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FOREWORD

The present issue of "Capsicum and Eggplant Newsletter" includes 39 scientific contributions, written by 107 Authors from 18 countries (Belarus, Belgium, Brazil, Bulgaria, China, Egypt, Hungary, India, Indonesia, Italy, Jordan, Japan, Korea, Moldova, Nepal, Spain, Tunisia and United Kingdom).

As in the past, we have not modified the accepted contributions. Therefore, the authors, not CENL, are responsible for the scientific content of their reports.

As usual, we would like to remind our readers that this Newsletter is highly dependent on the financial support of the recipients. Therefore, a subscription fee is highly appreciated. The subscription fee has not changed: 30 EURO for normal and 150 EURO for supporter subscribers. Remember that to make the payment less time-consuming and to reduce bank costs, we have introduced the option of a 3-year subscription. It is possible (and encouraged!) to order your own personal copy to hasten its delivery to you. Just fill in the order form on page 165 and send it to us, together with a copy of the payment order that must be made out to **Eucarpia**. In case you decide to pay by credit card, please use the voucher on page 167. Because **the cost to cash cheques is very high, you are kindly requested to avoid this method of payment: credit card payment is highly preferred**.

The deadline for submission of articles to be included in the next issue of the Newsletter (No. 23, 2004) is **February 29, 2004**. Please note that **contributions will be accepted only if submitted through electronic mail** (as attached file) **or on computer disk.** Suitable formats are as follows: operating system Windows 95-98-2000; word processing systems Word. **Please, note that the EUCARPIA Secretary is now in Vienna: you can find the address and banking directions on page 3 of this volume.**

We regret to report that several papers had to be rejected because of a lack of scientific rigor. Therefore, we would like to remind everyone that submitted articles must deal with genetics and/or breeding of pepper or eggplant. Reports on cultural practices, fertilisation, spacing studies, etc., will not be accepted. Most of the rejected articles had poor English grammar and syntax. Please, before submitting a manuscript, have it proofed by someone capable of editing it in the English language. **It is imperative that you follow the submission instructions very carefully. Otherwise your contribution will not be accepted.**

Luciana Quagliotti
(Director)

Piero Belletti
(Editor)

Turin, 30 June 2003

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GENETIC VARIABILITY AND PHYLOGENETIC ANALYSIS OF BRAZILIAN SPECIES OF *Capsicum*

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Introduction

The genus *Capsicum* includes approximately 20 to 25 species, all originated from the American continent. This estimate is, however, only approximate and it is possible that new species are discovered when germplasm collection expeditions in the wild are undertaken. Only five species of *Capsicum* are largely cultivated (*C. annuum, C. baccatum, C. chinense, C. frutescens* and *C. pubescens*) while the great majority are not yet domesticated. There are many taxonomic proposals for the wild species of the genus, with considerable controversies to be addressed (Prince et al., 1995). The taxonomic and genetic variability studies of *Capsicum* species native to Brazil have been based, mainly, on morphological characters, interespecific crossings and fertility of interspecific hybrids. Little is known about the Brazilian wild species, their genetic variability and phylogenetic relationships. The biological knowledge of this germplasm is important for the understanding of the genus in its totality. This knowledge is also fundamental for the development of appropriate strategies of germplasm collection and conservation. Additionally, it is possible that the wild germplasm harbors genes of agronomic importance, such as those related to disease resistance, yield and fruit quality.

Recently, a collection expedition to the Atlantic forest in Eastern Brazil and Roraima state sampled wild populations of *Capsicum* in areas prone to high anthropic pressure. The main objective of the study described here was to analyze the genetic variability and phylogenetic relationships at the DNA level of a sample of the new accessions in comparison with wild and cultivated *Capsicum* genotypes maintained in a germplasm collection.

Material and Methods

Genetic Material: Biological samples of 92 accessions of wild and cultivated *Capsicum* species were used for DNA extraction, including samples of 26 new wild *Capsicum* accessions recently collected in the Atlantic Forest and Roraima state. Part of the accessions were analyzed with morphological descriptors and tentatively classified at the species level. Additionally, four species were used as outgroups in the study: *Atropa belladona, Lycopersicon esculentum, Nicotiana tabacum and Physalis spp.*

DNA polymorphism analysis with RAPD markers: The genomic DNA was isolated from young leaves according to Doyle and Doyle (1987). The PCR conditions followed the protocol described by Ferreira e Grattapaglia (1998). Each 10 μl of reaction contained: 10 mM Tris-HCl pH 8,3, 1,5 mM $MgCl_2$, 1,0 μg/μl of BSA, 0,2 mM of each dNTP, 0,4 μM primer, 7,5 ng of genomic DNA and 1 unity of Taq polymerase.

Phenetic Analysis: Random amplified polymorphic DNA data were used to construct a binary matrix based on the presence or absence of the amplified fragment after separation of PCR products in electrophoresis gels. A genetic similarity analysis among accessions was performed using the Jaccard coefficient, and the resultant similarity matrix was used to group genotypes through UPGMA analysis (Sneath and Sokal, 1973). Dendrograms were produced using the NTSYS-pc 2.0 package for numerical taxonomy (Rohlf, 1992)

Cladistic Analysis: The character state was the fragment being either present in an accession (coded as 1) or absent (coded as 0). The final data set, therefore, consisted of a binary data matrix of the presence and absence of restriction fragments. Phylogenetic analysis was performed using PAUP 3.1.1 (Phylogenetic Analysis Using Parsimony; Swofford, 1993) by unweighted maximum

parsimony, considering each character as equally weighted and unordered. For tree searches Tree Bisection-Reconnection (TBR) branch swapping was selected with ACCTRAN (accelerated transformation) optimization. PAUP was also used for computing consensus trees (strict, semi-strict and 50% majority rule), performing bootstrap analysis to assess the degree of support for each branch and computing character statistics such as consistency index (CI) and retention index (RI).

Results and Discussion

Seventeen highly informative RAPD primers (Operon® primers AA1, AB8, AB9, AB11, AB17, C16, F13, O4, O7, O11, R1, R14, Y18, W4, W13, W19 e Z6) selected in previous experiments were used in the evaluation of the 96 accessions. A total of 277 polymorphic RAPD markers were used to analyze the 92 wild and cultivated *Capsicum* accessions and the four outgroup species. The phenetic and cladistic analyses (Figures 1 and 2) resulted both in dendrograms that are in general, compatible with the known *Capsicum* phylogeny (Prince et al., 1995). The cladistic analysis resulted in 20100 trees with maximum parsimony with length of 703 steps and CI=0,393 and RI=0,890. The clades were well defined and with good statistical support as indicated by the indices (CI e RI) and by the bootstrap values described in the tree.

The accessions were divided into two main groups: one group containing the cultivated, semi-domesticated and wild species collected in the Atlantic Forest and Roraima state and a second group with the outgroup species clustered.

The first group was divided in two subgroups. In the first subgroup, the *C. annuum, C. frutescens* e *C. chinense* accessions presented high relative similarity, while the *C. baccatum* e *C. baccatum* var. praetermissum accessions constituted another subgroup. Three accessions (94,95 e 96), recently collected in Roraima state and classified based on morphological and botanical descriptors as *C. chinense*, grouped with accessions of *C. chinenese* species (Figures 1 e 2). The second subgroup included the remaining accessions collected in the Brazilian Atlantic Forest, which clustered with samples of other wild accessions such as *C. buforum*, *C. villosum*, *C. dusenii*, *C. parvifolium*, *C. campilopodium*, *C. fexuosum* e *C. chacoense*. The phenetic analysis suggested a subgroup composed of *C. flexuosum* e *C. chacoense* accessions, what was not corroborated by the cladistic analysis. Similarly, *C. villosum* and *C. buforum* accessions formed a subgroup that in the phenetic analysis was genetically similar to the newly collected sp1, sp2 e sp3, but that clustered with sp4, sp6, sp7, sp8 e sp9 based on the cladistic analysis. However, both analysis indicated that the accessions sp1, sp2 e sp3 were genetically similar. The data obtained in this study allowed the estimate of genetic diversity and the establishment of phylogenetic relationships of the germplasm analyzed. Complementary studies of cpDNA sequence divergence are currently underway to further investigate the existence of new *Capsicum* species within the recently collected accessions.

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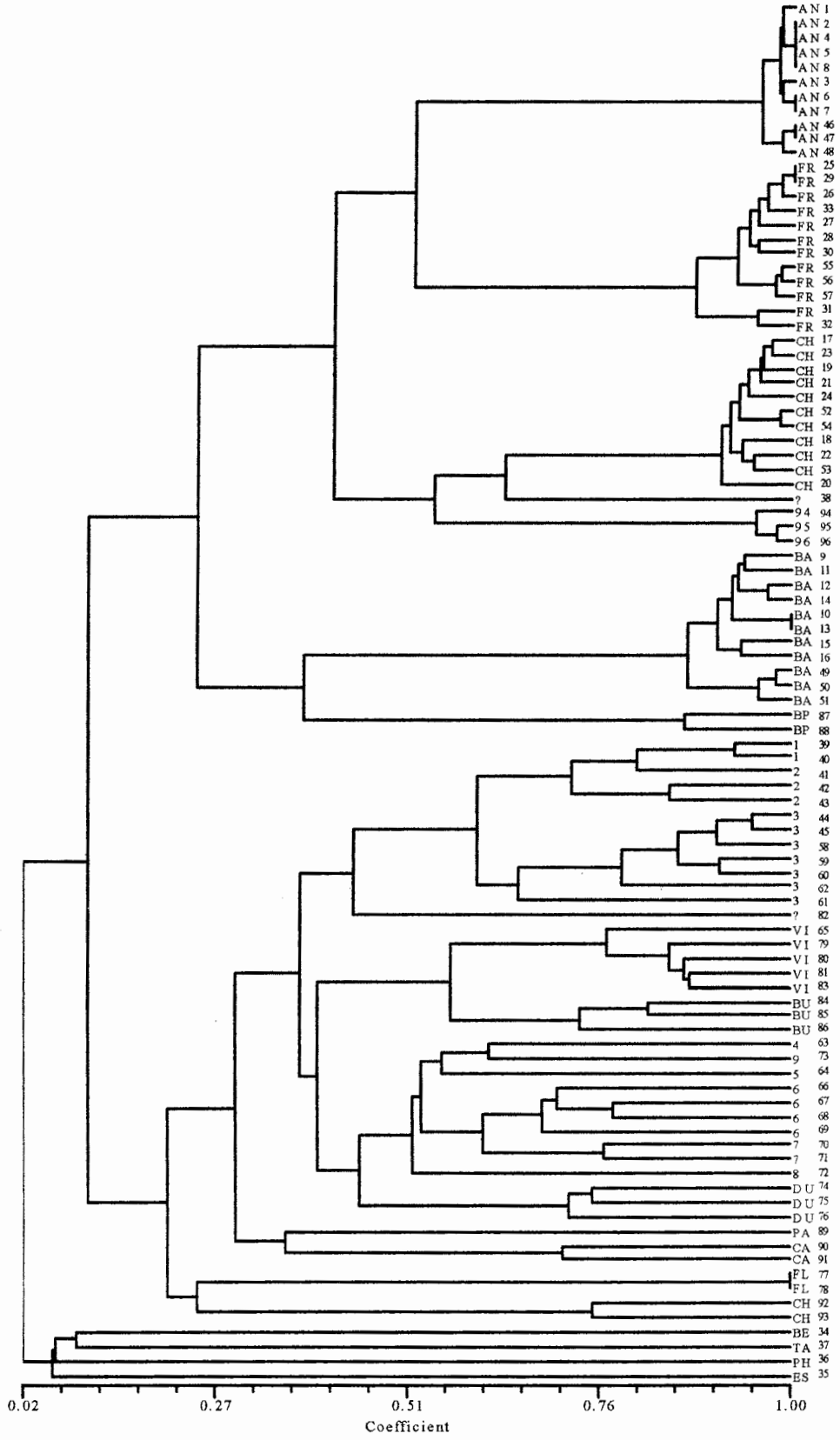

Figure 1. Genetic similarity of 92 wild and cultivated *Capsicum* accessions based on phenetic analysis of DNA fragment polymorphisms randomly amplified (RAPD). The dendrogram was constructed through the use of Jaccard coefficient and UPGMA. AN=*C. annuum*; FR= *C. frutescens*; CH= *C. chinense*; BA= *C. baccatum*; BP= *C. baccatum* var. praetermissum; 1,2,3,4,5,6,7,8,9,94,95,96= not described species; VI= *C. villosum*; BU= *C. buforum*; DU= *C. dusenii*; PA= *C. parvifolium*; CA= *C. campilopodium*; FL= *C. flexuosum*; CH= *C. chacoense*; BE= *Atropa belladona*; TA= *Nicotiana tabacum*; PH= *Physalis spp*; ES= *Lycopersicon esculentum.*

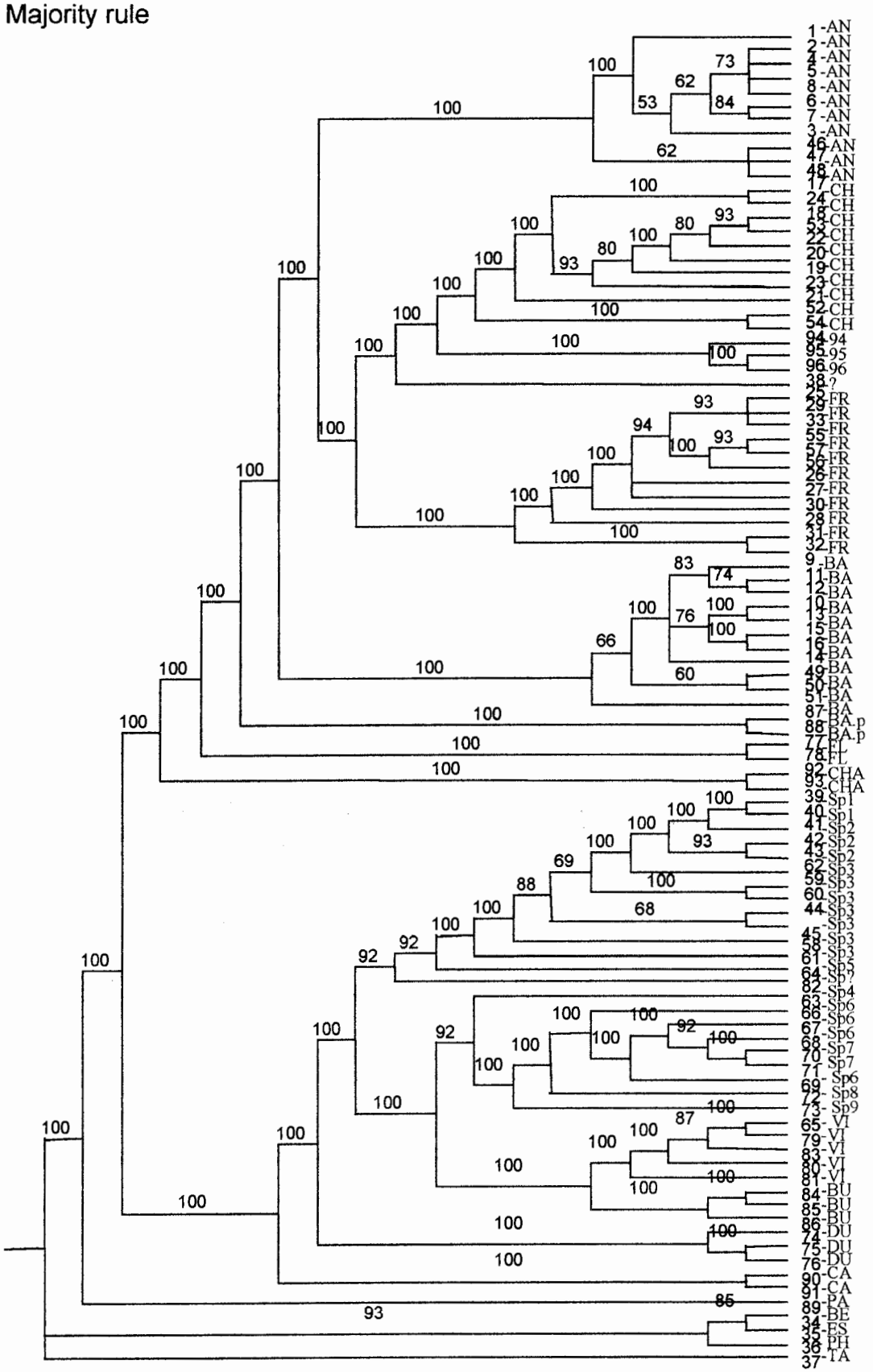

Figure 2. Cladistic analysis of 92 wild and cultivated *Capsicum* accessions based on 277 RAPD markers. The cladogram represents the 50% majority rule consensus tree of 20100 trees with maximum parsimony found by the heuristic search. The number in the branches refer to the number of times that a particular clade was obtained in the trees generated by PAUP program.

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GERMPLASM COLLECTION OF *CAPSICUM* SPP. MAINTAINED BY EMBRAPA HORTALIÇAS (CNPH)

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INTRODUCTION

The cultivation of sweet pepper and other *Capsicum* spp. species are of increasing relevance for the Brazilian agriculture. Annually, ca. 13,000 ha are cultivated yielding approximately 280,000 tons of fruits for fresh consumption and processing as sauces, preserves and other preparation forms.

The agribusiness activities involving *Capsicum* demand new cultivars, particularly genotypes for processing with higher yield and quality, and other important traits such as resistance to the most important diseases and insects. Breeding new cultivars is based on genetic variability, and for that a well-organized and characterized germplasm bank is essential. Embrapa Hortaliças (CNPH) has begin a *Capsicum* germplasm bank 23 years ago, in cooperation with other Brazilian and international institutions. In 1998, a research project financed by the World Bank (Prodetab) was established to enrich, characterize and maintain the germplasm bank, as well as to organize a database to supply relevant information to the *Capsicum* breeding program.

The Embrapa Hortaliças *Capsicum* germplasm bank was established in 1980 by germplasm exchange with other countries and domestic collect expeditions. Presently, the germplasm bank has 1,220 entries of open pollinization cultivars, hybrids, populations of cultivated types, lines and wild types of *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*.

The *Capsicum* research project developed by Embrapa Hortaliças has a multidisciplinary and interinstitutional team involving germplasm collection; multiplication; morphological, cytogenetic and molecular characterization; conservation; documentation; evaluation of disease resistance; and plant breeding. Presently, the breeding program is focused on the demands of the private sector and aims at new cultivars of paprika, jalapeños and hot peppers for processing.

COLLECTION CHARACTERIZATION AND MAINTENANCE

During the last five years 654 entries of the germplasm bank were multiplied and characterized at the Embrapa Hortaliças, Brasilia, Brazil. Five plants of each entry were grown under greenhouse condition. Five flowers of each genotype were manually auto pollinated, and properly identified at harvest to compare to open pollinated fruits. Seeds were extracted manually, washed in cold water, pre-dried at 32°C for 48h and then dried at 40°C for 48h. After that, seeds were packed in sealed aluminium packs and kept in a cold room at 4°C.

The characterization was done accordingly to the morphological descriptors for *Capsicum* of the International Plant Genetic Resources Institute (IPGRI). Each entry was characterized for 56 morphological and phenological traits, with some small changes. Plant descriptors: (18 traits): plant height; plant canopy width; leaf width, length, colour, shape, density, pubescence and lamina margin; stem shape, colour, length, diameter and pubescence stem; branching habit; nodal anthocyanin; growth habit; tillering. Inflorescence (13 descriptors): days to flowering; number of flowers per axil; flower position; corolla colour; corolla spot colour; corolla shape; plant male sterility; calyx margin; calyx pigmentation; calyx annular constriction; corolla spot colour; anther colour; filament colour. Fruit (22 descriptors): days to fruiting; anthocyanin spots or stripes; fruit colour at intermediate and mature stages; fruit position; fruit colour at mature stage; fruit shape; fruit length; fruit width; fruit weight; fruit pedicel length; fruit wall thickness; fruit shape at pedicel attachment; neck at the base of fruit; fruit shape at blossom end; fruit blossom end appendage; fruit cross-sectional corrugation; placenta length; number of locules; fruit surface; ripe fruit persistence; pungency and aroma. Seeds (3 descriptors): seed colour; number of seeds per fruit; seed surface.

RESULTS AND DISCUSSION

Since 1998, 654 of the 1,220 entries of the *Capsicum* germplasm bank were characterized following the IPGRI descriptors. Of these, 397 were classified as *C. annuum*, 75 of *C. baccatum*, 147 of *C. chinense* and 35 of *C. frutescens*. The morphological characterization following the above mentioned descriptors allowed the identification of similar species and groups of entries, besides the genetic variability within each species. Great variability was observed for all plant and fruit traits, especially in yield and fruit colour, shape, pungency and aroma. The frequency of 25 selected descriptors was used to characterize 148 genotypes of *Capsicum annuum* (Table 1). Corolla spot colour, calyx annular constriction, neck at base of fruit and male sterility were not detected in the evaluated

genotypes. Some IPGRI descriptors were considered as secondary or time-consuming and for these reasons suppressed, while other regarded as important for the breeding program were added, such as pungency, aroma and fruit position. Pungency, aroma and fruit position were included to characterize fruit quality traits more easily. Three persons tasted and smelled small portions of the fruits and made a subjective evaluation (aroma ranging from none to strong; pungency ranging from none to hot). Fruit position was characterized as pendant, erect (upright), intermediate and variable (plants with fruits in more than one position).

All information available (morphological traits, passport data and a digital photo) was grouped into a databank by using the ACCESS software. Some of the entries of the Embrapa Hortaliças *Capsicum* germplasm bank can be accessed through internet (*www.cnph.embrapa.br/projetos/capsicum/consulta/html*) at a homepage in Portuguese with a digital photograph, number of registration, origin and species identification. Other relevant information on *Capsicum* production, history, species and types (with photographs), diseases and pests, and recipes are also available in another homepage in Portuguese (*www.cnph.embrapa.br/capsicum*).

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Table 1. Frequency of some Capsicum descriptors (vegetative plant, inflorescence and fruit) of 148 genotypes of the *Capsicum annuum* germplasm collection maintained by Embrapa Hortaliças (CNPH), Brasília-DF, Brazil.

Descriptors	Characterization	Frequency (%)
Stem colour	Green	83
Nodal anthocyanin	Purple	76
Plant growth habit	Intermediate	82
Flower position	Erect	24
Corolla colour	White	93
Corolla spot colour	Spotless	100
Anther colour	Pale blue	58
Days to flowering (days)	32-55	74
Male sterility	Absent	100
Number of flowers per axil	One	95
Calyx annular constriction	Absent	100
Days to fruiting (days)	39-56	76
Fruit shape	Triangular	49
Fruit colour at mature stage	Dark red	62
Pungency	Hot	35
Aroma	Slight	98
Position fruit	Pendant	76
Fruit shape at pedicel attachment	Lobate	83
Neck at base of fruit	Absent	100
Fruit cross-sectional corrugation	Intermediate	64
Fruit surface	Smooth	51
Ripe fruit persistence	Persistent	95
Fruit wall thickness (mm)	1-4 – 3,4	52
Fruit length (cm)	6,0 – 10,0	55
Fruit width (cm)	1,8 – 4,1	41

Capsicum and Eggplant Newsletter 22 (2003): 21-24.

Evaluation and Characterization of Jordanian Pepper (*Capsicum annum* L.) Landraces

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Key words: Pepper, Capsicum annum L., salt stres, landraces

ABSTRACT

Eight accessions of local pepper (*Capsicum annum* L.) landraces were evaluated and characterized under greenhouse conditions in the Jordan Valley at Karama Station during 2001/2002 growing season. Average soil salinity of the experimental field ranged from 6 to 4 dS m^{-1} at zero to 30 cm and 30 to 60 cm soil depth, respectively. Considerable differences in plant growth parameters, yield and average fruit number $plant^{-1}$ were observed between the accessions. Accessions with higher shoot and root dry weights such as Jo.209, Jo.202 and Jo.207 and those with higher yields like Jo.204, Jo.209 and Jo.202 are considered less sensitive to stress conditions including salinity. These germplasm can be used by breeders in their breeding programs according to their objectives. Furthermore, The variation in fruit characters: weight, length, width, curvature, pungency and color might be useful for breeders in pepper selection.

INTRODUCTION

Pepper (*Capsicum annum* L.) is one of the most popular vegetable crops grown in Jordan. Most of pepper cultivars currently grown in Jordan are hybrids. However, some local pepper landraces are still grown in many small farms due to some consumer demand. Local pepper landraces were cultivated under different irrigation water quality including saline water and in different environments for several decades. Therefore, variation between local pepper landraces in response to stress conditions is expected. Wide range of variation was observed in pepper germplasm in several countries including India (Munshi *et al.*, 2000), Albania (Hallidri and Tome, 2000) and Nigeria (Denton *et al.*, 2000). Differences between pepper landraces due to genotype, environments or genotype-environment interaction were also reported (Zewdie and Bosland, 2000). Variations in response to stress conditions and disease and pest tolerance might be of great value for breeders (Zewdie and Zeven, 1997). In Jordan, most of the local pepper landraces are not characterized, Hence this study was initiated to evaluate and characterize some morphological and agronomical traits of eight accessions of local pepper landraces.

MATERIALS and METHODS

Eight accessions of Pepper (*Capsicum annum* L.) landraces collected from local farmers throughout the country during 1995, and conserved in the gene bank of the National Center of the Agricultural Research and Technology Transfer (NCARTT) were used in this study (Table 1). Plants were grown at Karama Station under greenhouse conditions. Seeds were sown in mid Sep. in 2001 and forty-five day old seedlings were transplanted into greenhouse in late Oct. 2001. Plants were spaced 0.5m apart in 5m raised beds 1.5 m apart. Black polyethylene mulch and a drip irrigation system were used. Analysis of soil from the experiment field and irrigation water samples is presented in table 2.

The trial was replicated 3 times with 30 plants per replicate and analyzed as a Randomized Complete Block Design (RCBD).

Average plant height, stem color and diameter were recorded at onset of first mature green fruits. Four plants per replicate were harvested; plants were separated into shoot and root and dried at 65°C to constant weight to determine average shoot and root dry weights per plant. Fruits were harvested for several times at commercial ripeness stage starting from Jan.15^{th} 2002 and yield, average fruit number $plant^{-1}$ and average fruit weight were calculated. The following fruit characters were recorded

according to the pepper descriptor published by the International Board for Plant Genetic Resources (IBPGR, 1988), average fruit length and width, fruit curvature, pungency and color.

RESULTS and DISCUSSIONS

Variations in vegetative growth parameters were observed between pepper landraces (Table 3). While there were no significant differences in stem diameter among all pepper accessions, plant height ranged from 87 in accession Jo.203 to 146 in accession Jo.209, The heaviest average shoot dry weight (204 gm) was observed in accession Jo.209 with no significant differences from those produced by accessions Jo.202, Jo.205 and Jo.207. The lowest weight, however, was observed in accession Jo.203. Average root dry weight plant^{-1} ranged from 37 mg in accession Jo.202 to 22.0 mg in accession Jo.109b. Root to shoot dry weight ratio was highest in accession Jo.204 (0.23) while the lowest ratio (0.15) was found in accessions Jo.109a and Jo.109b.

Significant variations in yield were among the landraces (Table 4). Pepper yield ranged from 8.5 ton ha^{-1} in accession Jo.109b to 27.2 ton ha^{-1} in accession Jo.204. Average fruit number plant^{-1} was 30 and 79 in Jo.109a and Jo.204 accessions, respectively. The respective average fruit weight was 8 and 34 gm for accessions Jo.203 and Jo.209. Average fruit length was lowest (6.2 cm) in accession Jo.109b and highest (15 cm) in accession Jo.204 and fruit width was 1.5 and 3.8 cm in accessions Jo.203 and Jo.209, respectively (Table 4).

Fruit curvature varied from slightly curved to curved (Table 4). Fruit pungency varied from intermediate to high and fruit color ranged from light green to dark green.

Considerable differences in plant vegetative growth, yield and fruit characters were observed among these accessions (Tables 3 and 4). Though, variation in shoot and root dry weights may be due to differences in genotype of pepper landraces, this does not negate the effect of stress conditions including water and soil salinity. Pepper has been cataloged as moderately sensitive to salt stress; fruit yield decreased by 14% for each unit increase in EC_e in the root medium with a threshold value of 1.5 dS m^{-1} (Maas and Hoffman, 1977). Therefore, pepper accessions with higher shoot and root dry weights such as Jo.209, Jo.202 and Jo.207 and those with higher yields like Jo.204, Jo.209 and Jo.202 are considered less sensitive to salt stress as compared with the other accessions. On the other hand, lower yields in accessions Jo.109a, Jo.109b and the appearance of blossom end-rot in fruits of accession Jo.205 indicate that these accessions are more sensitive to salt stress (Table 4). The variation in fruit characters: weight, length, width, curvature, pungency and color might be useful for breeders in pepper selection.

Seed requests:

Seeds of the Jordanian pepper landraces used in this study could be obtained from the Director General of the National Center for Agricultural Research and Technology Transfer (NCARTT) . P.O. Box 639 Baq'a 19381 Jordan.

Email: Director@ncartt.gov.jo

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Table 1: Local pepper landraces collected from different areas of Jordan during 1995 and preserved in the NCARTT gene bank.

Accession Jo. Number	Genus	Species	Location	Collecting date	Collector
109a	*Capsicum*	*Annum*	Balqa', Salt Wadi	1995	Syouf, Saifan, Tehabsem NCARTT
109b	*Capsicum*	*Annum*	Balqa', Salt Wadi	1995	University of Jordan
202	*Capsicum*	*Annum*	Balqa', Um- Jhash	1995	University of Jordan
203	*Capsicum*	*Annum*		1995	University of Jordan
204	*Capsicum*	*Annum*	Balqa', Um- Jhash	1995	University of Jordan
205	*Capsicum*	*Annum*	Zarqa, Sukneh	1995	University of Jordan
207	*Capsicum*	*Annum*	Zarqa, Zarqa	1995	University of Jordan
209	*Capsicum*	*Annum*	Irbid, N.Shoneh road	1995	University of Jordan

Table 2: Analysis of saturated paste extracts and irrigation water at the site of the experiment.

Sample	EC ($dS\ m^{-1}$)	pH	Meq L^{-1}			
			Ca	Mg	Na	Cl
Soil 0-30 cm	5.5	7.6	14	20	19	32
Soil 30-60 cm	3.4	7.8	11	8	15	17
Water	2.5	8.0	5.5	6.0	11.2	10.1

Table 3: Average plant height, stem diameter, stem color, shoot and root dry weights and root: shoot ratio in 8 accessions of local pepper landraces grown under greenhouse in the Jordan Valley during 2001/2002 season.

Accession Jo. number	Plant height (cm)	Stem diameter (cm)	Stem color	Shoot dry weight (gm $plant^{-1}$)	Root dry weight (gm $plant^{-1}$)	Root: shoot ratio
109a	125.0 ab	2.2 a	Green	154.5 b	23.5 ab	0.15 b
109b	93.7 cd	2.5 a	Green	152.9 b	22.0 b	0.15 b
202	115.3 bc	2.5 a	Green	195.6 a	37.0 a	0.19 ab
203	86.7 d	2.3 a	Green	133.8 b	24.4 ab	0.19 ab
204	121.0 ab	2.2 a	Green	148.7 b	35.2 ab	0.23 a
205	104.0 bcd	2.6 a	Green	193.3 a	34.8 ab	0.18 b
207	128.7 ab	2.4 a	Green	193.4 a	31.4 ab	0.16 b
209	146.0 a	2.7 a	Green	204.1 a	32.6 ab	0.16 b

Table 4: Yield, average fruit number per plant and some fruit characters in 8 accessions of local pepper landraces grown under greenhouses in the Jordan Valley during 2001/2002 season.

Accession Jo. number	Yield (ton ha^{-1})	Average fruit number $plant^{-1}$	Fruit characters						
			Average fruit weight (gm)	Average fruit length (cm)	Average fruit width (cm)	Fruit Curvature	Fruit pungency	Fruit color	Remarks
109a	10.4 d	30.0 c	17.8 bcd	13.6 ab	3.7 a	Slightly curved	Intermediate	Dark green	
109b	8.5 d	35.0 c	12.3 de	6.2 d	3.3 b	Slightly curved	Intermediate	Dark green	
202	22.0 b	51.8 b	22.2 b	11.7 c	3.1 b	Slightly curved	High	Dark green	
203	11.1 d	73.4 a	7.7 e	13.5 ab	1.5 e	Curved	High	Light green	
204	27.2 a	79.2 a	17.1 bcd	14.8 a	2.0 d	Curved	High	Dark green	
205	18.0 c	48.1 b	19.7 bc	14.0 a	1.9 d	Slightly curved	Low	Light green	Sensitive to blossom end rot
207	18.4 c	60.2 b	15.5 cd	11.4 c	2.4 c	Curved	Intermediate	Dark green	
209	24.5 ab	36.3 c	34.3 a	12.3 bc	3.8 a	Slightly curved	Low	Light green	

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'SERRANO CRIOLLO DE MORELOS' A GOOD EXAMPLE OF A LAND VARIETY

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In Mexico, pepper varieties are classified into types (tipos). One of those types is 'Serrano' and within this type we find the variety 'Criollo de Morelos' (SCM) collected in Morelos State (Mexico). Guerrero and Laborde (1980) detected several SCM lines resistant to *Phythophtora capsici* which have been the main source of resistance to that pathogen, particularly the line SCM-334. But they selected those lines within SCM where susceptibility to *P. capsici* was also found. Variability in SCM is a constant.

When we started to look for PVY (Potato Virus Y) resistance in SCM, we detected resistance to PVY-1-2 pathotype but also some plants susceptible to that pathotype (Pasko *et al.*, 1992). Within this susceptible material where *Pvr4,* one of the genes responsible for resistance to PVY-1-2, is not present, other genes are taking their opportunity to manifest themselves, conferring resistance at different levels (Arnedo Andres *et al.*, 1998). When *Pvr4* is absent, we agree with Dogimont *et al.* (1996) on the occurrence of a gene controlling the systemic necrotic symptoms and hose maximum expression is observed in homozigosity (Arnedo Andres *et al.*, in preparation). However, our results do not agree with the existence of the gene *pvr5* (it should confer only resistance to PVY-0) as proposed by Dogimont *et al.* (1996), but they can be explained by the simple presence of resistant alleles of the *pvr2 locus* (Gebre Selassie *et al.*, 1985). *Pvr4, Pn1* and *pvr2* are not all the *loci* for PVY resistance present in SCM. At this moment we are working with another additional *locus* for resistance to PVY.

More recently we have started to work with the trait pungency. Although SCM is considered a pungent variety again we have found variability for this trait. One out of 24 SCM plants repeatedly showed no presence of capsaicinoids together by HPLC and tasting methods. This situation seems to be not an exception. Recently Votava and Bosland (2002) have reported several accessions containing pungent and non-pungent individuals.

Another surprising fact was that although we have observed always hairiness on SCM stems, glabrous lines have also been detected. This is the case of the line maintained at the University of Cornell that we had recently the opportunity to test.

All this facts, the variability found in a variety endemic to the area of Morelos, drive us to the conclusion that SCM should in fact be considered a land variety in the sense of Briggs and Knowles (1967) which means that SCM behaves as a real gene pool may be unexploited for some traits of present and future interest.

Of course, not every trait will be found in SCM. Looking for resistance to TSWV (Tomato Spotted Wilt Virus), some plants selected as resistant were finally classified as escapes to infection as their progenies gave no increment on the presences of resistant plants (escapes) with respect to the original population (Gonzalo Pascual, 2001).

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A NEW ONCE-OVER HARVEST-TYPE VARIETY 'SAENG-RYEOG NO. 216' IN RED PEPPER (*CAPSICUM ANNUUM* L.)

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Hot pepper has been the second most important crop after rice in Korea for many years in terms of the cultivated acreage as well as source of farmers' income (Cho et al., 2000). In average, Koreans consume more than 2.5kg of hot pepper powder annually, which is probably the highest in the world. The main usage of hot pepper in Korea is as spice for kimchi (pickled vegetables) and as condiment for various meal preparations (Kim, 2002). The hot pepper production in Korea is still labor intensive. Therefore, the price of domestic hot pepper is about fivefold higher than that of imported one from China. To maintain competitiveness of the domestic pepper industry, it is inevitable to reduce the production cost. The present paper is to report a new pepper variety 'Saeng-ryeog No. 216', with which farmers can harvest mature peppers in one time. This variety also offers possibility of mechanized harvesting practice.

As an attempt to find out a way of reducing the labor cost required for harvesting, a breeding program was initiated to develop varieties, which can be harvested once-over. During 1990 to 1992, germplasm collected were evaluated for once-over harvest type (Yoon et al., 1995). In 1993, a cross between desirable resources, 'Jalapeno' and an unknown variety was initiated, followed by subsequent selfing generations in the pedigree breeding scheme from 1994 to 2001. Selection in the field was focused on the possibility of one-time harvesting and good fruit quality.

The first flowering date of 'Saeng-ryeog No. 216' is 87 days from seeding, which is a little later than that of a leading commercial variety, 'Manita'. It has upright fruits, determinate growth habit, and medium fruit size with approximately 10g in fresh weight. On harvesting, the percentage of fully matured and usable fruits was more than 88%, or 437g per plant. The mature fruit color is bright red, and it has no pungency. Field resistance to powdery mildew, anthracnose and virus diseases was medium level in the field experiment conducted in 2001 to 2002.

Table 1. Horticultural characteristics of 'Saeng-ryeog No. 216'

Variety	Days to flowering [z]	Fruit setting	Plant type	Fruit pericarp	Rate of once-over harvesting (%)	Diseased fruit (%)
Saeng-ryeog No. 216	87	Upright	Determinate	Plain	88	6.0
cv. Manita	74	Pendent	Indeterminate	Plain	66	9.8

[z] Days after sowing

Table 2. Fruit characteristics of 'Saeng-ryeog No. 216'

Variety	Fresh fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Mature fruit color	Fresh yield [z] (g/plant)	Yield [y] (MT/ha)
Saeng-ryeog No. 216	9.9	7.7	1.5	Red	437	5.7
cv. Manita	9.9	9.9	1.4	Red	626	5.6

[z] One-time harvesting yield for 'Saeng-ryeog No. 216', and three-times harvesting yield for 'Manita' were presented.

[y] Dried fruit yield by 55 x 20cm of planting distance

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LOW NIGHT TEMPERATURE EFFECT ON PEPPER OVARIES CHARACTERISTICS. HISTOLOGICAL STUDY.

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1. Introduction

For early season harvest, peppers grown during winter in unheated plastic greenhouses are usually affected by low night temperatures. These conditions have a considerable negative effect on pepper flower development and limit the crop yield.

Under optimal night temperature the flowers are small, whereas under low night temperatures pepper flowers have larger ovaries (Rylski, 1986). Pressman et al. (1998a) reported an increase in ovary size of sweet pepper flowers, as night temperatures decreased from 20 to 12 or 10 °C.

It is suggested that the increased ovary diameter results in the enlargement of cells in the various locations in ovaries developed under low night temperature regime (28/12°C) (Pressman et al.,1998b). Such an alteration of flower part growth is likely to be partly responsible for poor normal fruit set (Tarchoun et al., 2003).

Usually, fruits developed at low night temperature were malformed because of low or no seeds set (Rylski, 1986). Such fruits are without commercial value. Most studies on pepper attribute the lake of fruit set and fruit malformation to the poor pollen viability (Polowick and Sawhney, 1985; Mercado et al., 1997c), investigations on ovules involvement are, however, scarce .

The objective of the present study is to determine histologically the effect of low night temperature in pepper ovaries characteristics especially on the number of ovules and locules.

2. Materials and methods

Plants of hot pepper (*C.annuum* L.) cultivars Beldi and Baklouti are grown in chambers with day/night temperatures of either low night temperatures regime (LTR: 25°C/10°C) or optimum temperatures regime (OTR: 25°C/20°C).

About 50 flowers from each temperature regime and cultivar were sampled on the day of anthesis, their ovaries were separated and fixed in FAA during 24 hours. After dehydration in different alcohol concentrations and infiltration with paraffin, ovaries were cut transversely with a rotary microtone (Leica) into 10µm sections. These sections were stained with safranine and fast green. For each cultivar and temperature regime, about 20 slides presenting the equatorial sections (Tomer et al., 1998) were used to determine, under a microscope :

1. locules and ovules number per ovary,
2. longitudinal and transversal diameter (Ø1, Ø2) which were parallel and vertical to the ovary circumference, respectively.

To detect the effect of male and female gamete on fruit shape or size, artificial pollination was carried out on Beldi cultivar grow either in low or at optimal night temperature. Pollen, collected from opened flowers developed in the OTR of 20°C, was immediately used to

pollinating the flowers (after castration at a bottom stage) of plants grown under the LTR of 10°C (T1-2). A reciprocal pollination was applied. Pollen from lower night temperature was used to pollinating flowers of plants grown under the higher temperature regime (T2-1). About 20 flowers in each replicate (total 60) of each cultivar was treated. Data are means of these replications. Mature fruits characteristics were recorded for each pollination method: weight (g), length and diameter (mm) and number of seeds per fruit.

Data were analyzed using SAS system, ANOVA was applied at the 5% level.

3. Results and discussion

3.1. Ovaries characteristics

Under the lower night temperature regime (25/10°C) ovaries show a decrease of more than 50% of the number of ovules compared to those developed under the higher night temperature. Therefore, the number of locules records a significant increase ($P<0.05$)(Table1). Longitudinal and transversal diameters were dependent on the temperature regime. Ovaries developed under the lower night temperature were wider than those under the higher regime, since measurements of transversal and longitudinal diameters (Ø2, Ø1) revealed an increase of 14% and a decrease of 31%, respectively, resulting in the enlargement of ovaries (Table 1 and Fig.1). Pressman et al. (1998b) attributed the swelling of pepper ovaries to the increase of the size of all cells in the ovaries grown under low night temperature of 12°C or treated with triiodobenzoic acid (TIBA).

Table 1: The effect of night temperature on the number of ovules and locules per ovary, longitudinal and transversal diameter (Ø1, Ø2, respectively) in pepper flowers measured following transversal sections

	No. of ovules	**No. of locules**	**Ø 1 (µ)**	**Ø 2 (µ)**
25/20°C	40.23 a	2.17 b	182.42 a	159.85 b
25/10°C	15.50 b	2.83 a	151.87 b	184.25 a
LSD p=0.05	2.63	0.17	13.18	11.79

The effect of LTR on the ovaries characteristics was in a similar magnitude for both cultivars. A marked reduction (about 60%) in the number of ovules was recorded in ovaries of plants grown under 10°C night temperature compared to the control ovaries (Table 2). Tomer et al. (1998) pointed out that, at night temperature of 12°C, ovules of tomato cultivars were absent from cold affected locules, thus, no seed production could take place. In addition, for the number of locules, differential susceptibility of the cultivars was expressed under LTR. This number was more increased in Baklouti than Beldi ($p<0.05$) (Table 2). Figure 1 shows transverse sections of ovaries, presenting various degrees of deformation. Locules of ovaries grown under optimal night temperature of 20°C were normal and containing more ovules than those of deformed ovaries grown at night temperature of 10°C. The increase of the number of locules was associated to enlargement of ovary particularly in Baklouti. Under low night temperature, transversal diameter (Ø2) of both cultivars was increased, compared to the longitudinal one (Ø1), of about 22% and 16% for Baklouti and Beldi, respectively. Although, at optimal night temperature, the longitudinal diameter was more longer than transversal one resulting in elongated ovaries in both cultivars (Table 2). Cultivars susceptibility to the effect of low night temperature on ovary abnormalities was noted in others *Solanaceae,* tomato (Hosoki et al., 1990) and pepper (Pressman et al., 1998b).

Table 2: Number of ovules and locules per ovary, longitudinal and transversal diameter (Ø1, Ø2, respectively) (µ) of the hot pepper cultivars Beldi and Baklouti grown at optimal (25/20°C, day/night) or at low (25/10°C) night temperature regimes (Data are means ± SD, n=20).

		Beldi	Baklouti
	No. of ovules	43.4 ± 7.8	37.0 ±5.9
25/20°C	**No. of locules**	2.1 ± 0.4	2.2 ± 0.4
	Ø 1	162.8 ± 6.8	202.0 ± 9.6
	Ø 2	142.7 ± 9.6	176.9 ± 6.2
	No. of ovules	16.3 ± 5.1	14.7 ± 4.0
25/10°C	**No. of locules**	2.7 ± 0.4	3.0 ± 0.3
	Ø 1	149.3 ± 5.8	154.4 ±14.2
	Ø 2	178.5 ± 7.9	199.9 ± 12.2

3.2. Fruit characteristics

Fruit characteristics, except fruit diameter, were more elevated, in case of self pollination at OTR, than others treatments. Artificial pollination, using pollen from night temperature of 20°C, increase the number of seed per fruit in comparison to the self pollination at night temperature of 10°C and treatment using pollen from lower night temperature (T2-1) (Table 3). Similar results were reported by Polowick and Sawhney (1985) and Pressman et al. (1998a). Although, high significant differences were noted between the number of seeds following self pollination at higher night temperature and the artificial pollination (T1-2) using a pollen from night temperature of 20°C. Theses results indicate that the number of seed seems to be dependant not only to the pollen viability but also to the number of ovules since these ovules were negatively affected by low night temperature of 10°C (Table 1) and thus, artificial pollination with viable pollen (T1-2) was ineffective in improving fruit shape and size (Table 3).

Table 3: fruit characteristics of Beldi cultivar following the hand pollination using pollen collected from the OTR of 20°C, to pollinating the flowers of plants grown under the LTR of 10°C (T1-2), and reciprocal treatment: pollen from LTR was used to pollinating flowers of plants grown under the higher temperature regime (T2-1). Self pollination in each temperature regime is a control.

Treatment	Weight (g)	Length (mm)	Diameter (mm)	No. of seeds/fruit
Self pollination at 25/20°C	15.3* a	122.6 a	19.5 b	120.3 a
Self pollination at 25/10°C	8.9 b	81.2 b	22.5 a	26.6 c
T1-2	9.1 b	89.2 b	13.9 c	46.6 b
T2-1	10.2 b	66.2 c	18.5 b	27.8 c

*means followed by the same letter are not significantly different according to the Duncan test, P=0.05.

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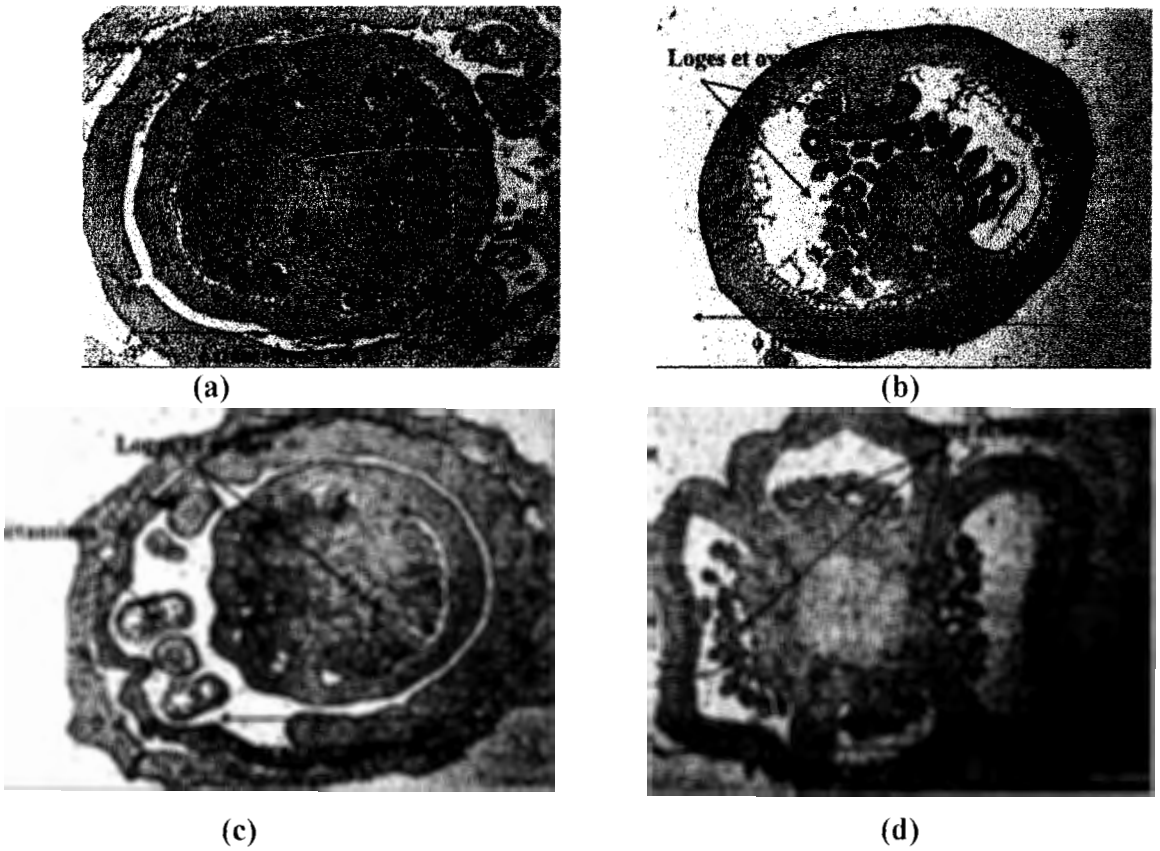

Figure 1: A transverse sections of pepper ovaries. Beldi ovary at 25/20°C (a), at 25/10°C (b) and Baklouti ovary at 25/20°C (c), at 25/10°C (d).

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GENETIC ARCHITECTURE OF CHILLI (*CAPSICUM ANNUUM* L.)

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INTRODUCTION

India is one of the largest producer of chilli (8,32,600 metric tons) occupying third place among the vegetable crops in area and production (Anon., 1997). A genotype with moderate pungency coupled with amenability for cultivation during most of the season is an ideal dual purpose type of commercial exploitation which could be achieved through intervarietal crossing and selection of improved strains in succeeding generations. For which, knowledge on the genetics and mode of inheritance of various economic traits, which influence the yield, is very much essential. Literature on this aspect is limited, hence the present study was undertaken to understand the genetic background in the expression of economic characters through diallel technique.

MATERIALS AND METHODS

Ten chilli genotypes comprising five pungent and five non-pungent types were crossed in 10 x 10 diallel design in all possible combinations excluding reciprocals during 2001-02. The resultant 45 F_1 hybrids were raised along with their parents in randomized block design, replicated thrice. Forty-five days old seedlings were transplanted at 60 x 60 cm spacing keeping 12 plants in each replication for each parents and crosses. Observations on 13 yield and its components were recorded in 5 randomly selected and tagged plants in each of crosses and parents from each replication. Genetic analysis was carried out according to (Hayman, 1954).

RESULTS AND DISCUSSION

The additive component 'D' was significant for all the characters except primary branches per plant (Table 1). Similarly, the two measures of dominance i.e. 'H_1' (dominance effect) and 'H_2' (proportion of dominance due to positive and negative effect of genes) were significant for all the

thirteen traits. The significant D, H_1, and H_2 indicated the existence of both additive and dominant gene actions in the expression of these traits. However, the additive component was in higher proportion than dominant factors for plant height, fruit volume, fruit weight, total chlorophyll and total capsaicin content, indicating preponderance of additive gene action. Similar results were also reported by (Ahmed *et al*, 1982; Rao and Chhonkar, 1983; Sekar, 1984 and Sarala Devi and Arumugam, 1999).

The estimate of 'F' value which indicates the relative proportion of the dominant and recessive alleles were positive for all the traits except fruits per plant and total capsaicin content. This indicated more proportion of dominant alleles in the control of these characters. The environmental factor 'E' was significant only for total chlorophyll which indicated environmental influences in the expression of this trait.

The ratios of different genetic parameters and heritability estimates are given in Table 2. The proportion of $(H_1/D)^{1/2}$ represents the degree of dominance was more than unity for, days to flowering, primary branches per plant, fruits per plant, fruit length, fruit girth, fruit shape index, days to maturity and fresh fruit yield per plant indicating the existence of over dominance for these traits and partial dominance for rest of the traits like, plant height, fruit volume, fruit weight, total chlorophyll and total capsaicin content (Sekar, 1984; Joshi, 1988 and Sarala Devi and Arumugam, 1999).

The ratio $H_2/4H_1$ was not equal to 0.25 for all the traits under study indicating unequal distribution of positive and negative alleles. The proportion of dominant to recessive genes in controlling the characters as indicated by the ratio KD/KR was more than one for all the traits except for fruits per plant and total capsaicin content revealing the presence of high proportion of dominant genes in the control of these yield components. The numbers of genes and gene groups controlling the trait (h^2/H_2) was the maximum for fruits per plant and fresh fruit yield per plant (2-3), while it was less than one for remaining characters.

Moderate to high heritability estimates were observed for all the traits except primary branches per plant. These indicated the larger proportion of additive genes for the inheritance of this traits with less environmental influences. The present study suggested that the recurrent selection followed by pedigree breeding would be helpful to exploit both additive and dominance genes controlling these traits.

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Table 2 : Ratio of genetic parameters and heritability in chilli

Character	$(H_1/D)^{1/2}$	$H_2/4H_1$	KD/KR	h^2/H_2	Heritability in narrow sense (%)
Days to flowering	1.32	0.18	2.03	0.53	46.20
Plant height (cm)	0.89	0.17	2.03	0.02	67.10
Primary branches per plant	2.78	0.21	1.49	017	22.10
Fruits per plant	1.43	0.19	0.42	2.12	76.60
Fruit length (cm)	1.07	0.17	1.31	0.34	72.30
Fruit girth (cm)	1.48	0.21	1.47	0.06	44.30
Fruit shape index	1.02	0.18	2.24	0.01	54.00
Fruit volume (cc)	0.81	0.15	2.69	0.09	69.40
Fruit weight (g)	0.63	0.22	1.26	0.59	82.20
Days to maturity	1.24	0.21	1.39	0.13	57.00
Total chlorophyll (mg/100 g)	0.35	0.20	1.13	0.03	93.00
Total capsaicin content (µg/g)	0.34	0.23	0.98	0.06	95.20
Fresh fruit yield per plant (g)	1.48	0.24	1.06	2.59	48.00

Table 1 : Estimates of genetic parameters in chilli

Character	D	F	H_1	H_2	h^2	E
Days to flowering	54.60** ± 8.74	48.76* ± 20.16	94.48* ± 18.60	69.66* ± 15.81	36.58* ± 10.58	0.41 ± 2.64
Plant height (cm)	224.31** ± 16.58	137.61* ± 38.27	180.62** ± 35.30	127.02* ± 30.04	-0.93 ± 20.03	2.66 ± 5.01
Primary branches per plant	0.54 ± 0.42	0.59 ± 1.05	4.23** ± 0.98	3.59** ± 0.83	0.61 ± 0.56	1.11** ± 0.14
Fruits per plant	10797.40** ± 3224.13	-12338.67 ± 7439.04	22078.13** ± 6862.86	17011.41** ± 5832.68	36157.83** ± 3904.17	64.67 ± 972.11
Fruit length (cm)	10.39* ± 0.75	2.94 ± 1.72	11.86* ± 1.59	8.03* ± 1.35	2.76* ± 0.90	0.16 ± 0.23
Fruit girth (cm)	1.11* ± 0.21	0.63 ± 0.48	2.45* ± 0.45	2.03* ± 0.38	0.13 ± 0.25	0.05 ± 0.06
Fruit shape index	2.11** ± 0.28	1.65* ± 0.64	2.20** ± 0.60	1.60** ± 0.51	-0.01 ± 0.34	0.05 ± 0.09
Fruit volume (cc)	7.36** ± 0.45	5.47** ± 1.03	4.85** ± 0.96	3.04** ± 0.82	0.27 ± 0.55	0.05 ± 0.13
Fruit weight (g)	3.32** ± 0.17	0.48 ± 0.41	1.31** ± 0.38	1.15** ± 0.32	0.67** ± 0.22	0.04 ± 0.05
Days to maturity	234.69** ± 32.57	95.92 ± 75.18	361.30** ± 69.33	299.47** ± 58.93	38.95 ± 39.45	0.89 ± 9.82
Total chlorophyll (mg/100g)	119.56** ± 1.42	5.36 ± 3.27	14.39** ± 3.02	11.45** ± 2.56	0.80 ± 1.72	1.54 ± 1.43
Total capsaicin content (µg/g)	298625.33** ± 4283.76	-2047.01 ± 9883.92	34648.41** ± 9118.38	30816.64** ± 7749.61	1978.89 ± 5187.28	18.60 ± 1291.6
Fresh fruit yield per plant (g)	24683.61** ± 2780.91	2239.07 ± 6416.39	54115.5** ± 5919.43	51479.74** ± 5030.89	133777.76** ± 3367.46	766.26 ± 838.47

*, ** significant at 5 % and 1 % level of significance, respectively.

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DETECTION AND CHARACTERISATION OF SNPs IN *Capsicum* spp.

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Key words: SNPs, SSCP, pepper, *Capsicum annuum,* polymorphism.

Introduction

In the past few years, SNP (single nucleotide polymorphism) discovery and marker development have increased exponentially across a wide range of plant species. SNP markers offer great potential for wide genome coverage and high throughput analysis in breeding through marker-assisted selection (MAS) and other studies. The identification of SNPs for targeting quality traits (QTLs) as well as disease resistance genes is already advanced in a number of major crops. We report on a preliminary study aimed at the detection of SNPs, by PCR-SSCP (single strand conformation polymorphism), in genes coding for well-studied enzymes in *Capsicum spp.* In a first screening, PCR products were analysed by SSCP for the presence of point mutations in amplicons from different samples. Polymorphic fragments were then sequenced for SNPs and validated by tetra-primer ARMS (amplification refractory mutation system) PCR (Ye *et al.*, 2001).

Materials and methods

Genomic DNA was extracted (Doyle and Doyle, 1987) from seven *Capsicum* species *(C. annuum* L., *C. chinense* L., *C. frutescens* L., *C. pubescens* L., *C. chacoense* L., *C. baccatum* L. and *C. tovari* L.*),* together with 17 *C. annuum* L. accessions. All the materials were obtained from the germplasm bank of Di.Va.P.R.A, Plant Genetics and Breeding. Fifteen genes from *C. annuum* were selected from the NCBI database for SNP mining. A set of primers were designed for each gene to amplify fragments of about 450 bp (Table 1). Each fragment was chosen from a region overlapping the intron-exon junctions.

PCR reactions were performed at 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C followed by 5 min at 72 °C. SSCP analysis (amplification and electrophoresis) was performed as described by Sunnucks *et al.* (2000) with slight modifications. Gels were silver stained using the procedure described by Bassam *et al.* (1991).

The polymorphic fragments were purified and directly sequenced in an ABI 3100 sequencer using BigDye™ terminator chemistry. The sequences were aligned with Megalign (DNASTAR) using Clustal algorithm. Putative SNPs were identified.

Genetic variability in DNA sequences was measured by the nucleotide diversity, π, with $\pi = K/L$ (K=number of differences per nucleotide site and L=sequence length on bp (Nei and Li, 1979)

SNPs discovered were validated by Tetra-primer ARMS-PCR. Primers were designed using the software made available on line (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). Four sets of primers were designed for validating 4 SNPs (1 in the *capsanthin/capsorubin synthase*, 2 in the *Eg2* and 1 in the *5-epi-aristolochene synthase* genes). Touchdown PCR was carried on as described by Ye *et al. (*2001).

Results and discussion

Thirteen of fifteen fragments were amplified as unique scorable bands. Eight of fifteen fragments were analysed by SSCP. Data obtained provided a preliminary estimation of the polymorphism present in both *Capsicum* species and *C. annuum* accessions. Sequencing, performed in samples shown to be polymorphic to SSCP analysis, gave evidence of putative SNPs (Figure 1). A total of 2719 bp was scanned, of which 1836 bp were derived from coding region (exons) and 883 bp from non coding region (promoters and introns) (Figure 2).

Fifty-seven SNPs were found, of which 34 were in coding region (cSNPs), however only 18 of them caused amino acid changes. Most of the mutations were at the third position of the codon (47%) while 38% and 18% were at the second and the first position respectively.

The frequency of base substitutions, between 5 of the 7 Capsicum species in study, was 1 SNP per 48 bp while 1 SNP per 1360 bp between *C. annuum* accessions. In previous studies it was observed a frequency of 1 SNP per 1 x 10^3 bp in mouse (Lindblad-Toh *et al.*, 2000) and 5 x 10^4 bp in man (Wang *et al.*,1998). Our results show that, among the detected SNPs, 50% were transitions and 50% transversions. This is not in full agreement with data obtained in *Beta vulgaris* (Schneider *et al.*, 2001), mouse and man (Wang *et al.*,1998; Lindblad-Toh *et al.*, 2000) but might be explained by the relatively low number of scanned nucleotides. Nucleotide diversity (π) ranged between 3.8 and 45.5 (average 19.2) among *Capsicum* species and between 0 and 1.9 (average 0.7) among *C. annuum* accessions.

Sequencing data were validated by tetra-primer ARMS-PCR procedure (Figure 3). Among the three SNPs detected in *C. annuum*, one SNP was confirmed at base 512 of the *capsanthin/capsorubin synthase* gene and two in *EG2* gene at 5182 and 5252 base position respectively. The SNP EG2-5252 showed to be heterozygous (Figure 3) and it made possible to distinguish the *C. annuum* L. Thai accession from all the others.

The two step strategy used for SNP mining using SSCP and sequencing followed by the tetra-primer ARMS-PCR genotyping proved to be a reliable and cost effective method for SNP characterisation. The use of SSCP offers some economic advantages as it is possible to identify and sequence only the polymorphic amplicons. Nevertheless a complete study of nucleotide variability in this species would require a large genonome coverage.

Conclusions and perspectives

This on-going research was performed to obtain a preliminary view of pepper nucleotide diversity. Since SNPs appeared to be present with a slightly higher frequency in non coding regions than in coding ones, future research will focus on a deeper analysis of this findings. SNP mining will provide invaluable tools for application in MAS, genetic diversity studies, germplasm characterisation and variety identification.

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Gene	GeneBank accession	Amplified region	PCR product
Capsanthin/capsorubin synthase	X77289.1	500-1000	405
Fibrillin	X77290.1	400-900	643
Geranyl-diphosphate synthase	X80267.1	250-750	616
Chromoplastic oxydo reductase gene	X78030.1	400-900	510
Defensin	X95730.1	1000-1500	504
Gamma thionin	X95363.1	680-1180	517
Eg2	AJ010950.1	4750-5250	528
Chromoplast chrA	L47242.1	2500-3000	527
Sn1	X79230.1	750-1250	541
Map kinase1 (MK1)	AF247135	100-600	528
5-epi-aristolochene synthase	AJ005588	2000-2500	582
Waxy (W24)	AF397131	100-600	594
Knolle protein(promoter)	AJ276631.1	500-1000	588
An.1	AJ130829.1	1500-2000	490
Pap gene	AJ131456.1	2500-3000	516

Table 1 - Summary of the loci considered for SSCP analysis and SNP mining.

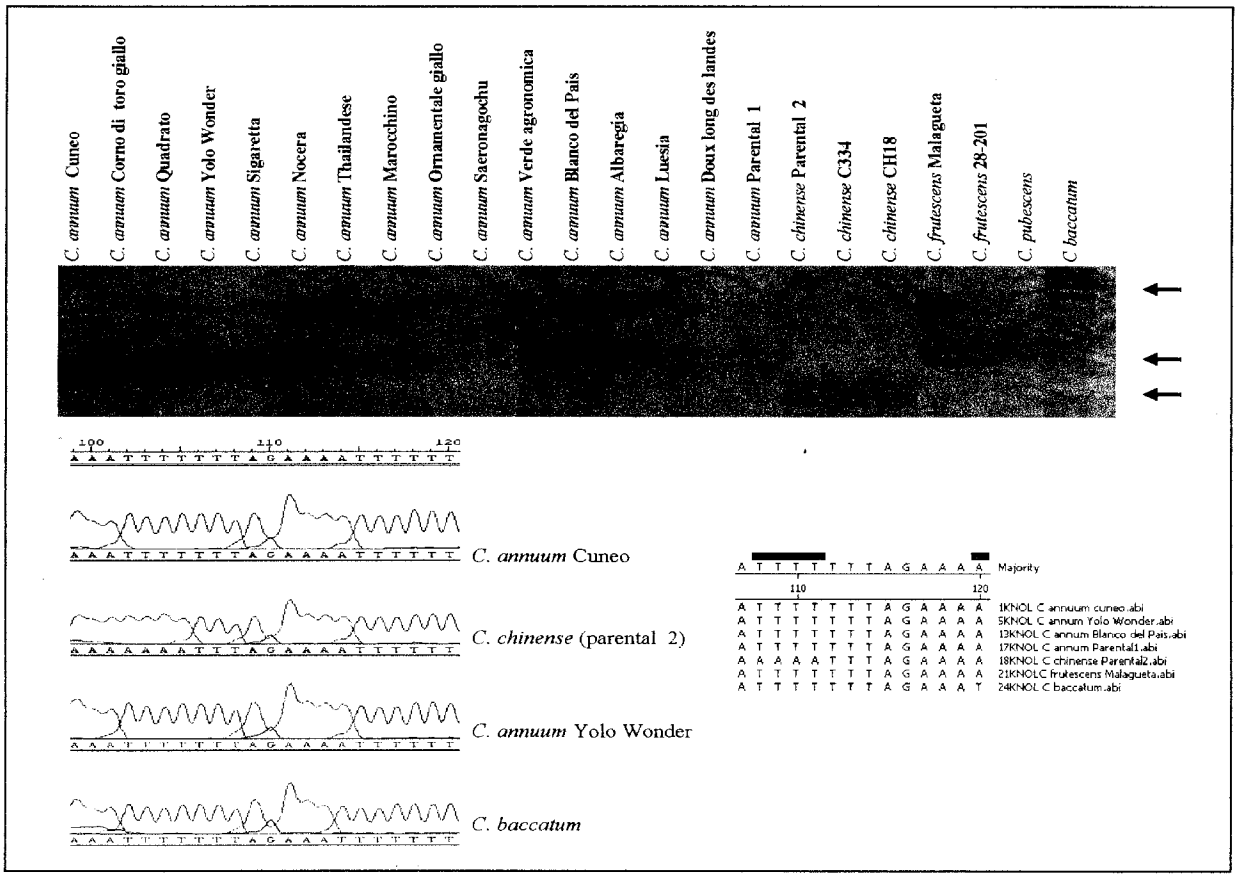

Figure 1 - A) SSCP gel of the *Knolle* promoter fragment; the arrows indicate the allelic variants. B) Chromatogram of 4 sequenced samples; traces show SNP presence in *C. annuum* L., *C. chinense* L., *C. baccatum* L. C) Partial alignment of the sequences described.

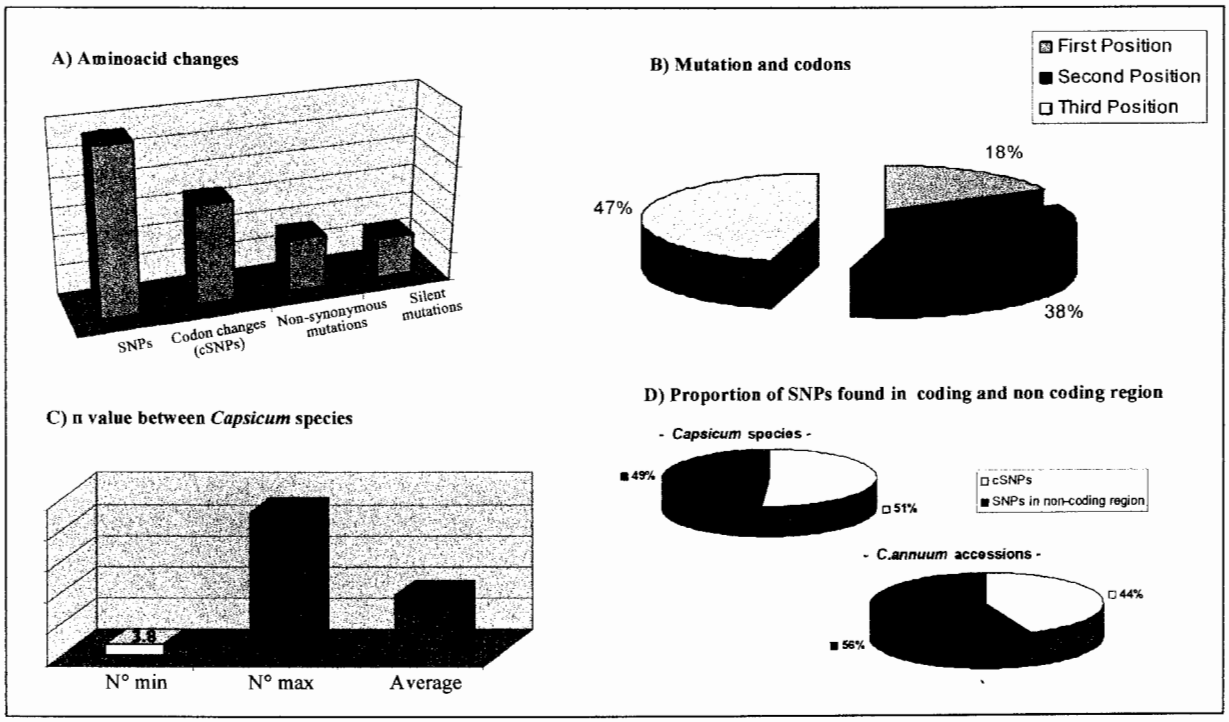

Figure 2 - Presence of SNP and other polimorphisms in the 5 fragments of pepper. A) Codon and amino acid changes produced by 57 SNPs found; B) Relative position of SNPs in codons; C) π value calculated between *Capsicum* species; π is calculated as number of SNP per bp (x1000); D) Proportion of SNPs found in coding and non coding region in *Capsicum* species (top) and in *Capsicum annuum* accessions (bottom)

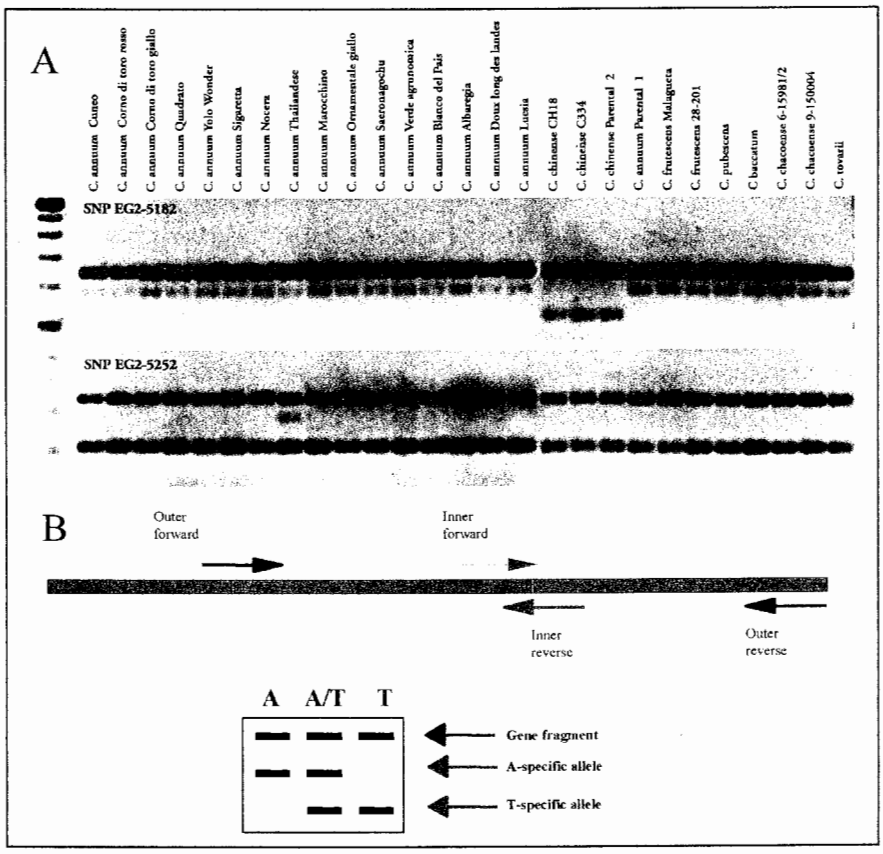

Figure 3 - A) Agarose gel electrophoresis of 7 *Capsicum* species, 17 accessions of *Capsicum annuum* L. SNP-EG2-5182 (top gel) and SNP-EG2-5252 (bottom gel) are showed. The arrows show the three products as described in Figure 3A. B) Diagrammatic representation of SNP identification using the Tetra Primer ARMS-PCR technique.

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NOTE ON EVALUATION OF CHILLI (*CAPSICUM ANNUUM* L.) GENOTYPES FOR BIOCHEMICAL CONSTITUENTS

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Chilli (*Capsicum annuum* L.) is the only source of capsaicin- an alkaloid that is a digestive stimulant, an important ingredient of daily diet and a cure for rheumatic troubles. Chilli also contains many other medicinal properties. Green chilli fruits are valuable on account of their richness in ascorbic acid, which is an important vitamin. The fruit colour is due to the presence of total carotenoids pigments, which mainly consists of capsanthin and capsorubin. The extent of colouring matter is important for spice industry. Furthermore the colouring matter is used to impart colour to the other food products. Keeping view of the above mentioned points, thirty chilli genotypes were evaluated in the present study for their biochemical constituents viz. capsaicin, ascorbic acid and total carotenoids.

The ascorbic acid content was determined by 2, 6 dichlorophenol indophenol method of AOAC (1970). Estimation of total carotenoids was done as per the method given by Ranganna (1997). Capsaicin content was found out as per the method given by Quagliotti (1971). Fruit width was estimated by using Vernier calipers.

The data regarding capsaicin, ascorbic acid and total carotenoids contents are given in the table 1.Capsaicin content showed a narrow range of variation from 0.33mg/100mg in Rajasthan local to 0.49mg/100mg in KDCS-810 with an overall mean of 0.41mg/100mg.Smaller sized fruits which are short and thin contained more capsaicin compared to long fruits. The mean ascorbic acid content was lowest in DCL 344 (78.30 mg/100g) and highest in ACS 2000-02 (188.0mg/100g). Ascorbic acid content had a grand mean of 130.01mg/100g of fresh fruit weight. Long fruits contained higher ascorbic acid content. Total carotenoids showed a range or variation from 1475.3μg/100g in DCL 008 to 4208.0μg/100g in Red star with an over all mean of 2732.4μg/100g.

From the results it may be concluded that significant variation exists among the selected chilli genotypes for the estimated biochemical constituents. The smaller sized fruits were associated with higher capsaicin content and large fruited genotypes contained higher ascorbic acid. Similar association was also reported by Sharma *et al* (1981).

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Table 1. Bio-chemical constituents of 30 genotypes of chilli

Genotypes	Fruit length (cm)	Fruit width (cm)	Capsaicin (mg/100mg)	Ascorbic acid (mg/100g)	Total carotenoids (µg/100g)
ACS 2000-02	12.84	1.38	0.34	188.30	2094.00
ACS 98-09	8.44	1.24	0.37	150.30	2036.10
DCL 408	7.02	0.92	0.46	100.30	1509.00
DKC 8	6.24	1.05	0.48	147.00	3146.70
Phule Sai	6.54	1.00	0.40	140.00	3095.20
KDCS-810	5.96	0.84	0.49	87.67	2034.60
RC 1-1	12.50	1.24	0.36	160.67	1899.60
DCL 228	10.72	0.96	0.40	150.33	2606.70
DCL 236	6.78	1.22	0.43	104.73	2507.10
DCL 335	7.36	1.02	0.42	130.00	4181.60
DCL 006	8.86	0.84	0.45	118.33	1724.40
DCL 358	7.34	1.02	0.41	115.33	2479.70
DCL 901	8.38	1.06	0.47	142.67	3550.10
DCL 001	8.52	0.82	0.47	113.33	3051.00
DCL 268	9.96	0.96	0.35	129.00	2497.00
DCL 270	10.92	1.16	0.39	143.30	2474.00
Red star	10.22	0.80	0.43	119.67	4208.00
DCL 008	11.40	1.36	0.34	157.67	1475.30
Soldier	8.00	1.04	0.38	120.00	3930.90
Green wonder	11.16	2.11	0.41	179.70	2598.70
PMR 57	6.96	0.90	0.42	114.70	1941.40
K 1	7.06	0.90	0.40	120.30	1796.70
A 8	8.28	1.04	0.39	79.70	1885.10
DCL 344	10.52	0.94	0.41	78.30	3956.60
DCL 266	10.50	1.10	0.48	147.70	3632.00
DCL 271	12.50	1.16	0.37	155.30	3167.00
Rajasthan local	8.26	1.30	0.33	90.70	2058.70
Sel.5	8.00	1.28	0.37	149.00	2625.90
Motikeera 39	12.28	1.28	0.37	159.70	3927.70
Pusa sadabahar	6.44	0.78	0.45	110.30	3879.50
Range	5.96 to 12.84	0.80 to 2.11	0.33 to 0.49	78.30 to 188.30	1475.30 to 4208.00
Grand mean	8.99	1.09	0.41	130.01	2732.50
SE ±	0.70	0.08	0.03	4.10	92.10
CD 5%	1.39	0.16	0.06	8.16	183.28
Coefficient of variation (CV)	23.51	23.75	11.21	21.61	31.25

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VARIABILITY, CORRELATION AND PATH ANALYSIS IN *KHARIF* GROWN CHILLI (*Capsicum annuum* L.) GENOTYPES FOR DIFFERENT CHARACTERS

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ABSTRACT: Estimates of variability, heritability, genetic advance, correlation and path co-efficient analysis were carried out in 26 chilli genotypes in transitional tract of Karnataka. High degree of genotypic and phenotypic co-efficient of variation was observed for number of primary branches, fruit length, pericarp thickness, number of fruits per plant and green fruit yield per plant. High heritability coupled with high genetic advance as a percentage of mean was observed in respect of fruit length and green fruit yield per plant. Yield per plant showed positive correlation with number of fruits per plant and pedicel length. Path co-efficient analysis revealed the importance of number of fruits per plant in determining selection criteria for improvement of chilli yields.

INTRODUCTION

Chilli is grown in arid and semi-arid regions of India. Therefore it is important to evolve varieties for maximize economic yield and consistent in their performance in a given environment. The gain from selection in crop breeding program is dependent on the amount of variability for economic characters in the germplasm. For rapid progress in selection of the germplasm study on association of yield with component characters shall help in elucidating the intrinsic nature of correlation and ease the selection procedures.

MATERIAL AND METHODS

The field experiment was conducted during *kharif* 1999 at the Department of Horticulture University of Agricultural Sciences, Dharwad with 26 genotypes of chilli using Randomized Block Design in three replications. A spacing of 60 x 30 cm. was followed. Observations were recorded on five randomly selected plants for 13 characters *viz.*, plant height (cm), number of primary branches, plant spread (cm), days to 50 per cent flowering, fruit length (cm), pedicel length (cm), pericarp thickness (cm), number of seeds per fruit, green fruit weight (g), number of fruits per plant, green fruit yield per plant (g) and fruit rot incidence (%). The mean values of five plants were used for analyzing genetic coefficient of variation, heritability (in broad sense), and genetic advance as per cent of mean were computed by using standard methods along with correlation and path analysis as per method followed by Dewey and Lu (1959).

RESULTS AND DISCUSSION

It is evident from the data (Table 1) there was wide range of variability for all the characters providing an ample scope for selecting the desirable types.

The characters *viz.*, number of primary branches, fruit length, pericarp thickness, number fruits per plant and green fruit yield per plant showed highest degree of PCV and GCV indicating broad genetic base for these traits (Rani and Singh, 1996). High variability for fruit

rot disease incidence indicate high scope for selection against the disease (Table 1). The highest estimates of heritability was obtained for plant height (93.7 %) , number of primary branches (91.7%), fruit length (95.7%) and green fruit yield (90.5 %) along with high genetic advance over mean. Thus, improvement through selection could be made for these characters while, days to 50 per cent flowering and plants spread showed high heritability coupled with low genetic advance over mean. This indicates predominance of non-additive gene effects which arises when variability of the traits is low (Kataria *etal.*, 1997).

Correlation studies revealed that green fruit yield had positive and significant association with number of fruits (Jose and Khader, 2002) and pedicel length. Green fruit weight was significantly correlated with number of fruits per plant in negative direction. On the contrary number of fruits per plant seem to be major character contributing to the yield per plant. Hence, care to be taken to strike balance while selecting for the characters based on market demand and preference.

Path analysis provides information on real nature of association by separating into direct and indirect effects. Among the character studied number of fruits had maximum direct effect (0.671) on yield followed by pedicel length and pericarp thickness. Low negative direct effect (-0.073) of fruit weight was observed with yield per plant due to indirect negative association with number of fruits per plant. However, overall correlation remained positive due to the influence of other characters studied (Sharma and Roy, 1995). Hence from the investigation it could be concluded that number of fruits per plant and pedicel length deserve greater weightage during selection for yield in chilli varieties.

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Table 1. Variability in 26 chilli varieties for characters

Sl. No	Characters	Mean	Range		Genotypic coefficient of variation (GCV) (%)	Phenotypic coefficient of variation (PCV) (%)	Heritability (h^2) (%)	Genetic advance over mean (GAM) (%)
			Min.	Max.				
1	Plant height (cm)	56.85	34.21	85.83	23.44	24.21	93.70	46.74
2	Number of primary branches	6.15	3.33	11.73	43.04	44.94	91.70	84.99
3	Plant spread (cm)	42.77	24.73	54.52	18.76	20.46	84.10	35.44
4	Days to 50% flowering	33.70	28.33	41.00	11.73	12.60	86.70	22.52
5	Fruit length (cm)	7.73	3.77	18.95	45.62	46.80	95.00	91.58
6	Fruit diameter (cm)	1.12	0.77	1.93	23.61	26.51	79.30	42.93
7	Pedicel length (cm)	3.18	1.77	4.56	22.84	24.81	84.70	43.41
8	Pericarp thickness (cm)	0.14	0.08	0.23	31.74	41.28	59.10	51.85
9	Number of seeds per fruit	70.34	14.33	113.00	30.76	31.79	93.60	61.31
10	Green fruit weight (g)	2.44	1.13	3.93	33.11	33.84	95.70	66.83
11	Number of fruits per plant	170.33	75.57	333.33	36.02	40.31	79.80	66.30
12	Green fruit yield per plant (g)	296.11	111.00	548.17	39.02	41.02	90.50	76.47
13	Fruit rot incidence (%)	34.47	9.50	60.67	38.41	39.33	95.30	77.25

Table 2. Genotypic correlation coefficient for different pairs of characters in pooled analysis of chilli.

Characters	1	2	3	4	5	6	7	8	9
Days to 50% flowering (1)	1.000	0.257*	0.033	-0.233*	0.251	-0.063	-0.311**	-0.039	-0.038
Fruit length (2)		1.000	-0.155	0.236*	0.273*	-0.051*	0.237*	0.269*	0.208
Fruit diameter (3)			1.000	-0.178	-0.122	0.209	0.017	-0.091	-0.088
Pedicel length (4)				1.000	-0.192	-0.085	0.389**	0.247*	0.429**
Pericarp thickness(5)					1.000	0.189	0.117	0.188	0.133
Number of seeds per fruit(6)						1.000	0.019	-0.186	0.061
Green fruit weight(7)							1.000	-0.227*	0.109
Number of fruits per plant (8)								1.000	0.714**
Green fruit yield per plant(9)									1.000

*significance at 5% probability, ** significance at 5% probability

Table 3. Genotypic path co-efficient analysis for green gruit yield per plant in chilli.

Characters	Days to 50% flowering	Fruit length	Fruit diameter	Pedicel length	Pericarp thickness	Number of seeds per fruit	Green fruit weight	Number of fruits per plant	Green fruit yield per plant
Days to 50% flowering	**0.019**	0.011	0.00	-0.071	0.015	-0.012	0.023	0.026	-0.038
Fruit length	0.005	**0.042**	0.010	0.072	0.016	-0.010	-0.017	0.181	0.208
Fruit diameter	0.001	-0.006	**-0.005**	-0.054	-0.007	0.040	-0.001	-0.061	-0.088
Pedicel length	-0.004	0.010	0.001	**0.306**	-0.012	-0.016	-0.028	0.166	0.429**
Pericarp thickness	0.005	0.011	0.001	-0.059	**0.306**	0.036	-0.0009	0.126	0.133
Number of seeds per fruit	-0.001	-0.002	-0.001	-0.026	0.011	**0.190**	-0.001	-0.125	0.061
Green fruit weight	-0.006	0.010	0.00	0.119	0.007	0.002	**-0.073**	-0.152	0.109
Number of fruits per plant	0.001	0.011	0.00	0.075	0.011	-0.035	0.016	**0.671**	0.714**

Residual effect = 0.364

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VARIABILITY, HERITABILITY AND GENETIC ADVANCE FOR YIELD, QUALITY AND BACTERIAL WILT INCIDENCE IN *Capsicum chinense* JACQ.

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Indroduction

The diverse climatic and soil conditions prevailing in different parts of Kerala have helped in the development of different ecotypes in chilli. Though Kerala is not a major chilli producing state , the variability existing in this crop is tremendous. *Capsicum chinense* with its perennial nature, ability to yield substantially under shaded conditions and tolerance to diseases like bacterial wilt, collar rot etc is ideal for homestead conditions of Kerala. The species is characterized by the presence of typical annular constriction at the junction of calyx and pedicel , two to five flowers per node and variously incurved peduncles . The fruits are highly pungent and fleshy in nature. The production of this crop in small holdings by individual farmers, in diverse environmental conditions substantially contributed to the vast variability of this crop. Hence there is good scope for its selection and improvement . The present study was taken up to determine the genetic variability, heritability and genetic advance existing in *Capsicum chinense* for yield, quality and reaction to bacterial wilt.

Materials and Methods

The experiment was conducted a t College of Horticulture, Vellanilkkara, Thrissur in two seasons, October 1997 - June 1998 and September 1998 – April 1999. Twenty eight accessions of *Capsicum chinense* collected from different parts of Kerala were used for the study (Table 1).

The experiment was laid out in randomized block design with two replications The crop was raised according to the package of practices recommendation of KAU. Observations were recorded on biometric characters like plant height , number of branches per plant, days to first flowering , days to harvestable maturity pedicel length , fruit length , fruit girth , number of seeds per fruit, fruit weight yield and driage. Quality characters like capsaicin , colour value and oleoresin content were studied based on standard analytical methods. Bacterial wilt incidence was also recorded.

Results and Discussion

The analysis of variance showed significant differences for all the characters during the two seasons. The range, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) , heritability , genetic advance and genetic gain (as percentage of mean) for the 16 characters studied are presented in tables 2and 3. The yield per plant ranged from 12-75.5g during first season and 14 - 185g during second season. The genotype CC8 was the highest yielder during both seasons, followed by CC5 and CC37 during first season and CC23 and CC10 during second season.

The number of fruits per plant ranged from 4 – 52.5 during the first season and from 5 –63.5 during the second season . CC37 had the maximum number of fruits during first season but CC8 recorded the maximum during second season . Days to harvestable maturity was minimum in CC 23 during first season (147 days) while it was only 138 days during second season in the genotype CC10 . Kataria *et al.* (1997)observed a range of 94 – 133 days for first harvest in *Capsicum chinense* . In the present study a range of 147 –192 days during first season and 138 –170 days during second season revealed that accessions of *Capsicum chinense* are late maturing when compared to *C. annum.*

Among the biotic stresses bacterial wilt caused by *Ralstonia solanacearum* was observed more serious. During the first season incidence of wilt ranged from zero (CC32) to 46 per cent (CC2, CC3 and CC17)and from zero(CC28) to fifty per cent (CC16) in the second season. The quality parameters like oleoresin , colour value and capsaicin showed much variability. CC17 recorded the highest oleoresin content while the colour value was maximum in CC37 and CC51. CC5 was the most pungent genotype during both seasons.CC20 was the less pungent genotype during both seasons. The green fruited accessions of *Capsicum chinense* had a higher capsaicin content compared to white fruited accessions. Similar results were reported by Theymoli *et al.* (1982) in *Capsicum annum* and Sheela (1998) in *C. frutescens.*

Existence of high variability for yield and yield attributes in *Capsicum chinense* was observed in the present study. The genotypic coefficient of variation was of high magnitude for fruit length , fruit weight , fruit number per plant and fruit yield per plant during both seasons resulting in high heritability. Sheela (1998) observed higher coefficients of variation for fruit size, mean fruit weight , yield per plant and fruit length in the related species *C. frutescens*. High GCV and PCV for the characters suggest very high variability which in turn offers good scope for selection. High environmental effects on phenotype for the characters like bacterial wilt incidence, yield and fruit girth were evident from their higher PCV as compared to GCV . High heritability along with high genetic gain was observed for fruit length, fruit weight, fruit number per plant and fruit yield per plant. This is in conformity with that of Ahmed *et al.* (1990) and is indicate of additive gene action. So these characters can be improved by selection.

On the basis of present study fruit length, fruit weight, fruit number per plant, fruit yield per plant and number of primary branches per plant appears to be the characters of major importance and should be given due weightage while formulating selection strategies for improvement of yield in *Capsicum chinense.*

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Table 1. Genotypes used during first and second seasons

First season			Second season	
Sl. No.	Accession number	Source	Accession number	Source
1.	CC 1	Karunagappally	CC 2	Karunagappally
2.	CC2	Karunagappally	CC5	Karunagappally
3.	CC3	Karunagappally	CC 6	Karunagappally
4.	CC5	Karunagappally	CC8	Alappuzha
5.	CC6	Karunagappally	CC9	Kottayam
6.	CC8	Alappuzha	CC10	Alappuzha
7.	CC9	Kottayam	CC 14	Wayanad
8.	CC10	Alappuzha	CC15	Kottayam
9.	CC 11	Alappuzha	CC16	Mavelikkara
10.	CC 14	Wayanad	CC17	Wayanad
11.	CC 15	Kottayam	CC18	Thrissur
12.	CC 16	Mavelikkara	CC20	Muvattupuzha
13.	CC17	Wayanad	CC 21	Muvattupuzha
14.	CC18	Thrissur	CC22	Kottayam
15.	CC20	Muvattupuzha	CC23	Thiruvananthapuram
16.	CC 21	Muvattupuzha	CC25	Thiruvananthapuram
17.	CC 22	Kottayam	CC26	Thrissur
18.	CC23	Thiruvananthapuram	CC28	Thrissur
19.	CC25	Thiruvananthapuram	CC30	Wayanad
20.	CC26	Thrissur	CC 32	Thrissur
21.	CC28	Thrissur	CC37	Thrissur
22.	CC 30	Wayanad	CC 38	Ernakulam
23.	CC 32	Thrissur	CC42	Thrissur
24.	CC 37	Thrissur	CC46	Thrissur
25.	CC 38	Ernakulam	CC 51	Kayamkulam

Table 2. Range, Mean, P±CV, GCV, heritability, genetic advance, and genetic gain (as percentage of mean) of 16 characters in *C. chinense* during first season

Sl. no.	Characters	Range	Mean ±SE	PCV	GCV	Heritability	Genetic Advance	Genetic gain (as % of mean
1.	Plant height(cm)	29 - 57.5	43.46 ± 2.88	14.68	13.10	0.795	10.46	24.07
2.	Number of primary branches	2.8 –10.8	4.24 ± 0.39	37.10	35.94	0.938	3.05	71.79
3.	Days to first flowering	104.5 –121.5	114.8 ± 2.62	4.66	4.05	0.758	8.35	7.27
4.	Days to harvestable Maturity	147 –172	156.66 ± 2.62	3.75	3.35	0.799	9.66	6.166
5.	Pedicel length (cm)	1.35-4.25	3.19 ± 0.16	25.93	25.39	0.959	1.63	51.09
6.	Fruit length (cm)	0.90-6.20	3.71 ± 0.12	41.52	41.38	0.993	3.16	85.08
7.	Fruit girth (cm)	3.05-7.7	6.16 ± 0.73	20.89	17.21	0.679	1.80	29.18
8.	Number of seeds/fruit (g)	20.5- 36.5	30.56 ± 1.99	13.77	12.14	0.776	6.73	22.02
9.	Fruit weight (g)	1.05-6.6	3.84 ± 0.19	44.08	43.79	0.987	3.44	88.91
10.	Number of fruits/plant	4.0-32.5	10.3 ± 1.64	60.52	58.22	0.925	10.89	115.36
11.	Yield (g)	11.90-75.5	25.54 ± 4.69	50.53	47.23	0.874	24.16	91.06
12.	Driage (%)	20.75-24.85	22.67 ± 0.31	5.13	4.93	0.926	2.22	9.79
13.	Bacterial wilt Incidence (%)	0-46	22.08 ± 0.88	69.28	58.70	0.718	2.46	102.5
14.	Oleoresin (%)	9.0-25.75	14.53 ± 0.55	29.59	29.34	0.984	8.71	59.94
15.	Colour value	579.57-1433.5	991.86 ± 72.08	22.50	21.30	0.896	411.83	41.52
16.	Capsaicin (%)	0.82-1.85	1.44 ± 0.11	21.45	19.80	0.852	0.54	37.5

Table 3. Range, Mean, PCV, GCV, heritability, genetic advance, and genetic gain (as percentage of mean) of 16 characters in *C. chinense* during second season

Sl. no.	Characters	Range	Mean ± SE	PCV	GCV	Heritability	Genetic Advance	Genetic gain (as % of mean
1.	Plant height(cm)	35-5.7	46.88 ± 4.98	15.74	11.61	0.544	8.27	17.62
2.	Number of primary branches	2.8-11.8	4.58 ± 0.54	39.51	37.70	0.910	3.40	74.17
3.	Days to first flowering	100-122	107.34 ± 2.18	4.71	4.25	0.814	8.48	7.90
4.	Days to harvestable Maturity	138-170	147.98 ± 2.56	5.20	4.90	0.889	14.09	9.52
5.	Pedicel length (cm)	1.40-4.35	3.13 ± 0.23	28.19	27.17	0.929	1.69	53.85
6.	Fruit length (cm)	0.95-6.15	3.45 ± 0.49	44.51	42.09	0.894	2.83	81.98
7.	Fruit girth (cm)	2.50-8.95	6.11 ± 0.26	27.52	27.17	0.975	3.38	55.30
8.	Number of seeds/fruit (g)	28.5-53.0	36.78 ± 5.15	18.28	11.73	0.412	5.70	15.49
9.	Fruit weight (g)	0.9-7.2	3.71 ± 0.15	49.69	49.51	0.993	3.77	101.5
10.	Number of fruits/plant	15-63.5	16.92 ± 2.59	92.99	91.72	0.973	31.53	186.35
11.	Yield (g)	14-185	46.36 ± 5.68	92.69	90.68	0.957	85.5	182.39
12.	Driage (%)	20.3-24.65	22.20 ± 0.24	5.96	5.85	0.965	2.63	11.84
13.	Bacterial wilt Incidence (%)	0-50	19.83 ± 1.12	77.09	60.94	0.625	2.36	99.15
14.	Oleoresin (%)	9.25-24.0	15.61 ± 0.64	30.51	30.23	0.982	9.64	61.73
15.	Colour value	579.5-1535.5	1022.36 ± 73.32	26.22	25.22	0.925	510.84	49.96
16.	Capsaicin (%)	0.88-1.75	1.38 ± 0.09	20.54	19.34	0.886	0.52	37.62

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VARIABILITY, HERITABILITY AND GENETIC ADVANCE IN BIRD PEPPER (*CAPSICUM FRUTESCENS* L.)

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INTRODUCTION

Bird pepper (*Capsicum frutescens* L.) is an economically important species, valued for its perennial nature and highly pungent small fruits with characteristic flavour. It has high capsaicin content and ideal for extraction of high pungency oleoresin. Almost all the bird pepper types are indigenous and characterized by a wide range of observable variability. Breeding works in *Capsicum* have so far been concentrated mainly on *C. annuum* with little emphasis on *C. frutescens*. Considering the importance of bird pepper in industry and export an experiment has been taken up to estimate the genetic variability, heritability and genetic advance in the available accessions of bird pepper.

MATERIALS AND METHODS

This experiment was carried out at the College of Agriculture, Kerala Agricultural University, Thiruvananthapuram, Kerala, India, during the period from 1998 –1999. The materials for the study consisted of 20 accessions of bird pepper collected from different parts of Kerala. The experiment was conducted in randomised block design with two replications. Ten plants were maintained per plot. All the recommended cultivation practices were followed to raise the crop under irrigated condition. Five plants were selected randomly from each accession and observations were recorded on plant height, internodal length, stem girth, leaf area, petiole length, days to first flower, node to first flower, height of node to first flower, fruits per plant, fruit length, fruit girth, fruit weight, yield per plant and incidence of mite. The analysis of variance (Panse and Sukhatme, 1978), variability for different quantitative characters (Burton, 1952) and expected genetic advance at 5.0 per cent intensity of selection (Johnson *et al.*, 1955) were calculated

RESULTS AND DISCUSSION

Analysis of variance showed significant differences among the accessions for plant height, stem girth, leaf area, leaf petiole length, fruits per plant, fruit length, fruit girth, fruit weight and yield per plant.

The present investigation revealed considerable amount of variation for all the characters studied. Higher values of genotypic (3250.51) and phenotypic (3333.35) variances were recorded for yield per plant (Table 1).

Higher phenotypic and genotypic coefficients of variation were observed for yield per plant, fruit weight, fruits per plant, fruit length and fruit girth indicating the higher magnitude of variability for these traits and consequently more scope for their improvement through selection. Days to first flower and node at first flower had low phenotypic and genotypic coefficients of variation. High values of GCV have been reported both for fruit size (Arya and Saini, 1976; Nandi, 1992; Sarma and Roy, 1995) and for fruit length (Nandi, 1992) in *C. annuum.*

High values of heritability were also observed for most of the characters studied. Higher magnitude of heritability (>90%) was registered for fruits per plant, fruit length, fruit girth, fruit weight and yield per plant. High values of heritability were also reported earlier for fruit weight (Gopalakrishnan *et al.*, 1984), fruit size and yield per plant (Arya and Saini, 1977; Jabeen *et al.*, 1998 and Munshi and Behra, 2000) in *C. annuum.*

High heritability does not mean a high genetic advance for a particular quantitative character. Johnson *et al.*, (1955) reported that heritability estimates along with genetic gain would be more rewarding than heritability alone in predicting the consequential effect of selection to choose the best individual. The expected genetic advance was high for fruits per plant, fruit weight, fruit length, fruit girth and yield per plant. High expected genetic advance was also reported earlier in *C. frutescens* for fruit size, mean fruit weight, yield per plant and fruit length by Sheela (1998) .

High heritability coupled with high genetic advance obtained in the present study for fruits per plant, fruit weight, fruit length, fruit girth and yield per plant can be considered as the favourable attributes for the improvement through selection. Similarly, the high heritability combined with high genetic advance could be treated as an indication of additive gene action and the consequent high-expected genetic gain from selection for

these characters. High heritability in conjunction with high genetic advance reported for fruit size in *C. annuum* by Gopalakrishnan *et al.* (1984) and in *C. frutescens* by Sheela (1998) supports the present finding.

On the basis of the present study it is evident that characters *viz.* fruits per plant, fruit weight, fruit length and fruit girth deserve due weightage while formulating selection strategies for the improvement of yield in bird pepper.

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Table 1. Variability parameters for biometrical characters in bird pepper (*Capsicum frutescens*)

Characters	Range	Mean ± SE	Genotypic variance	Phenotypic variance	GCV %	PCV %	Heritability %	Genetic advance %
Plant height	37.05 - 61.22	51.08±1.29	31.36	34.69	10.94	11.53	90.41	21.48
Internodal length	2.45 - 2.60	2.55 ± 0.06	0.01	0.01	2.26	4.30	28.62	2.35
Stem girth	3.95 - 4.75	4.41 ± 0.06	0.04	0.05	4.54	5.07	83.34	8.84
Leaf area	25.49 – 35.03	40.60 ± 0.78	6.67	7.89	6.36	6.92	84.55	12.04
Petiole length	3.05 – 3.75	4.45 ± 0.08	0.03	0.04	3.89	4.49	70.48	6.74
Height of node to first flower	24.15 - 30.34	27.57 ± 1.12	1.24	3.76	4.04	7.03	32.02	4.61
Node to first flower	20.13 - 21.00	20.73 ± 0.28	0.02	0.14	0.68	1.80	14.62	0.53
Days to first flower	50.63 - 53.63	51.93± 0.56	0.05	0.68	0.43	1.59	7.27	0.24
Fruits per plant	102.15 -219.20	131.51 ±3.87	1252.96	1282.86	26.92	27.23	97.69	54.79
Fruit length	2.05 – 4.30	3.25 ± 0.08	0.51	0.52	21.97	22.19	97.46	44.62
Fruit girth	1.50 - 3.10	2.34 ± 0.06	0.26	0.27	21.79	22.21	96.72	43.59
Fruit weight	0.50 - 1.98	1.43 ± 0.05	0.22	0.23	32.80	33.54	97.15	66.43
Yield per plant	65.88 - 269.63	162.83 ± 6.43	3250.51	3333.35	35.01	35.46	97.52	71.23
Incidence of mite attack	0.32 - 1.20	0.87 ± 0.004	0.04	0.05	22.99	25.70	90.21	45.98

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GENE ACTION AND COMBINING ABILITY FOR FRUIT YIELD AND ITS COMPONENT CHARACTERS IN SWEET PEPPER

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INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is an important vegetable cultivated in most parts of the world for its green, immature fruits used in various preparations and salads. The crop grows well in cool weather, which makes Kashmir valley the most ideal place for cultivation of not only fresh green fruits but the seeds of varieties and hybrids could be produced during summer on large scale for export. Despite such potential only limited cultivars are under cultivation, which are either low yielders or poor in quality. This necessiated search for high yielding varieties or hybrids with superior quality. Prior to initiation of any improvement programme, the knowledge of combining ability of parents and crosses and the gene effects involved in the inheritance of various traits is a prerequisite as it provides a guideline for selecting elite parents or combiners which may later be hybridized either to exploit heterosis or to accumulate fixable genes through selection and also helps in understanding inheritance of various quantitative characters which are essential for choosing appropriate breeding methods. The current investigation is an attempt to get information on nature and magnitude of gene action and combining ability by using line x tester analysis designed by Kempthorne (1957).

MATERIALS AND METHODS

The experimental material comprised eight diverse lines of sweet pepper (KSPS-464, HC-202, KSPS-4, World Beater (WB) KSPS-461, KSPS-13, Vinedale and HC-201) each crossed to three testers namely California Wonder (CW), Oskash and KSPS-2 during 1996 tc develop 24 F_1^s. The 11 parents (8 lines and 3 testers) along with 24 F_1^s were evaluated during 1997 at Vegetable Experimental Farm, SKUAST, Srinagar in a randomized block design with three replications. In each replication 10 plants each of F_1^s, and parents were planted in a single row at a spacing of 60 cm between rows and 45 cm between plants within the row. The observations were recorded on five randomly selected plants of each entry for ten different characters (Table-1). The combining ability was carried out as per the method given by Kempthorn (1957). The additive (σ^2_A) and non additive (σ^2_D) genetic variances were estimated from the mean squares for general combining ability (gca), specific combining ability (sca) and error. Average degree of dominance (ADD) was worked out as$(\sigma^2_D)/(\sigma^2_A)½$ given by Comstock and Robinson (1952)

RESULTS AND DISCUSSION

The significant gca and sca variances and the estimated genetic components of variances (Table 1) revealed the importance of both additive (σ^2_A) and non-additive (σ^2_D) genetic variances in the inheritance of various characters. However the additive component of variance (σ^2_A) was more prominent in the genetic control of days to first fruit set which was further supported by degree of dominance whose value was less than one (0.5015). Whereas non additive gene effects (σ^2_D) played a greater role in the inheritance of plant height, plant spread, branch number, fruit girth, fruit number, fruit weight and fruit yield. Importance of non-additive component of variance has been reported by Sharma and Saini (1977) and Ahmad *et al* (1994). The average degree of dominance being more than one for these characters was also in agreement with the above results which indicated over dominance coupled with non additive gene effects and such genes could be successfully exploited by employing heterosis breeding. For fruit length and pericarp thickness

both additive and non additive gene effects were almost of equal magnitude and their degree of dominance value being 0.92 and 0.99 respectively, indicated partial to complete dominance which could be exploited by following recurrent selection as it is most ideal for utilizing both type of gene actions.

The estimates of gca effects of eleven parents presented in Table 2 revealed that none of the parents either among testers or among lines was good general combiner for all the traits. For different characters, different parents proved as good combiners. Among parents HC-202 and Oskash for days to first fruit set. KSPS-461, KSPS-464 and Vinedale for plant height; Vinedale, KSPS-4 and KSPS-464 for plant spread; KSPS-461, KSPS-4 and KSPS-13 for number of branches per plant; KSPS-461, KSPS-464, and W.B. for fruit length , KSPS-13, W.B. and C.W. for fruit girth KSPS-13, KSPS-464 and KSPS-2 for pericarp thickness; KSPS-461, Vinedale and Oskash for number of fruits per plant; KSPS-461, KSPS-13 and KSPS-2 for weight per fruit and KSPS-461, KSPS-13 and KSPS-2 for fruit yield exhibited desirable significant gca effects and proved to be good general combiners. An overall view of the gca effects (Table 2) indicated that the parents viz KSPS-461, KSPS-13 and KSPS-2 in general proved as good general combiners for most of the traits and could be used in hybridization as they possessed high gca effects for fruit yield and yield attributing characters such as weight per fruit, fruit number, fruit length and branch number. It was also evident that for most of the characters the parents with good general combining ability possessed high mean values but some parents like KSPS-2 and KSPS-4 though highest yielders but were poor general combiners for yield. This revealed that combining ability cannot be always judged accurately only by *per.se* performance especially like fruit yield which is dependent on many metric traits and has a polygenic control. For selection of parents one should therefore consider both gca effects as well as *per.se* performance for achieving desired results.

Specific combining ability effects presented in Table-3 revealed that none of the hybrids exhibited desirable significant sca effects for all the traits. Out of 24 cross combinations, 10 crosses had significant desirable sca effects for plant height, 9 for plant spread, 6 for number of branches per plant, 7 for fruit length, 9 for fruit girth, 5 for pericarp thickness 9 for fruit number, 12 for weight per fruit and 8 for fruit yield. Besides revealing significant desirable sca effects most of these crosses also exhibited more or less high *per se* performance.

Out of 24 hybrids, the best specific combinations were KSPS-461 x KSPS-2 and Vinedale x C.W for plant height, KSPS-464 x Oskash and HC-201 x KSPS-2 for ps; HC-201 x KSPS-2 and Vinedale x Oskash for number of branches; KSPS-13 x CW and HC-201 x KSPS-2 for fruit length; HC-201 x Oskash and W.B x KSPS-2 for fruit girth; W.B x KSPS-2 and KSPS-4 x Oskash for pericarp thickness; Vinedale x CW, KSPS-13 x C.W and W.B x Oskash for fruit-number; HC-201 x KSPS-2, KSPS-461 x Oskash and W.B x KSPS-2 for weight per fruit and HC-201 x KSPS-2, vinedela x C.W. and KSPS-461 x Oskash for fruit yield. Besides revealing significant desirable sca effects, most of these crosses also exhibited more or less high *per se* performance which is result of either poor x poor or poor x high general combining parents. In the highest yielding crosses KSPS-461 x Oskash, Vinedale x C.W. and HC-202 x Oskash, the parents Oskash, HC-202 and Vinedale were the poor combiners while the parents viz KSPS-461 and C.W. had good general combining ability. Since most of the high yielding crosses had one parent with high gca effect, it seems both additive and non-additive genetic components are playing an important role in the inheritance of fruit yield. This is in agreement with the observations made by Gopalakrishnan *et al* (1987) and Ahmed *et al* (1997) who indicated suitability of such cross combinations for extracting transgressive segregants. It was also evident from the results that the crosses namely KSPS-461 x Oskash, HC-201 x CW; Vinedale x CW; HC-202 x Oskash and KSPS-461 x KSPS-2 besides having high sca effects also recorded superior *per se* performance inrespect of fruit yield and most of the yield attributing traits and thus could be considered for the commercial exploitation of hybrid vigour under temperate region of our country.

Table1: Estimates of components of variance for combining ability, genetic components of variance and average degree of dominance for yield and yield related traits in sweet pepper.

Source	Days to first fruit set	Plant height (cm)	Plant spread (cm)	Branch number	Fruit length (cm)	Fruit girth (cm)	Pericap thickness (mm)	Fruit number	Fruit weight (g)	Fruit yield (g)
σ^2_{gca}	3.1504	-0.4347	-0.1162	0.0236	0.4845	0.0566	0.1010	1.2023	20.3391	8303.58
σ^2_{sca}	- 1.5845	17.7186	13.4878	0.4902	0.8355	0.2787	0.2003	4.2148	51.8709	24529.63
σ^2_A	6.3008	-0.8694	-0.2323	0.0472	0.9689	0.1133	0.2019	2.4046	40.6781	16607.16
σ^2_D	-1.5845	17.7186	13.4878	0.4902	0.8355	0.2787	0.2003	2.2148	51.8709	24529.68
σ^2_A/σ^2_D	-3.9765	-0.0491	-0.0172	0.0963	1.1598	0.4065	1.0080	0.5705	0.7842	0.677
ADD	0.5015	4.5144	7.6195	3.222	0.9286	1.5684	0.9960	1.3239	1.1292	1.215

Table 2: General combining ability effects of parents for yield and yield components in sweet pepper.

Parents	Days to first fruit set	Plant height (cm)	Plant spread (cm)	Branch number	Fruit length (cm)	Fruit girth (cm)	Pericap thickness (mm)	Fruit number	Average Fruit Weight (g)	Fruit yield (g)
A. Lines										
KSPS-464	2.934**	3.849**	1.478**	0.011	1.354**	-0.243**	0.262**	-2.083**	-1.591**	-128.192**
HC-202	-2.591**	0.382	-3.137**	0.344**	1.724**	-0.421**	0.040	-2.083**	-7.474**	206.225**
KSPS-4	0.856	-2.385**	2.496**	0.500**	-1.068**	0.135**	0.196**	-0.528**	0.237	-21.269*
W.B.	0.250	-3.096**	-2.715**	0.300**	0.299**	0.268**	-0.204**	0.361*	-3.319**	-70.269**
KSPS-461	0.895	4.471**	-0.682*	0.700**	2.743**	-0.065	-0.049	3.472**	15.470**	462.408**
KSPS-13	-1.272	-0.051	0.763*	0.367**	0.254**	0.490**	0.996**	0.250	8.448**	143.086**
Vinedale	-0.889	1.460**	3.300**	-0.278**	-1.390**	-0.321**	-1.071**	1.250**	-13.454**	-174.225**
HC-201	-0.183	-4.629**	-0.656**	-0.656**	-0.468**	0.157**	-1.171**	-0.1695	1.681**	-5.314
SE_{gi}	1.1496	0.4943	0.3996	0.1014	0.0835	0.0502	0.0493	0.1695	0.5542	12.5993
SE_{gi-gj}	1.6258	0.6990	0.5651	0.1434	0.1181	0.0710	0.0697	0.2397	0.7837	17.8181
B.Testers										
C.W.	1.424**	-0.136	1.213**	0.293**	-0.071	0.286**	0.032	-0.319**	1.520**	6.667
Oskash	-2.251**	0.289	-0.298	-0.261**	0.214**	-0.368**	-0.272**	-1.347**	-3.796**	-6.329
KSPS-2	0.823	-0.153	-0.915**	-0.032	-0.142**	0.082**	0.240**	-1.028**	2.276**	-0.339
SE_{gj}	0.7040	0.3027	0.2447	0.0621	0.0511	0.0307	0.030	0.1038	0.3394	7.7155
SE_{gi-gj}	0.9956	0.4280	0.3461	0.0878	0.0723	0.0435	0.0427	0.1468	0.4799	10.9113

***, ** significant at 5% and 1% level of significance respectively.**

Table 3: Best 5 crosses in respect of *per se* performance and significant desirable sca effects for ten characters of Sweet pepper.

S.No	Character	Crosses with *per se* performance	Crosses with significant sca effects.
1.	Days to first fruit set.	7 x 10(23.75), 2 x 10(30.27) 2 x 11(31.53), 6 x 10 (31.53) 2 x 9 (31.66)	8 x 9 (-1.927), 7x10(-1.861) 1 x 10 (-1.849),4 x 9(-1.494), 6 x 11 (-1.468).
2.	Plant height	5 x 11 (54.46), 7 x 9 (50.53), 1 x 10 (50.17), 5 x 10(49.26) 1 x 11(48.20)	1 x 11(5.675), 7 x 9(4.736), 6 9(3.314), 8 x 10(3.200), 2 x 9 (3.181).
3.	Plant spread	7 x 9 (41.33), 1 x 10 (40.66), 7 x 10 (38.26), 6 x 9 (37.80), 3 x 11(37.46)	1 x 10 (5.662), 8x11(5.582), 7x9(2.984), 4 x 9(2.465), 5 x 10 (2.342).
4.	Branch number	3 x 9(7.60), 5 x 1 (7.46), 5 x 9 (7.40), 6 x 11(7.40), 1 x 9 (6.68).	8 x11(1.010), 7 x 10(0.928), 6 x11(0.787), 5 x10 (0.750), 6 x 9(0.463)
5.	Fruit length	5 x 9(11.16), 5 x 10(10.60) 1 x 10(10.03), 5 x 11(9.43) 6 x 9 (9.20)	6 x 9(1.337), 8 x 11(1.264) 4 x 11(1.197), 1 x 10(0.821) 5 x 9(0.815)
6.	Fruit girth	4 x 11((6.16), 6 x 9 (6.13), 4 x 9(6.00), 5 x 9(5.63), 6 x 11(5.60)	8 x 10(0.701), 4 x 11(0.640) 3 x10 (0.524), 1 x 10(0.446) 1 x 9(0.414).
7.	Pericarp thickness	6 x 9(5.60), 6 x 10(4.86), 6 x 11(4.80), 1 x 9(4.53) 4 x 11(4.26)	4 x 11(0.738), 3 x 10(0.583) 8 x 11(0.504), 6 x 9(0.479) 2x10(0.406)
8.	Fruit Number	5 x 11(20.66), 5 x 10(20.03) 4 x 10(20.00), 7 x 9(19.03), 6 x 9(18.03).	7 x 9 (2.875), 6 x 9(2.208), 4 x 10(2.028), 4 x 10(2.097) 1 x 11(1.917).
9.	Fruit weight	5 x 10(67.46), 6 x 11(61.66) 5 x 11(61.40), 5 x 9(60.76), 5 x 11(59.10)	8 x11(10.479), 5 x10(8.830) 4 x11(7.279), 3 x 10(6.396) 4 x 9(4.702)
10.	Fruit yield	5 x 10(1377.00),5 x11(1221.70) 5 x 9(1093.00), 6 x 9(1021.60), 8 x 11 (1002.60)	8 x11(242.026), 7 x9(172.00) 2 x 10(150.196),5 x10(148.696) 3 x 10(141.707)

1). KSPS-464 2). HC-202 3). KSPS-4 4). W.B. 5). KSPS-461 6). KSPS-13
7). Vinedale 8). HC-201 9). C.W. 10). Oskash 11). KSPS-2.

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ESTIMATION OF HETEROSIS, COMBINING ABILITY AND *PER SE* PERFORMANCE IN SUMMER GROWN CHILLI (*Capsicum annuum* L.) FOR YIELD AND RESISTANCE TO LEAF CURL COMPLEX

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ABSTRACT

Fifteen hybrids evolved from 6 X 6 diallel design excluding reciprocals revealed predominance of non-additive gene effects for six of the ten characters studied including yield and leaf curl complex resistance. Among the parents, 'Pant C-1', 'KTPI-19' and 'RHRC-Cluster-Erect', were good general combiners for green fruit yield per plant while 'RHRC-Cluster-Erect', 'Pant C-1' and 'PMR-52/88/K' showed significant *gca* effects for resistance to leaf curl complex . The top crosses involved parents with low and high *gca* effects implying heterosis breeding would be appropriate for green fruit weight, green fruit yield per plant and leaf curl complex resistance in summer evaluated chilli hybrids.

INTRODUCTION

The productivity of chilli is hampered by poor quality and susceptibility to leaf curl complex. Among the three seasons during which the crop is cultivated, summer yields are low on account of above said problems.

Substantial solution to the constraints is the necessity to have a security of the crop and quality of the produce through heterosis breeding. Therefore, the recognition of the genetic attribute available in some of the genotypes used in the experiment and evaluation of parents and F_1's was carried out during summer season. This would help in selection of superior parents and *sca* effects for superior hybrids.

MATERIAL AND METHODS

Six diverse chilli genotypes were crossed in diallel mating fashion to obtain 15 hybrids. The hybrids were evaluated along with their parents and commercial check in a randomized block design with three replications at Department of Horticulture, UAS, Dharwad during summer 1999 with a spacing of 60 X 45 cm. Observations were recorded on days to 50% flowering, fruit length (cm), fruit diameter(cm), pedicel length (cm), pericarp thickness(cm), number of seeds per plant, green fruit weight (g), number of fruits per plant, green fruit yield per plant(g) and leaf curl complex (%).

Statistical analysis of combining ability based on mean values was done as per method I and method II of Griffing (1956). The F_1 hybrid performance was calculated as the estimate of heterosis over better parent. Pooled *gca* status of the parents was analyzed by compiling *gca* effects of different characters studied as per the method suggested by Arunachalam and Bandopadhay (1979). Top crosses were considered based on *per se* values.

RESULTS AND DISCUSSION

The perusal of analysis of variances revealed significant differences among all the characters thus, indicating wider genetic variability among the genotypes in the present investigation (Table 1). GCA and SCA variances for ten characters were studied (Table 2). The GCA and SCA variances were significant for all the characters except pedicel length and pericarp thickness respectively.

The magnitude of estimated components of dominant variance was more than additive variance for days to 50% flowering, fruit diameter, pedicel length, green fruit weight, number of fruits per plant, green fruit yield and resistance to leaf curl complex indicating predominance of additive gene effects. This is in conformity with the findings of Basavaraj (1997) and Mulge (1992). Therefore, heterosis may be rewarding for the aforesaid characters. Since the estimates of additive gene effects were higher in fruit length and number of seeds per fruit .Progeny selection would suffice in accumulation of desirable genes for these characters. Additive and dominance variances were of equal magnitude for pericarp thickness, in such situation reciprocal, recurrent selection may be adopted for population improvement.

From the study on *gca* effects none of the parents were good general combiners for all the characters however, KTPI-19 (6.20, Pant C-1 (14.41) and RHRC-Cluster-Erect (-8.80) exhibited highest *gca* effect for green fruit weight, green fruit yield per plant and resistance to leaf curl complex, respectively (Table 3).

In the present investigation, fruit weight, fruit yield and resistance to leaf curl complex were given more emphases as a measure of productivity in summer grown chilli. *Per se* performance, heterosis and *sca* effects were considered together(Table 4). In case of fruit weight the heterosis and *sca* effects were negative though *per se* values of the top crosses were high. The negativity or insignificance was due to wide variability involved in the parents of the crosses for the trait *viz.*, RHRC-Cluster-Erect X KTPI-19 and Pant C-1 X KTPI-19 (Lohithaswa *et al.*,2001). Similarly, insignificant heterosis was observed when LCA 206 (105.53g /plant) was crossed with KTPI-19 (182.3 g/plant) for green fruit yield per plant. Hence, the chances of F_1 heterosis enhanced when parents were selected from intermediate classes of *per se* values. This was evident from the mean values of the parents of top two crosses for yield per plant as they showed significant positive heterosis and *sca* effects (Table 4).

With regard to resistance to leaf curl complex, the heterosis values when considered alone were found misleading but there was a correspondence between *sca* effect and *per se* values in required (negative) direction. Therefore, selection for yield and leaf curl complex in chilli could be simplified by selecting the parents based on mean values.

The study revealed that the frequency of heterotic hybrids were comparatively high when one of the parents(female) involved in the crosses was of low combining ability status (Table 4). This could be due to complementation of differing alleles or genes and implies that mostly non additive variation plays a major role in expression of green fruit weight, green fruit yield per plant and leaf curl complex resistance.

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Table1. Analysis of variance for ten characters in 6x6 half diallel in chilli

Sl No.	characters	Source of Variation		
		Replications	Treatments	Error
	Degree of freedom	2	20	40
1	Days to 50 % flowering	8.400	92.80**	13.61
2	Fruit length	15.93	22.72**	14.72
3	Fruit diameter	0.041	5.35**	0.04
4	Pedicel length	0.74	1.09*	0.43
5	Pericarp thickness	0.012	0.022**	0.01
6	Number of seeds per fruit	40.22	2008.16**	228.04
7	Green fruit weight	0.13	89.69**	0.33
8	Number of fruits per plant	11.41	4722.74**	73.83
9	Green fruit yield per plant	20.37	9977.52**	254.64
10	Leaf curl complex	2.15	815.31**	15.95

* significant at 5 % probability ** significant at 1 % probability

Table 2. Analysis of variance for combining ability in chilli

Sl No.	characters	SOURCE			Additive Variance (σ^2A)	Dominant variance (σ^2D)	σ^2A/σ^2D
		GCA	SCA	Error			
		14	21	40			
1	Days to 50 % flowering	21.99**	33.92**	4.53	-2.98	29.39	-0.100
2	Fruit length	86.02**	5.73	4.91	20.08	0.82	24.480
3	Fruit diameter	2.98**	1.38**	0.031	0.40	1.37	0.292
4	Pedicel length	0.28	0.39*	0.14	-0.028	0.25	-0.112
5	Pericarp thickness	0.014**	0.005	0.003	0.002	0.002	1.000
6	Number of seeds per fruit	1741.89**	311.89**	76.01	357.50	235.88	1.515
7	Green fruit weight	74.23**	15.12**	0.12	14.78	15.00	0.985
8	Number of fruits per plant	2525.09**	1257.300**	24.61	316.94	1232.69	0.257
9	Green fruit yield per plant	1250.12**	4017.75**	84.88	-691.900	3932.87	-0.180
10	Leaf curl complex	375.58**	237.03**	5.31	34.74	231.72	0.150

* significant at 5 % probability ** significant at 1 % probability

Table 3. Estimates of *gca* effects and pooled *gca* effects of the parents used in diallel crosses of chilli

Sl.No	Characters	Parents						SE(gi)	CD at 5%	CD at 1%
		LCA 206	RHRC-CLUSTER-ERRECT	PANT C-1	PMR-52/88/K	UJWALA	KTPI -19			
1	Days to 50 % flowering	0.78	-1.95**	-0.81	0.83	-1.34	2.49**	0.68	1.37	1.84
2	Fruit length	0.80	0.59	-0.88	-1.42	0.96	1.87*	0.71	1.43	1.92
3	Fruit diameter	-0.35**	-0.29**	-0.42**	-0.42**	0.42**	1.06**	0.038	0.077	0.10
4	Pedicel length	0.30*	0.06	-0.01	-0.27*	0.00	-0.09	0.12	0.24	0.32
5	Pericarp thickness	-0.03	-0.01	-0.02	-0.03	-0.01	0.09**	0.02	0.04	0.05
6	Number of seeds per fruit	-8.45**	-0.17	0.62	-5.43	-14.51**	27.87**	2.81	5.68	7.60
7	Green fruit weight	-1.46**	-0.99**	-1.38**	-1.47**	-0.90**	6.20**	0.11	0.22	0.29
8	Number of fruits per plant	8.44**	4.49**	20.81**	-2.97	-1.51	-32.29**	1.60	3.23	4.38
9	Green fruit yield per plant	-5.94	6.32*	14.41**	-16.99**	-9.08**	11.28**	2.90	5.99	8.03
10	Leaf curl complex	5.35**	-8.80**	-3.56**	-2.46**	-1.53*	7.00**	0.74	1.49	2.00
	GCA status	**L**	**L**	**L**	**L**	**L**	**H**			

* significant at 5 % probability ** significant at 1 % probability L = Low combiner, H = High combiner

Table 4. *Per se* performance, heterosis and combining ability effects of top four crosses (Based on mean value).

Sl.No	Crosses	*Per se* value of P_1	*Per se* value of P_2	*Per se* value of F_1	Heterobeltiosis	*sca* effects	*gca* status
Green fruit weight (g)							
1	Ujwala X KTPI - 19	2.83	26.93	5.54	-79.43**	-3.50**	L x H
2	Pant C-1 X KTPI -19	1.50	26.93	4.83	-82.06**	-3.64**	L x H
3	PMR 52/88/K X KTPI - 19	1.13	26.93	4.49	-81.47**	-3.58**	L x H
4	RHRC-Cluster-Erect X KTPI - 19	3.56	26.93	3.70	-86.26**	-5.25**	L x H
S E ±				0.48	0.58	0.30	
Green fruit yield per plant (g)							
1	RHRC-Cluster-Erect X Pant C-1	93.00	100.00	346.73	246.73**	182.29**	L x L
2	LCA 206 X Pant C-1	105.53	100.00	186.87	77.07**	34.69**	L x L
3	LCA 206 X KTPI - 19	105.53	182.30	180.10	-1.21	31.05**	L x H
4	LCA 206 X Ujwala	105.53	170.17	166.07	36.08**	37.38**	L x L
S E ±				13.03	10.99	8.17	
Leaf curl complex (%)							
1	LCA 206 X RHRC-Cluster-Erect	39.9(39.16)	19.9(24.65)	10.00(18.44)	-25.15*	-17.71**	L x L
2	RHRC-Cluster-Erect X PMR 52/88/k	19.8(26.45)	13.20(21.34)	10.00(18.44)	-13.59	-5.91**	L x L
3	RHRC-Cluster-Erect X Ujwala	19.8(26.45)	11.60(19.89)	10.00(18.44)	-7.29	-6.84**	L x L
4	RHRC-Cluster-Erect X KTPI - 19	19.8(26.45)	80.00(63.44)	11.70(20.05)	-24.20*	-13.76**	L x H
S E ±				3.26	3.26	2.04	

* significant at 5 % probability ; ** significant at 1 % probability ; figures in paranthesis indicate transformed values

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CORRELATION STUDIES FOR QUANTITATIVE CHARACTERS IN RED PEPPER CULTIVARS FOR GRINDING (*CAPSICUM ANNUM L.*)

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Introduction

Many of the characters describing pepper are in particular correlation. The quantitative characters such as number of fruits per plant, length, diameter and average weight of the fruit have a great economic importance but their evaluation and analysis impede the work of the breeder. The evidence of strong correlation between them made easy the breeding process. Many researchers look for correlation between different characters in pepper (Depestre et al., 1989; Ahmed et al., 1997; Jose & Khader, 2002; Pinaki et al., 2002).

The objective of this study is to establish what is the correlation between the morphological characters in the main cultivars of red pepper for grinding.

Material and Methods

The study is carried out in the experimental field of the Institute of Horticulture and Canned Foods, Plovdiv (former the "Maritsa" Vegetable Crops Research Institute) during the period 1996-1999. Six cultivars of red pepper for grinding: the Bulgarian ones 'Gorogled 6' and 'Buketen 50', the Spanish 'Belrubi' and 'Negral' and the Hungarian – 'Kaloscai 801' and 'Mihalytekeli' were tested. The trial is performed in alluvial-meadow soil by block method in four replications and experimental plot of 4,8 m^2 according to the adopted technology for cultivation of red pepper for grinding (Veselinov et al., 1984). Twenty plants and fruits from each treatment have been analyzed. The following morphological characters: plant height (1), stem height (2), fruit number on the main stem (3), fruit number on the branches (4), fruit length (5), fruit diameter (6), pericarp thickness (7), locules (8), average fruit weight (9) and usable fruit part (10) have been observed. The cultivars are sweet peppers (paprika) intended for grinding and distinguish by growth type, habit, orientation and shape of the fruits (Table 1).

Table 1. Morphological characters of the studied red pepper cultivars

Characters	Gorogled 6		Buketen 50		Negral		Belrubi		Kalochai 801		Mihalyteleki	
	min	max	min	max	min	max	Min	max	min	max	min	max
Plant height (cm)	36.8	52.6	27.4	38.8	48.5	75.2	53.7	68.4	39.8	49.6	46.2	60.1
Stem height (cm)	15.2	20.8	11.2	27.0	17.4	25.8	17.0	23.0	10.2	19.2	12.8	22.2
Fruits per stem (number)	0.9	4.0	1.4	11.2	1.0	5.2	0.8	1.4	0.5	2.6	0.7	3.2
Fruits per brunches (number)	8.9	12.0	0.0	9.9	9.1	16.6	9.5	18.5	7.5	12.7	8.2	13.0
Fruit length (cm)	7.4	9.5	8.6	10.4	3.4	4.1	13.4	15.4	8.7	11.0	9.3	11.7
Fruit diameter (cm)	2.4	3.5	2.2	3.3	3.6	4.5	2.3	2.8	2.0	2.4	2.0	2.6
Pericarp thickness (mm)	1.0	1.6	0.5	2.1	1.0	3.5	1.0	2.2	0.8	2.4	0.9	2.3
Locules (number)	2.6	3.2	2.4	3.0	2.9	3.6	2.8	3.3	2.5	2.8	2.8	3.0
Average fruit weight (g)	13.6	23.9	12.0	21.7	16.0	27.8	33.2	41.7	10.3	21.4	8.1	22.4
Average pericarp weight (g)	10.6	17.7	9.5	15.8	14.4	20.0	25.6	33.0	8.8	19.6	6.4	20.0

The correlation coefficients are calculated separately for each cultivar and totally for all cultivars for each year and for the whole experimental period.

Results and Discussion

The correlation coefficients for the studied period of morphologic characters separately for each cultivar in most cases are insignificant during the separate years of investigation. Probably this is due to very little variability in the studied characters in each cultivar. Only the correlation between the average fruit weight and the weight of the usable part in all cultivars and all years of cultivation is strong (Fig. 1). The total correlation for these two characters logically is very strong, r = 0.951** (Table 2). In this case could be considered that the link is universal. The presence of such correlation of tested cultivars, which are comparatively uniform in morphology, is a proof that small changes in one character cause adequate alteration in the other. This correlation has also very important practical aspect because it gives an opportunity with comparatively good accuracy by the weight of the harvested produce to be predicted the weight of the usable part which will enter for drying and further processing.

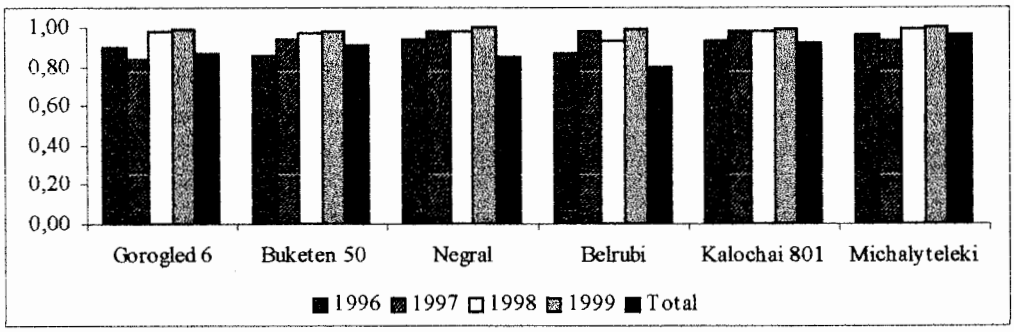

Figure 1. Correlation coefficients between the average fruit weight and the weight of the usable part

He & Wang (1989) established positive correlation between weight and diameter of the fruit, between weight and thickness of the pericarp and negative one between weight and length of the fruit. We did not establish such correlation in our study.

Table 2. Total correlation coefficients in red pepper cultivars for grinding

♦	1	2	3	4	5	6	7	8	9	10
1	♦	0.223**	-0.517**	0.864**	-0.028	0.487**	0.154**	0.502**	0.554**	0.625**
2		♦	0.370**	-0.141**	-0.097*	0.065	0.352**	0.015	0.287**	0.302**
3			♦	-0.764**	-0.082	-0.056	0.336**	-0.115**	-0.137**	-0.202**
4				♦	0.103*	0.371**	-0.155**	0.418**	0.434**	0.530**
5					♦	-0.628**	-0.094*	-0.272**	0.502**	0.482**
6						♦	0.093*	0.684**	0.030	0.057
7							♦	0.167**	0.463**	0.270**
8								♦	0.268**	0.274**
9									♦	0.951**
10										♦

In inclusion of all studied cultivars in the total correlation analysis is found also three well expressed correlations (Table 2). The link between length and diameter of the fruit is

inversely proportional (r = -0.628**). The correlation is defined as moderate to strong and is expressed unidirectional during the all years of study (Table 3 and 4). Our results do not confirm the positive correlation between these two characters established by Legg & Lippert (1966) in chilli peppers.

Table 3. Correlation coefficients in red pepper cultivars for 1996 and 1997 separately

1996 ←

♦	1	2	3	4	5	6	7	8	9	10
1	♦	-0.279**	-0.446**	0.801**	-0.199*	0.529**	0.291**	0.187*	0.471**	0.437**
2	0.317**	♦	0.475**	-0.421**	-0.117	-0.160	-0.099	0.007	-0.385**	-0.367**
3	-0.192*	-0.004	♦	-0.604**	-0.302**	0.106	0.091	0.102	-0.308**	-0.278**
4	0.336**	0.058	0.049	♦	0.050	0.354**	0.158	0.088	0.581**	0.532**
5	0.112	-0.207*	-0.089	0.235**	♦	-0.642**	-0.413**	-0.305**	0.426**	0.466**
6	0.102	0.450**	-0.016	-0.034	-0.492**	♦	0.405**	0.432**	0.240**	0.167
7	0.410**	0.106	-0.122	0.249**	-0.135	0.289**	♦	0.203*	0.168	0.142
8	0.159	0.256**	-0.016	0.108	0.065	0.235**	0.099	♦	0.227*	0.203*
9	0.454**	0.310**	-0.080	0.350**	0.441**	0.033	0.300**	0.299**	♦	0.966**
10	0.459**	0.308**	-0.063	0.352**	0.430**	0.000	0.297**	0.279**	0.994**	♦

1997 →

During the whole experimental period is proved also the correlation between plant height and average fruit weight (r = 0.554**), as well as between the plant height and weight of usable part (r = 0.625**).

Table 4. Correlation coefficients in red pepper cultivars for 1998 and 1999 separately

1998 ←

♦	1	2	3	4	5	6	7	8	9	10
1	♦	-0.373**	-0.586**	0.291**	0.210*	0.272**	0.313**	0.343**	0.670**	0.647**
2	0.875**	♦	0.508**	-0.196*	0.136	-0.094	0.151	-0.134	-0.206*	-0.160
3	-0.653**	-0.622**	♦	-0.263**	-0.082	-0.014	-0.091	-0.251**	-0.416**	-0.308**
4	0.755**	0.701**	-0.629**	♦	-0.002	-0.047	-0.106	0.245**	0.122	0.112
5	-0.501**	-0.427**	-0.019	-0.333**	♦	-0.594**	0.071	-0.063	0.624**	0.530**
6	0.472**	0.416**	-0.094	0.356**	-0.861**	♦	0.332**	0.162	0.047	0.158
7	0.465**	0.530**	-0.400**	0.365**	-0.093	0.144	♦	0.199**	0.282**	0.314**
8	0.324**	0.265**	0.030	0.203*	-0.360**	0.273**	0.199**	♦	0.250**	0.278**
9	0.467**	0.574**	-0.542**	0.379**	0.080	0.101	0.540**	0.039	♦	0.967**
10	0.497**	0.601**	-0.540**	0.393**	0.000	0.181*	0.558**	0.048	0.991**	♦

1999 →

Some authors find statistically significant link between plant height and fruit number in other pepper cultivar types (Nguen K. Huan, 1990). In our study the correlation between plant height and fruit number on main stem is moderate to strong during the three years, while in 1997 practically it lacks. This year was more unfavourable in climatic aspect for development of red pepper in Bulgaria. Data from tables 3 and 4 show that during this year the correlation coefficients as a whole are with lower values i.e. weaker expression of interlinks between the studied morphological characters. The lack of correlation during the more unfavourable years shows that the correlation is not universal and cannot help the breeder. Basavaraja N. & Hulamani (2001) in testing chili pepper cultivars establish positive

moderate correlation between plant height and fruit length which in our study we fail to confirm.

The analysis of correlations in years is an important element from formation of significant conclusions. The use only of total correlation coefficient in interpretation can lead sometimes to incorrect statements. For example, in our study the correlation between the plant height and fruit number on the branches from total correlation analysis is strong (r = 0.864**), but in reality during 1997 and 1998 it is low (Table 3 and 4). Similar is the example also with the correlation between fruit number on the main stem and fruit number on the branches.

Conclusions

From the performed four year studies with Bulgarian and foreign cultivars of red pepper for grinding can be made the following conclusions:

In some of the studied morphological characters the correlation is unstable and is expressed depending on the year of cultivation.

The strongest and stable correlation is established between average fruit weight and weight of usable part (r = 0.951**).

The correlation between the length and the diameter of the fruit is inversely proportional (r = -0.628**).

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CORRELATION AND PATH COEFFICIENT ANALYSIS FOR YIELD AND BIOCHEMICAL CHARACTERS IN CHILLI (*CAPSICUM ANNUUM* L.)

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INTRODUCTION

Chilli (*Capsicum annuum* L.), although being an introduced crop has adapted very well to the Indian conditions. Being cultivated throughout the country as a spice crop as well as a vegetable crop, it has accumulated a wide range of variability due to its cross-pollinated nature. This variability will be helpful in the improvement of this crop. In any crop improvement programme it is a prerequisite to critically assess the interrelationship for yield and its contributing characters. Correlation studies provide information about the nature and magnitude of various associations among the traits. Path coefficient analysis assesses the direct and indirect associations and facilitates in selecting the more reliable yield contributing characters. The present investigation was undertaken to determine the nature and degree of associations among the characters and their direct and indirect effects on yield.

MATERIALS AND METHODS

The correlation and path coefficient analysis in chilli was carried out at the Indian Agricultural Research Institute, New Delhi during the kharif season of 2001-2002.Thirty genotypes collected from different sources were evaluated in the study. Experiment was laid out in randomized block design with three replications. The seeds were sown in the nursery during June 2001 and thirty days old seedlings were transplanted at a spacing of 60cm x 30cm in individual plots of 3m x 1.2m size. The crop was raised under irrigated conditions with all recommended agronomic practices. The observations were recorded in 14 characters viz. Plant height (cm), number of primary branches, number of secondary branches, days to first fruit harvest, fruit length (cm), fruit width (cm), number of fruits per plant, fruit weight (g), yield per plant (g), ascorbic acid (mg/100g), total carotenoids (µg/100g), capsaicin content (mg/100mg) and Total soluble solids (TSS) (%). The ascorbic acid content was determined by 2,6 dichlorophenol indophenol method of AOAC (1970). Estimation of total carotenoids was done as per the method given by Ranganna (1997). Capsaicin content was found out as per the method given by Quagliotti (1971). The phenotypic and genotypic correlations were studied as described by Al-Jibouri *et al* (1958). Path coefficient analysis was carried out according to Dewey and Lu (1959).

RESULTS AND DISCUSSION

In majority of the characters (Table 1) studied, genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlations coefficients indicating a strong inherent relationship between various characters. Munshi *et al* (2000) also observed similar results. Yield per plant expressed significant positive correlation with number of primary branches, number of secondary branches and number of fruits per plant indicating

that selection for bushy plants with more number of fruits per plant could lead to higher yield. This result is in agreement with Rao and Chhonkar (1981), Bavaji and Murthy (1982) and Sarma and Roy (1995),.Fruit length and fruit width showed significant positive correlation with fruit weight but all these three characters expressed significant negative correlation with number of fruits per plant. This indicates that the increase in fruit size was associated with reduction in the number of fruits. Munshi *et al* (2000) also observed similar results. Capsaicin content exhibited significant positive association with number of fruits per plant and negative association with fruit width, fruit length and fruit weight whereas ascorbic acid content exhibited significant negative association with number of fruits per plant and positive association with fruit width, fruit length and fruit weight. Moreover, capsaicin content and ascorbic acid content showed strong negative association with one another. These results show that pungency is related to more number of fruits per plant with smaller size while ascorbic acid is related with larger sized fruits. This result is in accordance with the findings of Sharma *et al* (1981) and Sathe and Phadnavis (1977).

Path analysis (Table2) with yield as the dependent variable revealed that fruit length and number of fruits per plant had higher degree of direct effects, followed by fruit width and days to first fruit harvest. The direct effect of number of fruits per plant was high in magnitude that counteracted its negative indirect effects through fruit length and fruit width to render a significant positive correlation with yield. Also number of fruits per plant showed a positive indirect effect through fruit weight. Dahiya *et al* (1991) also have expressed a similar view. Fruit length and fruit width recorded positive direct effect towards yield and their indirect effect through each other were also positive. Sharma *et al* (1981) observed similar result. Though fruit weight had a negative direct effect on yield, its genotypic correlation was positive due to its positive indirect effects through fruit length, fruit width and days to first fruit harvest. Deka and Shadeque (1997) observed similar negative direct effect of the fruit weight on yield. However, Rao and Chhonkar (1981) reported positive direct effect of fruit weight towards yield. The negative correlation of capsaicin content with yield was due to its negative indirect effect through days to first fruit harvest, fruit length and fruit width that even nullified the insignificant positive direct effect of capsaicin towards yield as suggested by Sharma *et al* (1981). Both number of primary branches and number of secondary branches showed low direct effects but they have recorded significant level of positive correlation with yield. Also they have expressed considerable amount of positive indirect effect through number of fruits. This reveals that selection of plants for more number of primary as well as secondary branches could lead to increased fruit yield. Deka and Shadeque (1997) have also expressed a similar view. The residual value was low, indicating that most important yield contributing characters were included in the study. It can be concluded that traits like number of fruits per plant, fruit length, fruit width and days to first fruit harvest could be considered as most important yield contributing characters since they have expressed positive direct and indirect effects towards yield and also showed substantial positive correlation with yield. The two biochemical characters, capsaicin content and ascorbic acid were inversely related to each other and any improvement in one of the trait would always affect the other.

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Table 1. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients for different pair of characters

Characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
Plant height (X_1)		0.194	0.006	-0.014	-0.029	0.081	-0.006	-0.047	-0.013	0.144	0.048	-0.181	-0.247	0.128
No. of primary branches (X_2)	0.171		0.348	-0.345	-0.119	-0.063	-0.059	0.387	-0.068	0.531	-0.119	0.029	-0.271	-0.073
No. of secondary branches (X_3)	0.010	0.328		-0.553	-0.377	-0.393	-0.287	0.732	-0.323	0.568	-0.460	-0.001	0.012	-0.288
Days to flowering (X_4)	-0.010	-0.312	-0.497		0.829	0.556	0.632	-0.884	0.703	-0.320	0.550	-0.097	-0.426	-0.103
Days to first fruit harvest (X_5)	-0.014	-0.096	-0.296	0.715		0.845	0.878	-0.729	0.928	0.226	0.765	-0.118	-0.681	-0.233
Fruit length (X_6)	0.075	-0.081	-0.345	0.498	0.660		0.491	-0.610	0.835	0.331	0.620	0.042	-0.607	0.068
Fruit width (X_7)	-0.022	-0.042	-0.268	0.531	0.710	0.423		-0.593	0.876	0.178	0.613	-0.174	-0.501	-0.351
No. of fruits per plant (X_8)	-0.036	0.352	0.650	-0.781	-0.569	-0.504	-0.507		-0.636	0.431	-0.520	0.119	0.500	-0.008
Fruit weight (X_9)	-0.007	-0.070	-0.302	0.641	0.855	0.745	0.805	-0.613		0.346	0.701	-0.110	-0.666	-0.234
Yield per plant (X_{10})	0.106	0.444	0.455-	-0.275	0.153	0.271	0.152	0.362	0.274		0.149	-0.003	-0.299	-0.078
Ascorbic acid (X_{11})	0.045	-0.115	0.444	0.501	0.662	0.561	0.562	-0.467	0.678	0.146		0.025	-0.468	0.058
Total carotenoids (X_{12})	-0.174	0.027	-0.004	-0.084	-0.101	0.047	-0.151	0.115	-0.111	0.000	0.025		0.280	0.131
Capsaicin (X_{13})	-0.150	-0.189	0.010	-0.308	-0.422	-0.410	-0.374	0.338	-0.492	-0.216	-0.273	0.228		0.146
Total soluble solids (X_{14})	0.128	-0.071	-0.286	-0.086	-0.171	0.075	-0.304	-0.021	-0.215	-0.093	0.052	0.130	0.134	

Table 2. Genotypic path coefficient analysis

Characters	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}	Genotypic correlation coefficient
Plant height (X_1)	**0.048**	0.021	0.002	0.000	-0.012	0.085	-0.004	-0.048	0.011	-0.002	0.014	0.009	0.019	0.144
No. of primary branches (X_2)	0.009	**0.108**	0.109	0.009	-0.049	-0.066	-0.046	0.399	0.056	0.004	-0.002	0.010	-0.011	0.531
No. of secondary branches (X_3)	0.000	0.038	**0.312**	0.015	-0.153	-0.411	-0.226	0.755	0.265	0.016	0.000	0.000	-0.042	0.568
Days to flowering (X_4)	-0.001	-0.037	-0.173	**-0.027**	0.337	0.582	0.498	-0.912	-0.577	-0.019	0.008	0.016	-0.015	-0.320
Days to first fruit harvest (X_5)	-0.001	-0.013	-0.118	-0.023	**0.407**	0.885	0.692	-0.752	-0.827	-0.026	0.009	0.026	-0.034	0.226
Fruit length (X_6)	0.004	-0.007	-0.123	-0.015	0.344	**0.946**	0.387	-0.629	-0.685	-0.021	-0.003	0.023	0.010	0.331
Fruit width (X_7)	0.000	-0.006	-0.090	-0.017	0.357	0.514	**0.588**	-0.611	-0.719	-0.021	0.014	0.019	-0.051	0.178
No. of fruits per plant (X_8)	-0.002	0.042	0.229	0.024	-0.297	-0.639	-0.467	**0.931**	0.522	0.018	-0.009	-0.019	-0.001	0.431
Fruit weight (X_9)	-0.001	-0.007	-0.101	-0.019	0.410	0.873	0.691	-0.656	**-0.221**	-0.024	0.009	0.026	-0.034	0.346
Ascorbic acid (X_{10})	0.002	-0.013	-0.143	-0.015	0.311	0.649	0.483	-0.536	-0.575	**-0.034**	-0.002	0.013	0.008	0.149
Total carotenoids (X_{11})	-0.009	0.003	0.000	0.003	-0.048	0.044	-0.137	0.123	0.091	-0.001	**-0.079**	-0.011	0.019	-0.003
Capsaicin (X_{12})	-0.012	-0.029	0.004	0.012	-0.277	-0.635	-0.395	0.516	0.546	0.012	-0.022	**0.038**	0.021	-0.299
Total soluble solids (X_{13})	0.006	-0.008	-0.090	0.003	-0.095	0.071	0.277	0.008	0.192	-0.002	0.010	0.006	**0.145**	-0.078

[Residual effect = 0.0762 (Direct effect in bold figures)]

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CORRELATION AND PATH COEFFICIENT ANALYSIS IN BIRD PEPPER (*CAPSICUM FRUTESCENS* L.)

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INTRODUCTION

Yield in plants is the end product of interaction of many correlated characters. Selection for these characters will be more effective when it is based on component characters that are highly heritable and positively correlated. When more number of variables are considered in correlation, the association becomes more complex and less obvious. The use of path coefficient analysis is helpful under such situation. This analysis shows the direct and indirect associations and reveals the most reliable yield contributing characters. In bird pepper (*Capsicum frutescens* L.), a wide range of variability is available which provides a great scope for improving fruit yield through a systematic and planned selection programme for one or more direct or indirect yield components. Keeping in view the above facts, the present investigation was conducted to determine the nature and degree of association among the characters and their direct and indirect effects on yield.

MATERIALS AND METHODS

Twenty accessions of bird pepper (*Capsicum frutescens*) collected from different parts of Kerala were evaluated at the College of Agriculture, Thiruvananthapuram, Kerala, India during 1998 – '99. The experiment was conducted in randomised block design with two replications. Ten plants were maintained per plot. Five plants were selected randomly from each accession and observations were recorded on plant height, stem girth, leaf area, days to first flower, fruits per plant, fruit length, fruit girth, fruit weight and yield per plant. Correlation of various biometrical characters was undertaken as per the procedure suggested by Singh and Choudhary (1979). Path coefficient analysis using genotypic correlation coefficient was carried out according to Dewey and Lu (1959).

RESULT AND DISCUSSION

In all the characters, genotypic correlation coefficient was found to be higher in magnitude than phenotypic correlation coefficient indicating a strong inherent association among various characters (Table 1). Similar observations were made by Sundaram and Ranganathan (1978), Rao and Chonkar (1981) and Choudhary *et al.* (1985). The phenotypic and genotypic correlation coefficient revealed that the association of plant height with other traits was low. The positive association of leaf area with days to first flower, fruit length, fruit girth, fruit weight and yield per plant suggested that the selection of genotypes with high leaf area would help in isolating lines with early flowering and high yield. Days to first flower were found to be negatively correlated with fruits per plant. The results are in conformity with the findings of Ahmed *et al.* (1997). Yield per plant showed highly significant positive correlation with fruits per plant, fruit length, fruit girth and fruit weight suggesting that these characters are the most important yield components and that effective improvement in yield can be achieved through selection based on these characters. Fruit

length and fruit girth showed significant positive correlation with fruit weight. Sheela (1998) reported significant positive association of economic traits like number of harvests, fruit girth, fruit length, mean fruit weight and fruit size with yield in *C. frutescens.*

The results of path analysis revealed that fruits per plant, fruit length, plant height and stem girth had shown positive direct effect on yield while leaf area, days to first flower, fruit girth and fruit weight had exerted negative direct effect on yield (Table.2). The direct effect of fruits per plant was positive and much higher than its genotypic correlation with yield. It exerted positive indirect effect through fruit length while its contribution through fruit girth and fruit weight was negative. Positive direct effect of number of fruits was supported by Gill *et al.*, (1977), Sundaram and Ranganathan (1978) and Munshi *et al.*, (2000) in chilli. The direct effect of fruit length was also positive. Its indirect effect through fruits per plant was high and positive indicating that direct selection for fruit length and indirect selection for fruits per plant can increase yield. Though the direct effect of fruit girth and fruit weight was negative, its indirect effect through fruits per plant and fruit length was high and positive indicating that indirect selection for fruit length and fruits per plant can increase yield.

The direct effect of plant height was positive and much higher than its genotypic correlation with yield. It exerted high and positive indirect effect through fruits per plant and fruit length. The low residual effect (0.1057) indicated that all the important characters are correlated with yield. Rao and Chonkar (1981) and Munshi *et al.*, (2000) also observed low residual value on their study. Based on correlation and path analysis studies, it could be concluded that selection for fruits per plant, fruit weight, fruit length and fruit girth might lead to increase in yield.

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Table 1. Genotypic and phenotypic correlation co-efficient among the characters in bird pepper

Character		Stem girth	Leaf area	Days to first flower	Fruits/ plant	Fruit length	Fruit girth	Fruit weight	Yield / plant
Plant height	P	-0.0276	0.1408	0.2271	-0.0426	-0.2531	0.0872	0.2035	0.1218
	G	-0.0232	0.1732	0.5821**	-0.0525	-0.2737	0.1136	0.2300	0.1260
Stem girth	P		0.4604**	0.3474*	0.3792*	0.6080**	0.4945**	0.6399**	0.6606**
	G		0.5171**	1.1622**	0.4249**	0.6652**	0.5450**	0.7172**	0.7235**
Leaf area	P			0.1297	0.5723**	0.5607**	0.5276**	0.4649**	0.5880**
	G			0.5586**	0.6232**	0.6399**	0.5923**	0.4965**	0.6176**
Days to first flower	P				-0.0487	0.1938	0.2199	0.2814	0.1952
	G				-0.1900	0.7134**	0.6952**	1.1580**	0.9155**
Fruits/ plant	P					0.5614**	0.6262**	0.4484**	0.7061**
	G					0.5835**	0.6517**	0.4626**	0.7202**
Fruit length	P						0.7054**	0.8339**	0.8504**
	G						0.7181**	0.8490**	0.8784**
Fruit girth	P							0.7340**	0.8108**
	G							0.7619**	0.8500**
Fruit weight	P								0.9238**
	G								0.9376**

*, ** Significant at 5% and 1% level respectively. P – Phenotypic correlation coefficient
G – Genotypic correlation coefficient.

Table 2. Direct and indirect effect of characters on yield per plant in bird pepper

Character	Plant height	Stem girth	Leaf area	Days to first flower	Fruits/ plant	Fruit length	Fruit girth	Fruit weight	Genotypic correlation coefficient
Plant height	**0.6191**	-0.0159	-0.1360	-0.0819	0.3216	0.0648	-0.2515	-0.0098	0.1260
Stem girth	-0.0144	**0.6874**	-0.4059	-0.0915	0.7815	0.3108	-0.7843	-0.0564	0.7235
Leaf area	0.1072	0.3555	**-0.7850**	-0.0820	0.7518	0.3378	-0.5430	-0.0481	0.6176
Days to first flower	0.3595	0.4461	-0.4561	**-0.1411**	1.0875	0.4826	-1.0384	-0.0618	0.9155
Fruits/ plant	0.1695	0.4573	-0.5023	-0.1306	**1.1749**	0.4096	-0.9284	-0.0685	0.7202
Fruit length	0.0703	0.3747	-0.4649	-0.1194	0.8437	**0.5704**	-0.8330	-0.0662	0.8784
Fruit girth	0.1424	0.4930	-0.3897	-0.1340	0.9975	0.4344	**-1.0936**	-0.0731	0.8500
Fruit weight	0.0780	0.4974	-0.4848	-0.1118	1.0320	0.4848	-1.0253	**-0.0779**	0.9376

(Residual, R = 0.1057). Figures in bold are the direct effects.

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STABILITY ANALYSIS FOR YIELD AND QUALITY CHARACTERS IN HOT PEPPER (*Capsicum annuum* L.).

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Introduction

Hot pepper (*Capsicum annuum* L.) is an indispensable condiment contain a pungent principle known as capsaicin which is a digestive stimulant and an important ingradient of daily diet and a cure for many rheumatic troubles (Anon, 1964). In addition to capsaicin it is also rich in vitamin A and C and contain carotenoid pigment capsanthin responsible for red colour. Recently pepper has acquired a great importance for oleoresin, which permits better distribution of colour and flavour. In breeding programme emphasis is generally placed on the simultaneous improvement of both yield and quality and these characters being quantitative in nature are highly influenced by the environment. During the process of development such superior varieties, genotype-environment interactions are of major consequences to the breeder as it has masking effect on the performance of genotypes and the relative ranking of the genotypes do not remain same when tested over number of environments. Therefore, a genotype possessing considerable high yield potential coupled with stable performance in different environments has greater value. Such stable cultivars with high yield potential can be directly released for commercial cultivation or utilized in the development of elite gene pool through combination breeding programme. In the present study an attempt was made to identify promising genotypes with stable performance both for yield and quality characters in hot pepper under temperate conditions of Kashmir Valley.

MATERIALS AND METHODS

The investigation on stability analysis of yield and quality traits in thirty hot pepper genotypes were carried-out at three locations viz. Shalimar, Shuhama and Haran, representing three different agro-climatic conditions of the Kashmir Valley. The experiment was laid out in randomized block design with three replications during 1998-99. The experimental plot comprised four rows of 3.6m length with spacing of 45 cm x 45 cm across all the three locations. Standard and uniform package of practices were followed to raise a healthy crop. The observations were recorded on fruit yield (q/ha), ascorbic acid content (mg/100g fresh weight), red fruit colour (ASTA units) and capsaicin (mg/100g dry weight). The ascorbic acid content was estimated by 2,6-dichlorophenol-indophenol method of AOAC (1970). The capsaicin content was determined by the colorimetric method as reported by Quagliotti (1971) and the procedure described by AOAC (1980) was used to determine the extractable red fruit colour from the dried hot pepper fruits. The stability of the genotypes for different characters was worked out following the linear model proposed by Eberhart and Russel (1966).

RESULTS AND DISCUSSION

Pooled analysis of variance revealed the presence of significant genetic variability in the materials for all the traits. Similarly, the environments differed significantly from each other for all the characters except ascorbic acid content, indicating the differential effect of each environment (Table-1). Such a variability has been suggested to determine, to some extent, the usefulness of regression response parameter. The component of G x E interaction was also

significant for all the characters indicating differential response of genotypes that were grown under different environmental conditions where relative merits of different genotypes changed with change in environment. The differential response of genotypes when grown under different environments has also been reported by Sooch *et al.* (1981). The components of environment + (genotype x environment) was further partitioned into linear components of G x E interaction and non-linear pooled deviation. The genotype x environment (linear) component was non-significant for ascorbic acid content and fruit colour while it was significant for fruit yield and capsaicin with linear component having greater magnitude in most of the characters than the corresponding non-linear component. The magnitude of linear components and the corresponding non-linear components for fruit yield, ascorbic acid content, fruit colour and capsaicin were 76.48, 87.10, 0.44, 0.002 and 50.19, 28.21, 0.25, 0.002 respectively. The pre ponderance of G x E linear component suggested that the performance of genotypes across environments for these traits could be predicted with greater precision. Sooch *et al.* (1981) also reported the importance of linear component of genotype x environment interaction in hot pepper under Punjab conditions.

Estimates of mean performance, regression coefficient (bi) and deviation from regression (S^2 di) are presented in Table-3. The estimates of regression coefficient for thirty genotypes ranged from –1.76 to 6.18 for fruit yield, -14.18 to 12.84 for ascorbic acid content 2.62 to 5.62 for fruit colour and –2.36 to 4.81 for capsaicin. Variation among regression coefficients indicated that the genotypes differed for degree of response to environmental changes owing to presence of different set of alleles for stability in each genotype for various traits making it essential to give more importance to genotypes having stability for a number of traits in future improvement of the crop species

The comparison of mean yield performance among genotypes indicated SC-108, SC-103, SC-109, SC-107, SC-106, SC-114, SC-291, SC-100 and SC-111 to be significantly superior to all other genotypes. The genotypes SC-103, SC-109, SC-107 and SC-111 had a regression coefficient approximating to unity and high mean yield indicating their good performance and adopting to all the environments. The genotypes SC-618 and SC-114 were identified to be adapted to favourable environments as indicated by their regression coefficient which were significantly greater than unity. The genotype SC-106, however, demonstrated above average stability and its specific adaptation to poor environments. The genotypes SC-108 and SC-100 having high value of regression coefficient and significant deviations indicated their uncertainty in stable performance under different environments. For ascorbic acid content the genotypes SC-11, SC-120, SC-102, SC-291 and SC-104 recorded high ascorbic acid content and had average stability and found suitable for all the environments whereas genotypes SC-218, SC-101 and SC-19 had above average stability and genotypes SC-106, SC-14, SC-522 and SC-112 exhibited below average stability. For fruit colour five genotypes namely SC-109, SC-106, SC-101, SC-114 and SC-304 performed better than the population mean and possessed average stability and suited over all the environments. The genotype SC-102 revealed below average stability indicating its adaptability to favorable environments. For capsaicin the genotype SC-192 revealed its adaptability to all the environments for higher pungency whereas genotypes SC-108 and SC-106 were observed to be specifically adopted to unfavourable and favourable environments respectively for more pungency. The genotypes SC-372, SC-218, SC-405, SC-502, SC-102, SC-522 and SC-104 recorded low pungency but had average stability indicating their adaptation to all the environments.

The overall perusal of the results revealed that the genotypes SC-106 and SC-114 besides having high yield potential and superior ascorbic acid content, fruit colour and fruit pungency also exhibited stability. These genotypes would, therefore, be useful for commercial cultivation over wide range of environments or could be exploited as elite genepool in future breeding programme.

Table-1: Analysis of variance for phenotypic stability with regard to different traits in hot pepper.

Source of Variation	d.f.	Fruit yield	Ascorbic acid	Fruit colour	capsaicin
Genotypes (G)	29	833.946**	7009.46**	4.27**	0.1077**
Environments (E)	2	1805.890**	30.93	3.20**	0.0145**
G x E	58	64.204**	59.15**	0.38*	0.0025*
Environment (Linear)	1	3611.780**	61.96	6.48*	0.0289**
G x E.(Linear)	29	76.482	87.10**	0.44*	0.0020
Pooled deviation	30	50.196	28.21	0.25	0.0027
Pooled Error	180	36.605	19.28	0.06	0.0001

***, ** significant at 1% and 5% level of significance, respectively.**

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Table-2: Stability parameters namely mean, regression Co-efficient (bi) and deviation from regression (S^2d_i) for different traits of hot pepper.

S. No	Genotype	Fruit yield			Ascorbic acid			Fruit colour			Capsaicin		
		Mean	bi	S^2di	Mean	bi	S^2di	Mean	bi	S^2di	Mean	bi	S^2di
1	**SC-192**	56.01	1.247	99.16	122.79	0.195	-40.78	91.82	0.448	0.314*	0.720	0.872	0.0001
2	**SC-105**	37.92	0.124*	-34.91	54.76	-0.056	-33.96	109.43	2.660*	0.251*	0.258	3.332*	0.0024**
3	**SC-108**	73.38	2.870*	312.24**	111.92	3.180	-38.14	81.76	0.437	0.000	0.915	0.985*	-0.0000
4	**SC-372**	57.91	0.794	-28.46	104.53	0.250	-29.98	111.94	1.289	-0.062	0.135	0.872	0.0001
5	**SC-103**	64.51	1.157	56.30	116.92	3.064	-14.93	97.48	-0.899*	-0.000	0.315	-0.901*	-0.0001
6	**SC-109**	67.13	1.403	36.43	108.23	-14.182*	161.43*	183.64	0.103	0.001	0.302	0.385	0.0015**
7	**SC-107**	81.43	1.570	-9.07	103.66	11.902*	97.00	96.22	-0.113	0.001	0.280	0.844	0.0010**
8	**SC-11**	52.09	0.667	-36.50	143.44	0.511*	-36.92	105.03	3.513*	0.000	0.282	-0.489	0.0017**
9	**SC-48**	35.39	6.189*	32.65	6.30	0.315	-33.78	109.43	3.216*	0.125	0.285	0.028	0.0002
10	**SC-618**	63.73	1.922*	-36.03	94.97	6.858*	34.80	114.46	5.628*	-0.001	0.298	3.868*	0.0115**
11	**SC-106**	83.89	-1.076*	8.79	177.77	9.780*	-38.54	125.58	0.784	1.083**	0.345	4.813*	0.0003
12	**SC-218**	43.16	0.799	-36.53	151.04	-4.692*	-32.61	95.59	0.182	1.551**	0.143	1.917	0.0001
13	**SC-237**	47.34	1.452	-3.01	87.15	-0.173	-40.74	99.37	-0.088	0.251*	0.343	-0.046	0.0011**
14	**SC-405**	16.58	0.251*	-21.62	25.64	1.766	-23.51	67.29	1.540	-0.062	0.223	0.967	0.0001
15	**SC-101**	59.93	0.981	-29.07	158.87	-9.834*	56.78	155.34	0.718	-0.000	0.278	3.840*	0.0078**
16	**SC-502**	55.93	1.455	-35.96	164.30	-4.199*	187.93*	120.12	0.566	0.817**	0.205	0.281	-0.0000
17	**SC-110**	57.70	0.547	-35.96	111.05	-4.185*	-27.22	86.16	0.032	-0.000	0.277	0.243	0.0026**
18	**SC-120**	52.85	-0.515	-34.74	150.17	3.065	-14.91	62.29	1.023	0.000	0.287	0.947	0.0008*
19	**SC-114**	97.16	2.134*	16.77	144.52	5.740*	33.09	152.83	1.162	0.062	0.232	1.538	0.0010**
20	**SC-102**	53.26	0.469	-33.64	168.65	0.132	-33.77	180.50	2.324*	0.063	0.215	0.816	0.0001
21	**SC-291**	70.35	0.965	157.80*	154.30	2.804	70.00	105.03	1.781	0.001	0.900	1.690	0.0009**
22	**SC-277**	35.89	1.332	-36.32	40.42	1.081	-39.12	86.79	1.145	0.188*	0.267	0.385	0.0015**
23	**SC-522**	56.41	0.922	-0.92	191.03	12.847*	-28.13	101.25	0.161	0.503**	0.150	1.661	0.0000
24	**SC-19**	58.06	0.771	-36.40	138.22	-10.662*	-15.28	69.81	1.441	0.062	0.343	0.064	0.0077**
25	**SC-100**	84.63	1.757*	253.40**	173.43	6.925*	124.63*	90.56	0.214	-0.001	0.338	0.545	0.0008**
26	**SC-111**	84.63	0.967	-20.17	175.17	-3.741*	231.90	110.06	0.014	0.126	0.218	-0.443	0.0162**
27	**SC-112**	47.29	0.779	-34.72	161.04	12.471*	112.16	96.22	1.239	-0.062	0.277	1.147	0.0099**
28	**SC-104**	60.63	0.791	-1.26	167.56	3.756	-0.03	70.44	0.870	0.189*	0.178	1.023	-0.0001
29	**SC-302**	52.38	2.161*	-36.58	113.44	-5.116*	-9.80	111.32	-2.621*	0.126	0.292	1.679	0.0015**
30	**SC-304**	41.17	0.818	-36.54	53.89	-0.063	-4.27	135.22	1.230	-0.002	0.262	-2.366*	0.0071**
	Pop. mean	**57.09**			**122.51**			**107.42**			**0.319**		
	±S.E	**5.00**			**3.75**			**9.30**			**0.037**		

***,** Significant at 1% and 5% level of significance respectively.**

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GENETICS OF FERTILITY RESTORATION AND IDENTIFICATION OF RESTORERS AND MAINTAINERS IN PEPPER (*CAPSICUM ANNUUM* L.)

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Abstract

In pepper (*C. annuum* L.), genetics of fertility restoration was studied in a cross between stable cms line 'CCA-42 61' and restorer line 'Pant C-1'. The fertility restoration was inherited as monogenic trait in 'Pant C-1'. A total of 48 hot pepper lines and 5 sweet pepper lines (all *C. annuum*) were screened for the presence or absence of restorer/maintainer genes and results pertaining to identification of restorer and maintainer lines have been discussed in the light of transfer of sterile cytoplasm in more desirable hot pepper line.

Key words: *Capsicum*, cms line, Fertility restoration, Inheritance

Introduction

Unstable (temperature sensitive) expression of male sterility in cytoplasmic-nuclear male sterile (cms) lines of hot pepper (*Capsicum annuum* L.) has been a major limitation in their commercial exploitation in producing commercial hybrid seeds. Therefore, commercial hybrid seed production programs at most of the Indian public and private sectors are based on manual emasculation and pollination (Berke, 1999). However, in Punjab State, many farmers are producing hot pepper (chilli) hybrid seeds exploiting nuclear male sterile line ('MS-12'; *ms-10; ms-10*) and natural cross pollination (Dash *et al.,* 2001). With the development of stable cms lines in the recent past, cms based hybrid seed production of hot pepper has been commercialized in many countries (Shifriss, 1997; Kumar *et al.,* 2000). At this institute, a stable cms line ('CCA-4261') introduced from Asian Vegetable Research and Development Center, Taiwan is presently being utilized to identify potential male parent(s) for commercial hybrid development and their seed production. Nevertheless, for long-term perspective, sterile cytoplasm from 'CCA-4261' is required to be transferred in several lines with more desirable fruit types, which are being predominantly cultivated by the Indian growers. To facilitate such nuclear diversification of sterile cytoplasm, experiments were conducted to study genetics of fertility restoration and to screen inbred lines for the presence of restorer (*Rf*)/maintainer (*rf*) genes.

Materials and methods

Plant materials

A total of 48 hot pepper and 5 sweet pepper lines were utilized during this study along with a stable cytoplasmic-nuclear male sterile line ('CCA-4261'). Most of the lines utilized during this study were the inbreds derived after at least two successive generations of selfing.

Development of F_1s and evaluation

Season 1999-2000

A cross between cms line 'CCA-4261' and 'Pant C-1' was developed and F_1 seeds were harvested.

Season 2000-2001

The F_1 plants of 'CCA-4261' x 'Pant C-1' developed during 1999-2000 were utilized to develop seeds of F_2 and test cross ['CCA-4261' x ('CCA-4261' x 'Pant C-1')] generations. The F_1 between 'CCA-4261' and 'Pant C-1' was again developed. Thirty-seven inbred lines of hot and sweet pepper were crossed on cms line ('CCA-4261').

Season 2001-2002

The F_1, F_2 and test cross generations derived from 'CCA-4261' x 'Pant C-1' developed during 2000-01 were raised along with the parental lines and individual plant in segregating generations were examined for male sterility trait. The F_1 plants of 'CCA-4261' x 'Pant C-1' were again utilized to develop F_2 and test cross seeds. The 37 'CCA-4261' based F_1s developed during 2000-01 were evaluated for the expression of male fertility/sterility along with the parental lines. Twenty-eight inbred lines of hot and sweet peppers were crossed on 'CCA-4261' line, from which17 were derived from the new inbred lines) (Table 2).

Season 2002-2003

The F_1, F_2 and test cross progenies derived from 'CCA-4261' x 'Pant C-1' developed during 2001-02 were raised along with the parental lines. The 28 F_1s developed during 2001-02 were evaluated for the expression of male fertility/sterility (Table 2).

Determination of male fertility

In the segregating populations, individual plant and in non-segregating populations 5 to 10 plants were examined for male fertility/sterility expression. The fertility restoration was determined by following two methods: (i) through visual (eye site) observation of anthers from 5-10 plants for the presence (male fertile) or absence (male sterile) of pollens and (ii) through selfing observations (upon bagging) on ability of plant to produce selfed seeds (male fertile) or non-ability of plants to produce selfed seeds (male sterile). In addition, cytological analysis for pollen fertility percentage was carried out during 2001-02 based on counting of stained pollens (male fertile) and non-stained pollens (male sterile) in 1.5 % acetocarmine.

Results and Discussion

Genetics of fertility restoration

The F_2 populations derived from the cross 'CCA-4261' x 'Pant C-1' were successfully raised during two consecutive seasons. During 2001-02, among the total of 106 F_2 plants, 82 plants were male fertile and 24 plants were male sterile. During 2002-03, among the 76 F_2 plants, 57 were male fertile and 16 plants were male sterile (Table 1). The segregation test pertaining to male fertile and male sterile plants in F_2 generation revealed good agreement between the observed and expected number of male sterile and male fertile plants, as the estimated value of χ^2 for goodness of fit (based on monogenic control of fertility restoration) was non-significant for both the seasons (Table 1). Thus in 'Pant C-1' fertility restoration is controlled by a major dominant (*Rf*) gene.

Table 1. Segregation test for fertility restoration in cross 'CCA-4261 x 'Pant C-1'

Season	O/E	F_2 segregation (# of plants)			$\chi^2_{(3:1)}$
		Male fertile	Male sterile	Total	
2001-02	O	82	24	106	0.314
	E	79.5	26.5		
2002-03	O	60	16	76	0.631
	E	57	19		

Peterson (1958) reported a major dominant gene controlling fertility restoration in pepper. However, in several test crosses, a dihybrid ratio for male sterile and fertile plants was also observed, suggesting thereby two independent loci controlling the fertility restoration. Contrasting results with respect to genetics of fertility restoration were reported by Novak *et al.* (1971), wherein one segregation ratio was in agreement with 3:1 ratio as also described by Peterson (1958) and the other ratio was 9:7, suggesting complementary gene action of fertility restorer genes. During this study, although F_2 segregation ratio revealed presence of single dominant gene for fertility restoration in 'Pant C-1', the observation on test cross progenies (data not shown) revealed involvement of minor gene(s) in the expression of fertility restoration. Such discrepancy with respect to results on genetics of fertility restoration can be explained on the basis of differences in the utilized paternal genotypes at fertility restoration locus.

Identification of restorer and maintainer lines

Forty-eight hot pepper and five sweet pepper inbred lines were successfully developed and evaluated for the presence or absence of fertility restorer gene. On the basis of fertility restoration in the cms based F_1 derived from all the lines, they were classified in three different categories: (i) inbreds with restorer allele, (ii) inbreds with maintainer allele and (iii) inbreds still segregating for restorer and maintainer alleles (Table 2).

Table 2. Evaluation and characterization of cms based F_1s with respect to fertility restoration

S. N.	Name of lines crossed on 'CCA-4261'	Pungent or non-pungent (sweet)	Fertility restoration based on: Season 2001-2002		Season 2002-2003	
			Visual/ (Pollen fertility %)	Selfing test	Visual test	Selfing test
Lines identified as restorers						
1	'EC-345629'	Pungent	YES (71.05)	YES	NT	NT
2	'EC-391097'	Pungent	YES (83.72)	YES	NT	NT
3	'EC-491097'	Pungent	YES (78.24)	YES	NT	NT
4	'EC-119457'	Pungent	YES (71.05)	YES	NT	NT
5	'Local Collection-4'	Pungent	YES (54.51)	YES	NT	NT
6	'Local Collection-2'	Pungent	YES (84.54)	YES	NT	NT
7	'EC-345625'	Pungent	YES (75.33)	YES	NT	NT
8	'EC-301075'	Pungent	YES (86.33)	YES	NT	NT
9	'Phue Sai'	Pungent	YES (75.32)	YES	NT	NT
10	'IC-119378'	Pungent	YES (58.35)	YES	NT	NT
11	'EC-119321'	Pungent	YES (72.65)	YES	NT	NT
12	'DC-3'	Pungent	YES (85.49)	YES	NT	NT
13	'PDC-50'	Pungent	YES (81.57)	YES	NT	NT
14	'EC-343159'	Pungent	YES (76.45)	YES	NT	NT
15	'NO-18'	Pungent	YES (85.14)	YES	NT	NT
16	'JCA-21'	Pungent	YES (63.91)	YES	NT	NT
17	'DC-5'	Pungent	YES (80.75)	YES	NT	NT
18	'EC-257216'	Pungent	YES (61.87)	YES	NT	NT
19	'EC-268216'	Pungent	YES (82.86)	YES	NT	NT
20	'DC-28'	Pungent	YES (66.66)	YES	NT	NT
21	'PDG-1'	Pungent	YES (53.60)	YES	NT	NT
22	'PBC-873'	Pungent	YES (52.59)	YES	NT	NT
23	'G-4'	Pungent	YES (45.55)	YES	YES	YES
24	'Taiwan-1'	Pungent	YES (80.70)	YES	YES	YES
25	'Pant C-1'	Pungent	YES (75.11)	YES	YES	YES
26	'PDC-53'	Pungent	YES (81.54)	YES	YES	YES
27	'KA-2'	Pungent	YES (87.71)	YES	YES	YES
28	'EC-257716'	Pungent	YES (63.01)	YES	YES	YES
29	'9852-173'	Pungent	NT	NT	YES	YES
30	'97-7125-2'	Pungent	NT	NT	YES	YES
31	'97-7116'	Pungent	NT	NT	YES	YES
32	'KDCS-810'	Pungent	NT	NT	YES	YES
33	'P-1649'	Pungent	NT	NT	YES	YES
34	'PBC-1512'	Pungent	NT	NT	YES	YES
35	'PBC-535'	Pungent	NT	NT	YES	YES
36	'Perennial-2A'	Pungent	NT	NT	YES	YES
37	'Pusa Jwala'	Pungent	NT	NT	YES	YES
38	'PBC-367'	Pungent	NT	NT	YES	YES
39	'K. Chanchal'	Pungent	NT	NT	YES	YES
40	'EC-257710'	Pungent	NT	NT	YES	YES
41	'Japani Longi'	Pungent	NT	NT	YES	YES
42	'Local (M)'	Pungent	NT	NT	YES	YES
43	'Punjab Lal'	Pungent	NT	NT	YES	YES

S. N.	Name of lines crossed on 'CCA-4261'	Pungent or non-pungent (sweet)	Fertility restoration based on: Season 2001-2002		Season 2002-2003	
			Visual/ (Pollen fertility %)	Selfing test	Visual test	Selfing test
Lines identified as maintainers						
44	'California Wonder'	Sweet	NO (34.6)*	NO	NO	NO
45	'ISPN-2-4'	Sweet	NO (36.22)*	NO	NT	NT
46	'LCA-206'	Pungent	NO (35.44)*	NO	NT	NT
47	'KSPS-501'	Sweet	NO (34.64)*	NO	NT	NT
‡Lines segregating for both the traits						
48	'JCA-9'	Pungent	NO (33.05)*	NO	YES	YES
49	'EC-491094'	Pungent	YES (51.73)*	NO	YES	YES
50	'KSPS-202'	Sweet	NO (37.17)*	NO	YES	YES
51	'PDC-49A'	Pungent	NO (35.19)*	NO	YES	YES
52	'ISPN 2-3'	Sweet	NT	NT	NO	YES
53	'G-5'	Pungent	YES (82.14)	YES	NO	NO

NT = not tested; * Very few pollen grains were formed

‡These lines may serve purpose of both restorers as well as maintainers following individual plant purifications for *Rf* and *rf* alleles.

Among the 48 hot pepper lines, 43 lines had restorer allele and four lines ('JCA-9', 'EC-491094', 'PDC-49A' and 'G-5') were suspected to had both restorer and maintainer alleles segregating in different plants. Hence, from these four hot pepper lines, restorer and maintainer plants can be purified through developing and evaluating test cross progenies on individual plant basis. The remaining one hot pepper line ('LCA-206') was found to be maintainer, which can be utilized for more rapid transfer of sterile cytoplasm (without maintainer breeding) in order to develop new pair of A and B line in the nuclear back ground of 'LCA-206'. Among the five sweet pepper lines, three lines had maintainer alleles and the remaining two lines were suspected to had both restorer and maintainer alleles segregating in different plants. None of the sweet pepper line had restorer allele (Table 2).

Based on these results, it may be suggested that the distribution of restorer (*Rf*) allele is more frequent in the hot pepper lines, while the distribution of maintainer (*rf*) allele is more frequent in sweet pepper lines. This is the confirmation of previous reports, wherein more frequent occurrence of *Rf* allele in small-fruited hot pepper lines and more frequent occurrence of *rf* allele in large fruited sweet pepper lines have been reported (Shifriss, 1997).

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STUDIES ON GERMPLASM INNOVATION IN HOT PEPPER BREEDING*. I. ANALYSIS OF THE SEGREGATION AND DISTRIBUTION OF SOME TRAITS FROM AN F_2 PROGENY IN HOT PEPPER

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Abstract

The segregation and distribution of four quantitative traits from an F_2 progeny of a single cross in hot pepper (*Capsicum annuum* L var. *longum*) were studied. The results showed that transgressive segregation occurred in fruit length, fruit width and the number of fruits/plant in F_2 progeny. The phenotype of fruit weight, fruit length and fruit width better fit normal distribution, while frequency distribution of the number of fruits/plant showed extremely significant positive skewness. Many kinds of plant patterns appeared in the F_2 population, in which some exhibited similar to F_1, some showed better performance than F_1, and some performed better than female parent P1 for the above four traits.

Key words: *Capsicum annuum* L var. *longum*, F_2 progeny, traits, segregation, distribution

Introduction

Plant breeding has reached to the higher level at home and abroad. In order to breed crops for higher yield, better quality and stronger resistance, plant breeders usually introduce the elite homozygous germplasm according to the breeding objectives. In fact, it becomes more and more difficult to introduce the materials from other research units or breeders. An important reason is lack of the elite germplasm with better bio-agronomic traits besides the intellectual property. Therefore, the breeders generally crossed between one breeding line, with better performance of one or several traits, and another line possessing one to some excellent characters, then select the optimum recombination from the F_2 segregating generation, aiming at germplasm innovations. Germplasm innovation has been the key and spirit of plant breeding.

The main breeding objectives of hot pepper are high yield, superior quality and resistance to *Phytophthora* blight and virus diseases in China. Every breeding objective all are correlated with a group of objective traits. The achievement of high yield in breeding objectives depends mainly on fruit weight and the number of fruits/plant, and that of superior quality lies on fruit nutrient quality and market quality characters, such as fruit length and fruit width.

There are lack of the elite germplasm with well branched plant, excellent fruit setting, more fruit weight (fruit weight > 20 grams) and bigger fruit size (fruit length > 15 cm) in hot pepper breeding practice. The authors crossed one breeding line with another line possessing large fruit, aiming at innovating this kind of germplasm from the selection. Here the segregation and distribution of four quantitative traits from an F_2 progeny of this single cross were studied at first.

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Materials and Methods

Hybridazation was undertaken between the female parent line P1 (*Capsicum annuum* L var. *longum*) and male parent line P2 (*Capsicum annuum* L var. *longum*). The line P1 originated from an F_8 selection from 'PBC 830', a breeding material from Asian vegetable Research and Development Center, possessing vigorous, well branched plant, excellent fruit setting and uniform fruit size The line P2 originated from an F_6 selection out of an open-pollinated Guangzhou local variety, and had bigger fruits. Characters of the two parents are listed in Table 1. F_1 hybrids were identified on the basis of fruit length and fruit color, then single F_1 plant was selfed to obtain F_2 seeds.

Seeds were sown in the middle of July, 2002, and eight-to-nine leaf seedlings were transplanted to the field with a spacing of 30 cm x 60 cm. Four characters, fruit weight, fruit length, fruit width and the number of fruits/plant were recorded according to IBPGR (IBPGR, 1984), on ten individual plants in the P1, P2 parent and F_1 generation, respectively, and ninety five individual plants in F_2 generation.

According to the performance of P1, P2 and F_1, the segregation patterns of single trait in F_2 progeny were divided into seven kinds using t test, i.e. over-P1, P1, P1-F_1, F_1, F_1-P2, P2 and over-P2 type.

For the trait distributions of ninety five individual plants in F_2 generation, the test of normality, skewness and kurtosis were conducted according to Wang(1984).

Results and Discussion

Segregation types of the traits in F_2 progeny

According to the performance of P1, P2 and F_1 (Table 1), the segregation types of the traits in F_2 progeny were analyzed and presented in Table 2.

Table 1 The mean values of four traits of the parents and their F_1

Material	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Number of fruits/plant
Female parent P1	13.55(1.82*)	11.01(0.58)	1.69(0.04)	57.3(9.9)
Male parent P2	59.59(3.94)	19.15(0.55)	3.38(0.26)	15.6(4.1)
F_1	30.21(3.96)	15.84(0.99)	2.35(0.09)	27.0(6.1)

*Values represent standard deviations.

It was shown that there were only three kinds of segregation types in the segregation of fruit weight in F_2 progeny, i.e. P1- F_1 type, F_1 type and F_1-P2 type. In the segregation of fruit length, transgressive segregation occurred, although the percentage was very small; the percentages of female parent P1 type and male parent P2 type plants all were low. Low percentage of the plants, obtained from transgressive (female parent) segregation, appeared in the segregation of fruit width, and the plants of male parent P2 type and over-P2 type were not observed; most plants fell into F_1-P2 type. It was noteworthy that high percentages of female parent P1 and male parent P2 type plants were obtained in the segregation of the number of fruits/plant.

Table 2 The percentage of segregation types of the traits in F_2 progeny

	Over-P1	P1 type	P1- F_1 type	F_1 type	F_1-P2 type	P2 type	Over-P2
Fruit weight	0	0	43.16%	33.69%	23.15%	0	0
Fruit length	1.06%	1.06%	50.53%	29.48%	12.60%	3.16%	2.11%
Fruit width	1.06%	1.06%	25.27%	21.03%	51.58%	0	0
Number of fruits/plant	0	15.78%	21.06%	31.57%	2.11%	20.00%	9.48%

Frequency distributions of four traits in F_2 progeny

Frequency distributions of four traits in F_2 progeny were shown in Figure 1, and the statistical test results of frequency distributions were gave in Table 3.

U test showed that there were not significant deviations from the normal distribution for fruit weight, fruit length and fruit width. In fact Figure 1 had already indicated the symmetry of the distributions of these three traits. Extremely significant deviations from the normal distribution existed for the number of fruits/plant, and strong positive skewness was evident.

The phenotype of fruit weight, fruit length and fruit width better fit normal distribution, while frequency distribution of the number of fruits/plant showed extremely significant positive skewness. This suggested that fruit weight, fruit length and fruit width were quantitatively inherited, and the number of fruits/plant was controlled by major and polygene (Wang & Gai, 1997).

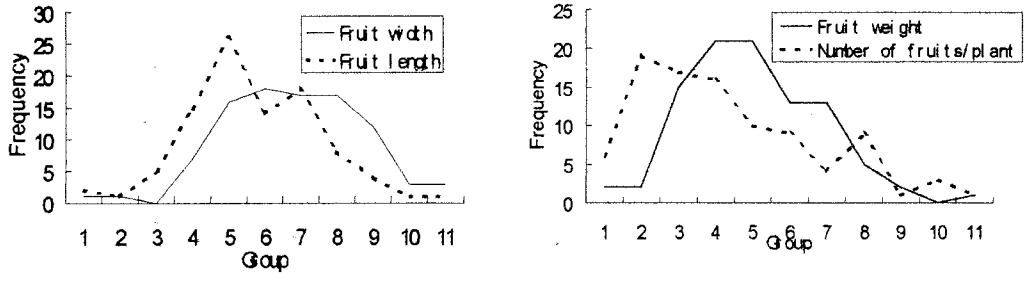

Fig 1 Frequency distributions of four traits in F_2 progeny

Table 3 Test on normal distributions of four traits in F_2 progeny

Traits	Minimum	Maximum	Mean	Standard deviation	Skew coefficient	Kurtosis coefficient
Fruit weight	15.85	47.70	28.80	5.92	0.402	0.104
Fruit length	10.06	21.08	15.22	2.02	0.235	0.296
Fruit width	1.57	3.06	2.44	0.28	-0.201	0.051
Number of fruits/plant	7	74	30.43	16.43	0.732**	-0.277

**:P<0.01

Plant patterns appeared in F_2 progeny

Many kinds of plant patterns appeared in the F_2 population. Interestingly, three F_2 plants showed similar as F_1 plants in these four traits (Table 4, plant pattern I).

In fact, F_1 cross P1 X P2 in the study was 'Yuejiao No. 3' F_1 hybrid released by our unit in 2000. To breed for higher yield and superior quality than this new variety, ten F_2 plants (Table 4, plant pattern II, III, IV, V) were selected according to the following criteria: the number of fruits/plant > that of F_1 plant, fruit weight ≥ that of F_1 and fruit length ≥ that of F_1. Among them fruit length of two plants reached to that of male parent P2 or over-P2 level, the number of fruits/plant of another two plants showed no difference from that of female parent P1. Besides, selection for the female or male parent line might be conducted from these plants, according to the requirements of the female or male parent line in other heterosis breeding program.

In addition, it was found that four plants (Table 4, plant pattern VI, VII, VIII) showed better than female parent P1 for these traits in the F_2 plants. These plants exhibited similar number of fruits/plant as F_1, more fruit weight than P1, and longer fruit than P1 for three plants and same fruit length as P1 for another one. Selection for the new female parent line will be undertaken from these plants according to the requirements of the female parent in heterosis breeding.

Table 4 Some plant patterns in F_2 progeny

Plant patterns	Fruit weight type	Fruit length type	Fruit width type	Number of fruits/plant type	Number of plants	Percentage
I	F_1	F_1	F_1	F_1	3	3.16
II	F_1	P2	P1-F_1	P1-F_1	1	1.06
III	F_1	Over-P2	F_1- P2	P1-F_1	1	1.06
IV	F_1	F_1	F_1- P2	P1	2	2.11
V	F_1 or F_1- P2	F_1 or F_1- P2	F_1 or F_1- P2	P1-F_1	6	6.32
VI	F_1-P2	F_1 or P2	F_1- P2	P1	2	2.11
VII	P1-F_1	P1	P2	P1	1	1.06
VIII	P1-F_1	P2	P1-F_1	P1	1	1.06

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POLLEN CRYOPRESERVATION IN *CAPSICUM* SPECIES-A FEASIBILITY STUDY

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Introduction

Hot pepper is one of the most important commercially grown vegetables in the tropics, and is probably most important after tomato (Grubben, 1977). The fruits are popular for its nutritional value and used in cuisines all over the world. One of the important aspects concerning crop improvement of capsicum is disease resistance. Many of the domesticated capsicum varieties are susceptible to an array of diseases such as bacterial wilt, root knot, viruses and bacterial leaf spot. The strategy of a capsicum breeder is to assemble into cultivars with genetic potential for yield, disease resistance and improved quality. Modern pepper breeding has relied on a relatively narrow gene base within various cultivars groups, despite the morphological genetic diversity apparent both intraspecifically and interspecifically (Poulos, 1994). Under proper storage conditions pepper pollen can maintain viability for extended periods (Alexander *et al.*, 1991; Kristoff and Barnabas, 1983, Benzdic kova 1988). Sources of resistance are available for creating diverse genepools (Poulos, 1994). Hitherto no reports are available on pollen cryopreservation in *Capsicum* species. Rylski (1986) reported that in *Capsicum*, seed set and production could be improved by pollinating with large quantities of pollen. A consolidated stock of viable pollen belonging to different species/cultivars kept conserved in a pollen cryobank will help breeders in achieving this objective. This study reports the feasibility of cryopreserving pollen belonging to 4 capsicum species namely *C. annuum* L., *C. baccatum* L. *C. chinense* Jacq. and *C. praetermissum* Heiser & Smith.

IIHR Contribution No.14/2003

MATERIALS AND METHODS

i) *Pollen collection:* Flowers were collected between 10 AM and 11 AM from field grown healthy plants and brought to the laboratory. Pollen was extracted by keeping the flowers in a BOD incubator attached with fluorescent light at 25^0C for dehiscence, scooping out the pollen mass through lateral sutures of the anther with a spear needle. Pollen thus extracted is collected in a clean butter paper.

ii) *Viability& sterility assessment:* Methods followed are described in detail (Alexander *et al* 1991). Pollen was germinated by improved cellophane method. The preparations were incubated at 25 ± 2^0C for a durations of 4-6 hours, after which staining was accomplished using the versatile stain (Alexander, 1980), pollen stained green are recorded sterile.

iii) *Cryopreservation:* Pollen samples assorted specie-wise were bulked and transferred into empty gelatin capsules and packed in a laminated aluminum pouch and sealed air tight. The pouches were put in 'Eppendoff' tubes, capped and sealed with adhesive tape and held in liquid nitrogen vapours in a Cryogenic container (MVE- Mach SM-33) with the help of a Teflon string. After 48 hours of cryogenic exposure, viability of pollen samples was re-assessed.

The data was analyzed by Factorial CRD with arcsine conversions of percentages for germination values and ANOVA was performed.

RESULTS AND DISCUSSION

The initial germination profiles recorded for the 4 capsicum species in this study was below 55 per cent (as per arcsine conversions). Pollen samples responded well to the 48-hour cryogenic exposure. There was no significant difference in germination percentages of fresh and stored pollen (presented in table-1) except in *C.chinense* ($p=0.05$) where a reduction in post-cryogenic exposure viability was observed. *C. praetermissum* pollen although low in fresh pollen viability, recorded a significant increase in germination profile.

As can be seen in table-1, sterility was minimum in *C. baccatum* and *C .chinense*, having a relatively higher fresh pollen germination profile. Conversely, highest pollen sterility was recorded for *C. annuum*. Wit low germination value. With increasing trend in sterility, lower fresh viability profiles were observed.

The results described above have special significance to capsicum breeders and Gene bank curators. Breeders can avoid growing a crop exclusive by for pollen collection, once sufficient NGD is kept accumulated in a pollen bank. Asynchrony in flowering will no longer be a problem. The frequency of cumulative pollinations could be increased, which will result in more number of seeds set. In capsicum, pollen fails to effect normal fertilization after 3 days of anthesis (Dempsey, 1966). The dry and powdery pollen grains of capsicum are extremely susceptible to even mild variation in temperature and humidity. Pollen desiccates very rapidly under field conditions and do not germinate at all if collected 2 hours after dehiscence. So, if pollen is collected and stored immediately after anthesis at -196^{0}C, the viability can be preserved and extended over a period of time. Since many of these species are widely used as source of resistance, a pollen cryobank for these species will increase breeding efficiency in capsicum.

Table I. Viability of fresh and stored pollen of capsicum species.

Species	**Sterility (%)**	**Fresh**	**Stored**	**Mean**
C.annuum	26.05	37.63 (37.82)	39.57 (38.95)	38.6 (38.38)
C.baccatum	2.2	57.28(49.17)	55.57(48.18)	56.43(48.67)
C.chinense	2.85	61.58 (51.76)	55.65 (48.22)	58.61 (48.79)
C.praetermissum	7.35	38.93(44.32)	43.88(41.46)	41.41(40.01)
Mean		48.86(44.32)	48.67(44.20)	
		SEM	LSD	
Species (A)		1.1029	*3.306	
Treatment (B)		0.7798	NS (2.33)	
Interaction (AXB)		1.5597	NS (4.615)	

- Significant at p=0.05

(Figures in brackets indicate transformed values)

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CHANGES IN THE SEED PROTEIN PATTERNS OF ISOGENIC PEPPER LINES (*Capsicum annuum L.)* OBTAINED BY GAMMA RAYS IRRADIATION OF THE CULTIVAR 'ZLATEN MEDAL'

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The alterations of gene expression resulting in the induction of new proteins and the repression of at least some normally expressed proteins are considered as a typical response of plants to various abiotic and biotic factors (Mladenova et al., 1998). A factors of mechanical, physical and biological origin like tissue cutting, osmotic stress, low temperature, UV irradiation and gamma irradiation have been used (Edreva, 1991). The methods of irradiation influence have a wide application at different economically important plants for obtaining a cultivars with better qualities, as an earlier ripening and resistance to wide-spread diseases. Pepper (*Capsicum annuum L.)* is an important vegetable crop grown in Bulgaria. The morphological markers observed, however, did not reflect completely the changes in plant genome after different external factors. One of the most reliable criteria of their registration on molecular level is the expression of the primary gene products as the proteins by electrophoretic methods.In this study the alterations in the electrophoretic patterns of the soluble seed proteins of isogenic pepper lines developed after gamma irradiation of cultivars are presented.

Materials and methods

Three average samples from ripe pepper seeds were prepared for investigation of soluble proteins of the cultivars 'Zlaten medal' and 'Pazardjishka kapiya' and the respective isogenic lines, developed after gamma rays irradiation (Daskalov, 1998) - 'Zlaten medal *ms8'*, 'Zlaten medal *al'* , 'Zlaten medal *al sw'* - from cv. 'Zlaten medal' and '215 *ms3'* - from 'Pazardjishka kapiya'. The line 'Zl. medal *ms8'* carried the gene *ms8* for pollen male sterility, the line 'Zl.medal *al'* - with the gene *al* for anthocyanin - less, the line 'Zlaten medal *al sw'* - with the genes for anthocyanin - less and sulfury white immature fruit color and line '215 *ms*3' - with the gene *ms3* for pollen male sterility.

The seed protein extracts were prepared according to Reisfeld et al. (1962) with and without urea in the extraction media, according to Cooper (1987) and by Smith and Payne (1984) at SDS conditions. The supernatants were analyzed by vertical block electrophoresis on the apparatus Biotech Hoefer SE 600 (Pharmacia, Uppsala, Sweden) and electrophoretic power source EPS 600.

Results and Discussion

The electrophoretic patterns of unreduced pepper soluble seed proteins of the cultivars 'Zl. medal' and 'Pazardjishka kapiya' and the respective male sterility lines

are presented in Fig. 1. The differences between the phenotypes of cv. 'Zl. medal' and the line Zl. medal *ms8* are quantitative. The components with low and

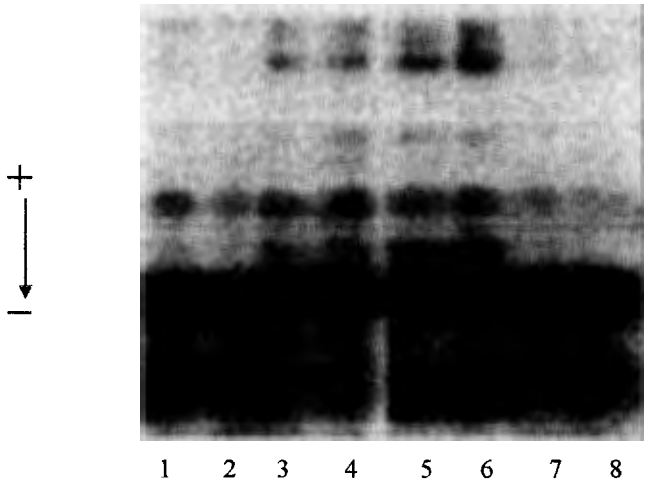

Fig. 1. Electrophoretic patterns of unreduced seed proteins (Reisfeld, 1962) of pepper cultivars and lines: 1, 2 - cv. 'Zl. medal'; 3, 4 - line Zl. medal *ms8*; 5, 6 - cv. 'Pazardjishka kapiya'; 7, 8 - line '215 *ms3*'.

average electrophoretic mobility of the line 'Zl. medal *ms8*' are with higher staining intensity compared with cv. 'Zl. medal'. Some of the components of cv. 'Zl. medal' in these areas are expressed in tracks. The pattern of the cv. 'Pazardjishka kapiya' indicates high staining intensity of the proteins with low and middle mobility in comparison with its isogenic line '215 *ms3*'. In the pattern of the cv. 'Pazardjishka kapiya' a component with middle electrophoretic mobility is expressed that is absent in the line '215 *ms3*'.

Fig. 2. Scheme of the electrophoretic patterns of reduced seed proteins (Reisfeld, 1962) of pepper cultivars and lines: 1, 2, 3 - cv. 'Zl. medal'; 4, 5, 6 - line 'Zl. medal *ms8*'; 7, 8, 9 - line 'Zl. medal *al*'; 10, 11, 12 - line 'Zl. medal *al sw*'.

According to electrophoretic separation of the reduced proteins a large number of components with nearly identical mobility and intensity in cv. 'Zl. medal' and the isogenic lines 'Zl. medal *ms8*', 'Zl. medal *al*' and 'Zl. medal *al sw*' are expressed. The only difference is observed in the slowest moving zone where in the

line 'Zl. medal *ms8*' a doublet of components is expressed, while in the patterns of cv. 'Zl. medal' and lines 'Zl. medal *al'* and 'Zl. medal *al sw'* there is one component (Fig. 2).

The SDS electrophoretic patterns (Fig. 3) reveal two slowest moving components in the line 'Zl. medal *al'* while in the same zones of the other lines and the cv. 'Zl. medal' as well only one component is observed.

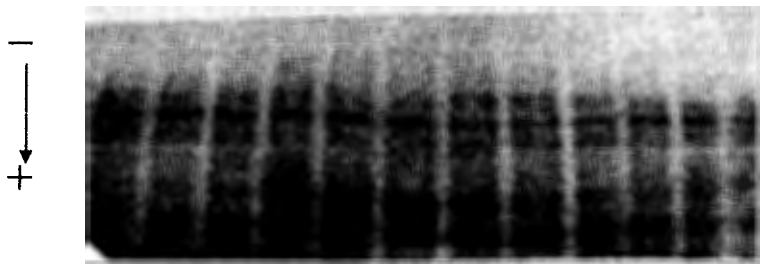

Fig. 3. SDS electrophoretic patterns of seed proteins (Smith and Payne, 1984) of pepper cultivars and lines: 1, 2, 3 - cv. 'Zl. medal'; 4, 5, 6 - line 'Zl. medal *ms8'*; 7, 8, 9 - line 'Zl. medal *al'* ; 10, 11, 12 - line 'Zl. medal *al sw'*.

The patterns of the reduced soluble proteins are shown in Fig. 4. Clearly expressed phenotype qualitative and quantitative differences between cv. 'Zl. medal' and the isogenic lines are seen in the two zones of the spectrum. A high staining intensity component in the zone of slow moving proteins is observed in

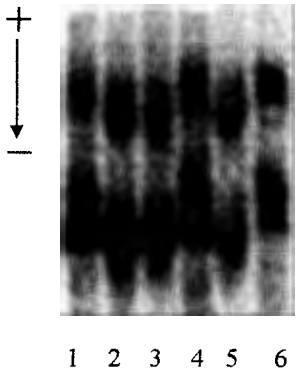

Fig. 4. Electrophoretic patterns of reduced seed proteins (Cooper, 1987) of pepper cultivars and lines: 1 - cv. 'Zl. medal'; 2 - line 'Zl. medal *ms8'*; 3 - line 'Zl. medal *al'*; 4 - line 'Zl. medal *al sw'*; 5 - 'Pazardjishka kapiya'; 6 - line '215 *ms3'*.

the line 'Zl. medal *ms8*' that is absent in cv. 'Zl. medal'. The fastest component of 'Zl. medal *ms8*' has higher electrophoretic mobility than the respective one in cv. 'Zl. medal'. The line 'Zl. medal *al'* differs from cv. 'Zl. medal' by a slow moving component, while the differences between the cultivar and the line'Zl. medal *al sw'*

are only qualitative. In the line '215 *ms3*' lacks the fastest moving component that is specific for cv.'Pazardjishka kapiya'. Besides that a quantitative differences are visible between the cultivar and isogenic line.

Our result confirm the well known from literature fact that after different external factors influence, a changes take place in the nucleotide sequence of DNA molecule. The alterations of the patterns of the proteins as primary gene products find expression in lack of definite one, or appearance of a new componens that reveal a new qualities on morphological level.

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NO SEASONAL DIFFERENCE OF IMMATURE FRUIT DEVELOPMENT OF TETRAPLOID PEPPER (*Capsicum annuum* L. 'Shishitoh')

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Abstract

Fruit development and seed number of diploid and tetraploid 'Shishitoh' pepper were compared at two growing seasons. In April, when maximum/minimum temperature is low, it took three weeks for the diploid 'Shishitoh' fruits to grow to market standard, while in June when max./min. temperature is high, fruit reached the market standard in just two weeks after flowering. On the other hand, tetraploid 'Shishitoh' fruits reached the market standard in just two weeks after flowering in either April or June. The total seed number of both diploid and tetraploid 'Shishitoh' was not significantly different in April and June.

Introduction

Capsicum. annuum L. 'Shishitoh' was a popular cultivar in Japan. Typical fruits of 'Shishitoh' is elongate, having sunken apex (Fig. 1 A). The immature fruits of 5 cm long have high commercial value. It is often observed in the production field that fruit development of 'Shishitoh' varies at different season, hence, causes difficulty in fruit production program. Generally, it is suggested that fruit development of pepper was affected by mean temperature (Bakker, 1989, Ali and Kelley, 1993), and seed number (Marcelis and Baan Hofman-eijer, 1997). In this report, we compared fruit development and total seed number between diploid and tetraploid 'Shishitoh' at two growing seasons. And our result showed that fruit development of tetraploid pepper was not affected by growing season.

Materials and Methods

Plant materials

Tetraploid plants of *C. annuum* L. 'Shishitoh no.562' (Nihon Horticultural Production Institute, Japan) were produced by colchicine treatments of seeds (Ishikawa, K., et al. 1997, 2000). The seeds of self pollinated tatraploid and diploid 'Shishitoh' were sown in trays in August, 2000 in a greenhouse under natural light condition and at minimum soil temperature of 18°C. After two weeks, the seedlings were transferred to pots (soil volume 600 ml) and grown for one month under the same conditions. Later, the plants were transplanted into the ground. Three diploid and tetraploid plants were transplanted side by side and grown in a greenhouse. The height of the plant after six months was about 170 cm and nearly 200 flowers and fruits were growing per plant. The max./min. ambient (mean±standard error) temperatures in April and June were 20.8±0.8/ 12.4±0.7 and 27.3±0.8/17.5±0.4 °C, respectively.

The length, diameter and seed number of the fruits

The length of the fruits was measured from the end of the sepal to the bottom of the fruits, while the diameter was measured at the widest part of the fruits.

Flower buds were selected and tagged in April and June from each among three diploid and tetraploid plants. After one, two and three week(s) from flowering, the length and diameter of the fruits were measured. In April, one and two fruits per plant were sampled from diploid and tetraploid plants, respectively. The number of samples was later made from two and three plants per plant during June sampling.

Counting of seeds from thirteen mature fruits of both diploid and tetraploid plants was made in April and June.

Results and Discussion

In April, when mean temperature is low, it took three weeks for the diploid 'Shishitoh' fruits to grow to market standard of 47.1mm long and 15.4mm in diameter (Fig. 1A and 2A ,B), while in June, when max./min. temperature is high, fruits reached the market standard of 50.2mm long and 14.7mm in diameter in just two weeks after flowering (Fig. 1A and 2A, B).

On the other hand, the mean length of tetraploid fruits two weeks after flowering were nearly 5cm, while the diameter reached about 18mm in April and June (Fig. 1B and 2C, D). This showed that tetraploid 'Shishitoh' fruits grow faster even in April when max./min. temperature is low.

Our result also showed that the total seed number is not related to seasonal difference in growth of early stage, since the total seed number of 'Shishitoh' was not significantly different in April and June (Table 1). In both seasons, diploid fruits had twice to three-fold more seeds than tetraploid fruits. Marcelis and Baan Hofman-Eijer (1997) showed a linear increase in individual fruit weight and seed number, however, Ali and Kelly (1993)showed that large, medium and small fruits were obtained at the intermediate, high and low-pre-anthesis temperatures, respectively. Our results suggest that if the fruits have the sufficient number of the seeds, the growth of the fruits in early stage can be affected by temperature.

Acknowledgement

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A)

B)

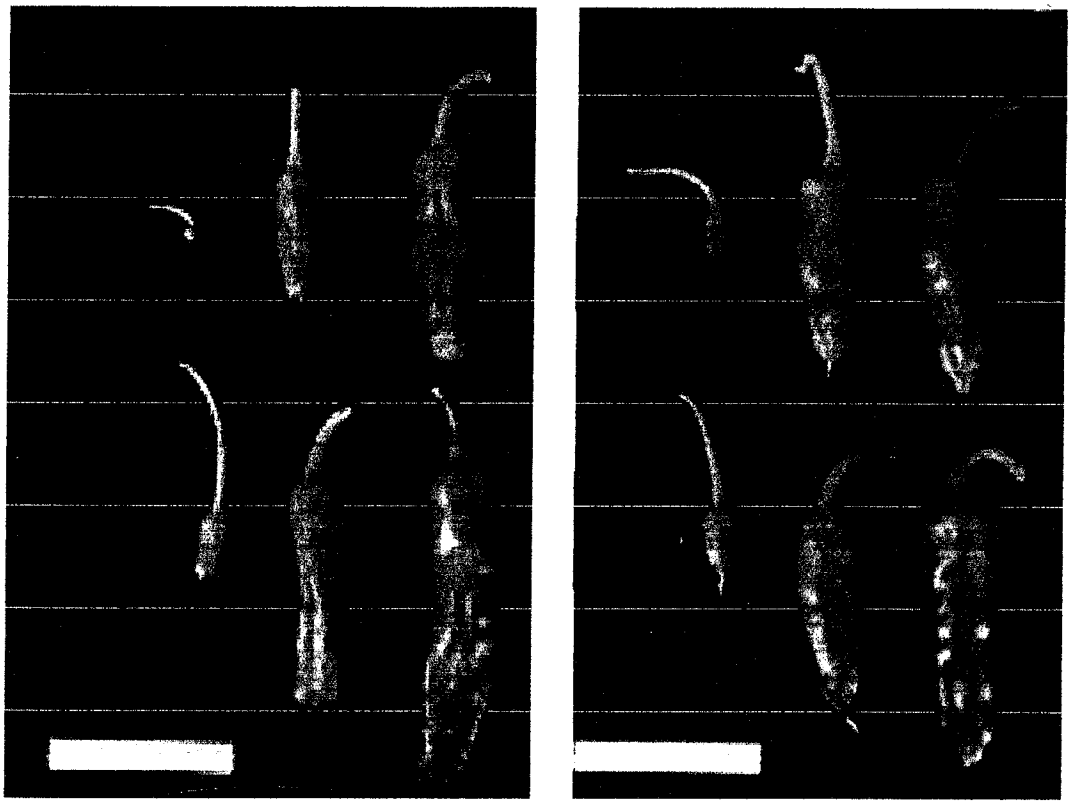

Fig.1 Immature fruits of *Capsicum annuum* L. 'Shishitoh'
A) Diploid and B) Tetraploid

In both figures, upper and lower three fruits from left to right were obtained in April and June, 1, 2 and 3 weeks after flowering. Bars indicate 5 cm.

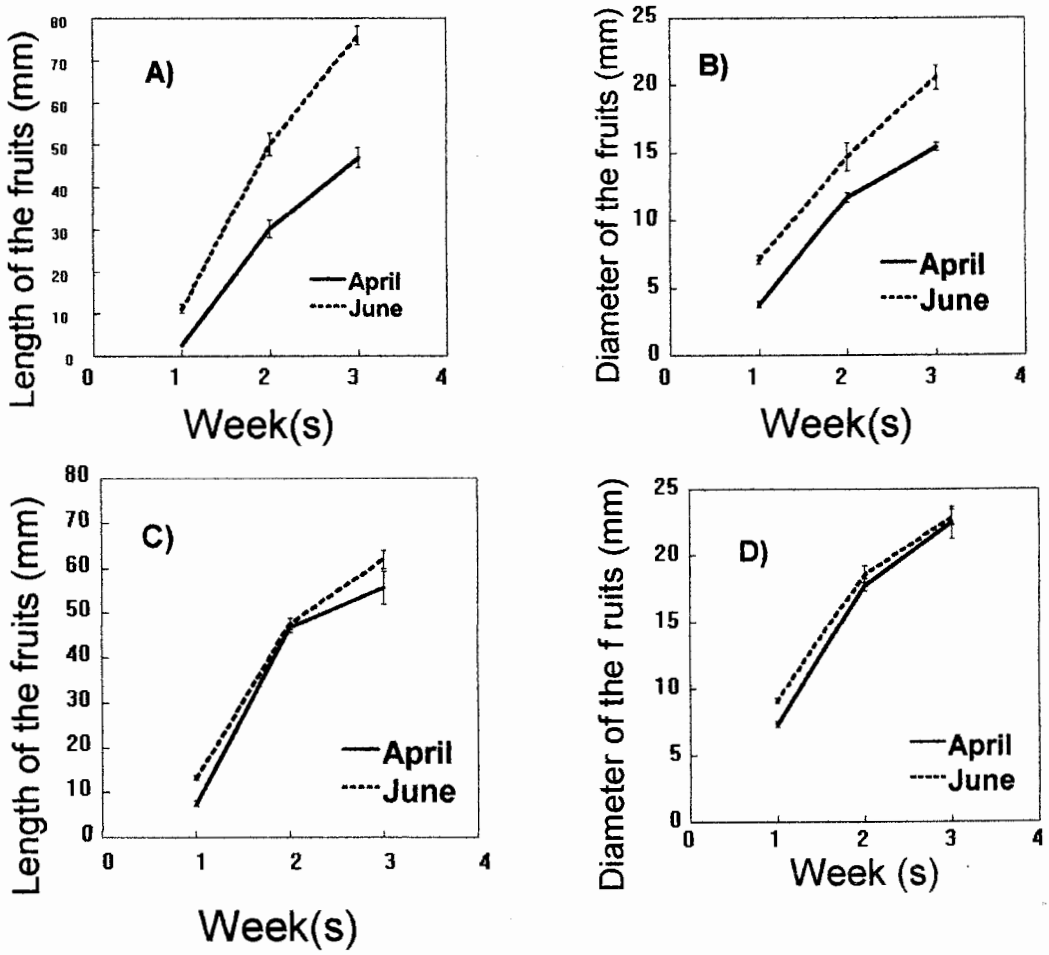

Fig.2 Development of the diploid and tetraploid fruits of *Capsicum annuum* L. 'Shishitoh' in April and June.

Length (A) and diameter (B) of the diploid fruits.

Length (C) and diameter (D) of the tetraploid fruits. Means ±standard error.

Table 1 Total seed number of diploid and tatraploid fruits of *Casicum annuum* L. 'Shishitoh' in April and June.

	Polyploidy	
Seasons	Diploid	Tetraploid
April	84±16	36±4
June	77±15	23±3

n=13, mean±standard error. Within each column, non significant at p□0.01.

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ENABLING *EX-SITU* CONSERVATION OF LANDRACE FIELD-COLLECTIONS FROM MATURE PEPPER PLANTS VIA BUD CULTURE

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Abstract

Shoot-buds were excised from the basal 5 to 7 stem nodes of mature field grown landrace pepper (*Capsicum annuum* L.) collections. They were aseptically grown on Murashige and Skoog (MS) medium with or without supplements of different benzyladenine (BA) alone or plus Indole-3-acetic acid (IAA) concentrations. The greatest number of shoots were produced in cultures on the medium containing 3 µM benzyladenine (BA) alone or plus 0.9 µM Indole-3-acetic acid (IAA) and on the medium with 5 µM BA alone or plus 1.5 µM IAA. Cultures on the medium without BA and IAA supplements produced single shoot per culture bud. However, efficient cloning may be achieved without plant growth regulators since 5 to7 explants could be excised from single mature plant in the field. This *in vitro* method would be useful in enabling conservation of desirable mature variants of pepper landrace germplasm collected in grower fields and in gamete sorting/double-haploid biotechnology based breeding of collected germplasm. **Nomenclature:** *N*-(phenylmethyl)-1*H*-purin-6-amine [benzyladenine, BA], 1*H*-indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA).

Keywords: breeding resources, (*Capsicum annuum*), germplasm, *in vitro*, tissue culture.

Introduction

Breeders have a renewed interest in landraces of crop species (Ehdaie and Waines, 1989) since they usually exhibit useful genotypic variation for tolerance to biotic and abiotic stresses prevailing under environmental conditions where they are grown (Chang, 1985). Usefulness of the genotypic variation for local vegetable cultivars and landraces in Egypt has been the subject of studies by many researchers (Ahmed, 1996; Hussein, 1994; Khalil, 1996; Mohamed, 1996). Ryder (1991) used the lettuce (*lactuca sativa* L.) accession 251245 introduced from Egypt as a source for resistance to lettuce mosaic virus in breeding program for the lettuce cv. 'Salinas 88'.

Substantial amount of green pepper (*Capsicum annuum* L.) in southern Egypt is still produced using seeds of a highly heterogeneous local cultivar. The cultivar is highly variable for fruit size, shape and pungency. However, it is favorable for the local consumers who use its fruits both as fresh vegetable and as pickle. High per cent (up to 70%) of outcross may occur in pepper (George, 1985; Todorov and Csillery, 1990). This local cultivar, therefore, could be considered mixture of heterozygous genotypes originated in the past from varietal intercrosses that occurred in the grower's farms where they produce their own seeds without adequate isolation and no practising for plant roguing. In southern Egypt, this cultivar is usually produced under conditions of low farming inputs, interrupted schedules of irrigation, and temperatures higher than optimum for production of pepper. Therefore, ex situ conservation of such landraces may be a useful tool to maintain breeding resources for improvement of the pepper.

Towards enabling ex situ conservation, the bjective of this study, therefore, was to investigate the response of shoot-buds exised from mature plant of landrace field collections to cloning and multiplication *in vitro*.

Materials and Methods

Shoot-buds in leaf axils of the basal 5 to 7 stems node were excised from field grown mature plants (at the end of the growing season) of Egyptian pepper landrace (*Capsicum annuum* L.) collections grown in the Agricultural Research Station, Assiut University, Egypt. These shoot-bud explants were stirred for 10 sec in 70% ethanol followed by 10 min in 0.5% sodium hypochlorite (10% Clorox plus two drops of Tween-20 per liter). Then they were rinsed three times with sterile distilled water. The disinfested explants were cultured on MS (Murashige and Skoog, 1962) medium under aseptic conditions. The medium contained 3% sucrose and 0.7% agar. The following supplemented of benzyladenine (BA) and Indole-3-acetic acid (IAA) were used (in μM): 1) 1 BA alone or plus 0.3 IAA, 2) 3 BA alone or plus 0.9 IAA, and 3) 5 BA alone or plus 1.5 IAA. The medium without these supplements was used as reference treatment. The pH of the medium was adjusted to 5.7 before being autoclaved (1.4 $kg.cm^{-2}$ at 121°C).

The seven treatments were arranged in randomized complete-blocks with four replicates. Each replicate contained ten culture vessels from each treatment. The axenic cultures of the explants were kept at 25°C±0.5 under light (16h/day) from cool-white fluorescent tubes. Two consecutive experiments were conducted using randomly sampled plants within the field grown landrace plants. Data were recorded after 4 weeks of the culture on percentage of explants developing shoots and number of shoots per cultured bud. Individual shoots were separated from the multiple shoot-buds developed on primary medium containing BA alone or plus IAA. They were pooled and randomly assigned to culture on the medium either lacking or containing 2 μM α-naphthaleneacetic acid (NAA). Single shoots developed in the primary cultures from the control treatment were kept separate on the medium lacking or containing 2 μM NAA. Data were recorded after 4 weeks on the number of shoots forming roots.

The rooted shoot-buds were transplanted into 5-cm plastic pots containing sterile mixture of equal volumes from sphagnum peat, washed sand, and soil. These pot cultures were acclimatized under plastic chamber. Two weeks later, acclimatized plantlets were transplanted in the plastic house. All data of the study were analyzed according to the statistical methods explained by Gomez and Gomez (1984).

Results and Discussion

No multiple shoot-buds were observed in the explant cultures on the medium lacking BA alone or plus IAA (Table 1). Single shoot developed in 60 to 70 per cent of these cultures 3 to 4 weeks after incubation. Forty to fifty per cent of the developed single shoots had roots. The remaining shoots formed roots after additional 3 to 4 weeks when they were transferred to the medium containing 2 μM NAA. Multiple shoot-buds developed in cultures on the medium supplemented with BA alone or plus IAA. Seventy to eighty per cent of these cultures had multiple shoot-buds. Greater number of shoots-buds were obtained from cultures on primary medium containing 3 or 5 μM BA with or without IAA than those which incubated on the medium with 1 μM BA alone or plus IAA. Proliferation of callus tissues associated with the shoot-bud development in all cultures when IAA was added to the medium containing BA. However, callus proliferation did not affect the per cent of the explants forming multiple shoot-buds and the number of shoot-buds produced in these cultures.

Sun and Wang (1990) reported that the optimal medium for shoot-bud multiplication contained 1 mg/L BA (i.e., 4.4 μM) plus 0.5 mg/L IAA and GA (i.e., 2.85 and 1.44 μM, respectively). Also, IAA stimulated shoot-bud regeneration from different seedling explants in 9 cvs./lines of chili pepper (Fari et al., 1990). However, Ebida and Hu (1993) regenerated the greatest number of shoot-buds from shoot tip explants on BA containing medium with no auxin

1993) for producing anther explants to use in gamete sorting and developing double-haploid lines.

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supplements. They observed only callus and roots when 0.4 mg/L of NAA (i.e., 2.15 µM) was added to the BA culture medium. Genotypic differences (Fortunato and Tudisco, 1991) and/or explant type (Ebida and Hu, 1993) affect the response of pepper cultures *in vitro*.

Table (1): The percentages of explants developing multiple shoot-buds and average number of shoot-buds produced in two consecutive in vitro experiments on medium containing different concentrations of benzyladenine (BA) alone or plus Indole-3-acetic acid (IAA) using shoot-bud explants excised from leaf axils on the basal 5 to 7 nodes of the mature field plants of pepper landrace collections from mid-southern Egypt.

Supplements (µM)	Explants developing multiple Shoots-buds (%)		Average number of shoot-buds per explant	
	1st Experiment	2nd Experiment	1st Experiment	2nd Experiment
BA IAA				
1.0 0.0 (treat 1)	70.0	75.0	2.5	2.3
1.0 0.3 (treat 2)	72.5	70.0	2.2	2.2
3.0 0.0 (treat 3)	80.0	77.5	4.2	3.8
3.0 0.9 (treat 4)	72.5	75.0	3.8	3.5
5.0 0.0 (treat 5)	77.5	80.0	4.5	4.0
5.0 1.5 (treat 6)	82.5	77.5	4.2	4.2
0.0 0.0 (control)	00.0	00.0	1.0	1.0
Contrasts	**Mean squares[1]**		**Mean squares[1]**	
Control vs. others	19.71[2]**	19.71[2]**	0.0	2.21[3]**	1.86[3]**
1&3&5 vs. 2&4&6	1	ns	66.67 ns	1.04 ns	0.0 ns
vs. 3&5	3	20.42[3]ns	37.5 ns	12.5	9.37 **	7.04**	0.12
vs. 5	2	ns	12.5 ns	ns	0.12 ns
vs. 4&6		66.6 ns	10.41[3]ns	7.04 **	7.04**
4 vs. 6		20.0[3] ns	12.5 ns	0.12 ns	1.12 ns

[1]ns,** Nonsignificant and significant at $P < 0.01$, respectively.
[2,3] Should be multiplied by 10^3 and 10, respectively.

Twenty to thirty per cent of shoots that were separated from multiple shoot-buds readily formed roots on secondary medium lacking plant growth regulators. Similar to findings by others (Ebida and Hu, 1993), significantly ($P < 0.01$) higher percentage (70 to 80%) of these shoot-buds formed roots when they were transferred to secondary medium containing 2µM NAA. *Ex vitro* acclimatized plants obtained from the *in vitro* propagated plantlets showed normal growth, fruit development, and seed set.

Utilization of shoot-buds excised from mature pepper plants may be advantageous for conservation of this landrace. This is because explants can be collected without interruption for the production in grower's fields. Five to seven shoot-bud explants can be excised from single plant and, therefore, several copies can be simply produced on medium lacking plant growth regulator supplements. Also, it offers a flexible schedule for the interested scientists in collecting this germplasm. Additionally, variants and elite genotypes under stress conditions in the grower fields can be propagated and then grown under favorable conditions (Kristiansen and Andersen,

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SOMATIC EMBRYOS OF *CAPSICUM ANNUUM* L., GENETIC SPECIALITIES OF FORMATION

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It is well known that the process of mass production of pepper regenerants, in vitro is far from its complete understanding. The main difficulties, researchers face with, are the follows: low output of regenerants per explant or absence of regeneration at all, weak repetition of the results, low rooting capability during passaging in culture. It is true for practically all in vitro directions but especially actual for organogenesis and somatic embryogenesis. In this connection according to our point of view, not empirical elaboration of protocols of different methods for concrete genotypes is perspective but fundamental researches connected with genetics of biological development of studied object and gene expression. In the case of somatic embryogenesis of pepper the following strategy of research is suggested:

1. Estimation of gene bank of studied species in order to single out different donors of a trait;
2. Detailed characteristic of trait expression during ontogeny;
3. Revealing biotic and abiotic factors, which modulate expression;
4. Studying laws of variability and inheritance of a feature in different types of donors revealing possible pleiotropic effects;
5. Working out methods of controlling expression of a character in the necessary direction.

Systemic approach to solution of the problem of formation somatic embryos lets to make a prognosis on probability of presence a gene family in population of a species and responsiveness of defined genotype within studied character. Also it lets to optimise media for concrete type and finally not only the expression within the frame of reaction norm of the genotype, but qualitative change it.

Earlier (Timina et. al. 2001) we identified different donors of formation of somatic embryos of pepper. The goal of given work was to study the expression and inheritance of the character in some donors during ontogeny.

Materials and methods

Studied gene pool of Capsicum annuum L. was presented by varieties and lines of var. annuum, according general classification of the genus Capsicum L. (Baral, Bosland, 2002) and 35 genotypes, among them 12 F_1 hybrids and 1 interspecific with C frutescent L were compared. We studied formation of general quantity of embryos, development of the embryos up to the stage of tulip, dividing received structures into normal (green) and chimerical (albino). Immature zygotic embryos on the different stages of development were used as explant. We also used other types of explants: hypocotyls, cotyledons and leaves from plants, which were sterile grown on the medium containing ingredients according Murashige and Skoog double diluted, without hormonal additions. The most responsive genotypes were used in semidiallel crosses, for studying of inheritance of different stages of embryogenesis. Plants were grown in plastic unheated greenhouse during spring-summer rotation according to general technologies. Pollination of maternal form was done by redundancy of paternal form. Embryos of parents and F_1 hybrids were excised aseptically on all stages of their development: globular, heart, torpedo, stick, semi-ring and ring and then with other explants raised on medium supplemented by 2.4 D according Binzel et al., 1996 in double repetition. In each repetition at least 10 tubes with defined type of explant were used. Data were worked out using the degree of dominance h_p (Zhuchenko et. al., 1973) and genetic parameters of diallel crosses (Fedin et. al., 1980).

Results and discussion

1. Expression of formation of somatic embryos in several donors.

Screening of C. annuum gene bank carried out earlier has made it possible to reveal donors of both direct and indirect embryogenesis (Timina et. al., 2001). The exact timing of induction of somatic embryos connected with concrete stage of explant development in ontogeny (torpedo and stick) has been

the distinctive feature of direct embryogenesis of studied donors and F_1 hybrids received on their ground.

Depending on genetic background, responsive ones are explants even on the stage of globe and heart, that we could see studying F_1 of interspecific hybrid C. frutescens x C. annuum. Probably the data received are evidence of different embryo's adaptation ability depending on genotype and its stage of development and is the result of its different surviving capacity in artificial medium. Different embryo's surviving capacity probably could become a basis for working out express-methods of adaptation ability to abiotic stressors and connected pleiotropic gene effects.

Data've been received indicate that the process of switching on the sporophytic way of embryos development is the irreversible one the case of direct embryogenesis using 2,4D as the inductor in the favourable in vitro conditions thanks to what we suppose there is a special gene system in C. annuum sooner referring to gene regulator one. The work of this supposed gene is highly depended on physiological stage of explant's donor.

Thus, explants that were excised from fruits at the beginning of technical ripeness were characterized by most intensive formation of cluster bipolar structures. Explants from plants at the full technical ripeness – by solid decreasing of induction process and explants from plants at the ending of vegetation – mainly by not embryogenic callus. Ageing of an organism and changing of an endogenous balance of hormones-inductors on the one hand and also formation of enzymes-repressors could be the cause of such modulation of expression. Use of zygotic embryo explants at the stage of semi-ring and ring induced among majority of responsive genotypes only callus formation. At the same time there was one cross combination F_1 Kolobok x L-48, explants of which on the same stage of development formed embryos directly. Received results prove data of Harini, Lakshmi Sita (Fari, 1995), who had watched the analogical process among formed mature zygotic embryo of the variety of California Wonder and probably the results gave an evidence of another mechanism of cascade activation of somatic embryogenesis of pepper.

Indirect formation of embryos was watched using donors L-49, Gogoshari and Kolobok. Callus was received from seeds with damaged seed peal, which contained both endosperm and embryo (semi-ring, ring). In three months of cultivation, callus was subcultivated on fresh medium with the same concentration of hormone. The culture was raised for two weeks illuminated with intensity of 3000 Lk and 16-hour photoperiod and then in darkness at the temperature 25°C during one month.

Thus, necessity of dedifferentiation and subcultivation and also stress impact of callus are specialities of indirect embryogenesis of C. annuum.

2. Inheriting stages of formation of somatic embryos.

Disconnection and different formation of somatic embryos among studied donors were indirect evidence of that in vitro, embryogenesis could be divided into a number of stages: induction, formation general quantity of embryos and also albinos and green structures, being taking under account by us at the stage of tulip. And the induction capability was a characteristic for the process as a whole, and other stages were its details.

Defined degree of dominancy of F_1 hybrids changed differently in dependence on cross combination or genetic background and stage of embryogenesis (tabl. 1). In general, though for the whole process we indicated prevalence of over negative and negative dominancies, the stage of general quantity of embryos was characterized by total prevalence of over positive and intermediate dominancies. The stage of albino forming was characterized by total equality of negative and positive dominancies and the stage of green structure formatting – by over expressed negative dominancy. Such differentiation gave an evidence of that each stage of embryogenesis was controlled by independent genetic systems. There were the presence of cross combinations that had h_p in absolute figures higher than 1 that was an evidence of epistatic types of genetic interactions and of different genetic systems controlling stages of embryogenesis of studied donors. It is interesting to mark one more type of genetic interactions characterizing 4^{th} stage of embryogenesis – complementary or new formation. Besides these are most valuable identified gene systems stipulating further regeneration of plants, they are different at donors L-48, L49 and variety of Prometey as we can understand from the functional test of allelism.

Found gene interactions did not let to define genetic parameters on the basis of semidiallel crosses because of not matching of experimental material to suggested model and this was confirmed by insignificant coefficients of regression of each stage. That is why for more detailed genetic identification of donors, in future, genetic analysis using of F_2, F_3 and becrosses will be fulfilled.

Repetition of genetic analysis of F_1 for the same combinations but with using explants on the stage of stick showed for number of combinations complete decreasing of formatting of somatic embryos, changing of types of gene interaction and dominancy, which stipulated different expression of the trait during ontogeny. (tabl. 2). Thus, investigations have been fulfilled by us give evidence of the presence of at least two types of gene regulation of formation of somatic embryos of C. annuum. The first one is connected with the functioning of supposed gene regulation switching on the sporophytic way of development on the earliest phases of ontogeny of immature zygotic embryo.

The second – is connected with changes of gene interaction and the degree of dominancy functioning on later phases of ontogeny. Probably the first type is stipulated by the influence of inductor during transcription, the second type - during processing, cotranslational transfer or translation. Although we have not known yet the mechanisms of cascade activation and repression of the process of somatic embryogenesis in vitro, but found specialities of expression and influence of gene background let us work out aimed decisions for improving of expression of the trait by synthetic selection just today.

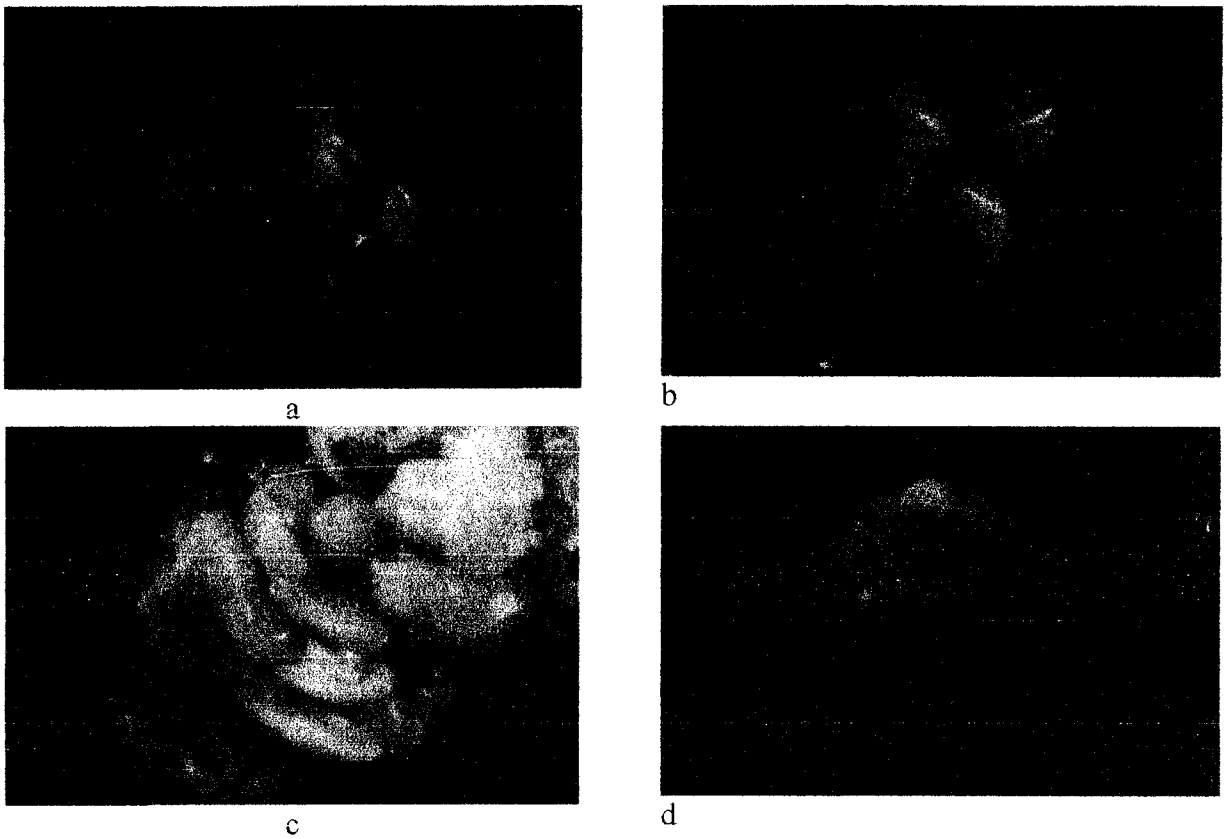

Figure 1. Stages of direct embryogenesis at donor of this sign (variety Tolstiy Baron).
a. Torpedo-shaped structures
b. Tulip-shaped structures
c. Clusters of chimerical structures
d. Forming of green structures beginning to callus under durable cultivation on the medium with 2.4D

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Table 1. The inheritance of stages of somatic embryogenesis in explants of hybrids F_1 Capsicum annuum in vitro (in torpedo phase)

Nr	Hybrid combination	Stage's of embryogenesis															
		induction of somatic embryos, %				total number of found embryos				the number of albinos				the number of green structures			
		P_1	P_2	F_1	h_p1	P_1	P_2	F_1	h_p2	P_1	P_2	F_1	h_p3	P_1	P_2	F_1	h_p4
1	L48 x Prometey	6.7	8.3	12.5	6	15	25	24	0.8	15	25	23	0.6	0	0	1	-
2	Kolobok x Prometey	9.1	8.3	0	-23	29	25	0	-13.5	27	25	0	-26	2	0	0	-1
3	L49 x Prometey	8.3	8.3	22.7	-	21	25	125	51	21	25	113	45	0	0	12	-
4	Dobrinya x Prometey	18.18	8.3	12.5	-0.15	51	25	49	0.85	49	25	49	1	2	0	0	-1
5	Kolobok x L48	9.1	6.7	10	1.75	29	15	33	1.57	27	15	33	2	2	0	0	-1
6	L49 x L48	8.31	6.7	0	-9	21	15	0	-6	21	15	0	-6	0	0	0	-
7	Dobrinya x L48	18.18	6.7	4.2	-1.4	51	15	6	-1.5	49	15	6	-1.53	2	0	0	-1
8	L49 x Kolobok	8.31	9.1	0	-23	21	29	0	-6.25	21	27	0	-8	0	2	0	-
9	Dobrinya x Kolobok	18.18	9.1	8.33	-1.2	51	29	40	0	49	27	40	0.18	2	2	0	-
10	Dobrinya x L49	18.18	8.31	9.3	-0.8	51	21	26	0.67	49	21	24	-0.78	2	0	1.8	0.8

Table 2 The inheritance of stages of somatic embryogenesis in explants of hybrids F_1 Capsicum annuum in vitro (in stik phase)

Nr	Hybrid combination	Stage's of embryogenesis															
		induction of somatic embryos, %				total number of found embryos				the number of albinos				the number of green structures			
		P_1	P_2	F_1	h_p1	P_1	P_2	F_1	h_p2	P_1	P_2	F_1	h_p3	P_1	P_2	F_1	h_p4
1	L48 x Prometey	30,77	0	4,25	-0,72	225	0	34	-0,7	225	0	34	-0,7	0	0	0	-
2	Kolobok x Prometey	0	0	8,33	-	0	0	11	-	0	0	11	-	0	0	0	-
3	L49 x Prometey	0	0	0	-	0	0	0	-	0	0	0	-	0	0	0	-
4	Dobrinya x Prometey	0	0	4,17	-	0	0	8	-	0	0	8	-	0	0	0	-
5	Kolobok x L48	0	30,77	0	-1	0	225	0	-1	0	225	0	-1	0	0	0	-
6	L49 x L48	0	30,77	11,1	-0,28	0	225	6	-0,95	0	225	6	-0,95	0	0	0	-
7	Dobrinya x L48	0	30,77	0	-1	0	225	0	-1	0	225	0	-1	0	0	0	-
8	L49 x Kolobok	0	0	8,33	-	0	0	2	-	0	0	0	-	0	0	2	-
9	Dobrinya x Kolobok	0	0	0	-	0	0	0	-	0	0	0	-	0	0	0	-
10	Dobrinya x L49	0	0	0	-	0	0	0	-	0	0	0	-	0	0	0	-

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EFFECTS OF CRYOPRESERVATION OF PEPPER AND EGGPLANT SEEDS

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1. Introduction

Cryopreservation at –196 °C in liquid nitrogen (LN_2) can be used to conserve reproductive plant material and minimize its deterioration in storage, and has been successfully employed for seeds, ovaries, dormant buds, pollen, meristems, tissue cultures, embryonic axes, somatic embryos, excised embryos, etc. Results have been encouraging for conserving orthodox seed of cultivated and wild plants, and even recalcitrant seeds (IRIONDO *et al.* 1991, JIN HU *et al.* 1996, STANWOOD 1985). Several factors have been shown to exert a positive, although somewhat variable influence on the outcome; these are seed moisture content, lipid content, cooling rate, length of time under LN2, and conditioning, after thawing but before germination, of recalcitrant seeds.

This paper concludes a series of tests conducted at the Vegetable Germplasm Bank, University of Turin, on pepper and eggplant (BELLETTI *et al.* 1990, LANTERI *et al.* 1990, QUAGLIOTTI and LOTITO 1995), to investigate factors such as seed moisture, cooling rate, and cycles of freezing and thawing.

2. Materials and methods.

Four experiments (Table 1) were conducted, to analyse the effects of seed moisture, number of cooling and warming cycles, and cooling rate.

The seeds used were those of pepper (*Capsicum annuum* L.) cv. 'Corno di toro' and eggplant (*Solanum melongena* L.) cv. 'Prospera'. Control samples were held at - 20 °C in our germplasm bank. Samples to be cooled in LN2 were first placed in plastic cryovials.

Each freeze-thaw cycle (cooling to - 196 °C, warming to ambient temperature, and return to LN2) was either *rapid* (from LN2 to + 30 °C for 15 min, ambient temperature for 60 min, return to LN2) or *slow* (from LN2 to - 20 °C for 60 min, - 10 °C for 60 min, 0 °C for 60 min, ambient temperature for 60 min, and direct return to LN2).

Seed viability after treatment was checked using 8 replicates of 100 seeds each, performed according to International Seed Testing Association rules (1999), at 20 °C for 16 h/d plus 30 °C for 8 h/d.

In experiments 1 and 2, seeds germinated each day up to the fourteenth day were counted, and we determined percentage germination (PG), mean germination time (MGT) and percentage of abnormal seedlings (AS). In later experiments, attention was focussed on seed vigour rather than on germinability. Thus in experiment 3 an ageing test was introduced such that all seed, after the freezing treatments, was held for 48 h at 40 °C and 20 % relative humidity, before the germination tests. In experiment 4, a controlled deterioration test was similarly introduced. This consisted of a period of 48 h at 45 °C and 28 % RH (*S. melongena*) or 24 h at 45 °C and 18 % RH (*C. annuum*).

3. Results

3. Results

Experiment 1 showed that the two methods of cooling and warming (slow or rapid) gave similar results. Thus in the later experiments only the rapid method was used. The number of cycles (from 0 to 25) had a statistically significant effect on PG in both pepper and eggplant. However, even the maximum number of cycles had relatively little effect on PG (3-4 %). In addition, unexpectedly, the lowest mean PG values (80.5 % versus 86.7 control) for pepper were found for the seeds with the lowest moisture content (4.7 %) (Fig.1).

Experiment 2 showed that both the number of cycles and the moisture content did not influence PG in pepper. In contrast, for eggplant, the negative effect of greater seed moisture (10.5 %) was already significant after 10 cycles.

Experiment 3 showed the negative effect of high seed moisture content both in eggplant and pepper. In the latter, significantly more abnormal seedlings appeared at high humidity and with increasing numbers of freeze-thaw cycles, with synergism between these two factors. Above 15 % RH, the viability of pepper seeds was strongly depressed by numerous freeze-thaw cycles.

Experiment 4 also showed that high seed moisture was the main factor in reducing PG (fig.2). In pepper, the number of cycles and the interaction number of cycles x seed moisture content also had a significant effect (fig.3).

4. Conclusions

The two kinds of seed tested proved to be equally resistant to the changes in temperature consequent upon interruption of cryoconservation. Unexpectedly, different rates of cooling did not appreciably affect subsequent germinability, and at least at low values of seed moisture content, the negative effects of cooling and rewarming on viability were relatively slight.

Damage sporadically appeared in seeds with the lowest RH. This could mean that survival under cryoconservation and resistance to repeated thermal cycling, which were normally high, could be reduced by strong initial dehydration used as a preliminary to conservation. Our results also showed that, at least for the dryest seeds, cell damage consequent on numerous freeze-thaw cycles was minimal.

Seeds of pepper were more easily damaged than those of eggplant. We do not know, however, how much of this was due to the genotypes used or to the previous histories of these particular seed lots.

Table 1 Experimental plan

Esperiment	Seed moisture(%)	No. of freeze-thaw cycles	Method of cooling
1	4.7, 6.3, 10.5 or 11.5*	0**, 1, 3, 5, 10, 25	slow, rapid
2	4.8, 6.5, 10.5	0**, 1, 3, 5, 10, 25, 50	rapid
3	4.8, 6.5, 10.5, 15, 20	0**, 25	rapid
4	4.8 , 6.5, 10.5, 20	0**, 25	rapid

* 10.5 for pepper; 11.5 for eggplant. ** control

Figure 1 – Experiment 1: Pepper seeds

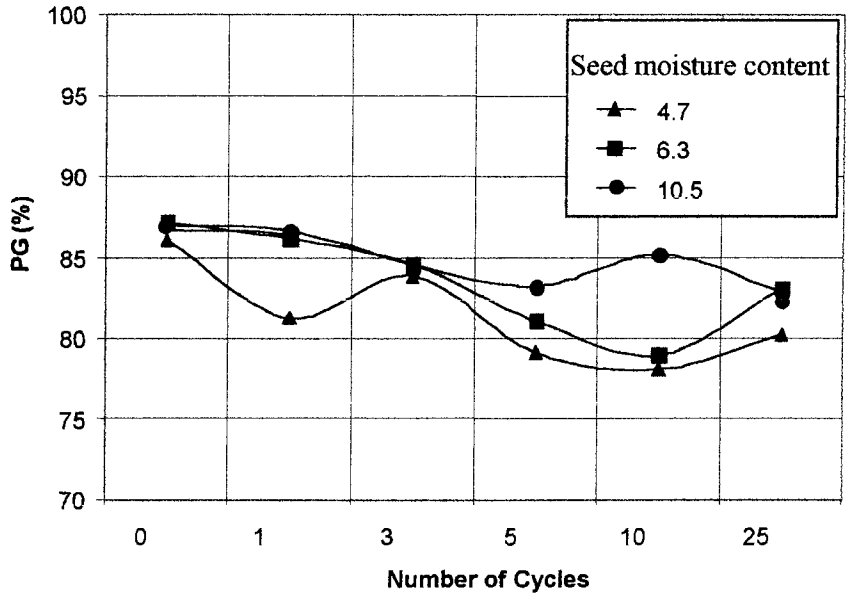


Figure 2 – Experiment 4: Eggplant seeds

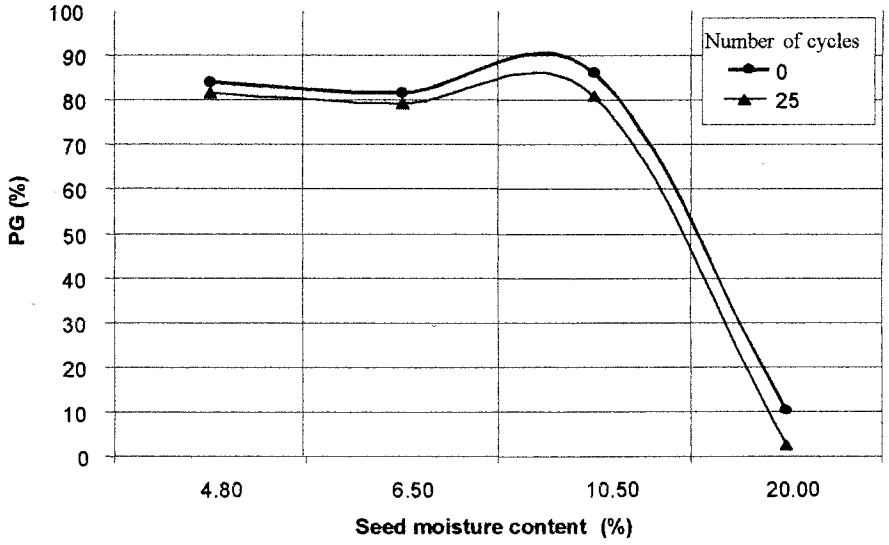
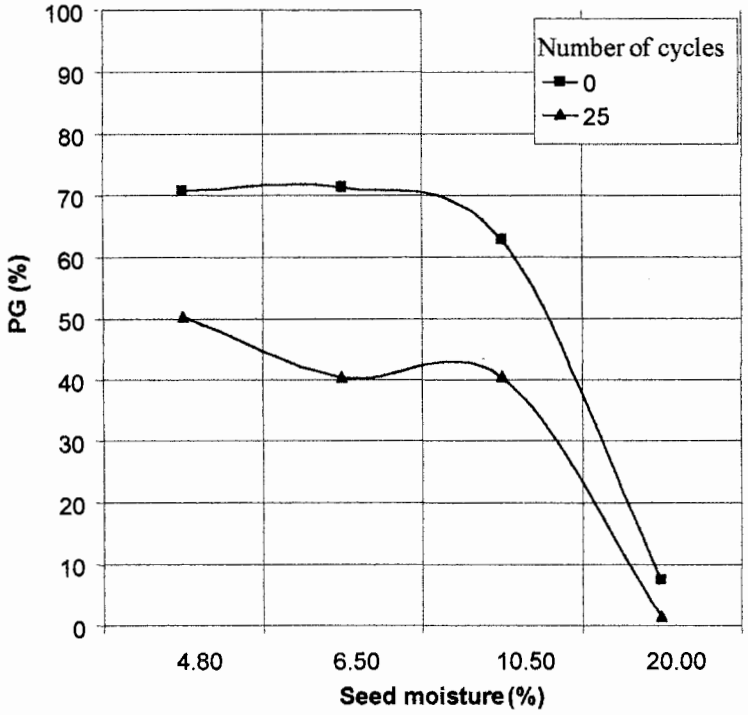


Figure 3 – Experiment 4: Pepper seeds



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SCREENING OF 69 HOT PEPPER LINES FOR RESISTANCE AGAINST CUCUMBER MOSAIC VIRUS BY MECHANICAL INOCULATION

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Keywords: virus resistance, *Capsicum annuum*, CMV, germplasm evaluation

Introduction

Hot pepper is the most important vegetable crop in Indonesia. One constraint hampering hot pepper production is disease due to viruses. CMV is the most widespread virus infecting hot pepper in Indonesia (Duriat, 1996) and its infection have caused major yield reduction (Sudarsono et al. 1998, Nilam Sari et al. 1998; Florini and Zitter, 1987). Under permissive condition, CMV may spread to most of the hot pepper planting in the field and cause total loss of harvest. Many strains of CMV have existed in Indonesia and CMV-02 is the most severe strain identified (Duriat, 1996).

Planting of CMV resistance cultivars is the most effective control measure against virus infection in hot pepper (Green and Kim, 1994) and especially in Indonesia since strains of CMV and insect vector spreading it always exist in the field. Therefore, breeding for CMV resistance hot pepper is necessary and identification of donor lines carrying CMV resistance characters need to be conducted. The objectives of this study were to evaluate response of 69 hot pepper lines against the most severe CMV strain reported in Indonesia and to identify the resistance lines.

Materials and Methods

Severe isolate of CMV from Indonesia (CMV-02) was obtained from Research Institute for Vegetable, Lembang, Indonesia and maintained by mechanical inoculation in tobacco var. *Xanthii*. For hot pepper evaluation, CMV was prepared by extracting leaf sap of virus infected tobacco showing symptoms of CMV infection. For each of 69 lines, 25 seedlings grown individually in 200 ml plastic pot containing sterilized potting mix were evaluated. Seedlings were maintained under an insect proof glasshouse and were mechanically inoculated twice with CMV-02 at cotyledon and two leaf stage, respectively. The inoculated cotyledons and leaves were dusted with carborundum (600 mesh) prior to rubbing the sap of CMV infected tobacco.

Number of seedlings showing symptoms, period of incubation, and types of symptoms of CMV infection in the inoculated seedlings was observed weekly up to four weeks after the second inoculation. Representative of symptomatic and all symptomless seedlings were verified for the presence of CMV particles using DAS-ELISA (AGDIA). Disease intensity (DI) was calculated using the following equation

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$DI = \frac{\Sigma (n \times v)}{N \times V} \times 100\%$, with n, v, N, and V were number of seedlings with symptoms, score of symptom severity in individual seedlings, number of seedlings evaluated and the highest score (5) of symptom severity, respectively. Symptom severity was scored as follow: **score 0**: no symptoms observed; **score 1**: necrotic local lesion, no systemic invasion or very mild chlorosis; **score 2**: mild to moderate mottle or mosaic without significant leaf distortion; **score 3**: moderate to severe mosaic with significant leaf deformation; **score 4**: severe mosaic with severe stunting and leaf deformation; and **score 5**: very severe mosaic, leaf deformation, and stunting. Subsequently, the tested lines were grouped into six response groups (Table 1) based on the calculated DI and results of DAS-ELISA

Results and Discussions

Mechanical inoculation of CMV on 69 hot pepper lines resulted in ranged of response from symptomless to severe mosaic and leaf deformation. Lines grouped into moderately to highly susceptible showed 100% symptomatic seedlings after inoculation with CMV. For seedlings showing symptoms, the incubation period ranged from 8 to 26 days after inoculation (Table 2). Lines with shorter incubation period (IP) showed severe mosaic and leaf deformation while one with longer IP showed mild mosaic or mottle symptoms.

Some of the moderately resistance and all resistance lines were segregated for symptomless and symptomatic seedlings after inoculation with CMV. Symptomless seedlings remained symptomless after reinoculation with CMV but most of them showed positive results of DAS-ELISA.

The calculated disease intensity, based on score of symptoms in the inoculated seedlings, among 69 hot pepper lines ranged from 0 to 98%. Four lines (C1024, KA-2, PBC495, and C1042) showed DI = 0 while three lines (LV2323, Tit Paris, and PBC068) showed DI $\leq$ 10%. The rest of the lines evaluated showed DI ranged from 11 to 98%. The C1024, KA-2, PBC495, and C1042 lines showed 100% symptomless seedlings after twice inoculation with CMV. Moreover, DAS-ELISA using leaf sap showed negative results indicating the absence of CMV particle in the inoculated seedlings. The tested seedlings remained symptomless and gave negative results of DAS-ELISA after reinoculation with CMV.

In this evaluation we have identified four hot pepper lines that were symptomless and showed negative results for DAS-ELISA. According to Dolores (1996), such lines were classified as immune against CMV. However, further testing is needed whether these lines were actually immune, unable to support replication, or unable to support systemic spread of CMV.

Identification of hot pepper lines with resistance to local strain of CMV is necessary for breeding program. In this evaluation we have been able to identify four different lines showing immunity to CMV. Since the selected lines is immune against the most severe isolates of CMV from Indonesia, they may be used to overcome major problem associated with severe CMV infection in the field. Although screening of hot pepper lines against CMV have been conducted (Nilamsari et al. 1998; Duriat and Gunaeni, 1996; Dhawan et al. 1996; Green and Kim, 1994), none reported lines with resistance character against CMV such as the one identified in this report.

These four lines can be used as donor parents for developing CMV resistance hot pepper varieties. Studies on the genetics factors governing the resistance character will be beneficial for setting up breeding program for CMV resistance cultivars. Moreover, C1024 and C1042 were originated from Indonesia, PBC495 was from India, and KA-2 was from Sri Langka, respectively. It is possible that some of these lines carry different resistance

mechanisms against CMV. In such cases, the different resistance mechanisms can be pyramided into single genotype to develop more durable CMV resistance hot pepper cultivars.

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Table 1. Grouping of response of hot pepper lines against CMV infection based on calculated disease intensity (DI)

Response against CMV	Disease Intensity (%)	Hot pepper lines
Immune	0 and (-) ELISA	C1024, KA-2, PBC495, and C1042
Resistance	0 – 10 and (+) ELISA	LV2323, Tit Paris, PBC068
Moderately resistance	10.1 – 20	19 hot pepper lines
Moderately susceptible	20.1 – 30	10 hot pepper lines
Susceptible	30.1 – 50	22 hot pepper lines
Highly susceptible	> 50.1	11 hot pepper lines

Table 2. Reaction of hot pepper lines after twice inoculation with CMV-02.

Lines	Origin	IP *) (dpi)	DI (%)	Symp-toms	Lines	Origin	IP *) (dpi)	DI (%)	Symp-toms
Immune:									
C1024	Indonesia	-	0	-	I-7-1	Indonesia	18	30	MS
KA-2	Sri Langka	-	0	-	C1080	Indonesia	13	30	MS
PBC495	India	-	0	-	Zao Feng	China	14	31	MS
C1042	Indonesia	-	0	-	**Susceptible:**				
Resistant:					C1059	Indonesia	18	32	MS
LV2323	Indonesia	26	8	MS	Num	Thailand	14	33	MS
Tit Paris	Indonesia	24	8	MS	C Giant	Sri Langka	14	36	MS
PBC068	Taiwan	26	10	MS	CNPH703	Brazil	20		MS
Moderately resistant:					Chin Fair	Taiwan	14	37	MS
H Sithon	Thailand	23	11	MS	IR	Indonesia	12	38	MS
MC5	Malaysia	26	12	MS	PBC569	USA	14	38	MS
PBC145a	Taiwan	24	12	MS	PBC455	India	17	40	MS
Pusa Sada-bahar	India	24	12	MT	IAC Uba-tuba	Brazil	18	47	MS
PBC384	Malaysia	20	13	MS	C1058	Indonesia	14	40	MS
HAD832	France	24	13	MS	PBC581	Malaysia	14	40	MS
Punjab	India	24	13	MS	PBC371	Thailand	16	42	MS
C1079	Indonesia	22	15	MS	Bulan 39	Thailand	16	42	MS
M Scarlet	Korea	24	17	MS	PSR67085	USA	13	42	MS
Kalmicho	Korea	22	17	MS	Hot Long	Korea	13	45	MS
Ludhiana	India	24	18	MS	PBC578	Taiwan	12	46	MS
KKu cluster	Thailand	22	18	MT	LC Serdang	Malaysia	14	43	MS
Tiwari II	India	22	18	MS	U kimba	Nigeria	16	47	MS
Szechwan	Taiwan	22	18	MS	Banglen	Thailand	12	48	MS
RI-26(17)	Malaysia	18	18	MS	Long chili	Taiwan	16	50	MS
PBC516	AVRDC	18	18	MS	Cipanas	Indonesia	14	50	MS
PBC592	Thailand	15	18	MS	**Highly susceptible**				
Hot Beauty	F1 hybrid	19	20	MS	KA 11	Sri Langka	16	57	MS
Wonder hot	F1 hybrid	19	20	MS	Cili langkap	Malaysia	10	75	MS
Moderately susceptible:					Saegochu	Korea	12	65	MS
Huaruar	Thailand	24	23	MS	Atarodo	Nigeria	11	70	MS
Pant C-1	India	20	24	MS	Jawahar	India	14	73	MS
LV1092	Indonesia	22	24	MT	PBC622	Taiwan	18	73	MS
Extra long	India	16	25	MS	L. Rosso	Italy	12	75	MS
Twist green	Korea	19	27	MS	Sakaraho	Nigeria	14	77	MS
Prapadaeng	Thailand	18	27	MS	Matikas	Philipine	10	78	ML
LV1592	Indonesia	20	28	MS	Chilli	Malaysia	10	88	MS
MC-4	Malaysia	18	29	MS	CA87067	USA	8	98	ML

Note: * **IP:** incubation period; **dpi:** days post inoculation; **MS:** mosaic, **MT:** mottle, and **ML:** mosaic and leaf deformation symptoms. **DI:** disease intensity; immune: DI= 0 & (-) ELISA, resistance: DI=0-10 & (+) ELISA, moderately (mod) resistance: DI=10.1-20, mod. susceptible: DI=20.1-30; susceptible: DI=30-50; and highly susceptible: DI≥ 50%.

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RED PEPPER (PAPRIKA) VARIETIES RESISTANT TO BACTERIUM AND THEIR ROLE IN CULTIVATION

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The gene stock of the population of pepper developed in the Carpathian basin, as a result of long selection work, proved to be suitable to make Hungarian pepper (paprika) world famous. Producer and consumer requirements with pepper varieties necessitated the expansion of the gene stock of Hungarian varieties. Incorporation of genes ensuring resistance against bacterium disease of pepper lasting almost a decade has also set this aim. Under the Hungarian conditions only the *Bs-2* gene causing hypersensitive reaction, coming from *Capsicum chacoense* wild species and the *gds (general defense system)* gene redeveloping the general defense system of the host plant found in *C. annuum* PI. 163 192 line ensure resistance against *Xanthomonas vesicatoria* bacterium .

Resistance improvement of vegetable plants so far has been based on reaction concomitant with fast tissue destruction following the entry of pathogens. In course of resistance improvement of eating pepper varieties against bacterium, *Bs-2* gene ensuring satisfactory resistance level was built into spice pepper (paprika) varieties, too. Among the undesirable, hardly penetrable characteristics, closely connected to the gene ensuring hypersensitive reaction, primarily small fruit size delayed the production of resistance types. After almost a decade long work it was possible to unite the adequate resistance with economic characteristics required from pepper varieties. **Kaldom** and **Kalorez** varieties candidates represent this result. (Márkus et. al. 2001).

The other trend of our resistance improvement program is the incorporation of the gene, the *gds* gene we described, localizing the pathogen with entirely different strategy than the hypersensitive reaction (Szarka – Csilléry, 2001a., 2001b, Szarka et al.2002). The reaction determined by the *gds* gene is aimed at keeping alive the cells affected by the pathogen by all means. The reaction based on cell growth and cell wall thickening provides

satisfactory defense both against pathogen bacterium and fungus species and against environmental effects. In addition, it provides great growth strength for the varieties.

As a result of our work, we plan a significant role for varieties resistant to *Xanthomonas vesicatoria* bacterium in making Hungarian spice pepper (paprika) cultivation profitable. Saving the costs of repeated and inefficient chemical defense against the bacterium, itself ensures reasonable profit for the producers. We have also taken into consideration that disease resistant varieties might become fundamental elements of organic cultivation.

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SCREENING OF SWEET PEPPER GERMPLASM FOR RESISTANCE TO BACTERIAL WILT (*Ralstonia solanacearum*)

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Introduction

Bacterial wilt in capsicum caused by *Ralstonia solanacearum* E.F. Smith has become a serious problem in India (Gowda *et al.*, 1974; Gopalkrishnan and Peter, 1991). The commercial varieties are susceptible to this disease and chemical control through treatment of soil is cumbersome and uneconomical (Madalageric *et al.*, 1983). That is why, breeding varieties for bacterial wilt resistance combined with high yields and acceptable quality is the present day need. Occurrence of this disease is associated with high temperature (above 32°C) and sufficient moisture in the soil. Yield losses up to 100 are reported in wilt prone areas (Wang *et al.* 1997). Keeping this in view an investigation was undertaken to test a wide range of germplasm collection of sweet pepper, with the objective that they can either be utilised directly for cultivation to be find out germplasm sources of resistance to bacterial wilt to be utilised ultimately in breeding resistant cultivars.

Materials and Methods

A set of thirty genotypes of sweet pepper comprising exotic and indigenous genetic stock collections as well as a few commercial lines with good performance was planted in a completely randomised block design with three replications. The disease intensity was recorded under natural sick plots maintained at the vegetable experiment farm of the Department, under field conditions. The wilting of the susceptible check indicated the presence of virulent inoculum in the soil. Bacterial ooze test was carried out on all the wilted plants to confirm bacterial wilt. The disease rating was done as per the scale suggested by Mew and Ho (1976).

Resistant: < 20 wilting

Moderately resistant: 20 to 40 wilting

Moderately susceptible : 41 to 60 wilting

Susceptible : > 60 wilting

Results and Discussion

As presented in Table 1, the genotypes IHR-546 and PBC-631 were highly wilt resistant genotypes of capsicum. The lines Arka Gaurav, PBG 5005, Cap B, Bastidon and Cap C were observed to be moderately resistant and remaining entries ranged from moderately susceptible to susceptible disease attack against bacterial wilt. Other workers have also reported the resistance against bacterial wilt in sweet pepper. The resistance has been observed by Matsunaga *et al.*, 1993 and Wang *et al.*, (1997).

The wilt resistant accession, IHR-546 was dark green fruited, compact growth habit with pungent fruits. Fruit length is about 6-7 cm. Line PBC-631 had long fruits like paprika type,

light green coloured and sweet in taste. Lines Cap B and Cap C (moderately resistant) were having erect conical type of fruits. Bastidon had normal bell shaped fruits.

The present studies indicate that the resistant gene for bacterial wilt is available in the capsicum strains and same may be incorporated into otherwise suitable commercial cultivars. Therefore, a breeding programme involving genotypes viz., IHR-546 and PBC-631 can be envisaged to transfer bacterial wilt resistance in a single genotype coupled with high yield potential.

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Table 1 – Disease rating of *Capsicum annuum* genotypes.

S.No.	Name of the line	Number of the wilted plants	Number of the plants survived	Percent Incidence /Mortality	Disease rating
1	IHR-546	2	41	4.81	Resistant
2.	California Wonder	35	3	91.42	
3	Yolo Wonder	35	0	100	
4.	Arka Mohini	27	15	64.68	
5	Arka Gaurav	15	33	31.25	Moderately resistant
6.	HS201	29	30	49.15	
7.	HS202	21	15	58.33	
8.	EC-143570	47	2	95.91	
9.	PBC-631	0	35	0.00	Immune/Resistant
10.	PBG-505	15	23	40.00	Moderately resistant
11.	EC-143567	52	0	100	
12.	EC-174852	41	6	87.23	
13.	EC-203602	44	8	84.61	
14.	EC-240610	15	25	37.50	
15.	EC-160093	29	12	70.73	
16.	EC-175959	47	2	95.91	
17.	EC-175963	27	15	64.68	
18.	EC-175965	39	8	82.97	
19.	EC-279074	42	4	91.30	
20.	EC-464483	43	7	86.00	
21.	CapB	15	33	31.25	Moderately resistant
22.	CapC	11	32	25.58	Moderately resistant
23.	Bharat	35	2	94.28	
24.	EC-464110	19	21	47.50	
25.	EC-464111	42	7	86.0	
26.	Bastidion	12	35	25.54	Moderately resistant
27.	Pusa Deepati	20	25	44.44	
28.	Marvel	17	21	44.73	
29.	EC-464113	25	16	60.93	
30.	EC-464117	17	18	48.57	

Table 2 – Classification of *Capsicum annuum* based on bacterial wilt incidence.

Reaction to wilt	Wilt incidence (%)	Number of accessions	Name of accessions/varieties
Resistant	20	2	IHR-546,PBG-631
Moderately resistant	20-40	5	Arka Gaurav, PBG-5005,Cap B, Cap C. Bastidon
Moderately susceptible	40-60		
Susceptible	60		

Note: Moderately susceptible and susceptible genotypes being of no consequence, not indicated in the last columns.

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Development of resistant pepper lines against anthracnose using interspecific crossing between *Capsicum baccatum* and *C. annuum*

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Introduction

Fruit rot of chili pepper caused by anthracnose (*Colletotricum gloeosporioides*) is one of the most serious diseases in Korea. Resistance was identified only in different species, *Capsicum baccatum* and *C. chinensis*, by Pae *et al.* (1995) and Black (1998), respectively. Unfortunately, these two species cannot be easily crossed with *C. annuum*. In order to overcome interspecific crossing barrier and to restore agronomic characteristics at the levels of commercial chili pepper, we used several breeding strategies including embryo rescue, backcross, and advancement of generation based on phenotype selection. We report the results of embryo rescue, and the performance of some lines, resistant to anthracnose with good agronomic characteristics.

Materials and Methods

Interspecific cross & embryo rescue: Four hundred and fifteen seeds of 62 days old immature fruits derived from reciprocal crosses between *C. baccatum*, 'UK1', and *C. annuum*, 'L.F.', were sown on 1/2 MS medium under conditions of 25□ and 12hr day length. Seedlings were transplanted in greenhouse 14 days after germination in 1/2 MS medium.

Bioassay: A *Colletotricum gloeosporioides* isolate was prepared from infected fruits collected in Korea on potato dextrose agar (PDA) medium by tissue isolation technique. Inoculum of 5×10^5 spores/ml was sprayed with $1.5 kg/cm^2$ pressure. The anthracnose resistance was investigated in parental cultivars and two hundred and twenty three progeny plants derived from single-, three way-, and back-crosses. Five fruits from each plant were inoculated and kept at 25±2□ in moist chamber. The disease symptoms were recorded seven days after inoculation. The degree of disease incidence on fruit was judged by visual observations, using the scale 1 to 9. Plants falling in scale 1 to 3 were determined as resistant.

Results and Discussion

F1 seeds derived from *C. baccatum* x *C. annuum* ('UK1' x 'L.F.') showed 4.6% survival rate in greenhouse condition. Through embryo rescue in 1/2 MS medium, we finally obtained nine F1 plants, which were highly sterile. However, the reciprocal crosses did not produce any survival plant. These results were similar with the previous report (Consoli *et al.*, 1992).

Progenies from three-way crosses involving *C. annuum* as either female or male parent showed very low fertility, *i.e.*, 10.5 - 14% in F1. On the contrary, F2 generation from backcrosses gave reasonably high rate of fertility, 29.7 and 73.5%. In F1 generation, most of the fertile plants were resistance to anthracnose, whereas, half of F2 and F4 plants were susceptible (Table 2).

Fourteen were selected from one hundred and thirty progenies as having the high degree of resistance to anthracnose with good fruit characteristics. The fruits of these lines were similar with *C. annuum* ('LF') in length and width of fruits, whereas the weight of fruits had an intermediate value (Table 3).

Only one report has been published on interspecific hybridization between *C. baccatum* and *C. annuum* in relation to disease resistance. Dumas de Vaulx and Pitrat (1977) reported resistance of PVY and *Phytophthora capsici* in F2 progenies of *C. baccatum* and *C. annuum*, but so far no report has been found on the resistance to anthracnose.

Table 1. Embryo rescue of interspecific crosses between *C. baccatum* and *C. annuum*.

Crosses[z]	No. of fruits (seeds)[y]	No. of F1 plants on MS medium	No. of F1 plants in greenhouse
'UK1' x 'L.F.'	11(197)	14(7.1%)[x]	9(4.6%)
'L.F.' x 'UK1'	5(218)	0(0%)	0(0%)

z 'UK1': *C. baccatum*; 'L.F.': *C. annuum*.
y Number of fruits survived after 62 days. Numbers in the parentheses are the number of seeds sown in 1/2 MS medium.
x Percent germination

Table 2. Fertility and disease reaction of the progenies from single-, back-, and three way-crosses.

Classification of cross	Cross[z]	Generation	No. of plants	No. of fertile plants[y]	No. of resistant plants	No. of selected plants
Three-way cross	'Jungang' x ('UK1' x 'L.F.')	F1	220	31(14.1)	25	1
	(UK1 x L.F.) x 'Suwon 1R'	F1	19	2(10.5)	2	1
Backcross	'UK1' x ('UK1' x 'L.F.')	BC1F2	136	100(73.5)	56	5
	'L.F.' x ('UK1' x 'L.F.')	BC1F2	128	38(29.7)	26	6
Single cross	'UK1' x 'L.F.'	F4	92	52(56.5)	22	1
Total			595	223(37.5)	130	14

z Cultivars, 'Jungang', 'Suwon 1R' and 'L.F.' are *C. annuum.*
y Percent of fertile plants in the parentheses

Table 3. Agronomic characteristics of progenies showing highly resistant to anthracnose.

Name of lines	Genera-tion	Plant type[z]	Direction of flower[y]	Fruit characteristics			Color of corolla spot[x]	Anther color[w]	Resistance to anthracnose[v]
				Weight (g)	Length (mm)	Width (mm)			
SPRT-9-28-4	BC2F1	C	P	3.4	58.8	12.6	G-Y	PB	2
SPRT-9-8	BC1F1	C	P	4.1	52.9	14.3	G-Y	PB	1
SPRT-9-26-6	BC1F2	C	E	3.8	78.5	12.6	G-Y	PB	1
SPRB-9-159-1	BC1F2	C	I	4.0	74.3	13.2	G-Y	PB	1
SPRB-9-163-1	BC1F2	P	E	3.3	73.2	12.2	G-Y	PB	1
SPRB-9-169-1	BC1F2	C	P	4.1	77.7	11.2	G-Y	PB	1
SPRB-9-169-2	BC1F2	C	P	4.9	70.5	14.0	G-Y	PB	1
SPRB-5-148-1	BC1F2	P	I	4.9	80.9	18.2	G-Y	PB	1
SPRB-6-150-1	BC1F2	C	P	7.5	91.5	16.2	G	PB	3
SPRB-6-150-2	BC1F2	C	P	5.7	95.5	13.1	G	PB	3
SPRB-6-150-4	BC1F2	C	P	5.9	86.9	11.8	G	PB	3
SPRB-6-150-5	BC1F2	C	P	9.8	104.1	14.7	G	PB	2
SPRB-6-150-6	BC1F2	C	P	11.0	113.2	15.3	G	PB	3
SPRB-8-2-1-151	F4	C	P	3.8	66.7	11.6	G-Y	PB	2
Mean±SD		-	-	5.4±2.4	80.3±16.8	13.6±2.0	-	-	1.8±0.9
'UK1' (*C. baccatum*)		P	E	2.7	53.4	11.8	G-Y	Y	1
'L.F.' (*C. annuum*)		C	P	14.1	130.0	16.6	A	B	9

z C: Compact; P: Prostrate.
y I: Intermediate; E: Erect: P: Pendant.
x G-Y: Greenish yellow; G: Green; A: Absent.
w B: Blue; Y: Yellow; PB: Pale blue.
v 1 : Resistant □ 9 : Highly susceptible.

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EVALUATION OF *CAPSICUM* SPP. GENOTYPES FOR RESISTANCE TO *PHYTOPHTHORA CAPSICI* IN BRAZIL

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Introduction

Phytophthora wilt is the most important fungus disease of *Capsicum* spp. in Brazil, particularly destructive under high humidity and high temperature. Most of the Brazilian cultivars and hybrids are susceptible to *Phytophthora capsici* and resistance to phytophthora wilt is important for Capsicum breeding programs. The use of resistant cultivars is the most effective method of disease control (Fernandez, 1988; Bosland & Lindsey, 1991). Chemical control is relatively efficient but is expensive and can cause genetic mutations on the pathogen.

For the last twenty years, the Embrapa Hortaliças *Capsicum* breeding program has been working with multiple resistance and some of the released resistant lines are been used in Brazil and other countries (Reifschneider et al., 1998).

The objective of this work was to evaluate part of the Embrapa Hortaliças *Capsicum* spp. germplasm collection for resistance to phytophthora wilt in order to identify news resistant genotypes.

Materials and Methods

During the period from 1998 to 2001, 363 Capsicum genotypes of the germplasm collection (245 *C. annuum*, 42 *C. baccatum*, 36 *C. chinense*, 28 *C. frutescens* and 12 wild *Capsicum*) were evaluated to phytophthora wilt. Four different experiments were carried out under greenhouse conditions in a randomized experimental design with three replications (7 plantlets/replication). Plantlets were inoculated 40 days after emergence by placing 3 ml of 5×10^4 *P. capsici* zoospore suspension per plant of the CNPH 08 isolate (Reifschneider et al., 1986). Genotypes CNPH 148 and 192 were used as resistant and susceptible checks, respectively. Evaluations were made 7 and 15 days after inoculation by determining the percentage of dead plants.

Results and Discussion

Among the genotypes tested, those showing plantlet survival ranging from 75 to 100% were rated as resistant. Only 2.7% of the genotypes evaluated were identified as resistant sources to phytophthora wilt, nine genotypes of *Capsicum annuum* and one single accession of the *C. parviflorum* (Table 1). The susceptible (CNPH 0192) and resistant (CNPH 0148) genotypes were included in all four experiments and performed as expected (Table 1). The determination of new resistance sources and the introgression of *Phytophthora capsici* resistance genes in other domesticated species is strategic to the development of *C. baccatum*, *C.*

chinense and *C. frutescens* resistant cultivars. This method is particularly important for those materials that do not have satisfactory resistance level.

Embrapa Hortaliças *Capsicum* collection is one of the largest in Brazil (Reifschneider et al., 1998), and has more than 1,200 accessions, mostly of *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens*. Additionally, ten wild *Capsicum* species recently founded in the remains of the Atlantic forest in Brazil were characterized and maintained at the germplasm bank (Carvalho, personal communication, 2003). The search for new sources of resistance to *Phytophthora capsici* in the *Capsicum* germplasm collection of Embrapa Hortaliças will continue to test new entries, especially wild and semidomesticated species collected in Brazil, since disease resistance is one of the most important goals of the Embrapa Hortaliças *Capsicum* breeding program.

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Table 1. *Capsicum* genotypes resistant to *Phytophthora capsici* selected among 363 *Capsicum* genotypes at the Embrapa Hortaliças, Brasilia-DF, Brazil.

Genotypes (CNPH)	Species	Reaction	Plantlet survival (%)
CNPH 0192	*C. annuum*	susceptible (check)	0
CNPH 0148	*C. annuum*	resistant (check)	100
CNPH 0146	*C. annuum*	resistant	75
CNPH 0149	*C. annuum*	resistant	100
CNPH 0187	*C. annuum*	resistant	100
CNPH 2171	*C. annuum*	resistant	100
CNPH 2172	*C. annuum*	resistant	100
CNPH 2175	*C. annuum*	resistant	100
CNPH 2541	*C. annuum*	resistant	100
CNPH 4504	*C. annuum*	resistant	75
CNPH 3331	*C. parviflorum*	resistant	75

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ANALYSIS OF DNA METHYLATION DURING GERMINATION OF PEPPER SEEDS BASED ON METHYLATION-SENSITIVE AFLP MARKERS

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Key words: DNA methylation, isoschizomers, AFLP, MSAP, *Capsicum annuum* L.

Introduction

DNA methylation in plants is related to a number of epigenetic phenomena among which the regulatory mechanisms during development and differentiation (Lund *et al.* 1995, Finnegan *et al.* 1993).

Here we report our preliminary results on the analysis of cytosine methylation during pepper seed germination using MSAP (methylation-sensitive amplified polymorphism) (Reyna-Lòpez *et al.* 1997, Xiong *et al.* 1996), an adaptation of the AFLP (amplification fragment length polymorphism) technique (Vos *et al.* 1995).

The technique is based use of the isoschizomers *Hpa*II and *Msp*I, that differ in their sensitivity to methylation of their recognition sequences. Both enzymes recognize the tetranucleotide sequence 5'-CCGG, but their action is affected by the methylation state of the external or internal cytosine residues. *Hpa*II is inactive when either or both of the two cytosines is fully methylated (both strands methylated) but cleaves the hemimethylated sequence (only one strand methylated), while *Msp*I cleaves hemi or fully methylated $C^{5m}CGG$ but not $^{5m}CCGG$ (McClelland *et al.* 1994).

Material and Methods

DNA was extracted every 48 h from embryo tissues of germinating F_1 pepper seeds (cv. "Corno di Toro"). We used two consecutive PCRs to selectively amplify the *Eco*RI-*Hpa*II (*H*) and *Eco*RI-*Msp*I (*M*) DNA fragments. The pre-selective amplification (first PCR) was performed using *Eco*RI and *Hpa*II/*Msp*I adapter-directed primer, each possessing a single selective base (E+A; HM+T). Selective amplification (second PCR) was carried out using the 12 primer combinations listed in Table 1.

Within each AFLP primer combination patterns obtained in 10 lanes were compared, corresponding to five different sources of pepper genomic DNA (i.e. from dry seeds and seeds germinated for 2, 4, 6 and 8 days) each restricted with one of the pairs of enzymes *Eco*RI/*Hpa*II (lane *H*) or *Eco*RI/*Msp*I (lane *M*) (Figure 1)

Results and discussion

Comparison of the AFLP patterns revealed two main kinds of polymorphism: (i) bands always appearing after digestion with *H* but not with *M* or vice versa (i.e. 131 out of 936); these bands were the result of DNA methylation at the 5'-CCGG sites but did not highlight changes in methylation pattern during germination (Figure 1a, Table 1), (ii) bands showing polymorphism at different stages of germination (i.e. 328 out of 936); these bands resulted from changes in DNA methylation status (Figure 1b-f, Table 1).

Changes in DNA methylation status were mainly displayed as: (i) fragments not found in dry seeds but present after digestion with *H* and *M* at a certain stage during germination, i.e. 215 out of the 328 polymorphic bands (pattern D1 - Table 2); (ii) fragments present after digestion with *H* and *M* in dry seeds but no longer detected at a certain stage during germination, i.e. 44 out of 328 polymorphic bands (pattern C1 - Table 2).

Demethylation preferentially originates short fragments, as a previously methylated 5'-CCGG recognition site near an *Eco*RI recognition site, can come within reach of *Msp*I or *Hpa*II or both enzymes (Figure 2). As reported by Xu *et al.* (2000) and Reyna-Lopez *et al.* (1997), short DNA fragments, ranging from 50 to 1500 bp, can be more effectively amplified by AFLP or detected in a sequencing gel.

During germination, the observed high frequency of fragments originated *de novo* (i.e. 215) and the concomitant disappearance of fragments which were detected in dry seeds (i.e. 44) may thus be attributed to demethylation events. Presumably, the fragments originated *de novo* were observed at higher frequency since, being shorter, they had a higher chance of amplification and detection. The hypothesis that we were detecting demethylation events is further supported by the fact that, during germination, after digestion with both *Msp*I and *Hpa*II, we observed a progressive increase in the frequency of fragments detected together with a progressive decrease in the frequency of fragments no longer detected. By the same interpretation, the appearance at higher frequency (pattern D2 and D3 – Table 2) and the disappearance at lower frequency (pattern C2 and C3 – Table 2) of some fragments, after digestion with only one of the two isoschizomers, might also be attributed to demethylation events occurring during germination.

Interestingly 49 of the 215 fragments detected at a certain stage of germination underwent a second change in methylation status, since they were no longer observed in a subsequent phase of germination. This may be further on interpreted as the occurrence of a second demethylation event, originating shorter fragments which were differently positioned in the sequencing gels.

The interpretation of our data, as reflecting progressive DNA demethylation occurring during germination, is supported by the fact that in plant nuclear genomes cytosine methylation is known to play an important role as regulator of gene expression in development (Siroky *et al.* 1998), and methylation in particular regions of genes or in their vicinity can inhibit the expression of these genes, while artificial demethylation of genes is known to result in reactivation (Grunau *et al.* 2001).

The results here reported show this technique to be highly efficient for large scale detection of cytosine methylation in germinating seeds. The ability to isolate and amplify these MSAP fragments may thus make possible direct identification of sequences (or genes) which play a key role at different stages of germination.

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Primer combinations	Total bands	Class I	Class II
E+AAC/HM+TAA	87	14	31
E+AAC/HM+TCC	50	7	19
E+AAC/HM+TTC	92	11	26
E+ACG/HM+TAA	78	10	29
E+ACG/HM+TCC	72	6	42
E+ACG/HM+TTC	81	8	31
E+ACT/HM+TAA	98	15	26
E+ACT/HM+TCC	71	12	20
E+ACT/HM+TTC	60	10	22
E+AGT/HM+TAA	88	18	32
E+AGT/HM+TCC	82	9	27
E+AGT/HM+TTC	77	11	23
Total	936	131	328

Table 1. Number of bands observed after amplification with the 12 AFLP primer combinations used. Class I: bands observed only after digestion with *Eco*RI and *Hpa*II and not after digestion with *Eco*RI and *Msp*I or vice versa, which did not show polymorphism in dry seeds as well as germinating seeds. Class II: bands showing polymorphism at different stages of germination.

class	Dry seeds H	Dry seeds M	pattern	Germinated seeds H	Germinated seeds M	Number of bands	total
A	+	-	A1	+	+	7	
			A2	-	-	7	
			A3	-	+	0	14
B	-	+	B1	+	+	8	
			B2	-	-	10	
			B3	+	-	0	18
C	+	+	C1	-	-	44	
			C2	+	-	4	
			C3	-	+	3	51
D	-	-	D1	+	+	215*	
			D2	+	-	12	
			D3	-	+	8	245

* 49 of these 215 sites showed a second change in methylation status revealed as disappearance of the corresponding bands at a different stage of germination

Table 2. Changes in patterns of cytosine methylation during germination of pepper seeds revealed by MSAP. Column H: pattern after digestion with *Eco*RI and *Hpa*II; Column M: pattern after digestion with *Eco*RI and *Msp*I. Symbols + and - indicates respectively presence and absence of a fragment. Four classes of pattern were detected in dry seeds and classified as A, B, C and D. The 3 possible changes in AFLP pattern within each class, identified as progressive numbers (e.g. class A, pattern A1, A2, A3) as well as the number of bands observed are reported.

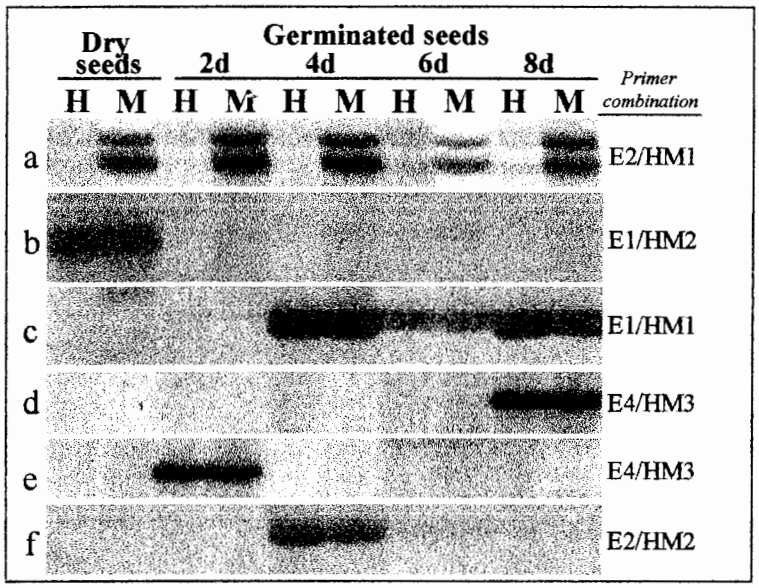

Figure 1. Examples of some AFLP profiles obtained using MSAP on dry and germinated seeds. H lanes: fragments obtained after digestion with *Eco*RI-*Hpa*II; M lanes: fragments obtained after digestion with *Eco*RI-*Msp*I. **a:** example of fragment whose methylation pattern does not change during germination; **b:** example of fragment detected after both digestion with *H* and *M* in dry seeds and disappearing at different stages of germination (pattern C1); **c-d:** examples of fragments not detected in dry seeds but appearing after digestion with both *H* and *M* at different stages of germination (pattern D1). Fragments **e-f** shows two changes in methylation status during germination (appearance and disappearance of a band).

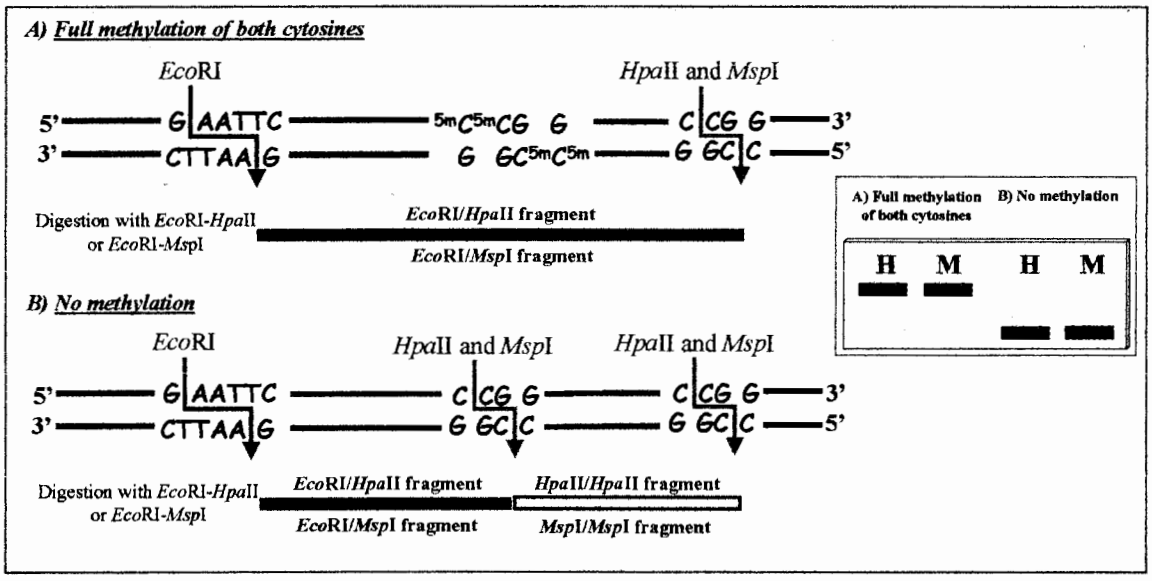

Figure 2. Example of the result of DNA demethylation at the 5'-CCGG site. (A): when full methylation occurs at both external and internal cytosines 'large' fragments are originated with both *Hpa*II and *Msp*I. (B): when the *Hpa*II/*Msp*I restriction site in unmethylated, both *Hpa*II and *Msp*I can cut and originate 'short' fragments.

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AGRONOMICAL VARIATION IN JORDANIAN EGGPLANT (*Solanum melongena* L.) LANDRACES

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1. INTRODUCTION

Eggplant (*Solanum melongena* L.), also known as aubergine, brinjal or guinea squash (Collonnier *et al.*, 2001), is one of the most important vegetable crops grown in Jordan. Most of the eggplant cultivars currently grown in Jordan are hybrids. However, some eggplant landraces are still grown in many small farms due to some consumer demand. Farmers have given various local names to some landraces; "Batteiri" and "Ajamy" are the most popular local eggplant landraces. Genetic diversity in local eggplant landraces is particularly high in Jordan (Saifan *et al.*, 1999; Saifan *et al.*, 2002). These landraces are of a great value due to their wide range of variability in morphological and physiological characters (Collonnier *et al.*, 2001). Most of local eggplant landraces has unknown characters, therefore, the aim of this study was to evaluate productivity and characterize some morphological and agronomical traits of twenty-nine accessions of local eggplant landraces.

MATERIALS and METHODS

Twenty-nine accessions of eggplant (*Solanum melongena* L.) landraces collected from local farmers throughout the country during 1995, and conserved in the gene bank of the National Center of the Agricultural Research and Technology Transfer (NCARTT), were used in this study (Table 1). The experiment was performed in Karama station (NCARTT station in the Jordan Valley). Forty-five day old seedlings were transplanted into the field in late Sep. 2001. Plants were spaced 0.5m apart in 5m raised beds 1m apart. Black polyethylene mulch and a drip irrigation system were used. Analysis from saturated paste extracts from the experimental field and water samples from the irrigation water are included below:

Sample	EC ($dS\ m^{-1}$)	pH	Meq L^{-1}			
			Ca	Mg	Na	Cl
Soil 0-30 cm	21.7	7.6	68	123	71	154
Soil 30-60 cm	12.2	7.7	39	97	53	126
Water	2.5	8.0	5.5	6.0	11.2	10.1

The treatments were arranged in a Randomized Complete Block Design (RCBD) and replicated 3 times. Each replicate included 30 plants.

Average number of days to first flower opening and first harvest at commercial ripeness stage, growth habit (upright, intermediate and prostrate) were recorded. Four plants from each treatment in each replicate were separated into shoot and root, dried at 65°C to a constant weight to determine average shoot and root dry weights $plant^{-1}$. Fruits were harvested for several times at commercial ripeness stage starting from Jan.2nd 2002 and yield, average fruit weight and

average fruit number plant^{-1} were calculated. The following fruit and seed characters were recorded according to the eggplant descriptor published by the International Board for Plant Genetic Resources (IBPGR), 1988: average fruit length and breadth, fruit curvature, color, flesh density and taste, seed number per fruit, color and 100 seed weight.

RESULTS and DISCUSSION

Variations in vegetative growth parameters were observed between eggplant landraces (Table1). Growth habit was upright, intermediate and prostrate for 14, 10 and 5 accessions, respectively. Average number of days from seed sowing to first flowering ranged from 78 to 85 days and to first harvest ranged from 144 to 177 days. Wide and significant differences in shoot and root dry weights were revealed among the eggplant landraces. Average shoot dry weight plant^{-1} ranged from 164 g in accession 118 to 33 g in accession 123. Average root dry weight plant^{-1} ranged from 19 g in accession 118 to 7 g in accession 135.

Significant variations in yield of the eggplant landraces were also observed (Table 1). Eggplant yield ranged from 40 ton ha^{-1} in accession 118 to 13 ton ha^{-1} in accession 123. Yield less than 20 ton ha^{-1} was produced by 7 accessions, between 20 and 28 ton ha^{-1} by 11 accessions and between 30 and 40 ton ha^{-1} by 11 accessions. Average fruit number per plant ranged from 27 to 10 in accessions 121 and 123, respectively. Eleven accessions had average fruit number less than 15, ten accessions between 15 and 18 and 8 accessions between 20 and 27 fruit plant^{-1} (Table 1). Average fruit length ranged from 22 to 8 cm in accessions 125 and 139, respectively, and fruit breadth from 11 to 3.5 cm in accessions 141 and 119, respectively. Fruit breadth to length ratio varied from 1.0 in accession 139 (fruit length as long as broad) to 0.2 (fruit length several times as long as broad) like in accession 125 (Table 1).

Fruit curvature varied from none to snake shaped (Table 2). Fruit color ranged from purple green in accession 126 to milk white in accession 121 and to black in accessions 116, 117, 123, and 131 (Table 2). Fruit taste varied from excellent in accession 122 to very bitter in accession 140 and fruit flesh density from very loose in accessions 117, 127 and 141 to very dense in accessions 119, 124, 132 and 137 (Table 2). Seed number per fruit varied from very few in accessions 139, 140, 141 to many in 14 accessions, seed color ranged from light yellow to brown and 100 seed weight from 0.27 g in accession 136 to 0.52 g in accession 125 (Table 2).

Considerable differences in plant vegetative growth, yield and fruit characters were observed among the accessions (Tables 1and 2). Accessions with higher yields (118, 117, 127 and 121) could be recommended for further studies. The wide range of variation in morphological and agronomical characters in the local eggplant landraces might be of a grate value for breeders according to their objectives.

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Table 1: Some agronomical characters of 29 accessions of local eggplant landraces grown in the Jordan Valley during 2001/2002 season.

Accession Jo. Number	Growth habit	Average No. of days		Shoot dry weight g plant^{-1}	Root dry weight g plant^{-1}	Yield Ton ha^{-1}	Ave. fruit No. plant^{-1}	Ave. fruit weight (g)	Ave. fruit length (cm)	Average fruit breadth (cm)	Fruit breadth to length ratio
		First flowering	First harvest								
113	Upright	78	157	58.9 e-j *	11.3 b-f	24.7 d-h *	15.0 b-f	140.4 b-f	14.0 c-f	7.5 c-f	0.6 de
114	Upright	78	144	52.9 f-j	8.8 b-h	24.6 d-g	16.9 a-f	105.0 d-g	14.3 c-f	4.7 I-m	0.4 f-i
115	Upright	83	144	60.4 e-j	9.8 b-h	24.2 d-g	15.6 b-f	123.3 d-g	10.7 g-j	5.7 g-k	0.6 de
116	Upright	78	144	89.0 b-e	10.3 b-h	26.7 c-f	20.0 a-f	111.4 d-g	13.0 c-h	6.0 f-j	0.5 e-g
117	Prostrate	78	172	143.2 a	17.8 a	38.5 ab	18.2 a-f	152.6 a-d	13.0 c-h	8.9 bc	0.7 cd
118	Intermediate	84	144	163.6 a	19.1 a	40.1 a	23.8 a-d	141.4 b-f	12.0 e-i	6.4 e-i	0.6 de
119	Upright	83	144	112.5 b	11.0 b-f	27.9 c-e	25.1 a-c	87.7 g	14.3 c-e	3.5 m	0.3 hi
120	Intermediate	83	144	41.8 ij	7.9 e-h	21.9 e-h	15.7 b-f	104.6 d-g	13.8 c-g	5.4 h-l	0.4 e-i
121	Upright	83	144	102.4 bc	11.3 b-e	36.3 ab	27.2 a	109.4 d-g	12.7 d-i	4.2 k-m	0.3 g-i
122	Upright	78	144	106.2 bc	11.0 b-f	33.0 a-d	26.0 ab	116.8 c-g	12.2 e-i	4.7 j-m	0.4 e-i
123	Intermediate	78	167	32.7 j	7.5 f-h	13.0 i	9.6 f	100.7 e-g	12.9 d-h	7.5 c-f	0.6 de
124	Prostrate	85	144	50.6 g-j	12.0 b-d	17.7 g-i	13.5 d-f	102.7 d-g	11.7 e-i	5.6 h-l	0.5 e-g
125	Upright	78	172	49.3 g-j	8.2 e-h	20.0 e-I	13.3 d-f	104.1 d-g	21.7 a	5.0 I-m	0.2 i
126	Upright	78	144	88.3 b-e	11.6 b-e	32.2 a-d	22.2 a-e	108.0 d-g	12.2 e-i	6.0 f-j	0.5 e-g
127	Prostrate	78	177	104.7 bc	12.0 bc	36.5 ab	21.8 a-e	142.0 b-f	15.8 b-c	7.7 c-e	0.5 e-g
128	Intermediate	85	152	59.0 e-j	9.1 b-h	15.9 hi	13.2 d-f	106.9 d-g	16.2 bc	6.1 e-j	0.4 e-i
129	Intermediate	78	144	54.1 f-j	8.6 c-h	19.2 f-i	15.0 b-f	92.1 fg	13.5 c-h	5.4 h-l	0.4 e-h
130	Upright	79	152	93.4 b-d	10.5 b-h	34.9 a-c	16.2 a-f	146.2 a-e	10.4 h-j	8.2 b-d	0.8 bc
131	Upright	78	144	114.0 b	10.2 b-h	33.0 a-d	18.3 a-f	136.2 b-g	13.0 c-h	7.0 d-h	0.5 ef
132	Intermediate	85	152	45.6 h-j	7.2 gh	20.3 e-I	15.6 b-f	94.7 fg	14.5 c-e	5.6 h-l	0.4 e-i
133	Upright	83	147	82.8 b-f	9.1 b-h	21.6 e-h	15.0 b-f	104.8 d-g	18.0 b	5.4 h-l	0.3 g-i
134	Intermediate	79	168	79.5 c-g	9.6 b-h	28.0 c-e	13.0 d-f	162.8 a-c	13.0 c-h	9.3 ab	0.7 c
135	Upright	78	144	76.5 c-h	6.8 h	18.5 f-i	13.9 c-f	98.6 e-g	11.0 f-i	4.6 j-m	0.4 e-h
136	Intermediate	85	147	53.4 f-j	8.2 d-h	19.1 f-i	14.9 b-f	100.0 e-g	11.5 e-i	4.6 j-m	0.4 e-h
137	Upright	78	144	112.7 b	11.0 b-f	34.9 a-c	23.7 a-d	126.7 c-g	10.5 h-j	5.5 h-l	0.5 d-f
138	Intermediate	83	152	99.2 b-d	11.7 b-e	33.4 a-d	14.1 c-f	176.3 a-b	10.7 g-h	9.5 ab	0.9 ab
139	Prostrate	83	152	69.2 d-I	10.8 b-g	20.3 e-i	11.1 ef	147.2 a-e	7.7 j	7.7 c-e	1.0 a
140	Intermediate	78	167	60.4 e-j	12.5 b	15.1 hi	10.0 f	122.0 d-g	10.9 f-i	4.5 j-m	0.4 e-i
141	Prostrate	83	167	102.7 bc	10.9 b-g	30.4 b-d	11.2 ef	192.4 a	13.5 c-h	10.5 a	0.8 bc

** Means within columns having different letters are significantly different according to DMRT($p<0.05$)*

Table 2: Fruit curvature, color, taste, seed number and size seed color and seed weight in 29 accessions of local eggplant landraces grown in the Jordan Valley during 2001/2002 season.

Accession Jo. number	Fruit Curvature	Fruit color	Fruit taste	Fruit flesh density	Seed number fruit^{-1}	Seed color	Seed weight (gm 100^{-1})
113	None	Purple black, uniform	Good	loose, good	Few	Brown -yellowish	0.35
114	Slightly curved	Purple black, uniform	Sweet	Dense	Many	Brown	0.41
115	Slightly curved-curved	Purple,uniform	Not good	Loose	Many	Brown	0.46
116	None- slightly curved	Black, uniform	Good	Loose	Many	Brown	0.46
117	None	Black, uniform	Good	Very loose(spongy)	Many	Brown-yellowish	0.40
118	None	Purple black	Bitter	Dense	Many	Brown-yellowish	0.53
119	Slightly curved	Purple,striped	Good	Very dense	Many	Brown	0.47
120	None- slightly curved	Purple black, striped	Bitter	Dense	Many	Brown-black	0.38
121	Slightly curved- curved	Milk white	Sweet	Dense	Many	Light-yellow	0.36
122	Slightly curved	Purple black	Excellent	Dense	Few	Light-yellow	0.34
123	None- slightly curved	Black	Good	Dense	Many	Brown-yellow	0.43
124	None-slightly curved	Purple grey	Bitter	Very dense	Many	Brown-yellow	0.46
125	Curved- snake shaped	Purple black	Bitter	Dense	Few	Brown-yellow	0.52
126	Slightly curved	Purple green	Good	loose	Few	Brown-yellow	0.46
127	None	Purple-black	Good	Very loose (spongy)	Few	Brown-yellow	0.34
128	None- slightly curved	Purple-black, not uniform	Good	Dense	Many	Brown-yellow	0.51
129	Slightly curved	Purple, grey	Bitter	Dense	Many	Brown	0.41
130	None	Purple black	Good	Loose	Few	Brown-yellowish	0.41
131	None	Black	Bitter	Dense	Intermediate	Light-brown	0.41
132	Slightly curved	Purple black	Bitter	Very dense	Intermediate	Light-brown	
133	None- slightly curved	Black	Good	loose	Many	Brown	0.48
134	None	Purple black	Good	Spongy	Intermediate	Brown-yellowish	0.51
135	Slightly curved	Purple grey	Good	Dense	Intermediate	Brown	0.46
136	None	Purple	Bitter	Spongy	Few	Brown grey	0.27
137	Slightly curved-curved	Purple grey	Bitter	Very dense	Many	Light-brown	
138	None	Black	Bitter	Spongy	Few	Brown-yellow	0.41
139	None	Purple black	Bitter	Med. Dense	Very few	Light-yellow	0.31
140	None- slightly curved	Purple black	V. Bitter	Spongy	Very few	Light-brown	0.40
141	None	Purple black	Good	V.loose (spongy)	Very few	Brown grey	0.35

* Very few < 10, few ~ 150, intermediate ~ 100 and many ~ 300.

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CHARACTERIZATION AND TYPIFICATION OF SPANISH EGGPLANT LANDRACES

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Introduction

Evidence indicates that eggplant (*Solanum melongena* L.) was brought to the Iberian Peninsula by Muslims (Nuez *et al.*, 2003). The effects of both artificial and natural selection, together with other evolutionary forces, like mutation, genetic drift or migration, and the amplifying effect on diversity of recombination resulting from occasional crossings between different genotypes, has resulted in the development of many Spanish eggplant landraces (Prohens and Nuez, 2001).

As early as in the XIIth century, agronomist Abu-Zacaría distinguished four eggplant varieties in Spain (Prohens and Nuez, 2001). In the XVIth century, Alonso de Herrera also makes reference to varieties of different sizes and colours. Later, in the XIXth century, other authors state that in Spain more than 30 eggplant varieties are grown and make a description of some of them. Most of these materials have been replaced by modern varieties. However, as a result of several collecting expeditions (Cuartero *et al.*, 1985; Nuez *et al.*, 1987) an important part of these landraces has been collected and is preserved in the germplasm bank of the "Centro de Conservación y Mejora de la Agrodiversidad Valenciana".

Here, we present the results of a morphological characterization of Spanish eggplant landraces and make a grouping of the accessions in types of commercial interest.

Material and methods

A total of 67 accessions of eggplant collected in Spain were included in the trial. The commercial Spanish variety 'Listada de Gandía' was used as a control. Between five and ten plants per accession were grown in 2000 in the open air in the locality of Turís (Valencia), in the Spanish Mediterranean. The common cultural practices for eggplant in our region (Baixauli, 2000) were followed. The accessions were characterized following the recommendations of the IPGRI's eggplant descriptors list (IBPGR, 1990) and the primary characterization descriptors list of EGGNET (European network for management, characterisation and valorisation of genetic resources of eggplants).

Traits studied were the following: stem colour, leaf blade lobing, leaf prickles, number of flowers per inflorescence, mean fruit weight, fruit shape, fruit curvature, fruit predominant colour at commercial ripeness, fruit additional colour distribution at commercial ripeness, fruit flesh colour, flesh density, length of fruit covered by the calyx and fruit calyx prickles.

Accessions have been grouped in types on the basis of three traits of great commercial interest: fruit shape, predominant colour at commercial ripeness, and fruit predominant colour distribution at commercial ripeness.

Results and discussion

A wide range of variation was found for fruit traits (Table 1). However, for the vegetative traits studied, there was a lower variation. In this way, although in most accessions stem colour is green, in some is purple. Leaf blade lobing is also variable, ranging between weak and intermediate. For other vegetative traits, like leaf prickles or number of flowers per

inflorescence, diversity is very limited. In all cases, Spanish eggplant landraces have very few or no leaf prickles. Also, all accessions only have one flower per inflorescence, a typical trait of *S. melongena* (Prohens *et al.*, 2003).

Regarding fruit traits, we have found an important variation for the mean fruit weight (Table 1), with values ranging between 195 y 567 g. We have also found many different fruit shapes, although ovate and elongated types are prevalent. Regarding the predominant fruit colour, in most accessions it is purple black or purple, although some accessions have less common colours, like lilac grey, green or white (Table 1). In the same way, there are different types of fruit predominant colour distribution at commercial ripeness, even though most varieties have a uniform coloration pattern. Fruit curvature depends to a great extent of the fruit length/breadth ratio; as a rule, the more elongated the fruit is, the greater the possibility of having some fruit curvature. Therefore, it is possible to find some accessions that bear elongated and snake- or sickle-shaped fruits (Table 1). Flesh density is not a very variable trait, as in all Spanish landraces the flesh is compact and dense. Regarding the flesh colour at commercial ripeness, it is white, or more commonly intermediate (white greenish). With respect to the length of fruit covered by the calyx, there is a considerable variation between accessions. In general, the longer the fruit, the smaller the fruit surface covered by the calyx. It is worth mentioning that in Spain there is a traditional variety ('de Almagro') that belongs to the *depressum* botanical variety and in which at least 3/4 parts of the fruit must be covered by the calyx to be commercial (Prohens and Nuez, 2001). Finally, for fruit calyx prickles, a trait with great commercial importante in eggplant, as, there is variation too. While some accessions have no calyx prickles, most have few (between 1 and 5) or many (between 6 and 20). Only one accession has more than 20 prickles in the calyx (Table 1).

Because of their morphological characteristics, Spanish eggplant landraces belong to the so-called H group within the "eggplant complex", which is characterized by vigorous plants with fruits relatively big and with a low spinosity (Daunay *et al.*, 1997). On the other hand, the other group of cultivated eggplants (group G), typical of Southeast Asia, has low vigour and their fruits are small, and generally green with white stripes or vice versa.

It has been possible to group the 67 accessions in 21 groups with different commercially important fruit characteristics. This is a high number of types if compared with the variation found among the prevailing modern varieties of eggplant. Furthermore, within each of the types made up of several accessions, it is also possible to find a broad variation for other traits of interest.

A remarkable fact concerning fruit shape is that there are appreciable differences among regions in the mean length/breadth ratio of eggplant accessions. In this way, in the region of Catalonia (North) eggplant varieties have fruits, as a mean, more elongated than in Andalucia (South), while in Valencia (Center) they are intermediate between the former regions (Table 3). Probably, these differences are associated to different types of uses in different regions. In Andalucia it is more common to use eggplant in fryings and stews than in Catalonia, where a common typical dish is to prepare stuffed halves of elongated eggplants.

The characterization performed here shows that Spanish landraces represent an important source of variation for eggplant breeding and that they can contribute to diversification of commercial types. In the same way, these varieties can be of great interest for ecological agriculture, as they have a good adaptation to the local conditions where they have evolved.

Acknowledgements

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Table 1. Number of Spanish eggplant landraces corresponding to each of the descriptors states for the traits in which variation was detected. Asterisks (*) indicate the state of the descriptors corresponding to the reference variety 'Listada de Gandía'.

Stem colour		*Fruit shape*		*Fruit curvature*	
Green	60*	Broader than long	1	None (fruit straight)	39
Purple	7	As long as broad	2	Slightly curved	12
Leaf blade lobing		Slightly longer than broad	22	Curved	10*
		Twice as long as broad	14*	Snake shaped	2
Weak	41	Three times as long as broad	17	Sickle shaped	4
Intermediate	26*	Several times as long as broad	11	*Flesh colour*	
Fruit weight (g)		*Fruit predominant colour*		Blanco	20*
		Purple black	24	Intermediate	47
100-200	1	Purple	33*	*Fruit length covered by calyx*	
200-300	21	Lilac grey	7	Less than 20%	37*
300-400	23*	Green	1	Between 20% and 70%	26
400-500	15	White	2	More than 70%	4
500-600	7	*Predominant colour distribution*		*Fruit calyx prickles*	
		Uniform	45	None	9
		Mottled	13	Few (1 to 5)	32
		Striped	9*	Many (6 to 20)	25*
				Very many (>20)	1

Table 2. Number of Spanish eggplant landraces corresponding to each of the types established. Types are based on the combination of fruit shape, fruit predominant colour at commercial ripeness and fruit predominant colour distribution at commercial ripeness.

Fruit shape	Predominant colour	Colour distribution	Accessions (n)
Broader than long	Purple	Uniform	1
As long as broad	Purple black	Uniform	1
As long as broad	Green	Striped	1
Slightly longer than broad	Purple black	Uniform	5
Slightly longer than broad	Purple	Uniform	2
Slightly longer than broad	Purple	Mottled	8
Slightly longer than broad	Purple	Striped	6
Slightly longer than broad	Lilac grey	Uniform	1
Twice as long as broad	Purple black	Uniform	4
Twice as long as broad	Purple	Uniform	2
Twice as long as broad	Purple	Striped	1
Twice as long as broad	Lilac grey	Uniform	2
Twice as long as broad	Lilac grey	Mottled	4
Twice as long as broad	White	Uniform	1
Three times as long as broad	Purple black	Uniform	9
Three times as long as broad	Purple	Uniform	5
Three times as long as broad	Purple	Mottled	1
Three times as long as broad	Purple	Striped	1
Three times as long as broad	White	Uniform	1
Several times as long as broad	Purple black	Uniform	5
Several times as long as broad	Purple	Uniform	6

Table 3. Number of Spanish eggplant landraces from each of the three regions from which a greater number of accessions has been characterized grouped by fruit shape. Percentage of accessions belonging to each category is indicated between brackets.

Fruit shape	Catalonia (North)	Valencia (Center)	Andalucia (South)
Broader than long	1 (4%)	0 (0%)	0 (0%)
As long as broad	0 (0%)	0 (0%)	2 (8%)
Slightly longer than broad	5 (21%)	3 (21%)	13 (52%)
Twice as long as broad	0 (0%)	4 (29%)	9 (36%)
Three times as long as broad	10 (42%)	4 (29%)	1 (4%)
Several times as long as broad	8 (33%)	3 (21%)	0 (0%)
Mean length/breadth ratio	2.81	2.61	2.17

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CHANGES IN PHYSICAL CHARACTERS OF BRINJAL (*Solanum melongena* L.) FRUIT DURING DEVELOPMENT

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Introduction

Although brinjal is indigenous to India, it is grown in many countries where climatic conditions are congenial for their growth and development. Among the different fruit characters, weight, texture and dry matter content are important in determining quality of the fruit. Very little work has been done so far on changes in these characters during fruit development. Hence, information generated on these, aspects will be of immense help to producers, breeders, consumers and food technologists.

Materials and methods

A field experiment was conducted with ten popular Indian cultivars of brinjal at Indian agricultural Research Institute, New Delhi, India, to determine fruit weight, dry matter content, texture and seed hardening during fruit development. Fruits were tagged just after fruit set, and were harvested at 10, 15, 20, 25 and 30 days after fruit set (DAS). The firmness or texture of fruits was measured in terms of Newton (N) by using Instron Universal Testing Machine. For measurement of texture, the load cell of the instrument was one kg and speed was 50 mm per minutes and a probe of 35 mm diameter was used. The dry matter content was determined by drying chopped fruit at 65^0C in a hot air oven till constant weight was obtained. Seed quality was categorized as per the method used by Singh *et al.* (1990). Data were analysed as per procedure of Completely Randomized Design.

Results and discussion

The data in Table 1 revealed that varieties differed significantly with respect to fruit weight. With advancement of maturity there was a gradual and significant increase in weight. The interaction between variety and stage showed significant difference and on 30^{th} day, maximum and minimum weight was observed in 'Pusa Uttam' (504.00g) and 'Pusa Purple Cluster' (67.33 g) respectively. The increase in fresh weight in each variety with advancement of maturity was due to more accumulation of metabolites and was a varietal character. These findings are in accordance with those of Reddy and Santhadmath (1975)and Singh *et al.* (1990).

Significant difference was observed among varieties with respect to force required to compress the fruit (texture) at different stages of maturity. Maximum force (98.53 N) was recorded in variety 'Pusa Purple Long' at 25 DAS whereas minimum in 'Pusa Hybrid-9' (34.49) at 10^{th} day. Increment in compression force was noted, which indicates decrease in quality, with advancement of maturity (Table 2). This increment may be due to maturation of seeds inside the fruit, thickening of cell wall and formation of fibre. Hardening in fruit texture due to fibre formation has also been reported by Kaur and Bains (1988) and Ekka (1998) in okra fruit.

Dry matter content of all the varieties except 'Pusa Ankur', 'Pusa Purple Long' and 'Pusa Bindu' decreased gradually with advancement of growth and after reaching a minimum level, it increased again (Table 3). Maximum dry matter (13.50%) was recorded in 30 days old fruit of 'Pusa Purple Long' and minimum (8.05%) was recorded in 'Pusa Purple Cluster' after 10 DAS. In three of the varieties *viz.*, 'Pusa Ankur', 'Pusa Purple Long' and 'Pusa Bindu' a gradual increase in dry matter content was observed. It might be possible that the decreasing trend in dry matter content in these fast growing varieties ended before starting of observation of this character on 10^{th} day of fruit set. Increase in dry matter at later stage may be due to accumulation of metabolites in the fruit and seed and lesser rate of its utilization in the other biochemical processes. The decrease in respiration rate at later stage of fruit growth might have contributed to non-utilization of metabolites and thereby increase in dry matter content (Jones & Corner, 1962).

Seed remained soft and white in color up to 15 days in all the varieties except 'Pusa Bindu' & 'Pusa Ankur' (Table 4). At 20 DAS all the varieties became unacceptable as the seed became yellow and hard except 'Pusa Uttam' and 'Pusa Upkar' which were rendered unacceptable on or after 25^{th} day of fruit set (Table 4). Hardening of seeds with advancement of growth may be attributed to migration of calcium to seed coat and the loss of moisture from seed associated with seed maturity (Singh *et al.*, 1990).

The above studied characters were found to be reliable maturity indices. The fruits of small round fruited varieties ('Pusa Bindu' & 'Pusa Ankur') should be harvested around 10 DAS, 'Pusa Hybrid-5', 'Pusa Hybrid-6', 'Pusa Hybrid-9' and 'Pusa Purple Long' between 15-20 day and 'Pusa Uttam', 'Pusa Upkar' and 'Pusa Kranti' between 20 to 25 days after fruit set.

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Table 1: Fruit weight of brinjal cultivars at different stage of maturity

Cultivar	Fruit weight (g) on days after fruit set					
	10	**15**	**20**	**25**	**30**	**Mean**
Pusa Hybrid-5	42.00	152.00	174.67	192.00	247.67	161.65
	(7.30)	(9.51)	(3.00)	(7.30)	(6.55)	(6.73)
Pusa Hybrid-6	57.33	124.33	293.33	321.33	334.00	226.06
	(9.97)	(7.78)	(5.04)	(12.23)	(8.84)	(8.77)
Pusa Hybrid-9	61.00	136.67	302.67	368.67	434.00	260.60
	(10.60)	(8.55)	(5.20)	(14.03)	(11.40)	(9.97)
Pusa Uttam	125.33	201.33	243.67	450.00	504.00	304.86
	(21.79)	(12.60)	(4.19)	(17.72)	(13.95)	(13.81)
Pusa Upkar	78.00	285.33	342.66	420.67	495.33	324.39
	(13.56)	(17.85)	(4.17)	(16.01)	(13.11)	(12.94)
Pusa Ankur	61.66	169.33	180.33	186.00	205.67	160.59
	(10.72)	(10.59)	(3.10)	(7.08)	(5.44)	(7.39)
Pusa Purple long	72.66	94.00	100.67	120.33	181.33	113.79
	(12.63)	(5.88)	(1.73)	(4.58)	(4.80)	(5.97)
Pusa Bindu	31.00	107.00	170.37	179.00	181.33	133.80
	(5.39)	(6.69)	(2.93)	(6.81)	(4.80)	(5.33)
Pusa Kranti	79.33	189.38	418.33	438.00	449.20	314.84
	(13.79)	(11.84)	(7.19)	(16.67)	(11.89)	(12.28)
Pusa purple Cluster	14.00	31.00	57.67	57.00	67.33	44.20
	(2.43)	(1.94)	(0.88)	(2.16)	(1.78)	(1.84)
Mean	62.23	149.03	227.87	273.30	309.99	
	(10.82)	(9.32)	(3.74)	(10.40)	(8.2)	

C.D. at 5%; Variety:0. 72;Days:0. 51;VarietyX Days:1.60

Note: Figure in parenthesis pertains to transformed mean values (i.e. actual mean divided by corresponding square root of EMS). Decrement in transformed value at any stage does not indicate actual decrease in character as it is influenced by EMS at that stage. Difference has been considered to observe the significant change with the help of critical difference of the transformed data. However, trend depends on the original mean of character.

Table 2: Texture of brinjal cultivars at different stage of maturity

Cultivar	Force (N) on days after fruit set				
	10	**15**	**20**	**25**	**30**
Pusa Hybrid-5	70.94	74.69	88.66	98.27	-
Pusa Hybrid-6	54.53	62.78	68.32	90.98	-
Pusa Hybrid-9	34.49	54.76	81.67	93.82	-
Pusa Uttam	37.71	56.89	71.58	-	-
Pusa Upkar	67.29	73.89	78.46	97.66	-
Pusa Ankur	47.80	71.34	97.69	-	-
Pusa Purple long	39.98	65.32	80.88	98.53	-
Pusa Bindu	71.13	90.20	-	-	-
Pusa Kranti	69.00	77.76	97.91	-	-
Pusa purple Cluster	74.40	80.87	94.71	-	-
C.D. at 5%:	6.72	4.71	8.24	2.24 ;	**- :>100 N**

Table 3: Dry matter content of brinjal cultivars at different stage of maturity

Cultivar	Dry matter (%) on days after fruit set					
	10	**15**	**20**	**25**	**30**	**Mean**
Pusa Hybrid-5	11.24	10.83	11.21	12.53	13.56	11.87
Pusa Hybrid-6	11.13	10.76	10.98	11.52	12.26	11.33
Pusa Hybrid-9	11.48	10.95	12.37	13.26	13.40	12.29
Pusa Uttam	11.62	11.13	12.30	12.98	13.23	12.24
Pusa Upkar	11.57	10.56	10.37	11.39	12.43	11.26
Pusa Ankur	10.43	11.22	12.01	12.28	12.62	11.73
Pusa Purple long	9.20	10.66	11.70	12.76	13.58	11.58
Pusa Bindu	10.19	12.31	12.78	13.14	13.37	12.36
Pusa Kranti	11.57	11.47	11.80	12.16	12.54	11.91
Pusa purple Cluster	8.08	9.88	10.12	10.74	11.66	10.10
Mean	10.66	10.97	11.56	12.27	12.86	

C.D. at 5%;Variety:0.42;Days:0.29;VarietyX Days:0.94

Table 4: Number of days taken for seed hardening and browning in different cultivars of brinjal

Cultivar	Force (N) on days after fruit set				
	10	**15**	**20**	**25**	**30**
Pusa Hybrid-5	-	+	+	++	++
Pusa Hybrid-6	-	+	+	++	++
Pusa Hybrid-9	-	-	+	++	++
Pusa Uttam	-	-	+	++	++
Pusa Upkar	-	-	+	++	++
Pusa Ankur	-	++	++	++	++
Pusa Purple long	-	-	+	++	++
Pusa Bindu	-	++	++	++	++
Pusa Kranti	-	+	+	+	++
Pusa purple Cluster	-	-	+	++	++

- : Seed soft and white (fruit edible)
+ : Seed semi arid and light brown(non acceptable)
++: Seed hard and deep brown(non edible)

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POLLINATION BEHAVIOUR AND NATURAL HYBRIDIZATION IN *SOLANUM MELONGENA* L. TO UTILIZE THE FUNCTIONAL MALE STERILE LINE FOR HYBRID SEED PRODUCTION

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1. Introduction

Heterosis has been amply exploited in egg plant for developing high yielding hybrids possessing number of other valuable characters. However, genetic emasculation has not been efficiently used in developing hybrids (Kumar *et al.*, 2000). Bumble bees (*Bombus* spp.) are by far the most efficient pollinators of egg plant (Abrol, 1991; Amoako and Yeboh-Gyan, 1991; Abak *et al.*, 1995). However, those reports did not mention the extent of natural hybridization which is necessary for hybrid seed production in open pollinated condition utilizing the functional male sterile line UGA-1MS developed by Phatak and Jaworski (1989).

The aim of this study was to investigate floral biology, pollination behaviour and natural hybridization in eggplant in main cropping season (autumn-winter) of West Bengal (eastern state of India).

2. Materials and Methods

The studies were conducted under field growing conditions during 2000-01. Five diverse genotypes grown in autumn-winter season (September to March) under average day and night temperature ranging from 24.8 to 33.4° C and 10.2 to 25.1° C, respectively were employed for floral biology study. The stigma receptivity was studied by fruitset method employing 5 diverse parental lines and the functional male sterile line UGA-1 MS under two different environmental conditions : winter E_1(November-December under day and night temperature range of 26.2-30.7° C and 11.5-18.3° C) and rainy season E_2(July-August when day and night temperature lay between 32.3-33.0° C and 25.8-26.1° C). Emasculated flowers in the 6 female parents were hand pollinated with the mixed pollens between 8.30 to 12.30 a.m. in 4 stigmal stages: one day before anthesis (S_1), day of anthesis (S_2), one day after anthesis (S_3) and two days after anthesis (S_4). Thirty randomly emasculated flower buds in 5 plants per genotype were hand pollinated in each stigmal stage in one-month span.

Influence of insect pollinators on fruit set was studied through growing 5 plants each of the 5 diverse genotypes both under 2 x 2 x 3 ft nylon net cage and unprotected condition in autumn-winter season. Frequency of natural hybridization was determined through use of three homozygous genotypes possessing dominant

(pigmentation in stem, petiole, vein and fruit, *P* and presence of prickle in stem, petiole, vein and calyx without any pigmentation, *gS*) and recessive (prickleless and green, *gs*) marker characters. Sixty each of *P* + *gs* and *gS* + *gs* plants were planted alternatively in 4 rows in symmetrical design in two separate and isolated blocks in such a manner that the row started with *P* or *gS* plants. Single fruit from each of the 30 *gs* plants in each planting block was harvested at maturity and the seeds were sown in the seed bed. Any progeny possessing pigment and prickle must have issued from hybridization and expressed in percentage. Natural fruit set in 30 plants of the functional male sterile line UGA-1 MS surrounded by different normal male fertile genotypes was also recorded.

3. Results and discussion

Anthesis started in the morning from 6.45 a.m. and prolonged upto 9.45 a.m. Anther dehiscence began 34 to 110 minutes after anthesis. Stigma became receptive before anthesis and remained so after anthesis also. However, stigma became fully receptive, as revealed by the highest percentage fruit setting upon hybridization, on the day of anthesis and declined there after (Table 1). Much higher crossability and less decline in stigma receptivity after anthesis was recorded in autumn-winter compared to rainy season condition.

Table 1. Crossablity (% fruit set) in two environments(autumn – winter,E_1 and rainy season, E_2)

Female line	Stigmal stage for hand pollination			
	S_1	S_2	S_3	S_4
BCB-11	75.2(61.3)	85.4 (71.3)	74.6 (53.9)	61.3 (48.4)
BCB-18	71.3 (53.4)	89.6 (62.8)	80.2 (50.4)	58.7 (42.7)
BCB-34	74.8 (43.9)	90.4 (63.5)	73.4 (47.6)	64.7 (40.8)
BCB-67	62.4 (60.2)	81.3 (65.7)	78.6 (46.8)	55.8 (41.7)
BCB-74	61.3 (51.5)	85.2 (71.5)	75.3 (49.2)	60.2 (51.6)
UGA-1MS	63.4 (42.6)	82.6 (75.2)	72.8 (53.4)	51.5 (46.8)

% fruit set in E_2 in parenthesis

Floral morphology of the fertile flowers though generally favour self-pollination and fertilization yet fruit set in the caged plants of the 5 genotypes was reduced by 14.3 to 71.0% compared to that occurred in the unprotected plants. Reduction of fruitset by 50-75% (Amoako and Yboh-Gyan, 1991) and even 85-90% (Pal and Singh, 1943) under insect proof condition was reported earlier. In the present insect proof growing condition, fruit yield reduction ranged between 66.3 to 83.5% in the genotypes due to marked reduction in both fruitset and fruit weight (Table 2).

Table 2. Fruit characters of the genotypes under unprotected (U) and caged (C) growing in field.

Genotypes used	Fruit number/plant		Av. fruit weight (g)		Fruit yield/plant (kg)	
	U	C	U	C	U	C
BCB-11	19.82	5.47	144.41	93.40	2.64	0.51
BCB-18	28.85	17.18	66.45	29.32	1.92	0.53
BCB-34	60.83	21.39	61.20	25.65	3.34	0.58
BCB-67	56.75	35.16	85.81	24.80	4.46	0.88
BCB-74	21.33	18.28	128.45	49.36	2.78	0.93
Mean difference	*		**		**	

*** Significant at P = 0.05; ** Significant at P = 0.01**

Our result on prominent role of insect pollinators on fruit set and yield could be explained by the requirement of sonication due to buzz foraging of bees from most bee families to release the pollens even for self-pollination when pollens are liberated by the common apical opening of anther as in eggplant (Westerkamp, 1987; Buchmann and Nabhan, 1996; Westerkamp and Gottsberger, 2000).

Table 3. Natural hybridization in eggplant

Planting combination in two blocks	Seedlings from *gs* plants examined	Seedling characters			Estimated natural hybridization (%)
		Green and non-spiny	Pigmented	Green and spiny	
gs + *P*	4209	3919	290	-	6.88
gs + *gS*	4740	4352	-	388	8.18
				Average	**7.53**

Altogether 8949 seedlings of 30 days old of the recessive marker parents were examined in the seed bed and average estimated natural hybridization as evidenced by the presence of seedlings having pigment and prickle was only 7.5 percent (Table 3). In the 30 plants of the functional male sterile line, only one fruit was set naturally. High crossing success of 82.6% could be achieved in UGA-1MS by hand pollination (Table 1). So, two contradictory pictures emerged: marked role of insect pollinators particularly bees in pollen liberation, fruit set and yield and very low natural hybridization.

Flowers of eggplant do not possess nectar so chance of pollen contamination to the insect visitors foraging for the light and dry pollens, which are liberated from the top of the anther, is low. This might have resulted for low natural hybridization despite overwhelming role of insect pollination on fruitset. Insect pollinators chiefly bees basically act as pollen releaser for self-pollination. In this situation, open

pollination will not result appreciable hybrid seed set. So, hand pollination obviating emasculation is suggested to utilize the functional male-sterile line UGA-1 MS in hybrid development.

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IN VITRO INDUCTION OF HAPLOID IN EGGPLANT (*SOLANUM MELONGENA* L.)

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Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop of Indian origin holds a coveted position in Indian sub-continent in summer and rainy season. Due to good nutritional value and its suitability to grow under a wide range of growing conditions, it has been extensively cultivated in different parts of world. Immense variability exists in this crop, which provides an opportunity to the researchers to develop plants with good quality and productivity (Daunay *et al.* 2000). Recently much work has been done on development of hybrids with good yield and quality. But availability / development of homozygous / inbred line is a bottleneck in efficient exploitation of heterosis as the development of inbred takes 3-4 generations of selfing. Another alternative way is to develop homozygous lines using in vitro method such as anther or pollen culture.

Haploids produced through anther/pollen culture is of great importance in plant breeding programmes as well as in basic genetic studies. Haploid and doubled haploid lines obtained by efficient anther culture technique are now opened up new possibilities for rapid fixation of genotypes and genetic analysis. Moreover it can be an excellent support for molecular analysis and location of quantitative traits. Among the various methods available for haploid production, anther culture is the most practical, viable and widely used technique. Anther culture has been applied to many species and it is recognized that the production of haploid lines greatly assist in to subsequent production of F_1 hybrids (Rotino *et al.* (1992), Arnison *et al.* (1990). However, usefulness of this approach is limited in solanaceous species because some respond very poorly to anther culture (Yadav *et al.* 1989).

The culture response is affected by numerous factors, such as genotype, donor plant culture conditions, pretreatment of flower buds or anthers, stage of development of microspore, culture medium composition and culture conditions (Arnison *et al.* 1990). Haploid production for practical application in breeding programme of several solanaceous crops such as tomato and potato were well documented. However, very little progress has been made in case of anther culture for haploid development in eggplant. In present study an attempt was made to standardize protocol for anther culture in eggplant to obtain haploid plants.

Materials and methods

The experimental material comprised of three eggplant F1 hybrids. Young anthers of *Solanum melongena* were cultured on MS medium (Murashige and Skoog medium, 1962) and GD medium (Gresshoff and Doys, 1972) containing various growth regulators like 2-4 D, BA, NAA (0.5, 1.0, 2.0 mg/l) for callus induction. The anther was crushed in acetocarmine to test the stage of pollen development.

The young isolated buds were pretreated in cold for 2-3 hrs. These buds were surface sterilized with 0.1 % $HgCl_2$ for 3 minutes and washed thrice with sterile water. The anthers were aseptically cultured on MS as well as GD medium. Anthers alongwith their filaments were excised under aseptic condition and placed on a sterile petri dish horizontally and sealed with parafilm. The cultures were incubated in dark for initial 20-30 days at 25 ± 2 °C and after callus initiation all the cultures were transferred to light for 16 hour photoperiod. A minimum of 200 anthers from 5 plants were cultured for each treatment. After four week of incubation the anthers were observed for callus initiation. Cultures were scored for frequency of callus induction at the end of four weeks. The frequency was calculated as the ratio between the numbers of anthers responded to callus induction or regeneration to that of total number of anthers inoculated. The developed plantlets from the anther culture were transferred to small pots. After hardening (20-30 days) they were transplanted to the green house.

Results and discussion

Culture of anthers to raise haploids followed by chromosome doubling is an easy method of developing homozygous lines in relatively less time than traditional methods. In eggplant callus initiation from anther has been reported earlier by Vaulx and Chambonnet (1982), Yadav et al. (1989) and Karakullucku and Abak (1993). However, regeneration was poor in these cases. In present study, in vitro regeneration was carried out to produce haploid through anther culture. Selection of anther/correct stage of pollen development were found to be the most critical factor for haploid production.

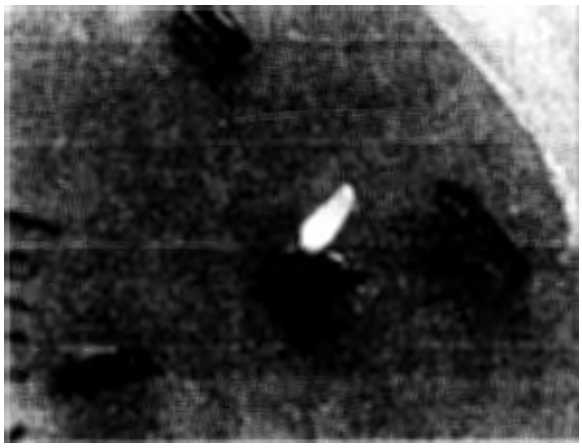


Fig. 1. Direct embryogenesis from anther

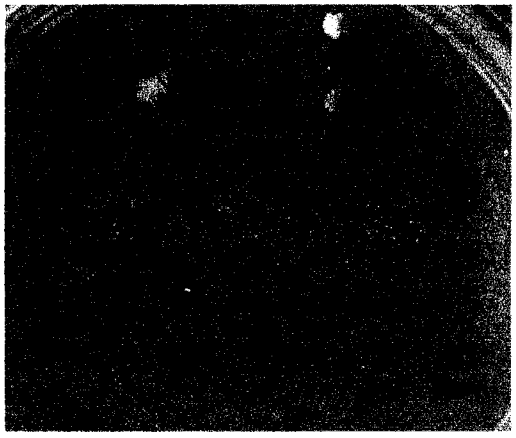
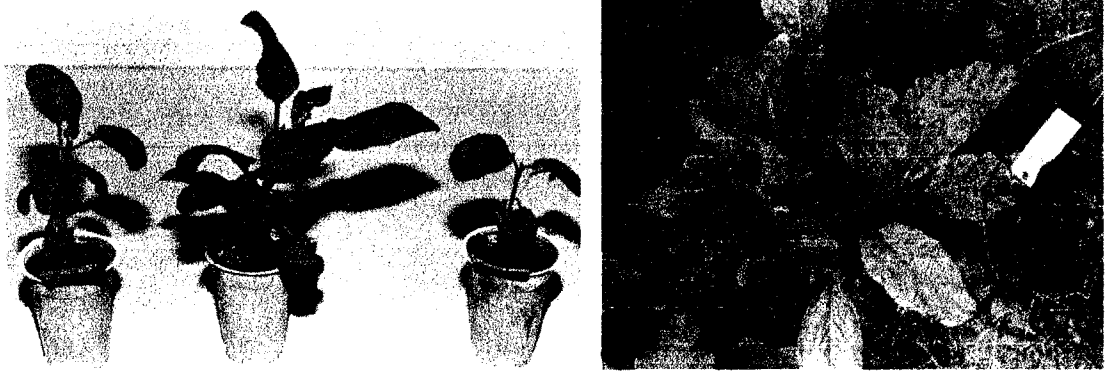


Fig. 2. Callus initiation from anther



Plantlets derived from anther culture (in pots and in field)

In responsive anthers, the wall tissue gradually turned brown and depending on the genotype after 3-8 weeks they burst open due to pressure exerted by growing callus (Figure 1). After 4 weeks interval the anthers from all the three F_1 hybrids produced callus. However the anther of F_1 MS x Pant Rituraj showed produced maximum embryogenic callus (Figure 2). The average number of anthers giving callus initiation was between 11.8 to 20 % depending on the genotype and pretreatment. Two types of response were recorded; some anthers produced embryogenic callus, while some produced embryos directly. These embryogenic calli were again multiplied on same medium and then transferred to different media having varying concentration of NAA (0.1, 0.2, 0.5, 1.0, 2.0 and 2.5 mg/l). The embryos were taken directly for germination and embryogenic callus was cultured to stimulate development of embryos. The embryogenic callus initiation was observed from the anthers on medium containing 2.0 mg/l 2,4-D. Karakullukcu and Abak (1993) also used 2,4-D for callus initiation in eggplant in combination with kinetin. A cold treatment of anthers at 5°C for 2-3 hours gave better frequency of callus induction. The cold or mild heat treatment has been reported to be effective for initial response of anther culture. Earlier Valiux and Chambonnet (1982) used heat treatment at 35 °C for eggplant anthers. The regeneration from the callus was initially observed as green spot in the callus. Shoot initiation from the callus was observed on the MS medium containing 0.2 – 0.5 mg/l NAA. These shoots were multiplied on 1.0 mg/l BAP with 0.2 mg/l IAA. The explants bearing several shoot buds were transferred to GR-free MS medium for elongation. The individual elongated shoots were cultured on half strength MS medium to induce rooting. The putative haploid plants were acclimatized under conditions of high humidity and transferred to field (Figure 3,4). The regenerated plants were found to be sterile with stunted morphology as compared to normal fertile plants. The further evaluation of these plants is still under progress. The variability observed in the callus induction of anthers of three hybrids suggests that it is better to consider behaviour of each genotype on different induction medium. Considering the frequency of callus initiation and plantlet regeneration the further work on factors involving the initial response may permit to achieve a higher frequency.

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RELATION BETWEEN BACTERIAL POPULATION AND WILT INCIDENCE IN EGGPLANT

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INTRODUCTION

Eggplant (*Solanum melongena* L) is one of the common fruit vegetables in Nepal. It is grown extensively in Eastern and Central Terai regions of Nepal. To some extent it is also cultivated in the foot hill of the country. Bacterial wilt caused by *Ralstonia solanacearum* Smith is the most important disease responsible for the limitation of its cultivation in the country. It is wide spread and encountered to be the most devastating disease for eggplant. Complex infection of plant parasitic nematodes and *R. solanacearum* causes increased wilt severity reducing the bacterial wilt resistance in solanaceous crops (Yen et al., 1998). However the disease incidence was observed as high as 50 %, but in some of the locations of eastern terai bacterial wilt has been observed in high incidence (Timila et al., 1996). There is no more cultivation of eggplant in one of the locations named Indrapur due to this disease and replaced by wheat crop (as found during field visit,1998). The disease is reported to be specific to location, strain and the environment (Mew and Ho, 1977). Being soil borne nature of the pathogen, the inoculum level present in soil plays an important role in the out break of disease in the field. With the purpose of finding out the role of bacterial population in disease incidence, an experiment was conducted.

MATERIALS AND METHODS

The experiments were conducted during 1998 and 1999 in two consecutive years in experimental field (infested with *R.solanacearum*) of Regional Agricultural Research Station at Tarahara, Eastern Nepal. The cropping period of eggplant in that region is starting from October (seeding start) and ending at May (ending). Before transplanting , the first soil samples were collected from different points and made composit sample. Three replicates of working sample was made from it. During 1998, the second samples were collected after 6 weeks of transplanting of the susceptible variety, Pusa purple long then followed by the collection at an interval of 4 weeks until 18 weeks after transplanting. But in 1999, the second sample was collected after 8 weeks of transplanting followed by 4 weeks interval until 20 weeks. The working samples were made in the same way mentioned above. Ten gram of soil was suspended in 90 ml of sterile distilled water and shaked for half an hour. One ml. of the soil solution was drawn and mixed with 9 ml of sterile distilled water. In this way it was serially diluted to 10^{-4} for each sample. Hundred microliter of suspension from each dilution was plated in SM1 medium in two replications. The plates were incubated at 28° C for 48 hours. The typical fluidal colonies were counted on each plate . Bacterial population per gram dry soil were calculated for each sample by using the formula, Colony forming unit per gm. soil is equal to number of colonies X 1000 X dilution factor/dry weight of 1 gm.soil X 10.

For comparing disease incidences with bacterial population present in soil 'Pusa purple long' variety was transplanted in the field. Observations were taken for the disease appearance and for the incidence at corresponding times of soil samples taken.

RESULTS AND DISCUSSION

The data revealed that the disease incidences were found to be increased as the bacterial population increased in soil. The result (table 1) also showed that for the appearance of disease in

the field, certain level of inoculum must be there. When the bacterial population was 10^3 cfu per g soil, disease symptom was not obvious. As the temperature increased, the colony forming units (cfu) per gram (g) soil was also increased. During 1998, wilt appearred when the log (x+1) value of bacterial population was 5.12. It was It was ten weeks after transplanting. Whereas, in 1999, the wilt symptom appeared when the log (x+1) value of bacterial population was 2.3 (12 weeks after transplanting). In 1998, cfu per g soil was reached to 10^5 after 10 weeks of transplanting whereas it was only 10^4 even after 20 weeks of transplanting. The disease incidence was also found to be gradually increased as the bacterial population increased. Though the bacterial population was lesser in 1999 than in 1998, the disease incidence reached to 85 % after 20 weeks of transplanting (table 2).

Table 1. Effect of bacterial population in wilt incidence of eggplant, 'Pusa purple long' during 1998.

S.No	Weeks after transplanting	Cfu/g.soil	Log (x+1) transformation	Wilt incidence percent
1	Before transplanting	$0.27X10^3$	1.43	-
2	Six weeks	$2.9 X 10^3$	3.46	no disease
3	Ten weeks	$1.4 X 10^5$	5.12	20.00
4	Fourteen weeks	$1.6 X 10^5$	5.20	32.50
5	Eighteen weeks	$3.2 X 10^5$	5.50	72.50

It could be due the effect of the variation in temperature and rainfall during cropping period of the years. In 1999, the temperature and total rainfall were slightly higher than in the year 1998 (Timila et al., 2000). The disease incidences were higher even in other resistant varieties during that particular year than in 1998 as revealed by Timila and Shrestha (2001). It showed that certain bacterial population (inoculum) must be in soil to develop disease in the field. Thus the results of both years showed the direct relationship between the bacterial population and the disease incidence in the field at Tarahara condition of Nepal.

Table 2. Effect of bacterial population in wilt incidence of eggplant, 'Pusa purple long' during 1999.

S.No	Weeks after transplanting	Cfu/g.soil	Log (x+1) transformation	Wilt incidence percent
1	Before transplanting	$0.5 X10^3$	2.70	-
2	Eight weeks	$5.1 X10^3$	3.71	no disease
3	Twelve weeks	$1.7 X 10^4$	4.23	12.5
4	Sixteen weeks	$2.4X 10^4$	4.38	62.0
5	Twenty weeks	$2.9 X 10^4$	4.46	85.0

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EFFECT OF HOST NUTRITION ON PHOMOPSIS DISEASE OF BRINJAL

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Abstract

A field trial was conducted during 2000 and 2001 to assess the effect of fertilizers on Phomopsis disease of brinjal at Palampur. An increase in nitrogen fertilization resulted in increased severity of leaf blight and fruit rot and nitrogen in the form of ammonium sulphate suppressed the progress of disease. Increased doses of potassium fertilizer decreased the disease significantly. Application of phosphorus @ 64 kg P ha^{-1} drastically decreased the severity of fruit rot only but not the severity of leaf blight.

Introduction

Phomopsis leaf blight and fruit rot of brinjal (*Solanum melongena* L.) caused by *Phomopsis vexans* (Sacc. & Syd.) Harter is a serious disease causing 10-25% loss in marketable fruits (Panwar *et. al.*1970) Although the disease has been investigated by several workers (Pawar and Patel, 1957; Vishunavat *et. al* 1992), information on the role of host nutrition, a primary component of disease control, on the disease is lacking. Since manipulation of plant nutrients play a significant role on the outcome of disease, the present study was conducted on the effect of fertilizer on the disease and crop yield.

Materials & Methods

Field trials in randomized block design with three replications on the effect of forms of nitrogenous fertilizers and different doses of nitrogenous, phosphorus and potassium fertilizers were conducted at the experimental farm of the department during 2000 and 2001 crop season with highly susceptible brinjal cv. 'PPC' following recommended package of practices for vegetable crops (Anonymous, 1998) except application of fertilizers. One-month-old seedlings of brinjal were transplanted in the first week of June during both the years with row-to-row and plant-to-plant spacing of 60 and 45 cm, respectively in 2 x 2 m^2 Plots. Data on leaf blight and fruit rot were scored on 1-12 point scale (Horsfall and Barratt, 1945) with the appearance of disease and thereafter at weekly intervals till the completion of experiment. The percent disease severity was determined, pooled at the end for determining the relative effect of each treatment. To ascertain the role of forms of nitrogenous fertilizers, calcium ammonium nitrate, ammonium sulphate and urea were applied @ of 100, 102.5, 97.5 kg N, P & K /ha respectively in three splits with Phosphorus and potassium @ of 60 and 45 kg P & K /ha respectively as basal dose . Plots which did not receive N, P, K fertilizers served as check. In other field trials, Calcium ammonium nitrate (CAN) @ of 87.5, 100, 112.5 kg Nha^{-1}, single super phosphate @ 56, 60, 64 Kg P ha-1 and murate of potash @ 30, 45, 60 kg K ha^{-1} with normal doses of P & K, N & K,and N & P, respectively were applied for determining the role of different doses of N, P & K fertilizers on disease and crop yield.

Results and Discussion

All nitrogenous fertilizers caused significant increase in disease severity and yield over unfertilized plots (Table 1). The average severity of leaf blight and fruit rot was more in plots fertilized with urea and CAN than those with ammonium sulphate. The severity of leaf blight and fruit rot did not differ significantly in plots fertilized with urea or CAN but differed significantly with NH_4SO_4. Although, fertilization with CAN or urea significantly increased the leaf blight and fruit rot of brinjal, there existed no significant differences in crop yield between plots fertilized with CAN and ammonium sulphate and those of ammonium sulphate and

urea. Thus, application of ammonium sulphate to brinjal crop can play a significant role in the suppression of disease and in ameliorating the crop yield. A progressive increase in the severities of leaf blight and fruit rot was noticed with an increase in the levels of nitrogen over plots which received either P & K or no fertilizer (Table 2). There existed no significant differences in severities of leaf blight in plots, which received 87.50 or 100 kg N ha^{-1}. However, at these levels of nitrogen there were significant differences in the severity of fruit rot. Plots which received 112.50 kg N ha^{-1} showed the maximum development of leaf blight and fruit rot and differed significantly from other two doses of N fertilizer. Leaf blight severity also differed significantly between plots fertilized with potassium, phosphorus, and those, which did not receive any fertilizer. In these plots though no difference in severity of fruit rot was observed, but there existed significant difference in crop yield. Plots, which did not receive any fertilizer, showed the minimum development of disease but with less crop yield. An increased dose of N caused non- significant effect on crop yield. Thus fertilization with lower dose (87.50 kg N ha^{-1}) of nitrogen will help in maintaining the disease to the minimum without any appreciable effect on crop yield. The different doses of phosphorus showed variable response on disease severity (Table3). No significant differences in severities of leaf blight were observed with an increase in levels of phosphorus. However, increased doses of phosphorus caused significant effect on fruit rot severity. Although, there was more leaf blight at 64 kg P ha^{-1} but had minimum fruit rot and significantly higher crop yield. Plots which were fertilized with nitrogen and potassium, though developed comparatively more leaf blight and fruit rot but were at par in their effect on crop yield with those of 56 and 60 kg P ha^{-1}. The plots, which did not receive any fertilizer, developed less disease but had poor crop yield. Progressive decrease in severity of leaf blight and fruit rot was noticed with an increase in doses of potassium (Table 4). At 60 kg K ha^{-1} there was minimum leaf blight and fruit rot and maximum crop yield which differed significantly from other treatments. Plots, which received nitrogen and phosphorus, developed maximum leaf blight. However, in these plots there was less fruit rot than those plots which received 30 kg K ha^{-1}. Although significant differences were observed in severities of leaf blight and fruit rot at all levels of K fertilizers, no differences in yield were observed between plots, which did not receive K, and those with 30 and 45 kg K ha^{-1}. Plots with no fertilizers though developed less disease but yielded poorly.

Nitrogen applied to brinjal as calcium ammonium nitrate (CAN) and urea enhanced the severity of leaf blight and fruit rot where as nitrogen in the form of ammonium sulphate reduced it drastically. Fertilization with ammonium sulphate also supplies sulphate in addition to nitrogen and fungitoxic potential of sulphur is well documented (Horsfall and Cowling, 1980), thereby could be less disease. These results are in agreement with Daly(1949) , William(1965) who reported reduced severity of stem rust of wheat and fruit & root rot of tomato, respectively with application of NH_4–N. Similarly Huber and Watson (1974) have cited several examples where disease severity decreased with ammonical nitrogen and enhanced by nitrate nitrogen and vice-versa. An increased susceptibility of brinjal to phomopsis disease at higher nitrogen nutrition is probably due to luxuriant growth of the host thereby affecting the microclimate of crop canopy favouring the pathogen. Alternatively, it could be possible that at higher nitrogen nutrition there is enhanced exudation of nutrients which inturn enhance the inoculum potential and aggressiveness of the pathogen resulting in more disease. Huber (1980) have reported higher disease with an increased N-nutrition in Tomato- *Corynebacterium michiganense,* Potato- *Streptomyces scabies,* Potato- *Alternaria solani* host-patho systems. Phosphorus nutrition in majority of cases behaves like nitrogen. In the present studies, it reduced the fruit rot significantly at higher doses. (Huber & Watson, 1974) reduced the severity of early and late blight of potato with potassium fertilization. Potassium nutrition in general is negatively correlated with disease in many host-Patho systems (Walker, 1946). Thus application of ammonical nitrogen at recommended doses, phosphorus and potassium at higher doses will help in reducing the phomopsis leaf blight and fruit rot of brinjal and in ameliorating the crop yield especially under the Himachal Pradesh conditions.

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Table 1: Effect of different forms of nitrogenous fertilizer on the Phomopsis leaf blight and fruit rot of brinjal.

N. Forms	Dose (kg/ha)	Disease severity (%)		Yield (q/ha)
		Leaf blight	Fruit rot	
CAN	100	60.1(50.8)	55.1(47.9)	211.7
Ammonium Sulphate	102.5	39.2(38.7)	29.3(32.7)	213.1
Urea	97.5	61.1(51.4)	55.2(47.9)	214.2
None		22.3(28.1)	17.1(24.4)	110.6
CD (*P*=0.05)		4.9	8.3	2.9

Arc sine transformed values in the parentheses

Table 2: Effect of different doses of nitrogen (CAN) on the Phomopsis leaf blight and fruit rot of brinjal.

Nitrogen Dose (CAN, kg/ha)	Disease severity (%)		Yield (q/ha)
	Leaf blight	Fruit rot	
87.50	44.4(41.7)	32.5(34.7)	213.9
100	46.6(43.1)	54.6(47.6)	213.4
112.50	61.9(51.8)	66.1(54.4)	211.5
P and K	27.4(31.5)	20.9(27.2)	159.7
No fertilizer	22.1(28.0)	18.5(25.4)	104.7
CD (*P*=0.05)	3.2	4.4	8.5

Arc sine transformed values in the parentheses

Table 3: Effect of different doses of phosphorus (SSP) on the Phomopsis leaf blight and fruit rot of brinjal.

Phosphorus (SSP,kg/ha)	Disease severity (%)		Yield (q/ha)
	Leaf blight	Fruit rot	
56	44.5(41.8)	36.7(37.3)	212.9
60	42.7(40.8)	68.7(55.9)	213.7
64	43.7(41.3)	27.1(31.4)	248.7
N and K	48.4(44.0)	51.5(45.8)	211.2
No fertilizer	22.6(28.4)	21.1(27.3)	105.7
CD (*P*=0.05)	(4.2)	(3.9)	7.3

Arc sine transformed values in the parentheses

Table 4 : Effect of different doses of potassium (MOP) on the Phomopsis leaf blight and fruit rot of brinjal.

Potassium (MOP, kg/ha)	Disease severity (%)		Yield (q/ha)
	Leaf blight	Fruit rot	
30	59.0(50.2)	72.1(58.1)	213.4
45	48.1(43.9)	61.9(51.8)	214.1
60	29.3(32.7)	34.5(35.9)	240.1
N and P	60.6(51.1)	64.8(53.6)	212.4
No fertilizer	22.9(28.6)	19.8(26.4)	104.7
CD (*P*=0.05)	(2.7)	(2.9)	15.5

Arc sine transformed values in the parentheses

ANNOUNCEMENTS

XII[th] EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant 17 – 19 May 2004, Noordwijkerhout – the Netherlands

Organized by

- Plant Research International
- NAK Tuinbouw

In cooperation with

- Plantum NL
- De Ruiter Seeds
- Enza
- Nunhems
- Rijk Zwaan
- Seminis
- Syngenta
- Western Seed

At the last Capsicum and Eggplant Eucarpia meeting in Antalya, Turkey it was decided to organise the next meeting in the Netherlands. The meeting will be held in Noordwijkerhout, the Netherlands in May 2004. It will be organised by the members of the technical working group of Capsicum and Eggplant Breeders of the Netherlands assembled in Plantum NL, Plant Research International in Wageningen and the Leids Congres Bureau (LCB) in Leiden.

Scientific Committee

- Alain Palloix
- Roeland Voorrips
- Jair Haanstra
- Ietje Boukema
- Kazim Abak
- Paul Bosland
- Marie-Christine Daunay
- Piero Belletti
- Istvan Nagy

All researchers, scientists, breeders and specialists involved or interested in Capsicum and Eggplant genetics and breeding are invited to participate in this meeting. Please, make this announcement available to your colleagues working in this area all over the world.

Scientific Program

All participants are urged to submit a presentation or poster about topics related to the genetics and breeding of Capsicum and Eggplant. Abstracts should be submitted by e-mail **before December 1, 2003** in MS Word format to *eucarpia@plant.wag-ur.nl.* Please indicate on the abstract whether it is intended as a presentation or poster. The scientific committee will review all abstracts. Accepted papers and posters will be published in a book of proceedings. All papers, posters and abstracts should be in English. Abstracts should be formatted as follows:

QTL MAPPING OF ANTHRACNOSE (*COLLETOTRICHUM SPP*) RESISTANCE IN *CAPSICUM*

R.E. Voorrips[1], L. Sanjaya[2], R. Groenwold[1] and H.J. Finkers[1]

[1] Plant Research International, P.O.Box 16, 6700 AA Wageningen, The Netherlands. Email: R.E.Voorrips@plant.wag-ur.nl. [2] Research Institute for Vegetables, Lembang, Indonesia

Text (maximum 400 words)

Preliminary Program

Monday May 17	*Tuesday May 18*	*Wednesday May 19*
Registration from 8:30h a.m.	Oral Presentation Session	Oral Presentation Session
Opening/Welcome	Excursion:	Plenary Discussion
Oral Presentation Session	*- De Ruiter Seeds*	Excursion:
Poster Session	*- Capsicum/Eggplant grower*	*- NAK Tuinbouw – Field trials*
Round Table Discussions	*- Capsicum/Eggplant market*	*- Guided Tour at the Keukenhof*
	Dinner at De Ruiter Seeds	Dinner at the Keukenhof

Accommodation

The Leids Congres Bureau (LCB) will arrange hotel accommodations in the conference building of the NH Hotel Leeuwenhorst in Noordwijkerhout.

The conference hotel is set in a lovely area, surrounded by trees, dunes and tulip fields. Amsterdam, Schiphol, Leiden and The Hague can be reached by car within 30 minutes. Complementary parking is available for 600 cars. Monday through Friday, a shuttle bus travels to and from Leiden Railway Station (during rush hour).

The hotel rooms are equipped with bath, separate shower, toilet, radio, television, telephone, and modem connection. A number of rooms have a mini bar, and non-smoking rooms are also available. In the "Internet Corner" in the hall, you can surf the Internet and e-mail.

Costs are approximately:

- 107,59 Euro per night, one person per room
- 123,18 Euro per night, two persons per room

Information about alternative Hotel accommodations are available on the web site: www.eucarpiacapsicum.nl

Registration Fees

The participation fee will be approximately 490 Euro for early registration/520 Euro for late registration. It includes lunches, dinners, refreshments during scientific sessions, excursions and the meeting proceedings.

Further Information

The official language will be English. No simultaneous translation is planned.

We will visit the Capsicum and Eggplant registration trials at the NAK Tuinbouw in Roelofarendsveen. A wide range of the commercial Capsicum and Eggplant varieties for the European market is included in these trials. This visit is organised as alternative for the traditional demonstration trial, which is too expensive to organise in the Dutch heated greenhouses.

The second circular will be sent in October 2003.

In order to receive the second announcement please complete and return by mail, fax or e-mail the preliminary registration form to the contact address. The form can be found linking on the Web site of the Meeting.

Contact Address

EUCARPIA - Capsicum & Eggplant – 2004
Leids Congres Bureau BV
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Email: info@leidscongresbureau.nl (subject: Eucarpia – Capsicum and Eggplant – 2004)

Website: www.eucarpiacapsicum.nl

RECIPES

Recipes in which pepper or its derivates are used follow. As usual they have been supplied by Terry Berke. Thank you, Terry.

Chekchouka, from Ali Boussaffara, a traditional dish in Tunisia

- 2 tomatoes, choppped
- 1 hot pepper, chopped
- 2 sweet peppers, chopped
- 3 cloves garlic, minced
- Dill leaf to taste
- 2 Tb. olive oil
- 2 eggs

Heat oil in skillet. Add tomatoes, peppers, and garlic and cook until slightly soft. Add eggs gently on top, cook until egg yolks are not quite hard, sprinkle dill leaf on top and serve with toast. This dish provides balanced nutrition, with protein, vitamins, and fiber.

Bar-B-Q Nutria

Nutria (also called coypu) are large South American rodents whose meat is low in fat and cholesterol and higher in protein than beef or chicken.

- 1 nutria, skinned, cleaned, and cut up into serving pieces
- 1 cup barbecue sauce, your choice
- 1 onion
- 1 rib of celery
- ½ bell pepper
- 2 cloves garlic
- 1 jalapeno
- 1 TB vegetable oil

Chop vegetables and sauté them in oil until brown. Add barbecue sauce and nutria; cook for 45 minutes and serve. If you live in Lousiana, you can buy state-certified nutria meat, or just take an ax handle to the bayou and whack you one. If you don't live in Louisiana, you may substitute any other large South American rodent, such as the four-foot-long capybara.

Caribbean Red Island Seasoning (from the January 2002 issue of Seminis Garden News)

- 3 garlic cloves, peeled and left whole
- 1 carrot, peeled and cut into thin strips
- 4 branches of fresh thyme
- 3 whole Caribbean Red chiles
- 2 tablespoons chopped chives
- 6 whole black peppercorns
- 1 pint white vinegar

Place all of the ingredients except for the vinegar in a sterilized jar. Pour the vinegar over the mixture, and allow it to steep for one week before using. Then sprinkle on various foods.

Melinda's Costa Rican "Perked" Coffee Barbecue Sauce, the winning recipe (from the 2002 Más Melinda's Recipe Contest)

- 1 cup molasses
- 1 cup packed light brown sugar
- 3 cups tomato ketchup
- 1 teaspoon seasoning salt
- 1/4 cup Worcestershire sauce
- 1/3 cup apple cider vinegar
- 1 cup yellow onion, finely chopped
- 1 tablespoon garlic powder
- 1/3 cup Melinda's Habanero Pepper Sauce
- 1 teaspoon ground chili powder
- Fat rendered from 4 slices hickory smoked bacon
- 2 cups Costa Rican strong perked black coffee

Combine all ingredients in a large heavy saucepan. Bring to a simmer over medium heat. Reduce to low and simmer, stirring occasionally for 30 minutes. Remove from heat. Brush sauce on grilled chicken, pork or beef during last 10 minutes of grilling. For added flavor brush additional sauce on meat immediately after removing from grill. Refrigerate any left over sauce. Yield: About 2 quarts, Heat Scale: Medium

The Prime Minister's Hot Sauce

From the famous Errol W. Barrow, who was Prime Minister of Barbados from1961-76 and again from 1986 until his death in 1987. He was also an accomplished cook, and published Privilege: Cooking in the Caribbean (Macmillan Caribbean) in 1988.

- 6 large bonney peppers, seeds and stems removed, chopped (substitute habaneros)
- 1 large onion, coarsely chopped
- 2 small cloves garlic
- 1 tablespoon mustard
- 1 tablespoon white vinegar
- 1 tablespoon vegetable oil
- 1/2 cup chopped carrots
- 1 cup water
- Salt and pepper to taste

Combine all ingredients in a saucepan and boil for about 15 minutes. Adjust the consistency with water. Puree in a food processor or blender and bottle in sterilized bottles. Yield: About 2 cups; Heat Scale: Hot.

Pimientos de Padrón

From the sun-drenched region of Padron, located along the Atlantic coast of Spain. http://www.fiery-foods.com/dave/fried.asp. These are small, horn-shaped, conical chiles with a heat level that is usually mild, with about one in five pods spicy. Substitute serranos.

- 2 tablespoons olive oil
- 12 pimientos de Padrón or serranos, stems removed
- Sea or rock salt and freshly ground black pepper

In a medium skillet, heat the olive oil hot. Add the chiles and fry, stirring well, until they blister and start to turn brown. Remove the chiles from the pan, drain on paper towels, place in a bowl and add salt and pepper to taste. Stir or toss the chiles to cover. Yield: 6 appetizer servings. Heat Scale: Medium.

Chile Rellenos, from Mexico

- 12 large poblano chiles with stems
- 1 pound cheese, cut in 12 strips
- 1 pound lean ham, cut in 12 strips

Roast and peel chilies. To stuff, cut a small slit below the stem of each chile and remove the seeds. Place a strip of cheese next to a strip of ham and slip into the slit in the green chile.

Batter

- 4 eggs, separated
- 4 tbsp all-purpose flour
- 3/4 tsp baking powder
- 1/4 tsp salt

Beat egg whites until stiff, set aside. Beat yolks until thick, set aside. Sift together dry ingredients and add yolks, blending well. Carefully fold beaten egg whites into the yolk mixture. Dip the stuffed chiles into the batter. Then fry in deep fat (360-365 degrees Fahrenheit) until golden brown.

Smoky Mayonnaise, from http://www.fiery-foods.com/dave/chipotle_flavors1.asp
Use this interesting variation on mayonnaise whenever the bland kind is called for. Also use this as a topping for cold, cooked shrimp and hard-boiled eggs or as a dip for raw vegetables.

- 2 chipotle chiles, rehydrated, seeds and stems removed, or substitute 2 chipotle chiles in adobo sauce plus 2 teaspoons of adobo sauce
- ½ cup prepared mayonnaise
- ½ cup sour cream
- ¼ teaspoon dried cilantro flakes

In a blender or food processor, combine the rehydrated chipotles and 2 teaspoons of the rehydrating water and puree. Alternately, if using chipotles in adobo, puree the chipotles with 2 teaspoons of the sauce. Add the pureed chipotles to the mayonnaise, sour cream, and cilantro flakes and mix well. Heat Scale: Medium

Israeli Sabra Dip, from The Hot Sauce Bible, by Dave DeWitt and Chuck Evans.
Sabra is the Hebrew word for cactus, and also slang for a person born in the state of Israel.

- 1 large ripe avocado, peeled and pitted
- 2 jalapenos, chopped
- 1 onion, chopped
- 3 Tb. Lemon juice
- 1 1/2 cups cream cheese
- milk as required
- salt to taste

Chop avocado, jalapeno, and onion together in a blender. Add lemon juice and cream cheese and puree. Add milk to get the desired texture. Add salt to taste.

Nohn Mai Phai (bamboo worm), also known as "Jungle French Fries" in Thailand
From http://www.fiery-foods.com/dave/thaifood.asp

- 25 bamboo worms (substitute night crawlers)
- 2 cups vegetable oil
- 1/2 cup Maggi hot sauce (substitute your favorite hot sauce)
- salt to taste

Deep fry them until crunchy, slather with Maggi sauce and a generous shake of salt, and masticate while washing the bugs down with lots of your favorite drink. You could savor a Styrofoam cup cooked this way!
Below is a note received from our reporter in the field in China:
"They're a delicacy in SW China too. I like them a lot -- we eat them deep fried but just with salt in Yunnan. Personally, I favor bee larva over bamboo worms. I do believe night crawlers would be a bit over-flavored to function as a true substitute! Recommend traditional use as bait to catch fish."

Sun-Cured Pickled Jalapeños, from http://www.fiery-foods.com/dave/pickle.html
These pickled chiles have an East Indian flavor because of the mustard seeds and ginger. Any small green chiles can be substituted for the jalapeños. Serving Suggestions: Serve these unusual chiles on sandwiches, hamburgers, or as a side relish for grilled or roasted meats.

- 1 cup jalapeño chiles, stems and seeds removed, cut in 1/4-inch strips
- 1 tablespoon coarse salt
- 1 tablespoon mustard seeds
- 1 teaspoon cumin seeds
- 1/4 cup oil, peanut preferred
- 1 teaspoon chopped fresh ginger
- 1/4 cup freshly squeezed lemon juice

1. Sprinkle the chile strips with the salt; toss and let them sit for 10 minutes.
2. Toast the mustard and cumin seeds on a hot skillet, stirring constantly, for a couple of minutes until the seeds begin to crackle and "pop."
3. Heat the oil to 350 degrees F., remove from the heat, stir in the ginger, and let it simmer for 2 minutes. Remove the ginger and discard.
4. Stir in the chiles, cumin seeds, lemon juice, and pack in a sterilized jar.
5. For 5 days, set the jar in the sun in the morning on days when it is at least 70 degrees, and bring it in at night. Shake the jar a couple times each day.
Yield: 1 pint

PEPPER TRIVIA

Some new pieces of information and curiosities about pepper world, kindly supplied by Terry Berke.

The hottest habanero record is held by Frank Garcia of GNS Spices, and certified by the Guinness Book of World Records at 577,000 Scoville Heat Units (dry fruit basis). To my knowledge, this record has never been duplicated or verified. The same variety, 'Red Savina', was grown out several times by horticulturists at New Mexico State University and never measured beyond 300,000 S.H.U. Pungency is affected by the environment, if a plant is grown under stress it will produce more pungent fruits. Stress can be caused by drought, flooding, heat, cold, insect attack, etc.

An Indian cow shelter is selling its animals' urine for people to drink as a cure for indigestion and skin cancer. The business in Jaipur has a hospital attached to it which also sells soap containing cow dung. Its most popular product is undiluted cow urine. A 78-year-old patient says a daily dose keeps him fit. The Gau Seva Sangh centre also sells a digestive mixture that combines urine with hot pepper and another urine-based mix which claims to cure skin cancer.

Farmers in Japan are using red chilli powder bombs to keep monkeys from pilfering their produce. The bombs, which propel the powder into the eyes and noses of the monkeys when they pass in front of sensors, replace electrified fences and wires, which the monkeys outwitted while stealing a half-million dollars worth of produce. Source: Fiery Foods Magazine

Most dogs do not like hot sauce. To stop a puppy from chewing on a lamp cord, try coating it with hot sauce. If he stops chewing, it worked. If he covers it with cheese and a floured tortilla, you have to try something else.....

Richard Lignon, in his History of the Barbadoes (1647), described the two varieties of peppers he found on the island: "The one so like a child's corall, as not to be discerned at the distance of two paces, a crimson and scarlett mixt; the fruit about three inches long and shines more than the best pollisht corall. The other, of the same colour and glistening as much but shaped like a large button of a cloak; both of one and the same quality; both violently strong and growing on a little shrub not bigger than a gooseberry bush." Source: Fiery Foods Magazine

Researchers at Virginia Tech added capsaicin to the diet of commercial meat chickens to see if it protected them against Salmonella, a common intestinal pathogen. They reported that capsaicin increased resistance to Salmonella without adversely affecting weight gain or the taste of the chicken when cooked. If further research validates this study, this may provide an antibiotic-free method to produce chicken and give a whole new meaning to the term 'hot wings'..... Source: Virginia Tech http://www.technews.vt.edu/

Rodents have an aversion to capsaicin-coated seeds. Feeding poultry feed to which capsaicin has been added could be very beneficial in poultry houses. Rodents love to get into poultry house, where they eat the feed, destroy buildings, and spread Salmonella and other diseases. Snyder Seed, a New York-based company, developed a line of wild birdseed coated with chili pepper oil, which they call Hot Pepper Treat. Squirrels and other rodents won't eat the food. Source: Virginia Tech http://www.technews.vt.edu/

Smoked chiles, called chipotles, had their origin in the ancient civilization of Teotihuacán, north of present-day Mexico City. It was the largest city-state in Mesoamerica and flourished centuries before the rise of the Aztecs. Chipotles also made an appearance in the marketplaces of Tenochtitlán, the capital city of the Aztecs that is now called Mexico City. The most commonly smoked chiles are jalapeños, named for the city of Jalapa in the state of Veracruz. Jalapeños will not dry properly in the sun—their thick flesh would rot first. However, like meats, they can be preserved by the process known as smoke-drying. Source: http://www.fiery-foods.com/dave/chipotle_flavors1.asp

CAMBRIDGE, MA-Jon Rosenblatt, 27, a Harvard University English graduate student specializing in modern and postmodern critical theory, deconstructed the take-out menu of a local Mexican restaurant "out of sheer force of habit" Monday."What's wrong with me?" Rosenblatt asked fellow graduate student Amanda Kiefer following the incident. "Am I completely losing my mind? I just wanted to order some food from Burrito Bandito. Next thing I know, I'm analyzing the menu's content as a text, or 'text,' subjecting it to a rigorous critical reevaluation informed by Derrida, De Man, etc., as a construct, or 'construct,' made up of multi-varied and, in fact, often self-contradictory messages, or 'meanings,' derived from the cultural signifiers evoked by the menu, or 'menu,' and the resultant assumptions within not only the mind of the menu's 'authors' and 'readers,' but also within the larger context of our current postmodern media environment. Man, I've got to finish my dissertation before I end up in a rubber room." At approximately 2 a.m., Rosenblatt was finishing a particularly difficult course-pack reading on the impact of feminism, post-feminism, and current 'queer' theory on received notions of gender and sexual preference/identity. Realizing he hadn't eaten since lunch, the Ph.D candidate picked up the Burrito Bandito menu. Before he could decide on an order, he instinctively reduced the flyer to a set of shifting, mutable interpretations informed by the set of ideological biases-cultural, racial, economic, and political-that infect all ethnographic and commercial "histories." "Seeing this long list of traditional Mexican foods-burritos, tacos, tamales-with a price attached to each caused me to reflect on the means by which capitalist society consumes and subsumes ethnicity, turning tradition into mass-marketable 'product' bleached of its original 'authentic' identity," Rosenblatt said. "And yet, it is still marketed and sold by the dominant power structure in society as 'authentic' experience, informed by racist myths and projections of 'otherness' onto the blank canvas of the alien culture." Source: http://www.theonion.com/onion3826/grad_student.html

Did you know that.... October is National Pickled Pepper Month in the U.S.

News Flash! Archaeologists have found the oldest Tabasco® bottle. A 130-year-old Tabasco® bottle has been recovered and reconstructed from 21 glass fragments found in the archaeological site of the historic Boston Saloon in Carson City, Nevada. The recovery of the artifact was announced by Nevada state historic preservation officer Ron James and McIlhenny Company historian Shane Bernard. Ashley Dumas, a graduate student at the University of Alabama who directed the excavations at the original Tabasco® factory said that the bottle found in the Comstock mining district of Carson City is a Type 1a bottle, one of the earliest forms know. Edmund McIlhenny began bottling Tabasco® in 1868, and the Boston Saloon operated between 1864 and 1875 and the probable date of the bottle is 1870. Since the proprietor of the Boston Saloon was William A.G. Brown, a prominent free African-American from Massachusetts, the find has interesting meaning for Western food history. "The Tabasco® bottle is particularly intriguing because of what it implies about African-American cuisine and the development of the West," said supervising archaeologist Kelly Dixon. "This was an exotic product and Comstock African-Americans were apparently breaking new ground. Source: http://www.fiery-foods.com/whatsnew/indnews1002.asp#Tabasco

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No. 23, 2004
(to be published in the summer of 2004)

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To "Capsicum and Eggplant Newsletter" Editorial Board

Please, when available, send me a copy of "Capsicum and Eggplant Newsletter" No. 23 (2004). I am sending the subscription rate directly to EUCARPIA Secretariat (c/o Plant Breeding Dept., University of Agricultural Sciences, Gregor Mendel Str. 33, A-1180 VIENNA, Austria, fax +43 1 47654 3342).

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Eggplant *(Solanum spp)*

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