cluster I and cluster IV, followed by IV and V, I and III and II and IV minimum inter cluster distance was between cluster II and cluster V.

The inter cluster distances were a measure of genetic distinctness among the clusters based on observed variation in all the characters studied. This indicates that members of these groups were very distinct from each other. Similar studies were conducted by Manjula *et al.* (2001) on 46 non-oil seed sunflower genotypes based on 14 characters, Reddy and Devasanamma (2004) in sunflower.

A better way to exploit genetic correlation with several traits having high heritability is to construct an index, called selection index, which combines

information on all the characters associated with yield. This technique provides information on yield components and thus aids in indirect selection for the improvement of yield.

The selection index involving all the yield components namely plant height, stem girth, number of primary branches, leaf length, leaf width, pedicel length, days to flower, duration of the crop, number of flowers, flower diameter, number of whorls of ray florets, fresh weight of flowers, seed setting per cent, maximum efficiency compared to direct selection based on yield. Smith (1937) model with all the yield components was selected for ranking the genotypes. Ranking based on selection index showed that Ka was the most superior one followed by A and S. It indicated that superiority of these genotypes were more stable and reliable since the selection index value was calculated considering other yield contributing factors also. Selection through index values in sunflower was also reported by Patil *et al.* (1997) and Ojha and Roy (1998).

The study revealed that the accessions Kapugal (Ka), Ananthagiri (A) and Sreemannarayana nagar (S) were the most promising ones the salient characters of which are given below.

Accession Kapugal (Ka) was identified as the most superior one, with an average yield of 114.69 g plant^{-1}. It took 56.66 days for flowering and produced an average of 24.66 flowers plant^{-1}, Average fresh weight of flowers was 4.64 g and average diameter of flower was 4.11 cm.

Accession Ananthagiri (A) with an average yield of 113.54 g plant^{-1} took 65.00 days for flowering and produced an average of 31.16 flowers plant^{-1}, average fresh weight of flower was 3.64 g and average flower diameter was 4.51 cm.

Sreemannarayana nagar (S) was the next high yielding accession with 103.75 kg plant^{-1} and it flowered in 61.83 days, producing on an average of 25.83 flowers per plant. Average fresh weight of flowers was 4.04 g and average flower diameter was 4.78 cm.

5.2 Reproductive biology and compatibility

Study of floral biology is quite essential for crop improvement programme and especially in heterosis breeding where emasculation and pollination at appropriate time should be carried out to obtain greater percentage of seed set.

5.2.1. Floral features

In the present study time of anthesis in different accessions ranged from 6 am to 10 am, which is in agreement with the results obtained by Mohanty *et al.* (2002). Time of anther dehiscence was from 9 am to 12 noon and completed by 2 pm. Anther dehiscence occurred at the time of opening of first whorl of ray floret which was in agreement with the results of Mohanty *et al.* (2002). Pollen grains were spiny with 98-99 percent fertility, spiny nature of pollen was also reported by Srivastava (1976). Time of stigma receptivity ranged from 4 to 6 days, with maximum receptivity during the first 2-3 days of anthesis of each floret as indicated by the fleshy succulent papillae which turned brown and dried after 5th day. Since it takes 10-17 days for the complete florets to open in a flower (Mohanty *et al.*, 2002) for maximum seed set, pollination has to be continued for several days.

5.2.2. Self and cross compatibility studies

All the plants tested for compatibility studies were found to be self and cross compatible. There was no difference in compatibility due to reciprocal crossing. In general, the seed set percentage was less in selfing (10 %) compared to crossing indicating that the crop is better adapted to cross pollination (74 %). The presences of different insects in the field also substantiate this observation.

The seed set under selfing and artificial selfing using two types of bags were on par. Similar results were reported by Beher and Debner (2004) in dhalia. The average seed set for both selfing and artificial selfing in nylon net bags was higher than butter paper bags. This may be because of the lesser humidity inside the nylon bags. Patil (2003) in his work reported that bagging

of individual buds in sunflower adversely affected the seed set percentage and butter paper bags showed superior seed setting in comparison to the muslin cloth bagging.

5.3. Heterosis

In the present investigation, critical analysis of the results obtained for the heterobeltiosis and relative heterosis for the various trait revealed that the heterosis breeding programme has great potential in improving the various economically important characters like flower size, fresh weight of flowers, flowering duration in this crop. Similar results of heterosis were obtained by Kumar *et al.* (1989), Reddy *et al.* (1989), Gupta *et al.* (2001), Mohanty *et al.* (2003), Sreekala and Raghava (2003), Velmurugan *et al.* (2003) in marigold.

The prospect of improvement in the various characters are summarized below and based on the values superior hybrids were selected for further analysis.

5.3.1. Heterobeltiosis and relative heterosis for the various characters

Only two intra specific and two inter specific hybrids showed negative heterobeltiosis for plant height whereas two hybrids (Ko-2 X A-5, Vk1-5 X Pil.O-4) showed negative relative heterosis. This indicates that in general hybrids were taller than their parents, the tallest among them being the hybrid of the cross Pil.O-3 X Tr-5. This hybrid ranked second in heterosis for fresh weight of flowers and third in flower yield also. All the hybrids from direct crosses showed higher heterobeltiosis in comparison to those from reciprocal crosses except Th-6 X Ka-3.

For stem girth, seven hybrids showed negative heterosis over better parent and eight hybrids over that of mid parent value. Thirteen hybrids recorded negative heterobeltisis and two hybrids showed negative relative heterosis for leaf length. Out of 18 hybrids only two showed negative heterosis over better parent. And in only one hybrid negative relative heterosis was recorded for leaf width.

As there is positive correlation between number of branches and number of flowers the hybrids with positive correlation were selected for the character number of branches only three hybrids showed negative heterosis over better parent and mid parental value. Reciprocals recorded higher heterosis in comparison to direct crosses. Highest value of relative heterosis was recorded in the hybrid Vk1-5 X Pil.O-4, which had highest heterosis for number of flowers also as both these characters were found related. This hybrid ranked second in fresh weight of flowers and percentage seed set also.

Earliness in the flowering is desirable character, except one hybrid (Th-1 X C-6), all the hybrids were late in flowering in comparison to their better parent and mid parent value. Duration of the crop increased in case of 14 hybrids over mid parent and better parent. For number of flowers which ultimately results in increased flower yield nine hybrids recorded heterosis over better parent and 12 hybrids recorded heterosis over mid parent value.

Shortening of pedicel was observed in eight hybrids as compared to corresponding better parent and in seven hybrids in comparison to mid parental value. Flower diameter was reduced in nine hybrids as compared to their better parents and in six hybrids as compared to their mid parental value.

Flower colour ranged from light yellow to orange, for number of whorls of ray florets seven hybrids showed negative heterobeltiosis and ten hybrids showed negative relative heterosis value.

Fresh weight of flowers is the desirable character as it influences the flower yield directly, fresh weight of flowers recorded positive heterosis in 11 hybrids over better parent and in 14 hybrids over mid parent. Ten hybrids showed positive heterobeltiosis for flower yield and 13 hybrids showed positive relative heterosis.

5.3.2. Selection of superior hybrids

Hybrid Th-1 X C-6 recorded average heterosis for all the characters and did not show any significant negative heterosis for any of the traits. It showed a positive heterobeltiosis value of 13.10 per cent for flower yield.

Hybrid Tr -5 X Pil.O-3 ranked first in fresh weight of flowers and seed set percentage and second for plant height and number of branches. Also it showed positive heterosis for all the characters except for days to flower which is a desirable character. It showed positive heterobeltiosis for number of whorls of ray florets, seed set percent recorded a heterobeltiosis of (10.58 %) which was highest among all the hybrids, highest heterobeltiosis for flower yield also recorded (53.83).

Th-1 X A-3 cross-recorded highest hetrobeltiosis for flower number and ranked third for number of branches and fresh weight of flowers and plant height. It showed a positive heterobeltiosis of 19.21 per cent for flower yield.

Vk1-5 X Pil.O-4 was showing highest heterobeltiosis for flower yield, 3 rd in fresh weight of flowers, it showed a positive heterobeltiosis value of 31.93 per cent.

The hybrid Th-6 X Ka-3 recorded highest heterobeltiosis for flower diameter (13.46 %), which is a desirable character in the market, 4th highest in seed set percentage and second in flower diameter.

Cross Tr-3 X Vk2-3 recorded second highest heterobeltiosis for number of flowers and highest heterobeltiosis for pedicel length and leaf length. It showed a positive heterobeltiosis of 12.82 per cent for flower yield. And this hybrid was the shortest among all the hybrids.

Because of the superior characters exhibited by these six hybrids they were selected for biochemical analysis and molecular characterization (Table 20).

5.4. Biochemical and molecular characterization

5.4.1. Total carotenoid content

Six crosses along with three hybrids from each were selected for carotenoid and xanthophyll analysis. The carotenoid content of parents ranged from 3.44mg to 7.30mg with an overall mean of 5.56mg. The range (4.13mg to 5.63mg per gram of fully opened flowers) and mean value (4.93mg) were found to be greater than that reported earlier (Velmurugan *et al.*, 2003) in African marigold. In hybrids the range was 4.52mg to 7.64mg and overall mean was 5.44mg per gram of fully opened flowers whereas in earlier studies the mean value of the hybrids (4.93 mg/g of fully opened flowers) was on par with that of the parents though the range (4.73 mg to 6.13 mg) was different (Velmurugan *et al.*, 2003). In the present study only hybrid recorded higher values than over all mean of the parents (5.6 mg). Kasemsap *et al.* (1990) recorded a maximum of 15249.5 ppm of carotenoid in African marigold.

5.4.2 Molecular characterization of selected parents and hybrids

RAPD analysis:

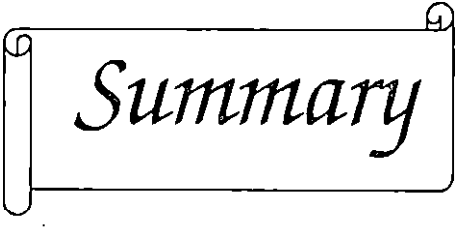
To characterize the F_1 hybrids in relation to the parents RAPD assay was carried out. Initially 20 decamer primers of OPA, OPS and OPY series were screened for amplification of genomic DNA using the parents and F_1 s of a single cross. Out of these three primers *viz.*, OPA-2, OPY-9 and OPY-10 that gave good amplification and showing polymorphism were selected for further characterization. The RAPD products generated by each of three selected primers gave characteristic bands in both parents and the three hybrids. Altogether the three primers produced a maximum of 20 bands the size of which ranged from approximately 500 bp to 5148 bp. The extent of variability was in conjunction with the morphological variability exhibited by parents.

Altogether the three primers produced a maximum of 20 bands the size of which ranged from approximately 500 bp to 5148 bp. The average number of bands per primer for each cross is 5.8. Kumari *et al.* (2006) reported that out

of 25 RAPD bands using four random decamer primers in African marigold 13 were polymorphic. The hybrids in three crosses (Th-1 X C-6, Tr-5 X Pil.O-3 and Th-6 X Ka-3) shared more bands with the female parents whereas in two crosses (Th-1 X A-3 and Vk1-5 X Pil.O-4) they shared more bands with the male parent. In the remaining one cross, the number of monomorphic bands was same between the hybrids and the female as well as the male parent. When the hybrids of each cross were compared between themselves, it was found that in three crosses (Tr-5 X Pil.O-3, Th-1 X A-3 and Vk1-5 X Pil.O-4) polymorphism was high, in two crosses (Th-1 X C-6 and Tr-3 X Vk2-3) polymorphism was moderate and in one (Th-6 X Ka-3) it was low. The high polymorphism between the parents indicated that they are differing from each other.

The bands of approximate size 1375 bp and 1584 bp were present in all the parents whereas 1904 bp band was found in all except Th-6 and A-3. Similarly the bands of approximate size 1375 bp and 1584 bp were shared by all the hybrids except two and 1904 bp was present in all the hybrids except three.

The result of RAPD analysis confirmed the variability in the genetic make up of the parents and hybrids of African marigold and the phylogeny of the hybrids. The detection of entirely new RAPD fragments (5148 bp and 1250 bp) specifically found in the hybrids indicated the formation of new primer recognition sites through recombination during hybridization. In conclusion, the results from present study indicated that variability could be induced in African marigold through hybridization.


Summary

6. SUMMARY

The present study on "Assessment of variability and compatibility in *Tagetes* spp." was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during 2006 – 2007.

The programme envisages assessment of genetic variability and divergence, formulation of a selection index based on major characters contributing to yield, studying the compatibility between different genotypes, estimation of heterosis as well as biochemical and molecular characterization of superior hybrids along with their parents.

The experimental material consisted of 14 accessions of African marigold and seven varieties of French marigold, collected from different agroclimatic conditions. Observations on different quantitative and qualitative characters were recorded and the data were subjected to suitable statistical analysis. The salient findings of the study are summarized below.

1. Fourteen accessions of African marigold collected from Andhra Pradesh, Karnataka and Kerala showed significant differences for most of the vegetative and floral characters studied *viz.* plant height, leaf length, leaf width, number of primary branches, pedicel length, number of whorls of ray florets, fresh weight of flowers, yield and seed set percentage. The accessions of French marigold also showed variation for all the 14 characters studied.

2. Accession Kapugal (Ka) had maximum flower yield of 114.69g per plant. It produced 24.66 flowers per plant with maximum fresh weight of flowers (4.64g) and flower diameter (4.11cm). It was closely followed by the accession Ananthagiri (A) with an average yield of 113.54g per plant. The number of flowers per plant (31.16) and flower diameter (4.51cm) was higher in this accession. In French types variety French Vanilla had the highest yield of

29.76g per plant. It produced 14.67 flowers per plant, followed by Boy O Boy with an average yield of 27.13g per plant.

3. Highest genotypic and phenotypic coefficients of variation were observed for number of whorls of ray florets followed by yield per plant indicating the strong influence of environment on genotype in the expression of these traits.

4. High heritability and genetic gain were noted for the characters such as number of whorls of ray florets, fresh weight of flowers and plant height indicating the scope of improvement of these characters through direct selection.

5. Correlation studies revealed strong association between yield and the traits number of flowers per plant and fresh weight of flowers whereas flower diameter and number of whorls of ray florets, plant height and fresh weight of flowers, stem girth and fresh weight of flowers are significant only at genotypic level.

6. Results of path coefficient analysis brought out the fact that fresh weight of flowers had the highest positive direct effect on yield followed by number of flowers per plant and days to flower. Plant height imparted highest negative effect on yield followed by number of branches.

7. Genotypes were grouped into five clusters based on genetic distance. There was no parallelism between geographical distribution and genetic diversity. Intra cluster distances were much lesser than inter cluster one, suggesting the homogenous and heterogenous nature of the strains within and between the clusters respectively.

8. A selection model was formulated based on the characters with good efficiency over direct selection such as plant height, stem girth, number of flowers, number of whorls of ray florets, fresh weight of flowers, flower yield. Comparison of different genotypes based on the selection index value revealed

the superiority of genotypes Kapugal (Ka) followed by Ananthagiri (A) and Thammara (T) all from Andhra Pradesh.

9. Compatibility studies showed that there was no self or cross incompatibility within African marigold. In case of inter specific crosses seed set as well as germination was low due to the difference in ploidy level. In the present study only those inter specific crosses in which African types were used as female parent were successful.

10. Heterosis studies conducted in all the successful crosses showed that there was no much difference between direct and reciprocal crosses except for the character seed set percentage. Six superior hybrids were selected based on heterobeltiosis and relative heterosis values for further analysis.

11. Biochemical analysis of the selected hybrids and parents for the total carotenoid content showed that overall mean for parents was 5.56 mg and that for the hybrids was 5.54 mg per one gram of fully opened flowers. Only one hybrid in the cross Th-1 X A- 3 showed higher mean value than that of parents

12. Molecular characterization with three primers produced a maximum of 20 bands the size of which ranged from approximately 500 bp to 5148 bp. The bands of approximate size 1375 bp and 1584 bp were present in all the parents whereas 1904 bp band was found in all except Th-6 and A-3. Similarly the bands of approximate size 1375 bp and 1584 bp were shared by all the hybrids except two and 1904 bp was present in all the hybrids except three.

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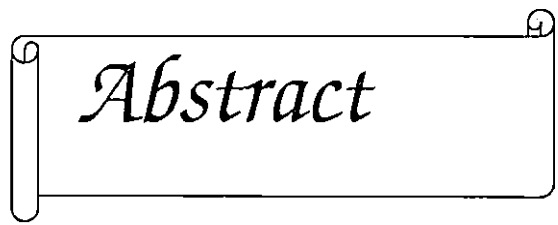
Abstract

ASSESSMENT OF VARIABILITY AND COMPATIBILITY IN *Tagetes* spp.

By

KISHORE BODDU

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

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VELLANIKKARA, THRISSUR - 680 656
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2007

ABSTRACT

The present investigation on "Assessment of variability and compatibility in *Tagetes* spp." was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during 2006 – 2007. The experimental material consisted of 14 accessions of African marigold and seven varieties of French marigold, collected from Andhra Pradesh, Karnataka and Kerala.

The major objectives include assessment and interpretation of genetic variability and the inter relationship between different traits, investigation of the reproductive biology and compatibility behaviour between different genotypes, formulation of an efficient selection method for identifying superior genotypes, estimation of the heterotic potential as well as characterization of superior hybrids at biochemical and molecular level along with their parents.

A local cultivar from Kapugal (Ka) in Andhra Pradesh had maximum flower yield of 114.69g per plant. On an average, it produced 24.66 flowers per plant with maximum fresh weight of flowers (4.64 g) and flower diameter (4.11 cm). It was closely followed by the accession collected from Ananthagiri (A) (Andhra Pradesh) with an average flower yield of 113.54g per plant. For this accession the number of flowers (31.16) and flower diameter (4.51 cm) were higher than Ka. In French types variety French vanilla had the highest flower yield (29.76 g per plant), followed by Boy O Boy (27.13 g per plant).

Highest genotypic and phenotypic coefficients of variation were observed for the character number of whorls of ray florets followed by yield per plant. Heritability and genetic gain were maximum for the characters such as number of whorls of ray florets, fresh weight of flowers, yield and plant height. Correlation studies revealed the traits number of flowers per plant and fresh weight of flowers had strong correlation with yield. Comparison of different genotypes based on the selection

index value revealed the superiority of genotypes Kapugal (Ka) followed by Ananthagiri (A) and Thammara (T). The 14 genotypes were grouped into five clusters based on genetic distance. Reproductive biology and compatibility studies showed that there was no self or cross incompatibility in African marigold but in inter specific hybridizations with French types, seed set and germination was lower.

Six superior hybrids Th-1 X C-6, Tr-5 X Pil.O-3, Th-6 X Ka-3, Th-1 X A-3, Vk1-5 X Pil.O-4 and Tr-3 X Vk2-3 for the characters plant height, number of whorls of ray florets, number of flowers, fresh weight of flowers and flower yield were selected based on heterobeltiosis and relative heterosis values. There was no much difference between direct and reciprocal crosses except for the character seed set percentage. Biochemical analysis of the selected hybrids and parents for total carotenoid content showed that overall mean of parents was 5.56 mg and that for the hybrids it was 5.54 mg per one gram of fully opened flowers.

Molecular characterization of the selected hybrids and parents produced a total of 20 bands with three primers, The bands of approximate size 1375 bp and 1584 bp were present in all the parents whereas 1904 bp band was found in all except Th-6 and A-3. Similarly the bands of approximate size 1375 bp and 1584 bp were shared by all the hybrids except two and 1904 bp was present in all the hybrids except three.

Appendices

Appendix 1

Reagents for DNA isolation by Doyle and Doyle (1987) method:

I. Extraction buffer (10 x): 2.5 g sorbitol, 4.8 g Tris

0.74 g EDTA- sodium salt

80 ml sterile Milli Q water

pH adjusted to 7.5 with Hcl, water to 100 ml

(Added 3.8 g sodium metabisulfate (0.38 %) prior to extraction)

II. Lysis buffer: 15.76 g 1 M Tris- pH 8

9.305 g 0.25 M EDTA

100 ml sterile Milli Q water

2g CTAB, stirred to dissolve

29.22g 5 M Nacl

III. TE buffer: 10mM tris- pH 8

1mM EDTA

(Water to 100 ml, autoclaved and stored at room temperature)

IV. Isopropanol

V. Chloroform: isomyl alcohol mixture (24:1) v/v

VI. Sarcosine 5%

VII.Ethanol 100 % and 70 %

Appendix 2

Operon decamer primers used for the investigation

Sl. No	Primer code	Primer sequence
1	OPA 1	5' CAGGCCCTTC 3'
2	OPA 2	5' TGCCGAGCTG 3'
3	OPA 3	5' AGTCAGCCAC 3'
4	OPA 4	5' AATCGGGCTG 3'
5	OPA 5	5' AGGGGTCTTG 3'
6	OPA 6	5' GGTCCCTGAC 3'
7	OPA 7	5' GAAACGGGTG 3'
8	OPA 8	5' GTGACGTAGG 3'
9	OPA 9	5' GGGTAACGCC 3'
10	OPA 10	5' GTGATCGCAG 3'
11	OPS 1	5' GTTTCGCTCC 3'
12	OPS 2	5' TGATCCCTGG 3'
13	OPS 3	5' CATCCCCCTG 3'
14	OPS 4	5' GGACTGGAGA 3'
15	OPS 5	5' TGCGCCCTTC 3'
16	OPY 6	5' AAGGCTCACC 3'
17	OPY 7	5' AGAGCCGTCA 3'
18	OPY 8	5' AGGCAGAGCA 3'
19	OPY 9	5' AGCAGCGCAC 3'
20	OPY 10	5' CAAACGTGGG 3'