GREGOR MENDEL FOUNDATION PROCEEDINGS 2009

(PROCEEDINGS OF THE NATIONAL SEMINAR ON GENETICS, BREEDING AND BIOTECHNOLOGY HELD AT UNIVERSITY OF CALICUT ON 11 & 12 DECEMBER 2009)

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Breeding for hybrid sesame suited for summer rice fallow

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Abstract: *Sesame (Sesamum indicum L.) is usually grown in summer rice fallows as catch crop by utilizing the residual moisture. The area under this crop is getting reduced day by day due to its low yield. In this study, an attempt was made for the development of male sterile lines through mutation and wide crosses so as to exploit heterosis. In order to find out the best specific combiners, first a diallel analysis was carried out. The best combiners were reciprocally crossed with a wild species Sesamum malabaricum L. Male sterility was observed in F_1s of the cross in which S. malabaricum was used as the female parent, while the reciprocal was fertile. This indicates the presence of cytoplasmic male sterility in the cross with the source of sterility as S. malabaricum cytoplasm. The F_1s were found to resemble more the wild parent than the cultivar. Flower colour and appearance were similar to that of S. malabaricum. Seed coats were rough, showing its resemblance with the wild parent. Oil content was low. The F_1 was later backcrossed repeatedly with the cultivar to develop cytoplasmic male sterile line with the genome of the cultivar so as to use it in hybrid seed production programme.*

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the oldest oil seed crops known to and used by man. It yields oil and protein of high quality and holds tremendous potential for export. However the average per hectare yield of sesame in India is very low as compared to other producing countries. This is mainly due to the lack of improved cultivars. The potential of sesame breeding is amply demonstrated by the achievements in South Korea (Kang, 1994), in its traditional areas of production in India (Sharma, 1994) and in China (Zhao, 1994). There is an urgent need to augment its productivity through exploitation of heterosis which is a quick and convenient way of combining desirable traits from diverse parents. There are two basic prerequisites for the successful economic exploitation of heterosis on a commercial basis. First, a significant heterotic effect must be present in a hybrid over its parents. Second, production of large scale hybrid seed should be possible economically. Though sesame is a self pollinated crop, it is very easy for hand emasculation and pollination and a single attempt gives about 50-60 seeds. It is therefore easier to exploit its heterosis and high level of heterosis has been observed in sesame hybrid populations (Dixit, 1976). In all the early works, F_1 populations were developed by hand emasculation and pollination which is time consuming and laborious and it could not be used for the production of hybrid sesame seeds for commercial planting.

True male sterility has not been described in sesame. This phenomenon will be much useful in hybrid sesame only if a stable male sterility, either cytoplasmic or genetic is identified and utilized. The discovery of cytoplasmic male sterility (CMS) in wild relatives of numerous cultivated plants has contributed enormously to agriculture by making possible large scale production of hybrid seeds. With this background the present study was formulated with a view to assess the variability and to estimate the combining ability of selected genotypes. Exploitation of male sterility in sesame through mutagenesis and wide hybridization for future development of hybrid sesame was also aimed at.

MATERIALS AND METHOD

Eight diverse genotypes representing accessions and varieties of various eco-geographical conditions throughout India constituted the materials for the present study. The field trials were laid out at Regional Agricultural Research Station, Kayamkulam, Kerala of Kerala Agricultural University (KAU). Soil in this region is predominantly sandy loam and it constitutes the main sesame growing area in the state. Cultural operations were carried out as per the package of practices recommendations of KAU.

The present investigation consisted of two sets of experiments: *Experiment 1* was aimed at production of intervarietal hybrids through crossing selected lines to study the combining ability in order to identify the best general combiners using diallel analysis. *Experiment 2* was aimed at induction of male sterility through physical (gamma radiation) and chemical mutagens (EMS) and study of the inheritance of male sterility. It also aimed at

interspecific crosses of the general combiners with the wild species *Sesamum malabaricum* L. to explore the possibility of development of CMS lines.

Eight diverse genotypes representing accessions and varieties procured from different eco-geographical regions of India were selected based on multivariate analysis of yield components. These genotypes were raised in a crossing block for diallel analysis. 56 cross combinations between these eight genotypes in all possible combinations including reciprocals were effected. Emasculation and hybridization were carried out as per Thankavelu and Nallathambi (1982). Best general combiners were then utilized for the development of male sterility through induced mutagenesis and wide hybridization with *S. malabaricum*. For induced mutagenesis seed samples each consisting of 600 seeds of the two selected varieties were introduced into Co^{60} gamma cell installed at Radio Tracer Lab, KAU, Vellanikkara, Thrissur, Kerala and exposed to radiation for appropriate periods to irradiate with gamma rays from 100gy to 600gy. For chemical mutagenesis seeds presoaked in water for two hours were treated by keeping immersed in mutagen solutions (aqueous) of concentrations 0.2% to 1.0% for four hours with intermittent shaking.

RESULTS AND DISCUSSION

The success of a plant breeding programme greatly depends on the choice of parents for hybridization and the type of gene action in the case of different agronomic traits. Combining ability analysis provides such information so as to frame the breeding programme effectively. Combining ability studies in general reveal the nature of gene action and lead to identification of parents with general combining ability effects (gca) and the cross combinations with high specific combining ability (sca) effects. In order to study the combining ability effects eight genetically diverse parents were subjected to 8x8 full diallel analysis. Based on the general combining ability of yield and related components, two best combiners, Thilak and OS2 were selected and were subjected to induced mutagenesis and wide hybridization.

Induced mutagenesis

Under induced mutagenesis, in M_1 generation, the percentages of germination and survival at 30 days were adversely affected by both the physical and chemical mutagens. Reduction was drastic in higher doses of the chemical mutagen (Table. 1). Percentage of pollen fertility was also decreased with increase in doses of mutagens. Reduction in fertility was more drastic in chemical mutagen. Similar reduction in seed fertility was also noticed in both the genotypes.

In M_2, study of viable sterile mutations was confined to those doses in which pollen fertility got reduced to less than 50%. All mutants in which pollen fertility was completely affected were classified as viable sterile mutants. These viable mutants were screened in M_1 plant progenies. The frequencies of these were estimated as mutations per M_1 plants and 100 M_2 plants (Table. 2).

Table. 1. Effect of mutagens on germination and survival on 30^{th} day in M_1 generation.

Mutagen/Dose	% germination	% survival on 30^{th} day
1.Gamma rays (Gy)		
100	88.94	93.09
200	81.06	73.79
300	73.94	61.18
400	63.19	53.07
500	49.26	38.71
600	38.83	33.11
2.EMS (%)		
0.2	82.49	90.18
0.4	64.00	76.38
0.6	50.16	64.24
0.8	26.38	51.10
1.0	22.59	35.21

Table. 2. Frequency of viable mutants in the M_1 and M_2 generations.

Mutagen/dose	No. of plants scored in M_1	% frequency	No. of plants scored in M_2	% frequency
Gamma 500	30	0	250	0.0
Gamma 600	30	3.3	250	0.8
EMS 0.8	30	3.3	250	0.8
EMS 1.0	30	10.0	250	0.4

On sibmating and open pollination capsule set was observed in three out of six sterile plants. The sibmating and open pollination failure in the remaining plants showed cent percent pollen sterility. This was indicative of total female sterility in these plants along with complete male sterility resulting in the total absence of capsules. All floral parts except anthers were normal in size and colour in male sterile plants. Anthers were flatter and greenish than normal which is whitish and plumy.

The decrease in germination contributed mainly to the lethality of the seeds from physiological injuries, chromosomal aberrations and toxic effects of the hydrolytic products of the mutagen (Freese-Gertzen *et al*., 1964). Konzak *et al.* (1965) reported that the alkyl sulphonates and alkyl sulphates form strong acids upon hydrolysis. Since hydrolysis may occur both externally and internally in the cells, significant amount of acid may become available which cause toxicity. The reduction in germination with EMS treatment can be attributed to these hydrolytic products. The reduction in survival on the 30^{th} day is an index of post germination mortality in the treated material. In the present study both physical and chemical mutagens induced pollen sterility. A linear increase in sterility with increased doses of mutagens was also reported earlier (Nair and Nair, 1978; Ganesan, 1995).

The crossing success on male sterile plants was normal indicating female fertility. The inheritance study indicated that male sterility observed in the present case was also governed by a single recessive allele. Similar green anther mutants with complete male sterility were isolated by Brar (1982) and Osman and Yermanos (1982). They used this trait to differentiate male sterile and fertile plants.

The present study has also shown the feasibility for the generation of CMS line in sesame through interspecific hybridization between *Sesamum indicum* and *S. malabaricum* as *S. malabaricum* was found to be more cross compatible compared to two other wild species of sesame with *S. indicum*. F_1, BC_1 and BC_2 generations were observed. The interspecific hybrids both direct and reciprocals resulted in successful capsule and seed set indicating cross compatibility between the species. The hybrids with *S. malabaricum* as ovule parent resulted to be male sterile while the reciprocals were fertile. This pointed out the cytoplasmic difference of the crosses which resulted in male sterility. In several species CMS results from nuclear cytoplasmic interaction which fails to produce functional pollen but maintains female fertility as reported by Newton (1988). CMS has also been exploited for the production of hybrid seeds in many crops such as maize, sorghum, sunflower *etc*. Yuan (1993) reported a wild abortive (WA) cytoplasm derived from *Oryza sativa* f. *spontanea* as a source of sterility in rice. Here in the present study the cytoplasm of *S. malabaricum* may be the factor which induced sterility system. It was evident from the studies that the F_1 hybrids of both direct and reciprocals exhibited the dominance of wild parent characters-stem colour, branching pattern, leaf shape, corolla colour, seed texture, etc. But on fertility status only a difference was noted. *S. indicum* cultivar x *S. malabaricum* showed high percentage of pollen fertility resulting in fairly good capsule set on selfing whereas reciprocals showed very high percentage of sterility leading to very few or no capsule set on selfing. Though F_1 was similar to wild parent, in the progenies of back crosses there was an increased resemblance to the qualitative characters of *Sesamum indicum* which indicates the accumulation of cultivar genome along with the sterility factor from the donor.

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Genetic and non genetic factors affecting callus induction and regeneration in sugarcane

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Abstract: *Four species of Saccharum (S.officinarum, S.barberi, S.sinense, S.robustum), one intergeneric hybrid (Saccharum x Zea) and five sugarcane varieties (Co 86032, Co 99004, Co 94012, Co 1148, CoJ 64) were tested for their response to callus induction and regeneration. Young leaf explants of each plant were inoculated in callus medium having different gelling agents like agar, agarose, phytagel, clarigel and bactoagar. It was found that different gelling agents responded differently for callus induction. In another experiment they were inoculated on MS medium containing different concentrations of 2,4-D (0, 1.0, 2.0, 3.0, 4.0 mg/l) for callus induction. Maximum of 95% callus induction was observed in the variety Co 99004 and Saccharum x Zea showed the lowest callus induction (40%). The actively growing calli were transferred to MS medium supplemented with different concentrations of 6- Benzyl Amino Purine (BAP), kinetin and combination of BAP and kinetin for shoot regeneration. Most of the genotypes showed maximum shoot regeneration in MS medium supplemented with a combination of BAP and kinetin (0.5 mg/l each). S. officinarum and Co 86032 showed maximum number of regenerated shoots in medium supplemented with 0.5 mg/l BAP + 0.5 mg/l kinetin and 1.0 mg/l kinetin respectively. Though the induction and shoot regeneration was satisfactory in all the clones, differences could be observed for these characters between the genotypes and media combinations. From the study it was concluded that the somatic embryogenesis and organogenesis in sugarcane is significantly influenced by genetic and non genetic factors like genotypes, gelling agents and auxins and cytokinins in the culture media.*

INTRODUCTION

In sugarcane breeding programmes, increased disease resistance and high sucrose content are the main areas targeted. In recent years, biotechnological techniques such as tissue culture and gene transfer systems have been used for the improvement of sugarcane. While considering the improvement programme through *in vitro* culture, it is necessary to develop efficient procedures to increase the regeneration rate of plantlets through the production of more friable calli.

Callus is an unorganized mass of plant cells and its formation is controlled by growth regulating substances present in the medium (Shah *et al*., 2003). The specific concentration of plant regulators, needed to induce callus formation varies from species to species and can even depend on the source of explant (Charriere *et al*., 1999). Furthermore, genotype is one of the most important factors for callus induction (Sarrafi *et al*., 1996; Punia and Bohorova, 1992).

The major area of tissue culture in sugarcane improvement is the production of somaclones from callus cultures of commercially important varieties to rectify their specific defects. The highly polyploid nature of sugarcane coupled with chromosome numerical variation in different cells of the same tissue is an added advantage in sugarcane for creating somaclonal variation for a wide spectrum of characters. Research on sugarcane tissue and cell culture was started in Hawaii in 1961. Later several somaclones were developed through tissue culture with improved productivity and eliminating certain minor defects like spines, leaf drying and disease susceptibility. This approach was utilized to rectify specific defects of the widely adapted sugarcane varieties which were rejected at the final stage of selection. Production and utilization of somaclonal variants from selected species and interspecific and intergeneric hybrids of sugarcane have a great scope in the improvement programmes. Extensive application of this method in sugarcane improvement is awaiting the standardization of optimum culture conditions of these genotypes to maximize the regeneration efficiency.

The present study was carried out to determine the effects of different gelling agents like agar, agarose, phytagel, clarigel and bactoagar and also different concentrations of 2,4-D on callus induction and different BAP and kinetin concentrations on shoot regeneration in different species and hybrids of sugarcane. Using more diverse genotypes (species and hybrids) in one tissue culture study may be useful, showing similarities and/or differences in factors such as gelling agents, concentrations of growth regulators in the medium. Thus the same steps may be used for callus production or plant regeneration in other tissue culture and transformation programmes.

MATERIALS AND METHOD

Shoots from 4-6 months crops of sugarcane species (*S. officinarum*, *S. barberi* and *S. sinense, S. robustum*) and intergeneric hybrids (*Saccharum* x *Zea*) and five varieties of sugarcane (Co 86032, Co 99004, Co 94012, Co 1148, CoJ 64) were collected from germplasm collection and experimental fields of Sugarcane Breeding Institute, Coimbatore. The outer leaves were removed and surface of shoots wiped with alcohol. Two or three outer whorls were removed and the apical portion of the stem (10cm) was cut and taken into laminar air flow chamber. The developing leaves encircling the growing points were dissected out and bits of about 0.5 cm x 0.5 cm were cut with the help of sterile forceps and scalpel. The excised explants (10/ culture bottle) were immediately inoculated on MS medium (Murashige and Skoog, 1962) supplemented with different gelling agents and various concentrations of 2,4-D (Table 1). Three replications of each treatment were done and incubated in dark at 25±1ºC temperature. Subculturing was done at 15-20 days intervals. Observations were made on callus induction time, callus weight, callus induction percentage, callus morphology and embryogenic callus production.

RESULTS AND DISCUSSION

Time taken for callus induction in each clone was recorded in general without considering the gelling agent effect. Normally callus induction takes place within two weeks. In this experiment all the species and hybrids have produced callus within two weeks. While comparing, the species took long time to produce calli than hybrids.

While considering the role of gelling agent in callus induction, the species showed good callus growth in clarigel where as the intergeneric hybrids responded well in all the callus induction media irrespective of gelling agents. The varieties showed high callus induction percentage than the species and intergeneric hybrids. From this experiment it is proved that callus induction is genotype dependant and the role of gelling agents is minor in callus induction (Fig.1).

Callus induction time was found to be different in different genotypes. Most of the varieties showed callus initiation within 12-14 days. Callus initiation in *S. sinense* was observed within 10 days. *S. barberi* took 32 days to produce callus and the intergeneric hybrid, *Saccharum* x *Zea* took the maximum time (36 days) for callus induction. Data presented in Table 2 indicate clear genotypic variation in time taken for callus initiation in clones. Similar observations have also been made in *Sorghum* (Patil *et al*., 1998; Baskaran *et al*., 2005). It was also observed that use of lower concentration of 2,4-D generally required more days to initiate callus formation from explants. A minimum period of 10-14 days was required to initiate callus in medium supplemented with 3.0 and 4.0 mg/l 2,4-D.

Average callus weight of clones was recorded. Among the species, *S. officinarum* showed 1.26g, *S. barberi* 1.13g and *S. sinense* 1.70g callus weight. The intergeneric hybrid, *Saccharum x Zea* showed the lowest callus weight of 0.83g. Among the varieties Co 99004 produced the maximum (2.81g) and Co 1148 produced the minimum (1.03g) callus weight. It is reported that the varieties produced more callus than the species and the intergeneric hybrid and callus weight is dependent on the genotype of the explants used (Gandonou *et al*., 2005). Co 99004, which produced the highest callus weight showed the highest callus induction percentage (95%) also. A positive relationship between callus weight and callus induction percentage was observed in the case of Co 99004. (Melahat *et al*., 2001). Callus growth was influenced by 2,4-D concentration used. High callus weight was recorded in medium containing 3.0 mg/l or 4.0 mg/l 2,4-D and callus weight was less in medium with lower concentration of 2,4-D.

The actively growing calli of a set of species and hybrids were transferred to MS medium supplemented with different concentrations of BAP and kinetin. The line diagram (Fig. 2) indicates that regeneration is affected by the concentration of growth regulators. *S. officinarum* showed the maximum regeneration (100%) in both S4 and S5 media. *S. barberi* produced the maximum regeneration in S4 and S5. *S. sinense* showed 90% results in S2, S3 and S5 media. *Saccharum* x *Zea* showed 80%results in S2 and S5 media. Co 86032, Co 99004 and Co 94012 produced the maximum shoot regeneration in S5 medium. Co 1148 showed the weakest response to regeneration (20%) and CoJ 64 produced 90% regeneration in S5 medium. The results indicate that a combination of BAP and kinetin (0.5 mg/l each) induced more regeneration in cultures.

The number of shoots regenerated in the sugarcane species and hybrids in each treatment was counted (Table-3). In *S. officinarum*, maximum shoots (250) were regenerated in the SDM medium supplemented with a combination of BAP and kinetin (0.5 mg/l BAP + 0.5 mg/l kinetin). In Co 86032 also 250 shoots were regenerated from medium supplemented with 0.5 mg/l BAP and 100 shoots from medium containing 1.0 mg/l kinetin. Co 1148 showed only less capacity of regeneration. The highest amount of shoot (20) was obtained in medium containing

0.25 mg/l BAP and no shoots were produced in medium with 1.0 mg/l BAP. Though the regeneration of calli and shoot formation was satisfactory in almost all clones, differences could be observed for these characters between the genotypes and media combinations. Data were statistically analyzed with SPSS software and it has revealed that significant difference was obtained for callus induction and regeneration in genotypes studied and hence in sugarcane these characters are highly dependent on genotype and phytohormone.

Table 1. Different media used for callus induction

Sl. No.	Medium	2,4-D concentration	Gelling agent
1	Co	CM + 0 mg/l 2,4-D	CM+8 g/l agar.
2	C_1	CM + 1 mg/l 2,4-D	CM+4g/l agarose.
3	C_2	CM + 2 mg/l 2,4-D	CM+2.5 /l phytagel.
4	C_3	CM + 3 mg/l 2,4-D	CM+2.5 g/l clarigel
5	C_4	CM + 4 mg/l 2,4-D	CM+8 g/l bactoagar.

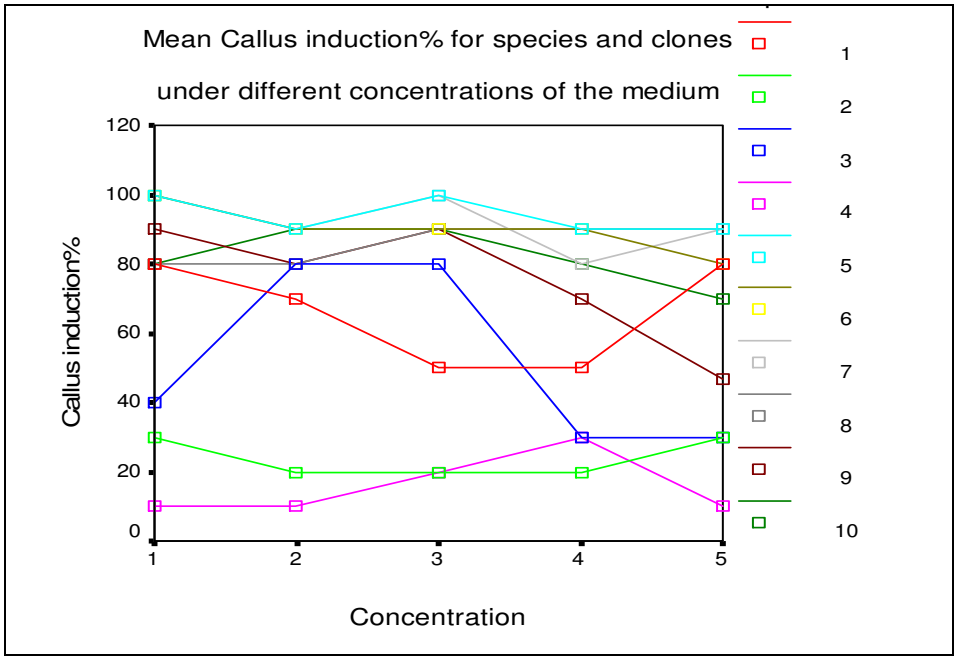
Table 2. Time taken for callus induction (in days)

Sl. No	Clones	Time taken(in days)
1.	*Saccharum officinarum*	12
2	*Saccharum barberi*	32
3	*Saccharum sinense*	10
4	*Saccharum robustum*	15
5	*Saccharum x Zea*	36
6	Co 86032	12
7	Co 99004	11
8	Co 94012	12
9	Co 1148	13
10	Co J64	11

Table 3. Number of shoots regenerated from sugarcane species and hybrids

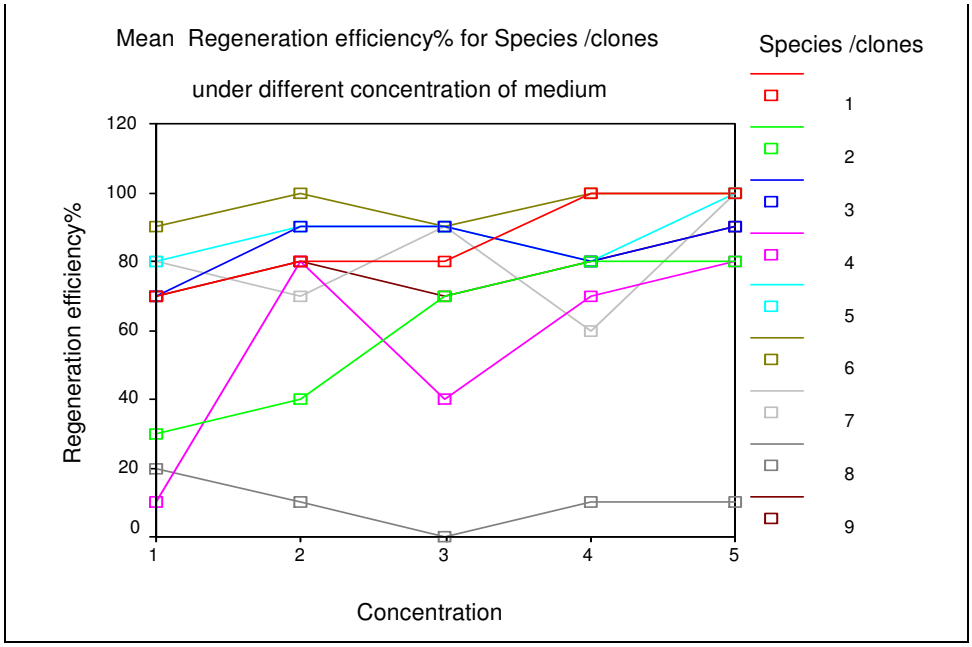
Sl. No.	Clones	No. of shoots regenerated				
		S1	S2	S3	S4	S5
1	*Saccharum officinarum*	100	200	110	160	250
2	*Saccharum barberi*	120	140	150	160	130
3	*Saccharum sinense*	110	130	120	140	150
4	*Saccharum robustum*	120	140	150	150	160
5	*Saccharum x Zea*	90	170	200	150	170
6	Co 86032	150	250	150	100	140
7	Co 99004	170	160	140	150	160
8	Co 94012	140	130	120	130	180
9	Co 1148	20	10	0	8	5
10	CoJ 64	90	80	120	140	160

Fig. 1. Line diagram showing mean callus induction percentage for clones in different gelling agents.



Clones: 1. *S. officinarum*; 2. *S. barberi*; 3. *S. sinense*; 4. *S. robustum*; 5. *Saccharum* x *Zea*; *6.* Co 86032; 7. Co 99004; 8. Co 94012; 9. Co 1148; 10. CoJ 64.

Fig. 2. Line diagram showing mean callus induction percentage for clones in different gelling agents.



Clones: 1. *S. officinarum*; 2. *S. barberi*; 3. *S. sinense*; 4. *Saccharum* x *Zea*; 5. Co 86032; 6. Co 99004; 7. Co 94012; 8. Co 1148; 9. CoJ 64

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Morphology and cytology of a novel intergeneric hybrid *Saccharum* x *Tripsacum* and its derivatives

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Abstract: *The present day commercial sugarcane varieties are derivatives of man made interspecific hybrids between cultivated Saccharum officinarum and wild S. spontaneum of the family Gramineae. Interspecific and intergeneric hybrids with wild related species are being developed for the genetic base broadening of sugarcane. The present study is on the morphology and cytology of a novel hybrid between sugarcane and Tripsacum, a robust perennial grass native of the western hemisphere. The sugarcane variety CoC671 (2n= 108) when pollinated with Tripsacum sps. hybrid (2n= 54) gave rise to hybrids with chromosome number ranging from 2n= 108 to 116 and resembling sugarcane in morphology. In pollen mother cells there were predominance of bivalents and a few multivalents at metaphase I, meiotic abnormalities such as lagging chromosomes and disoriented spindles in anaphase I and asynchronous division and disoriented spindles in anaphase II. These hybrids were fertile and were further crossed with sugarcane and Tripsacum to obtain backcross progeny. The Saccharum x Tripsacum hybrid backcrossed with the sugarcane variety Co 775 gave rise to two distinct morphological categories of plants, one with high tillering (Mean = 41) and thin canes (1.44 cm) and another with low tillering (13.39) and thick canes (2.39 cm). Their leaf width also varied significantly. Most of the broad leaved plants were non-flowering. The back cross hybrids with Tripsacum were with less cane height and with broad and long leaves. Among the backcross hybrids a few plants had the Tripsacum morphological features on leaf and stem. The inflorescence of all the hybrids had resemblance with that of Saccharum. Fertility of the hybrids and variability in the backcross progeny had shown that these hybrids can be further used in genetic base broadening of sugarcane.*

INTRODUCTION

Sugarcane is an important agro industrial crop of tropical and subtropical regions and is cultivated on close to 20 million hectares in more than 90 countries. Sugarcane belongs to the grass family (Gramineae), an economically important seed plant family that includes cereals such as maize, wheat, rice, and sorghum as well as many forage crops. The modern commercial cultivars of sugarcane are interspecific hybrids between cultivated *Saccharum officinarum* and wild *S. spontaneum.* In order to widen the genetic base for its diversified use for production of sugar, ethanol, fibre and electricity and to improve the productivity interspecific and intergeneric hybridization with wild related species is being performed (Sreenivasan *et al.*, 1987). Intergeneric hybrids between *Saccharum* with members of Gramineae such as *Erianthus* (Janaki Ammal, 1941; D'Hont *et al*., 1995; Premachandran and Lalitha, 2007), *Imperata* (Janaki Ammal, 1941), *Miscanthus* (Li *et al.*, 1948), Bambusa (Venkatraman, 1937), *Sorghum* (Thomas and Venkatraman 1930; Nair, 1999), *Zea* (Janaki Ammal, 1938) have been reported. The wide hybrids in sugarcane can be maintained by clonal propagation and due to the high ploidy level many of the hybrids are fertile also, thereby it can be used in gene introgression studies.

Tripsacum is a perennial grass native to the western hemisphere, belonging to the Maydeae sub tribe of Andropogoneae tribe, closely related to *Zea mays*. The *Tripsacum* species are with predominantly diploid (2n = 2x = 36) or tetraploid (2n = 4x = 72) ploidy levels. The diploids reproduce sexually whereas tetraploids reproduce by diplosporous apomixis. Sexual allotriploids (2n =3x=54) were also developed from crosses of sexual diploid *Tripsacum dactyloides* (2n = 36), and apomictic tetraploid (2n = 72) *T. maizar* (Li *et al*, 2000). The present study is on the hybrid between a commercial sugarcane variety (*Saccharum* spp. hybrid) and the triploid *Tripsacum* species hybrid.

MATERIALS AND METHOD

The plant materials used in this study consist of sugar cane commercial varieties CoC 671 and Co 775 and *Tripsacum* sps. hybrid being maintained at Sugarcane Breeding Institute, Coimbatore which was originally received from Genetics Division, Indian Agricultural Research Institute, New Delhi. The inflorescence of field grown sugarcane variety CoC 671 was covered with a cloth bag before the start of spikelet opening and was pollinated with *Tripsacum* pollen for five days from the start of spikelet opening. The seedlings were raised in the glass house and were transplanted to poly bags after 25 days. These seedlings were transferred to field after 45 days. The hybrids obtained were pollinated with the sugarcane variety Co 775 or was selfed and seedlings were raised. One of the

(sugarcane x *Tripsacum*) x sugarcane hybrids was pollinated with *Tripsacum* and the backcross progenies were raised. All the hybrids were maintained clonally in the field. The tillering data on the plants were taken at the age of three months and leaf length, width as well as juice brix were recorded at ten months after planting. The somatic chromosome number of parental as well as the hybrid clones were determined by root tip squash technique. Single budded sets were planted for rooting in poly bags with river sand. After 5-7 days young growing roots were collected and pretreated in alpha bromo naphthalene for 1 hr. After washing thoroughly in water the roots were transferred to 3:1 ethanol: acetic acid and stored at 4°C for at least 16 hrs. The roots were kept in 1N HCl at 60°C for hydrolysis, for about 12 minutes. After hydrolysis the roots were transferred to leuco basic fuschin and kept for half an hour in dark. The well stained root tips were cut and squashed in 1% aceto carmine. Well spread chromosomes were counted and photographed under microscope.

For meiotic studies immature inflorescences at short blade stage were fixed in 3:1 ethanol-acetic acid with ferric acetate for 14 to 18 hrs. Then they were stored in 70% ethanol at 4◦C. Anthers from individual spikelets were smeared on glass slide in a drop of 1% acetocarmine. Chromosome behaviour at different meiotic stages of pollen mother cells was observed. For determining pollen fertility mature anthers were teased out in a 1:1 mixture of acetocarmine and glycerol and observed under microscope after one hour. Well stained pollens were counted as fertile and incompletely stained or unstained pollens were counted as sterile.

PCR amplification of 5s rDNA region of the parental clones and the hybrids was done. PCR reactions were performed on total DNA extracted from leaf tissues of the parental as well as the hybrid progeny samples. The primer sequences reported by D'Hont *et al*. (1995) were used. The amplification reaction mix consisted of 5ng of genomic DNA, 0.2µM of each primer P1 (5'TGGGAAGTCCT(C/T)GTGTTGCA3') and P2 (5'-(T/G)T(A/C)G(T/C)GCTGGTATGATCGCA-3'), 200µM of dNTP mix, 1X PCR buffer and one unit of Taq polymerase in a 25µl final volume. The PCR was carried out for one 3-min cycle at 95◦C, 30 cycles of 55s at 93◦C, 15s at 55◦C and 30s at 72◦C. The amplification products were separated by electrophoresis in 2% agarose gels in TAE buffer at 70V.

RESULTS AND DISCUSSION

Fifteen hybrids were raised from the cross between the sugarcane variety CoC 671 and the *Tripsacum* sps. All the hybrids resembled sugarcane in general plant morphology. Data on tillering, stalk diameter, leaf length and leaf width data of the parental sugarcane clone and the hybrids are given in Table 1. The mean stalk diameter, leaf length and leaf width of the hybrid were 2.71cm, 127.6 cm and 5.34 cm, respectively. The inflorescence was an open panilcle as that in sugarcane in all the hybrids. All the hybrids observed were fertile and their floral morphology also resembled sugarcane. The chromosome number of the sugarcane variety CoC 671 is 2n = 108 and that of the *Tripsacum* clone was determined to be 2n = 54. The chromosome number of the five hybrid clones determined ranged from 2n = 108 to 114 (Fig. 1a, 1b). In pollen mother cells of the hybrids at metaphase I predominant bivalent formation was observed. In the hybrid CYM 06-1416 with 2n = 108 there were 54 bivalents in many cells. The anaphase segregation was

PCR amplification of 5s rDNA region of the parents and the hybrid progeny revealed the hybridity of the progeny studied. *Tripsacum* showed two bands of size ~200bp and 450 bp. CoC 671 showed a band of 250 bp while the hybrids showed bands of ~250 bp and 500 bp (Fig. 3). Polymorphism of 5s rDNA region is useful for the identification of intergeneric hybrids (D'Hont *et al*., 1995). PCR amplification of the 5s rDNA region of the parental as well as F1 clones proved the hybridity of the progenies. *Tripsacum* distorted due to lagging chromosomes and disoriented spindles (Fig. 2).showed two bands of size 200bp and 450bp and CoC 671 is having a single band of size 250bp. The amplified fragment sizes of the hybrids were different from that of the parental clones which may be due to the modification of the 5s rDNA region in hybrids when the genomes of two distant species are brought together.

The *Saccharum* x *Tripsacum* hybrid CYM 06-1416 was crossed with sugarcane variety Co775. The backcross hybrids obtained were with two distinct morphological categories, with considerable difference in leaf width. The narrow leafed plants were with 2.4 to 3.3 cm leaf width whereas the broad leaved ones were having leaf width from 4.5 to 7.5 cm. Those with narrow leaves were with thin canes, less leaf length and high tillering compared to that of broad leaved category (Table 1). Most of the broad leaved plants were non-flowering, whereas the narrow leaved were early and profusely flowering. The somatic chromosome numbers of four narrow leaved and four broad leaved BC1 plants were determined. The broad leaved plants had the chromosome number ranging from 2n=104 to 106, whereas the narrow leaved plants were with 2n= 84 to 87 (Table 2). The two distinct morphological

categories of plants which differ in their chromosome number also drastically indicate that these two categories of plants are with different genome constitution. The {[(sugarcane x *Tripsacum)*] x sugarcane} x *Tripsacum* hybrids were also with two categories of plants, with narrow leaves and profuse tillering and with broad leaves and less tillering. Some of these plants had tuft of hairs at the base of the leaf sheath as that in *Tripsacum*, which was not observed in *Saccharum* clones. The selfed progeny of the sugarcane x *Tripsacum* hybrid CYM 06-1417 were with broad leaves only.

PCR amplification of 5s rDNA region of the parents and the hybrid progeny revealed the hybridity of the progeny studied. *Tripsacum* showed two bands of size ~200 bp and 450 bp. CoC 671 showed a band of 250 bp while the hybrids showed bands of ~250 bp and 500 bp (Fig. 3). Polymorphism of 5s rDNA region is useful for the identification of intergeneric hybrids (D'Hont *et al.*, 1995). PCR amplification of the 5s rDNA region of the parental as well as F1 clones proved the hybridity of the progenies.*Tripsacum* showed two bands of size 200bp and 450bp and CoC671 is having a single band of size 250bp. The amplified fragment size of the hybrids were different from the parental clones which may be due to the modification of the 5s rDNA region in hybrids when the genomes of two distant species are brought together.

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Intergeneric hybridization is being done in sugarcane with related genera of grasses in order to introgress economically important characters such as disease resistance, ratoonability, high biomass production, etc. Distant hybridization of sugarcane with members outside Saccharastrae such as *Bambusa* (Venkatraman, 1937; Rao *et al.,* 1967) and *Zea* (Janaki Ammal, 1938) was also reported. *Tripsacum* belongs to the subtribe Maydeae and there are many reports of intergeneric hybridization of *Tripsacum* with its related genus *Zea mays* (deWet and Harlan, 1974; deWet *et al*., 1972; Stalker et al., 1977). In the present study the hybrids from the cross between *Saccharum* variety CoC 671 (2n= 108) and triploid *Tripsacum* (2n=54) were similar to the female parent in plant morphology. The chromosome number 2n= 108 to 114 observed in the hybrids varied from the expected chromosome number of 2n= expected from n+n 81 (*ie*. 54 +27) transmission. In interspecific and intergeneric hybrids of *Saccharum* functioning of 2n gametes was well documented (Sreenivasan *et al*., 1987; Lalitha and Premachandran, 2007). In *Zea* x *Tripsacum* crosses also the 2n gametes function generally (deWet and Harlan, 1974).

The chromosome number of the progenies ranges from 108-114. When an F1 hybrid was back crossed with sugarcane variety Co775 the progeny could be put in two distinct morphological categories. One type of progenies is having broad leaves and the other group is having narrow leaves. The broad leaved progenies were nonflowering and the narrow leaved were early flowering .There is a considerable variation in their tillering as well as cane. The two distinct morphological categories of plants differ in their chromosome number also drastically indicating that these two categories of plants are with different genome constitution.

Saccharum is able to tolerate alien genome in hybrid combinations as seen from the large number of intergeneric hybrids reported by various authors. The fertility of the hybrids between the genera *Saccharum* and *Tripsacum* as found in the present study and the variability in the backcross progeny had shown that such hybrids can be used for gene introgression from the distantly related *Tripsacum* to sugarcane.

Table 1. Comparison of leaf width, number of tillers, stalk diameter and leaf length of *Saccharum* x *Tripsacum* hybrids and its back cross progenies

Category of hybrid	No. of plants	Leaf width (cm)		Number of tillers		Stalk diameter (cm)		Leaf length (cm)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
F1 progeny	8	3.5-6.2	5.34	8-65	27.75	1.4-3.3	2.71	100-142	127.63
Narrow leaved BC1	9	2.4-3.3	2.7	20-56	41	1.3-1.6	1.4	100-142	116.1
Broad leaved BC1	18	4.5-7.5	6.4	2-26	13.4	1.8-3.1	2.4	105-193	153.7

Table 2. Chromosome number of some (*Saccaharum x Tripsacum*) x *Saccharum* hybrids

Clone	Morphological category	Chromosome number (2n)
CYM 07-766	Narrow leaved	87
CYM 07-768	Narrow leaved	84
CYM 07-789	Narrow leaved	85
CYM 07-792	Narrow leaved	85
CYM 07-767	Broad leaved	104
CYM 07-770	Broad leaved	105
CYM 07-791	Broad leaved	108
CYM 07-832	Broad leaved	106

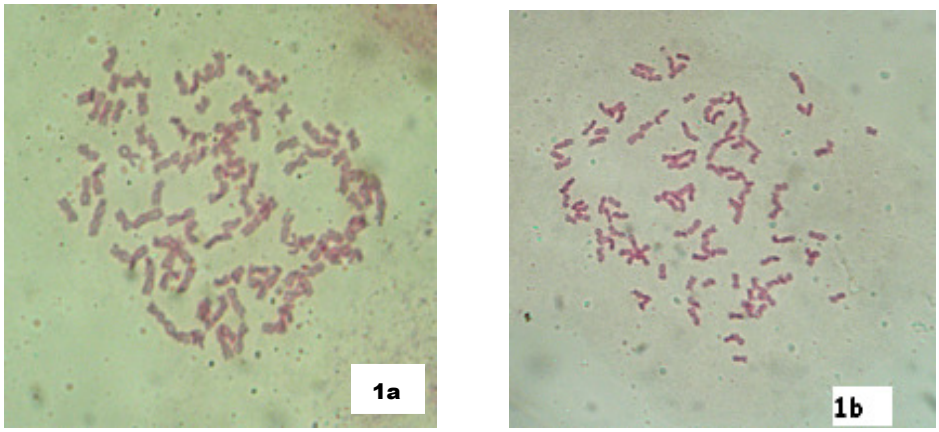


Fig 1. Somatic chromosome number in *Saccharum* x *Tripsacum* hybrids.
1a.CYM 06-1416 (2n = 108) 1b. CYM 06-1429 (2n = 114)

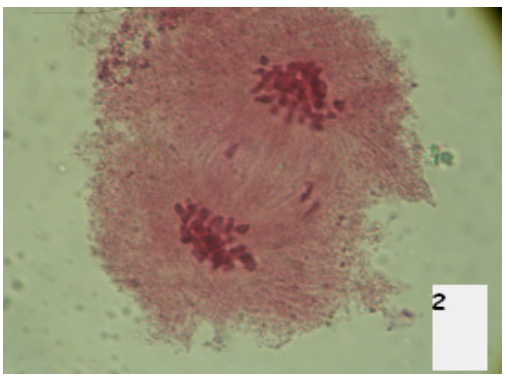
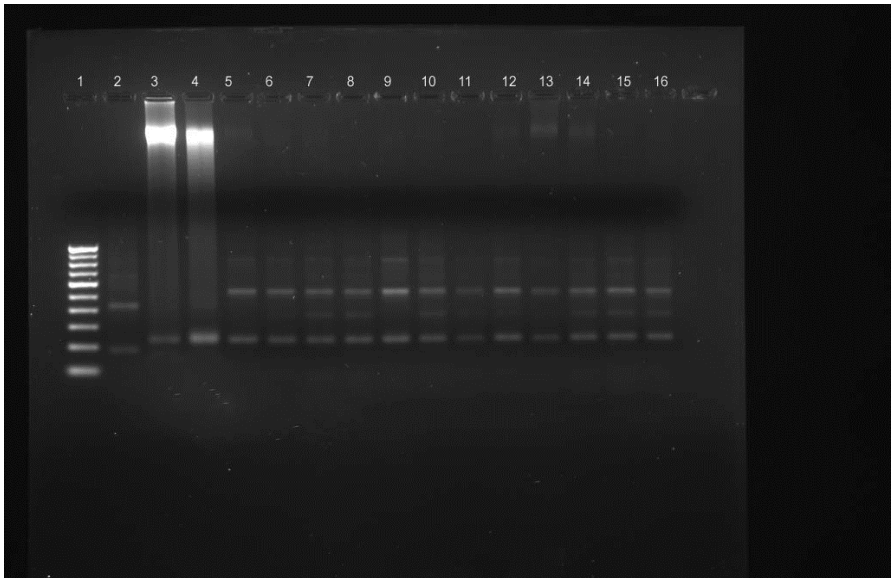


Fig 2. Lagging chromosomes in anaphase I of *Saccharum* x *Tripsacum* hybrid



Lane 1: 100bp marker, lane 2: *Tripsacum*, lane 3: CoC 671 lane 5-16 *Tripsacum* x CoC671 progenies
Fig 3. 5s r DNA amplification of parental and hybrid clones

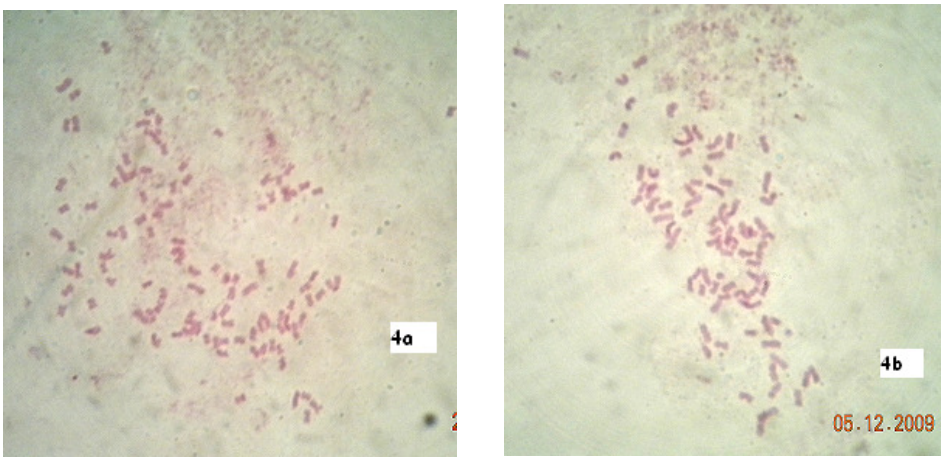


Fig. 4. Somatic chromosome number of (*Saccharum* X *Tripsacum*) X *Saccharum* backcross progenies.4a.CYM 07 – 791 (2n=108) 4b. CYM 07 – 768 (2n = 84)

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Substitution of cytoplasm of sugarcane with that of the wild grass *Erianthus arundinaceus*

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Abstract: *Sugarcane is an important agro-industrial crop in the tropical and subtropical parts of the world cultivated for sugar, ethanol and fibre. The sugarcane varieties commercially grown at present are derived from man made interspecific hybrids involving very few Saccharum officinarum L. clones, and thereby with a very narrow cytoplasmic diversity. Due to extensive cultivation of sugarcane varieties with same or very similar cytoplasmic background, the possibility of them becoming susceptible to various diseases and stress factors is high. To enhance the cytoplasmic diversity and to broaden the genetic base, variability could be introduced from other species and related genera. The wild related species S. spontaneum L. and Erianthus species are good candidates for such transfer of cytoplasm to sugarcane. The intergeneric hybrid between E. arundinaceus (Retz.) Jesw. clone IK 76-62 (2n=60) as female and S. spontaneum clone Iritty-2 (2n=64) which was confirmed to have Erianthus cytoplasm by chloroplast DNA polymorphism was used in crosses with sugarcane varieties Co 775 and CoC 671. The progeny raised was also confirmed to have Erianthus cytoplasm and the yield and quality characters of the plants were analyzed. These hybrids were further crossed with sugarcane and the backcross hybrids had Erianthus cytoplasm. These hybrids were having cane characters similar to commercial sugarcane varieties and had the sucrose % juice comparable to sugarcane clones. The chromosome number of the E. arundinaceus x S. spontaneum was 2n=62 and its hybrid with sugarcane varied from 2n=102 to 120. The backcross hybrids with sugarcane had the chromosome number ranging from 2n=102 to 114. The chromosome number of the backcross hybrids attained the level of chromosome number in commercial sugarcane varieties. Many of the backcross hybrids of sugarcane were male sterile, so that it could be easily made use of in crosses as female parent to evolve sugarcane varieties with Erianthus cytoplasm.*

Key words: sugarcane, *Erianthus arundinaceus* x *Saccharum spontaneum*, *Erianthus* cytoplasm, cytoplasmic substitution, chloroplast DNA, male sterility.

INTRODUCTION

Sugarcane is a large grass belonging to the genus *Saccharum* L. of family Gramineae. It is being cultivated in more than 20 million hectares in the tropical and subtropical regions across the world mainly for sugar and ethanol. In India sugarcane is grown in nearly 4.5 million hectares to meet the annual sugar requirement of 22 million tonnes at present in the country. The alternate use of sugarcane as an energy crop is gaining momentum. The ethanol from cane juice for blending with petrol as motor fuel besides that for potable alcohol and as feed stock for the chemical industry, and the cogeneration of electricity from sugarcane biomass necessitates increased sugarcane production in the coming years. Sugar production and productivity in the country experiences wide fluctuations in different years, mainly due to vagaries of nature, which increase the biotic and abiotic stress factors. New sugarcane varieties with resistance or tolerance to the diseases and pests as well as drought, water logging and salinity are being evolved to improve the productivity of sugarcane. The commercial sugarcane varieties under cultivation are derivatives of man made hybrids of *S. officinarum* with the wild *S. spontaneum*. The 'nobilization' as a breeding method involved the backcrossing of the *S. officinarum* x *S. spontaneum* hybrid with *S. officinarum* clone as female parent, thereby having the *S. officinarum* cytoplasm in all the sugarcane varieties. Only three or four *S. officinarum* clones used in Java and India in the early part of the 20th century appear in the pedigree as the cytoplasm donor of almost all the important sugarcane varieties under cultivation across the world. This narrow cytoplasmic diversity in sugarcane poses vulnerability of it to disease epidemics and susceptibility to varying abiotic stress factors (Mangelsdorf, 1983).

The cytoplasm of many of the crop plants such as wheat, maize, rice, oats, barley, sorghum, etc. were substituted with those of their wild related species to study the nuclear-cytoplasmic interactions and to have new genetic combinations with added agronomic features. The most important effect of alien cytoplasm substitution in plants was male sterility and it was effectively utilized in many breeding programmes. Plant height, flowering time, drought tolerance, heat tolerance, tolerance to iron deficiency, disease resistance and grain quality also were reported to be affected by cytoplasm substitution (Kihara, 1951; Shonnard and Gepts, 1994; Tsunewaki *et al.*, 2002; Liu *et al.*, 2002; Allen, 2005; Hariprasanna *et al.*, 2006; Atienza *et al.*, 2008). The wild species *S. spontaneum* and

those belonging to the related genus *Erianthus* are considered to be potential source of genes for biotic and abiotic stress resistance in sugarcane. Bakshi Ram *et al.* (2007) reported that there was no significant contribution of the *S. barberi and S. spontaneum* cytoplasm in hybrids with sugarcane for cane yield and juice quality traits. The present study is on the substitution of the sugarcane cytoplasm with that of *Erianthus arundinaceus*, a cane forming wild robust grass by repeated crossing of sugarcane with an *E. arundinaceus* x *S. spontaneum* hybrid confirmed to have *Erianthus* cytoplasm (Premachandran *et al.*, 2006).

MATERIALS AND METHOD

The *Erianthus arundinaceus* (IK 76-62) x *Saccharum spontaneum* (Iritty-2) hybrid CYM 04-420 (INGR No. 08039; IC 556972) with *Erianthus arundinaceus* cytoplasm was crossed with sugarcane commercial varieties Co 775 and CoC 671 as pollen parent. The seedlings raised in glasshouse were transplanted to field. Selected hybrids from CYM 04-420 x Co 775 and CYM 04-420 x CoC 671 were further crossed with sugarcane varieties Co 775, CoC 671, Co 62198, Co 89029 or BO 130 and the seedlings were raised. All the parental material and the hybrids were clonally maintained at Sugarcane Breeding Institute, Coimbatore.

The morphological features of the hybrids such as plant height, cane diameter, leaf length and leaf width were observed at 8 months and were compared with that of parental material. The plant height was measured from the base to the top visible dewlap. The cane diameter was measured using vernier caliper, at the middle of the 5th internode from the base. Leaf length and leaf width were measured from the third leaf below the topmost visible dewlap leaf. Cane juice quality was determined by hand refractometer (HR); brix reading of juice taken at the middle of the cane with a cane piercer.

The somatic chromosome numbers of the plants were determined from mitotic cells in the root tip. The cane cuttings were planted in sand and root tips from the actively growing roots were taken. The root tips were kept in saturated aqueous solution of α-bromo naphthalene for one hour and were fixed in 3:1 ethanol-acetic acid. After hydrolysis in 1N Hydrochloric acid for about 13 minutes the root tips were stained in leuco basic fuchsin for about 30 minutes. The stained meristem region was excised and squashed in 1% acetocarmine. The chromosome counts were made under the microscope from well spread cells. The meiotic studies were done from pollen mother cells in the immature inflorescence fixed in 3:1 ethanol- acetic acid. The young anthers were smeared in 1 % acetocarmine and pollen mother cells with meiotic division were observed under the microscope. Pollen fertility was determined by squeezing the fully matured anthers in 1:1 glycerine-acetocarmine mixture on glass slides. The fully stained pollen grains were considered fertile and the partially stained and unstained pollen grains sterile.

The cytoplasmic background of the hybrids was determined by polymorphism between *Saccharum* and *Erianthus* at the chloroplast DNA segments *psb*C-*trn*S and *trn*L intron as reported by Premachandran *et al*. (2006). The genomic DNA was isolated from freshly collected young leaves using CTAB method (Sambrook *et al.*, 1989). The chloroplast DNA segment *psb*C-*trn*S was amplified by the primer pairs: F 5'-GGT CGT GAC CAA GAA ACC AC-3'; R 5'-GGT TCG AAT CCC TCT CTC TC-3' (Demesure *et al.,* 1995) as described by Parani *et al*., 2000. The temperature profile consisted of 4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 63°C and 2 min at 72°C and the final extension for 15 min at 72°C. About 200 ng of PCR product in 10 µL reaction mix was digested with *Hae*III restriction enzyme (GENEI, Bangalore). The restricted product was separated on 1% agarose gel stained with ethidium bromide along with the 1 Kb DNA ladder at 80 m.amps for 2 hours. The chloroplast DNA region *trn*L intron was PCR amplified using a pair of universal primers: F 5'-CGA AAT CGG TAG ACG CTA CG-3'; R: 5'-GGG GAT AGA GGG ACT TGA AC-3' (Taberlet *et al.,* 1991). The PCR profile consisted of 5 min at 95°C, 30 cycles of 15 sec at 94°C, 1 min at 50°C and 1 min at 72°C and the final extension for 10 min at 72°C. The amplified PCR product was digested with *taq*I restriction enzyme (GENEI, Bangalore). The restricted product was separated on 1.5 % agarose gel along with the 100bp DNA ladder at 60 m.amps for 2-3 hours.

RESULTS AND DISCUSSION

The PCR-RFLP of the chloroplast DNA of the hybrids between *E. arundinaceus* x *S. spontaneum* hybrid CYM 04-420 and sugarcane commercial varieties Co 775 or CoC 671 as pollen parent had shown that all such hybrids were with *Erianthus* cytoplasm as that in female parent CYM 04-420. The PCR products of *psb*C-*trn*S and *trn*L intron had products of 1.5 kb and 520 bp respectively. The *psb*C-*trn*S amplified product when restricted with *Hae*III restriction enzyme showed 4 bands, in which *Erianthus* type of restriction pattern had one band at 600 bp whereas in case of *Saccharum* all the bands are less than 400 bp only. In case of *trn*L amplified product, when restricted with *taq*I, out of 4 visible bands, the higher sized band in the case of *Saccharum* is approximately 210 bp,

whereas in case of *Erianthus* type of restriction the higher sized band is approximately 160 bp. The backcross hybrids of CYM 04-420 x Co775 or CYM 04-420 x CoC 671 with sugarcane varieties Co 62198, Co 89029, BO 130, etc. also were with *Erianthus* cytoplasm as indicated in the PCR-RFLP of *psb*C-*trn*S and *trn*L intron segments (Fig. 1).

The morphological characters of the parental varieties and the hybrids observed are presented in tables 1 and 2. Plant height and cane diameter of the between *E. arundinaceus* x *S. spontaneum* hybrid CYM 04-420 was low compared to that of the sugarcane clones used in the study, whereas in CYM 04-420 x sugarcane hybrids and (CYM 04-420 x sugarcane) x sugarcane hybrids it was high, nearer to that of the sugarcane parent. The backcross hybrids were having mean cane diameter as that of the sugarcane varieties. The mean leaf length and width of the CYM 04-420 x sugarcane hybrids and the backcross hybrids were also nearer to that of the commercial sugarcane varieties used as parents. This indicated that by further backcrossing with sugarcane varieties the cane morphological characters could be brought at the level of the sugarcane varieties. The Brix was improved by backcrossing the (*Erianthus arundinaceus* x *S. spontaneum*) x sugarcane. The sucrose percentage of juice can be improved further by backcrossing the hybrids with sugarcane.

Most of the CYM 04-420 x sugarcane hybrids and (CYM 04-420 x sugarcane) x sugarcane hybrids were male sterile (Table 4 and Fig. 2). Pistillody, the homeotic transformation of stamen in to pistil was also observed in many hybrids. Only less than 5% of the hybrids had more than 20% fertile pollen. The maximum pollen fertility among the hybrids studied was in (CYM 04-420 x CoC 671) x Co 62198. The female fertility of the hybrids was not affected and seed set was found to be good in all the (*E. arundinaceus* x *S. spontaneum)* x sugarcane hybrids studied. Male sterility is a manifestation of the nuclear-cytoplasmic interaction where the nucleus is brought to an incompatible cytoplasm background. In alien substitution lines of wheat, barley, rice, oats, maize, sorghum, etc. male sterility was observed and in many such crops it was effectively used in hybrid seed production (Kihara, 1967; Tang *et al*., 2007; Hariprasanna *et al*., 2006).

The chromosome number of the *E. arundinaceus* x *S. spontaneum* hybrid CYM 04-420 is 2*n* = 62, resulted from normal reduced (*n*) gametes from both the parents. The chromosome number in progeny from CYM 04-420 x Co 775 (2n = 116) cross ranged from 2n = 102 to 120 and that of CYM 04-420 x CoC 671 (2n = 108) ranged from 2n = 108 to 120 (Table 5). The increase in chromosome number in the progeny than that expected from n + n transmission (31 + 58 = 89 or 31 + 54 = 85) indicated that 2*n* gametes had functioned from either male or female gamete. The chromosome number of the (CYM 04-420 x sugarcane) x sugarcane hybrids ranged from 2*n* = 102 to 120 (Table 5) indicating that only *n* + *n* gametes functioned in the backcross progeny. The range in chromosome number in the backcross progeny is as that observed in commercial sugarcane varieties and hence the use of the *Erianthus* cytoplasm hybrids in crosses with sugarcane may not alter the level of chromosome number in further backcross generations.

The present study has shown that substitution of cytoplasm of sugarcane varieties with that of the wild species *Erianthus arundinaceus* is possible. Male sterility in the hybrids and the backcross hybrids favour the hybridization with sugarcane varieties for repeated backcrossing to develop new sugarcane varieties with *Erianthus* cytoplasm.

Table 1. Morphological and quantitative characters of the parental clones.

Sl. No.	Parent	Plant height (cm)	Cane diameter (cm)	Leaf length (cm)	Leaf width (cm)
1	IK 76-62	286	1.5	151	4.1
2	Iritty-2	200	0.7	100	0.9
3	Co 775	128	2.9	113	6.2
4	CoC 671	165	3.2	129	5.4
5	Co 62198	240	2.2	107	4.9
6	Co 89029	185	2.2	126	4.2
7	BO 130	138	2.6	136	3.4
8	CYM 04-420	110	0.8	88	1.1

Table 2. Mean values of plant morphological characters and hand refractometer Brix in the progeny of hybrids with *Erianthus* cytoplasm

Sl. No	Parent	No. of plants observed	Plant height	Cane diameter (cm)	Leaf length (cm)	Leaf width (cm)	HR Brix (8 months)
1	CYM 04-420 x Co 775	96	134.00	1.64	122.70	3.54	12.60
2	CYM 04-420 x CoC 671	32	149.10	1.71	112.12	3.24	13.90
3	(CYM 04-420 x Co 775)– 871 x BO 130	100	115.64	1.94	126.88	3.52	14.79
4	(CYM 04-420 x Co 775)– 882 x Co 89029	61	126.77	1.93	131.57	3.67	14.74
5	(CYM 04-420 x CoC 671)– 1008 x Co 62198	91	123.85	1.95	132.59	3.13	15.11

Table 3. Chromosome number and pollen fertility in parental clones

Sl. No	Clone	Chromosome number (2*n*)	Pollen fertility (%)
1	*S. spontaneum* 'Iritty-2'	64	98.1
2	*E. arundinaceus* 'IK 76-62'	60	33.9
3	Co 775	116	82.5
4	CoC 671	108	74.2
5	Co 62198	120	68.0
6	Co 89029	110	73.3
7	BO 130	116	28.7
8	CYM 04-420	62	-

Table 4. Pollen fertility in [(*E. arundinaceus* x *S. spontaneum*) x sugarcane and [(*E. arundinaceus* x *S. spontaneum*) x sugarcane] x sugarcane hybrids

Parentage	Pollen Fertility (%)	No. of plants	Frequency
CYM 04-420 x Co 775	0	32	0.67
	01-10	9	0.19
	10-20	5	0.10
	20-30	1	0.02
	30-40	1	0.02
	>40	0	0.00
	Total	48	
CYM 04-420 x CoC 671	0	12	0.70
	01-10	4	0.24
	10-20	1	0.06
	>20	0	0.00
	Total	17	
(CYM 04-420 x Co 775)-871 x BO 130	0	18	0.72
	01-10	5	0.20
	10-20	1	0.04
	20-30	1	0.04
	>30	0	0.00
	Total	25	
(CYM 04-420 x Co 775)-882 x Co 89029	0	25	0.81
	01-10	5	0.16
	10-20	1	0.03
	>20	0	0.00

	Total	31	
(CYM 04-420 x CoC 671)-1008 x Co 62198	0	10	0.66
	01-10	3	0.20
	10-20	1	0.07
	20-30	0	0.00
	30-40	1	0.07
	>40	0	0.00
	Total	15	

Table 5. Chromosome number in [(*E. arundinaceus* x *S. spontaneum*) x sugarcane and [(*E. arundinaceus* x *S. spontaneum*) x sugarcane] x sugarcane hybrids

Parentage	Chromosome No. (2*n*)	No. of plants observed
CYM 04-420 (2n = 62) x Co 775 (2n = 116)	102	1
	108	4
	110	1
	112	3
	116	2
	118	3
	120	6
Total		20
CYM 04-420 (2n = 62) x CoC 671 (2n = 108)	108	1
	110	4
	112	1
	114	1
	116	1
	120	4
Total		12
(CYM 04-420 x Co 775)– 871 (2n = 102) x BO 130 (2n = 116)	108	1
	110	2
	112	1
	118	1
Total		5
(CYM 04-420 x Co 775)– 882 (2n = 112) x Co 89029 (2n = 110)	102	1
	108	3
	112	1
	114	2
Total		7
(CYM 04-420 x CoC 671)– 1008 (2n = 110) x Co 62198 (2n = 120)	106	1
	110	2
	120	1
Total		4

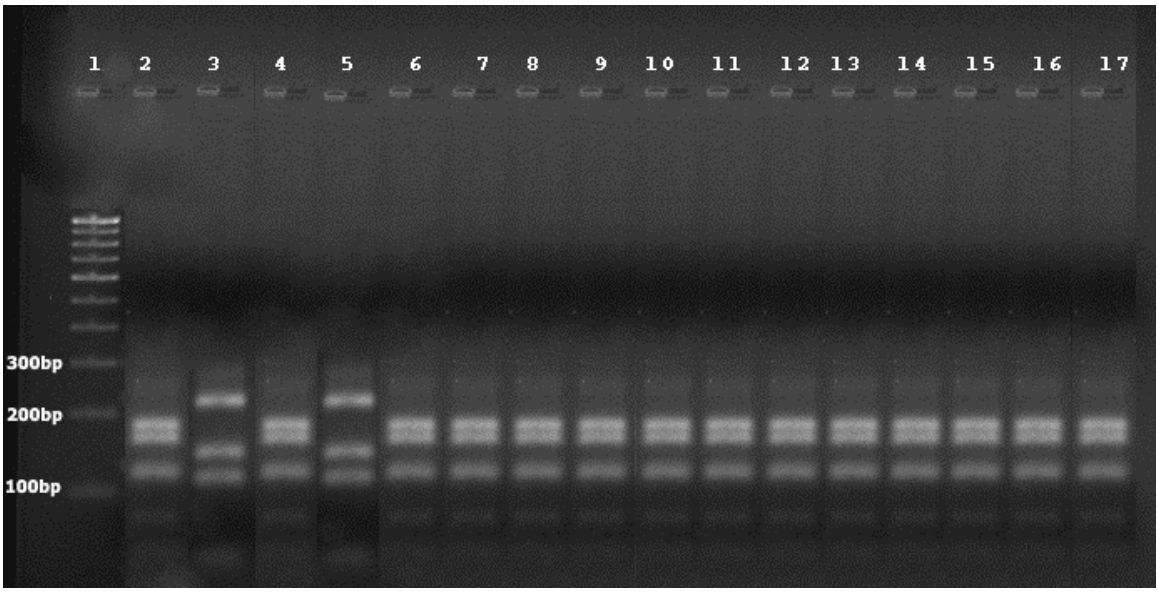

Fig 1: **Restriction pattern of chloroplast DNA segment *trn*L intron with *taq*I in parental clones and hybrids. Lane 1:** 100bp DNA marker; **Lane 2:** *E. arundinaceus* 'IK 76-62'; **Lane 3:** *S. spontaneum* 'Iritty-2'; **Lane 4:** CYM 04-420 (IK 76-62 x Iritty-2); **Lane 5:** Co 775; **Lane 6:** (CYM 04-420 x Co 775)– 871; **Lane 7 - 11:** (CYM 04-420 x Co 775)– 871x Bo 130; **Lane 12:** (CYM 04-420 x Co 775)– 882; **Lane 13 - 17:** (CYM 04-420 x Co 775)– 882 x Co 89029.

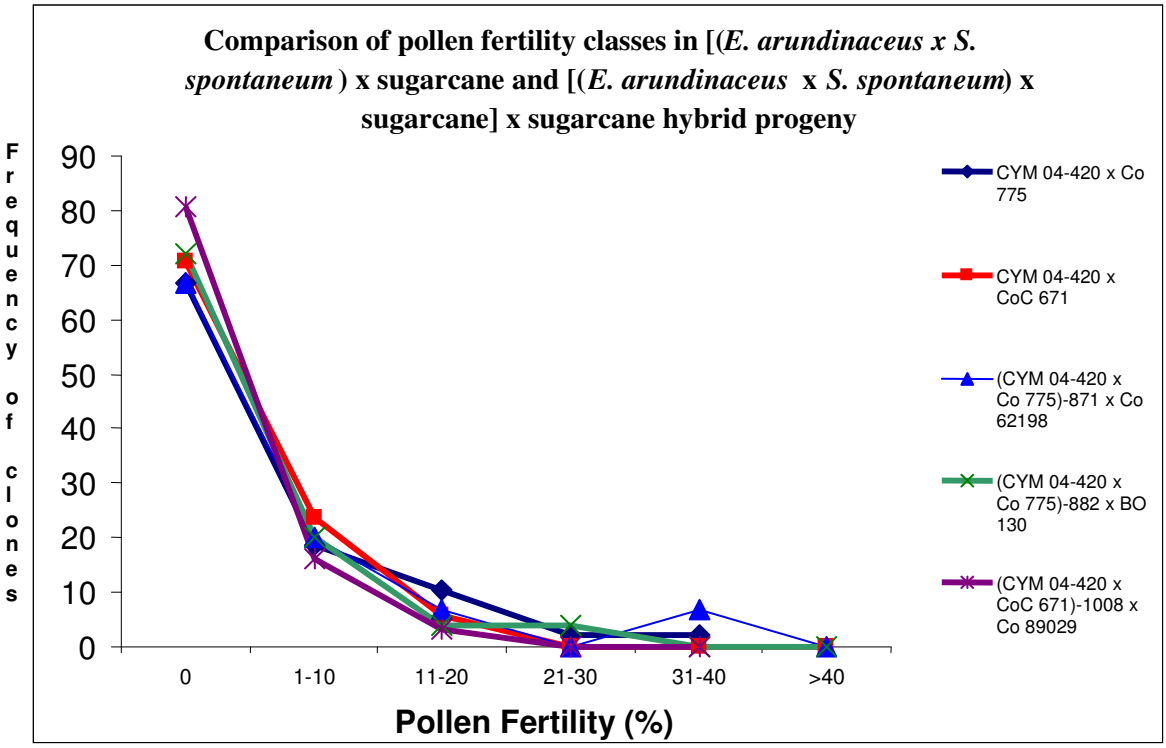

Fig. 2. Comparison of pollen fertility classes in *E. arundinaceus x S. spontaneum* x sugarcane and [(*E. arundinaceus* x *S. spontaneum* x sugarcane] x sugarcane hybrid progeny

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High yielding cardamom clones for Wayanad

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Abstract: *A sizeable area in Wayanad District of Kerala is under cardamom cultivation. However, production and productivity of the crop in Wayanad is very low compared to that of other areas of the cardamom tract in the country. One of the reasons for this situation could be the non availability of clones suited to the agro-climatic conditions of Wayanad. In this background, an evaluation trial incorporating nine improved cardamom clones evolved by the Indian Cardamom Research Institute (Spices Board) was conducted in a planter's field in Kalpetta and compared with the released clone ICRI-2 and the local variety Clone-37 for growth, yield and quality parameters with a view to isolate suitable clones for Wayanad. On analysis of data on growth characters and stabilized yield for three consecutive crop seasons, it is found that the hybrid MHC-18 performed the best with a yield of 1400 kg/ha followed by MCC-73 and MCC-21 indicating their suitability for large scale cultivation to obtain higher yield. MHC-18 is found to be superior with respect to quality characters also.*

Key words: Cardamom, *Elettaria cardamomum*, evaluation, Wayanad

INTRODUCTION

Small cardamom (*Elettaria cardamomum* Maton) is a spice crop cultivated on plantation scale in the tropical rainforests of Western Ghats. It is valued for its dried fruits (capsules), the cardamom of commerce. In Kerala, cardamom is cultivated in an area of 41,588 ha and out of which 4,106 ha are in Wayanad. The productivity of cardamom in Kerala is 294 kg/ha. Though a sizeable area in Wayanad is under cardamom cultivation, productivity of the crop is only 124 kg/ha, which is very low compared to that of other zones of the cardamom tract especially the Idukki zone (Spices Board, 2009). One of the reasons for this situation could be the non availability of improved clones suited to the agro-climatic conditions of Wayanad. In this back ground, an evaluation trial incorporating nine improved cardamom clones evolved by the Indian Cardamom Research Institute (Spices Board) was conducted in Wayanad with a view to isolate suitable clones for that area.

MATERIALS AND METHOD

The study was conducted in Scholar Estate located at Kalpetta in Wayanad during 2003-2009. The experiment was laid out in randomized block design (RBD) with three replications and twelve plants per plot adopting 2.7 m X 2.7 m spacing. Nine improved clones including hybrids and selections evolved by the Indian Cardamom Research Institute *viz*., MHC-10, MHC-13, MHC-18, MCC-21, MCC-40, MCC-73, MCC-200, MCC-260 and MCC-346 were incorporated in the trial alongwith the released variety ICRI-2 (Madhusoodanan *et al*., 1993) and the local variety Clone-37 as check. Package of practices recommendations of the Spices Board was followed for cultivation (Spices Board, 2001). Observations on growth and yield parameters such as total tillers per clump, tiller height, number of leaves on the tallest tiller, number of bearing tillers per clump, number of panicles per clump, number of racemes per panicle, number of capsules per raceme, number of seeds per capsule and stabilized yield for three consecutive crop seasons obtained after three years of planting were recorded and subjected to pooled analysis. Data on quality characters such as recovery percentage, percentage of 7 mm and above sized capsules, volatile oil content and oleoresin content were also recorded and analyzed.

RESULTS AND DISCUSSION

The performance of cardamom clones varied with regard to growth, yield and quality attributes (Tables 1, 2, 3, 4 and 5). All the parameters except panicles per clump showed significant difference between the clones. Cardamom being a commercial crop, more attention was paid for yield evaluation (George *et al*., 1981). High variability with regard to yield parameters in cardamom has been reported earlier (Korikanthmath *et al*., 1997). The yield differed significantly between the clones and the hybrid MHC-18 performed the best with a yield of 1400 kg/ha followed by selections MCC-73 (1159 kg/ha) and MCC-21 (1131 kg/ha). However, the performance of MCC-73 and MCC-21 was on par. Based on the yield performance MHC-18, MCC-73 and MCC-21 are considered to be the high yielders suited to the agro-climatic conditions of Wayanad area of the cardamom tract. An increasing trend in yield as well as consistency in performance could be observed in all the three high yielders over the three crop seasons studied. Performance of MCC-40 and MHC-10 was poor compared to the local check. All other

clones evaluated except MHC-18, MCC-73 and MCC-21 were found to be on par. Since cardamom is highly heterozygous, vegetative propagation by sucker multiplication is suggested to produce uniform planting materials (Nadgauda *et al*., 1983). Large-scale cultivation of these identified improved clones by adopting high production technology (Johny and Ravindran, 2002) would substantially enhance the production and productivity of cardamom in Wayanad.

Table 1. Growth characters of cardamom clones- pooled data (2006-07 to 2008-09)

Sl. No.	Clone	Tillers/ Clump	Tiller height (cm)	Leaves/ Tiller	Bearing tillers/Clump
1	MHC-10	35.91	370.18	18.27	18.94
2	MHC-13	44.34	368.49	18.66	24.60
3	MHC-18	54.86	381.15	19.45	30.30
4	MCC-21	43.48	384.66	18.59	25.00
5	MCC-40	34.94	345.99	18.85	21.13
6	MCC-73	41.46	349.99	19.00	22.86
7	MCC-200	43.36	348.59	18.71	23.29
8	MCC-260	34.50	337.99	18.25	19.74
9	MCC-346	43.40	373.27	17.99	23.22
10	ICRI-2	38.31	353.14	17.92	21.05
11	Clone-37 (check)	38.07	342.22	17.46	21.26
CD (5%)		10.50	11.32	0.98	5.34

Table 2. Yield attributes of cardamom clones- pooled data (2006-07 to 2008-09)

Sl. No.	Clone	Panicles/ Clump	Racemes/ Panicle	Capsules/ Raceme	Seeds/ Capsule
1	MHC-10	34.51	14.96	7.48	18.00
2	MHC-13	37.81	16.12	8.18	17.38
3	MHC-18	48.53	21.47	9.34	21.16
4	MCC-21	42.51	19.94	8.84	18.99
5	MCC-40	35.11	13.59	7.31	15.33
6	MCC-73	42.65	20.79	8.79	19.11
7	MCC-200	39.51	18.18	8.13	17.66
8	MCC-260	34.49	16.75	7.74	18.38
9	MCC-346	39.46	17.24	8.44	17.66
10	ICRI-2	36.09	15.82	7.68	17.38
11	Clone-37 (check)	39.01	17.37	7.54	17.16
CD (5%)		NS	2.33	1.19	3.03

Table 3. Yield of cardamom clones- pooled data (2006-07 to 2008-09)

Sl. No.	Clone	Yield/Plant (kg)	Yield/ha (kg)
1	MHC-10	0.589	765.33
2	MHC-13	0.646	840.00
3	MHC-18	1.077	1400.33
4	MCC-21	0.870	1131.66
5	MCC-40	0.552	718.00
6	MCC-73	0.891	1159.66
7	MCC-200	0.674	877.00
8	MCC-260	0.635	825.33
9	MCC-346	0.670	871.00
10	ICRI-2	0.627	814.33
11	Clone-37 (check)	0.668	868.00
CD (5%)		0.076	108.50

Table 4. Quality characters of cardamom clones- pooled data (2006-07 to 2008-09)

Sl. No.	Clone	Recovery (%)	7 mm capsules (%)	Volatile oil (%)	Oleoresin (%)
1	MHC-10	19.66	66.89	8.09	6.98
2	MHC-13	20.11	55.46	7.85	5.93
3	MHC-18	22.24	69.87	8.84	7.99
4	MCC-21	19.65	61.74	6.20	7.14
5	MCC-40	18.79	58.12	7.85	6.33
6	MCC-73	20.41	67.33	8.65	6.67
7	MCC-200	20.00	59.75	7.04	7.07
8	MCC-260	20.39	67.89	6.56	6.69
9	MCC-346	19.61	61.99	7.93	6.81
10	ICRI-2	19.59	64.00	8.95	7.63
11	Clone-37 (check)	19.99	57.41	6.25	5.75
	CD (5%)	1.35	6.29	1.27	0.45

Table 5. Season wise yield performance (kg/ha) of cardamom clones

Sl. No.	Clone	I	II	III	Pooled Mean
		2006-07	2007-08	2008-09	
1	MHC-10	698.66	764.83	833.66	765.33
2	MHC-13	774.66	836.33	911.33	840.00
3	MHC-18	1246.66	1414.33	1542.33	1400.33
4	MCC-21	1038.33	1128.83	1229.33	1131.66
5	MCC-40	660.66	715.00	779.66	718.00
6	MCC-73	1047.33	1163.50	1268.66	1159.66
7	MCC-200	789.00	881.83	961.33	877.00
8	MCC-260	765.33	819.00	894.00	825.33
9	MCC-346	807.00	864.50	942.33	871.00
10	ICRI-2	702.33	834.16	909.00	814.33
11	Clone-37 (check)	830.33	849.33	926.00	868.00
	CD (5%)	326.70	155.91	169.60	108.50

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Genetic divergence studies in grain amaranth (*Amaranthus spp.* L.)

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Abstract: *Euclidean cluster analysis was used for the characterization of germplasm of different geographical origin of grain amaranth (Amaranthus spp, L.). A quantitative assessment of genetic divergence for thirteen characters using Mahalonobies D^2 statistics revealed the presence of considerable genetic diversity. The 100 genotypes were grouped into sixteen clusters with variable number of genotypes. The clustering pattern revealed cluster II to be the largest consisting 31 genotypes followed by Cluster IX with 20 genotypes. Intra cluster distance was the highest in Cluster VIII followed by Cluster IX and Cluster I. The inter cluster D values ranged from 15.12 between Cluster XI and XV to 186.98, indicating presence of high divergence among the genotypes. Seed yield per plant contributed more towards divergence with 35.35% followed by dry weight of panicle (25.43%) and fresh weight of panicle (20.08%). Among the plant attributes, seed yield per plant, dry weight of panicle, panicle length, harvest index and number of spikes per panicle were found to be important. Accessions GP-BGA-3, IC-38127 and IC-423400 from cluster VIII and IC-452224, IC-415250, IC- 519558, IC-415387 and IC-415318 from the cluster XV were identified as potential parents in future hybridization programmes for genetic improvement in grain amaranth.*

Key Words: Grain amranth, genetic divergence, cluster, cluster means

INTRODUCTION

Grain amaranth belongs to the family Amaranthaceae and genus Amaranthus. In relation to major crops in many developing areas of the world the carrying capacity of the land is rapidly being exceeded because of increasing population growth. In maintaining sustainability in production and food security, underutilized pseudocereals like grain amaranth plays a very important role through its high nutritional value. Archeological findings in Thuacan in Puebbla, Mexico show that they were cultivated over 6000 years ago. The crude protein content of grain amaranth is reported to vary from 8% to 22%, which is comparable to any other common grains except soybean. Since the food uses of grain amaranth are similar to that of cereal grain grasses like wheat and oat, grain amaranth is also called a 'pseudocereal'. Though there has been an increasing trend in area, production and productivity of grain amaranth in the country from decades, concerted efforts are still required towards breeding highly productive cultivars to bridge the gap between the domestic and international level of productivity. Study of genetic divergence among a set of genotypes will therefore enable a plant breeder to choose suitable parents and plan an appropriated hybridization programme. D^2 statistics has proved to be a powerful tool in determining genetic divergence based on multiple growth characters, in asserting relative characters and assessing relative contribution of different components to total divergence (Bhatt, 1973). The present study was conducted to determine the magnitude of variability in yield and its attributing traits for identifying the promising genotypes of grain amaranth.

MATERIALS AND METHOD

The material used in the current study comprises of 100 genotypes of three different species of grain amaranth of different geographical origin obtained from AICRIP on Underutilized Crops, Main Agricultural Research Station, Hebbal, Bangalore, India. The experiment was laid out at Main Agricultural Research Station, Hebbal, Bangalore in 10 x 10 simple lattice design with two replications and the investigation was carried out during *kharif* 2006, to assess the genetic divergence of the crop.

RESULTS AND DISCUSSION

The results from the analysis of variance revealed highly significant differences among genotypes for all the 13 characters studied. The 100 genotypes were grouped into sixteen clusters with variable number of genotypes. The clustering pattern revealed cluster II to be the largest of all consisting of 31 genotypes followed by cluster IX with 20 genotypes and cluster XVI with 14 genotypes. The distribution pattern of genotypes into various clusters is shown in Table 1. Intra cluster distance was the highest in Cluster VIII with D^2 value of 7706.24 followed by Cluster IX (7175.65) and Cluster I (5875) (Tables 2&3). The study of intracluster distances indicated that, the maximum amount of heterosis was expected in the cross combination involving the genotypes of most divergent cluster. These results were in accordance with results of Lohithaswa (1992) and Asthana *et al.* (1998).

The minimum inter cluster D value (15.12) was observed between cluster VII and XI, indicating close genetic association between the genotypes of these two clusters. Cluster XV was the most diverse cluster as many clusters except VIII showed maximum inter cluster distance with it. Hence, it would be logical to incorporate genotypes from these clusters in further breeding programmes. These observations are also in accordance with the reports of earlier workers like Khumkar and Singh (2002) and Indra Singh and Garg (2003). Seed yield per plant contributed the highest towards divergence with 35.35% followed by dry weight of panicle (25.43%) and number of leaves (0.02%) was the least contributing trait towards total divergence (Table.3).These observations are also in accordance with the reports of earlier workers (Joshi and Rana, 1995; Bergale *et al.,* 2001; Shiv Datt and Mani, 2003). Greater emphasis should be laid on those characters contributing maximum to the D^2 values for the purpose of further selection and choice of parents for hybridization.

The genotypes in cluster IV were the earliest to flower within 43 days, while the genotypes in cluster XV were late to mature in 98 days. The lowest number of leaves per plant (35) was found in cluster IV. The genotype with low mean value for number of branches per plant (16) was found in cluster XI, while highly branched genotypes with the highest mean value (20) were accumulated in cluster VII. The shortest genotype (176cm) was included in cluster XV. Cluster XV recorded the highest (44.32g) seed yield per plant (Table.5). The same results were noticed by the earlier workers Waghmode *et al.* (1997) and Datta and Mukherjee (2004).

Rational choice of parents on the basis of their genetic diversity can provide the scope for rapid improvement. Hybridization between the genetically divergent genotypes will result in accumulation of favourable genes and produce a wide spectrum of variation in the segregating progeny (Shiva Datta and Mani, 2003). In the present study, among the plant attributes, seed yield per plant, dry weight of panicle, panicle length, harvest index and number of spikes per panicle were found to be important. Accessions GP-BGA-3, IC-38127 and IC-423400 from cluster VIII and IC-452224, IC-415250, IC-519558, IC-415387 and IC-415318 from cluster XV were identified as potential parents in future hybridization programmes for genetic improvement in grain amaranth.

Table 1. Composition of grain amaranth genotypes in different clusters

Clusters	Numbers	Genotypes
I	7	EC-519544,IC-42311, IC-37316, IC-415448, IC-415449, IC-415322, GP-BGA-16
II	31	IC-415290, IC-415266,IC-415272,IC-415264, IC-423448, EC-519554, IC-415318, IC-415297, IC-415262, IC-413426, EC-519549, EC-519526, EC-519522, IC-423408, GP-BGA-19, GP-BGA-20, GP-BGA-14, GP-BGA-12, GP-BGA-11, GP-BGA-27, GP-BGA-28, GP-BGA-24, GP-BGA-18, GP-BGA-9, GP-BGA-25, GP-BGA-8, GP-BGA-22, GP-BGA-5, GP-BGA-21, IC-415314, IC-415462
III	2	IC-415433, IC-519542
IV	2	IC-519549, EC-519532
V	2	GP-BGA-21, IC-415252
VI	2	IC-415271, IC-415274
VII	2	IC-415290, Annapurna
VIII	3	GP-BGA-3, IC-38127, IC-423400
IX	20	IC-GP-BGA-6, GP-BGA-1, GP-BGA-23, GP-BGA-26, GP-BGA-17, GP-BGA-7, GP-BGA-4, IC-415258, IC-98312, IC-415232, KBGA-1, IC-415317, IC-423408, EC-519526, IC-415258, IC-415284, IC-415331, IC-403548, IC-423398, IC-519512
X	2	IC-519543, EC-524457
XI	2	IC-415282, IC-415243
XII	2	IC-415266, IC-415316
XIII	2	IC-415220, IC-415320
XIV	2	IC-415236, IC-415466
XV	5	IC-415224, IC-415250, IC-519558, IC-415387, IC-415318
XVI	14	EC-519554, IC-519527, EC-519517, IC-415272, EC-519531, IC-415254, IC-423410, IC-415297, EC-519592, IC-519548, IC-423544, IC –421885, IC-415284, IC-415448

Table 2. Average inter cluster (above diagonal) and intra-cluster (diagonal) D^2 values for 16 clusters in grain amaranth.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	IX	XV	XVI
I	**5875.43**	5465.52	5797.57	7203.60	7647.82	9104.78	3182.05	14414.10	6183.94	4160.64	3517.09	3084.93	3754.12	6248.19	15760.07	5875.81
II		**5109.72**	3928.46	4910.81	5136.01	6256.13	3002.02	18428.23	6346.66	3519.80	3026.68	2776.79	2959.68	4329.99	20321.15	4964.87
III			**84.10**	500.27	471.11	1152.78	1776.41	24952.43	6888.12	1411.14	1157.96	1733.07	892.00	348.77	27500.73	2667.23
IV				**102.03**	874.39	1738.00	2722.90	26794.81	7975.72	1600.63	2008.99	2480.25	1922.24	241.96	29932.11	3396.40
V					**108.70**	294.73	3454.44	29200.31	8508.95	3010.34	2665.31	3328.55	1743.07	904.40	32001.70	3696.71
VI						**123.**50	4888.75	32164.67	9874.59	4611.81	3941.82	4736.94	2672.84	1795.22	34961.62	4733.31
VII							**128.33**	14896.94	4486.08	520.65	228.63	238.44	580.36	2131.59	16628.88	2596.85
VIII								**7706.24**	15422.96	17294.48	16907.04	14769.29	18473.80	25038.20	5105.36	20882.54
IX									**7175.65**	5423.50	4896.00	4129.18	4969.15	7250.38	17060.37	7062.52
X										**180.10**	410.08	530.28	967.62	1239.57	19564.93	2641.21
XI											**196.99**	317.63	474.17	1424.60	18785.17	2342.92
XII												**210.79**	688.11	1957.69	16696.19	2576.66
XIII													**250.94**	1389.85	20350.41	2326.63
IV														**284.87**	27863.95	2914.06
XV															**5670.30**	22963.57
XVI																4549.49

Table 3. Inter and intra cluster distances in grain amaranth

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	**76.65**	73.93	76.14	84.87	87.45	95.42	56.41	120.06	78.64	64.50	59.31	55.54	61.27	79.05	125.54	76.65
II		**71.48**	62.68	70.08	71.67	79.10	54.79	135.75	79.67	59.33	55.02	52.70	54.40	65.80	142.55	70.46
III			**9.17**	22.37	21.71	33.95	42.15	157.96	83.00	37.57	34.03	41.63	29.87	18.68	165.83	51.65
IV				**10.10**	29.57	41.69	52.18	163.69	89.31	40.01	44.82	49.80	43.84	15.56	173.01	58.28
V					**10.43**	17.17	58.78	170.88	92.24	54.87	51.63	57.69	41.75	30.07	178.89	60.80
VI						**11.11**	69.92	179.35	99.37	67.91	62.78	68.83	51.70	42.37	186.98	68.80
VII							**11.33**	122.05	66.98	22.82	15.12	15.44	24.09	46.17	128.95	50.96
VIII								**87.79**	124.19	131.51	130.03	121.53	135.92	158.24	71.45	144.51
IX									**84.71**	73.64	69.97	64.26	70.49	85.15	130.62	84.04
X										**13.42**	20.25	23.03	31.11	35.21	139.88	51.39
XI											**14.04**	17.82	21.78	37.74	137.06	48.40
XII												**14.52**	26.23	44.25	129.21	50.76
XIII													**15.84**	37.28	142.66	48.24
XIV														**16.88**	166.93	53.98
XV															**75.30**	151.54
XVI																**67.45**

Values in bold are intra-cluster distances

Table 4. Contribution of the characters towards genetic divergence in grain amaranth

Sl. No.	Characters	Rank	Contribution in per cent
1.	Days to 50 % flowering	13	0.26
2.	Days to maturity	0	0.00
3.	Stem girth at collar region (cm)	0	0.00
4.	Number of leaves	1	0.02
5.	Number of branches	0	0.00
6.	Plant height (cm)	16	0.34
7.	Panicle fresh weight (g)	994	20.08
8.	Panicle length (cm)	436	8.80
9.	Number of spikelets per panicle	19	0.38
10.	Dry weight of panicle (g)	1259	25.43
11.	Dry weight of stem (g)	164	3.32
12.	Harvest index (%)	298	6.02
13.	Seed yield per plant (g)	1750	35.35

Table 5. Cluster mean values for 13 different characters in grain amaranth

Clusters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}
I	52.91	93.44	5.74	41.85	17.26	175.27	248.15	83.69	76.69	137.74	106.59	12.43	30.12
II	51.03	94.41	6.01	38.39	18.97	170.36	225.79	80.15	73.34	127.59	99.85	12.13	27.37
III	47.14	93.13	5.93	42.65	19.63	165.22	199.29	79.26	65.64	91.76	103.40	12.64	24.65
IV	42.66	94.75	5.53	34.53	19.56	159.68	195.82	59.68	65.33	89.40	102.50	12.72	24.40
V	51.60	96.35	5.85	41.03	17.02	160.13	183.78	82.27	48.86	89.42	98.67	10.51	19.75
VI	51.60	89.44	5.31	39.28	19.04	133.72	173.28	89.93	45.93	87.33	96.25	9.33	17.07
VII	47.15	96.35	6.28	41.39	20.41	174.80	245.81	78.92	65.48	114.48	91.80	15.44	31.83
VIII	48.31	93.78	5.82	41.19	18.43	178.19	351.92	67.44	90.09	197.89	93.00	15.06	43.94
IX	48.95	95.23	5.94	38.04	18.98	168.11	241.66	77.29	74.99	148.27	94.27	12.03	29.11
X	48.21	91.63	5.96	34.90	16.11	180.02	238.95	62.01	75.10	103.90	102.36	14.98	30.89
XI	48.39	96.33	5.95	46.56	15.65	164.57	235.85	78.58	76.51	106.14	100.07	14.48	29.86
XII	48.34	96.36	5.48	40.75	17.87	172.74	239.68	73.42	81.61	120.06	91.01	14.33	30.17
XIII	52.56	89.38	6.11	37.25	19.51	167.34	227.95	85.28	53.46	110.05	98.67	12.22	25.43
XIV	46.39	94.29	6.20	41.20	16.98	155.02	200.54	64.99	67.45	91.92	116.08	11.76	24.38
XV	48.60	97.75	5.64	39.89	16.45	175.63	362.47	79.92	87.65	201.22	99.83	14.68	44.32
XVI	49.74	91.61	5.88	40.57	17.53	164.52	222.94	78.30	67.86	107.23	107.83	12.42	26.73

X_1 - Days to 50% flowering X_2 - Days to maturity X_3- Stem girth at collar region (cm) X_4 - Number of leaves X_5- Number of branches per plant X_6- Plant height (cm) X_7- Panicle fresh weight (g) X_8- Panicle length (cm) X_9- Number of Spikelets per panicle X_{10}- Dry weight of panicle X_{11}-Dry weight of stem(g) X_{12}-Harvest index (%) X_{13}- Seed yield per plant (g)

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Genetic variability created through biparental mating in bhendi (*Abelmoschus esculentus* (L.) Moench).

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Abstract: *The study was also aimed to know the relative efficiency of BIPs over F_3 in turns of release of genetic variability and to know the shift in the association pattern of components of traits with fruit yield in okra (Abelmoschus esculentus (L) Monech)., an important vegetable crop grown in the tropical and sub-tropical parts of the world. The present investigation was carried out during kharif and summer seasons of 2007-08 to study the nature and magnitude of variability generated in the case of different quantitative traits in the population obtained by attempting crosses in the F_2 generation of 4 commercial single cross private bhendi hybrids namely safal, rasi, seminis and ph101. Biparental mating design was attempted. The plants involved in the cross were also selfed simultaneously to obtain F_3 progenies. The effectiveness of biparental mating was compared with conventional breeding method. The range, variance, heritability and genetic advance were higher in BIPs for all the characters studied except hundred seed weight. The utility of biparental matings in early segregating generations in okra is emphasized.*

Keywords: Biparental, okra, heritability, genetic advance

INTRODUCTION

Okra (*Abelmoschus esculentus* (L) Monech) is an important vegetable crop grown in the tropical and sub-tropical parts of the world. India is the largest producer of okra covering an area of 3.58 lakh ha with an annual production of 35.24 lakh tonnes (Anon., 2006). Okra belongs to family Malvaceae with 2n =130 chromosomes and amphidiploid nature. The tender fruits are used as vegetable, eaten boiled or in culinary preparations as sliced and fried pieces. It has good nutritional value, particularly high content of vitamin-C (30 mg/100 g), calcium (90 mg/100 g) and iron (1.5 mg/100 g) (Pal *et al.*, 1952). Pedigree method of breeding is the most common in the improvement of self-pollinated crops. However, this method has certain limitations. The rate of homozygosity is very high, which reduces chances of recombination. This also retains tight and undesirable linkages and utilizes only fixable effects. Since, the routine pedigree method of breeding was considered inadequate to exploit the useful genetic variability for complex characters like yield (Humphery *et al.*, 1969), intermating in early segregating generations was suggested to pool the desirable genes from the selected plants in self-pollinated crops (Jensen, 1970). The present study aims to evaluate the efficiency of intermating in F_2 generation of a cross in effecting improvement and to compare it with the pedigree method of breeding.

MATERIALS AND METHOD

The experimental material for the present investigation comprised of F_2 generation of four commercial single cross private hybrids *safal*, *rasi*, *seminis* and ph101. About 25 plants were selected in each F_2 populations on visual basis keeping in view the vigour for selective intermating. The F_2 plants used in biparental mating were also selfed to yield F_3 progenies. The experiment was conducted at Agricultural Research Station, Hanumanmatti, Karnataka during *kharif* 2007-08. The BIP population and their corresponding F_3 population were sown in the field with a row length of 5m. The rows and plants were spaced at 60 cm and 30 cm respectively. The data were recorded on five plants in BIP and F_3 for 11 characters *viz.,* days to first flowering, fruit length, fruit diameter, average fruit weight, number of fruits per plant, plant height, internodal length, number of branches per plant, 100 seed weight, stem diameter and fruit yield per plant. The means and ranges in respect to each character were worked out in the biparental as well as F_3 progenies. The phenotypic and genotypic variances, phenotypic and genotypic coefficients of variation, heritability in broad sense and genetic advance were computed following the method of Burton and Devane (1953).

RESULTS AND DISCUSSION

The comparison of mean and range of expression of different characters (Table 1) between BIP and F_3 indicated that F_3 populations showed high mean values than BIPs for the characters like plant height,

internodal length, number of branches per plant, days to first flowering and fruit yield per plant. It was due to wide range observed in BIPs for the character under study. The present findings are in agreement with the findings of Singh and Sahu (1981), Kadlera (1997) and Parameshwarappa *et al.* (1997) in safflower for plant height and number of branches. BIP populations recorded the highest mean values for characters such as 100 seed weight and number of fruits per plant than the selfing generations. High mean values of BIPs compared to selfing generations were reported by Yunus and Paroda (1983) for traits like 100 grain weight and grain yield in wheat and by Singh and Sahu (1981) for 100 seed weight and grain yield in safflower. The range of expression of characters in biparnetal progenies was wider. It is noteworthy that especially the lower limit of range was lower in BIP for all the characters. At the same time, the lower limit was higher compared to that of F_3 progenies for most of the characters suggesting that intermating has helped in releasing more variability than selfing. The higher variability in the BIP population could have resulted from the additional opportunity for genetic recombination. General shifts in the values of range of expression of characters by biparental approach were also reported by Nematullah and Jha (1993) in wheat and Parameshwarappa *et al.* (1997) in safflower.

The estimates of variability, heritability and genetic advance for various characters in BIP and corresponding F_3 progenies are presented in Table 2. The BIP had greater GCV, PCV, heritability and genetic advance (% of mean) in respect of all the characters except 100-seed weight. The characters which showed wider range were also characterized by higher magnitudes of GCV and PCV. Generally, BIP population had higher GCV and PCV for all the characters except for 100 seed weight. Higher GCV and PCV in the BIP as compared to F_3 were also reported in wheat by Srivastava *et al.* (1989) and Kadlera (1997) in safflower. Among the characters GCV and PCV were high for number of branches per plant (20.93 and 30.34, respectively) and fruit yield per plant (23.62 and 28.08, respectively) in BIP. This suggests that, there is more scope for selecting better segregants in BIP population on the basis of number of branches per plant and fruit yield per plant.

In the case of BIP, heritability was higher in respect of yield and its component characters than in F_3s. This suggested that the variation due to environment played a relatively limited role in influencing the inheritance of these characters and thus the expected response to selection is higher in BIP. High heritability in the case of BIP over F_3 has also been reported by Yunus and Paroda (1983) in wheat and Kadlera (1997) in safflower. Like for heritability, BIP also showed relatively high expected genetic advance (as percentage of mean) estimates for all characters as compared to selfed progenies. Among the characters, fruit yield per plant and number of fruits per plant showed higher genetic advance. This suggested that, the gain from selection based on these two traits would be higher in BIP than in their corresponding selfed progenies.

The comparison of biparnetal mating and selfing shows that whatever additional variability realized with biparental mating in the early segregating generations has been the consequence of release of concealed variability in the segregating generation which is probably brought about by rare recombination between the tightly linked genes. In addition to this, it is also expected to help in maintaining a greater variability for selection to be effective for a longer period. Okra is an often cross pollinated crop where lack of variability has been implicated as one of the important causes for lack of desired progress in breeding. Hence, the present report on the use of biparental mating in an early segregating generation like F_2 of an appropriate cross, could be of much use in widening variability and consequently in making considerable gain in improving productivity.

Table 1. Mean and range of expression in respect of 11 quantitative traits in intermating (BIP) and selfed (F_3) populations of okra

Characters	Mean		Range	
	BIPs	F_3	BIPs	F_3
Days to first flowering	45.28	45.70	39.50-51.00	40.0-51.0
Fruit length (cm)	13.70	13.62	10.87-17.50	11.20-16.0
Fruit diameter (cm)	1.46	1.47	0.97-2.00	1.12-1.76
Average fruit weight (g)	17.27	18.00	12.50-21.80	14.25-21.75

Number of fruits per plant	24.94	24.74	12.75-38.25	14.0-42.75
Plant height (cm)	89.67	92.63	64.0-115.0	68.75-124.20
Internodal length (cm)	6.43	6.90	3.70-9.33	4.60-9.0
Number of branches per plant	2.32	2.56	1.0-3.68	1.5-3.52
100 seed weight (g)	5.77	5.88	4.53-7.10	4.57-3.52
Stem diameter (cm)	0.86	0.88	0.65-1.12	0.65-1.19
Fruit yield per plant (g)	428.70	448.50	181.20- 712.80	212.25-759.75

Table 2. Estimates of genetic variability parameters in respect of 11 quantitative traits in F_3 and BIP populations of okra

Sl. No.	Characters	Populations	GCV (%)	PCV (%)	h^2bs	GA	GAM (%)
1	Days to first flowering	BIP	4.91	5.52	79.44	3.10	6.87
		F_3	4.84	8.31	13.88	0.78	1.72
2	Fruit length (cm)	BIP	6.97	8.81	61.65	1.56	11.44
		F_3	4.49	7.16	39.38	0.79	5.80
3	Fruit diameter (cm)	BIP	12.42	16.51	56.38	0.28	19.05
		F_3	5.80	8.63	45.05	0.12	8.13
4	Average fruit weight (g)	BIP	9.28	11.79	62.17	2.58	14.89
		F_3	5.35	8.95	36.03	1.21	6.72
5	Number of fruits per plant	BIP	19.07	23.17	67.63	8.15	32.39
		F_3	14.19	21.63	45.88	4.80	19.46
6	Plant height (cm)	BIP	10.20	11.38	79.97	16.85	17.32
		F_3	9.05	12.35	54.23	12.71	13.70
7	Internodal length (cm)	BIP	15.65	19.74	61.19	1.62	25.70
		F_3	12.70	15.99	58.84	1.38	21.05
8	Number of branches per plant	BIP	20.93	30.34	47.89	0.71	30.13
		F_3	11.94	15.32	59.38	0.48	18.88
9	100 seed weight (g)	BIP	7.32	8.32	76.38	0.77	13.17
		F_3	11.62	12.98	77.10	1.22	22.31
10	Stem diameter (cm)	BIP	11.22	12.43	81.20	0.18	20.44
		F_3	8.23	11.42	52.28	0.11	12.15
11	Fruit yield per plant (g)	BIP	23.62	28.08	70.69	176.35	40.88
		F_3	18.96	25.81	54.70	128.93	28.82

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Histological changes in the cerebral neurosecretory cells during larval-pupal transformation of *Spodoptera mauritia Boisd.* (Lepidoptera: Noctuidae).

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Abstract: *The structure of neuroendocrine organs of sixth instar larvae of Spodoptera mauritia Boisd. (Lepidoptera:Noctuidae) consists of the neurosecretory cells of the brain as well as the retrocerebral organs like the corpora cardiaca and corpora allata. Neurosecretory cells are intact cells in the brain. Neurosecretory cells have been identified by their characteristic staining properties with histochemical stain (Paraldehyde-Fuschin). Three types of neuroscretory cells are present in Spodoptera mauritia which includes medial, lateral and posterior group. Different sub types of medial and lateral neurosecretory cells are also present in Spodoptera mauritia . These different sub types of cells show difference in cell volume in different days of sixth instar larva. The present study shows that in Spodoptera mauritia the median neuroscretory cells of the brain show an increase in volume and cellular contents on day two and day four of sixth instar larval stadium. The increase in the synthetic activity of neurosecretory cells on day two and day four of last larval stadium of Spodoptera mauritia possibly represent the biphasic release of neurohormones needed for larval –pupal metamorphosis.*

Key words: larval-pupal transformation, *Spodoptera mauritia*

INTRODUCTION

Endocrine system plays an influential role in the sequential events taking place during insect metamorphosis. The major hormones controlling insect development and metamorphosis are the pro thoracicotropic hormone secreted by the neurosecretory cells of the brain, ,juvenile hormone secreted by the corpora allata and ecdysone secreted by prothoracic glands. The neurosecretory cells of the brain in Lepidoptera have been described by several authors (Mc Leod and Beck, 1963; Singh and Arif, 1978). The main objective of the present investigation was to study the structure of neuroendocrine organs of sixth instar larvae of *Spodoptera mauritia* and the changes undergone by the neurosecretory cells in different days of development of the sixth instar larvae.

MATERIALS AND METHOD

The studies were conducted on rice army worm *Spodoptera mauritia* which is a widely distributed sporadic pest of paddy in South India. The adult moths were collected at night using fluorescent lamps and kept in glass chimneys, closed at both ends by muslin cloth. The first instar larvae hatched from the eggs after three days. Larvae were fed daily with fresh tender leaves of *Ishaemum aristatum* which was collected from paddy fields. The first instar larvae molted into sixth instar larvae within 2-3 weeks. The sixth instar larvae transformed into a wandering stage during which they did not feed. These wandering larvae became prepupae on the fifth day and pupated after 24 hrs. The sixth instar larvae of *Spodoptera mauritia* were chosen for the present study. The larvae used for the experiments were taken from the laboratory stock culture. The age of the larvae was designated as day n where day 0 indicates the day of ecdysis to this stage. Newly ecdysed larvae were considered as day 0, the larvae 24hrs old as day 1 and so on. Larvae were anaesthetized in specimen tubes containing diethyl ether and they were dissected in cold insect saline using sterilized instruments. The brain was separated together with the suboesophageal and prothoracic ganglia under a stereozoom dissection microscope. The dissected out tissue was fixed in Bouin's fluid for 24-48 hrs. The fixative was washed out in running water and brought to 70%ETOH.Then the tissue was hydrated and oxidized for thirty minutes by using $KMnO_4H_20$. The oxidant was removed with 4% aqueus sodium bisulphate. Then the tissue was brought to 70% ETOH and stained with Paraldehyde-Fuschin for 24 hrs. The stained tissue was differentiated in tap water, dehydrated through different grades of ETOH, passed through 100% ETOH-Acetone mixture, Acetone cleared in Methyl benzoate-acetone mixture and Methyl benzoate for 20 minutes and the mounted in DPX.

RESULTS AND DISCUSSION

In sixth instar larvae of *Spodoptera mauritia*, the major neuroendocrine organs consist of the neurosecretory cells of the brain as well as the retrocerebral neuroendocrine organs like the corpora cardiaca and corpora allata. The corpora cardiaca of the larvae are paired, slender fusiform and oblong organs lying on each side ventral to the brain and lateral to the oesophagus. The corpora allata are discrete spherical bodies lying ventrolateral to the oesophagus.

Neurosecretory cells can often be seen as intact cells in brain. Neurosecretory cells have been identified by their characteristic staining properties with specialized histochemical stain *ie*.,Paraldehyde Fuschin. This dye stains a different group of neurosecretory cells. In the present study it is found that the neurosecretory cells are located in three groups *ie*., medial group in the pars intercerebralis, lateral group in the pars lateralis and the posterior group in the posterior region of the brain. In the stained whole mount preparation of the brain, all the three groups of neurosecretory cells could be classified into subtypes like A (medial) cells L(lateral) cells and P (posterior) cells.

The neurosecretory cells could be further classified based on the topographical features, volume of cytoplasm and nucleus as well as on the amount and stainability of the neurosecretory material. In the larvae each hemisphere of the brain contained ten stainable materials in the medial group. Of these two were distinguished as A1 cells, two as A2 cells, two as A3 cells and four as A4 cells. In the newly molted sixth instar larvae, A1 cells were darkly stained neurons and were the largest volume of 1.322*10-6 mm^3. A2 cells were small irregularly shaped and moderately stained with a volume of 0.6721*10-6 mm^3.A3 cells were smaller and darkly stained neurons with a volume of 0.2938*10-6 mm^3. A4 cells showed least sensitivity and had a volume of 0.918*10-6 mm^3.

The lateral group contained five cells of L1 type which were smaller in size and nuclear volume. The other five cells were of L2 type which were larger in size and cell volume. There were 4 posterior groups of P cells with two on each side of the medial tissue and they are moderately stained.

In the present study, medial, lateral and posterior groups of neurosecretory cells differed in cell and nuclear volumes as well as in the amount of stainable material in the perikaryon. In the day 0 larvae A1, A2, A3 and A4 cells were found to have smallest nuclear and cell volumes and also the amount of stainable material.

The amount of neurosecretory material in the perikaryon was sparse in day 0 larvae as compared to the other days of the instar. Day 1 and day 2 larvae showed moderate amount of neurosecretory material. A considerable increase of cell volume and nuclear volume were observed in day 3 larvae. The maximum accumulation of neurosecretory material was observed in day 4 larvae. A decrease in cell and nuclear volume was observed in day 5 (prepupa). The neurosecretory cells of day 2 larvae showed an initial increase in volume and an exponential increase in the volume of neurosecretory cells was observed in the brain of day 4 larvae.

During larval-pupal development of all the Lepidoptera examined, ecdysteroid release occurs twice. The first small peak of ecdysteroids is involved in the change of developmental commitment of epidermal cells and in the induction of gut purge and wandering behaviour. A second major peak of ecdysteroids preceeds pupal development (Bollenbacher *et al*.,1975) since PTTH released by the neurosecretory cells of the brain stimulate the release of ecdysteroids, a biphasic secretion of PTTH has also been reported during larval pupal development. Earlier studies have demonstrated that increase in cell volume and stainability of neurosecretory material in the cytoplasm represent higher synthetic activity of neurosecretory cells (Granger and Bollenbacher, 1981; Santha and Nair, 1991).The increase in the synthetic activity of neuroseccretory cells on day 2 and day 4 of last larval stadium of *Spodoptera mauritia* possibly represent the biphasic release of neurohormones needed for larval –pupal metamorphosis.

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Variability of seed related characters in teak (*Tectona grandis* L.f.) from Western Ghat region

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Abstract: *The present study analyses the variability of nineteen seed related characters, with respect to morphological, physiological and biochemical aspects, in ten teak (Tectona grandis L.f.) populations from Kerala, Karnataka and Tamil Nadu part of Western Ghats. The data were subjected to multivariate analysis. Correlations were estimated between all the traits and also between the latitude, longitude, altitude and mean annual rain fall at the areas of the seed sources. Significant variability is found to exist in all the studied characters except mesocarp thickness and length- breadth ratio of shell diameter. Positive correlations were found between germination percentage and longitude where as germination is negatively correlated with latitude and endocarp ash. Rainfall has positive correlations with mesocarp lignin content. Altitude is positively correlated with fruit filling percentage. A multiple regression equation was fitted to predict germination using seed viability (through tetrazolium test), endocarp ash and mesocarp lignin (R^2 = 0.56). Clustering was done to look in to the over all similarity between the ten populations and the results show three different groups where seed source from Doddaharve (Mysore) stands separately.*

INTRODUCTION

Teak (*Tectona grandis* L.f), a member of the family Verbenaceae, is one of the leading tropical timbers in the world and is well known for its aesthetic beauty, strength and durability. Teak is naturally found in the peninsular India below 24 degree latitude. India is considered as a centre of genetic diversity of teak. Information on the extent, nature and distribution of genetic diversity in a species is important for effective breeding and conservation strategies. Naturally distributed in different climatic and edaphic zones, teak has developed different ecotypes during the processes of evolution. Wide variation in the performance of different ecotypes has been previously recognized (Keogh, 1982; White, 1993). Teak exhibits a great variability between provenances and land races in various quantitative and qualitative traits (Keiding *et al*., 1986). Variation in physical characteristics of the drupes and germination was reported in seven provenances from the Kerala state of India (Jayasankar *et al*., 1999). Germination of teak fruits was observed to be highly variable from source to source (Indira and Basha , 1999; Sivakumar *et al*., 2002).

Kertadikara and Prat (1995) opined that studies on the variability in seed characters and germination behaviour of a species will help in identifying the seed lots for a planting program. Molecular marker studies have shown that Western Ghat region forms a separate genecological zone and teak from this region is more diverse when compared to central Indian region (Nicodemus *et al*., 2005). The present study investigates the variability in various morphological, biochemical and physiological traits of teak drupes from the western Ghat region. When the seeds were put in the nursery, many of the provenances did not give enough germination. Hence, it was decided to develop a model so as to predict the germination of a particular seed source by analyzing any of the biochemical/physiological/ morphological variations.

MATERIALS AND METHOD

Ten different provenances (seed sources) covering Karnataka, Kerala and Tamil Nadu were selected (Fig.1) and the longitude and altitude of the area were measured using a GPS (Table 1). The annual rainfall data was compiled from the website http://www.worldclim.org as per Hijmans *et al*. (2005). From each locality, seeds were collected from 10 randomly selected trees and the seeds were bulked. Nineteen characters related to the seeds were noted down (Table 2). From each lot of bulked seeds three replications with twenty seeds each were taken and physical measurements were taken for shell diameter (vertical and transverse here after length and breadth respectively), drupe diameter (vertical and transverse here after length and breadth respectively), fruit weight, shell weight, mesocarp weight, mesocarp thickness, maximum possible germination and drupe filling percentage. Measurements of shell were taken after removing the mesocarp from the seed. The difference between the drupe and shell measurement gave the values for mesocarp measurement. For 25 seed weight, drupes were broken and undamaged seeds were collected. Twenty five seeds with three replications were weighed using highly precise electronic balance. 100 drupe weight was taken using seed lots containing hundred seeds with three replications.

Seed viability

Twenty seeds with three replications were soaked in 1% solution of 2,3,5 triphenyl tetrazolium chloride for 4 hours in the dark. Completely stained seeds were counted as viable and expressed in percentage.

Maximum possible germination

The drupes having at least one locule filled with visually healthy seed are considered as germinable and the percentage of these drupes was given as MPG percentage.

Germination test

Three replications of hundred seeds were taken and the seeds were pretreated with water i.e., alternate wetting (1 day) and drying (1 day) for 4 cycles. They were sown in trays filled with sand. Multiple seedlings from one fruit were counted as one. Germination was expressed in percentage after one year long observation.

Ash Content

Ash content of the mesocarp and endocarp were gravimetrically estimated. One gram of finely powdered mesocarp and endocarp were treated at 600^{o}C in a muffle furnace for four hours. The remaining ash was weighed and expressed as percentage of ash content.

Lignin

The lignin content of wood and pulp is generally determined as Klason lignin in accordance with the standard method TAPPI T 222 om-88 (Schwanninger and Hinterstoisser, 2002) and the same method is used here for mesocarp and endocarp.

Statistical analysis

The data were subjected to analysis of variance and correlation was estimated between all the traits and the geo-climatic data to know their relationship. Multiple linear regression equations were worked out to predict the germination from other variables. Clustering was carried out to understand the overall similarity between the provenances. All the analyses were carried out using SPSS 16.0.

RESULTS AND DISCUSSION

The seeds from different provenances were phenotypically different in colour, shape, size etc. (Fig. 2). The Mean performance of different variables is given in Table 2. Provenances are significantly different for all characters except for length- breadth ratio of the shell diameter and mesocarp thickness. Seed characters like maximum possible germination, drupe length and breadth, shell weight, drupe filling percentage and hundred drupe weight were highest in the seeds from Doddaharve and lowest in Mandagadde. Twenty five seed weight and mesocarp weight were highest for seeds from Parambikulam and lowest in Hudsa and Mandagadde. Drupes and shells from Wayanad showed maximum roundness where as seeds from Asahmbu showed minimum roundness as interpreted from the drupe and shell length-breadth ratio. Shell length and breadth was highest for Wayanad and Ashambu respectively where as it was lowest for Mandagadde. Wayanad seeds were found to have a bigger average shell length when compared to breadth. Endocarp ash was highest in Nilambur and was lowest in Konni where as mesocarp ash was highest in Hudsa and lowest in Arienkavu. Lignin content of the endocarp was highest in Mandagadde and lowest in Ashambu where as mesocarp lignin was highest in Vazhani and lowest in Mandagadde. The ashambu provenance showed maximum seed viability and the seeds from Parambikulam, Hudsa and Arienkavu showed the lowest seed viability. Germination was highest for seeds collected from Konni where as it was lowest for seeds from Hudsa (Table 3).

Table 1. Details of the selected provenances

No	Provenance	Longitude (°)	Latitude (°)	Altitude(m)	Rainfall (mm)
1	Hudsa	74° 37’	15° 15’	480	1860
2	Mandagadde	75° 29’	13° 46’	592	1536
3	Doddaharve	75° 57’	12° 24’	877	1258
4	Wynad	76° 05’	11° 53’	752	2137

5	Nilambur	76° 21’	11° 20’	68	2353
6	Vazhani	76° 13’	10° 46’	58	2730
7	Parambikulam	76° 48’	10° 26’	560	1890
8	Konni	76° 57’	9° 10’	220	2229
9	Arienkavu	77° 07’	8° 59’	381	1694
10	Ashambu	77° 18’	8° 22’	200	1226

Fig.1. Map showing the sites of seed collection

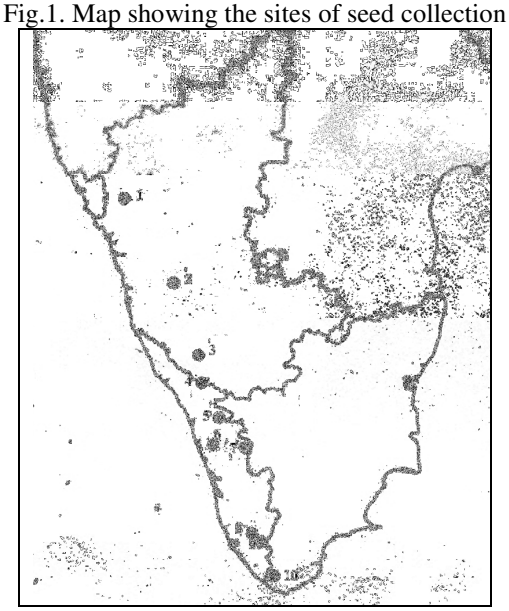

Table 2. Mean performance of different variables

No.	Parameters	Mean	SD
1	25 seed weight(g)	0.45**	0.05
2	Endocarp ash(%)	1.97**	0.24
3	Endocarp lignin(%)	34.54**	1.65
4	Mesocarp lignin(%)	42.05**	2.53
5	Mesocarp ash(%)	4.45**	1.05
6	MPG(%)	37.5**	16.89
7	Shell diameter (L)(mm)	0.93**	0.07
8	Shell diameter (B) (mm)	0.98**	0.07
9	Shell diameter (L/B)	0.95^{ns}	0.05
10	Drupe diameter(L)(mm)	1.23**	0.10
11	Drupe diameter (B)(mm)	1.36**	0.12
12	Drupe diameter (L/B)	0.91**	0.04
13	Shell weight(gm)	0.38**	0.08
14	Mesocarp weight(g)	0.18**	0.06
15	Mesocarp thickness(mm)	0.17^{ns}	0.03
16	Drupe filling %	12.17**	7.53
17	100 drupe weight(g)	53.01**	13.16
18	Seed viability (%)	25.5*	9.06
19	Germination (%)	12.67**	5.73

*Note: **, *, significant at 0.01level, 0.05 level respectively; ns -non-significant*

Table 3. Provenance mean for each of the fruit characters

Provenance	Parambi kulam	Vazha ni	Ashamb	Hudsa	Konni	Waya nad	Arien kav	Madda gadde	Dodda harve	Nilamb ur
25 seed weight (g)	0.53	0.40	0.45	0.36	0.48	0.44	0.45	0.48	0.50	0.42

Endocarp ash (%)	1.59	2.09	1.97	2.08	1.52	2.02	2.04	2.21	1.95	2.22
Endocarp lignin(%)	33.04	34.63	32.11	34.29	35.69	34.61	34.88	37.02	32.47	36.67
Mesocarp lignin(%)	45.30	45.88	39.71	43.05	42.46	43.11	42.35	37.58	40.30	40.80
Mesocarp ash(%)	5.55	4.58	4.75	5.64	3.49	4.59	2.51	3.24	5.47	4.67
MPG(%)	45.00	20.00	43.33	23.33	36.67	58.33	30.00	18.33	70.00	30.00
Shell diam (L) (mm)	0.97	0.88	0.92	0.87	0.86	1.05	0.88	0.83	1.03	0.96
Shell diam (B) (mm)	1.04	0.89	1.07	0.95	0.97	1.011	0.93	0.87	1.07	1.00
Shell diam (L/B)	0.93	0.99	0.87	0.92	0.89	1.04	0.95	0.96	0.97	0.96
Drupe diam (L) (mm)	1.37	1.15	1.19	1.18	1.17	1.36	1.15	1.10	1.38	1.23
Drupe diam (B) (mm)	1.51	1.24	1.42	1.33	1.33	1.39	1.32	1.16	1.57	1.33
Drupe diam (L/B)	0.91	0.93	0.84	0.89	0.88	0.98	0.87	0.95	0.88	0.93
Shell weight (g)	0.41	0.29	0.46	0.39	0.36	0.42	0.31	0.24	0.53	0.40
Mesocarp weight(g)	0.29	0.13	0.21	0.15	0.16	0.22	0.13	0.15	0.26	0.14
Meso.thickness (mm)	0.22	0.16	0.16	0.17	0.16	0.17	0.17	0.14	0.21	0.15
Drupe filling %	14.17	5.42	13.75	6.25	9.58	19.17	9.17	5.42	29.58	9.17
100 drupe weight (g)	65.75	39.72	64.75	49.94	48.96	55.19	42.92	33.84	77.10	51.96
Seed viability(%)	16.67	35.00	40.00	16.67	38.33	23.33	16.67	20.00	23.33	25.00
Germination (%)	20.00	18.67	14.00	1.00	22.00	14.00	10.00	7.00	9.00	11.00

Correlations were estimated between all the traits and the geo-climatic data. Positive correlations were found between germination percentage and longitude where as germination was negatively correlated with latitude and endocarp ash. A higher endocarp ash may be due to the presence of higher inorganic components like Calcium which help in increasing cell wall rigidity and thickness (Schroeder,1982) and it may affect germination adversely. Altitude is positively correlated with fruit filling percentage. Rainfall has positive correlations with mesocarp lignin content. The lignin is found to help in withstanding the deterioration during weathering and it increases water impermeability in many seeds and thus an increase in mesocarp lignin might be an adaptation which is helpful to evade the chances of attack of seed pathogens which are active during continuous rains. The role of lignin in disease resistance in plants is well established (Hijwegen, 1963; Milosevic and Slusarenko,1996). Previous works in this species are of various opinions on the effect of seed size on germination. Kumar (1979) and Syam (1988) support the use of bigger seeds for better germination where as Indira *et al*., (2000) and Sivakumar *et al*., (2002) are of the opinion that seed size does not have significant effect on germination. Jayasankar *et al.* (1999) have found that teak seed size has got negative relation with germination. However, the present study did not show any significant correlation between seed size and germination and it is in agreement with the views of Indira *et al.* (2000) and Sivakumar *et al.* (2002).

Fig. 2. Seeds from different seed sources

To find out the factors which affect the germination of seeds, multiple linear regression equation was fitted considering germination percentage as dependent variable and all other variables as independent variables. Results show that the three variables which affect the germination are endocarp ash, seed viability and mesocarp lignin.

$y = -7.730 - 11.201x_1 + 0.219\ x_2 + 0.883\ x_3$ (Adj.$R^2 = 0.56$),
where y= germination (%) , x_1 = endocarp ash (%), x_2 = see viability (%) and x_3 = mesocarp lignin(%)

Jayasankar *et al.* (1999) have obtained a multiple regression equation to predict germination from seed parameters were the R^2 values is 0.366 only but Sivakumar *et al*. (2002) have obtained a non linear polynomial regression model with the R^2 value 0.599. Clustering was done by computing Euclidean distances between every pair of the seed source to see over all similarity in the seeds. Three clusters were obtained (Table 4). The seeds from Doddaharve were found to stand separate.

From the above study it is concluded that the teak seed related characteristics exhibit large variability. Seed characters like maximum possible germination, drupe length and breadth, shell weight, drupe filling percentage, 100 drupe weight were highest in the seeds from Doddaharve whereas Mandagadde was found to be inferior in many of the studied characters. Seeds from Doddaharve are entirely different from other teak provenances of Western Ghats included in the present study. Konni provenance showed the highest germination. The multiple regression equation developed is useful to predict germination using the seed characters such as endocarp ash, mesocarp lignin and seed viability determined through tetrazolium test.

Table 4. Different clusters

Cluster	Provenances
1 (5 provenances)	AKV, NBR, HUD, MDG, VZNI
2 (4 provenances)	ASB,KNI, PRM, WYN
3 (only 1 provenance)	DRV

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Studies on general combining ability, specific combining ability, heterosis and their relationship in FCV tobacco (*Nicotiana tabacum* L.)

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Abstract: *An investigation was carried out to evaluate seven lines and four testers to asses combining ability using Line × Tester mating design. The 28 hybrids along with 11 parents were grown in a randomized block design with three replications at Zonal Agricultural Research Station, Navile, Shimoga, during Kharif 2007. Overall status of a parent or cross with respect to gca and sca/heterosis respectively was determined following a method suggested by Arunachalam and Bandopadyaya (1979) and modified by Mohan Rao et al. (2004). Combining ability analysis and the heterosis, mid parent heterosis and standard heterosis (taking Kanchan as standard parent) an attempt was made to deduce a systematic relationship if any between gca, sca and heterosis. It was interesting to note that, given a cross with high overall sca status, the probability of it to be a H × L and H × H combination was higher than the probability of finding it to be a L × L. Similarly for heterosis, given a cross with high overall heterotic status, the probability of it to be an H × L and H × H combination was higher than the probability of finding it to be an L × L. Thus the present study clearly established the superiority of H × L and H × H category of crosses. Whenever a female parent with high gca status was involved, it produced crosses with high overall sca/heterotic status over a number of characters. This suggested the ability of these female parents in transmitting additive alleles with increasing effects to their progeny, again suggesting the emphasis to be laid on the gca effects for the selection of parents for hybridization. It is worthwhile to start with H × L and H × H for realizing hybrids with high heterosis and sca effects.*

Key words: General combining ability, specific combining ability, tobacco, *Nicotiana tabaccum*

INTRODUCTION

Tobacco is one among the commercial crops of national importance after sugar cane and cotton. It has been playing a prominent role in the development of nation's economy. More than 98% of the area under FCV tobacco is in Andhra Pradesh and Karnataka. The quality of tobacco produced in Karnataka light soils (KLS) is on par with the best in the world and is in great demand for export purpose. Even though FCV tobacco is growing in Karnataka yield levels are lower than the national average. To achieve this, heterosis breeding is one of the tools which can be effectively used to improve yield. In this context, it is worth mentioning the role of combining ability concept, which reiterates the importance of choosing suitable parents to nick well in the expression of heterosis. Thus the evaluation of genotypes for their nicking ability is a prerequisite for the final selection of parents in hybridization programme. This is because the *per se* performance of a parent is not always a true indicator of its potential in hybrid combination. Further, predicting the magnitude and frequency of heterotic hybrids assumes greater importance. Under these premise the present investigation was undertaken.

MATERIAL AND METHOD

The experimental material consisted of seven females and four males crossed in Line × Tester design to generate 28 hybrids. The experiment was laid out in a Randomized Block Design with three replications at the Zonal Agricultural Research Station, Navile, Shimoga, during *kharif* 2007. The observations were recorded on sixteen morphometric traits in five plants selected randomly for each genotype and the average value was computed. Heterosis, mid parent heterosis and standard heterosis (taking Kanchan as standard parent) were estimated as per standard formulae in vogue and the mid parent heterosis values were used to calculate the overall status of a parent or cross with respect to gca and sca/heterosis. Combining ability analysis was done according to Kempthrone (1957).

Overall gca status of parents, sca and heterotic status of crosses

Since yield and its component characters are correlated either positively or negatively, it is usual to find, for particular parent and cross gca and sca/heterosis, respectively in the desirable direction for some character and undesirable direction for the others. The problem of ascertaining the status of a parent with

respect to gca and sca/heterosis for crosses over a number of characters assumes importance under present context. Therefore, overall status of a parent or cross with respect to gca and sca/heterosis respectively was determined following a method suggested by Arunachalam and Bandopadyaya (1979) and modified by Mohan Rao *et al*. (2004).

General combining ability effects of parents, specific combining ability effects and estimates of mid parent heterosis (MPH) of crosses were ranked by giving highest rank for the parent or a cross which manifested highest gca effects and sca effects or heterosis, respectively. The lowest rank was given for parents or the cross with lowest gca effects and sca effects or heterosis for a character, respectively. This was repeated for all characters except for days to 50 percent, midrib to lamina ratio and nicotine per cent, ranking was given in a reverse order because parents or hybrids which are low value for these characters were required. The ranks obtained by parents/ crosses were summed up across all characters to arrive at total score for each of the parent or a cross. Further, the mean of the total scores thus obtained was used as the final norm to ascertain the status of a parent or a cross for their gca and sca/heterosis. The parents and crosses whose total rank exceeded the final norm were given as high (H) overall gca and sca/heterotic status, respectively. On the other hand, the parents or crosses which secured a total rank less than the final norm were given as low (L) overall gca and sca/heterotic status, respectively.

RESULTS AND DISCUSSION

Analysis of variance (Table 1) revealed that mean squares due to parents, crosses and parents vs crosses (modified line × tester) were significant indicating the presence of substantial variation among the parents and also among hybrids thereby justifying that appropriate material has been involved in the research. Four out of seven lines viz,. KST-27, LV-2, KST-29 and SBS-1 and two of testers FCH-197 and KANCHAN had high (H) overall general combining ability status (Table 2), while the remaining parents had low (L) overall general combining ability status implying that 50 percent of the lines and testers were high over all general combiners suggesting their ability to transmit additive genes in desirable direction for the traits under study. The overall sca status of each hybrid was determined. From the results (Table 3), it is evident that 13 out of 28 hybrids had high (H) overall sca and the remaining had low (L) overall sca status across the traits. It was found that 50 percent, *i.e*., 14 out of 28 crosses (Table 4) were high (H) overall heterotic and the remaining had low (L) overall heterotic status across the traits. Based on overall gca effects, the crosses were classified into HH (both the parents in a cross with high overall gca status), HL (one parent with high and the other parent with low overall gca status) and LL (both the parents in a cross with low overall gca status).

Relationship between overall gca, sca and heterosis

Encouraged by a definite relationship between gca effects of parents with sca and heterosis of their crosses, an attempt was made to deduce systematic relationship if any between gca, sca and heterosis. The method suggested by Arunachalam and Bandopadyaya (1979) and modified by Mohan Rao *et al*. (2004) was used in the present investigation and employed to arrive at such a relationship.

A perusal of Table 5 indicated that the number of hybrids with high (H) overall specific combining ability status were more in H × H and H × L (both 5) than L × L (3) category of crosses. Conditional probability that a cross with high overall sca status is found in HH, HL or LL category was worked out. From the results, it was interesting to note that, given a cross with high overall sca status, the probability of it to be a H × L (0.38) and H × H (0.38) combination was higher than the probability of finding it to be a L × L (0.23). Similarly for heterosis, given a cross with high overall heterotic status, the probability of it to be a H × L (0.47) and H × H (0.47) combination was higher than the probability of finding it to be a L × L (0.07). Thus the present study clearly established the superiority of H × L and H × H category of crosses. This type of observation was also brought out by the studies of similar nature in *Brassica campestris* (Bandyopadhyay and Arunachalam, 1980), sesame (Ramesh *et. al.,* 2000) and in sunflower (Mohan Rao *et. al.,* 2004). It is worth mentioning here that, whenever a female parent with high gca status was involved, it produced crosses with high overall heterotic status over a number of characters. This suggested the ability of these female parents in transmitting additive alleles with increasing effects to their progeny, again suggesting the emphasis to be laid on the gca effects for the selection of parents for hybridization. Thus, the established superiority of H × L and H × H crosses, in the present study in producing crosses with high sca and heterotic status is of practical utility to a breeder, when he has to

attempt successful hybridization economically in terms of time, cost and the number of crosses. It is worthwhile to start with H × L and H × H for realizing hybrids with high heterosis and sca effects.

Table 1: Analysis of variance for yield, quality and other quantitative traits in FCV tobacco

Source of variance	df	Plant height (cm)	Leaves per plant	Inter nodal length (cm)	Leaf Length (cm)	Leaf breadth (cm)	Leaf area (cm^2)	Days to 50% flowering	Green leaf yield (t ha^{-1})
Replications	2	26	7.14	0.03	99.07	11.72	28192	24.0	5.34
Genotypes	38	1591**	27.16**	1.64**	99.79**	10.07**	48130**	314.7**	12.21**
Parents	10	1945**	26.75**	1.09**	133.1**	9.06**	55580**	237.5**	8.63**
Crosses	27	1500**	28.29**	1.87**	83.54**	10.21**	43155**	339.8**	13.19**
Parents vs. Crosses	1	511**	0.91	0.73**	206.2**	16.24*	107946**	412.2**	21.42**
Lines	6	2764*	38.70	2.02	59.48**	17.95**	48408	619.6**	21.91
Testers	3	1658**	45.64	3.68	176.6**	9.09*	77820**	219.8**	14.22
Line × Testers	18	1052**	21.93**	1.52**	76.05**	7.82**	35627**	266.5**	10.11**
Error	76	19	0.89	0.02	2.41	2.69	3125	2.7	0.02

Source of variance	df	Cured leaf yield (t ha^{-1})	Top grade equivalent (t ha^{-1})	Midrib to lamina Ratio		Nicotine (%)		Reducing sugar (%)	
				X leaves	L leaves	X leaves	L leaves	X leaves	L leaves
Replications	2	0.01	3417	0.006	0.009	0.64	0.01	8.60	7.38
Genotypes	38	0.23**	34579**	0.031**	0.034**	1.36**	0.39**	8.74**	10.39**
Parents	10	0.17	30382**	0.037**	0.014**	0.03	0.07	9.19	6.36**
crosses	27	0.24*	34529**	0.030**	0.042**	1.35**	0.45**	8.87**	11.61**
Parents vs. Crosses	1	0.42**	77885**	0.011*	0.012*	14.89**	1.80**	0.59	17.85**
Lines	6	0.40	54598	0.029**	0.043**	1.50**	0.44**	10.94**	22.96*
Testers	3	0.26	31409	0.023**	0.023**	1.74**	0.69**	6.69**	3.14
Line × Testers	18	0.18**	28360**	0.031**	0.045**	1.24**	0.42**	8.54**	9.23**
Error	76	0.01	16	0.002	0.003	0.02	0.04	0.33	1.02

*significant at P= 0.05 level **significant at P= 0.01 level

Table 2: Overall general combining ability status of parents in FCV tobacco

Lines	Total rank	Overall GCA status
KST-29	77	H
SBS-1	69	H
SMG-02-4-9	55	L
SMG-02-1-6	27	L
SMG-02-1-3	49	L
LV-2	78	H
KST-27	93	H

Final norm: 64

Testers	Total rank	Overall GCA status

FCH-197	58	H
A-1	29	L
A-3	33	L
KANCHAN	40	H

Final norm: 40

Table 3: Overall specific combining ability status of hybrids in FCV tobacco

Testers Lines	FCH-197 (H)	A-1 (L)	A-3 (L)	KANCHAN (H)
KST-29 (H)	H (340)	L (194)	L (125)	H (256)
SBS-1 (H)	L (213)	L (219)	H (255)	H (256)
SMG-02-4-9 (L)	H (334)	L (160)	L (186)	L (224)
SMG-02-1-6 (L)	L (147)	H (343)	H (276)	L (159)
SMG-02-1-3 (L)	L (132)	H (309)	L (169)	L (197)
LV-2 (H)	L (173)	L (167)	H (354)	H (234)
KST-27 (H)	H (367)	L (126)	H (292)	H (287)

Final norm: 231.93

Values in the parenthesis indicate total score secured by the crosses across 16 traits

H: Overall high combiner L: Overall low combiner

(H): overall high general combiner (L): overall low general combiner

Table 4: Overall heterotic status of hybrids in FCV tobacco

Testers Lines	FCH-197 (H)	A-1 (L)	A-3 (L)	KANCHAN (H)
KST-29 (H)	H (336)	L (225)	L (159)	H (243)
SBS-1 (H)	H (270)	H 269	H 306	H 269
SMG-02-4-9 (L)	H (254)	L (122)	L (157)	L (135)
SMG-02-1-6 (L)	L (132)	L (207)	L (189)	L (104)
SMG-02-1-3 (L)	L (164)	H (309)	L (140)	L (150)
LV-2 (H)	H (276)	L (225)	H (364)	H (267)
KST-27 (H)	H (324)	H (232)	H (343)	H (320)

Final norm: 231.82; Values in the parenthesis indicate total score secured by the crosses across 16 traits

Table 5: Distribution of heterotic crosses in relation to overall gca of parents and sca of crosses in FCV tobacco

Parental GCA	Number of crosses under the category	Number of crosses with high overall sca status	Number of crosses with high overall heterotic status	Conditional probability of given cross belonging to high sca status	Conditional probability of given cross belonging to high heterotic status
H × H	8	5	7	0.38	0.47
H × L or L× H	14	5	7	0.38	0.47
L × L	6	3	1	0.23	0.07

H × H: Both the parents are high in their overall general combining ability
H × L: one parent is high and the other low in their overall general combining ability
L × L: Both the parents are low in their overall general combining ability

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Stubble cropping as a method of conservation of native rices

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Abstract: *Production of an additional crop from ratoon plants is being practiced in some rice farming areas of the world to produce a second crop from the stubbles of a harvested rice crop. Tiller production from stubbles can be enhanced by splitting the stubbles and planting them separately. While ratoon cropping can be used to produce an additional crop with lesser investment, planting of separated stubbles can be used as a conservation technique both under normal and critical conditions. Under normal conditions, this technique can be used to enhance the availability of seeds from a crop especially in conservation protocols where the number of plants raised in the crop of each variety is low. Under critical condition of drought, flood or other cases of habitat destruction, this technique can be used to develop more number of plants and seeds from the few materials rescued from the stress or disaster. Regneration of stubbles is a varietal character. Many nativerice varieties of Kerala show good response to stubble cropping. The present study was carried out with five native rice varieties Kunhukunheu, Thondi, Ponmani, Chitteni and Ponnariyan which show comparatively good ratoon performance by stubble cropping. The number of days to flower and duration of the stubble crop showed significant reduction indicating the possibility of early harvest of the stubble crop.*

INTRODUCTION

Ratoonability is an ancestral character of rice inherited from its wild progenitors which helps to perennate even under adverse conditions. Ratoon cropping from stubbles can be used to produce a second crop with lesser investment under normal and critical conditions. Tiller production from stubbles can be enhanced by splitting the stubbles and planting them separately since stubble cropping can be considered as an advanced method in ratoon cropping (Richharia and Pavithran, 1987). This technique can be used to enhance the availability of seeds from a crop especially in conservation protocols and critical conditions of draught, flood and insect and pest damages. Unexpected pests and viruses often devastate entire areas, leaving the farmer with neither food nor income and sometimes with debt. While conservation of native rices is important as they are potential sources of rare and valuable genes, this method can be utilized among small farming communities to get an additional income.

MATERIALS AND METHOD

Rice seeds collected from farmers were grown under experimental conditions in the net house of the Genetics and Plant Breeding Division of Department of Botany, University of Calicut during the first crop season of 2005-2006. Five native rice varieties *Kunhukunhu*, *Thondi*, *Chitteni*, *Ponmani* and *Ponnariyan* were used for stubble cropping. The plants for the mother crop were grown in pots of 20cm diameter filled with paddy soil + sand + enriched compost in 4:1:1 proportion under wet land condition with one plant per pot applying 1g factomfos per plant at monthly intervals starting from the 30th day onwards till flowering. Observations on growth and yield characters were recorded and the crop harvested at maturity. After harvest, the stubbles of the parent crop were separated and planted under same planting conditions to raise a crop from the stubbles. Data on vegetative and yield characters of the stubble crop were recorded and analyzed in comparison with the seed crop (Table 1).

Table 1. Vegetative and yield characters of seed crop and stubble crop in the case of the varieties studied.

Varitey	Character	Seed crop/stubble crop	Mean	SD	CV	Level of Significance
Kunhukunhu	Days to flower	Seed crop	89.67	1.53	1.70	1%
		Stubble crop	41.67	10.21	24.50	
	Tiller number	Seed crop	11	1.73	15.73	NS
		Stubble crop	7.33	2.71	36.97	
	EBT%	Seed crop	85.63	7.56	8.83	NS
		Stubble crop	83.8	14.67	17.51	

	Duration (days)	Seed crop	119.67	10.94	9.14	1%
		Stubble crop	71.67	8.47	12.33	
	Plant height (cm)	Seed crop	65.8	3.45	5.24	NS
		Stubble crop	56	7	12.5	
	Panicle length (cm)	Seed crop	21.67	0.86	3.97	NS
		Stubble crop	14.33	2.95	20.59	
	Panicle density	Seed crop	5.88	0. 76	12.93	NS
		Stubble crop	2.87	0.93	32.40	
	Spikelets per panicle	Seed crop	127.67	18.93	14.83	1%
		Stubble crop	42.33	19.86	46.92	
	Seeds per panicle	Seed crop	110.67	14.57	13.17	1%
		Stubble crop	39	19	48.72	
	Hundred grain weight (g)	Seed crop	1.22	0.14	11.15	1%
		Stubble crop	1.79	0.12	6.70	
	Fertility %	Seed crop	86.84	1.59	1.83	NS
		Stubble crop	91.54	2.85	3.11	
	Yield per plant (g)	Seed crop	15.45	5.91	38.25	NS
		Stubble crop	5.16	4.77	92.64	
Thondi	Days to flower	Seed crop	127.33	5.51	4.33	1%
		Stubble crop	81	10.39	12.83	
	Tiller number	Seed crop	21.67	5.51	25.43	NS
		Stubble crop	5.33	3.21	60.23	
	EBT%	Seed crop	65.9	8.12	12.32	NS
		Stubble crop	81.5	9.03	11.08	
	Duration (days)	Seed crop	119.67	10.94	9.14	1%
		Stubble crop	71.67	8.47	11.82	
	Plant height (cm)	Seed crop	101.67	4.04	3.97	NS
		Stubble crop	102.67	31.57	30.75	
	Panicle length (cm)	Seed crop	25.07	3.09	12.38	NS
		Stubble crop	24.4	4.69	19.22	
	Panicle density	Seed crop	2.65	0.29	11.06	NS
		Stubble crop	5.85	1.38	23.59	
	Spikelets per panicle	Seed crop	65.67	0.58	0.88	NS
		Stubble crop	147	57.19	38.91	
	Seeds per panicle	Seed crop	45.67	0.58	1.27	NS
		Stubble crop	130.33	48.01	36.84	
	Hundred grain weight (g)	Seed crop	1.67	0.09	5.21	1%
		Stubble crop	2.19	0.1	4.57	
	Fertility percentage	Seed crop	63.38	5.06	7.98	1%
		Stubble crop	89.40	3.83	4.28	
	Yield per plant (g)	Seed crop	15.21	1.0	6.58	NS
		Stubble crop	14.50	12.10	83.45	
Ponmani	Days to flower	Seed crop	185.33	7.51	4.05	1%
		Stubble crop	65.33	2.80	4.29	
	Tiller number	Seed crop	39.33	10.02	25.48	NS
		Stubble crop	13	5.2	40	
	EBT%	Seed crop	80.9	8.64	10.68	NS
		Stubble crop	69.3	11.07	15.97	
	Duration (days)	Seed crop	215.33	7.50	3.48	1%
		Stubble crop	95.33	2.08	2.18	
	Plant height (cm)	Seed crop	76.9	9.65	12.55	NS
		Stubble crop	77.33	9.02	11.66	

	Panicle length (cm)	Seed crop	16.25	0.88	5.42	NS
		Stubble crop	17.6	2.33	13.24	
	Panicle density	Seed crop	2.02	0.40	19.80	NS
		Stubble crop	2.62	0.85	32.44	
	Spikelets per panicle	Seed crop	33	7.94	24.06	NS
		Stubble crop	50.33	20.00	39.74	
	Seeds per panicle	Seed crop	28.67	6.03	21.03	NS
		Stubble crop	45.67	21.13	46.27	
	Hundred grain weight (g)	Seed crop	1.67	0.09	52.10	1%
		Stubble crop	2.19	0.10	4.57	
	Fertility percentage	Seed crop	87.78	9.18	10.46	NS
		Stubble crop	89.37	6.83	7.64	
	Yield per plant (g)	Seed crop	15.29	3.12	20.41	NS
		Stubble crop	8.09	2.27	28.06	
Chitteni	Days to flower	Seed crop	196.67	2.89	1.47	1%
		Stubble crop	27.33	10.21	37.36	
	Tiller number	Seed crop	35.33	0.58	1.64	1%
		Stubble crop	21	3.61	17.20	
	EBT%	Seed crop	63.13	5.35	8.48	NS
		Stubble crop	58.87	5.32	9.04	
	Duration (days)	Seed crop	226.67	2.89	1.27	1%
		Stubble crop	57.33	10.21	17.91	
	Plant height (cm)	Seed crop	102.77	5.50	5.35	1%
		Stubble crop	79.17	2.47	3.12	
	Panicle length(cm)	Seed crop	16.93	0.4	2.36	NS
		Stubble crop	16.3	2.0	12.27	
	Panicle density	Seed crop	2.15	0.05	2.33	NS
		Stubble crop	2.17	0.52	23.96	
	Spikelets per panicle	Seed crop	36.33	1.53	4.21	NS
		Stubble crop	39.67	15.7	39.58	
	Seeds per panicle	Seed crop	32.67	3.51	10.74	NS
		Stubble crop	36.67	16.65	45.41	
	Hundred grain weight (g)	Seed crop	1.98	0.17	8.59	NS
		Stubble crop	2.06	1.44	69.90	
	Fertility percentage	Seed crop	85.27	2.12	2.49	NS
		Stubble crop	90.43	7.59	8.39	
	Yield per plant (g)	Seed crop	13.98	2.67	19.1	NS
		Stubble crop	9.33	4.85	51.98	
Ponnariyan	Days to flower	Seed crop	99	2.65	2.68	1%
		Stubble crop	75	3.46	4.61	
	Tiller number	Seed crop	15	3.46	23.07	NS
		Stubble crop	6.67	2.52	37.78	
	EBT%	Seed crop	80.97	9.94	12.28	NS
		Stubble crop	84.27	13.7	16.26	
	Duration (days)	Seed crop	131.33	5.51	4.2	1%
		Stubble crop	105	3.46	3.3	
	Plant height (cm)	Seed crop	109.07	4.76	4.36	NS
		Stubble crop	106	8	7.55	
	Panicle length (cm)	Seed crop	23.27	2.1	9.02	NS
		Stubble crop	21.63	1.3	6.01	
	Panicle density	Seed crop	4.39	0.44	10.02	NS
		Stubble crop	4.31	0.27	6.27	

	Spikelets per panicle	Seed crop	102.33	16.43	16.06	NS
		Stubble crop	92	6.25	6.79	
	Seeds per panicle	Seed crop	93.67	14.36	15.33	NS
		Stubble crop	82.67	6.81	8.24	
	Hundred grain weight (g)	Seed crop	1.93	0.19	9.85	NS
		Stubble crop	2.17	0.31	14.29	
	Fertility percentage	Seed crop	93.6	2.88	3.08	NS
		Stubble crop	88.83	4.32	4.86	
	Yield per plant (g)	Seed crop	21.63	5.25	24.27	NS
		Stubble crop	9.98	4.61	46.19	

RESULTS AND DISCUSSION

Days to flower and duration showed significant reduction in all the varieties in the case of the ratoon crops indicating the possibility of earlier harvest. The earliest flowering variety among stubble plants was *chiteeni* (27 days) and latest was *thondi* (81days). Number of tillers in the stubble crop ranged from 5.33 – 21 with minimum in *thondi* and maximum in *chitteni.* Percentage of ear bearing tillers ranged in the stubble crop from 58.87 to 84.27. Plant height was the minimum in *kunhukunhu* (56 cm) and maximum in *ponnariyan* (106 days). Panicle length was the maximum (24.4 cm) in *thondi* and minimum (14.33cm) in *kunhukunhu*. Only the variety *chitteni* showed significant reduction in plant height and tiller number and the other varieties showed no significant reduction. Spikelets per panicle was minimum (39.67) in *chitteni* and maximum in *thondi* (147). Seeds per panicle was the minimum (36.67) in *chitteni* and maximum in *thondi* (130.33). Spikelets/ panicle and seeds per panicle showed significant reduction in *kuhukunhu* only. Hundred grain weight showed significant reduction in *kunhukunhu*, *thondi* and *ponmani*. Yield per plant ranged from 5.16 g – 14.60 g with a minimum in *kunhukunhu* and maximum in *thondi*, but with no significant reduction in the ratoon crop. Regeneration of stubbles is a varietal character (Mohanan and Pavithran, 1993) and from stubbles seed production can be enhanced as it can be used as a method of seed recovery especially when the conserved germplasm faces threat of habitat destruction, natural calamities and stress resulting in reduction of available quantity of seeds for conservation.

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Effect of the explants from differently aged mother plants on callus induction and direct regeneration in jatropha(*Jatropha curcas* L.)

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Abstract: *Explants from one year and five year old mother plants of Jatropha (Jatropha curcas L.) have been evaluated for their response to callus induction and direct regeneration. Leaf disc, petiole and nodal explants were cultured on a range of concentration of Kinetin, BAP and NAA in combination with BAP for callus induction. Shoot tip was cultured on various concentration of GA_3 in combination with IAA for direct regeneration. Leaf discs from one year old mother plant cultured on BAP showed maximum callus induction frequency (56.15%) as compared to leaf disc from five year old mother plant (37.76%). The nodal explants from one year old mother plant cultured on kinetin is more effective in callus initiation compared to nodal explants from five year old mother plant cultured on BAP. Similarly, the age of mother plant had more effect on days to initiation of callus and callus induction frequency in petiole explants. Callus induction frequency was the maximum in petiole cultured in NAA (1.0 mg/l) + BAP (0.5 mg/l) (51.95%) and it was the minimum in petiole explants from five year old plant (31.56%). Shoot tip from one year old plant cultured on GA_3 (3.0mg/l) + NAA (5.0 mg/l) resulted in maximum shoot length and direct regeneration frequency as compared with shoot tip from five year old mother plant. Results are indicative to juvenility factor of mother plant having an effect on response to callus induction and direct regeneration in Jatropha.*

Key words: Jatropha, Callus, Direct regeneration, IAA, GA_3.

INTRODUCTION

Techniques for the regeneration of *Jatropha curcas* L. from various explants have been developed. Not much work has been reported on protoplast, cell, tissue and organ cultures of *Jatropha spp* compared to other crop species. But the accumulated literature on plant regeneration through tissue culture, especially during this decade demonstrates the potential use of *in vitro* techniques for Jatropha improvement. In recent years, plantlet regenerations from different *J. curcas* explants have been reported (Sujatha and Mukta, 1996; Sardana *et al*., 2000; Rajore and Batra, 2005).

MATERIALS AND METHOD

Different explants (nodes, petioles and leaves) of *Jatropha curcas* L. were collected from the one year and five year old mother plants maintained at K- block, GKVK, University of Agricultural Science, Bengaluru. The leaves at $3^{rd} – 4^{th}$ node from the apex were collected from 5- year and 1 year old plant were first thoroughly washed with tap water, then dipped in 0.5 per cent Bavistin (BASF India Ltd.) solution for 5 min and washed two to three times with sterile distilled water. The leaves were then surface sterilized with 0.1 per cent $HgCl_2$ (w/v) solution for 5–8 min and washed four to five times with sterile distilled water to remove any traces of the $HgCl_2$. Leaves were cut into 1.0 cm^2 segments and placed with the abaxial side in contact with the medium. Nodal explants (3 cm) collected from 5- year and 1 year old plant were first thoroughly washed with tap water for 20 mins, then dipped in 0.5 per cent Bavistin (BASF India Ltd.) solution for 5 min. The explants were surface disinfested with 0.1% mercuric chloride for 12 minutes, followed by rinses in sterile distilled water. The nodal explants were trimmed (1–1.5 cm) at the base and cultured with the cut surface in contact with the medium surface. Similar procedure was followed for petiole.

Shoot tips excised from 1- year and 5- year old plants were thoroughly washed with tap water for 20 mins, then dipped in 0.5% Bavistin (BASF India Ltd.). The explants were surface disinfested with 0.1% mercuric chloride for 2- 3 minutes, followed by washing three times with sterile distilled water. These explants were then introduced vertically into semisolid MS medium supplemented with various concentrations and combinations of growth hormones.

All the experiments were conducted in the laboratory, under well defined conditions of the culture room maintained at 25^{o} ±2^{o}c. Uniform light (Ca.1000lux) was provided by fluorescent tubes (7200K) over a light/dark cycle (18/6 hours). Experiments were set up in Completely Randomized Design with 3 replicates per treatment, which includes 2-3 explants per treatment. Observations on the number of explants forming callus, number of shoot tip responding for direct regeneration were recorded by visual observations.

RESULTS AND DISCUSSION

Use of leaf disc explant

Leaf disc from one year old plant cultured on kinetin and BAP showed more or less similar response towards initiation of callus (Tables1 and 2) but the callus induction frequency varied being with maximum in explants cultured on medium supplement with BAP (56.15 %) and minimum in explants cultured on medium supplement with kinetin (53.63%). On contrary to this Jha *et al.* (2007) recorded maximum frequency in explants cultured on medium supplement with in Kinetin.

Leaf disc from five year old plant cultured on kinetin had taken minimum days for initiation of callus while explants cultured on medium supplement with BAP took maximum days for initiation of callus. However, it showed maximum callus induction frequency (37.76%) compared to kinetin (28.38%). But this result recorded minimum callus induction frequency as compared to the result reported by Deore *et al.* (2008) in explants cultured on medium supplement with BAP. It may be due to the interaction effect of age of plant and treatment. Irrespective of explant and treatment, the explant from one year old plant showed minimum days for callus induction frequency than five year old plant. Similar observations were made by Sujatha and Mukta (1995), when they used leaf disc from third and fourth expanding leaf.

Use of petiole explant

The petiole explant from one year old plant cultured with the combination of NAA (5.5 m/l) + BAP (1.3 m/l) showed minimum days for callus initiation. However the maximum callus induction frequency was observed in petiole explant cultured on NAA (5.5 m/l) + BAP (1.7 m/l) with minimum days for initiation of callus induction (Table 3). Hence this treatment is better for the petiole explant compared to all other treatment combinations. The petiole explant from five year old plant has recorded maximum frequency of callus induction (42.53 %) in combination of NAA (5.5m/l) + BAP (1.7 m/l).

The age of plant had more effect on callus induction frequency as the callus induction frequency was maximum in explant from one year old plant (51.95%) and minimum in explants from five year old plant (31.56%). Comparable result was observed by Verma *et al*. (2008). Discoloration of media was more in callus from explants of five year old plant than callus from one year old plant. It appears that phenolic secretion is more from matured plant than early aged group; however there was no much difference in nature of callus from explants of different age group plant. Callus induction responses observed in this study reflects the degree of age of the plants as has been documented by George and Sherington (1984).

Use of nodal explant

Nodal explant collected from one year old plant cultured on MS medium supplemented with kinetin had taken minimum days for initiation of callus where as nodal explant cultured in medium supplemented with BAP had taken maximum days for initiation of callus. The maximum frequency of callus induction was observed in nodal explant from one year plant cultured in medium supplemented with kinetin (31.96%) whereas nodal explant cultured on medium supplemented with BAP recorded 19.78% (Table 4 and 5). Nodal explant from five year old plant cultured on kinetin took minimum days for initiation of callus (10.33 days) compared to that cultured on BAP (12.11days). Nodal explant from five year old plant cultured on kinetin showed maximum callus induction frequency (23.40 %) compared to explant cultured on BAP (16.91%). This indicates that explant from early age of plant with kinetin is more effective in callus initiation compared to explant from old age of plant with BAP. Datta *et al.* (2007) and Banerjee *et al*. (2007) recorded maximum frequency of callus induction as they used explant from *in vitro* grown plants. Probably this indicates the effect of juvenility of the mother plant on callus induction.

Use of shoot tip

In both the age groups, the explant cultured on medium supplemented with combination of GA_3 (3.0 mg/l) + IAA (5.0mg/l) treatment recorded maximum length of shoot and maximum callus induction

frequency for direct regeneration from shoot tip (Table 6). But when compared between age of the plant, the explant from one year old plant showed the maximum length of the shoot (3.46cm) and maximum direct regeneration frequency (59.15%), where as direct regeneration from shoot tip of five year old plant recorded minimum length of shoot (1.82cm) and minimum direct regeneration frequency (28.59%). Jyoti *et al*. (1998) observed maximum results as they excised shoot tip from *in vitro* grown seedling. Probably, here also the juvenility of the mother plant played an important role in direct regeneration.

Table 1. Effect of kinetin on callus induction from leaf disc explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	13.22	53.63	Soft friable, white
5 Year plant	13.66	28.38	Compact, light brown
SEm±	0.117	0.54	
CD at 5%	0.34	1.59	
Kinetin(mg/l)			
0.5	11.16	17.98	Soft, mushy, white
1.0	12.33	41.01	Soft friable, white
2.5	12.16	25.58	Soft friable, white
3.0	13.66	44.41	Soft friable, light white
4.0	14.50	57.83	Compact, white
4.5	16.33	59.23	Compact, brown
SEm±	O.20	0.94	
CD at 5%	0.59	2.76	
Interaction			
1 year + Kn(mg/l)			
0.5	10.66	25.83	Soft, mushy, white
1.0	12.66	51.83	Soft friable, light white
2.5	12.00	31.96	Soft friable, white
3.0	12.66	58.06	Soft friable, white
4.0	15.33	82.20	Compact, light brown
4.5	16.00	71.90	Compact, white
5 year + Kn(mg/l)			
0.5	11.66	10.13	Soft friable, light white
1.0	13.00	30.20	Soft friable, white
2.5	12.33	19.20	Compact, light brown
3.0	14.66	30.76	Compact, white
4.0	13.66	32.80	Compact, brown
4.5	16.66	47.23	Compact dark brown
CD at 5%	0.84	3.93	
CV %	3.92	5.70	
SEm±	0.28	2.33	
GM	13.44	41.01	

Table 2. Effect of BAP on callus induction from leaf disc explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	13.27	56.15	Soft friable, Light white
5 Year plant	15.27	37.76	Compact, light brown
SEm±	0.14	1.05	
CD at 5%	041	3.07	
BAP(mg/l)			
0.5	13.50	25.90	Soft friable, light white
1.0	13.16	38.00	Soft friable, white

2.5	14.00	46.76	Compact, light brown
3.0	13.83	51.58	Compact, white
4.0	15.50	58.41	Compact, brown
4.5	15.66	61.06	Compact dark brown
SEm±	0.24	1.82	
CD at 5%	0.71	5.32	
Interaction			
1 year + BAP(mg/l)			
0.5	13.66	30.53	Soft, mushy, white
1.0	13.33	42.50	Soft friable, white
2.5	11.66	56.66	Soft friable, light white
3.0	12.00	70.86	Soft friable, white
4.0	14.33	75.80	Compact, white
4.5	14.66	60.53	Compact, brown
5 year + BAP(mg/l)			
0.5	13.33	21.26	Soft, mushy, white
1.0	13.00	19.16	Soft friable, white
2.5	15.66	32.20	Soft friable, white
3.0	16.33	51.30	Soft friable, light white
4.0	16.66	41.03	Compact, white
4.5	16.66	61.60	Compact, brown
CD at 5%	1.01	5.32	
CV %	4.20	9.37	
SEm±	0.34	1.82	
GM	14.27	46.95	

Table 3: Effect of NAA and BAP on Callus induction from petiole explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	11.88	51.05	Soft mushy light yellow
5 Year plant	14.55	31.56	Compact, white
SEm±	0.26	0.61	
CD at 5%	0.78	1.80	
MS + GR (mg/l)			
N1.0 +B0.1	10.50	53.88	Soft friable, white
N1.0 +B0.5	11.16	56.48	Compact, light yellow
N1.0 + B1.0	12.33	51.21	Compact, white
N1.5+ B0.1	14.00	35.15	Soft, mushy, white
N1.5 + B0.5	15.33	29.56	Compact, light brown
N1.5 + B1.0	16.00	21.55	Compact, brown
SEm±	0.46	1.07	
CD at 5%	0.78	3.13	
Interaction			
1 year + NB(mg/l)			
N1.0 +B0.1	9.33	68.16	Soft friable, white
N1.0 +B0.5	9.66	70.43	Soft, mushy, white
N1.0 + B1.0	10.00	69.53	Compact, white
N1.5+ B0.1	12.66	40.66	Compact, white
N1.5 + B0.5	14.33	32.20	Compact, light brown
N1.5 + B1.0	15.33	25.30	Compact, brown
5 year + NB(mg/l)			
N1.0 +B0.1	11.66	39.60	Soft, mushy, white
N1.0 +B0.5	12.66	42.53	Soft friable, white

N1.0 + B1.0	14.66	32.90	Soft friable, white
N1.5+ B0.1	15.33	29.63	Compact, light brown
N1.5 + B0.5	16.33	26.93	Compact, white
N1.5 + B1.0	16.66	17.80	Compact, white
CD at 5%	1.92	4.42	
CV %	8.64	6.36	
SE±	0.65	1.51	
GM	13.22	41.30	

Note:- N- NAA, B- BAP

Table 4. Effect of Kinetin on Callus induction from nodal explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	10.16	31.96	Compact, light white
5 Year plant	10.33	23.40	Compact, Mushy, light brown
SEm±	0.124	0.30	
CD at 5%	0.362	0.898	
Kinetin (mg/l)			
00	00	00	Nil
0.5	10.16	18.4	Soft fleshy, light white
1.0	12.00	37.68	Soft friable, white
2.5	11.00	24.50	Compact, light yellow
3.0	13.33	44.23	Compact , light white
4.0	15.0	41.28	Compact , light white
SEm±	0.21	0.53	
CD at 5%	0.62	1.55	
Interaction			
1 year + Kinetin(mg/l)			
00	0.00	0.00	Nil
0.5	9.66	20.10	Soft friable, white
1.0	12.66	29.96	Soft fleshy, light white
2.5	11.66	45.90	Compact, light yellow
3.0	13.33	50.30	Compact , light white
4.0	13.66	45.53	Compact , light white
5 year + Kinetin (mg/l)			
00	0.00	0.00	Nil
0.5	10.66	16.70	Soft friable, white
1.0	11.33	18.90	Compact , light white
2.5	10.33	29.46	Soft friable, white
3.0	13.33	38.16	Compact, brown
4.0	16.33	37.03	Compact, dark brown
CD at 5%	0.888	2.20	
CV %	4.69	4.25	
SEm±	0.304	0.75	
GM	12.30	33.20	

Table 5. Effect of BAP on Callus induction from nodal explants of different age grouped plants

Treatment	Days for initiation of callus	Callus frequency (%)	Callus texture
1 Year plant	11.61	19.78	Compact friable, light white
5 Year plant	12.11	16.91	Compact friable, light

			white
SEm±	0.13	0.33	
CD at 5%	0.38	0.96	
BAP(mg/l)			
00	0.0	0.0	
0.5	12.0	11.38	Soft fleshy, light white
1.0	13.50	23.20	Soft friable, white
2.5	14.16	23.43	Compact, light yellow
3.0	15.00	26.98	Compact , light white
4.0	16.50	25.10	Compact , light white
SEm±	0.22	0.57	
CD at 5%	0.65	1.67	
Interaction			
1 year + BAP(mg/l)			
00	0.0	0.0	
0.5	11.66	12.16	Soft friable, white
1.0	13.66	31.60	Compact , light white
2.5	13.00	19.70	Soft friable, white
3.0	14.66	26.46	Compact, brown
4.0	16.66	28.80	Compact, dark brown
5 year + BAP(mg/l)			
00	0.0	0.0	
0.5	12.33	10.60	Soft friable, white
1.0	13.66	14.80	Soft fleshy, light white
2.5	15.00	27.16	Compact, light yellow
3.0	15.33	27.23	Compact , light white
4.0	16.33	21.40	Compact , light white
CD at 5%	1.07	2.37	
CV %	4.66	6.86	
SEm±	0.81	1.51	
GM	14.23	21.98	

Table 6: Effect of GA_3 and IAA direct regeneration plantlets from shoot tip of different age grouped plants

Treatment	Days for initiation of bud	Length of shoot	Survival %
1 Year plant	15.50	3.46	59.15
5 Year plant	15.20	1.82	28.59
SEm±	0.15	0.035	0.32
CD at 5%	0.43	0.10	0.92
MS+ GI(mg/l)			
$G_{1.0} + I_{3.0}$	12.83	1.45	33.71
$G_{3.0} + I_{3.0}$	13.16	1.73	37.23
$G_{5.0} + I_{3.0}$	13.66	2.08	37.73
$G_{8.0} + I_{3.0}$	14.66	2.31	38.80
$G_{1.0} + I_{5.0}$	15.83	2.70	47.21
$G_{3.0} + I_{5.0}$	17.83	4.05	54.83
$G_{5.0} + I_{5.0}$	17.83	3.83	53.63
$G_{8.0} + I_{5.0}$	17.00	3.21	49.33
SEm±	0.30	0.07	0.64
CD at 5%	0.86	0.20	1.84
Interaction			
1 year + GI(mg/l)			
$G_{1.0} + I_{3.0}$	12.33	2.16	41.20
$G_{3.0} + I_{3.0}$	13.66	2.93	52.93

$G_{5.0} + I_{3.0}$	12.66	2.60	47.26
$G_{8.0} + I_{3.0}$	15.33	3.06	58.90
$G_{1.0} + I_{5.0}$	16.00	3.46	63.76
$G_{3.0} + I_{5.0}$	18.33	4.83	72.53
$G_{5.0} + I_{5.0}$	18.33	4.56	74.13
$G_{8.0} + I_{5.0}$	17.33	4.10	62.53
5 year + GI(mg/l)			
$G_{1.0} + I_{3.0}$	13.33	0.73	26.56
$G_{3.0} + I_{3.0}$	12.66	0.53	21.20
$G_{5.0} + I_{3.0}$	14.66	1.56	27.53
$G_{8.0} + I_{3.0}$	14.00	1.10	18.36
$G_{1.0} + I_{5.0}$	15.66	1.93	30.66
$G_{3.0} + I_{5.0}$	17.33	3.26	35.13
$G_{5.0} + I_{5.0}$	1733	3.10	33.13
$G_{8.0} + I_{5.0}$	16.66	2.33	35.46
CD at 5%	1.22	0.28	0.92
CV %	4.70	6.75	3.59
SEm±	0.42	0.10	0.90
GM	15.35	2.64	43.87

Note:- G- GA_3, I- IAA

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Field evaluation of coffee mealy bug parasitoid, *Leptomastix dactylopii* (How.) in Wayanad district of Kerala, India

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Abstract: *Field releases of the exotic parasitoid, Leptomastix dactylopii were carried out on seven zones in Wayanad district of Kerala against coffee mealy bug Planococcus citri during the pest season of year 2006. The mean percentages parasitism by L .dactylopii in seven zones of Wayanad district of Kerala after 30,60 and 90 days of release were 51.18 ± 5.38, 63.61 ± 6.18 and 76.51 ± 5.56 respectively. The results indicate that L. dactylopii readily attacks, colonises, establishes and reduces the mealybug, P.citri population to substantial level under Wayanad condition. Hence it can be used in Wayanad for coffee mealy bug control.*

Key words: mealy bug; *Leptomastix dactylopii*; field evaluation, wayanad

INTRODUCTION

Planococcus citri (Risso) (*Homoptera: Pseudococcidae*) is one of the major economically important pseudococcids infesting coffee in Wayanad district of Kerala. A large number of native natural enemies attack *P.citri* in coffee and their contribution in its suppression is valuable (Reddy *et al.*, 1990). But, although they have substantial impact, late in the pest season, the natural enemies generally do not maintain the populations of the pest below the economic threshold level. Biological control of *Planococcus spp.* using *Cryptolaemous montrouzieri* (Muls.) (*Coleoptera: Coccinellidae*) was attempted since 1976 and the control achieved was partial to complete (Chacko *et al.*, 1983). Further observations on *C. montrouzieri* revealed that it could build up its population at high pest density only by which time the mealy bug would have caused severe damage to crop (Anonymous, 1987). In order to tackle *P.citri* more effectively through biological means, an encyrtid parasitoid, *Leptomastix dactylopii* (How.) (*Hymenoptera: Encyrtidae*) was first introduced to India through Indian Institute of Horticultural Research in 1983 from Trinidad, West Indies (Anonymous, 1983). *L. dactylopii* was earlier introduced to some other countries and the results varied from recoveries during the seasons of release to permanent establishment (Clausen, 1978). The study reported here was carried out to evaluate the performance of *L.dactylopii* in the coffee tracts of Wayanad district of Kerala, India.

MATERIALS AND METHOD

The field evaluations were carried out during the year 2006 (February to March) to assess the performance of the exotic parasitoid, *L. dactylopii* against coffee mealy bugs, *P. citri* in the coffee tracts of Wayanad district of Kerala. There are seven liaison zones in Wayanad viz. 1) Chundale 2) Kalpetta 3) Meenangadi 4) Sulthanbathery 5) Pulpally 6) Mananthavady and 7) Panamaram. These zones comprise groups of coffee growing villages with an average elevation of 900 meters. Kalpetta zone covers Kalpetta, Venganapally, Muttil and Kottathara villages. The villages Purakkadi and Poothadi constitute Meenangadi liaison zone. Chundale zone comprises Thariode, Achooranam, Kunnathidavaka, Mooppainad and Kottappadi villages. Panamaram zone covers Anchukunnu, Kuppathodi, Porunnannur and Kaniambetta villages. The villages Pulpally, Padichira and Irulam form Pulpally zone. Sulthanbathery zone covers Sulthanbathery, Kidanganad, Nenmeni, Noolpuzha and Ambalavayal. Mananthavady liaison zone covers Thirunelli, Thavinhal, Nellurnad, Edavaka, Venom, Peria, Vellamunda, Kuppadithara, Thondarnad and Padinjarathara villages. A total of 21 estates were selected for this study. Three estates were selected from each liaison zone. The details of the estates are furnished below.

i. Chundale zone

1. Cottanad Estate, Meppadi.
2. Aysha Plantation, Vythiri.
3. Padivayal Estate, Padivayal.

ii. Kalpetta zone

1. Warriat Estate, Muttil.

2. Edaguni Estate, Edaguni.
3. Plakandy Estate, Puthoorvayal.

iii. Meenangadi zone

1. Eden Vally, Vakery.
2. Georgia Estate, Krishnagiri.
3. Libra Garden, Appatt.

iv. Sulthanbathery zone

1. Beenachi Estate, Beenachi.
2. Vedankotta Estate, Cheeral.
3. Geetha Estate, Kaloor.

v. Pulpally zone

1. Dhanalakshmi Estate, Kurchipetta.
2. K. Krishnanunni Estate, Irulam.
3. Sreedharan Estate, Manalvayal.

vi. Mananthavady zone

1. Bisonfield Estate, Kartikulam.
2. Bhargiri Estate, Tholpetty.
3. Technical Evaluation Centre, Coffee Board, Kuzhinilam.

vii. Panamaram

1. Prasanthi Estate, Pachilakkad.
2. Ramdham Estate, Krishnamoola.
3. Muralivihar Estate, Kayakunnu.

The variety of coffee grown in these estates was S.274 (*Coffea canephora* var. *robusta*) and the plants were 25 to 60 years old. The spacing maintained between the plants was 10´X 10´ with medium shade pattern. The parasitoid, *L. dactylopii* was reared in the glass house on mealybug infested pumpkins as described by Chacko (1982) for *Cryptolaemous montrouzieri* (Muls.). About 25,000 adult parasitoids were released in a hectare plot of each estate during February 2006. There were 1087 plants in each experimental plot. The mealybug infestation levels were medium to high in the experimental plots. A mealybug count of 1-5 on a node was considered as low, 6-20 as medium, 21 to 40 as high and mealybugs over 40 as severe (Gokuldas Kumar *et al*., 1989). During the period of the study the average relative humidity was 75.4% and maximum and minimum temperatures were 30.6 ^{0} C and 18.9 ^{0} C respectively.

For sampling and observations the methods developed by Atwal and Singh (1990) and Reddy *et al*. (1988) were followed. For sampling, one hectare plot was divided into quadrants consisting of 16 plants. Five plants from the quadrant, one at centre and four from each corner of the quadrant were selected for sampling. 20 such quadrants were chosen for assessing the percentage parasitism of mealybugs by *L. dactylopii.* Follow up observations were made after 30, 60 and 90 days interval. From each plant one mealybug infested node with leaves was collected for observation. A total of 100 samples were collected from each plot for each observation. The collected nodes were taken to laboratory and the count of mealybug was made under microscope and classified as healthy and parasitised and calculated the percentage parasitism of the mealybug using the formula (Reddy *et al*., 1988).

$$\frac{\text{Number of parasitised mealybugs}}{\text{Total number of mealybugs}} \times 100$$

RESULTS AND DISCUSSION

The data on the evaluation of the mealybug parasitoid *L. dactylopii* in Wayanad district of Kerala is provided in Table I. The highest rate of parasitism was recorded in Mananthavady zone (80.45 ± 8.06) followed by Chundale zone (80.24 ± 3.90), Kalpetta zone (80.00 ± 4.05), Pulpally zone (77.14 ± 4.49), S. Bathery zone (77.03 ± 5.17), Panamaram zone (71.54 ± 8.10) and Meenangadi zone (69.14 ± 4.49) after 90

days of release. There was no significant difference in parasitism between zones. The mean percentage parasitism by *L. dactylopii* in the seven zones of Wayanad after 30, 60 and 90 days of release was 51.18 ± 5.38, 63.61 ± 6.18 and 76.51 ± 5.56 respectively. The results of the study clearly indicate that the parasitoid, *L. dactylopii* readily attacks, colonises, establishes and reduces the mealybug, *P.citri* to a substantial level.

In this direction the studies of various authors on various crops in India and other countries are noteworthy. Ortu and Prota (1983) reported 96 percent parasitism of *L. dactylopii* in citrus orchards in Sardinia. Barbagallo *et al*. (1982) claimed that the release of *L. dactylopii* on orange groves of Sicily for the management of *P. citri* gave very good results. *L. dactylopii* which was introduced into France in 1972, played an important role in the control of *P. citri* on citrus (Panis, 1983). Longo and Benefatto (1982) reported that *L. dactylopii* is able to control the citrus mealybugs in Italy. *L. dactylopii* was introduced into Cyprus from Italy in 1977, it has established and reported 15 percent parasitism of *P. citri* in citrus orchards (Krambias and Konzonis, 1980). Panis (1981) claimed that *L. dactylopii* has high searching ability and parasitizing scattered populations of mealybug nymphs. Meyerdirk *et al* (1978) reported that *L. dactylopii* was the most abundant parasite in grape fruits and lime trees in Texas and parasitizing 21 percent of *P.citri*. Luppino (1979) claimed that the citrus trees with parasite, *L. dactylopii* did not become re-infested by *P. citri*, where as infestation by *P. citri* re-appeared where pesticides had been used. Mineo and Viggiani (1975) claimed that the incidence of infestation, percentage of infested fruits and percentage of commercially damaged fruits were not significantly different in citrus plots treated with parasites only and plots treated with both parasites and insecticides in Sicily. In Belgium, the *P. citri* infestation in green houses was successfully controlled by *L. dactylopii* and use of pesticides was substantially reduced (Ronse, 1990). Pest infestation averaging 38 percent of citrus orchards were drastically reduced to 5 percent by *L. dactylopii* in Queensland (Smith *et al.*, 1988). *L. dactylopii* was an active natural enemy in the management of mealybug, *P. citri* in date palm gardens of Libiya (Bitwa and Binsad, 1988). *L. dactylopii* were released to control mealybug , *P .citri* in Israel and pest free house plants were thus produced at low cost, or equal to that of conventional pest control (Rubin, 1985). *L. dactylopii* was introduced into Queensland from California in 1980 to control citrus mealybug, *P. citri*, in citrus and custard apple and 50 to 80 percent parasitism was reported within three months (Smith, 1991). In Moracco, the *P. citri* was effectively controlled in the citrus orchards by the release of *L. dactylopii* (Abdel Khalek *et al*., 1998). In Ghana, the *L. dactylopii* was established in the cocoa gardens infested with *P .citri*, where it was introduced in 1949 (Ackonor, 2002). The citrus mealybug, *P. citri*, the main pest of citrus was controlled by *L. dactylopii* in Turkey (Ozkan *et al*., 2001). Reciti *et al.* (2001) observed 50 to 70 percent parasitism of *L. dactylopii* in citrus gardens of Italy. Hannekam *et al.* (1987) reported that the *L. dactylopii* was the most successful parasitoid of commercial green houses of Netherlands. The use of *L. dactylopii* for the control of *P. citri* in Sardinia led to drastic reduction in the use of synthetic insecticides against *P. citri* (Fronteddu *et al.*, 1996). Viggiani (1975) reported that the multiplication and parasitism of *L. dactylopii* were very good in citrus gardens of Italy.

Inoculative release of *L. dactylopii* was made in orange and lime orchards in 1984 in Karnataka, for the control of *P. citri* by Krishnamoorthy and Singh (1987). Prior to the release, infestation by the pest ranged from 38 to 68 percent, but establishment of the parasitoid resulted in complete control within 3 to 4 months. Mani and Krishnamoorhty (2000) claimed that *L. dactylopii* reduced the population of *P. citri* from 128.80 adults to 8.10 adults within 2 months on pomegranates in India. Nagarkatti *et al*. (1992) reported that *L. dactylopii* caused cent percent parasitism of *P. citri* on mandarins in Karnataka within 2 months. For the suppression of *P. citri* on citrus and grape, inoculative release of *L. dactylopii* has proved very effective (Singh, 1993). Krishnamoorhty and Singh (1987) reported that the mealybug problems on citrus and pomegranate can be tackled in India with the use of *L. dactylopii*. *L. dactylopii* proves to be very effective in suppressing the mealybug, *P.citri* on acid lime, guava and pomogranate (Mani and Krishnamoorthy, 2001). Mani (1993) reported that the release of *L. dactylopii* was very effective against mealybugs in ber orchards. Mani and Krishnamoorthy (1997) claimed that *L. dactylopii* is able to suppress the problem of *P. citri* on suppota gardens in India. Mani (1994) reported that the release of *L. dactylopii* gives effective and permanent control of *P. citri* in guava orchards. The *L. dactylopii* was released in Tamil Nadu on sweet orange, acid lime and lemon, within 4 months, complete control was achieved (Krishnamoorthy, 1990). *L. dactylopii* caused 85 percent parasitism of coffee mealybugs in Sathya estate, Mudigere, Karnataka (Anonymous, 1986). 27 percent parasitism of coffee mealybug was recorded in Honsala estate, Kalasa, Karnataka (Anonymous, 1986). *L. dactylopii* caused 40 to 80 percent parasitism of coffee mealybugs in

Ammanagiri estate and Badnekhan estate in Chikmagalur, Karnataka (Anonymous, 1987). From the foregoing discussion, it is concluded that the parasitoid, *L.dactylopii* is an effective bio agent of coffee mealybugs and it can be used in Wayanad district of Kerala for mealybug control.

Table 1. Evaluation of *L. dactylopii* in Wayanad district of Kerala

S.No.	Zones	Percent Parasitism		
		30 DAT Mean ± SD	60 DAT Mean ± SD	90 DAT Mean ± SD
1.	Chundale	53.31 ± 3.60	66.04 ± 3.83	80.24 ± 3.90
2.	Kalpetta	55.24 ± 4.20	65.87 ± 6.80	80.00 ± 4.05
3.	Meenangadi	48.18 ± 4.70	58.68 ± 3.87	69.14 ± 5.14
4.	S.Bathery	53.70 ± 4.85	65.67 ± 6.87	77.03 ± 5.17
5.	Pulpally	47.45 ± 6.28	62.42 ± 6.21	77.14 ± 4.49
6.	Mananthavady	52.73 ± 5.96	65.49 ± 7.69	80.45 ± 8.06
7.	Panamaram	47.68 ± 8.04	61.11 ± 8.01	71.54 ± 8.10
	Range	47.45 ± 6.28 to 55.24 ± 4.20	58.68 ± 3.87 to 66.04 ± 3.83	69.14 ± 5.14 to 80.45 ± 8.06
	Mean ± SD	51.18 ± 5.38	3.61 ± 6.18	76.51 ± 5.56

DAT – Days after treatment

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Cytomorphological studies in interspecific hybrids of finger millet.

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Abstract: *Study of the cytomorphology of interspecific hybrid between Eleusine coracana × E. africana along with the parents indicated that the hybrids were intermediate for productive tillers, finger length, finger number and days to 50% flowering and exhibited reduced pollen fertility over the parents. Chromosome pairing was normal in parents and hybrid. However, one chain quadrivalent at diakinesis and one lagging chromosome at metaphase I were observed in hybrid in most of the meiotic cells scored. Further, chiasma frequency was lower in the hybrid compared to their parents. The lower chiasma frequency in interspecific hybrid is suggestive of the fact that the genome of both the species might be differing for cryptic structural changes in the form of deletions and inversions. Presence of chain quandrivalent also supports the occurrence of translocation, which may reduce the chiasma frequency.*
Key words : Interspecific hybrids, cytomorphology, finger millet. chiasma frequency

INTRODUCTION

The genus *Eleusine* to which finger millet (*Eleusine coracana* (L.) Gaertn) belongs, contains 9 to 10 species and the basic chromosome number is n = x = 9. Among the reported species, three are tetraploids with 2n = 36 in *E. coracana* and *E. africana* and 2n = 38 in *E. kageziensis,* while *E. indica, E. tristachya, E. floccifolia* and *E. intermedia* are diploid species with 2n = 18. Contrary to this, *E. multiflora* and *E. jeageri* are reported to possess 2n = 16 and 2n = 20 chromosomes respectively (Hiremath S. C. and Chennaveeraiah M. S., 1982).

The studies pertaining to genome relations and molecular biology have not received much attention. Nevertheless, some attempts have been made to establish the chromosome relationship by crossing various species of genus *Eleusine*. Based on these studies it is concluded that the cultivated species *E. coracana* and others having 2n=36 chromosomes are allotetraploid (2n=4x=36) and genomic composition is AABB which have originated by the hybridization of two diploid taxa having AA and BB genomes followed by chromosome doubling. Based on genome studies, it is perceived that the donor of A genome is *E. indica* while the identity of B genome donor species is still obscure (Hiremath S. C. and Salimath S. S., 1992).

The interspecific hybridization studies so for attempted were from the angle of knowing genome relationship. However, such evaluation and utilization of wild gene pool from the point of crop improvement has received scant attention. There have been no efforts in the past for introgression of genes from wild relatives and deriving lines for utilization in finger millet crop improvement. *Eleusine africana* is a close relative of cultivated species of *E. coracana*. Earlier reports indicate that gene transfer from *E. africana* to *E. coracona* is feasible and useful in breeding. *E. africana* has more tillering ability (15-20) and high drought tolerance capacity. It matures early (95-100 days) also it has more fingers per ear and long finger length. So, utilization of *E. africana* as donor parent for present investigation is highly rewarding.

In the present study one of the wild tetraploid species *E. africana* (2n=4x=36) was crossed with three varieties *viz.,* Indaf 8, HR911 and PR 202 of cultivated species *E. coracana* with an objective of transferring some of the desirable characters from wild species to the popular cultivated varieties of finger millet and also to know the chromosome behavior at bivalent formation in interspecific hybrids.

MATERIALS AND METHOD

The material for the present study comprised of three crosses between wild species (*Eleusine africana*) with three cultivated popular varieties *viz*., Indaf 8, HR-911and PR 202. The crossed seeds were obtained from the Project Coordination Cell, All India Coordinated Small Millets Improvement Project, Indian Council of Agricultural Research, Gandhi Krishi Vignana Kendra, Bangalore. The crossed seeds

were sown during *kharif* 2005 along with parents. The F_1 plants were identified based on morphological characters. At the same time crossing programme was carried out to generate F_0 seeds.

Crossing technique employed

For crossing programme three cultivated popular varieties *viz*., Indaf 8, HR 911 and PR 202 were used as female parent while the wild species (*E. africana*) was used as male parent. Based on the days to flowering, sowing date of each parent was adjusted to achieve synchrony in flowering in all genotypes. Usually in finger millet, anthesis takes place from 1am to 4am and ends by 11 a.m. Male parent was sown in between female parents in 3m row. In female, only two fingers were allowed to pollinate with male and remaining were removed before pollination. The ear heads of both the parents were joined together and tied with cotton thread before anthesis taking place. After pollination, ear heads were separated and seeds collected only from female parent.

For assessing fertility, the pollen grains were stained in 2% acetocarmine and examined under the microscope. Deeply stained grains were counted as fertile and the unstained/ partially stained pollens were recorded as sterile. From each slide, five fields were chosen at random and the percentage of pollen fertility was calculated.

For meiosis study, flower buds of appropriate stage were fixed in freshly prepared Cornoy's II mixture made of 6 parts of ethyl alcohol, 3 parts of acetic acid and 1 part of chloroform. The flower buds were collected during 8-10 am on bright sunny days. The material was allowed to remain in the fixative for 24 hours and then transferred to 70% alcohol and stored for further use. The anthers were smeared in 1 or 2 drops of 2% acetocarmine and observed under microscope. Judicious warming over the flame before and after tapping helped to obtain good spread of chromosomes. Diakinesis and metaphase I for chromosome pairing and subsequently anaphase I for behaviour of chromosomes were studied. Chiasma frequency was estimated at diakinesis and a total of 20 cells were scored for this purpose. Total chiasma per cell and per bivalent was calculated by using the following formula. Cytological observations were made on both temporary and permanent mounts. Photomicrographs were taken. Chiasmata per cell and chiasmata per bivalent were calculated using following formulae (Manjula, 1998).

$$\text{Chiasmata per cell} = \frac{\text{Total number of chiasma}}{\text{Number of cells scored}}$$

$$\text{Chiasmata per bivalent} = \frac{\text{Total Chiasmata per cell}}{\text{Number of bivalents}}$$

RESULTS AND DISCUSSION

Comparisons of morphological characters of different interspecific hybrids with their parents are presented in Table.1. It indicated that most of the characters were intermediate in interspecific hybrids. There was considerable difference in respects of productive tillers per plant between the two species. The cultivated species *E. coracana var.* Indaf-8, HR911 and PR 202 showed less productive tillers (2 to 4) while *E. africana* had as many as 17 tillers. The F_1 was intermediate with 8-10 tillers per plant (Fig. 1). With regard to finger number, the interspecific hybrid was intermediate between parents in all the three crosses. Finger length in interspecific hybrid is more towards *E. africana* (13 cm) than cultivated species (Fig. 2). It indicated significant contribution of male parent (*E. africana*) to the F_1s in respect of this trait.

There was considerable difference for days to 50 per cent flowering between the two species. The varieties *viz.,* Indaf 8, HR911and PR202 took 86, 83 and 80 days for flowering, respectively whereas *E. africana* flowered early in 50 days. The F_1s were intermediate between parents with. 70, 68 and 67 days in cross I, cross II and cross III, respectively. Chennaveeraiah and Hiremath (1974) also observed that interspecific hybrids were intermediate between the parent species for most of the characters. With respect to grain yield per plant, the interspecific hybrid (8g) was inferior to cultivated varieties (26g), but they were superior to wild species (4.81g) in all the crosses. Table 2 indicates that interspecific hybrids are superior to cultivated species for the important traits like productive tillers, finger length, finger number and days to

50 % flowering in all the crosses. This shows that there is much scope for improvement of these traits in segregating generations.

Cytological studies

The interspecific hybrids showed low pollen fertility in the cross Indaf 8 × *E. africana* (35.50%), HR 911 × *E. africana* (47.25%) and PR 202 ×*E. africana* (42.25%). On the other hand, mean pollen fertility was 82.00 %, 84.17 % and 80 % in *E. coracana* var Indaf 8, HR911 and PR 202, respectively and 88.85% in *E. africana* (Table 3). The reduced fertility in the hybrid may be due to cryptic structural differences in the chromosome (Devarumath *et al*., 2005). Meiosis in the pollen mother cells of hybrid and parents was studied with special reference to pairing behaviour of chromosomes. Pairing between the homologous chromosomes was normal at diakinesis in cultivated species *E. coracana* variety Indaf 8, HR 911, PR 202 and *E. africana* (Fig. 3 and Fig. 4) and the bivalents showed mostly either one chiasma or two chaismata. The later stages of meiosis were normal resulting in high percentage of fertile pollen and good seed set. The hybrid also showed normal bivalents at diakinesis (Fig. 5) and normal movement of chromosomes during anaphase I (Fig. 6). It indicated the presence of good chromosomal homology between *Eleusine coracana* and *Eleusine africana.* The most striking observation in the meiotic plates of hybrid was the presence of one chain quadrivalent (Fig.7) at diakinesis in 90 per cent of the pollen mother cell studied. A lagging chromosome during metaphase-I was also seen (Fig. 8).

Further studies on chiasma frequency revealed that in the interspecific hybrid, chiasma frequency per cell (31.4) was lower compared to *E. coracana* (33.00) and *E. africana* (32.04) (Table 4). The lower chiasma frequency in interspecific hybrid is suggestive of the fact that the genome of both the species might be differing for cryptic structural changes in the form of deletions and inversions. Presence of chain quandrivalent also supports the occurrence of translocation, which may reduce the chiasma frequency.

Table 1. Comparative morphological features of three interspecific hybrids *E. coracana* × *E. africana* and their parents.

Sl. No.	Characters	Parents				F_1		
		P_1	P_2	P_3	P_4	$P_1 \times P_4$	$P_2 \times P_4$	$P_3 \times P_4$
1	Plant height (cm)	105.00	109.00	92.00	150.00	34.50	144.00	145.00
2	Culm thickness (cm)	1.20	1.10	1.00	0.60	1.10	0.70	0.80
3	Productive tillers / plant	2.40	3.00	4.00	17.00	8.00	8.00	10.00
4	Leaf number	12.00	13.50	13.00	16.00	13.00	14.00	12.00
5	Flag leaf length (cm)	26.00	25.50	26.00	40.00	35.00	42.50	30.00
6	Flag leaf width (cm)	1.10	1.10	0.90	0.76	1.20	1.00	0.80
7	Peduncle length (cm)	28.00	32.00	24.00	35.00	33.00	26.50	33.00
8	Finger number / ear	7.00	7.50	6.00	11.00	8.00	9.00	8.00
9	Finger length (cm)	7.25	7.00	5.10	13.00	12.50	11.50	10.00
10	Finger width (cm)	1.10	1.10	1.20	0.40	0.70	0.60	0.70
11	Days to 50% flowering	86.00	83.00	85.00	50.00	70.00	68.00	67.00
12	Days to maturity	125.00	120.00	117.00	95.00	120.00	115.00	112.00
13	Test weight (g)	2.67	3.33	3.20	1.03	1.92	1.97	1.43
14	Grain yield per plant (g)	18.00	25.40	26.00	4.70	8.20	7.50	8.75

Where, P_1 - Indaf 8 P_3 - PR 202 P_2 - HR 911 P_4 – *Eleusine africana* (wild species)

Table 2: Mean superiority / inferiority (%) of the interspecific hybrids over its parents for various characters

Sl. No.	Characters	Cross 1		Cross II		Cross III	
		P_1	P_4	P_2	P_4	P_3	P_4
1	Plant height (cm)	28.09	-10.33	32.11	-4.00	64.13	0.66
2	Culm thickness (cm)	-8.00	83.33	-36.36	16.66	-20.00	33.33
3	Productive tillers/plant	233.33	-52.94	166.66	-52.94	150.00	-41.17
4	Leaf number	8.30	-18.75	3.70	-12.50	-7.69	-25.00
5	Flag leaf length (cm)	34.60	-12.50	66.66	6.25	15.38	-25.00
6	Flag leaf width (cm)	9.09	57.80	-9.09	31.57	-11.11	5.26
7	Peduncle length (cm)	17.85	-5.71	-17.18	-24.28	37.50	-5.71
8	Finger number / ear	14.28	-27.27	20.00	-18.18	33.00	-27.77
9	Finger length (cm)	72.41	-3.84	64.28	-11.53	96.07	-23.07
10	Finger width (cm)	-36.36	75.00	-45.45	50.00	-41.66	75.00
11	Days to 50% flowering	-4.65	64.00	-6.02	30.00	-11.76	50.00
12	Days to maturity	-4.00	26.31	-4.16	21.50	-4.27	17.89
13	Test weight (g)	-28.08	86.40	-40.84	91.26	-55.31	38.54
14	Grain yield /plant (g)	-54.00	74.00	-70.00	53.00	-66.00	85.00

Where, P_1 - Indaf 8 P_3 - PR 202 P_2 - HR 911 P_4 – *Eleusine Africana*

Table 3. Mean pollen fertility (%) of the interspecific hybrids in comparison with parents

Parents and F_1 hybrids	Pollen fertility (%)
Indaf 8	82.00
HR 911	84.17
PR 202	80.00
E. africana	88.85
Indaf 8 × *E. africana*	35.50
HR 911 × *E. africana*	47.28
PR 202 × *E. africana*	42.25

Table 4. Chaisma frequency in the interspecific hybrid and their parents

	Mean bivalents/cell with				Mean xta per bivalent	Mean xta per cell
	1xta	2xta	3xta	4xta		
Parent 1 (*E.coracana* var. Indaf 8)	3.0	15.0	-	-	1.83	33
Parent 2 (*E. africana)*	4.0	14.0	-	-	1.77	32.02
F_1	4.3	13.0	0.3	0.05	1.74	31.4

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Fig. 1 **Fig. 2** **Fig. 3** **Fig.** 4

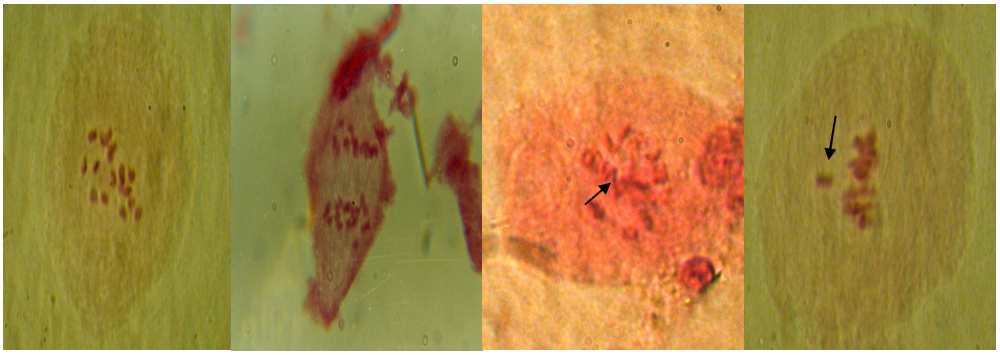

Fig. 5 **Fig. 6** **Fig. 7** **Fig. 8**

Fig.1: Comparison of productive tillers in interspecific hybrids and their parents
Fig.2: Comparison of ear characters among *E. carocona*, wild species *E. africana* with F_1
Fig.3 : Diakinesis in *Eluesine caracona* var Indaf 8 with 18 II
Fig.4 : Diakinesis in *Eluesine africana* with 18 II
Fig.5 : Diakinesis in interspecific hybrid *Eluesine caracona* var Indaf 8 × *Eluesine africana* showing norma18 II
Fig.6 : Anaphase I showing normal movement chromosome in interspecific hybrid of *Eluesine caracona* var Indaf 8 × *Eluesine africana*
Fig.7 : Diakinesis in interspecific hybrid *Eluesine caracona* var Indaf 8 × *Eluesine africana* showing 16 II + IV arrow mark indicates chain quadrivalent
Fig.8 : metaphase I showing lagging in interspecific hybrid of *Eluesine caracona* var Indaf 8 × *Eluesine africana*
P_1 - *Eluesine caracona* var Indaf 8 , P_2 - *Eluesine africana*

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Heterosis studies for yield and its attributing traits in sorghum (*Sorghum bicolor* (L.) Moench).

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Abstract: *Sorghum is one of the main staple food crop that sustains world's poorest people. India contributes 12% of the world's sorghum production but low productivity is the major concern. Although sustainable heterosis has been reported commercial exploitation to its full potential has not been possible. Heterosis helps to exploit the vigor with present genetic variability that helps to achieve a quantum jump in the yield. The present investigation was undertaken at Sorghum Research Station, Parbhani, Maharashtra with the aim to study heterosis for grain yield and its component traits. Five male sterile lines, eight male fertile lines and 14 crosses were evaluated with 3 replications. The female parent PMS 7B and PMS 37B and male parents KR 192 and RS 29 exhibited high mean value for grain yield/plant and other yield components. The crosses PMS 23A x C 43, and PMS 7A x RS 29 exhibited high mean, high average heterosis and high heterobeltiosis for grain yield and its component traits. So the crosses PMS 23A x C 43 and PMS 7A x RS 29 identified as good crosses can be evaluated for multilocation testing for commercial exploitation.*

Key words: Heterosis, Sorghum.

INTRODUCTION

Sorghum is one of the main staple food crops of the world's poorest people. It is the 4th important cereal crop in the world after rice, wheat and maize. Sorghum covers 39.6 million hectare area, producing 57.79 million tones with a productivity of 1.46 t/ha. India contributes 12% of world's sorghum production. Sorghum is the 3rd important cereal crop after rice and wheat in India. Sorghum covers 9.4 million hectare area in India producing 5.53 million tones with a productivity of 0.77 t/ha. Although sustainable heterosis has been reported, commercial exploitation to its full potential has not been possible. Hence study of heterosis helps to exploit the vigour with present genetic variability that helps to achieve aquantum jump in yield.

MATERIALS AND METHOD

The present study comprised of 5 female lines viz, PMS 7A, PMS 8A, PMS 9A, PMS 28A and PMS 37A and 8 males viz, RS 29, C 43, IB 12, KR 191, KR 192, KR 196, KR 199 and KR 200. The experiment was carried out at Sorghum Research Station, MAU, Parbhani during kharif 2004-05. Fourteen crosses and their parents were sown in a randomized block design with three replications adopting 45 x 15 cm spacing. Five competitive plants were selected randomly from each plot for recording observations on days to 50% flowering, plant height, number of leaves/plant, flag leaf area, third leaf area from top, ear head length, ear head breadth, grain yield/plant, fodder yield/plant, 1000 seed weight, weight of ear head, total chlorophyll content, relative water content, free soluble sugar content and protein content of seed. The data were subjected to analysis of variance for various characters, mean performance of parents and their crosses and heterosis.

RESULTS AND DISCUSSION

The analysis of variance revealed that the variation among the genotypes was highly significant for all the characters, overall heterosis of crosses tested by using parents. Mean performance of male parents, female parents and their crosses is represented in Table 1. Crosses with their mid parent and better parent heterosis are represented in Table 2. The manifestation of seed yield heterosis over mid parent and better parent in the present investigation ranged from –43.19 % (PMS 7A X KR 200) to 55.20% (PMS 28A X C 43) and –43.98% (PMS 7A X KR 200) to 28.11% (PMS 28A X C 43), respectively. For fodder yield, heterosis over mid parent and better parent ranged from -25.33% (PMS 7 A x KR 196) to 71.82% (PMS 28 A x RS 29) and -42.91 %(PMS 7 A x KR 196) to 45.43 % (PMS 28 A x RS 29), respectively. For relative water content, average heterosis ranged from -9.12 % (PMS 7 A x KR 199) to 9.28 % (PMS 28 A x IB 12) and

heterobeltiosis ranged from -9.40 %(PM 5 9 A x KR 196) to 7.91 % (PMS 28 A x IB 12). For protein content, average heterosis ranged from -0.79 % (PMS 8 A x KR 192) to 7.73 % (PMS 28 A x RS 29) and better parent heterosis ranged from -1.46% (PMS 28 A x KR 43) to 7.21 %(PMS 28 A x RS 29).

The range of mid parent heterosis and heterobeltiosis, and number of hybrids showing significant heterosis are represented in Table 3. Heterosis for grain yield is due to simultaneous heterosis in more than one components of yield. In the present study average heterosis and better parent heterosis for yield and its attributing traits are positive and significant. Ear head length is an important yield component trait. Significant positive average heterosis and heterobeltiosis were observed in eight and seven crosses, respectively. Earlier workers like Kaul *et al.* (2003), Hemalatha *et al.* (2003), also reported similar results. Ear head breadth is also important yield attributing character and eight and six hybrids showed significant positive average heterosis and better parent heterosis for this character and these results are in conformity with Kaul *et al.* (2003) and Hemalatha *et al.* (2003). For weight of ear head ten hybrids each exhibited significant positive average and better parent heterosis respectively. These findings are in accordance with earlier workers like Umakanth *et al.* (2003)and Kulkarni and Patil (2004). Six hybrids exhibited significant positive average heterosis for 1000 seed weight. This finding is in accordance with that of the earlier workers Umakanth *et al*. (2003) and none of the hybrids exhibited significant positive heterobeltiosis for 1000 seed weight. Similar results have been reported by Swarnalata and Rana (1988) and Thawari *et al.* (2000).

Heterosis for end product, *i.e*., fodder yield is being manifested as the cumulative effect of heterosis for the component traits. In the present investigation, the elaborative study of 14 crosses revealed this fact as most of crosses showed significant mid parent and better parent heterosis for fodder yield and its component traits *i.e*., number of leaves/plant, area of flag leaf and area of 3rd leaf from top. One and zero hybrids for number of leaves/plant, seven and four hybrids for area of flag leaf, nine and four hybrids for area of 3rd leaf from top and six and four hybrids for fodder yield exhibited positive significant on mid parent and better parent heterosis. Similar findings are also reported by Jey Prakash and Das (1994) and Desai *et al.* (1985). Earliness is a desirable character that helps to develop early varieties. Significant negative mid parental heterosis and heterobeltiosis for days to 50% flowering was observed in 13 and 11 hybrids respectively. Plant height is desirable to develop semi dwarf high yielding varieties that will be lodging resistant and fertilizer responsive. Three and four hybrids exhibited significant negative mid parent and better parent heterosis for plant height. These findings are in conformity with Kaul *et al.* (2003). Physiological characters like total chlorophyll content and relative water content are contributing traits of yield character. Eight and Seven crosses exhibited positive and highly significant average and better parent heterosis for total chlorophyll. Seven and Three hybrids exhibited positive and highly significant average and better parent heterosis for relative water content respectively. These findings are in accordance with Grewal *et al.* (2003) and Deshpande *et al*. (2003).

Protein and Free sugar content are quality characters. Four and one hybrids exhibited positive and significant average heterosis and better parent heterosis for free sugar content respectively and eight and seven hybrids for protein content respectively. Benerji. (1988) and Deshpande *et al.* (2003) reported similar results. The promising hybrids for characters like seed yield, fodder yield, physiological and quality characters are presented in Table 4. Overall, two hybrids PM 28A X C-43 and PMS 7A X RS 29 were identified superior for most of the seed yield, fodder yield, physiological and quality characters, which could be utilized commercially for the exploitation of heterosis of these characters.

Table 1. Mean performance of hybrids, male lines and female lines for the different characters.

Genotypes	Days to 50% flow.	Plant height (cm)	No. of leaves/ plant	Area of flag leaf (cm^2)	Area of 3^{rd} leaf (cm^2)	Ear head length (cm)	Ear-head breadth (cm)	Grain yield (gm)	Fodder yield (gm)	1000 seed weight (gm)	Weight of ear-head (gm)	Chlorophyll content	Relative water content	Free sugar content	Protein content
PMS 9 A x KR 196	69.00	162.33	9.80	401.17	780.64	36.00	7.90	76.60	305.00	27.52	131.60	3.10	79.87	1.30	8.20
PMS 7 A x KR 199	68.00	138.13	10.53	348.08	670.66	29.66	7.20	82.00	298.30	26.69	105.30	3.05	78.49	1.20	8.18
PMS 28 A x C 43	71.33	171.93	11.33	210.06	522.17	37.80	8.00	92.50	243.30	28.70	206.60	3.43	82.55	2.34	8.06
PMS 28 A x KR 191	75.00	158.80	12.20	281.88	674.76	35.93	7.80	68.20	336.60	24.84	262.00	3.08	82.22	1.31	8.45
PMS 7 A x KR 191	74.00	139.80	11.73	313.52	574.13	30.40	6.90	80.40	278.60	27.76	136.00	2.75	85.96	1.50	8.59
PMS 7 A x KR 196	70.33	134.80	9.33	395.91	673.33	27.93	6.00	64.50	211.00	26.77	86.66	2.35	80.23	1.43	8.65
PMS 28 A x RS 29	75.33	214.20	11.13	352.76	669.78	24.40	7.40	77.20	347.60	25.68	158.60	3.61	83.32	1.37	8.77
PMS 7 A x RS 29	74.00	161.87	10.06	400.80	674.96	29.33	8.00	89.50	368.00	27.77	185.30	3.54	82.43	1.43	8.65
PMS 37 A x RS 29	79.00	162.07	9.40	381.57	674.84	26.86	7.30	82.70	240.30	27.93	156.30	3.55	80.65	1.25	8.22
PMS 7 A x KR 200	72.00	152.67	10.33	304.68	688.18	27.86	6.20	48.40	261.60	28.22	97.30	3.61	83.92	1.54	8.28
PMS 8 A x KR 200	66.33	163.60	10.53	319.07	676.01	34.00	7.00	74.10	242.00	26.80	129.60	3.88	76.22	1.62	8.24
PMS 28 A x IB 12	73.33	157.07	10.46	277.36	639.94	35.93	6.90	86.20	258.00	26.70	155.00	3.49	86.16	1.36	8.18
PMS 28 A x KR 200	70.00	157.27	10.60	311.80	709.98	36.73	7.80	86.20	344.30	28.38	129.60	3.75	83.35	1.47	8.11
PMS 8 A x KR 192	66.00	154.87	9.30	329.07	728.79	33.46	7.10	74.30	237.60	29.17	156.30	3.80	78.63	1.30	8.10

PMS 7 B	78.00	144.20	11.00	315.79	623.15	30.13	7.40	84.00	369.60	28.15	122.30	3.45	82.52	1.07	8.16
PMS 8 B	72.33	145.53	10.46	205.26	523.15	31.26	6.70	65.60	287.00	28.88	80.60	3.41	77.80	1.23	8.21
PMS 9 B	75.66	140.00	9.60	262.41	546.97	27.06	6.00	53.30	199.00	24.24	77.60	3.25	88.16	1.06	8.02
PMS 28 B	79.33	145.47	11.33	161.72	433.24	27.93	6.00	47.00	239.00	20.95	94.30	3.16	79.84	1.24	8.18
PMS 37 B	75.00	144.43	9.60	315.30	617.51	23.33	6.70	79.10	139.00	23.32	99.60	3.27	79.99	1.27	8.16
C 43	74.00	137.07	10.93	430.10	721.28	30.13	7.10	72.20	216.10	27.86	112.00	2.71	86.74	1.97	8.05
RS 29	77.33	167.87	10.73	189.29	455.26	22.80	7.20	79.80	195.60	27.96	134.60	3.09	79.85	1.78	8.10
IB 12	79.33	128.07	10.73	431.78	670.64	28.73	6.10	79.60	248.60	26.76	101.00	3.70	77.84	1.43	8.06
KR 191	79.66	158.07	10.93	337.50	583.95	28.33	7.00	71.60	251.10	28.73	104.60	2.88	83.18	1.63	8.08
KR 192	75.00	177.80	9.86	327.60	647.53	27.43	7.00	84.40	189.30	27.38	110.00	3.11	86.07	1.52	8.12
KR 196	69.66	163.20	10.40	351.92	610.32	30.00	7.10	75.40	294.00	28.00	99.00	2.94	83.98	2.07	8.09
KR 199	74.00	162.00	10.30	319.67	616.83	29.20	7.70	87.30	265.60	28.00	107.30	3.08	84.22	1.81	8.10
KR 200	75.00	153.67	9.86	352.02	638.75	24.33	6.90	86.40	260.30	29.22	103.30	2.91	83.21	1.71	8.14
SE	0.91	2.60	0.35	20.85	26.60	0.50	0.12	2.25	5.84	0.44	6.02	0.032	0.33	0.017	0.037
CD	2.53	7.21	0.98	57.70	73.60	1.31	0.34	6.23	16.18	1.22	16.66	0.09	0.93	0.048	0.10

Table 2. Heterosis over mid parent (MP) and over better parent (BP) for different characters.

Crosses	50% flowering		Plant height		Number of leaves		Area of flag leaf		Area of 3rd leaf	
	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP
PM 5 9 A x KR 196	-5.03**	-0.94	7.07	15.95	-2.00	-5.76	30.60**	13.99	34.90**	27.90**
PMS 7 A x KR 199	-10.52**	-8.10**	-9.77**	-4.20	-1.12	-4.27	9.52	8.88*	8.17	7.62
PMS 28 A x KR 43	-6.95**	-3.60*	21.70	25.43	1.79	0.0	-29.01	-51.16	-9.54	-27.60
PMS 28 A x KR 191	-5.65**	-5.45**	4.63	9.16	9.61*	7.67**	12.92	-16.48	32.67**	15.55*
PMS 7 A x KR 191	-6.12**	-5.12*	-7.49**	-3.05	6.97	3.00	-4.10	-7.10	-4.87	-7.86
PMS 7 A x KR 196	-4.74**	0.96	-12.29**	-6.51	-12.80	-15.18	18.58*	12.5	9.17	8.05
PMS 28 A x RS 29	-3.82	-2.58**	36.72	47.24	-0.90	-1.76	100.99**	86.35**	50.76**	47.12**
PMS 7A x RS 29	-4.72**	-4.30*	3.73	12.25	-7.40	-8.54	58.70**	26.9**	25.17**	9.30
PMS 37 A x RS 29	-3.72**	-5.33**	3.79	12.21	-7.52	-12.39	51.23**	21.01*	25.81**	9.28

PMS 7 A x KR 200	-5.88**	-4.00**	2.50	15.87	-0.95	-6.09	-8.75	-13.44	9.07	7.73
PMS 8 A x KR 200	-9.95**	-8.29**	9.35	12.41	3.64	0.66	14.50	-9.30	16.36**	5.83
PMS 28 A x IB 12	-7.56**	-7.56**	14.84	-22.64	-5.16	-7.67	-6.53	-35.76	15.94**	-4.57
PMS 28 A x KR 200	-9.28**	-6.66**	5.14	8.11	0.04	-6.44	21.38*	-11.42	32.46**	11.15
PMS 8 a x KR 192	-10.40**	-8.75**	-4.20	-6.41	-8.46	-11.08	23.35*	0.44	24.50**	12.5**
SE ±	1.12	1.29	3.19	3.68	04.3	0.50	25.53	29.48	32.58	37.62
CD at 5%	2.19	2.52	6.25	7.21	0.84	0.98	50.03	57.78	63.85	73.53

Crosses	Ear head breadth		Grain yield		Fodder yield		1000 seed wt.		Ear head weight	
	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP
PM 5 9 A x KR 196	14.49**	11.26**	19.03**	1.59	23.75**	3.74	5.35**	-1.71	49.03**	32.92**
PMS 7 A x KR 199	-4.63	-6.49	-4.26	-6.07	-6.07	-19.29	-4.93	-5.18	-8.27	-13.90
PMS 28 A x KR 43	22.13**	12.67**	55.20*	28.11**	6.92	1.79	17.59**	3.01	100.29**	84.46**
PMS 28 A x KR 191	20.00**	11.42**	15.00**	-4.74	37.33**	34.02**	0.0	-13.53	163.44**	150.47**
PMS 7 A x KR 191	-4.16	-6.75	3.34	-4.28	-10.23	-24.62	-2.39	-3.37	19.87	11.20
PMS 7 A x KR 196	-17.24	-18.91	-19.07	-23.21	-25.33	-42.91	-4.64	-4.90	-21.68	-29.14
PMS 28 A x RS 29	12.12**	2.77	21.76**	-3.25	71.82**	45.43**	5.00*	-8.15	38.57**	17.83**
PMS 7A x RS 29	9.58**	8.10**	9.27**	6.54	30.21**	-0.43	-1.01	-13.49	44.25**	37.66**
PMS 37 A x RS 29	3.54	1.38	4.09	3.63	43.63**	22.85**	8.93**	1.07	33.47**	16.12**
PMS 7 A x KR 200	-13.28	-16.21	-43.19	-43.98	-16.93	-2.16	-1.62	-3.42	-13.74	-20.44
PMS 8 A x KR 200	2.94	1.44	-2.5	-14.93	-11.56	-15.67	-7.74	-8.28	40.94**	25.45**
PMS 28 A x IB 12	14.04**	13.11**	36.17**	8.29*	5.82	3.78	11.92**	-0.22	58.73**	53.46**
PMS 28 A x KR 200	20.93**	13.04**	29.23**	-0.23	37.91**	32.27**	13.13**	-2.87	31.17**	25.45**
PMS 8 a x KR 192	3.64**	5.97	-0.93	-11.96	-0.23	-17.21	3.69	1.00	64.00**	42.09**
SE ±	0.15	0.17	2.75	3.18	7.15	8.26	0.54	0.62	7.37	8.51
CD at 5%	0.29	0.33	5.39	6.23	14.01	16.18	1.05	1.21	14.44	16.67

Crosses	Total Chlorophyll content		Relative water content		Free sugar content		Protein content		Ear head length	
	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP
PM 5 9 A x KR 196	0.16	-4.6	-7.20	-9.40	-16.93	-37.19	1.80**	1.35*	26.18	20.00**
PMS 7 A x KR 199	-6.58	-11.59	-9.12	-6.80	-21.31	-33.71	0.61	0.24	-0.01	-1.55
PMS 28 A x KR 43	16.86**	8.54**	-0.88	-4.83	45.79**	18.78**	-0.67	-1.46	30.12**	25.45**
PMS 28 A x KR 191	1.9	-2.53	0.87	-1.15	-8.71	-19.63	3.93**	3.30**	27.72**	26.82**
PMS 7 A x KR 191	-13.11	-20.28	3.75**	3.34**	11.11**	-7.97	578**	5.26**	4.00	0.89
PMS 7 A x KR 196	-63.05	-31.88	-3.62	-4.46	-8.91	-30.91	6.46**	6.00**	-7.10	-7.30
PMS 28 A x RS 29	15.52**	14.24**	4.35**	4.34**	-9.27	-23.03	7.73**	7.21**	-3.80	-12.63
PMS 7A x RS 29	8.25**	2.60	1.53**	-0.10	0.35	-19.66	6.39**	6.00**	10.82**	-2.65
PMS 37 A x RS 29	11.63**	7.03**	0.91*	0.82	-18.03	29.77	1.10*	0.73	16.45**	15.13
PMS 7 A x KR 200	13.52**	4.63**	1.27**	0.85	10.79**	-9.94	1.59**	1.47*	2.31	-7.53
PMS 8 A x KR 200	22.78**	4.86**	-5.26	-8.41	10.20**	-5.26	0.79	0.36	22.32**	8.76**
PMS 28 A x IB 12	1.74	-5.67	9.28**	7.91**	1.87	-20.46	0.73	0.0	26.82**	25.06**
PMS 28 A x KR 200	23.55**	18.67**	2.23**	0.16	-0.33	-14.03	-0.61	-0.85	40.56**	31.50**
PMS 8 a x KR 192	16.56**	11.43**	-4.03	-8.64	-5.45	-14.47	-0.79	-1.33	14.02**	7.03**
SE $\pm$	0.038	0.044	0.41	0.47	0.021	0.024	0.046	0.053	0.61	0.71
CD at 5%	0.074	0.086	0.80	0.92	0.041	0.047	0.09	0.10	1.19	1.39

- and ** indicate significance at 5 and 1 per cent respectively

Table 3. Range of mid parent heterosis and better parent heterosis for yield and other characters and number of hybrids showing significant heterosis.

Characters	Range		No of hybrids showing desirable significant heterosis	
	MP	BP	MP	BP
1. Days to 50 % flowering	-10.52 to 3.72	-8.75 to -8.29	13	11
2. Plant height	-12.29 to 36.72	-6.51 to 47.24	3	4
3. Number of leaves per plant	-8.46 to 9.61	15.18 to 7.67	1	-
4. Flag leaf area	-29.01 to 100.99	-51.16 to 86.35	7	4
5. Third leaf area	-9.54 to 50.76	-27.00 to 47.12	9	4
6. Ear head length	-7.10 to 40.56	-12.63 to 31.5	8	7
7. Ear head bread	-17.24 to 22.13	-16.21 to 13.11	8	6
8. Grain yield / plant	-43.19 to 55.20	-43.98 to 28.11	7	2
9. Fodder yield per plant	-25.33 to 71.82	-42.91 to 45.43	6	4
10. Thousand seed weight	-7.74 to 17.59	-13.53 to 30.1	6	1
11. Weight of ear head	-21.68 to 163.44	-29.14 to 150.47	10	10
12. Total chlorophyll content	-63.05 to 23.55	-31.88 to 18.67	8	7
13. Relative water content (%)	-9.12 to 9.28	-9.4 to7.91	7	3
14. Free soluble sugar content	-21.31 to 45.79	-37.19 to 18.78	4	1
15. Protein content of seed	-0.79 to 7.73	-1.46 to7.21	8	7

Table 4. Crosses having high average heterosis and hetrobeltiosis for different characters are as follows

Sl. No	Characters	Crosses
1	Days to 50 per cent flowering	PMS 28 A x IB 12
2	Plant height	PMS 7A x KR 196, PMS 7A x KR 199 and PMS 7 A x KR 191
3	Number of leaves/plant	PMS 28A x KR 191
4	Area of flag leaf	PMS 7 A x RS 29
5	Area of 3rd leaf	MS 9A x KR 196
6	Ear head length	PMS 28 A x C 43, PMS 28 A x KR 200
7	Ear head breadth	PMS 28 A x C 43 and MS 9A x KR 196
8	Grain yield/plant	PMS 28 A x C 43 and PMS 28 A x KR 200
9	Fodder yield/plant	PMS 28 A x RS 29 and PMS 28 A x KR 200
10	1000 seed weight	PMS 28 A x C 43 and PMS 28 A x KR 200
11	Weight of ear head	PMS 28 A x C 43 and PMS 28 A x KR 191
12	Chlorophyll content	PMS 28 A x C 43, PMS 28 A x KR 200, and PMS 8 A KR 192
13	Relative water content	PMS 28 A x IB 12 and PMS 7 A x KR 191
14	Free sugar content	PMS 28 A x C 43
15	Protein content	PMS 28 A x RS 29, PMS 7 A x KR 196, PMS 7 A x RS 29

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Isolation and characterization of phthalate degrading bacteria

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Abstract: *Phthalates are esters of phthalic acid (PAE) and are mainly used as plasticizers, i.e., substances added to plastics to increase their flexibility, transparency, durability and longevity. There exists many phthalates of varying chain lengths. PAE with longer alkyl chains i.e., di-(2-ethyl hexyl) phthalate (DEHP) and di-octyl phthalate (DOP) are poorly degraded under aerobic and anaerobic conditions. PAE are synthesized in massive amounts to produce various plastics and became a menace to the environment, following their release as a result of extensive usage and production. PAE are known to cause hepatotoxic, teratogenic and carcinogenic effects in humans, apart from inducing sterility and disrupting endocrine system. These environmental and health concerns demand intervention of biotechnology to develop a suitable strategy for reduction and remediation of these toxic chemicals. With this purpose, employing biochemical techniques we have isolated 9 bacteria in pure form from the sludge and soil samples, which utilize DEHP or DOP as sole carbon source. Of them, six isolates were capable of degrading DOP and three degrading DEHP.*

INTRODUCTION

Phthalates (phthalic acid esters- PAEs) are used in a large variety of products, from enteric coatings of pharmaceutical pills and nutritional supplements to viscosity control agents, gelling agents, stabilizers, dispersants, lubricants, emulsifying agents and suspending agents. End applications include: adhesives and glues, building materials, personal care products, medical devices, detergents and surfactants, packagings, children's toys, modeling clay, waxes, paints, printing inks and coatings, pharmaceuticals, food products and textiles. The most widely-used phthalates are the di-2-ethyl hexyl phthalate (DEHP), the diisodecyl phthalate (DIDP) and the diisononphthalate (DINP). DEHP is the dominant plasticizer used in polyvinyl chloride (PVC), due to its low cost (Ling *et al*., 2008).

When used as plasticizers, phthalates are not chemically bonded to the plastic polymer and can therefore eventually migrate from the plastics into the environment. When the environment (air, oil water, blood, *etc*.) has a very high affinity for the plasticizer, the migration rate is dependent upon the ability of the plasticizer to diffuse through the resin to the attracting media (Graham, 1973).

Release of phthalates into the ecosystem or wastewater effluent occurs during the production phase and *via* leaching and volatilization from plastic products during their usage and after disposal (Chen *et al*., 2007). Potential adverse health effects of diisononyl phthalate (DINP) are due its migration from children's toys during mouthing activities, and concern has focused on their potential chronic effects on the kidney and liver (Matsumoto *et al*., 2008**).** Phthalate exposure can be through direct use or indirectly through leaching and general environmental contamination. Diet is believed to be the main source of DEHP and other phthalates in the general population. Fatty foods such as milk, butter, and meats are a major source. Low molecular weight phthalates such as di-2-ethyl phthalate (DEP), dibutyl phthalate (DBP), *etc*., may dermally be absorbed.

A number of studies on biodegradation of phthalates in natural water, wastewater and soil have been known to date. Many bacteria have been isolated from rivers, soil, and even marine regions for their ability to degrade phthalate both aerobically and anaerobically. To date it is well known that PAE with shorter alkyl chains (*i.e*., DEP, DBP) are very easily degraded, while PAE with longer alkyl chains (*i.e*., di-octyl phthalate (DOP), DEHP) are poorly degraded under aerobic and anaerobic conditions; and it is also confirmed that a correlation exists between increasing length of the ester side chain and decreasing biodegradability (Chen *et al*., 2007). Phthalates and their metabolites have been found to be potentially harmful for human and environment due to their hepatotoxic, teratogenic and carcinogenic characteristics (Liang *et al*., 2008).

Numerous studies have demonstrated that microorganisms play major roles in phthalate degradation in the environment. It was reported that complete degradation of phthalates can be achieved at a concentration as high as 2.6 g/l in 48h using *Comamonas acidovorans* strain FY-1 isolated from activated sludge .Two consortia using DEP as a substrate showed capability of degrading 500 mg/l dimethyl phthalate (DMP) to monomethyl phthalate (MMP) and phthalate as the intermediate before cleavage of the aromatic structure (Wang *et al*., 2003). The degradation pathways of phthalates will help to understand their materialization process and the toxicological behaviour of their metabolites. Generally, the phthalate biodegradation pathways consist of two processes: primary biodegradation from phthalate diesters to phthalate monoesters and then to phthalic acid and ultimate biodegradation from phthalic acid to CO_2 or CH_4 (Staples *et al*., 1997). Until the problem of endocrine disruption was proposed, the studies of phthalate esters degrading microorganisms were mainly focused on wastewater treatment and some microorganisms have been isolated for use in cleansing the phthalate ester contaminated wastewater. In the light of the new status of phthalate ester as endocrine disruptors, there is a current need for a technology for the reduction and remediation of these chemicals. For this purpose, isolation and characterization of microorganisms that utilize DEHP and DOP as their sole carbon source have been focused in this study (Nakemiya *et al*., 2005).

MATERIALS AND METHOD

Sample collection

Soil samples were collected from 10cm depth in $1m^2$ chosen areas. All the areas from where the samples collected were heavily contaminated with plastics. Samples were collected aseptically with the aid of sterile spatula, scalpels, gloves and plastic bottles and were fully labeled with description and date. Samples are representatives of a site. A total of five samples were collected during the months of March and April, 2009 which included samples from the following areas, near Canoly Canal (AKP), Calicut; Terumopenpol Limited, Puliyarakonam (ATP, BTP), Trivandrum (manufacturer of blood bank equipment and blood bags); and Njelianparambu (ANP,BNP) Calicut. Collected samples were used for the isolation and characterization of phthalate-degrading bacteria.

Screening for phthalate-degrading bacteria

Samples were examined immediately after they were brought to the laboratory. The samples were plated after serial dilution (up to 10^{-6} times). Pure cultures were obtained after repeated streaking and subculturing. By using the pure cultures, various staining and biochemical tests were conducted.

Serial dilution

Approximately 1g soil sample was suspended in 9 ml of sterile water with shaking. Serial dilution was made using standard protocol.

Spread plate technique

After the serial dilution of the soil samples collected from Canoly Canal and Trivandrum, 0.1ml of each sample was added to petri plates containing autoclaved solidified basal medium (1.0 g K_2HPO_4, 1.0 g NaCl, 0.5 g NH_4Cl, 0.4 g $MgSO_4$/l distilled water). DEHP and DOP were used as the carbon source. The concentration of DEHP and DOP added to the plates were 50µl. The pH was adjusted to 7. 2 (Chen *et al*., 2007). The plates were incubated at 37 ^oC in incubator.

Streak plate technique

Standard procedure was employed for the streak plate technique. Each Petri plate was observed for the growth of bacteria into colonies. Discrete colonies farthest away from the streak were selected for further studies.

Table 1. Descriptions of the isolates obtained by the culturing of the five samples collected.

Sl. No.	Isolates	Colony characters observed	Days taken for the appearance of colonies
1	AKP	Pinpoint, white, spherical colonies	3
2	ATP	Small cream coloured, slightly raised colonies	3
3	ANP	Cream coloured colonies	2
4	BTP1	Pinpoint, white colonies	3

5	BTP2	Spherical cream-white coloured colonies	4
6	BTP3	white coloured, spherical colonies	4
7	BTP4	White, spherical, raised colonies	4
8	BNP1	Cream coloured, spherical colonies.	2
9	BNP2	Cream coloured spherical colonies	2

Sub culturing

Pure colonies obtained were preserved for further studies by periodic sub culturing (bi-weekly) and maintained on agar slants. Specimens from these samples were taken for further studies.

Study of biochemical activities of microorganisms

The various biochemical tests performed for the characterisation of selected pure cultures were Gram-staining, spore-staining, starch hydrolysis, nitrate reduction test, urease test, carbohydrate fermentation, indole production test, methyl red test, Voges-Proskauer test, citrate utilisation test, hydrogen sulphide production test.

RESULTS AND DISCUSSION

The prime objective of this study was to isolate phthalate degrading bacteria. PAE with longer alkyl chains (*i.e*., DOP and DEHP) are poorly degraded under aerobic and anaerobic conditions (Chen *et al*., 2007). In this study, DEHP and DOP were used as the sole source of carbon and successfully isolated nine bacteria. Microorganisms are the only known organisms capable of completely degrading phthalate compounds. Most often, these microorganisms belong to the group of *Pseudomonas, Aspergillus, Micrococcus*, and *Bacillus* sp., and are found to be associated with the degradation of these products (Karegoudar *et al*., 1984). Six isolates capable of degrading DOP and three isolates capable of degrading DEHP were isolated from the soil. Biochemical tests were conducted with the nine isolates. Phthalate biodegradation in soil has been widely studied in the last decade (Liang, 2008). Previous studies suggest that the soil or the sludge samples used for the isolation were collected from regions heavily contaminated with plastic pollutants, *i.e*., mostly rivers and near factories (Feng *et al*., 2002, Ahn *et al.*, 2004, Chen *et al.*, 2007). Soil samples collected for the present study were also from highly plastic contaminated regions.

All the five samples collected were subjected to serial dilution, repeated spread and streak plate (Fig. 1) culture techniques. Pure cultures obtained by these techniques were subjected to Gram-staining (Fig. 2), spore staining and biochemical tests.

All the isolates were subjected to spore staining (Sherman and Cuppuccino, 1996). Three isolates were Gram positive (Table 2). Isolates showed the presence of endospores, which were ellipsoidal and centrally positioned (Table 2, and Figure 3).

Table 2. The results obtained with Gram staining of isolates from 5 samples collected from different places summarised.

Sl. No.	Sample	Gram (+ve / -ve)	Bacterial shape	Length (µm)	Width (µm)	Endo-spore (+ / -)	Spore shape	Spore position	Motility
1	AKP	- ve	Rod	0.1±1.5	0.1±0.5	-	-	-	motile
2	ATP	-ve	Rod	0.1±0.5	0.1±0.5	-	-	-	motile
3	ANP	+ve	Rod	0.2±0.5	0.2±0.5	+	E	C	non-motile
4	BTP1	+ve	Rod	1.5±1.5	1±0.5	+	E	C	motile
5	BTP2	-ve	Rod	0.5±1	1±1	+	E	C	motile
6	BTP3	-ve	Rod	0.1±2	0.1±1.5	-	-	-	motile
7	BTP4	-ve	Rod	1±0.5	1±0.5	-	-	-	motile
8	BNP1	-ve	Rod	1±1	0.5±0.5	+	E	C	non-motile
9	BNP2	+ve	Rod	0.1±0.5	0.1±0.2	+	E	C	motile

+ present, - absent, E- ellipsoidal, C-central.

All the isolates were subjected to 8 biochemical tests such as starch hydrolysis, carbohydrate fermentation test (glucose, lactose and sucrose), nitrate reduction test, urease test, indole production test, Voges-Proskauer test and citrate utilization test (Table 3) (Aneja, 1993).Test for citrate utilization was conducted to determine the ability of the bacteria to utilize citrate. The isolates that utilize citrate changed the indicator from green to blue. Five isolates showed positive results and the other 4 isolates showed negative result (Table 3, Fig. 4). Nitrate reduction test showed clear purple colour of the starch iodine paper, when immersed in culture broth (Table 3). Six isolates showed positive results and the other 3 isolates showed negative results. Test for starch hydrolysis was performed to determine the production of the exoenzyme amylase, by observing the clear zone around and beneath the bacterial growth on the agar plate flooded with Gram's iodine (Table 3; Fig. 5). Four isolates showed positive results and all the others showed negative results. Carbohydrate fermentation test was conducted and all the isolates showed negative results for lactose and sucrose fermentation. Five isolates showed positive results for glucose fermentation and the other 4 showed negative results (Table 3; Fig. 6). Urease test was conducted to determine the ability of the bacteria to produce the enzyme urease. Four isolates showed positive results and the other five isolates showed negative results (Table 3). All the isolates showed negative result for indole production, Voges-Proskauer and methyl red tests (Table 3).

Table 3: Summary of the results of eight biochemical tests performed with 9 isolates; citrate utilization test (CUT), nitrate reduction test (NRT), glucose fermentation (GF), lactose fermentation (LF), sucrose fermentation (SF), carbohydrate fermentation test (CFT), starch hydrolysis test (SHT), urease test (UT), indole production test (IPT), Voges-Proskauer test (V-PT), methyl red test (MRT).

Sl. No	Isolates	CUT	NRT	GF	LF	SF	SHT	UT	IPT	V-PT	MRT
1	AKP	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	ATP	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
3	ANP	-ve.	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
4	BTP1	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
5	BTP2	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
6	BTP3	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	BTP4	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8	BNP1	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
9	BNP2	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve

A gram positive *Microbacterium* sp. strain CQ0110Y with round morphology, smooth and glossy surface and yellowish colouring was isolated by Chen *et al*. (2007). Similar results were shown by the isolates ANP, BNP1 and BNP2 (Table 2). The morphological tests conducted in the nine isolates revealed that four isolates (AKP, ATP, BTP2, BNP2) are gram positive rods, and all of them produced endospore that are ellipsoidal in shape and centrally positioned.

Another gram positive, motile and rod *Bacillus* strain T-36 was isolated from sea mud in the Tagonoura harbor, Japan (Sakagami, 1982). The spores were ellipsoidal and central or paracentral. The growth on agar slants was abundant, rough, opaque, dull, waxy, spreading and off white. The growth in broth produced uniform turbidity. It produced acid but no gas from glucose, the pH in cultured glucose broth was 7.6 after 7 days incubation. Citrate was utilised and nitrite was produced from nitrate, but gas was not produced from nitrate broth under anaerobic conditions (Sakagami, 1982). Similarly various biochemical tests conducted in the nine samples revealed that, seven isolates (AKP, ATP, ANP, BTP1, BTP2, BTP4 and BNP1) reduced nitrates to nitrites (Fig. 7). Four isolates (AKP, BTP1, BTP2 and BTP3) utilized citrate (Fig. 6). Starch was hydrolyzed by four isolates (ATP, ANP, BTP1 and BTP2) (Fig. 5). Five isolates (BTP1, BTP3, BTP4, BNP1 and ANP) produced acid from glucose but no gas (Fig. 6). Five isolates (ATP, ANP, AKP, BTP1 and BTP2) are endospore forming. Endospores produced are ellipsoidal,

centrally positioned (Fig. 3). Seven isolates (AKP, ATP, BTP1, BTP2, BTP3, BTP4 and BNP2) are motile. In summary, out of nine isolates, three were DEHP degrading and the remaining six were DOP degrading bacteria. This preliminary information need to be confirmed by rDNA PCR.

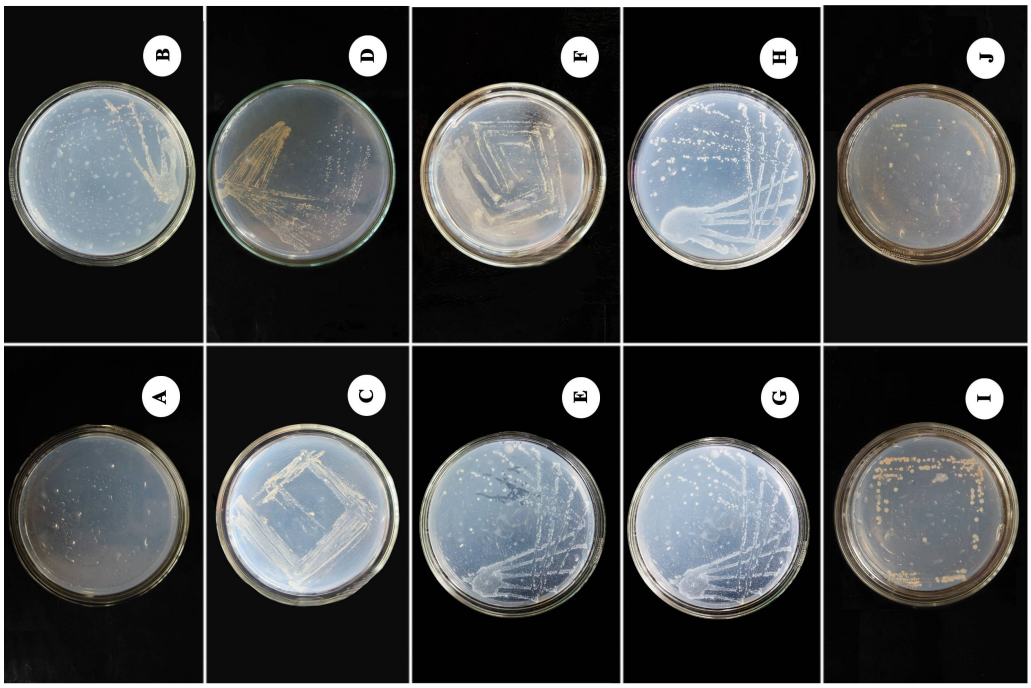

Fig. 1: Streak plate method of samples collected from different places, viz.; A.Control;B.Kanoli soil(AKP);C.TVM(ATP);D.Njeliyanparambu soil(ANP);E. TVM (BTP 1); F. TVM (BTP 2); G. TVM (BTP 3); F. TVM (BTP 4); I. Njeliyanparambu (BNP 1); J. Njeliyanparambu (BNP 2).

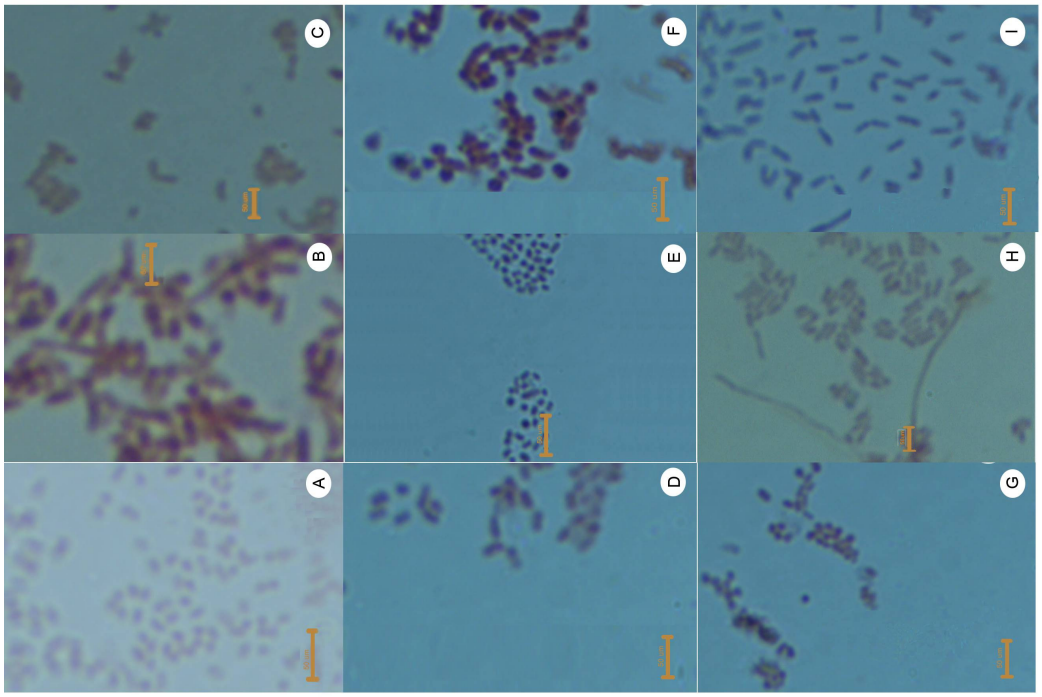

Fig. 2: Gram staining of sample collected from different places. A. Kanoli (AKP); B. TVM (ATP); C. Njeliyanparambu (ANP); D. TVM (BTP1); E. TVM (BTP2); F. TVM (BTP3): G. TVM (BTP4); H. Njeliyanparambu (BNP1); I. Njeliyanparambu (BNP2).

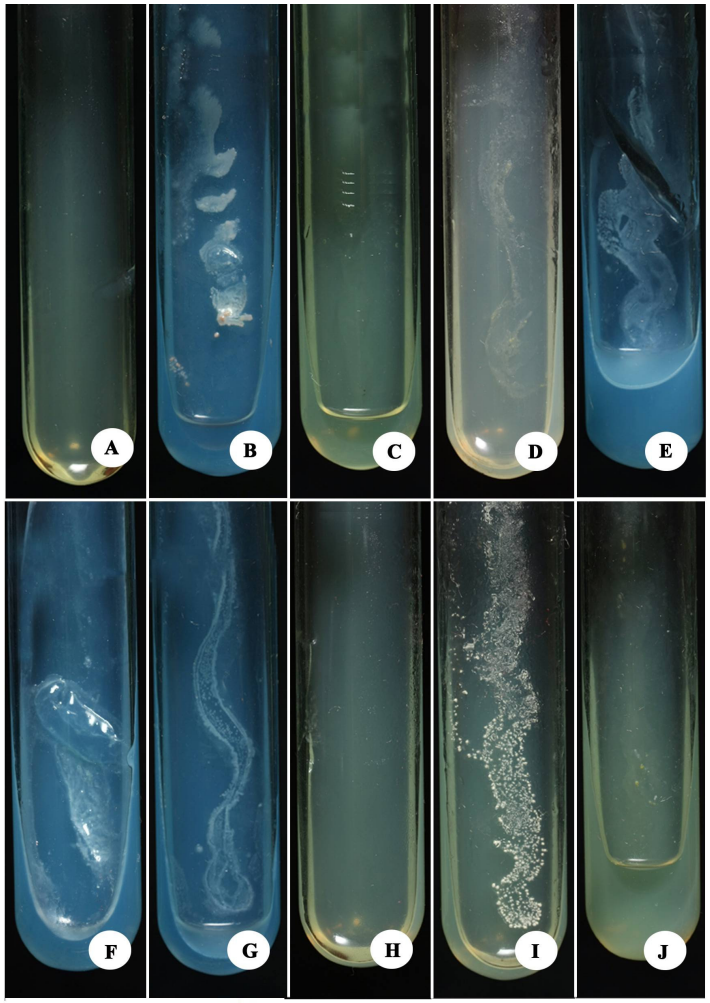

Fig. 4: Citrate utilization test of samples collected from different places viz., **A**. Control; **B**. Kanolisoil(AKP); C. TVM(ATP); D. Njeliyanparambu soil(ANP);E.TVM(BTP1);F.TVM(BTP2);G.TVM(BTP3);H.(BTP4);I.Njeliyanparambu soil(BNP1);J.Njeliyanparambu soil(BNP2).

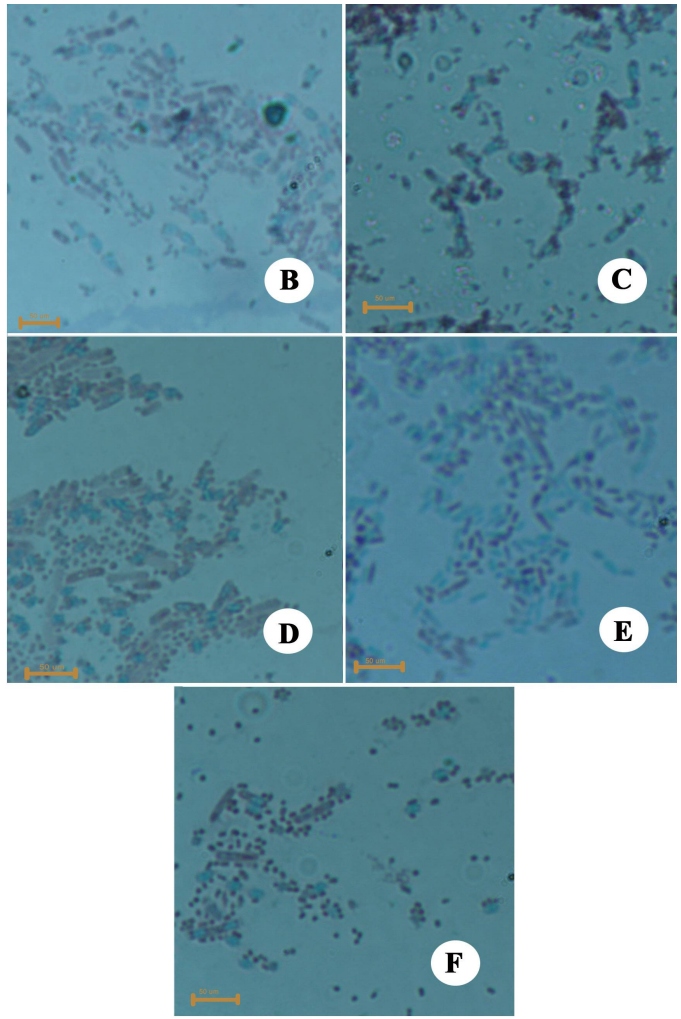

Fig. 3: Spore staining of samples collected from different places, viz.; A.Control;B. Kanoli soil(AKP);C.TVM soil(ATP);D.Njeliyanparambu soil(ANP);E.TVM soil(BTP1);F.TVM soil(BTP2).

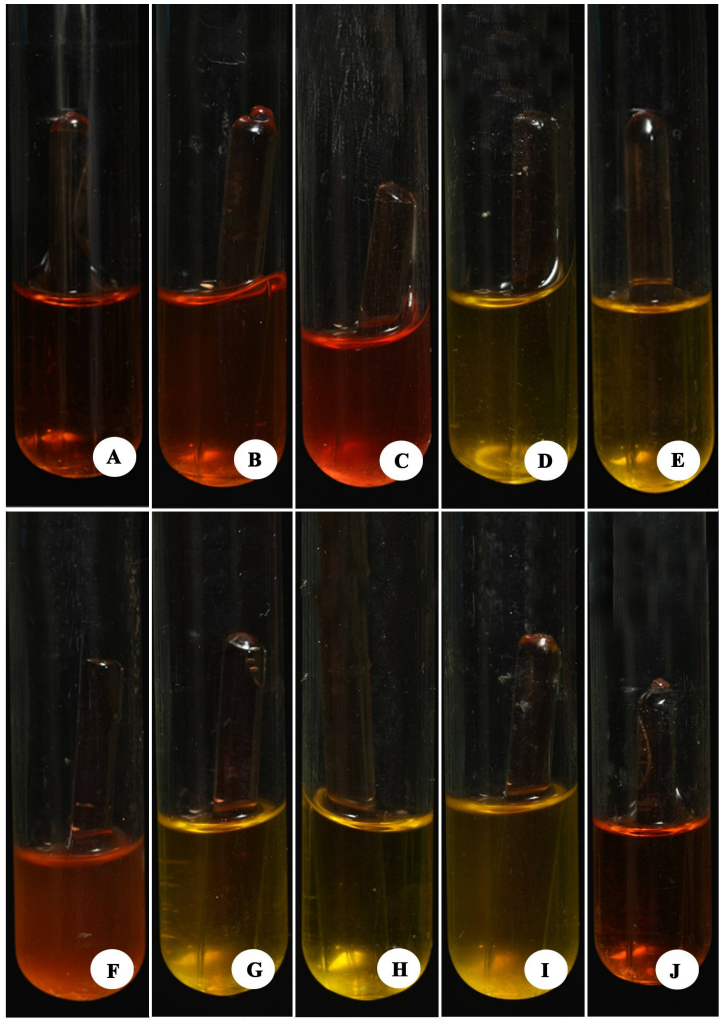

Fig. 6: Glucose fermentation of samples collected from different places viz., A. Control; **B.Kanoli soil(AKP);C.TVM soil(ATP);D.Njeliyanparambu soil(ANP);E.TVM soil(BTP1);F.TVM soil(BTP2);G.TVM soil(BTP3);H.TVM soil(BTP4);I.Njeliyanparambu soil (BNP1);F.Njeliyanparambu soil(BNP2).**

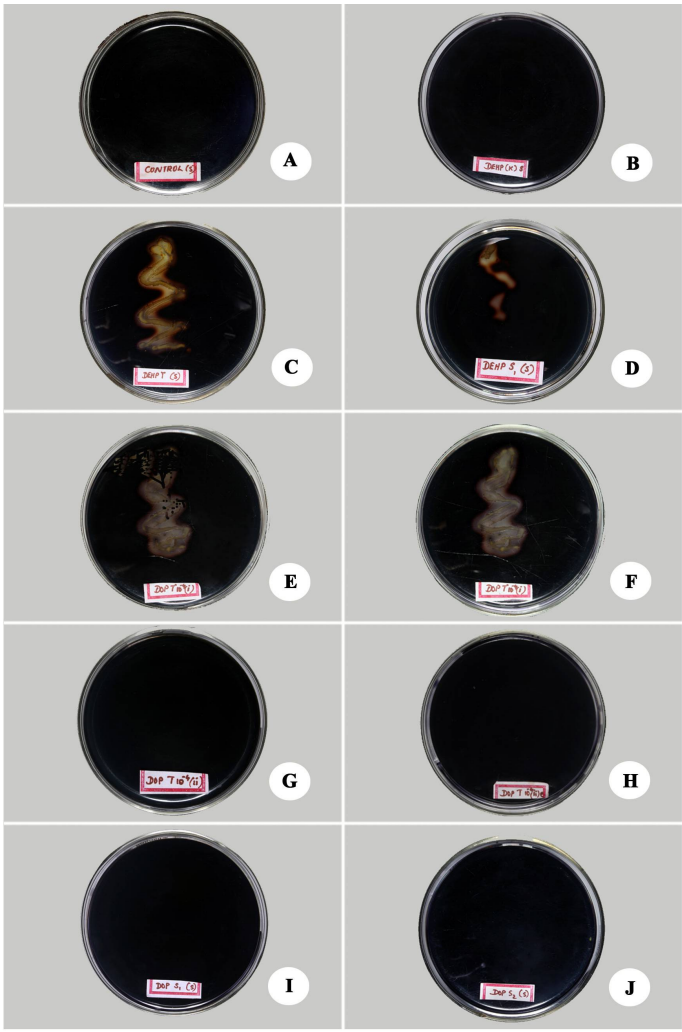

Fig. 5: Starch hydrolysis test of samples collected from different places, viz.; A.Control;B.Kanoil soil(AKP);C.TVM(ATP);D.Njeliyanparambu soil(ANP);E. TVM (BTP 1); F. TVM (BTP 2); G. TVM (BTP 3); F. TVM (BTP 4); I. Njeliyan-parambu (BNP 1); J. Njeliyanparambu (BNP 2).

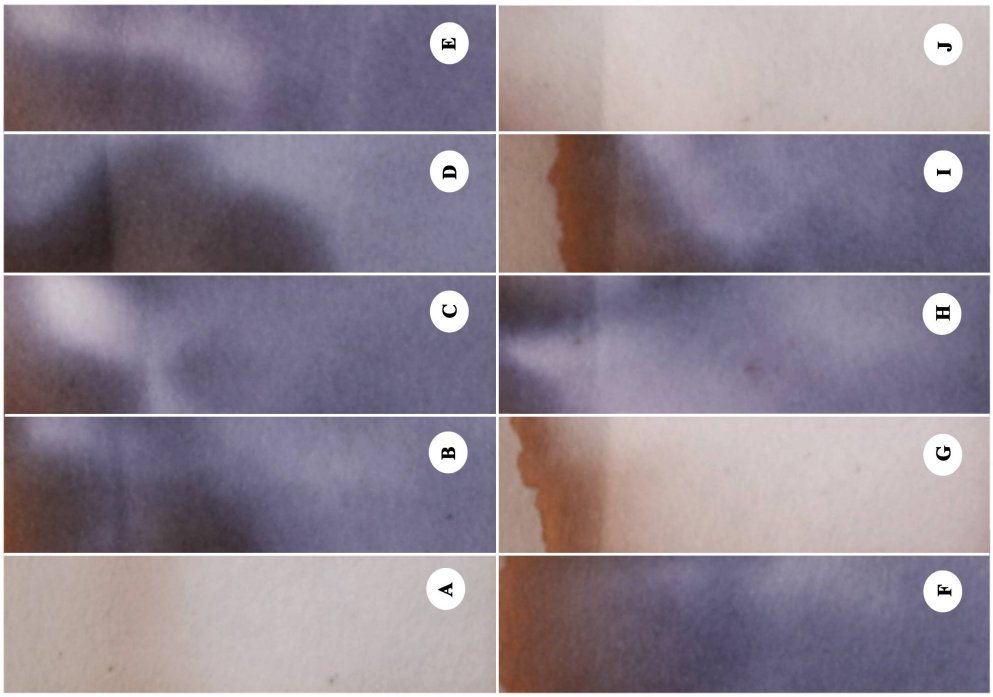

Fig. 7: Nitrate reduction test of samples collected from different places viz., **A**.Control;**B**.Kanolisoil(AKP);C.TVMsoil(ATP);D.Njeliyanparambu soil(ANP);E.TVM(BTP1);F.TVM(BTP2);G.TVM(BTP3);H.(BTP4);I.Njeliyanparambu soil(BNP1);J.Njeliyanparambu soil(BNP2).

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