

CAPSICUM & EGGPLANT

NEWSLETTER



University of Turin
DI.VA.P.R.A.
Plant Breeding and Seed Production



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FOREWORD

The fourteenth issue of "Capsicum and Eggplant Newsletter" includes two invited papers: the first one has been written by Chen Shifriss and deals with the male-sterility in *Capsicum*, while the second one refers to the gene nomenclature for Potyvirus resistance genes in pepper and has been prepared by Alain Palloix and Molly Kyle. Thank you very much to the above mentioned

Authors, for their kind willingness to increase the scientific value of our publication. As usual, the accepted contributions have not been modified and have been printed as received. So, only the Authors themselves are responsible for both the scientific content and the form of their own reports.

The co-operation between the Newsletter and the Food and Agriculture Organisation (FAO) has been renewed for this year. In this way we are able to distribute the Newsletter to Institutions in about 140 countries all over the world.

Please, remember that a subscription fee to the Newsletter will be much appreciated. The fees are the same as the last year. Remember also that it is possible to book your own copy, so quickening its delivery. Just fill in the order form on page 95 and send it to us, together with a copy of the payment order, which must always be made to Eucarpia. In case you decide to pay by credit card, please use the voucher on page 97. We prefer this way of paying because of the lower bank costs.

The deadline for the submission of articles to be included in the next issue of the Newsletter (No. 15, 1996) is February 28, 1996. Please note that it is also possible to submit the paper on diskette. Details can be found in the enclosed sample sheet.

Piero Belletti and Luciana Quagliotti

Turin, 1st June 1995

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Male sterility in Capsicum
Chen Shifriss

Male sterility in pepper, *Capsicum annuum* L., was first documented by Martin and Grawford (1951) and then by Peterson (1958). Since then, many reports dealing with the trait have appeared, including its isolation, induction inheritance, cytology and, particularly potential for hybrid seed production.

Interspecific hybridization

Sterility in pepper, as in other plants, can occur following interspecific hybridization due to chromosomal, or plasmon-genome incompatibility. Egava and Tanaka (14) suggested, based on chromosomal pairing in interspecific hybrids, the genetic distance between species. Hence, *Capsicum chinensis* and *C. frutescens* are considered close species (12 bivalents in the hybrids), while in *C. annuum* x *C. chinensis* and *C. annuum* x *C. baccatum* combinations both quadrivalent and hexavalent, due to reciprocal translocations were observed.

In these last cases F1 hybrids are practically male sterile, their anthers do not dehisce, containing few or no stainable pollen grains. Pollen fertility in such material can be restored by backcrossing to one or both parental lines. An extensive interspecific study was carried out (1, 3, 13, 14, 17, 22, 33, 37, 41, 42) to improve *C. annuum*, particularly with *C. chinensis*, *C. baccatum*, *C. frutescens* and *C. chacoense*.

With the *C. baccatum* x *C. annuum* combination, a developmental process of male sterilization followed repeated backcrossing to *C. annuum*, resulting in antherless (frequently combined with reduced female fertility) progeny. Such plasmon-genome interaction leads to the cytoplasmic male sterility (CMS) phenomenon.

The attempt of several workers to identify fertility restorer genes for this specific case was unsuccessful, as will be clarified later. F1 hybrids among *C. annuum* and the above mentioned species demonstrated different levels of male sterility, depending on the accessions used in each interspecific cross.

Contribution from The Agricultural Research Organization, ARO, The Volcani Center, Bet Dagan 50 250, Israel No. 1638-E, 1995 series.

Spontaneous and induced male sterile ms mutants

Over dozen single gene mutants were independently found or induced following mutagenesis (X-rays, gamma rays and EMS) (6, 10, 18, 24, 26, 30, 35, 38, 43, 51).

The ms-509 for example which was induced by mutagenesis in France (38) was found allelic to the msk allele isolated spontaneously in Korea (55). Looking for spontaneous mutants, it is advisable to select among “old” cultivars that contain more recessive mutants than in lines (cultivars) that pass strict selection for uniformity.

Generally, these ms mutants are similar in nature and function but few exceptional ones (26, 30, 35) are linked with additional marker traits which can help in early identification of the ms individuals for hybrid seeds production.

Cytoplasmic male sterility in *C. annuum*

Peterson (36) isolated a male sterile individual within accession 164835. He classified several degrees of sterility, from one or two to 10-15 viable pollen grains per anther. The trait was found to be controlled by a major ms gene interacting with specific S plasma type to generate (S) ms ms mother plants. A dominant Ms allele is necessary to restore pollen fertility. Hence, from the cross (S) ms ms x (N) Ms Ms we should obtain fertile (S) Ms ms progeny only. In addition, from several test cross pollinations, a dihybrid ratio was obtained (3 fertile: 1 sterile) suggesting additional locus, i.e. Ms1 and Ms2 independently restore fertility. Novak et al, working with Peterson's male sterile material (32), suggested two contradictory digenic interpretations to his data. Based on the 1:3 and, 9:7 (fertile: sterile) ratios in testcrosses and F2 populations, respectively, a complementary gene action was suggested. On the other hand, the 3: 1 test cross data suggested autonomous gene action of the expected Ms1 and Ms2 loci. With this last suggestion one would expect the 15:1 F2 ratio, but such F2 segregation was never documented. While Ms alleles were found in most wild hot pepper accessions, the ms alleles are present in most sweet, large-fruited lines (32,34,36,54, 55).

Further studies revealed that the trait is unstable with a tendency to restore pollen fertility under varying growing conditions (21, 22, 36, 48,

54, 55).

According to Peterson (36), "Warm temperatures present the most critical environment for sterility expression." The relative instability of the trait was attributed to an interaction between temperature and sterility modifier genes.

Woong Yu (55) in a study of 270 pepper lines, found 152 to be "stable B-lines", i.e., maintainer (N) ms mm, 66 restorer lines (N) MsMs and the remaining 50 lines were defined unstable leading to segregating progeny following crossing with Peterson's male sterile plants. In addition, the pattern of segregation demonstrated great deviation from year to year. These "unstable lines" were probably heterozygote to the Ms-modifiers complex and their progeny were exposed to yearly or seasonal variation. Eventually, selfing and selection in this material yielded both "stable" maintainer lines and fertility restorer ones.

Woong Yu (54, 55) did not find any maintainer line among pubescent phenotypes and among the sweet accessions; only one, Di Quneo, was a restorer.

Shifriss and Frankel (47) succeeded in isolating S plasma type following interspecific crosses. Eventually, this source of male sterility proved to be identical to that of Peterson's.

Cytoplasmic male sterility from interspecific crosses

Capsicum frutescens x *C. annuum* yielded cytoplasmic male sterile progenies following backcrosses with B-maintainer lines (3, 55). The *C. frutescens* plasmatype was found to be identical to Peterson's one in its sterilization and fertility restoration potential when crossed with Peterson's maintainer and restorer lines, respectively. Hence, three independent sources of S plasmatype, two in *C. annuum* and one in *C. frutescens*, were found to be identical. *C. baccatum* x *C. annuum* hybrids backcrossed to *C. annuum* yielded an extreme type of male sterility, viz, antherless flowers (1, 3, 13, 42). Efforts to isolate fertility restorer genes of a Ms nature were unsuccessful.

At this stage we should differentiate between the standard ms genes operating autonomously ms1' ms-509, etc. from the male sterility if genes interacting with N and S plasmatypes which were originally designated as ms genes (Peterson 36).

Hence, cytoplasmic male-sterile individuals will be marked (S) rf rf and the restorer allele as Rf. It was also clarified that ms mutants are non-allelic to the rf ones. A gradual feminization process which occurred in advanced backcrossing of *C. baccatum* x *C. annuum*, suggests a polygenic plasmon-genome nature of inheritance.

It is possible that pollen fertility in *C. baccatum* depends on (N) rf rf ((N) ms ms in previous nomenclature) and additional modifiers. Hence, no dominant alleles of Rf nature are expected to restore male fertility, but rather the recessive rf ones. Such an interspecific situation is useless for producing male-fertile hybrids.

Cytological aspects of meiotic breakdown

Except for one case of meiotic breakdown at the pachytene stage (30), all reported evidence on male sterility indicates that microspore breakdown, occurs after the tetrads stage of meiosis. Microscopic observation (19, 31) and electron microscopy (20) helped to clarify the functions of the tapetal layers and the callose during the process. In normal meiosis, the callose dissolves and normal microspores are released from the tetrads. In meiosis of cytoplasmic male steriles the microspores abort and both the outer and inner layers of the tapetum degenerate.

The aborted mass, late in anther development, consists of crushed microspore tetrads, primary walls of the sporogeneous cells and tapetum, callose and the collapsed tapetum.

Stability of the trait

All male-sterile ms mutants are highly stable and hence promising sources for hybrid seeds production. In exceptional cases (6), a few aborted pollen grains were found in anthers of male-sterile plants. The cytoplasmic source of male sterility is highly sensitive to temperatures, as found by Peterson and others (21, 22, 36, 48).

The variation among CMS lines in the expression of sterility relies on the difference among their maintainer lines, i.e., in their modifiers.

Hence, one can select B-lines highly resistant to seasonal (temperature) fluctuations.

Generally, under optimal and cool weather conditions meiotic breakdown in (S) rf rf plants is delayed, followed by a tendency to restore pollen

fertility. During the hottest part of the summer season (August-September in Israel), male sterility is complete. Hence, one can manipulate the different seasons for either hybridization during late summer or for selfing in (S) rf rf parental lines for seed increase during the cool season.

Maintenance of male sterile ms lines

Genic male sterility. Single gene ms mutants can be increased vegetatively but the resulting explants are non-juvenile, loaded with flowers at their young stage. The sibbing ms ms x Ms ms cross is a common procedure for seed increase of 50% msms plants while rouging the remaining 50% Ms ms individuals at anthesis. A seedling marker linked with the ms gene might be helpful for early identification and planting only the desirable ms ms individuals. Csillery (4) and Shifriss (50) succeeded in constructing the digenic system ms1 ms1 ms2 ms2 x ms1 ms1 ms2 ms2 which yielded 3 male-sterile vs. 1 fertile progenies, comparable to the 1:1 ratio in the previous system.

The digenic procedure, however needs vegetative maintenance of the two crossing components and close protection from viral contamination.

Shifriss (44) tried to build an XYZ system (12) in which an interspecific trisome, that is an alien addition line carrying a foreign univalent with the Ms gene. Such a line should serve as a maintainer - Y or B - line. Hence the cross ms ms x ms ms Ms should yield approximately 100% male sterile ms ms individuals. Using *C. annuum* (ann) ms ms and *C. chinensis* (chin) Ms Ms, we arrived at the following crossing stage:

12II Ms MS (ann) + 12 I Ms (chin) x 12 II ms ms (ann) + 12 II Ms Ms (chin)

This cross resulted in plants with large range of pollen fertility (sterility). Although selfed seeds were obtained, the desirable 12 II ms ms (ann) + I II Ms Ms (chin) Z line was not achieved.

CMS seed increase

The main advantage of the CMS system over the previous genic one, stems from the fact that one can obtain 100% male-sterile plants for direct use as females (S) rf rf x (N) rf rf. Alternatively, based on its relative instability, we can multiply the (S) rf rf plants under off-season conditions that induce pollen restoration for selfing. This procedure was

found applicable in the Arava region of Israel, during the winter season (Shifriss unpublished).

How to look for male sterility?

A. A search for ms ms mutant plants

Every open pollinated cultivar is a potential candidate in which we can find ms mutant plants. Exceptionally tall, poor fruiting plants are suspected of being ms ms.

B. A search for new plasmon genome interaction

This search is based on the suggestion of Frankel and Galun (15) that a standard ms ms plant may actually be a (S*) ms ms type and the fertile plants are (S*) Ms Ms. Such model should be tested through a search for an alternative (N*) type cytoplasm.

Crosses of tested accessions with the (S*) Ms ms male genotype, like (S*) Ms Ms x (S*) Ms ms, should yield F1 hybrids in which 50% will segregate for male sterility in their F2 generation. However, rare exceptional cases are expected to show fertile progenies only in their F2 generation. Such finding can be derived from the cross (N*) Ms Ms x (S*) Ms ms. Its F2 progenies, (N*) Ms Ms, (N*) Ms ms and (N*) ms ms are expected to be fertile and the new (N*) ms ms line will serve as a unique maintainer B-line to generate 100% male-sterile plants for hybrid seeds production. Such a system is ideal, since all pepper accessions may serve as restorer (S*) MS Ms – C lines.

We tested this assumption with a few dozen accessions and two different ms mutant genes but did not find the anticipated N*-type cytoplasm.

Male sterility and hybrid seed production

Different genetic and field procedures are being used on an experimental and a commercial scale in order to obtain both sweet and hot pepper hybrids. The ms mutants are widely used (2, 5, 7, 8, 17, 18, 27, 40, 49, 52, 53, 55) in spite of the limiting needs to identify the ms ms individuals at a rather late stage - anthesis - and to remove 50% fertile Ms ms.

The CMS system is particularly advantageous for hot pepper hybrids, since it supplies 100% male-sterile plants and, in addition, restorer, Rf genes are widely distributed in hot accessions (32, 34, 36, 47, 54, 55). Where sweet-fruited hybrids are concerned, we can use the (S) rf rf x (N)

rf rf scheme to obtain 100% females, but the Rf Rf C-lines should be reconstructed to through the Rf gene into sweet fertile lines. The only sweet accession, which was found to be Rf rf is Di Quneo (55). In practice the new backcrossed C-lines are of the (S) Rf Rf type.

The breeding procedure to build special sweet C-lines is a burden When compared with the genic system in Which each line is potentially a Ms Ms "restorer line". When (S) F1 's were compared with their isogenic (N) F1 IS, in both hot (55) and sweet groups, (Shifriss and Gazit, unpublished) no agronomical or other differences were found.

In addition, for breeding hot pepper hybrids, Shifriss and Sacks (52) suggested to use sweet-fruited CMS mother plants That contain a larger number of seeds per fruit than common hot varieties.

Woong Yu (55) suggested to combine both genic and CMS components of sterility in order to obtain double cross hybrids. Such a program can exploit the yielding heterosis well known among hot pepper accessions. Daskalof and Mihailov (9) demonstrated an elegant system in which a conditional female-sterile C-line continues to flower and to supply pollen. In addition, a CMS line Which contains a lethal gene 1 serves as the seed parent. True F1 seeds will be obtained from the cross (natural or manual): (S) rfrf 11Cfs Cfs x (N) RfRf LL cfs cfs.

Although the system is attractive, its applicability and the involvement of the lethal 1 gene remain unclear.

Following research on natural pollination of male-sterile plants, maximal yield of F 1 seeds per unit area was obtained within the 2:1, 3:1 ratios among rows of male-sterile and fertile ones (2, 5, 7, 8, 17, 27, 52).

Highest yield per plant was obtained from the 1: 1 ratio and under most experimental conditions hybrid seed yields from male sterile plants was lower than 50% comparable with fertile plants. Studies on insect pollination demonstrated that both honey bees (*Aphis melliferal*) and bumble bees (*Bombus terrestris*) prefer fertile plants When exposed to both fertile and male-sterile ones (40, 49).

Rabinovitch et ale (40) found that male fertile flowers produced more nectar and a higher sugar concentration than sterile ones. In addition "significant correlation between sugar quantity and number of honey bee visits per flower was evident." However, additional studies are needed, particularly on the effect of pollen on the selective attractiveness of bees to fertile vs. male-sterile flowers.

Economic importance of male sterility

Male sterility, genic and CMS sources are widely used on both experimental and commercial bases in the hybrid seed industry. Both manual and open pollination procedures are used for large scale hybridization. The increasing importance of male sterility stems from the growing involvement of seed companies in producing elite F1 hybrids.

Although sweet F1 's are considered the most important in the seed industry, more hot hybrids are becoming popular.

In Korea, for example, 197 pepper hybrids - mostly hot ones - were registered up to 1994, and all of them were multiplied with genic or cytoplasmic mechanisms of male sterility. Today the CMS system is the dominant one (Woong-Yu, personal commun.).

Besides the general importance of male sterility in the seed industry, it has been suggested as an instrument for selecting genotypes with high potential of parthenocarpy (45).

Parthenocarpy or pepper cultivars with low seeds dependence for normal fruit development might be of economic importance.

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PROPOSAL REVISION OF GENE NOMENCLATURE FOR POTYVIRUS RESISTANCE GENES IN *Capsicum* sp.

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Many different potyviruses infect pepper in different regions of the world. Several sources of resistance were characterized in *Capsicum* sp. and introduced in commercial varieties (for review, see Greenleaf 1986, Green and Kim 1991). Many of the resistance sources to potato virus Y (PVY), tobacco etch virus (TEV) and pepper mottle virus (PeMV) = were found to be monogenic and recessive, allowing gene denomination by the authors. An updated list of pepper genes was published by Daskalov and Poulos (1994). In this list, different symbols were given to the same gene or to alleles at the same locus involved in potyvirus resistance, probably because the different authors involved in gene denomination were interested in different potyviruses. This raised the necessity to clarify this nomenclature and to revise symbols for potyvirus resistance genes. Mapping of pepper genome is also underway (Prince et al 1993, Lefebvre et al 1995) and this revision would help for a better coordination and coherence in mapping projects.

The new nomenclature must be made according to the rules for gene nomenclature of *Capsicum* (Capsicum and Eggplant Newsletter 1994). This nomenclature must also easily accommodate new information about the known genes (new alleles, pleiotropic effects...), new loci for potyvirus resistance and revisions in viral taxonomy. As general rule, we propose the symbol *pr* for Qotyvirus resistance loci, followed by a number specific to each locus, according to the chronological precedent. Alleles at a single locus will be differentiated using superscript numbers. The proposed revision of the nomenclature described above can be summarized as follows in the table.

Present symbol	Source	Resistance to:	New symbol
<i>et^c, et^{c1}, et^{c2}</i>	PI 159236, PI 152225	TEV © (Greenleaf 1956, 1986) and TEV (Blauth 1994) PeMV (Zitter 1972)	<i>pr¹</i>
<i>y^a, vy¹</i> <i>ey^a, et^a, vy²</i>	Yolo RP10, yolo Y PI264281, SC46252 Florida VR2	PVY (Cook 1960), PVY (0) (Gebre Selassie et al 1983, 1985) PVY(0,1)+TEV (Cook and Anderson 1959) (Gebre Selassie et all 1983, 1985)	<i>pr²1</i> <i>pr²2</i>
<i>et^{av}</i>	Avelar	PeMV (Zitter and Cook 191973) PeMV, TEV, PVY (Greenleaf 1986).	<i>pr³</i>
<i>Cy2</i>	Cm 334	PVY (0,1,2), PeMV (Palloix 1992, Pasko et al 1992), (Chanie-Dogimont 1993), (Dogimont et al 1995).	<i>pr⁴</i>

Resistance to TEV was firstly discovered by Greenleaf (1956) in *C. chinense* PI 152225, who named this recessive resistance gene *ef*. Resistance to TEV (C) was later found in *C. chinense* PI 159236 and Greenleaf (1986) tentitatively named this allele *ef1* and renamed *ef2* the preceding allele, although allelism tests remained to be made. Zitter

(1972) reported that PI 159236 was also resistant to PeMV but did not look *for* cosegregation of the resistances to both viruses. More recently, Blauth (1994) performed the allelism test between the two Pis. They showed that TEV resistance cosegregated in F2 and BC1 populations. Resistance to PeMV in PI 159236 and tolerance in PI 152225 also cosegregated. Although they observed cosegregation *for* TEV/PeMV resistance in these two PIs no conclusive evidence *for* identity of TEV and PeMV resistance was obtained. Thus we propose the unique symbol pr1 *for* TEV and PeMV resistance in PI 152225 and PI 159236 (1 because it was historically the first discovered locus), although it remains to be determined whether resistance loci to TEV and to PeMV are identical or tightly linked.

Recessive resistance to pVY was first identified by Cook and Anderson (1959) in both *C. annuum* PI264281 and SC46252. These accessions were also resistant to TEV (C) and the authors named this locus *er* to differentiate it *from* the *C. chinense* source. Cook (1960) later discovered that the recessive resistances to TEV and PVY *from* SC46252 were cosegregating and suggested this resulted *from* a single locus. This TEV resistance allele complemented the recessive PVY resistance allele *from* PI264281 (the F1 hybrid was TEV resistant), suggesting these two alleles to be identical. He renamed it *eyli*. Cook (1961), further discovered a mutant (Yolo RP10) bearing a recessive resistance to PVY that was allelic to *ey* but was susceptible to TEV(C). He named the PVY -specific resistant allele *y* but this was not consistent with the preceding *et* or *ey* nomenclature. This allelic series was further renamed by Gebre Selassie et al (1983, 1985) *vy1* (for *y*) and *vi* (for *ey*) with the susceptible allele *vyi-*. These authors also showed that PVY isolates *from* Mediterranean regions could be classified into three pathotypes (0), (1) and (1-2), according to their virulence on *vyi-*, *vy1* and *vi* respectively. In order to simplify this complex nomenclature, we propose the symbol pr2 for this locus (the second potyvirus resistance locus discovered). The different alleles could be designated by prZ (susceptibility *from* Yolo Wonder), pr21 (instead of *y* or *vy1* *from* Yolo RP10 and Yolo Y) and prZ (instead of *ef*, *ey* or *vi* *from* SC46252 introduced in Florida VR2).

Monoaenic resistance to PeMV from Avelar was first reported by Litter and Cook (1973). Avelar is also resistant to TEV (C) and to PVY(O) and Greenleaf (1986) named *efv* the allele supposed to confer resistance to the three viruses. However, Litter and Cook (1973) had showed that resistance to PVY and TEV in Avelar were independent *from* resistance to PeMV. Subramanya (1982) tested the allelism between Avelar and PI 159236 *for* resistance to PeMV. He obtained susceptible F1 hybrid but F2 segregation did not allow to choose between 1/1 or 7/9 ratios corresponding respectively to the segregation of two alleles (with negative interaction as concluded) or two independent loci. Blauth (1994) further confirmed that PeMV resistance *from* *C. chinense* (PI 159236) was genetically distinct from that of Avelar. Thus we propose the symbol pr3 *for* this locus governing resistance to PeMV (the third locus discovered). Allelism between Avelar and *C. chinense* for TEV resistance remains to be made with an appropriate strain since Avelar was susceptible to the TEV isolate used by Blauth (1994), as well as with *C. annuum* (Yolo Y or SC46252) for PVY resistance.

A monoaenic dominant resistance to PVY was described in *C. annuum* Serrano Criollo de Morelos 334 (CM 334), (Pal16ix 1992, Pasko et al 1992). This resistance was effective against all the known strains of PVY and also against PeMV. Allelism tests were performed with the *C. annuum* sources of resistance to PVY and independent segregation was confirmed. Cosegregation of resistance to PVY and PeMV was also observed in doubled haploid and BC progenies (Dogimont et al 1995). We propose the symbol Pr4 for this locus.

Other monogenic resistances and new alleles will probably be discovered in a near future. New loci might be designated by new numbers and new alleles by superscripts if allelism tests are conclusive. Additional allelism tests should be performed to clarify relationships between PVY and TEV resistance from Avelar, the following for TEV resistance''': from SC 46252 and from *C. chinense*. Progress will also be obtained from cosegregation ' observations between resistance to different potyviruses. This is possible thanks to haploid: segregations or vegetative (graft) multiplication. When two resistances cosegregate in F2 or in successive BC, a single locus can be admitted from the segregating (breeding) point of view, although only gene sequencing will allow to chose between very tight linkage or pleiotropy. Some gene symbols may have to be changed, according to the results of these studies and to the increase of our knowledge in genetics of pepper/potyvirus interactions. We would expect the general nomenclature will allow these changes.

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NUCLEAR DNA CONTENT IN DIFFERENT SPECIES OF CAPSICUM MEASURED BY FLOW CYTOMETRY - P. Belletti, C. Marzachi, E. Nada and S. Lanteri DI.VA.P.R.A. - Plant Breeding and Seed Production University of Turin, via P. Giuria 15, 10126 Torino, Italy

Introduction

Flow cytometry (FCM) is a powerful technique for DNA analysis, originally developed for human cells and recently adapted to the analysis of plant cells. FCM analysis of nuclear DNA is based on the use of DNA-specific fluorochromes and on the measurement of the relative fluorescence intensity of stained nuclei. A possible application of FCM is for the determination of nuclear DNA content. Since thousands of cells can be analyzed within few minutes, FCM is more convenient, precise and rapid than other techniques, e.g. microdensitometry and static cytofluorometry (Galbraith, 1989).

The absolute DNA content (usually expressed as pg/nucleus) can be established through the inclusion of a suitable standard of known DNA content, as chicken red blood cells (CRBC), which have a DNA content of 2.33 pg (Galbraith *et al.*, 1983) or cells of species with previously determined DNA content.

The nuclear DNA content has been measured for a wide variety of species, using FCM (see for instance Arumuganathan and Earle, 1991). Only a few data are available for the species belonging to the genus *Capsicum*. Galbraith *et al.* (1983) found a DNA content of 5.52 pg/nucleus in *Capsicum annuum*. Arumuganathan and Earle (1991) reported a range of 5.6-7.51 pg/nucleus referred to 7 cultivars. Bennet and Smith (1976) found higher values, by using Feulgen microdensitometry: 8.0 and 10.8 pg/nucleus for wild and cultivated forms respectively. They also analysed the following species (DNA content in pg/nucleus are reported between brackets): *C. baccatum*, wild form (9.8), *C. baccatum*, cultivated form (12.7), *C. chinense* (11.8), *C. eximium* (12.3), *C. frutescens* (12.0) and *C. pubescens* (12.9). In this work we determined the nuclear DNA content in different species '.

of *Capsicum*, with the aim to detect variations which could be useful in a better understanding of systematic and evolutionary relationships within the genus.

Materials and Methods

Nine species and two sub-species of *Capsicum* (with two or more accessions each, with the exceptions of *C. baccatum baccatum*, *C. cardenasii* and *C. lovan/*) were analysed (Table 1). They represent all the *Capsicum*

species utilized by man (IBPGR, 1983), with the exception of *C. galapagoense*, whose seed at our disposal did not germinate at all.

For flow cytometric analysis, young leaves obtained from plants grown in climatic chamber were used. 20 mg of leaf tissue was homogenized in ice-cold nuclear extraction buffer (Saxena and King, 1989), filtered through a 25 μ m mesh nylon filter and centrifuged at 5,000 rpm. The intact released nuclei were stained with propidium iodide (PI) and treated with DNase-free RNase A (Sigma R 5000). For each accession, at least five different plants were used.

Fluorescence was measured using a FACScan flow cytometer (Becton and Dickinson, USA) equipped with a 488 nm light source (argon laser). Two filters were used to collect the red fluorescence due to PI staining the DNA, one transmitting at 585 nm and the other above 620. The flow rate was set at about 1,000 nuclei/sec and at least 5,000 nuclei were analyzed for each sample. Data were recorded in a Hewlett-Packard computer (HP 9000, model 300) using CellFit software (Becton and Dickinson).

Pea (*Pisum sativum*), with 2C nuclear DNA content of 9.07 (Dolezel *et al.*, 1992), was used as internal reference standard for the estimation of the nuclear genome size in *Capsicum annuum* 'Doux Long des Landes': the other accessions were measured with reference to the latter.

Clustering of the data was performed through the Ward method (SPSS, 1988) and the distances on the dendrogram were rescaled to numbers between 1 and 25.

Results and Discussion

The DNA amount of the studied species is reported in Table 1. Since no significant differences were found among accessions from the same species, only the mean values are reported. The general mean of the genus was 8.42 pg, and the values ranged between 7.65 (*C. annuum*) and 9.72 (*C. pubescens*). The DNA content values found in our study are lower than those of Bennet and Smith (1975), who used a different method of evaluation: in both cases, *C. annuum* (cultivated form) displayed the minimum amount of DNA and *C. pubescens* the highest. Referring to the species *C. annuum*, the value of 7.65 pg is practically in accordance with the findings of Arumuganathan and Earle (1991), but higher than the value obtained by Galbraith *et al.* (1983). This disaccordance could be explained by the fact that the latter Authors used a different staining fluorochrome, that is mithramycin, which binds selectively to guanine-cytosine rich regions of DNA. Moreover they used RBCS as internal standard: the genome size of RBCS is considerably different from that of *Capsicum* and this could amplify the risk of DNA content misestimation due to nonlinearity of the flow cytometer amplification system as well as to the zero level error (Bagwell *et al.*, 1989).

The dendrogram constructed with the data of DNA content is reported in Fig. 1. It is possible to observe two main groups of species, one including *C.*

cardenasii, *C. praetermissum* and *C. pubescens* and the other the remaining species. The latter group can be furtherly subdivided, having the two forms of *C. baccatum* and *C. eximium* a DNA content slightly higher than the other species.

In general, the white-flowered species showed a lower DNA content, with the exception of *C. praetermissum*, which displayed a value as high as 9.23 pg. On the other hand, *C. tovarii* and, to a lesser extent, *C. eximium*, although purple flowered-species, proved to have a DNA content very similar to that of white-flowered species. *C. annuum*, *C. chinense* and *C. frutescens*, which are known to be closely related species (IBPGR, 1983), displayed also very similar DNA contents. A similar consideration can be done with reference to *C. pubescens* and *C. cardenasii*.

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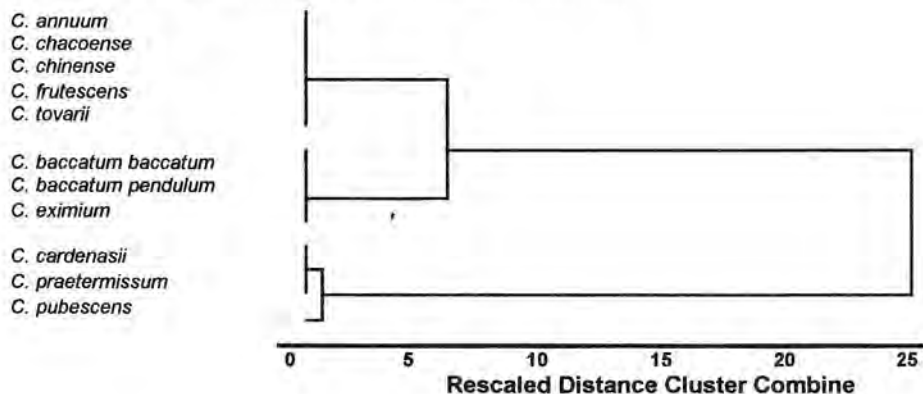
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Table 1 – Mean DNA content (\pm Standard error) in the species utilized in the study.

SPECIES	ACCESSION	DONOR*	DNA CONTENT (pg)
C. annuum	Cuneo	1	7.65 (\pm 0.14)
	Doux Long des Landes	2	
C. baccatum baccatum (wild forms)	10-14193	5	8.43 (\pm 0.14)
	C. baccatum pendulum (cultivated forms)	Campride aidida 15429	
C. cardenasii	40-15507	5	8.97 (\pm 0.20)
	Unnamed	6	
C. chacoense	6-15981/2	5	7.66 (\pm 0.08)
	9-15004	5	
C. chinense	Pimenta de Chien	3	8.04 (\pm 0.12)
	C 334	4	
C. eximium	Hawkes 3860	4	8.70 (\pm 0.08)
	Unnamed	6	
C. frutescens	28-201	5	7.94 (\pm 0.07)
	Malagueta	3	
	Tabasco	2	
C. pratermissum	4	6	9.23 (\pm 0.13)
	P 469/85	7	
C. pubescens	3	6	9.72 (\pm 0.14)
	42	6	
C. tovari	AC 2017	8	7.83 (\pm 0.10)
LSD (5% level)			0.258

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 5= Department of Horticulture, Tuscia University, Budapest Hungary
 6= Vegetable Crops Research Institute, Budapest Hungary
 7= Institute for plant Genetics, gatersleben, Germany
 8= CPRO-DLO, Wageningen, the Netherlands

Fig. 1 - The dendrogram constructed with the DNA content data



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HETEROSIS FOR CHIASMA FREQUENCY AND QUANTITATIVE TRAITS IN PEPPER F1 INTERVARIETAL HYBRIDS

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The genetic variability of species is to a large extent controlled by the recombination of genes or gene complexes between homologous chromosomes. The chiasma is a visible expression of genetical crossing-over and, as such as a potential marker for the degree of recombination between linked genes. The frequency and distribution of crossovers are measured directly by observing chiasmata at the diplotene or diakinesis stages of meiotic prophase. Although for a given species the number of chiasmata per nucleus deviates little from the average (Shaw et al. 1976), chiasma frequency differences between genotypes (Srivastava and Malik 1974, Murray 1976, Mirkova and Molhova 1983) and between male and female reproductive cells (Traut 1977) have been observed. Some individual differences can also be caused by mutations (Tease and Jones 1976), amount of heterochromatin (Rhoades 1978) or environmental factors (Couzin and Fox 1974). Significant genetic association of chiasma frequency in relation to heterosis (Srivastava 1979) has been demonstrated.

The present study was undertaken in six intervarietal F1 hybrids of *Capsicum annuum* to determine whether correlation exist between chiasma frequency in late diakinesis and quantitative traits. An attempt has been made to study correlation of chiasma frequency to a number of quantitative characters, such as plant height, pepper seed yield, fruit yield, fruit length, and thickness of pericarp.

The F1 hybrids (144 x Sivria, 144 x Ziaten medal, 144 x Pazardzishka ... kapia, Sivria x 144, Ziaten medal x 144 and Pazardzishka kapia x 144) showed high mean chiasma frequency per cell and per bivalent relative to the parental forms. They exhibited increase of chiasma frequency 18% in comparison with parental form, having the highest chiasma frequency. The data in the distribution of bivalents with different number of chiasmata for the F1 hybrids and parental forms revealed that the hybrids were additionally characterised to possess bivalents with three chiasmata.; thus feature was never observed in the parental forms. Bivalents with two chiasmata were, however a common feature in both

hybrid and parental populations, but in parental forms bivalents with one chiasma were also observed.

The increase in chiasma frequency and the regular and superior chromosomal behaviour of these hybrids were found to be positively associated with quantitative measures on seed and fruit yield, fruit length and thickness of pericarp.

There was no correlation between chiasma frequency and plant height. The negative correlation between height and chiasma frequency must be considered relative to the fact that height is not a yield-contributing trait (Quisenberry 1977).

There have been some reports from higher plants indicating that an ~ increase in chiasma frequency improves meiotic regularity (Hazarika and Rees 1967, Growley and Rees 1968, Hussain 1976). It is not clear whether the meiotic improvement was due to an increase in chiasma frequency or due to position distribution of chiasmata (interstitial and terminal). The amount of genetic recombination depends not only on chiasma frequency but also on chiasma position (Srivastava 1980); thus counting of chiasma position is a technique which could yield more information than studies of chiasma frequency alone.

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A FIELD STUDY OF ENVIRONMENTAL INTERACTION ON PUNGENCY

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Introduction

Pungency is one of the most characteristic attributes of the genus *Capsicum*. A consistent pungency level is important for processors and consumers. *Capsicum* pungency is the result of a genotype by environment interaction. Little is known to what extent the environment affects the level of pungency in *Capsicum*. It has been reported that high temperatures at time of maturity increase pungency (Cotter, 1980), and that irrigation practices can affect the level of pungency in a cultivar (Quagliotti, 1971). Quagliotti observed that pungency was influenced when plants were stressed for moisture. We have observed that pungency varies between cultivars, geographical areas and even between plots in the same field. The extent to which the environment affects pungency in a single field has not been explored.

Materials and Methods

A double haploid line generated from 'NuMex R Naky' provided a mechanism to quantify the environmental effect on pungency. These plants, being genetically identical, allowed for any variation measured in pungency to be attributed to the environment. In 1994, plots were planted at the Leyendecker Plant Science Research Center, located 10 miles south of Las Cruces. Throughout a 20 m x 183 m field, 5 plots of 1 m x 9 m were planted. Standard commercial and cultural practices were used during the course of the experiment. Mature fruits from individual plants were harvested within each plot. Pungency was quantified using High-Performance Liquid Chromatography (Collins *et al.*, 1995). The pungency measurements taken in parts per million were converted to Scoville heat units (SHU).

Results and Discussion

This study substantiates that the environment has a sizeable effect on pungency. Statistically significant differences ($P \sim .05$) in Scoville heat units were found among individual plants from the same plot (Table 1). The Scoville heat units of individual plants ranged from 2896 SHU to 9614 SHU. The overall field mean for individual plants was 5391 SHU. When individual plant SHU are compared to the mean, they ranged from 78% higher to 46% below the mean. The smallest difference between individual plants was 1.2%.

Table 1. Variation of pungency in Scoville heat Units (SHU) among individual plants.

PLANT - #	SHU ^z
PLANT - M	9614a
PLANT - B	8193b
PLANT - O	6894bc
PLANT - L	6203cd
PLANT - N	5936cde
PLANT - K	5543cdef
PLANT - H	5454cdef
PLANT - C	5237defg
PLANT - F	5170defg
PLANT - J	4608efgh
PLANT - A	4195fghi
PLANT - D	3832ghi
PLANT - E	3608hi
PLANT - I	3517hi
PLANT - G	2896i

^z Means are for individual plants with 2 replications. Mean separation within columns by Duncan test. Means followed by the same letter are not significantly different at $P \leq 0.05$.

When plot to plot variation for SHU was examined, statistically significant differences ($P \leq 0.05$) were found among plots. The Scoville heat units ranged from 7482 SHU to 3956 SHU (Table2). The range of Scoville heat units was 6% for the smallest difference and 89% for the largest difference from the mean.

Table 2. Variation of pungency in Scoville heat units (SHU) among plots.

Plot #	SHU ^z
5	7582a
1	5836ab
4	5452bc
2	4204bc
3	3956c

^z Means are calculated from plants within the plot. Mean separation within columns by Duncan test. Means followed by the same letter are not significantly different at $P \leq 0.05$.

Conclusion

The environmental effect on pungency is significant. The large environmental effect on pungency will impact breeding programs. It may be warranted that cultivars with a low genotype by environment interaction for pungency be selected, thus reducing the large variability observed for pungency.

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STABILITY ANALYSIS IN HOT PEPPER

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Introduction

Average yield alone may not be sufficient to describe the performance of a certain genotype, since it does not indicate the relative performance with other genotypes over different environments. A significant genotype x environment (GxE) interaction reduces the usefulness of genotype means for identifying superior cultivars (Magari and Kang, 1993). An ideal goal for breeders is to get environmentally buffered (stable) and high-yielding cultivar(s). Different stability estimates were proposed to measure the relative performance of genotypes under a wide range of environments. The different concepts and statistical implications were reviewed by Lin et. al. (1986) and Pritts and Luby (1990). This report summarizes the response of (INTHOPE 1) entries across seven testing environments by using three stability estimates. Some understanding was gained as to which cultivars did and did not contribute significantly to the GxE.

Materials and Methods

Data from the first set of the International Hot Pepper Trial Network (INTHOPE 1), which was evaluated at California, USA; Kamphaengsaen, Thailand; Los Banos, Philippines; Maha Illuppallama; Sri Lanka; Shanhua, Taiwan and Suweon, Korea were used for this analysis. Experimental designs were randomized complete blocks with three replications at each site. Data of total pod yield, g/plant were subjected to a combined analysis of variance to examine GxE interaction effects. After testing the significance level for GxE, the following stability parameters were estimated: a) stability variances, O^2 and (Shukla, 1972), b) coefficient of variation (CV) for determination of groupings I-N (Francis and Kannenberg, 1978), and c) regression coefficient b_1 (Finlay and Wilkinson, 1963). The stability variance statistics assigned to each genotype were calculated by the Proc IML SAS program (Kang, 1989). Regression coefficients and coefficients of variation were calculated using Basica programs for calculating adaptability and stability (Yap, 1989). Experiments conducted at Shanhua, Taiwan, during the summer (1990) and fall (1990/91) seasons were considered as two different environments. Similar analyses were conducted for the yield component, fruit number, over five sites but are not presented in this report.

Results

Combined analysis of variance for total pod yield showed highly significant F ratios for mean squares of environments, cultivars, and GxE interaction (data not shown). The interaction effect indicated that cultivars differed in their pattern of response relative to each other in various environments.

A significant stability variance (O^2) indicated the genotype was unstable across environments, and a significant adjusted stability variance (s^2) indicated a genotype was unstable even after removal of the environmental covariate (environmental index) effect from the GxE. Except cultivars 'MC 4', 'Atarodo' and 'JCJtilaba', all were rated as stable genotypes using the stability variance (O^2) estimate (table I). After removing the linear effect of the environment, 'Jatilaba' became stable.

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The CV analysis identified cultivars 'Jatilaba', 'Paris Minyak', 'Tit Super', 'Lv. 1092', 'Cipanas' and 'Long Fruit' as most stable (table 1). These had higher than average yield and smaller than average coefficient of variation (Group I). Cultivars 'Jatilaba', 'Paris Minyak', 'Tit Super', 'Lv. 1092', 'Cipanas' and 'Long Fruit' were identified to have above-average stability by the regression coefficient method (bl ranged - from 0.73 to 0.90 and mean yields were always above average). Similarly, cultivar 'Atarodo' had above-average stability, but produced above average yield mostly in poor environments. Cultivars 'Jawahar 218', 'Extra Long', 'Punjab Lal' and 'Keriting' had average stability with bl = 1.02, .03, 1.02, and 1.07, respectively, but their total yields were below average. Cultivars 'MC 4' and 'Huay Sithon' were unstable (bl = 1.43), but performed well in favorable environments, producing 17 and 35 g/plant at low-yielding environments and 651 and 417 g/plant at high-yielding environments, respectively. Taiwan-summer was considered a low-yielding environment (135 g/plant site mean), whereas California, USA, as a high-yielding environment (590 g/plant site mean).

Discussion

The use of a covariate in stability variance analysis provides information for the breeder to identify the underlying cause of the axE (Magari and Kang, 1993). This method rated more cultivars as stable. It rates based on the relative contribution of a cultivar to the axE variance, such that lower-yield cultivars are grouped as stable as far as they had low contribution to the axE. Well-adapted cultivars would be ones with low-stability variance (O_j^2) and high mean yield (Kang et al., 1991). With the CV method, however, for a genotype to be rated as stable, it has to yield above-average and have a smaller than average coefficient of variability. In the regression coefficient method, environments are grouped based on site mean. Cultivars adapted to specific environments were identified by their high or low bl values. Cultivars which had above-average phenotypic stability and above average yield in all environments are the most desired as they have general adaptability for all environments. Out of these three stability estimates, the CV method was the easiest method to apply. As discussed by Pritts and Luby (1990) and Negave and Bouwkamp (1993), however, a significant negative association between the CV and the mean would add bias in judging the cultivars.

In conclusion, cultivars 'Jatilaba' (except in stability variance, O_j^2), 'Paris Minyak', 'Tit Super', 'Lv. 1092', 'Cipanas', and 'Long Fruit' were stable by three of the stability estimates computed. They, therefore, might be important gemiplasm sources in a breeding program. These cultivars, except 'Long Fruit', all originated in Indonesia. It would be interesting to study the selection environments where these cultivars had been developed to define test sites for selection of widely adapted genotypes. A more extensive analysis will be conducted as additional data sets are received.

Acknowledgment

We would like to acknowledge and thank the following institutes for their collaboration in sharing complete data sets for these analyses: A VRDC headquarters, Tillwan; A VRDC Asian Regional Center, Thailand; A VRDC-Philippines Outreach Program, Bureau of Plant Industry, Los Bafios National Crop Research and Development Center, Philippines; Field Crops Research and Development Institute, Sri Lanka; Rural Development Administration-Horticultural Experiment Station, Korea and University of California-Davis, Department of Plant Pathology, USA. We also thank Prof. M.S. Kang (Louisiana State University, Department of Agronomy) for providing the Proc IML SAS program for calculating stability variances.

Table 1. Stability for total pod yield of 15 hot pepper cultivars evaluated at seven environments^a

Cultivar ^b	Origin	Yield g/plant	Stability estimates			
			Stability variances		Coefficient Of Variation	Regression Coefficient
			δ_i^2	s_i^2	Group ^d	b_i^e
MC4	MAL	270.83	55180.43	63263.70**	IV	1.43
Jawahar 218	IND	286.54	5835.51	7139.45	III	1.02
Ludhiana Long	IND	281.06	3177.50	1386.96	IV	1.13
Extra Long	IND	301.57	9137.57	6866.74	IV	1.03
Punjab Lal	IND	271.21	10551.93	12843.94	IV	1.02
Huaruar	THA	238.41	8306.48	5051.32	III	1.16
Huay Sithon	THA	201.20	5518.77	1684.79	IV	1.43
Atardo	NIG	285.98	117991.64	141804.23**	IV	0.86
Keriting	INI	266.89	6344.78	6483.16	III	1.07
Jatilaba	INI	441.03	20313.37	627.43	I	0.90
Paris Minyak	INI	379.11	5841.64	5757.03	I	0.72
Tot Super	INI	343.50	6656.00	7735.57	I	0.79
Lv. 1092	INI	317.45	7062.44	5041.87	I	0.75
Cipanas	INI	316.93	1341.73	1625.91	I	0.83
Long Fruit	THA	377.40	5541.61	4150.89	I	0.87
Mean		305.27				
Pooled Error				6154.31		

*, ** F-Test statistically significant at 5 and 1 % level, respectively.

A1) California, USA (March-Oct. 1993); 2) Suweon, Korea (feb-Aug 1991);

3) Los Banos, Phillippeans (Oct 1991-May 1992); 4) Maha Illuppallama, Sri Lanka (March-Aug1994); 5) Shanhua, Taiwan- Fall (Sept 1990 – Feb 1991); 6) Shanhua, Taiwan – summer (April-Oct 1990); 7) Kamphaengsaen, Thailand (May-Oct 1991).

BC annum except 'Atadoro' C, chinense.

CTHA = Thailand; INI = Indonesia; IND=India; Tai = Taiwan; NIG=Niger; MAL = Malaysia
Evalues after long transformation

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PLOIDY ASSESSMENT USING STOMATAL CHLOROPLAST NUMBER IN CAPSICUM
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Polyploids often serve as unique germplasm resources for specific traits of breeding significance. So there has been increasing interest of breeders for induction and utilization of polyploids in several crops. In chilli, an important cash and : condiment crop of India, work in this direction was initiated in our laboratory from 1989 onwards. Using the well-known alkaloid colchicine a good number of polyploids of Caosicum were developed in different species and as well as in divergent diploid cultivars of annum. In all these cases confirmation of ploidy is by counting of the chromosome number alone. The need for the development of a biological marker for rapid screening of tetraploids, without making cytological studies is realized. Since chilli is a transplanted crop and if there is a reliable marker to detect polyYPlddy in the nursery stage itself it will be of practical use.

Earlier literature in other crops revealed that there might have been a correlation between stomatal frequency and size together with pollen sterility and meiotic disturbances in detecting the polyploids. *t* However, in Capsicum work in this direction was completely absent. Hence, the utility of counting chloroplast number in stomatal guard cells and the development of a technique for rapid screening of treated populations is taken up.

The tetraploid stocks used in this experiment include seven divergent diploid strains of annuum (x180, x206, Santaka, Jawahar, Tc1, Se11 and Lec21) and one of chinense. Leaf samples of C2 generation tetraploids were collected from 6 week, old seedlings and the peels of lower epidermis were taken. These were stained in 2% iodine - potassium iodide solution and average number of chloroplasts per stomata, stomatal length and width of stomatal pore for diploids and tetraploids was recorded.

The mean number of chloroplasts in the guard cells of a different strains at both ploidy levels were counted and the results are presented in Table 1. Irrespective of the strain, the material can be classified into two groups 1) diploids and 2) tetraploids. In diploid group the mean number of chloroplasts per stomata was found to be 18.19 in contrast to 27.60 in tetraploid group. From the data it is apparent that the number of stomatal chloroplasts increased with the increase in ploidy level (Table 1). Considering diploid number as base, 49.3% increase is observed in tetraploids. The size of the stomata was also increased in tetraploids and the number per unit area was decreased (Table 1).

An increase in stomatal size along with a decrease in number per unit area was observed in Phlox (Rao et al. 1982), Trigonella (Arya et al. 1988) and Plantago (Asha Bhan et al. 1990). Butterfass (1958) and Rothacker et al. (1966) observed no significant change in the number of plastids in the stomata in diploids and tetraploids of Solanum tuberosum. Eigsti and Dustin (1955) pointed out that stomatal size and frequency

together with pollen sterility and meiotic disturbances have been taken as criteria of detecting polyploids.

The divergence that has been observed in the number of stomata between chinensis and annuum can be attributed to its different genomic constitution. However, confirmation of this may involve production of large number of polyploids of diverse genomic combinations and their testing. In India annuum is the most widely cultivated and economically useful species. Since, sufficient number of tetraploids from this species are tested in the study, it can be pointed out that in Capsicum too, the diploids and tetraploids can be identified on the basis of stomatal chloroplast number and size. The techniques seem to be accurate and it helps in screening large populations either in seedling stage itself or in early stages of plant growth.

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Table 1. Stomatal parameters in the diploids and tetraploids of 8 varieties of *Capsicum*

Character	C.annuum var. 180		C.annuum var. 206		C.annuum var. Santaka		C.annuum var. Jawahar		C.annuum var. Tc1		C.annuum var. Sell		C.annuum var. Lec21		C. chinense	
	Dip	Tet	Dip	Tet	Dip	Tet	Dip	Tet	Dip	Tet	Dip	Tet	Dip	Tet	Dip	Tet
No. of stomata/unit area	14.16	9.12	14.60	9.92	17.88	8.32	16.04	10.00	19.48	11.96	20.80	10.56	15.08	11.48	17.36	9.08
No. of epidermal cells/unit area	45.96	34.08	46.96	31.64	51.00	32.80	49.08	33.52	49.92	39.20	55.24	39.08	43.28	34.48	48.76	33.44
Length of stomata μ m	34.48	46.64	31.51	42.74	31.04	43.06	28.05	40.72	29.72	42.28	30.12	43.21	32.45	41.34	29.33	39.16
Breadth of stomata μ m	25.58	32.29	23.24	30.28	22.78	30.54	25.90	29.02	21.84	29.64	23.71	29.64	23.09	30.89	20.90	27.46
Width of stomatal pore μ m	6.24	10.30	6.40	7.80	5.62	7.29	3.90	7.80	4.68	5.62	4.37	6.55	6.55	7.80	3.90	5.30
No. of chloroplasts/stomata	19.42	26.38	19.06	26.13	18.24	25.08	18.20	24.00	18.00	28.50	18.10	31.45	17.50	29.00	19.40	30.28

NEW SWEET PEPPER CULTIVARS FOR CUBAN OFF SEASON PRODUCTION

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In vitro androgenesis has shown its usefulness in pepper breeding (Dumas de w Vaulx *et al.*, 1981). Haploidization technique gives the possibility, after hybridization, to regenerate hybrid gametic segregation. The doubled-haploid plants obtained give homozygotic genotypes when selfing progenies are z, definitively stabilized (Demarly, 1989). This operation made in 1-2 years would 7 require at least 6-8 self-pollinations.

In Cuba, climatic adaptation and disease resistance are main goals of pepper breeding program, since production in the isalnd is seasonal due to pepper limitant climatic conditions and pests during' the summer, mainly *Polyphagotarsonemus latus* Banks, which symptoms are often mistaken with those from virus. So it is necessary to develop new sweet pepper cultivars for off-season cuban production.

A population from the combination 'Lamuyo' x 'Medalla de Oro' has been submitted to anther culture technique (Gomez and Chambonnet, 1992) and compared during two years in a field trial to a coomercial variety ('SC-81 ') in order to establish its agronomic and disease performance, as well as its high temperature adaptation. Results are reported in this paper.

Mean temperature was 28°C during the growing season and 420 mm rainfall.

Nursery was sown in april, whereas the transplanting was done in the third ... week of May. A randomized complete block design with four replicates was used. Net plot size was 4 x 2 m; space between rows was 0.8 m and 0.25 m between plants in the row. Pest incidence in the field was measured by a scale (0 = healthy plant to 5 = stunting).

With yield there was significant variability for 'cultivar' but not for 'year x cultivar' interaction; similar occured with yield components number of fruits per plant and mean fruit weight.

Table 1 shows that the three doubled-haploid lines produced significantly higher yield per plant than the commercial control variety 'SC-81', which is commonly grown in Cuba during the summer season and used as fresh

condiment In meals. It has a good adaptation to high temperatures and humidity conditions but mean fruit weight is very low.

The increasing of mean fruit weight by breeding increased yield per plant in this case (Fig. 1). A possibility appears with the doubled-haploid lines which can be grown even in cuban extreme summer conditions. Mean fruit weight was higher in these lines than in 'SC-81' control variety as well as fruit quality; so they can be used for local consumption and not only as fresh condiment. These lines showed tolerance to *P. latus* while 'SC-81' was susceptible under field conditions.

Table 1 - Yield and yield components of pepper 'off season' cultivars.

Cultivar	Number of fruits/plants	Mean fruit weight (g)	Yield/plant (g) .
'HD-60'	11.13c	36.04 a	400 a
'HD-16'	12.63 b	30.46 b	381 b
'HD-12'	10.50 c	28.21 b	300 c
'SC-81' (control)	18.38 a	14.61 c	247 d
ES	2.23**	4.56*	5***

a b c d: Means without letter in common differ significantly at p = 0.05 (Duncan's Multiple Range Test)

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Fig. 1 - Linear regression between number of fruits/plant and yield/plant

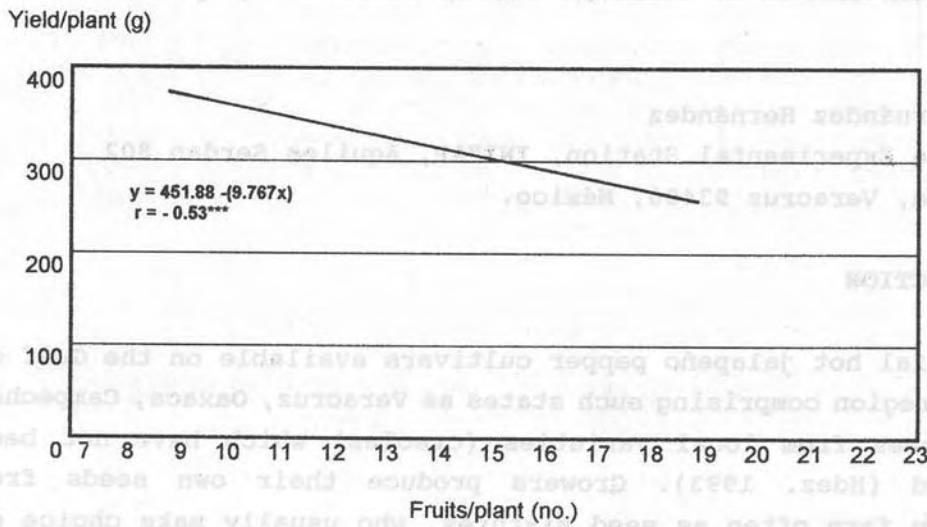
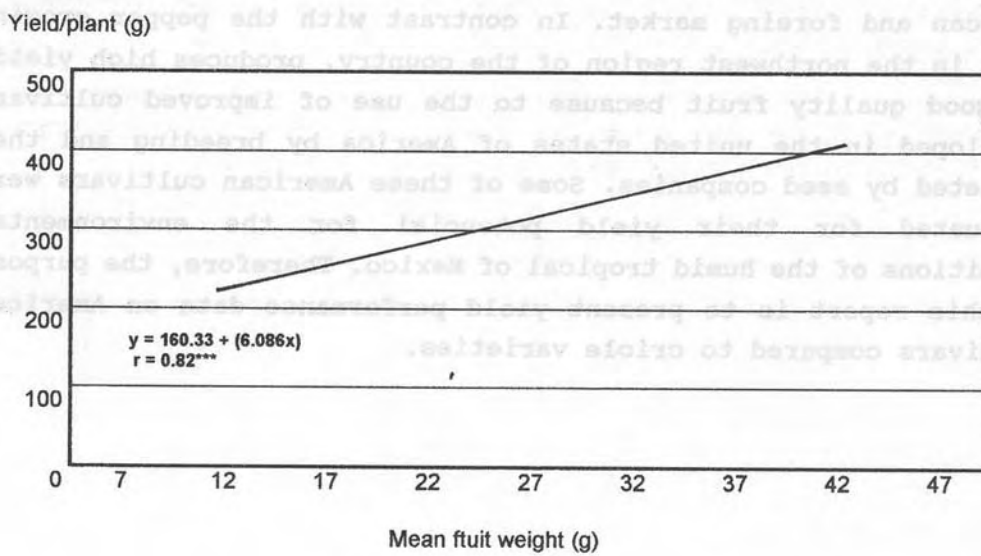


Fig. 2 - Linear regression between mean fruit weight and yield/plant



YIELD PERFORMANCE OF JALAPeO PEPPER CULTIVARS (*Capsicum. annum L.*)

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INTRODUCTION

Commercial hot jalapeno pepper cultivars available on the Gulf of Mexico region comprising such states as Veracruz, Oaxaca, Campeche, " etc. Comes from local varieties (creoles) which have not been improved (Hdez. 1993). Growers produce their own seeds from their own farm often as seed mixtures, who usually make choice of fruits for seeds without any breeding process. The use of this low genetic quality seed is one of the reasons for low yield and average yield range from 6 to 10 ton/ha. The most common creole varieties used are 'espinalteco, candelaria, tipico and tres lomos'. These cultivars shows high variability in terms of fruit shape, size, color and maturity, that are insuitable traits for the Mexican and foreing market. In contrast with the pepper growing area in the northwest region of the country, produces high yields of good quality fruit because to the use of improved cultivars developed in the united states of America by breeding and they marketed by seed companies. Some of these American cultivars were evaluated for their yield potencial for the environmental conditions of the humid tropical of Mexico. Therefore, the purpose of this report is to present yield performance data on American cultivars compared to criole varieties.

MATERIALS AND METHODS

Jalapeno entries evaluated in this trial included the cultivars Mitla (hybrid), Tam-Veracruz, Jalapeno M, Jumbo, Early-J, Jalapa (hybrid), from Peto seed Company and four creole varieties, Espinalteco, Candelaria, Tres lomos and Abel Salinas from local growers as local checks. The evaluation was conducted during the autumn-winter 1992-93 growing season at Espinal, Veracruz, Mexico. Seeds were sown in nursery trays and at 6 weeks seedlings were transplanted in the field at spacing of 60 and 30 cm between rows and plants respectively. The experimental material was planted in a randomized block design with four replications. Routine cultural practices (land preparation, cultivation, fertilization, pest control etc.) were carried out and plants were grown under open field conditions by rain. Plots were harvested for yield estimates in January and February 1993 at 95 to 131 days after transplanting. A cylindrical plastic box with transparent plastic on top was placed on plants for counting whitefly adults. The top side kept facing the sun so that the whiteflies being phototropic, migrated to it. Incidence of virus diseases were evaluated by estimating the percentage of plants showing characteristic symptoms in each plot (0-100%). Analyses of variance and Tukey's studentized range (HSD) test at the 5 percent level of significance were applied for statistical evaluation of yield, whitefly and virus diseases data.

RESULTS AND DISCUSSIONS

Comparative performance data on yield (ton/ha), whitefly /plant and virus diseases/plot are summarized in table 1. In general, all cultivars showed low fruit cumulative yield due to virus diseases and moisture stress mainly. Therefore with adequate irrigation and plant protection these cultivars may be better than the revealed

results. Tam-Veracruz and Mitla significantly out performed the rest of the cultivars including local checks at 6.28 and 5.32 ton/ha, respectively. As the result of virus diseases, the cultivars had low yield, small fruit size and low height plant. However fruits of Tam-Veracruz followed developing after virus infection. The most wide-spread virus disease was tigre disease (geminivirus complex) whose vector was the whitefly *Bemisia tabaci* Genn. La incidence of whitefly virus vector was strong and virus diseases symptoms were observed in all cultivars and none of them 5 showed resistance in their reaction. At the beginning of the harvest, plants were infected one-hundred percent and the ~4 difference among the cultivars was not statistically significant. other important data on vegetative and reproductive characters were not recorded due to virus diseases affected their characteristics. The Tam-Veracruz cultivar in the most seemed to the native jalapeno from Veracruz about their botanical and horticultural traits.

CONCLUSIONS

This preliminary study reflects the high yielding ability the Tam- Veracruz and Mitla with good pod characteristics. Overall performance data and other important observations indicate that these cultivars has tremendous potentialities. These cultivars should therefore produce much more fruit yield in optimal soil, protection plant and environmental conditions.

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TABLE 1. JALAPE&O PEPPER COMPARATIVE PERFORMANCE TEST, WINTER 1993, ESPINAL, VERACRUZ.

CULTIVAR	Total Fruit Yield Ton/HA	N* Of Whitefly Adult Per Plant	Virus Disease/Plant Score (0-100%)
TAM-Veracruz	6.28 a*	20.10	100
Mitla	5.32 a	23.62	100
Jalepano-M	1.97 b	18.40	100
Jumbo	1.97 b	16.92	100
Early-J	1.86 b	21.30	100
Abel Salinas	0.90 b	19.40	100
Jalapa	0.76 b	22.40	100
Espinalteco	0.72 b	25.80	100
Tres Lomos	0.58 b	17.10	100
Candelaria	0.27 b	15.40	100
ANOVA	**	NS	NS

** Significant al 1% level NS. Not significant

* Means in a column followed by the same letter are not significantly different at the 5% level of significance by Tukey's studentized range test.

CYTOLOGICAL INVESTIGATION ON PEPPER PLANTS INFECTED BY TOBACCO MOSAIC VIRUS (TMV) IN DIFFERENT STAGES OF DEVELOPMENT

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It is well known from the literature that diseases caused by viruses and other pathogens may induce deviation from normal cytological processes found in plants. Several investigators have shown that the different pathogens interfere with cell division and cause somatic polyploidy. Different abnormalities such like decrease of chiasma frequency, disturbances of cytokinesis, lagging chromosomes at Anaphase I and II, decrease of pollen fertility and other ones were reported by some authors in different virus-infected plants (Swaminathan et al. 1959, Kazimierski and Kazimierska 1969, Mirkova and Molhova 1983). The type of the disturbances observed in different phases of meiosis in bacterial infected tomatoes was similar to that described in virus diseased plants (Sotirova and Beleva 1978). The significant differences in mentioned meiotic aberrations between different plants were probably connected with different degree of infection in particular plants (Mirkova and Molhova 1983).

The present study was undertaken in some pepper plants from different species (*Capsicum annuum* - *C.annuum* var. *nigrum*, varieties: Sivria and Ziaten medal- and *Capsicum pendulum*) which were infected with Tobacco mosaic virus (TMV) in different stages of development (cotyledon stage and stage "second true life").

On the basis of studying microsporidogenesis in different pepper "-species and varieties the significant increase was estimated in the frequency of abnormalities at different meiotic phases (Metaphase I and II, Anaphase I and II, Telophase I and II and Tetrads) in plants infected by TMV, inoculated in different stages of their development comparing with respective healthy plants. In all virus-infected plants and healthy plants there were found some abnormalities of meiosis like univalents in Metaphase I, lagging chromosomes and bridges in Anaphase I and II, Telophase I and II and micronuclei in Tetrads, However, in all virus-infected plants we observed significant decrease of chiasma frequency

both per bivalent and per cell, pollen mother cells with different chromosome number in Anaphase I, non-synchronised division in Anaphase II, micronuclei in Telophases, formation of dyads, triads and polyads, pycnosis of chromosomes in all investigated phases.

The investigated virus-infected plants characterised with decreased pollen fertility and productivity (Table 1).

Table 1. Pollen fertility, seed and fruit productivity of pepper plants infected with TMV in stage

SPECIES	C. pendulum		C. annuum var. nigrum	
	healthy	infected	healthy	infected
Traits				
Pollen fertility	98,5	75, 45	92, 35	62,35
Number of Normal fruits	15-20	12-15	12-15	2-3
Number of Deformed fruits	-	-	-	10-12
Number of Normal Seeds per fruit	30-35	25-30	45-50	5-6
Number of deformed seeds per fruit	-	-	-	15-20

Plants from different varieties of *C.annuum* (Sivria and Ziaten medal) infected with TMV in cotyledon stage formed flowers that made possible cytological analysis but perished before fruit formation.

As a result of the present investigation the following conclusion can be made: The stage “second true life” is the most suitable early phase of plant development for infection with viruses and for analyses of cytological disturbances caused by these pathogens.

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EMERGENCE OF VIGOROUS-GROWING LATERAL SHOOTS FROM A CMV - INFECTED PLANT OF CAPSICUM FRUTESCENS L. FOUND ON A SUBTROPICAL ISLAND IN JAPAN

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ABSTRACT

We found that vigorous-growing lateral shoots emerged from a pepper plant (*C. frutescens L.*) infected with CMV growing in Irabu, a subtropical island located in the southwest part of Japan. This species can grow through the winter season there, and the plant mentioned above was about 5 years old. In these vigorous-growing lateral shoots, CMV could not be detected when checked by the ELISA, ;, method. The vigorous-growing cutting had resistance to the virus.

INTRODUCTION

CMV causes very severe damage and important economic losses in pepper. Once pepper plants are infected with CMV, their leaves often show mosaic or necrotic spots, their leaf width sometimes becomes narrow, and the plants become dwarfed. Furthermore, peppers (infected with CMV generally set few fruits, and their fruits are small and warped.

Vigorous-growing lateral shoots sometimes emerge, under greenhouse conditions from certain pepper cultivars infected with CMV several years after infection with the virus (Fig. 1) (Yazawa, 1989, 1991). No CMV V symptoms were exhibited nor was CMV detected in the Fig.1 The vigorous growing lateral shoots lateral shoots. Cuttings were not infected with CMV after emerged from the CMV -infected plant of inoculation by grafting and an aphid transmission method. *C. annum L.* 'M-3' growing in greenhouse.

We found that the vigorous-growing lateral shoots A vigorous growing lateral shoot emerged from a CMV -infected pepper plant not only B: CMV-infected lateral shoot under greenhouse conditions, but also in the field under weedy conditions, where peppers had reseeded themselves.

In this study, we investigated CMV infection and resistance in vigorous-growing lateral shoots that emerged from a CMV infected pepper plant (*C. frutescens L.*) growing under wild conditions.

MATERIALS AND METHODS

The presence of CMV in the infected pepper plant was confirmed with enzyme-linked immunosorbent assay (ELISA) method. To correlate the ELISA data to CMV infection, CMV concentration index was set up. The index was calculated by the following expression: index 0 = 0.000-0.099 (absorbance value at 405nm), 20 = 0.100-0.199, 50 = 0.200-0.499, 100 = 0.500-, CMV concentration index = L(number of samples at the given index value X the given value) number of samples. CMV was not detected in

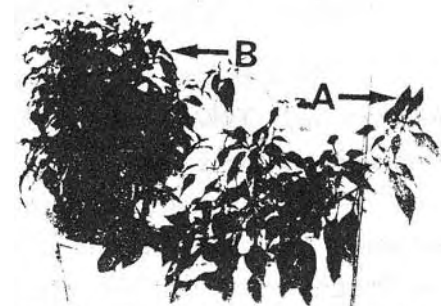


Fig.1 The vigorous-growing lateral shoots emerged from the CMV-infected plant of *C. annum L.* 'Af-3' growing in greenhouse
A: vigorous-growing lateral shoot
B: CMV-infected lateral shoot

index 0 plants. Most plants whose index values were greater than 50 displayed serious CMV symptoms.

A test for CMV -resistance in a cutting of the vigorous-growing lateral shoot was carried out. A vigorous-growing cutting was from the plant on November 3, 1992. The cutting was inoculated with CMV with aphids between September 24, 1993 and March 4, 1994. Presence of CMV in the vigorous-growing cutting was determined by the ELISA method on March 4, 1994. In this experiment, *C. annuum* L. cv. 'California Wonder' (a CMV-sensitive cultivar) seedlings were used as control plants. Vector aphids were maintained and propagated on CMV -infected tobacco plants.

Many plants of both *C. annuum* L. and *C. frutescens* L. growing on Miyako and Irabu island in southwest Japan exhibited ~ 1 symptom. And then, a test for CMV -resistance in seedlings of them was carried out in the field condition. Seedlings of *C. frutescens* L. introduced from the southwest part of Japan and 'California Wonder' were planted in the field on May 12, 1994. Concentrations of CMV were checked by ELISA method on October 26, 1994.

RESULTS

We found vigorous-growing lateral shoots emerged from a CMV -infected pepper plant (*C. frutescens* L.) growing in Irabu, a subtropical island located the southwest part of Japan (Fig2). Peppers often grow through the winter season there, and the pepper plant mentioned above was about 5 years old. Leaves of CMV -infected lateral shoots were small in size, twisted and displayed light green patches in a mosaic pattern. Furthermore, internodes were short, and few flowers and fruits developed on the infected shoots. In contrast, the vigorous-growing lateral shoots exhibited no CMV symptoms. Their leaves were large and uniformly deep green. Their internodes were long, and many flowers and fruits were set. After cutting off a vigorous-growing shoot from the infected pepper plant, the cutting still exhibited no CMV symptoms.

CMV was not detected in the vigorous-growing lateral shoots (Fig2-A) by the ELISA method in spite of a high concentration of CMV in the infected shoots (Fig2-B, Table 1). Furthermore, CMV was not detected in the vigorous-growing cutting.

The vigorous-growing cutting showed no CMV symptoms after the CMV -inoculation period of five months and CMV was not detected in the cutting by the ELISA method (Table 2). All of 14 'California Wonder' seedlings as control plants were infected with CMV. A test for CMV -resistance in seedlings of the pepper plants found on Irabu and Miyako island was carried out in the field condition CMV concentration index of the *C. annuum* L. seedlings introduced from Miyako island was about 30 (Table 3). CMV was not detected in the *C. frutescens* L. seedlings introduced from Miyako and Irabu island. The CMV concentration index in the seedlings of *C. annuum* L. 'California Wonder' was 59.

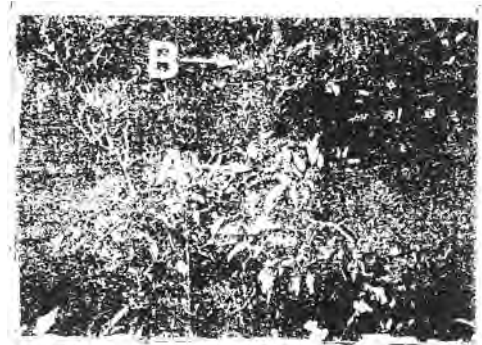


Fig.2 The vigorous-growing lateral shoots emerged from the CMV-infected plant of *C. frutescens* L. growing in Irabu island
A: vigorous-growing lateral shoot
B: CMV-infected lateral shoot

Table 1 CMV concentration in vigorous-growing lateral shoots that emerged from the infected plant of *C. frutescens* L. growing in Irabu island.

Shoot	No. of samples	Date of sampling	CMV conc. index ^Z
Vigorous-growing shoot	2	1992.11	0
Lateral shoot exhibiting CMV-symptoms	2	1992.11	100

Z: CMV conc. index = Σ (Number of samples at the given index value \times The given value) / Number of samples

Index: Absorbance value at 405nm

0:	0.000~0.099
20:	0.100~0.199
50:	0.200~0.499
100:	0.500~

Table 2 CMV concentration in the vigorous-growing cuttings of *C. frutescens* L. after inoculation with aphids.

Plant	Date of inoculation	Date of sampling	CMV conc. index ^Z
Vigorous-growing cutting No.1	1993.9.24~1994.3.4	1994.3.4	0
'California Wonder' seedling	1993.9.24~1994.3.4	1994.3.4	33

Z: Refer to Table 1

Table 3 Concentration of CMV in pepper plants growing in field.

Plant	No. of samples	CMV conc. index ^Z	Percentage of CMV-infected plants
Miyako-1 (<i>C. annuum</i> L.)	15	31	87
Miyako-2 (<i>C. annuum</i> L.)	15	36	60
'Shishito' (<i>C. annuum</i> L.)	15	15	13
'California Wonder' (<i>C. annuum</i> L.)	16	59	94
Miyako-3 (<i>C. frutescens</i> L.)	15	0	0
Irabu-1 (<i>C. frutescens</i> L.)	20	0	0
Irabu-2 (<i>C. frutescens</i> L.)	20	0	0

Z: Refer to Table 1

DISCUSSION .

In the southwest part of Japan, pepper plants grow in various places, because their seeds are dispersed by birds. These plants are not actively cultivated, but their fruits are harvested by the local people. Gradually, they have become nearly wild and can continue to grow for several years. Many pepper plants did not exhibit CMV symptoms. Considering this fact and our experimental results, it appears that many pepper plants growing in the area may obtain some way to overcome CMV.

It took several years for the vigorous-growing lateral shoots to emerge from CMV infected plants of *C. annuum* L. in the greenhouse (Yazawa ~, 1989). This suggested that vigorous-growing lateral

shoots could also emerge from the weedy plant of *C. frutescens* L. infected with CMV on Irabu island, because pepper plants can also live for several years.

In the work presented here, the vigorous-growing cuttings were shown to have resistance to CMV. This result was expected because the vigorous-growing lateral shoots must have been continuously infected with CMV by the infected lateral branches.

Dufour (1989) and Nono-Womdim (1991) reported that some inbred varieties of pepper had resistance to CMV migration. In the resistant pepper varieties, CMV was detected only in inoculated leaves. Although in our study, many leaves of the vigorous-growing cuttings were inoculated with CMV with aphids and tested by ELISA method, CMV could not be detected in the vigorous-growing cuttings. Furthermore the vigorous-growing cuttings showed resistance to CMV when they were exposed to CMV with infected rootstocks. In the grafting treatment, test plants were inoculated with the virus through their vascular bundles. This fact may show that the resistance vigorous-growing cuttings are not due to obstruction to CMV migration.

It was reported that pepper extract had inhibitory activity on plant virus infections (McKeen, 1956). CMV resistance of the vigorous-growing cuttings may be related to this inhibitive effect of pepper extract on some viruses.

Viruses are localized on mosaic-affected leaves. The green areas of mosaic-affected leaves of CMV -inoculated tobacco were virus-free and resistant to reinoculation with CMV in spite of high concentration of the virus in adjacent yellow areas (Loebenstein, 1971). Viral localization by the same mechanism operating in mosaic-affected leaves might also have occurred at the branch level in the pepper plants with the vigorous growing lateral shoots.

Vigorous-growing lateral shoots emerged from a weedy plant of *C. frutescens* L. infected with CMV. CMV could not be detected in the shoots and they had resistance to the virus. These facts may suggest that this phenomenon could be a way to introduce resistance in addition to traditional plant breeding methods.

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VARIETAL DIFFERENCES IN RESISTANCE TO BACTERIAL WILT IN RELATED SPECIES OF *CAPSICUM ANNUUM*

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Bacterial wilt is an important disease affecting sweet pepper (*Capsicum annuum* L.) in Japan. Breeding for disease resistance is the most effective means of controlling bacterial wilt. To discover sources of resistance to bacterial wilt, it is necessary to screen a wide range of *Capsicum* species. The resistance of sweet pepper and Chile pepper varieties to bacterial wilt has been reported previously (Matsunaga *et al.* 1993; Matsunaga and Monma 1994). The present study attempted to identify accessions resistant to bacterial wilt in related species of *C. annuum*.

A total of 64 accessions including 23 accessions of *C. chinense*, 14 accessions of *C. frutescens*, 25 accessions of *C. baccatum* and 2 accessions of *C. pubescens* were examined. As control varieties, 'White Khandari', 'Pant C-1' and 'California Wonder' were used. Ten seedlings per accession were transplanted to a field infested with *Pseudomonas solanaceamm* at NIVOT. Subsequently, an inoculum suspension of *P. solanaceamm* (isolated from a diseased pepper plant in this field) was poured into the soil at the base of each plant after root wounding. For evaluation of resistance, each plant was scored for bacterial wilt symptoms at 8 weeks after inoculation. The scale of resistance ranged from 0 = no symptoms to 4 = death.

At 8 weeks after inoculation, all the 'California Wonder' plants had died, whereas almost all the 'White Khandari' plants had no symptoms. All the plants of 50 accessions wilted and 21 of the 50 accessions died. Seven accessions of which the disease index was lower than 1 were considered to be resistant. These were 'Ranche Khorsani' belonging to *C. chinense*, 'Heiser 6240', 'LS2390', and 'LS1840' belonging to *C. frutescens*, and 'LS1716', 'Casali BGH 1761', and 'Pickersgill 277' belonging to *C. baccatum*. All the 3 resistant accessions of *C. baccatum* belonged to *C. baccatum* var. *baccatum*. Eleven accessions, that is 3 accessions of *C. chinense*, 2 accessions of *C. frutescens* and 6 accessions of *C. baccatum*, whose disease index was greater than 1 but lower than 2 were regarded as moderately resistant. The remaining 46 accessions were considered to be susceptible (Table 1).

As *C. chinense*, *C. frutescens* and *C. baccatum* are partly able to cross with *C. annuum*, the 7 accessions considered resistant in this experiment can be used as sources of resistance to bacterial wilt of pepper.

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Table 1. Resistance to bacterial wilt in related species of *Capsicum annuum*

variety or accession	8 weeks after inoculation		variety or accession	8 weeks after inoculation	
	wilted plant ^z (%)	disease index ^y		wilted plant ^z (%)	disease index ^y
[<i>C. chinense</i>]			[<i>C. baccatum</i>]		
Ranche Khorsani	40	0.40	LS1716	10	0.10
PI257084	50	1.50	Casali BGH 1761	10	0.10
Pimenta de Bode	80	1.60	Pickersgill 277	20	0.60
PI224412	80	1.90	3-4	100	1.10
PI215734	100	2.40	Smith SA 331	100	1.60
Habanero	100	2.50	PI439376	100	1.60
PI281444	100	2.67	LS1658	100	1.80
PI215734	100	2.80	PI260581	100	1.80
PI209589	100	2.90	PI260571	90	2.00
PI224443	100	3.50	Hawkes 4248	100	2.30
PI315023	100	3.90	PI439373	100	2.30
PI241678	100	4.00	Aji-colla	100	2.80
Heiser C334	100	4.00	Smith SA 304	100	3.00
PI260509	100	4.00	Chapeu de Frade	100	3.10
PI152222	100	4.00	Smith SA 353	100	3.20
Heiser C248	100	4.00	PI439396	100	3.44
Smith SA 260	100	4.00	Pickersgill 20	100	3.70
PI152225-1	100	4.00	SA 326	100	3.70
PI152225-2	100	4.00	PI439371	100	3.80
PI315008	100	4.00	CATIE 7417	100	4.00
PI315024	100	4.00	BIR/S. 0745	100	4.00
No.3341	100	4.00	PI370004	100	4.00
BIR/S. 0742	100	4.00	PI439372	100	4.00
[<i>C. frutescens</i>]			LS1634	100	4.00
Heiser 6240	10	0.40	Aji	100	4.00
LS2390	10	0.40	[<i>C. pubescens</i>]		
LS1840	20	0.60	Peran O Manzano	100	4.00
Smith SA 137	60	1.80	LS1659	100	4.00
Nioi Tougarashi	90	1.90	[Control Varieties *]		
LS1839	80	2.20	White Khandari	20	0.70
Malagueta	100	2.70	Pant C-1	90	2.70
LS2386	100	2.80	California Wonder	100	4.00
Tabasco-1	100	3.40			
Pickersgill 596	100	3.50			
Tabasco-2	100	3.60			
Tabasco-3	100	3.60			
LS4158	100	3.80			
BIR/S. 0845	100	4.00			

^z: percentage of wilted plants.

^y: rated on a 0 (no symptoms) to 4 (death) scale.

*: 'White Khandari' is a resistant control variety.

'Pant C-1' is a moderately resistant control variety.

'California Wonder' is a susceptible control variety.

PHYTOPHTHORA PROBLEMS ON CHILLIES AND ITS CONTROL.

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INTRODUCTION

Chillies, botanically known as *Capsicum annum* L. belongs to the N.O. SOLANACEAE. Due to its massive use in Indo-Pakistan, sometimes, some Botanists believed that perhaps its origin is in South Asia but in fact it is native to tropical America, West Indies and Brazil where it exists in wild form.

Chillies are grown all over the world for its dietic importance. The most important countries where this crop is grown are South America, (China, Turkey, Spain, India, Pakistan, Japan and Italy. In Punjab (1985-86) the total area under this crop was about 18.9 thousand hectares with the total production of about 31.8 thousand metric tons.

For the normal and satisfactory growth of the crop, the optimum temperature range is 24.27 C. If the temperature goes up the abscission of flower occurs and the growth of the plants is retarded.

Phytophthora (collar rot) is the major constraint for reduction in yield of chillies in Pakistan. When disease becomes severe especially during rainy season, the crop fails altogether.

Normal production of chillies in Pakistan was 98800 tons (1985-86) whereas the production figures during the year 1988-89 was 74400 tons.

Area and production of chillies.

YEAR	PUNJAB		PAKISTAN	
	Area (000) Hac.	Prod. (000)M.T.	Area (000) Hac.	Prod. (000) M.T.
1985-86	18.9	31.8	68.4	98.8
1986-87	14.7	24.7	64.6	92.4
1987-88	13.9	22.4	60.6	84.3
1988-89	12.5	20.1	57.6	74.4
189-90	17.7	28.6	71.0	125.5
190-91	17.1	27.7	61.6	100.9

SOURCE: Agri. Statistics of Pakistan, 1991. 62

The situation improved during 1989-90 but again went down during 1990-91. It can be controlled by strengthening the programmes in the fields of plant protection and Plant Breeding. To increase per acre yield it is important that the disease is controlled so that the losses caused are averted.

Phytophthora blight

Phytophthora blight caused by 'phytophthora capsici' is an important disease of chillies throughout the world. It was reported for the first time in Mexico in 1912. Since then the literature reflects its reporting from many countries of the world. Prior to the year 1986 this disease was not known in Pakistan. But during the crop season 1986 on account of excessive and prolonged rains during the months of April, May the disease was observed for the first time in major chillies growing areas of country. Heavy losses were faced by the growers and they were disheartened. Keeping in view the importance of the disease, studies were initiated in the plant Pathology Section, AARI, Faisalabad. The cause of the disease was established for the first time in Pakistan. As mentioned above the production of chillies in the country was reduced to 74,000 tonnes during 1988-89 as compared to the normal production of 92,000 tonnes and the country has to import chillies from other countries worth crores of rupees. This situation challenged the scientists for thorough investigation on the disease.

Symptoms.

All plant parts are affected by this disease. Seedlings soon after their emergence are killed in the nursery beds. On the

adult plants in the field, symptoms may develop on the stem at the soil line. Stem lesions are first dark green, later turn into dark brown, these lesions may extend up to 2-3 inches above the soil line and the plants may wilt and subsequently die within few days.

The branches are also affected and brown to dark brown

lesions appear and the portion further to the infection is killed and plants may give blighted appearance. The disease also attacks the fruit. The infection generally starts from the stem. The fungus grows from pedicel into the fruit. The invaded fruit tissue

becomes dark green, water soaked. Under high humidity white mold and fungus spores develop on the affected area and fruit can rot

completely in few days. Such fruits dry out rapidly, shrink and

wrinkle but remain attached to the plant. Epiphytotic are encouraged by rain and warm conditions. Crop nutrition is also important in determining the severity of the symptoms. Excess application of nitrogen increases the

incidence of the disease whereas

potassium retards it. The fungus remain viable in the soil for 2-8 years. An alternate source of infection is through diseased seed and infected nursery but the infection can be controlled by the use of seed dressing with Captan and Ridomil at 2.0 gms/ kg and by dipping nursery in 0.1% solution of Ridomil MZ for 5 minutes.

Recommendations for control.

The following control measures can help to reduce the damage due to this disease:

- Healthy seed obtained from healthy plants, healthy fields, should be used for nursery growing.
- Seed should be treated with Ridomil M.Z. or Captan @ 2.0 gms/kg
- Seed bed be sterilized with formalin or through solarization before sowing. The nursery or it should be virgin soil or there should have been no chillies crop for the last three years.
- Nursery plants should be dipped in 0.1% solution of Ridomil M.Z before planting for 5 minutes
- Transplanting should be done on the ridges on such a level so that the water does not touch the plant parts water should not be allowed to overflow the ridges. Earthing up should be done so that only the seepage water is available to the roots of the plants.
- Rotation should be followed. No chillies crop should be sown in such a field where there has been any solanaceae crop (Chillies, Brinjal, Tomato, Potato) for last 3 years.
- If the disease appears in the field, the chemical Ridomil M.Z should be sprayed @ 0.2% solution to the plants in such a way that the stems upto 10 - 15 cm from the soil level of the plants are also thoroughly covered with the chemical. It will be appropriate if 10-15 cm soil around the stem is also soaked with the solution of the chemical. Application of the chemical should be repeated after 2 weeks. The spray will further prove more beneficial if applied before irrigation
- Resistant varieties should be used if available.

ADDITIONAL SOURCES OF RESISTANCE TO VERTICILLIUM WILT AND PHYTOPHTHORA ROOT ROT OF *Capsicum*

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Verticillium wilt and Phytophthora root rot are economically important diseases of *Capsicum* in many areas of the world. No cultural or chemical controls have been found to be effective, leaving genetic resistance as the best technique for control of these diseases. New Mexico State University has developed a Verticillium wilt resistant line of *Capsicum annuum* that has reached a resistance level of approximately 75 percent (Gonzalez-Sa Ian and Bosland, 1993). Because no higher level of resistance could be achieved in this line, new sources of resistance to Verticillium wilt are being sought. The 'Criollo de Morelos 334' is an important source of resistance to Phytophthora root rot. Nevertheless, new sources of resistance to Phytophthora root rot will be useful as new pathotypes evolve.

Recently, *Capsicum* germplasm was obtained from the Asian Vegetable Research and Development Center (AVRDC), as part of the International Hot Pepper Trial Network (INTHOPE). The objective of INTHOPE is to evaluate popular land races and elite germplasm for adaptive qualities across the international test environments. Four sets of INTHOPE seed were screened for resistance to Verticillium wilt and Phytophthora root rot. In addition, wild species of *Capsicum* were investigated as sources of Phytophthora root rot resistance.

The Verticillium wilt screenings were performed in soil temperature tanks where temperature and lighting were strictly controlled. Soil was infested with *Verticillium dahliae* at a rate of 2000 microsclerotia per gram of soil. Seeds were planted in the infested soil in trays, where each tray contained four INTHOPE accessions and one susceptible control (B.G.1668). After 70 days, plants were scored for an interaction phenotype on a scale of 1 to 9, where: 1 = no disease symptoms and 9 = death. This screening method successfully differentiates between resistant and susceptible plants (Johnson, 1988). Statistical analyses were performed to determine disease severity (mean interaction phenotype score) and percent resistant plants, where resistant plants have a score of 1 or 3. All lines were statistically contrasted with the susceptible line for significant differences.

Of the INTHOPE accessions, ten lines were found to contain some level of resistance, although the disease severity (d.s.) of these accessions was fairly high (Table 1.). All plants from the susceptible line exhibited a high disease severity (mean d.s. = 7.48). Cuttings were taken from plants with a d.s. of 3 or less. Germplasm from these cuttings will be screened again, and accessions showing resistance will be selected and cuttings taken. This cycle will be repeated in an attempt to achieve a level of resistance greater than 75%. If this level of resistance can be reached, the line containing this

resistance can be used to introgress resistance to Verticillium wilt to commercial varieties.

Table 1. Disease severity and percent resistant plants for accessions showing resistance to Verticillium wilt.

AVRDC#	D.S.a	%RPb
PBC473	5.87	4
PBC580	6.33	0
PBC408	6.00	5
PBC518	5.36	23
PBC535	6.04	0
PBC485	6.14	0
PBC615	6.43	0
PBC731	6.33	0
PBC495	6.22	4
PBC370	6.70	11
PBC1668	7.48	0

a = Mean disease severity.

b = Percent resistant plants = individuals in Interaction phenotype classes 1 and 3.

The Phytophthora root rot screenings were done as described by Sosland and Lindsey (1991). Seedlings are inoculated at the 14-day-old stage and disease severity was scored 7 to 14 days later. An interaction phenotype scale, where: 1 = no disease symptoms and 9 = death, evaluated the accessions for resistance. From the INTHOPE accessions, five accessions (Table 2) had significant levels of resistance. The accession PBC408 exhibited some resistance to both Verticillium wilt and Phytophthora root rot.

A fascinating observation is that *C. ciliatum* was immune to Phytophthora root rot. The roots of *C. ciliatum* displayed no reaction to *Phytophthora capsici*. The roots were white and lacked even the slightest indication of host-parasite interaction. The controls were dead within the treatment, verifying that the parasite was presented to the host. Host-parasite interactions are generally believed to have evolved over a long period of time. Our observations have been that all Capsicums react in some way to *Phytophthora capsici*. These reactions range from a slight browning for highly resistant material to necrotic tissue for very susceptible material. *C. ciliatum* is an anomaly within the *Capsicum* genus with 13 chromosomes. Eshbaugh has suggested that it does not belong in the genus (Naj, 1992). Our results indicate that *C. ciliatum* is a non-host to *Phytophthora capsici*, which may support Eshbaugh's hypothesis that *C. ciliatum* does not belong in the *Capsicum* genus.

Table 2. Disease severity and percent resistant plants of accessions showing resistance to *Phytophthora* root rot.

AVRDC#	D.S. a	%RPb
PBC199	2.70	75
PBC408	3.18	70
PBC592	3.51	64
PBC612	2.71	71
PBC613	3.12	72
PBC474	9.00	0
PBC596	9.00	0
PBC595	9.00	0
<i>C. ciliatum</i>	1.00	100
<i>C. tovari</i>	3.22	72
NM 6-4	8.54	<1

a = Mean disease severity,

b = Percent resistant plants = individuals in Interaction Phenotype classes 1 and 2.

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SCREENING OF HOT PEPPER GERMPLASM FOR RESISTANCE TO FUSARIUM WILT (*Fusarium pallidoroseum* (Cooke) Sacc.)

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INTRODUCTION

Fusarium pallidoroseum (Cooke) Sacco has recently devastated the hot pepper crop in Kashmir, causing great concern to vegetable and seed growers. Ahmed *et al.* (1992) although found few lines and cultivars to be resistant under natural epiphytotic condition, however, for further confirmation; there is a need to screen genotypes under artificial inoculation. Therefore a material consisting of sixty six lines/cultivars were screened under controlled (artificial inoculation of soil and sick soil) and natural epiphytotic conditions.

MATERIALS AND METHODS

The seedlings of sixty six lines were raised at Vegetable Experimental Farm, S. K. University of Agricultural Sciences and Technology, Sri nagar, during Kharief 1992 and 1993. These seedlings were screened both under controlled and natural epiphytotic conditions as mentioned below.

I-a) Screening by artificial soil inoculations

Screening was carried by following the techniques suggested by Kesavan and Choodhury (1977) during Kharief 1993 and was performed as under:-

Culture of pathogenic fungus was subcultured on 2% PDA medium by transferring culture bits from culture and incubating at an ambient temperature ~ of 27...t. 1°C for one week for growth. For mass production of inoculum, the fungus was later grown on 150gm. Sand maize medium (4: 1) in 150ml. Erlenmeyer flasks maintained at room temperature (23-26°C) for 3-4 weeks. ~ Earthen pots of 22.5cm dia were filled with sterilized soil and the fungal inoculum was mixed thoroughly with the top layers at the rate of one inoculum flask per pot. Inoculated pots were placed under controlled conditions for 7 days for the development of fungus. Six week old healthy seedlings of 66 different lines/cultivars were transplanted in these inoculated pots and then placed under controlled conditions for the disease development. In each treatment 8 seedlings were planted in CRD with two replications along with 8 uninoculated seedlings as control. Periodical watering was done to provide requisite moisture for plant and pathogen growth. In order to make sure that

infection does take place and there is no disease escape a second inoculation was made after three weeks of transplanting by pouring water suspension containing spore and mycelium around the root zone as done earlier by Mishra and Bais (1987). The data on disease incidence was recorded upto fruit maturity and percentage wilt incidence (PWI) was worked out and rated as suggested by Kesavan and Choodhury (1977) as immune, highly resistant, resistant, moderately resistant, susceptible and highly susceptible having average PWI of zero, 1-10, 11-30, 31-50, 51-80 and 81-100, respectively.

b) Screening under sick soil conditions

Germplasm comprising of 64 lines/cultivars were screened in sick soil collected from farmers field where wilt incidence was cent per cent. Pots were " filled with sick soil and carried as under artificial soil inoculation with control during 1992 and 1993. The data on disease incidence was recorded. Pooled for both the years and average percentage wilt incidence worked out.

II) Screening under natural epiphytotic conditions

59 lines/cultivars were screened under natural epiphytotic condition for resistance to Fusarium wilt at Vegetable Experimental Farm, both during 1992 and 1993. Six week old seedlings were transplanted in RBD with two replications at a spacing 60x45cm. in a field where wilt incidence was almost cent per cent in susceptible lines in previous season. In each replication there were two rows of each genotype with ten plants in each row. The data on wilting percentage recorded during both 1992 and 1993 was pooled and average PWI was worked out.

RESULTS AND DISCUSSION

Results on screening have been presented in Table 1. Among the lines/ cultivars tested, cultivar 'Masalwadi' was found to be immune under all tested conditions. Under artificial soil inoculations only five lines viz. SC-120, SC-335, SC-415, SC-1 07 and' Phule C-5' were highly resistant, whereas 10 lines namely SC-348, SC-212, SC-172, SC-1 08, SC-407, 'LCA-305', 'LCA- 304', 'Arka Lohit', 'Pusa Jwala' and' Pant C-2' were however found resistant. Under moderately resistant group there were 26 lines with rest of the lines having susceptible to highly susceptible reaction.

In another experiment where pots were filled with sick soil, it was observed that among the lines tested, SC-212, SC-621, SC-407 'Arka Lohit' and' Phule C-5' were highly resistant, the lines SC-413, SC-371, SC-348,SC- 415, SC-372, SC-1 07, SC-404, SC-31, SC-451, SC-1 08, 'LCA-304', 'LCA- 248', 'DPLC-1' and' Pusa Jwala' were found resistant, 14 were moderately resistant while rest were susceptible to highly susceptible.

Under natural epiphytotic conditions also the lines SPC-2, SC-502, SC- 451, 'Arka Lohit', 'Phule C-5' were found highly resistant, 151ines namely SC-108, SC-413, SC-416, SC-348, SC-504, SC-404, Sc-31, SC-621, SC-

Table 1 Screening for resistance to *Fusarium pallidoroeseum* (Cooke) Sacc.

Grade	Percentage wilt Incidence	Under Controlled Conditions			Natural Epiphytotic Conditions
		Under Artificial Soil inoculation 1993	Under Sick Soil Conditions (Mean of two years 1992-1993)	Under Openfield Conditions Mean of two years 1992-1993)	
Immune	Zero	Masalawadi	Masalawadi	Masalawadi	
Highly	1-10	<u>SC-120, Phule C-5, SC-335, SC-415, SC-107.</u>	<u>SC-212, SC-621, SC-407</u> Arka Lohit, Phule C-5	<u>SC-502, SPC-2, SC-451</u> Arka Lohit, Phule C-5	
Resistant	11-30	<u>SC-348, SC-212, SC-172, SC-108, SC-407, LCA-304, Arka Lohit, Pusa Jwala, Pant C-2, LCA-305</u>	<u>SC-413, SC-371, SC-348</u> <u>SC-415, SC-372, SC-452</u> <u>SC-107, SC-404, SC-31, SC-451,</u> <u>SC-108, LCA-304, LCA-248,</u> <u>DPLC-1, Pusa Jwala</u>	<u>SC-108, SC-413, SC-416, SC-348,</u> <u>SC-504, SC-404, SC-31, SC-621,</u> <u>SC-131, LCA-304, LCA-248, Pusa</u> <u>Jwala, Pant C-2, Jawahar-218,</u> <u>LCA-206.</u>	
Moderately Resistant	31-50	<u>SC-101, SC-102, SC-413, SC-371,</u> <u>SC-504, SC-289, SC-137, SC-413,</u> <u>SC-419, SC-414, SC-452, SC-123,</u> <u>SC-451, SC-31, SC-621, SC-502,</u> <u>SC-418, SC-503, LCA-208, LCA-206,</u> <u>LCA-248, DPLC-1, Punjab Lal Jawahar-218, JCA-586, BC-212</u>	<u>SC-406, SC-101, SC-137,</u> <u>SC-335, SC-149, SC-419,</u> <u>SC-120, SC-245, SC-105</u> <u>SC-222, SC-508, SC-418</u> <u>Jawahar-218, LCA-586</u>	<u>SC-195, SC-103, SC-101, SC-137,</u> <u>SC-107, SC-187, SC-371, SC-501,</u> <u>SC-120, SC-419, SC-105, SC-222,</u> <u>LCA-208, Punjab Lal, TC-2,</u> <u>Mirch American.</u>	
Susceptible	51-81	<u>SC-501, SC-406, SC-222, G-4,</u> <u>SC-505</u>	<u>SC-501, SC-512, SC-187,</u> <u>SC-195, SC-103, SC-289,</u> <u>SC-16, SC-420, SC-412,</u> <u>SC-126, SC-505, SC-571,</u> <u>Punjab Lal, LCA-305,</u> <u>BC-212, G-4, LCA-235</u>	<u>SC-186, SC-245, SC-114, SC-181,</u> <u>SC-195, SC-109, SC-452, SC-512,</u> <u>SC-518, SC-158, SC-149, SC-503,</u> <u>SC-126, SC-403, KCS-1</u>	
Highly Suceptible	81-100	<u>SC-114, SC-518, SC-187, SC195</u> <u>SC-100, SC-453, SC-102, SC-149,</u> <u>SC-411, SC-417, SC-109, SC-508,</u> <u>SC-126, SC-412, S. Local Long,</u> <u>Mirch American, SC-42, SC-158, SC-16</u>	<u>SC-114, SC-158, SC-209,</u> <u>SC-407, SC-414, SC-42,</u> <u>SC-206, SC-100, SC-452,</u> <u>SC-109, SC-508, KCS-1</u>	<u>SC-174, SC-100, SC-411, JCA-586</u> S. Local Long, G-4, BC-212	

Genotypes underlined are resistant under all the three conditions

131, 'LCA-206, 'LCA-248,' 'Pusa Jwala', 'Pant C-2' and Jawahar-218' were found resistant, 16 were moderately resistant while the remaining twenty two lines were found to be susceptible to highly susceptible.

The lines namely 'Masalwadi', SC-120, 'Phule C-5', SC-335, SC-415, SC-1 07, SC-348, SC-108, 'LCA-304', 'Arka Lohit', 'Pusa Jwala', 'Pant C-2', SC-1 01, SC-371, SC-137, SC-419, SC-451, SC-31, 'LCA-248, 'Jwahar-218' and SC-502 showed resistance of various degrees varying from immune and highly resistant to moderately resistant in all the three screening procedures. Ahmed *et al.* (1991) under natural epiphytotic condition also reported 'Phule C-5', 'Masalwadi', SC-1 08, 'Arka Lohit', 'Pant C-2', SC- 101, SC-137, 'LCA-248' and 'Jwahar-218' as resistant. Further, it was observed that hot pepper lines such as SC-1 07, SC-108, SC-348, SC-502, SC- 451 and 'Pant C-2' in addition to high level of resistance have also been found ~ superior in yield and quality. Hence, these lines could be well identified for commercial growing under Kashmir conditions after necessary evaluation.

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FLOW CYTOMETRIC DETERMINATION OF NUCLEAR REPLICATION STAGES IN PEPPER SEEDS DURING PRIMING AND GERMINATION

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Introduction

At a certain stage during development seeds achieve optimal vigour. Seed vigour is defined as the ability to develop into normal seedling under field or greenhouse conditions. The inevitable process of seed ageing is related to a reduction in seed vigour, resulting in a decrease in rate of germination and stress resistance.

Priming, i.e. the pre-imbibition of seeds in osmotic solutions, can partly reverse the negative effects of ageing and may result in both accelerated germination rate and an improved seedling uniformity. Its beneficial effect has been related to the physiological changes occurring in the partially hydrated embryos, i.e. restoration of DNA, RNA and membrane integrity (Osborne, 1983) as well as to a more favourable metabolic balance of primed seeds at the start of germination in water (Dell' Aquila *et al.*, 1978).

In the present research, we analyse the relative nuclear DNA content in pepper seeds during germination and the effects of different priming treatments on germination characteristics and on relative DNA contents in tissues of pepper seeds belonging to different cultivars and seed lots. By combining results at the molecular and physiological level, we attempt to contribute in the definition of the various aspects of the seed quality concept.

Materials and Methods.

Two pepper (*Capsicum annuum* L.) seed lots of the cultivar 'Corno di toro' and one of the cultivar 'California Wonder' were used. Germination experiments were performed following ISTA Rules (1993).

Seed priming was carried out on filter paper wetted with PEG-6000 at the osmotic potential of -1.1, -1.3 and -1.5 Mpa for 14 days at 20°C in darkness. After priming, seeds were washed with running tap water to remove the osmotic agent and dried back to their initial moisture content (7.5% on a dry/weight basis).

Nuclear samples for flow-cytometric analysis were prepared as reported by Lanteri *et al.*, (1993). To detect DNA, 10mg/l of 4',6-diamidino-2-phenylindole (DAPI) was added to the isolation buffer. For each sample 20 seeds were used and determination was made in duplicate. A PAS II flow cytometer (Partec GmbH, MOnster, Germany) was used, equipped with a HBO-100 mercury arc lamp, a TK-420 dichroic mirror and GC-435 long pass filter. All analyses were performed using peak height detection and logarithmic amplification (Bino *et al.*, 1992). The fluorescent signals are presented as frequency distribution histograms over 500 channels, starting from channel number 20. The DNA amount is expressed as arbitrary C values in which the 1 C value comprises the DNA content of the unreplicated haploid chromosome complement.

Results

More than 90% of seedlings of all used pepper seed samples were normal. The osmotic treatments did not affect the percentages of normal seedling (data not reported) but significantly reduced the MGT as compared with untreated seeds. For all seed lots, the highest reduction of the MGT was observed after priming at the osmotic potential of -1.1 Mpa (Table 1).

DNA profiles of dry pepper seeds revealed three peaks: at the channel 100, which corresponded with the pre-replication diploid chromosomal configuration (2C) and at channel

144 and 220, which contained DNA amounts 1.5 and 3 times higher than the 2C value and corresponded with DNA levels of 3C and 6C respectively (Figure 1). Between 24 and 36 h of imbibition in water, a signal at channel 190, corresponding with 4C DNA, was induced in root tip cells of all samples of non primed seeds (Figure 2). This peak increased during subsequent times of imbibition and after 72 h the 4C/2C ratio was approximately 1. No changes in DNA content in the rest of the seeds were observed. After priming an induction of 4C signals was found in the pepper seed lots at all PEG concentrations. The induction of 4C signals was highest after treatment in PEG solution with an osmotic potential of -1.1 MPa and lowest after priming in -1.5 MPa (Table 1). For the three pepper seed samples primed at -1.5 MPa the amount of 4C cells ranged from 6.5 to 8.3% and the differences in the percentages of nuclei in G2 were not significant ($p > 0.05$) (Table 1). After priming at -1.3 and -1.1 MPa, however, significant differences between the individual seed samples were observed. Priming at -1.3 MPa induced about 14.5% of the cells to enter the G2 phase both in 'California Wonder' and 'Corno di toro' lot 1, compared with 26.0% of the cells in 'Corno di toro' lot 2 (Table 1). Priming at -1.1 MPa most effectively increased the number of 4C nuclei in all pepper seed samples. Again the largest effect was found for 'Corno di toro' lot 2 (Table 1). An inverse correlation was found between the percentage of nuclei in G2 stage (4C DNA) and MGT for each individual pepper seed lot (Figure 3)

Discussion

Flow cytometric determination of nuclear DNA contents in embryos of dry, fully matured pepper seeds revealed only 2C signals in all pepper seed lots. Therefore, pepper belongs to those species in which the quiescent embryo arrests in the presynthetic G1 phase of nuclear division, i.e. the stage before DNA synthesis.

In whole pepper seeds two other peaks were observed, which corresponded with the 3C and 6C DNA content and originated from the triploid endosperm tissue. Possibly the 6C peak arises from developmentally regulated cellular endoreduplication.

Upon imbibition in water an induction of the 4C signal, indicating DNA synthesis, was observed before visible germination; nuclear replication activity, therefore, precedes radicle protrusion.

Preconditioning of all the seed lots in PEG solutions induced DNA synthesis and considerably reduced the MGT. In each seed lot a strong positive correlation was found between the treatment effectiveness and the frequency of nuclei in 4C (Figure 3) These results are consistent with those previously obtained (Bino *et al.*, 1992; Lanteri *et al.*, 1993) and show that the induction of nuclear replication activity during priming sustains a more rapid germination upon subsequent imbibition in water. For all seed lots of pepper analysed, the lowest concentration of PEG (-1.1 Mpa) induced 4C signals and reduced MGT more efficiently than the higher PEG concentrations (-1.3 and -1.5 Mpa). The amount of induced DNA synthesis, as other processes in the seeds, is regulated by the water potential and it is directly correlated to the effect of the osmotic treatment. Nevertheless, the present results show that the amount of priming-induced DNA synthesis and the priming effect on seed performances may vary more between seed lots of the same cultivar than between lots of different cultivars. These variations apparently resulted from specific differences in the reaction to the priming treatments among seed lots and were previously observed by other researchers (Pearl and Feder, 1981; Ellis and Butcher, 1988).

The next step of our work will be the application of osmoconditioning to artificially aged pepper seed samples. They, together with a sample of unaged material, will provide an useful model to gather further information on the relationship between priming effect and nuclear replication activity of seeds at different degree of deterioration.

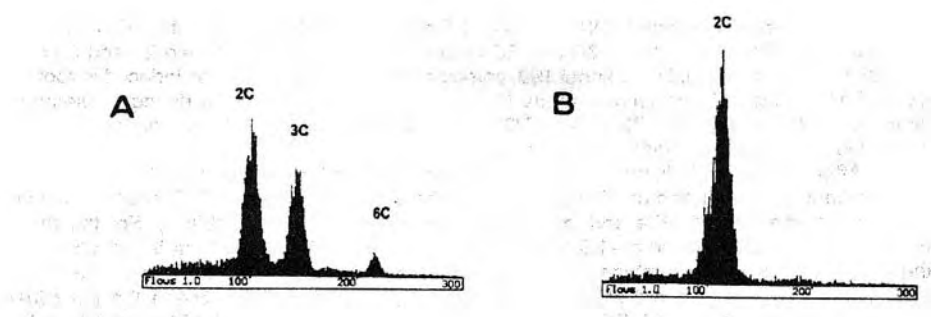


Figure 1. Histograms of flow-cytometric analysis of nuclei (A) from whole seeds, with peaks at the 2C (channel 100), 3C (channel 144) and 6C (channel 216) and (B) from embryo tissue with a peak at the 2C DNA level (channel 100)

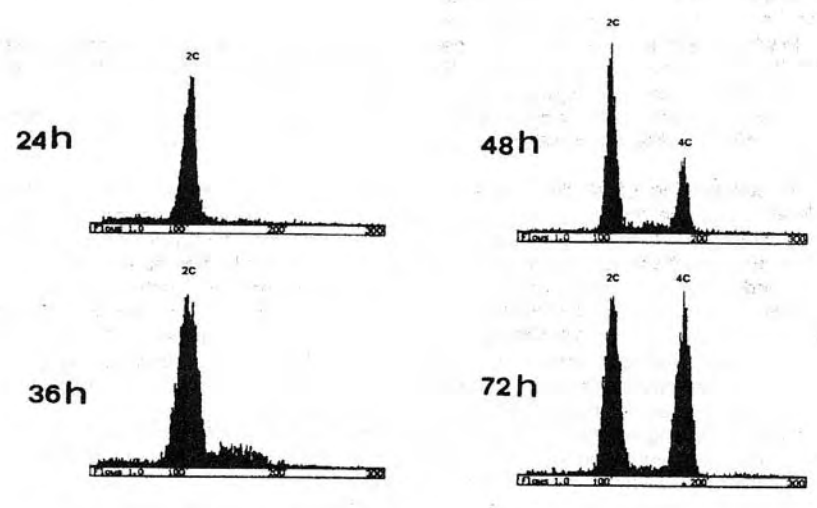


Figure 2. Histograms of flow cytometric analysis of nuclei in embryo root tips of pepper seeds (cv "Corno di toro" lot 1) after 24, 36, 48 and 72 h of imbibition in water.

FURTHER RESULTS ON STORAGE IN LIQUID NITROGEN OF PEPPER SEEDS

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Seed cryopreservation in liquid nitrogen (-196°C) of different species has shown to be a reliable technique for germplasm preservation (Hu *et al.*, 1994; Iriando *et al.*, 1992; Stanwood, 1984 and 1985).

In a previous work on pepper and eggplant (Bettetti *et al.*, 1990) it was observed that seeds subjected to 25 cycles of cooling and rewarming from 20 to -196°C and back, showed irrelevant loss of viability (3-4%) and seed viability was not affected by slow or fast cooling and rewarming rates. However the effect of seed moisture content was not clear since, in pepper, the lowest water content negatively influenced seed storability.

For a better understanding of the relationship between seed moisture content and seed viability and vigour after storage in liquid nitrogen, the following trial was carried out. Seeds of pepper at 5 different moisture contents, ranging from 4.7 and 20.1 %, were submitted to 25 cycles of cooling and rewarming. Afterwards, in order to magnify the effect of the treatment, seed vigour was evaluated by applying artificial ageing test (seed moisture content 18%, temperature 45°C and time 24 h).

The results, submitted to Response Surface Analysis, are reported in Fig. 1. As expected, on the whole, the decrease in the percentage of germination (Fig. 1A) and the increase in the mean germination time (Fig. 1B) was positively correlated with the number of cooling-rewarming cycles as well as the seed moisture content. However, as previously observed, in seeds subjected to a low number of cooling-rewarming cycles, a negative effect of low moisture content was observed.

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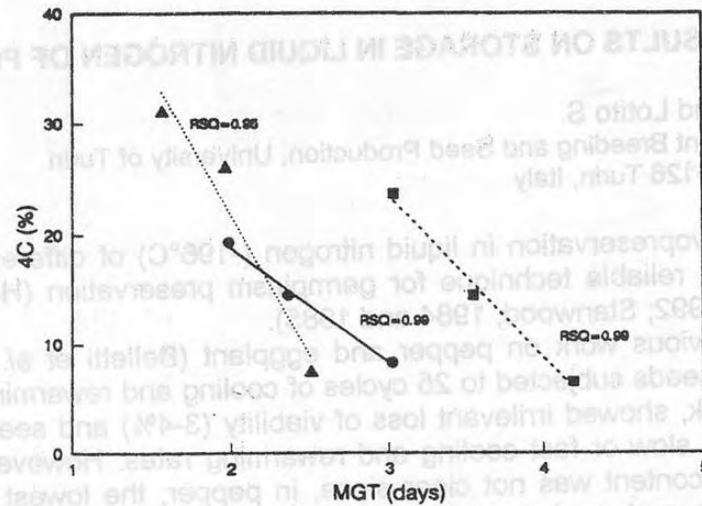


Figure 3. Relation between seed performance, expressed as mean germination time (MGT) and 4C percentage in pepper seeds 'California Wonder' (●), 'Corno di toro' lot 1 (■) and lot 2 (▲). For all seed lots, RSQ values for linear regression are significant at the $P < 0.01$ level.

Table 1 Germination percentage (GP, in % of normal seedlings), mean germination time (MGT, in days) and cell cycle stage (nuclear 4C in % of total nuclei) of untreated and primed seeds. Seeds were primed for 14 d in -1.5, -1.3 or -1.1 MPa PEG solution. Nuclear replication stages were analysed in root tips of embryos. MGT data are means \pm SE of four replicates of 100 seeds, 4C percentages are the duplicate means \pm SE of 20 seeds.

	UNTREATED			PRIMED					
	GP	MGT	4C	-1.5 MPa		-1.3 MPa		-1.1 MPa	
				MGT	4C	MGT	4C	MGT	4C
'C. Wonder'	90.1	4.7 \pm 0.1	0	3.0 \pm 0.0	8.3 \pm 0.2	2.4 \pm 0.1	14.5 \pm 0.2	2.0 \pm 0.1	19.3 \pm 0.2
'C. toro' lot 1	93.3	5.0 \pm 0.1	0	4.2 \pm 0.1	6.5 \pm 0.3	3.5 \pm 0.1	14.6 \pm 0.2	3.0 \pm 0.1	23.7 \pm 0.3
'C. toro' lot 2	95.3	4.7 \pm 0.1	0	2.5 \pm 0.0	7.5 \pm 0.2	1.9 \pm 0.1	26.0 \pm 0.2	1.6 \pm 0.0	31.0 \pm 0.3

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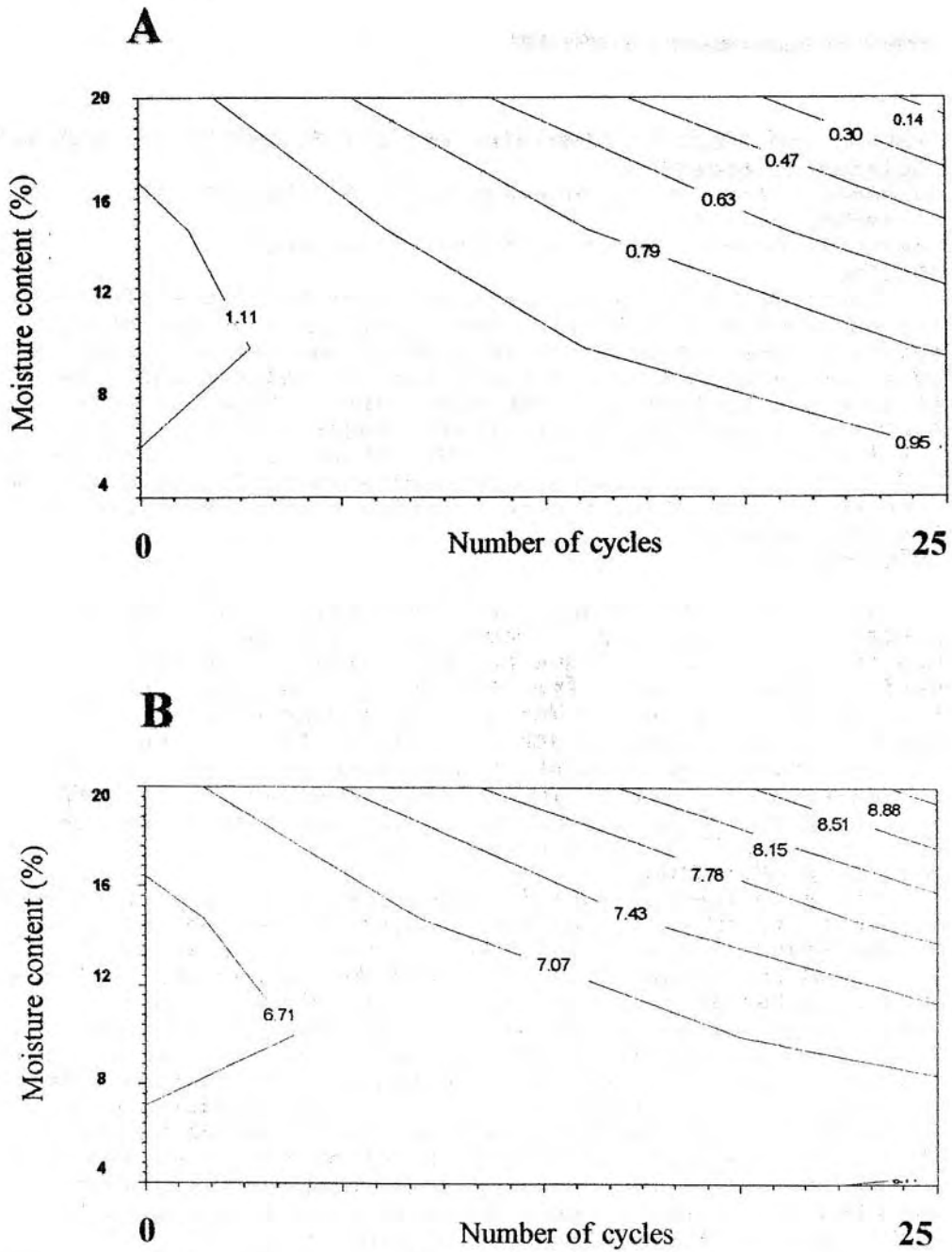


Fig. 1 Response surface analysis of percentage of germination (A, data expressed after angular transformation) and mean germination time (B) of pepper seeds at different moisture contents, after cooling and rewarming cycles.

GENERAL AND SPECIFIC COMBINING ABILITY ESTIMATES IN EGGPLANT (*Solanum melongina* L.)

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ABSTRACT

Combining ability analysis was done for fruit yield and its components in a diallel set involving four varieties of eggplant. Mean squares due to general combining ability (GCA) were highly significant for all the characters, while mean squares due to specific combining ability (SCA) effects for number of fruits per plant, fruit length, fruit yield per plant and plant height were highly significant. Both GCA and SCA effects contributed significantly to fruit yield and its components. SCA effects were relatively of greater importance than GCA effects.

INTRODUCTION

Griffing (1956) elaborated the diallel technique and presented a theoretical discussion on its use for estimating GCA and SCA. The technique has been extensively exploited in various field crops. A few studies have been made to explore this aspect in eggplant (Verma, 1986, Rashid 1988; Singh and Kumar 1988; Chadha and Hegde, 1989; Mishra and Mishra, 1990). The present studies were an attempt to obtain information on relative importance of GCA and SCA of different varieties for fruit yield and its various components for developing high yielding eggplant hybrid.

MATERIALS AND METHODS

The experimental material comprised of four varieties of eggplant viz; 'Nirala', 'Qaiser', 'Multan Selection' and 'Tranda Purple Round'. Nursery of these varieties was sown during July, and transplanted in the field during August, 1992 and crossed in a diallel set to obtain seed of six single crosses and their reciprocals. In the next growing season (February to July 1993) six single crosses and their reciprocals along with four parental varieties were planted in a Randomized Complete Block Design with three replications. The planting plan consisted of two rows (700 cm each) per experimental unit planted 100 cm apart with plant to plant distances of 50 cm. At flowering stage, ten randomly competitive plants were selected for recording data for various characters including number of fruits per plant, fruit width (cm), fruit length (cm), fruit weight (gm) fruit yield per plant (kg) and plant height (m). Analysis of variance was carried out to test the significance of differences. Analysis of combining ability was performed by, applying Griffing's (1956) Method-I, Model- II.

DISCUSSION

GCA effects of individual parents for each character are presented in Table-1. Variety 'Tranda Purple Round' had the highest positive GCA effects for fruit yield per plant, fruit weight and fruit width, an observation compatible with the findings of Singh and Kumar (1988) and Chadha and Hegde

(1989). Variety 'Multan Selection' showed negative GCA effects for most of the characters. This would indicate that certain varieties might contribute to higher fruit yield through their influence on individual yield components.

To study the performance of varieties in specific combinations, SCA effects were used and are given in Table-2.- The overall assessment for all the characters indicated that the highest specific effects for fruit yield per plant, fruit weight and plant height in cross combination 'Nirala'x'Oaiser' was accompanied by intermediate specific effects for number of fruits per plant, fruit width and negative for fruit length. These results are in agreement with Singh and Kumar (1988) and Mishra and Mishra (1990) studies. The cross 'Nirala'x'Multan Selection' showed the lowest specific effects for fruit yield per plant whereas it exhibited the ~ highest effects for fruit width, high for number of fruits per plant, negative for fruit length, fruit weight and plant height.

Estimates of reciprocal effects are presented in Table-3. For fruit yield per plant, maximum positive effect was noticed for the cross 'Multan Selection'x'Oaiser' followed by cross 'Tranda Purple Round'x'Oaiser.' Regarding number of fruits per plant cross 'Multan Selection'x'Oaiser', revealed the highest reciprocal effect. Reciprocal cross 'Multan Selection'x'Nirala' showed maximum negative effects for fruit yield per plant, fruit length and number of fruits per plant.

From the foregoing description of various characters, it may be concluded that both additive and non-additive gene effects contributed equally for the manipulation of genetic variability. However, the parents possessing high GCA value may be more useful in the hybridization programme of eggplant.

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TABLE 1
Estimates of relative GCA effects for fruit yield and its components in eggplant.

Varieties	No.of fruits/ plant	Fruit width	Fruit length	Fruit weight	Fruit yield/ plant	Plant height
Nirala	0.187	-0.239	0.299	0.251	0.037	2.163
Qaiser	4.234	-1.019	1.903	-5.031	0.151	0.816
M.S	-2.191	0.537	-1.019	-5.601	-0.364	-4.596
TPR	-2.229	0.722	-1.183	10.381	0.176	1.617

TABLE 2
Estimates of SCA effects for fruit yield and its components in eggplant.

Single crosses	No.of fruits/ plant	Fruit width	Fruit length	Fruit weight	Fruit yield per plant	Plant height
Nirala x Qaiser	1.955	0.102	-0.265	5.831	0.387	9.482
Nirala x M.S	3.513	0.231	-0.308	-3.055	0.141	-8.051
Nirala x TPR	0.251	-0.069	-0.361	2.347	0.142	-4.207
Qaiser x M.S	2.600	-0.039	0.531	1.186	0.255	3.417
Qaiser x TPR	0.254	-0.263	-0.897	2.118	0.151	-1.064
M.S x TPR	3.612	0.015	0.461	2.642	0.228	1.099

TABLE 3
Estimates of reciprocal effects for fruit yield and its components.

Single crosses	No.of fruits/ plant	Fruit width	Fruit length	Fruit weight	Fruit yield/ plant	Plant height
Qaiser x Nirala	0.183	-0.397	-0.182	0.121	0.003	8.252
M.S x Nirala	-4.017	-0.238	-0.414	3.547	-0.173	5.055
M.S x Qaiser	2.350	0.160	0.823	3.031	0.323	3.500
TPR x Nirala	1.250	-0.062	-0.260	-1.918	0.025	-0.677
TPR x Qaiser	0.500	0.634	0.929	0.218	0.100	15.827
TPR x M.S	-0.767	-0.030	0.205	-1.537	-0.124	-7.413

M.S = Multan selection
 TPR = Tranda Purple Round

SOURCES OF RESISTANCE TO VERTICILLIUM WILT IN SOLANUM MELONGENA AND ITS AFFINITIES IDENTIFIED BY IMPROVED ROOT DIP METHOD Baiqing Lin and Yunhua Xiao

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I. INTRODUCTION Fungi of the genus *Verticillium* are plant pathogens which cause vascular wilt disease in a large number of host plants (Pegg, 1974). Screening eggplant germplasm to verticillium wilt has been carried out in various countries. Unfortunately most investigations have dealt with a comparatively small number of accessions. Different degrees of susceptibility (Sivaprakasam, 1975) or resistance (Cox, 1956) have been observed in eggplant seedlings by root dip (Braveman, 1963; Nothmann, 1979) or by hypodermic spore injection (Sivaprakasam, 1975). No resistant orierarlt commercial eggplant cultivars are available in practice.

To evaluate eggplant accessions on mass, an improved root dip method had been developed to differentiate resistant to the disease (Lin, 1991). This report identifies sources of genetic resistance to verticillium wilt in eggplants.

II. MATERIALS AND METHODS

1. Accessions

1024 accessions of *Solanum* were involved in the experiments which included 983 landraces and cultivars, 26 introductions, 15 wild/semi-wild species. Jiuyeque, a landrace in Beijing and Qiqie No. 3, an improved commercial cultivar were used as a susceptible and resistance controls respectively.

2. Seedling culture

A two-layer tray system was used to prepare seedling and for inoculations. Germinated seeds were sown each in a reverted pyramid cell (3x4x4 cm) of a plastic tray (upper tray). Support media was perlite and peat, 2:1 v/v, supplemented with commercial fertilizer. The upper tray has 16x8 reverted pyramid cells. A row (8 cells) for an accessions as a replicate. 32 seedlings for each accessions were divided to 4 replicates and located randomly in a screening batch. Control accessions of Jiuyeque and Qiqie No.3 were appeared each in every 6 tray to delimited the influence of environment. The upper tray was placed in a rectangle flat (lower tray) which is slightly larger than the upper one and was filled with support media up to two third of its heights. The upper tray were watered when needed. During growing, radicula of seedlings had penetrated through a hole at the bottom of reverted cell to the media in the lower flat.

3. Inoculation and Resistance Evaluation

When seedlings have four well developed leaves (about 40 days in our greenhouse), upper tray was raised gently and thus resulted in some slight wounds in seedlings' rootlets. They were rinsed in a inoculation tank of 1.0 cm depth of inoculum suspensions (107 conidia of *Verticillium dahliae*, strain VD-02 from Beijing) for 15 minutes. After the treatment upper trays were placed back to its support flat. The media inside the flat were softened or changed during inoculation. Seedlings were watered thoroughly and placed in greenhouse benches at 25:20°C with 55-70% relative humid conditions. 4 weeks after inoculation, individual plants were rated for verticillium wilt symptom based on a six degree interaction phenotype scales, where: 0, no aerial symptom; 1, only first true leaf necrotic or curled; 2, first three leaves developed wilt symptoms; 3, only newest true leaves remain healthy,

older ones being necrotic and curled, defoliation happened; 4, all developed leaves had fallen out, plant having only One new-fonned leaf; 5, plant death. A plant scored as 1 or less was considered as resistant. Mean interaction phenotYPE, percentage of resistant plant and a disease severity were calculated for each accessions with the nearby control accessions as a covariance.

III. RESULTS

Of the 1024 Solanum accessions planted, Seeds of 11 accessions failed to gennjnated or seedlings were weak and died before inoculation. These 11 accessions were eliminated from experiments. The remaining 1013 accessions had excellent seedling vigor. No symptom of nutrient deficiency were observed among any of the accessions. The seedlings had lionnal groWth except some had be depressed in some degree by ovetheadof nearby fast groWth accessions after two true leaves developed.

Verticillium wilt disease'symptom appeared among accessions as early as 14 days after inoculation. The initial symptoms included a loss of turgor in the upper part of lower leaves, following by yellow 'L irregular spots on leaves. The yellow spots then developed on the upper ones. Eventually those spots became necrotic and leaf margins curled upward or laterally or rolled inward. The plants became epinastY. Some plants began to shed their lower leaves and after that the lower leaves were dropped. A few accessions developed stunting. Complete defoliation occurred in most susceptible accessions.

Table 1. The 10 Solanum accessions identified as having the highest level ofverticillium resistance when tested with the improved root dip methoda

Identification Number b	Identification	Number of Seedlings	Average Inter action Phenotype	Disease Severity c	Percentage of Resistant plants
II6B0301	<i>S. aethiopicum</i>	31	1.1	2.1	41.9
II6B0506	<i>S.melongena</i>	28	1.2	2.4	31.0
II6B0980	<i>S.sisymbriifolium</i>	32	1.4	2.7	28.1
II6B0345	<i>S.coagulans</i>	31	1.5	3.0	29.0
II6B0382	<i>S.melogena</i>	28	1.6	3.1	28.5
II6B0177	<i>S. melogena</i>	32	1.6	3.4	20.3
II6B0892	<i>S. melogena</i>	20	1.9	3.6	22.5
II6B0685	<i>S. melogena</i>	28	1.9	3.8	23.2
II6B0358	<i>S. melogena</i>	28	2.0	4.0	21.4
II6B0734	<i>S. melogena</i>	28	2.0	4.0	21.4
Jiuyeqie (sus. Ck)	<i>S. melogena</i>	88	5	10	–
Qiqie No.3 (res. Ck)	<i>S. melogena</i>	85	2.5	4.8	20.45

a Disease severity means for these accessions are st.atistically similar.

b Jiuyeqie(nine leaves eggplant, a landrace) as susceptible control, while Qiqie No.3, an improved commercial cultivar is used as resistance control. PI c Disease severity for each accessions was calculated by multiplying the number of plant belonging to each interaction phenotYPE class and dividing the sum of all scores by the total number of plant for each accessions multiplying of the highest interaction phenotYPE of scale(5). the data on table had been enlarged 10 times for easier identifying.

The disease severity of all of the accessions was among 2.1 to 10, showing obvious difference in resistant levels to verticillium wilt among *S. melongena* and its affinities. However, no accession was demonstrated as completely resistance, i.e. average interaction phenotype scored less than 1.0. Table 1 lists the 10 accessions identified with the lowest disease severity. II6B0301, II6B0506, II6B0980, II6B0345, had the lowest disease severity scores of 2.1, 2.4, 2.7 and 3.0 respectively. These 4 accessions had also highest percentage of resistant plants of 41.9, 31.0, 28.1, and 29.0 respectively. Some accessions had a few individuals of disease free or with an interaction phenotype score of 1.0. Among the total 1013 accessions screened 20 accessions did not contain any living plant while 15 accessions had more than 10 or more resistant plants. Generally speaking, wild species and semi-wild species demonstrated higher resistance to verticillium wilt. But it is noted that some wild affinities of eggplant also showing a high disease severity (Table 2).

Table 2. The resistance level of 15 affinities of *Solanum melongena* tested for verticillium wilt resistance with the improved root dip method

Identification number	Species	Average Interaction Phenotype	Disease Severity a
II6B0301	<i>S. aethiopicum</i>	1.1	2.1
II6B0980	<i>S. sisymbriifolium</i>	1.4	2.7
II6B0345	<i>S. coagulans</i>	1.5	3.0
II6B0403	<i>S. angulvi</i>	2.5	4.9
II6B0327	<i>S. aethiopicum</i>	2.8	5.5
II6B0414	<i>S. indicum</i>	2.9	5.7
II6B0586	Not Identified	2.9	5.7
II6B0312	<i>S. aethiopicum</i>	3.1	6.2
II6B0332	<i>S. aethiopicum</i>	3.2	6.4
II6B0983	<i>S. indicum forma album</i>	3.2	6.2
II6B0311	<i>S. aethiopicum</i>	3.3	6.5
II6B0728	<i>S. integrifolium</i>	3.5	6.9
II6B0333	<i>S. indicum</i>	3.5	6.9
II6B0936	<i>S. deflexicarpum</i>	3.7	7.4
II6B0987	<i>S. indicum forma album</i>	3.9	7.4
Jiuyeqiu (sus.ck)	<i>S. melongena</i>	5.0	10
Qiqie No.3 (re.ck)	<i>S. melongena</i>	2.5	4.8

a Disease severity for each accessions was calculated by multiplying the number of plant . belonging to each interaction phenotype class and dividing the sum of all scores by the J''f total number of plant for each accessions multiplying the highest interaction phenotype of scale(5). the data on the table had been enlarged 10 times for easier identifying.

IV DISCUSSIONS

Screening eggplant germ plasm resistant to verticillium wilt is one of the prerequisite for direct release of valuable crops cultivars in China because in some region the disease triggered the production of the vegetables. The results of evaluation are also valuable to resistance breeding programs. Sources of eggplant resistant to verticillium wilt were identified through use of an improved root dip method which could be used to screen the accessions in mass. The infection level of 107 cfu/ml of inoculum for root dip produced a simple and reliable evaluation for verticillium

wilt of *Solanum* germplasm. The reliability and differentiation among accessions depend on the stable soil temperature. Other environmental factors, such as light intensity, watering intervals, air humid had also effects on disease severity. Appreciate numbers of check/control accessions should appear in a replicate to eliminate environmental influence on interaction phenotype of accessions. This improved root dip method appeared to be more severe than traditional root dip method because it resulted in a more root wound on rootlets. It is also severe than that of soil drench and that of mixture inoculum (*microsclerotia*) with support media (Gonzalez-Salan, 1991).

Although some accessions of eggplant had an interaction phenotype scores for disease severity within 2.0-2.5, none of accessions had disease severity equal or lower than 1.0 or with disease free plantings more than 50 percents. So all of them could not been classified as resistant. However, some accessions had been considered as tolerance. We consider tolerant characteristics in *Solanum* spp mainly according to if the infected seedlings could setting fruits after transplanted to natural field. Most tolerant accessions mainly come from wild or semi-wild species of *Solanum*. Cargo eggplant .

(II6B030 1, *S. aethiopicum*(sens. lat)), had lowest disease severity of 2.1 and 41.9% disease free ~ plantings. But it is quite difficult to introduce the tolerance gene to cultivars because of sexual incompatibility between them. It had been used as a stock in grafting for over verticillium wilt and . - the application had gotten a preliminary success in Beijing. Another tolerant accession, II6B0506, Changtsing landrace with disease severity of 2.4 was a high production cultigen and with good quality in toast. Seed increase of this accessions was made with resistant individual plants and had released to the region suffering severely for wilt disease. Seeds increase had also been made for other accessions tolerant to verticillium wilt; e.g, II6B0177, II6B0685, II6B0358, II6B0734, II6B0144, II6B0565, II6B0582, Qiqie No.3, from resistant individual plant for further evaluation and distribution. We hope it be possible to select verticillium wilt resistant line within a commercial cultivar or landrace, rather than introgressing from alien gene sources.

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SCREENING OF GERMPLASM AGAINST INSECT PESTS OF BRINJAL CROP

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The nursery of thirteen varieties/lines viz; Multan Selection, Nirala, NuY'ki, White Egg Long, Sarhandi Long, 88006-2, Tranda Purple Round, No.6, White Egg Round, cPusa Purple Long, 89016-1, Pusa Purple Round and Qaisar were sown in nursery in beginning of July, 1993 and transplanted in the field on 22.7.93. No insecticide was sprayed throughout the crop season. The data regarding Jassid and Whitefly population was recorded at weekly interval starting from 20 days after transplanting. For this purpose three leaves i.e. upper, middle and lower were observed from five randomly selected plants in each variety/line. The fruit borer percentage was recorded by counting the infested fruits out of total yield.

The data given in the table reveals that none of the varieties/lines resisted against Jassid and Whitefly. In case of Jassid the attack was more than economic injury level in all the cases. The varieties Tranda Purple Round, No.6 and Pusa Purple Round were severely attacked by the Jassid i.e. 13.82, 11.29 and 11.07 Jassid per leaf respectively. Whereas, in Whitefly some of the varieties/lines like NuY'ki, Sarhandi Long, Tranda Purple Round, No.6, Pusa Purple Long, 89016-1 and Qaisar showed some tolerance while varieties Nirala and Pusa Purple Round were heavily infested i.e. 9.90 and 9.91 whitefly per leaf respectively.

Research Conclusion.

Out of 13 cultivars tested none of them was found tolerant to fruit borer. All were severely infested. The lowest attack of 19.20% was observed in 88006-2, while the highest value was 38.54% in White Egg Round.

Table-1: Showing average population of Jassid and Whitefly per leaf and borer attack (%) on fruit.

Varieties/lines	Jassid average.	Whitefly average.	Fruit borer %age.
Multan Selection	1.91	6.81	27.17
Nirala	2.51	9.91	29.82
Nurki	3.53	3.98	26.94
White Egg Long	4.87	5.44	21.08
Srahandi Long	2.07	4.24	27.16
88006-2	2.51	6.67	19.20
Tranda Purple Round	13.82	4.47	36.67
No. 6	11.29	4.49	36.30
White Egg Round	4.33	5.47	38.54
Pusa Purple Long	2.33	4.60	25.55
89016-1	2.31	4.78	32.06
Pusa Purple Round	11.07	6.91	29.82
Qaisar	1.47	4.87	27.20

ANNOUNCEMENT

12th National Pepper Conference, Las Cruces, New Mexico, USA

The 12th National Pepper Conference was held in Las Cruces (New Mexico, USA) on August 15-16, 1994, organized by Paul W. Sosland and Javier C. Vargas. During the Conference, 25 oral presentations and 23 posters were given. The oral presentations were the following.

- McGlashan D.H., Polston J.E. and Maynard D.N. Viruses affecting Scotch Bonnet pepper in Jamaica.
- Murphy J.F. and Kyle M.M. Restricted systemic infection of *Capsicum annuum* 'Avelar' by .. Pepper Mottle Potyvirus is overcome when co-infected with Cucumber Mosaic Virus.
- Liddell C.M. and Waugh M.E. The nature of *Phytophthora capsici* pathogenicity to pepper.
- Liddell C.M., Sollars J. and Jones T. Control of *Phytophthora capsici* blight and root rot of .. peppers by irrigation management.
- Matheron M.E., Matejka J. and Porchas M. Effect of Fluazinam on growth and sporulation of *Phytophthora capsici* and development of stem and root rot on chile pepper.
- Kousik C.S., Ritchie D.F. and Sanders D.C. Use of mixed genotypes combined with copper sprays to manage bacterial spot of bell peppers.
- Motsenbocker C.E., Gersch K.P. and Lang G;A. Differential Tabasco and Cayenne pepper fruit detachment studies.
- Marshall D.E. Mechanical pepper harvesting status-worldwide
- Davis J.H., Carson S.R., Flores A.M. and Sutherland C.A. Use of an aggregation pheromone to monitor pepper weevil (*Anthonomus eugenil*) in New Mexico during 1993.
- Sutherland C.A., Davis J.H., Eller F., Burke H. and Pakaluk J. The weevil fauna associated with chile in New Mexico (*Insecta: Coleoptera: Curculionidae*).
- Anciso J.R. and Chambers C. Utilizing pheromone traps in an integrated pest management approach for the pepper weevil in lower Rio Grande valley, Texas.
- Prostak D.J. and Neary P.E. Developing an economic threshold for European Corn Borer on bell peppers.
- Walter J.F. and Harris J.R. Neemix: a new tool in pepper pest control.
- Villalon B. Development of multiple disease and insect resistance Capsicums
- Blauth J.R., Murphy J.F. and Kyle M. Genetics of resistance to Pepper Mottle Potyvirus and Tobacco Etch Potyvirus in *Capsicum*.
- Poulos J.M., Green S. and Wang J. Genetics of resistance to Chili Veinal Mottle and Pepper Veinal Mottle Viruses in *Capsicum annuum*.
- Villalon B., Campos M. and Avirineni V.S. Plant regeneration from tissue culture and ... transformation of selected multiple virus resistant *Capsicum* genotypes.
- Kyle M., Prince J.P., Lackney V.K., Blauth J.R., Murphy J.F. and Angeles C. Molecular markers in *Capsicum*: fingerprint, mapping and tagging disease resistance genes.
- Castillo C., Fender S. and O'Connell M. Isolation of placental-specific cDNA from pungent and non-pungent Chiles.
- Roe N.E. and Stoffella P .J. Influence of 'uncured' compost on growth and yield of bell pepper. Grun P. and Orzolek M.D. Growth of *Capsicum chinense* landraces on contrasting soils in Pennsylvania: the wilt problem.
- Motsenbocker C.E., Mulkey W.A., Boudreaux J.E. and Buckley J.B. Influence of nitrogen rates and plant spacing on mechanical harvested Jalapeno and sport pepper.
- Shuler K.D. and Vavrina C.S. Effect of transplant depth on bell pepper growth and yield. Cantwell M. Storage conditions for intact and diced bell peppers.
- J. Singh. Performance of indian hot peppers in the International Hot Pepper Trial Network.

For information and Proceedings request

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The Proceedings with the abstracts of both the oral communications as well as the posters given during the Symposium have been published.

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BOOK ANNOUNCEMENT

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