# capsicum newsletter

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Section of Plant Breeding and Seed Production
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The picture in the cover is derived from the “Herbanrio nuovo di Castore Duante”, Venetia, MDCXXXVI

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## APPRECIATION TO DR. EDMOND POCHARD

Dr. E. Pochard recently retired from his activity in INRA, France. ~ We believe that all the members of the EUCARPIA Capsicum group will join us in whishing Dr. Pochard a challenging new post-Capsicum career.

Dr. Pochard is an outstanding pepper agronomist, geneticist, phytopathologist, pepper breeder and above all a generous person who inspired and helped many of us with scientific encouragement and valuable germplasm!

The late Dr. P. Smith named Dr. Pochard as number one pepper geneticist in Europe.

We need just to mention the 'Lamuyo' F1 hybrid and the haploids-dihaploids stories to appreciate the brilliance of his scientific leadership. It is our privilege to wish Dr. Pochard a pleasant retirement in the knowledge that he will always find fresh fields for creative expression.

The Scientific Committee and the Editorial Board

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## FOREWORD

Our faithful readers will find a surprise together with this issue of "Capsicum Newsletter". In fact, we have printed a special volume of our Newsletter. It includes the Proceedings of the EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant, which is going to be held next September in Rome, Italy. We hope that our decision to distribute the Proceedings using the mailing list of "Capsicum Newsletter" will be appreciated.

The invited paper included in this issue is particularly interesting: it deals with the molecular mapping in pepper and has been written by J. Prince and his collaborators. We thank them very much for their kind co-operation. As usual, any suggestions on the subjects and/or authors to be considered for the invited papers in the following issues of "Capsicum Newsletter" will be appreciated.

Although several contributions have not been accepted (the Scientific Committee has started doing its job we have not modified any of the published papers. Therefore, the authors themselves are not only responsible for the scientific content but also for the form of their own reports.

A ‘literature review' is again present in this issue. We hope it will be useful and we would like to remind you to send us a copy of your articles, especially those published in journals of limited circulation.

Please, remember that a subscription fee to the Newsletter is requested. The subscription fees have not been changed: 20 U.S.$ for normal subscribers and 100 U.S.$ for supporters. Remember also that now it is possible to book your own copy of the journal: just fill in the

order form on page 55 and send it to us. In the meantime your subscription fee should be paid directly to EUCARPIA Secretariat (please note that the address has been modified!). Please, do not send cheques to us in Turin, as we are not allowed by Italian law to run any financial activities.

Again we have to complain about the lack *of* attention paid by many authors to the instructions on the enclosed sample sheet. Please, cooperate with us and follow these instructions very carefully. Otherwise we will not accept the contributions and they will be sent back to the authors.

Lastly, we have to announce that the journal's Editorial Board has decided to enlarge the space available to each contribution. Starting from the next issue, you will have at your disposal four pages per article.

Piero Belletti and Luciana Quagliotti

Turin, 31st May 1992

**LIST OF THE AUTHORS**

**LIST OF THE CONTRIBUTIONS**

Capsicum Newsletter, 11 (1992), 11-18. Invited Paper.

# MOLECULAR MAPPING IN PEPPER:. AN UPDATE

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.
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Pepper (Capsicum L.) is an important vegetable crop worldwide. Much classical breeding work has been done to transfer disease resistances and desirable horticultural traits (see Greenleaf, 1986, for a thorough review of pepper breeding, and Pickersgill, 1989, for a review of genetic resources in Capsicum). Many traits, especially those conditioned by genes with incremental effects, are not amenable to classical breeding approaches. .In these cases, a marker that is linked and easily selectable could increase the efficiency of gene transfer and selection. Morphological genes as tags are often difficult to use, as major genes may control a wide range of responses, may affect expression of the gene of interest, and may not be dominant and scorable in all genetic backgrounds.

Phenotype-neutral molecular markers such as isozymes and restriction fragment length polymorphisms (RFLPs) can be used to tag genes and to construct genetic maps of the entire genome. Major genes can be tagged by one or more molecular markers for tracking in a breeding program (Tanksley et, al., 1989), complex genetic traits can be dissected into their component parts (Osborn et.al., 1987, Nienhuis .et al., 1987, and Paterson et. al., 1990), introgression of genes can be monitored (Young et. al., 1988), genetic variation within and between species can be examined (Figdore et al., 1988, Wang and Tanksley, 1989, and Miller and Tanksley, 1990a). and homologies among related genomes can be investigated (Tanksley et. al.,1988, and Bonierbale et. at.,1988). Livneh.et. al., (1990) have used an RFLP for hybrid identification in

pepper seed production. For recent reviews of the use of molecular markers in plant improvement, see Paterson et al., (1991) and Tanksley.et al., (1989).
For making a molecular map of any crop species, polymorphism between interfertile plants and the segregating population that results from a cross between those plants are needed (in the case of RFLPs, the polymorphisms are different restriction fragments detected by hybridization to cloned pieces of DNA). Many breeding populations are usually available, but they may have inadequate molecular polymorphism, and it is important to measure the amount of polymorphism at the outset of any mapping program. Molecular polymorphism in Capsicum has been detected for chromosomes (Pickersgill, 1977), isozymes et al., (Jensen .et.a1., 1979 and Loaiza-Figueroa etal., 1989), soluble seed protein gel mobility (Panda et al. 1986), and AFLPs (Prince et al., in preparation). The use of these measures of variation (except for RFLPs) and of classical taxonomic characters has been reviewed by Pickersgill (1988).

We present here a brief overview of the history of the development of the genetic map of Capsicum and the current status of some molecular mapping projects.

## Molecular maps constructed in pepper

In 1984, Tanksley published the first molecular map of pepper based on the segregation data of fourteen isozyme loci. Four linkage blocks were detected, and five hybrid primary trisomics made by Pochard (1970) were used in dosage analysis to determine the chromosomal locations of three of the isozyme loci. Data suggested that three of the markers were near the site of a reciprocal translocation between the two mapping parents.

Because of the relatively small number of available isozyme markers and the even smaller number that segregate in any given cross, Tanksley et al (1988) decided to use RFLPs, choosing a cDNA library constructed from whole tomato leaf mANA (Bernatzky & Tanksley, 1986). These clones represented expressed sequences in the genome. A package map consisting of 80 molecular markers in fourteen linkage groups was constructed using an interspecific backcross mapping population. Major results from this preliminary mapping study indicated that pepper and tomato shared the same gene repertoire in that all of the cDNA clones from tomato hybridized to pepper. The order of genes on

the chromosomes were different between the two genera. Based on a comparison of the linkage maps, a minimum of 32 chromosome breakage events had occurred since the pepper and tomato lineages diverged. Some large linkage blocks remain conserved, however, with the largest being 63 cM long. By contrast, the molecular maps of potato and tomato differ by only a few inversions (Bonierbale elal., 1988 and Tanksley m-al., in preparation).

In order to improve the linkage map of pepper, we (Prince, Pochard, and Tanksley, in preparation) have constructed a new map using an interspecific F2 mapping population. The use of an F2 population allowed improved resolution with a comparable population size. Random tomato genomic clones (Miller & Tanksley, 1990b) and cDNA clones from tomato leaf epidermal tissue (Yu .e1..al., unpublished) were placed onto this map in the hopes of coalescing the map into twelve linkage groups, corresponding to the haploid chromosome complement of pepper (Pickersgill, 1977). Presently (February 1992), eleven-large linkage groups and eight small linkage groups have been formed using one hundred and ninety-two molecular markers. The average density of the map is approximately one marker for every 3.5 cM. Again, similarities between the tomato and pepper maps are evident, with large linkage blocks conserved and considerable chromosomal rearrangements that may cluster around centromeric regions. The random tomato genomic clones did not hybridize- as readily to pepper as did the cDNA clones, with only about half of them being usable because of low homology or increased copy number in pepper.

Following are the strategies that we are using to attempt to coalesce the map into the expected twelve linkage groups.

**Improving the RELP map of pepper.**

Random. genomic pepper clones. We are currently developing a library of pepper genomic clones to use in more mapping (Blauth et al., unpublished). Based on data from our current mapping work, we have noticed that clones from several chromosome arms of tomato do not hybridize to pepper and that we may be missing corresponding regions of the pepper chromosomes (Prince, Pochard, and Tanksley, in preparation). These regions may be undergoing more rapid sequence divergence and should be detectable with the use of clones from the same species.

PCR-based markers. Gaps in our molecular map may be due to regions of rapid evolution, as noted above, or by stretches of repetitive DNA for which DNA crones cannot be used as markers. The RAPD technique (random amplification of polymorphic DNA, Williams 1990. and Welsh & McClelland. 1990) a polymerase chain reaction-based mapping technique. may allow us to pick up some of these regions. Ample polymorphism is detected and currently we are adding markers to the map (Prince et al. unpublished).

Pulling more clones off of the high-density tomato map. Now that a high density RFLP map of tomato has been completed with over 1000 markers (Tanksley et al., in preparation) we have a much larger pool of tomato clones to draw upon from all regions of the genome. We will target underrepresented regions to see if we can close the gaps between' some of our linkage groups.

**Current applications of molecular mapping in pepper**

We are currently working on mapping the genes for resistance to some of the most devastating viruses affecting pepper: cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), and the related potyviruses pepper mottle virus (PeMV), tobacco etch virus (TEV), and potato virus Y (PVY). Classical breeding to produce multiple virus-resistant plants remains difficult because of the multigenic nature of some of the resistance genes. The difficulty in scoring the phenotype. and the difficulty in monitoring more than one virus in a given plant at the same time. We hope to tag resistance genes from several sources to these viral diseases with molecular markers and use these markers to facilitate the breeding of superior lines. .

**CMV** High levels of resistance have been located in two accessions of .c frutescens from southern Mexico by loaiza-Figueroa and Prowidenti (unpublished). Resistance to CMV has been difficult to transfer despite reports of resistance in C. annuum cv. 'Perennial' and C. frutescens (Loaiza-Figueroa and Prowidenti, unpublished) We are working with several inter- and intra-specific populations in an effort to locate markers linked to resistance from these sources. Segregating populations are being screened for resistance and are being

mapped with our framework of seventy RFLP markers spaced at 5-cM intervals along the map of pepper.

**TMV** The mapping population used to construct our most recent map of 192 markers also segregates for TMV resistance. Screening of resistance in F3 families has just finished and data are being analyzed.

Potyviruses. A new population that is polymorphic at the DNA level and - that segregates for resistance to the potyviruses has been grown and is now being screened for resistance and being mapped with the same set of framework clones as are the CMV populations (Blauth et al., unpublished). In addition, a pepper genomic library has been constructed which should cover areas of the genome that are not well-represented with the libraries used to date.

Work by other groups in mapping projects in pepper. Two other groups are currently pursuing major mapping efforts in pepper. Lefebvre, Palloix, Pochard, and Rives at the Station d' Amelioration des Plantes Maraicheres, INRA, Avignon, France are currently working on resistance genes for several important fungal and viral diseases, and Massoudi, O'Connell, and Bosland at New Mexico State University in Las Cruces, New Mexico, United States, are currently working on Phytophthora resistance.

Coordinating mapping efforts. An effort is underway to coordinate mapping in the genus, and all interested workers are encouraged to contact Molly Kyle. Inter-institutional mapping efforts have been organized for a number of crops and have been especially successful in cereals. We hope that any groups interested in developing programs in this area and contributing to a collective effort will join this consortium, to be organized at the National Pepper Conference in August, 1992, in Monterey, California, USA.

## Conclusion

While mapping efforts are still in progress we are very optimistic that the map will develop quickly. A concerted effort is now possible thanks in part to support through the United States Department of Agriculture National Research

Initiative for the Plant Genome. The availability of the high-density tomato RFLP map offers a number of advantages in the construction of a map of pepper and in resolving the twelve linkage groups (Tanksley et al. submitted). The tremendous number of horticultural types in pepper and the difficulty in achieving the most important breeding objectives, despite considerable investment by workers around the world, suggest that marker-assisted selection may be especially useful in Capsicums.

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# INDUCED DOUBLE TRISD"IC IN CHILLI Capsicum annuumL.

L.D.Meshram, B.K. Kukade. M.B.Wadodkar, and R.A.Naphade.

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A double trisomic '[2n +1 +11] chili plant was recovered 30 Krad dose of gamma irradiated population of chilli cultivar Pusa Jwala' in $R^{l}$ generation.

The plant was identified due to its distinct difference. In it morpholol1cal characters as compared to normal untreated, plants. Morphologically the plant vas having dark green an leathery leaves, big flowers having 70-80 per cent pollen sterility. The plant borne the fruit. which were long shriveled in appearance. The fruits turned red in colour ripening and were having 3-4 seeds per fruit a. against *30-40* seeds in control.

The flower buds from the plant were fixed in acetic alcohol [1:3] and squashed in 1% aceto-carmine stain . At diakinesis 10 bivalents and two trivalents and $12^{11} + 2^{1}$ were observed. The trivalents showed three [pan, chain and y-shape] configurations. At metaphase 1 out of 1100 PMCs studied 45 showed 12 + 2 and quadrivalent was noted indicating that both the unicalents present in the plants were of different types. At anaphase 1 the chromosome disjunction was 13-13, 12-14 and 11-15, while in some PMC univalents remained as leggard. Meshram et al. [1980, 81] also reported that radiation induced trisomic and triploid in chilli.

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# INHERITANCE OF ANTHOCYANIN PIGMENTATION IN CHILLI (Capsicum annuum L.)

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To minimize varietal deterioration in a developed variety there is a need to have a marker to isolate true to type plants in seed production programmed. Since anthocyanin pigment which imparts purple colour in plant may be used as the marker for effective varietal identification, it is therefore important to know the type and number of gene (8) responsible for the trait.

Genetics of pigmentation was studied from six generations . (Pl, P2, 1'1, 1'2,BCl and BC2) ,of an intervarietal cross SPE-l (Purple stem) x 'Shalinar Long (Green stem) during kharief 1989 at Vegetable Experimental Farm, Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar, Srinagar, India. The anthocyainin pigmentation from the stem of each plant' was determined following the method as described by Swains and Hills (1959).

The mean anthocyanin content in Fl was slightly more than the mid parent value indicatino partial dominance of purple stem over green stem (Table 1). The continuous variation observed in F2 and Backcross generations suggested its polygenic nature. The simple additive-dominance model was inadequate, thereby indicatino the presence of non-allelic interactions. The six parameter model which included interaction components revealed only components additive(d) and dominance x dominance(l) to be significant. However in both perfect fit solution and 8imple additive-dominance model, the magnitude of adaitive component was much higher (Table 1) indicating the Importance of additive gene action in the inheritance of anthocyoin pigmentation. This fixable addition component can therefore be exploited through simple selection.

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Table 1: Mean anthocyanin content (mg/g) of different generation and content of generation means of cross SPE –1x Shelimar long

| Means (mg/m) | Three parameter Medal | Six parameter model |
|---|---|---|
| P1=1.32 = 0.015 | M=0.77 + 0.008 | M= 0.88= 0.135 |
| P2= 0.28+ 0.0010 | [d]= 0.48+0.008 | [d]= 0.52+0.009 |
| F1= 0.88+ 0.016 | [h]= 0.02+ 0.017 | [h]= 0.47+ 0.310 |
| F2 0.75+ 0.029 | | [I]= 0.08+ 0.134 |
| B1= 0.97+025 | | [j] = 0.06+0.069 |
| B2= 0.48+0.021 | (d.f)= 37.86 | [l]= 0.43+0.182 |

** Significant at 5% and 1 % level respectively

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# *A giant chilli plant as a transgressive variant of Capsicum Frutesecens*

N. Lakshmi, T. Srivalli and V.V ramachandra Rao

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Capsicum Frutecens popularly called 'seema mirapa' in Andhra Pradesh is a highly pungent species with very small fruits and maximum capasaicin content. Based on fruit colour, three cultivars are recognized namely green, white and yellow, the first two are immature fruit colours while the third represents fruit colour at maturity.

All the three cultivars are erect, tall, vigorous with good spread and big green ovate leaves. Flowers are small with greenish white corolla and bluish green anthers. Fruits are erect, tiny, spindle-shaped, green or white when immature turning into yellow or red at maturity depending on the cultivar.

Intraspecific hybridizations have been made in this laboratory in six combinations Involving the three cultivars in order to know the compatibility and cytogenesis relations of the three cultivars. In the hybrid progeny of the green and white cultivars a very tall chili plant with very good spread, profuse branching and high yield was noticed in the year 1990. The morphometrics of this plant along with the two parental cultivars is set out in Table 1. Data revealed that there is three to four fold increase in plant height, spread, number of branches and chlorophyll content, two fold increase in leaf area, and 5-6 fold increase in yield than those of the parental species. However there is decrease in. fruit girth and ascorbic acid content. Cytological studies revealed no appreciable change in meiotic metrics like Chiasma frequency per cell, per bivalent and percentage of ring and rod bivalents. Progeny studies revealed the recurrence of green and white cultivars suggesting that the parent plant is a hybrid involving the two cultivars. Since the hybrid exceeds the parents 1n several parametres it 1s considered as a transgressive variant formed as a result of recombination of genetic factors.

| TABLE 1. Morphometrics of parents and giant chili hybrid | | | | |
|---|---|---|---|---|
| S.No. | Character | C.f.g. | C.f.w. | Giant Hybrid |
| 1. | Plant Height (cm) | 59.60 | 61.33 | 205.00 |
| 2. | Plant Spread (cm) | 89.00 | 83.67 | 290.00 |
| 3. | No. of branches per plant | 149.00 | 112.00 | 448.00 |
| 4. | Leaf length (cm) | 7.50 | 7.14 | 10.50 |
| 5. | Leaf breadth (cm) | 2.80 | 2.65 | 3.42 |
| 6. | Leaf area (cm) | 8.65 | 8.49 | 18.80 |
| 7. | No. of fruits per plant | 125.00 | 148.00 | 750.00 |
| 8. | Fruit length (cm) | 6.00 | 5.17 | 6.02 |
| 9. | Fruit girth (cm) | 3.20 | 3.10 | 2.22 |
| 10. | Chlorophyll content mg/gm | 2.306 | 2.071 | 6.500 |
| 11. | Ascorbic acid in unripe fruits (mg/100gm) | 104.00 | 78.00 | 40.00 |
| 12. | Ascorbic acid in ripe fruits (mg/100gm) | 161.20 | 150.80 | 64.00 |
| 13. | Chaisma frequency per cell | 20.13 | 20.50 | 20.27 |
| 14. | Chiasma frequency per bivalent | 1.68 | 1.67 | 1.69 |
| 15. | Percentage of ring bivalents | 67.78 | 65.08 | 69.32 |
| 16. | Percentage of rod bivalents | 32.22 | 34.92 | 30.68 |
| C.f.g = Capsicum frutescens (green) | | | | |
| C.f.w = Capsicum frutescens (white) | | | | |

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# Induction of Callus and Organcogenesis In Pepper

R. Pundeva and N. SimeonovaInstitute of Genetics, BAS, Sofia 1113, Bulgaria

Donor material – Capsicum annuum F. Macrocarpum (cv.'Albena') C. annuum F. microcarpum (No 9), C.baccatum var. Pendulum (F.smith) Cultured explants – hypocotyl, cotyledon and root segments of in vitro grown seedling in cotylendon stage.

Nutrient media- PNS medium enriched with different supplements (Table 1). Growing conditions – temperature 25oC photoperiod – 16 h light. Table 1 shows the morphogenetic response of the explants treated with different hormone concentration. NAA induced direct root formation, sometimes attended with slight callus initiation. KEW with Bap stimulated shoot bud development. 2,4-D combined with KIN envoked abuntdant friable callus growth, while media containing IAA and Bap promoted the appearance of compact callus and differentiation of shoots and roots on it. Basal Hypoctlyl segments has higher predilection to rooting, while middle and apical ones preliminary preduced shoots. This tendency significantly decreased on more effective media (NO6,7,8) whole hypocotyl some. Three sectioned cotyledons also demonstrated different morphogentic response. Higher callus and oranogentic capacity has basal segment relatively good capability showed middle ones and less productive were apical sections.

Root explants possessed similar tendency. Basel segments were more effective regarding the rest of root parts but were comparatively less active towards the other tested organs.

The genotypes included in this study showed difference in the speed of their reaction to the corresponding nutrients media with respect to the callus mass and the number of meristemic buds per segment. C.baccatum var. pendulum was more effective than C.annuum accessions.

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Sripitist P., Nawata E., Shigenaga S., 1987. In vitro shoot- for ming capacity of cotyledon explants in red pepper (Capsicum annuum L. cv. Yatsursa) Japan J Breed, 37, No 2:133

Table 1. Morphogenetic response of meristematic explants to MS medium with different combinations of supplements (mg/l).

| Hormones | NAA 0.5 | IAA 0.5 | BAP 2.0 | DROP 0.5 | DROP 2 IAA 0.5 | IAA 1 BAP 2 | IAA 1 BAP 5 | IAA 0.5 BAP 1 | 2,4D 0.5 KIN 0.5 | KIN 1 BAP 3 |
|---|---|---|---|---|---|---|---|---|---|---|
| Explants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| cv.'Albena' | | | | | | | | | | |
| hypocotyl | R | C | C | C | C | C+R | C+R | C+R | C | C+R+S |
| cotyledon | R | C+R | C | C | C+S | C+S | C+S | C+R | C | C+S+R |
| root | R | C+R | C+R | C+R | C+R | C+R | C+R | C+R | C | C+R |
| No 9 | | | | | | | | | | |
| hypocotyl | R | C | C | | C | C+S+R | C+S+R | C+R+S | C+R | C+R+S |
| cotyledon | R+C | C+R | C | C | C+S | C | C+R+S | C+R+S | C | C+S |
| root | R | C+R | C | - | - | C | C+R | C+R | C+R | C+R |
| C.pendulum | | | | | | | | | | |
| hypocotyl | R | C | C | C+S | C | C+S+R | C+S+R | C+S+R | C+S | C+S+R |
| cotyledon | R | C+R | C | C | C | C+S+R | C+S | C+S+R | C+S | C+S+R |
| root | R | C+R | - | C | C+R | C+S+R | C | C+S+R | C+S | C+S+R |

C = callus; R = roots; S = shoots

# IN VITRO ANDROGENESIS IN CUBAN F1 HYBRIDS OF SWEET PEPPER

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During the summer of 1989 the technique proposed by Chambonnet in 1988 for the obtention of haploid plant through in vitro androgenesis was applied to seven cuban F1 hybrids of sweet pepper. The plants were grown in the field, no phytosanitary treatment was applied for aphyds control. The floral bottoms were removed in August at the morphological state in which the length of the petals and the sepals were equal, corresponding to the beginning of the first pollinic mitosis. The anthers were put on the induction medium (P) recommended in the technique; it was enriched with ampicillin (10 mg/l), ampicillin plus streptomicin (10 mg/l) or rifocin (5 mg/l).

The Petri dishes containing the anthers were placed at +35°C during 1-2 or 8 days in the darkness and then transferred .at +25°C and 12 hours of photoperiod; the technique was continued as e.stablished. We could prove feasibility of using in vitro androgenesis from cuban hybrids. Timing for treatment at +35°Cand darkness can be variable, from 1-2 to 8 days according to genotype.

A preliminary field trial was caried out in Cuba in 1991 including 19 doubled-haploid (DH) lines, outstanding for compact plant habit, earliness, productivity and disease tolerance.

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Capsicum Newsletter, 11 27-28.

# A Rapid Method For the Screening of Salinity resistance in Red Pepper Varieties

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Determination of salinity resistance is one of the thrust areas in any crop improvement programmes and there is greater need for the development of varieties resistant to saline soils. Sore conducting the field trails a rapid screening can be done by testing salinity resistance on the basis germination counts. In the present study an attempt has been made experimentally to screen some red pepper varieties or salinity resistance.

Seed materials of five different varieties of Capsicum annuum viz., Santaka, 197, 960, jawahar and 235 and one of Capsicum Chinese were used in the study of Nacl salinity effect on seed germination.

The seeds of these varieties were soaked in NaCl soulution in different concentration (1-10%) at four time intervals (6, 12,18 and 24 hours) Control were maintained for all samples by soaking them n distilled water. After Treatment the seeds were germinated in petriplates. The percentage interval in record.

Data revealed that in all varieties germination percentage is inversely related to increase in concentration.

Capsicum Chinense and Santaka variety of Capsicum annum shoed high salinity tolerance since the germination good even at higher concentrations (10%) and longer duration of saokng 24 hours Table I. Hence these two varieties are considered to be showing higher salinity resistance. However their stress tolerance is to be tested under field condition also.

The remaining four varieties are considered to be salt sensitive ones since their germination percentage is affected geately at higher concentrations of NaCl.

**TABLE 1.** **Percentage seed germination of some red pepper varieties under the influence of Nacl salinity**

| Name | Period of treatment (hours) | Concentration of Nacl | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | 1% | 2% | 3% | 4% | 5% | 6% | 8% | 10% |
| *C. annuumm* variety '960' | 6 | 92 | 96 | 92 | 84 | 80 | 84 | 72 | 68 | 68 |
| | 12 | 88 | 88 | 80 | 84 | 80 | 76 | 76 | 68 | 60 |
| | 18 | 92 | 84 | 92 | 84 | 80 | 76 | 72 | 72 | 64 |
| | 24 | 96 | 88 | 84 | 76 | 76 | 72 | 72 | 68 | 60 |
| *C. annuum* variety 'Jawahar' | 6 | 100 | 100 | 96 | 96 | 88 | 88 | 76 | 72 | 68 |
| | 12 | 96 | 96 | 92 | 84 | 84 | 80 | 76 | 76 | 72 |
| | 18 | 92 | 92 | 88 | 88 | 84 | 76 | 76 | 72 | 68 |
| | 24 | 92 | 88 | 88 | 84 | 84 | 80 | 76 | 68 | 64 |
| *C. annuum* variety 'Santaka' | 6 | 100 | 100 | 100 | 92 | 88 | 88 | 84 | 80 | 80 |
| | 12 | 100 | 96 | 92 | 92 | 84 | 84 | 80 | 76 | 80 |
| | 18 | 92 | 92 | 88 | 88 | 84 | 84 | 80 | 76 | 76 |
| | 24 | 92 | 92 | 84 | 88 | 84 | 80 | 80 | 80 | 76 |
| *C. annuum* variety '197' | 6 | 96 | 88 | 88 | 84 | 80 | 72 | 76 | 72 | 68 |
| | 12 | 96 | 96 | 92 | 84 | 84 | 80 | 72 | 68 | 64 |
| | 18 | 92 | 88 | 88 | 80 | 76 | 76 | 72 | 68 | 60 |
| | 24 | 76 | 80 | 80 | 72 | 76 | 72 | 68 | 64 | 60 |
| *C. annuum* variety '235' | 6 | 96 | 96 | 92 | 84 | 88 | 84 | 80 | 72 | 68 |
| | 12 | 92 | 88 | 88 | 80 | 84 | 76 | 76 | 72 | 64 |
| | 18 | 92 | 84 | 88 | 84 | 84 | 72 | 72 | 68 | 60 |
| | 24 | 88 | 88 | 84 | 76 | 76 | 72 | 68 | 64 | 60 |
| *C. chinense* | 6 | 100 | 96 | 92 | 96 | 92 | 88 | 84 | 80 | 80 |
| | 12 | 100 | 100 | 100 | 96 | 96 | 92 | 88 | 84 | 80 |
| | 18 | 96 | 88 | 88 | 84 | 76 | 80 | 76 | 76 | 72 |
| | 24 | 92 | 92 | 84 | 84 | 80 | 76 | 76 | 72 | 72 |

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# BIOLOGICAL CHARACTERIZATION OF SPANISH ISOLATES OF TOBAMOVIRUS.

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Characterization of nine tobamovirus isolates collected in Spain from pepper (seven) and tomato (two) was accomplished by studying the responses of Capsicum spp. genotypes which carry the L+, Ll, L2, L3 and L4 resistance genes (BOUKEMA, 1983)

According to biological reactions on a series of test plants (Table 1), three of the nine isolates were classified as ToMV (Tomato Mosaic Virus) while the other six belonged to the PMMV (Pepper Mild Mottle Virus). On the basis of the interactions with the L gene for resistance in pepper genotypes we have found that five of those PMMV isolates belonged to the Pl-2 pathotype and the sixth one to the Pl-2-3 pathotype (Table 2).

In Spain the number of commercial Fl hybrids which carry the L3 allele is still very low. That would be the reason for the scarcely proportion of Pl-2-3 pathotype found in this work and by other researchers in Spain (ALONSO et al., 1989; CUADRADO, 1991, personal communication).

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in Capsicum chacoense Hunz. and male sterility in progenies from the cross chacoense x annuum L. Vth Eucarpia Capsicum Meeting. Plovdiv (Bulgaria):

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Table 1. Response of a set of test species to nine tobamovirus isolates.

| Test species | Virus isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | T-1-75 | T-5-84 | P-3-85 | P-10-85 | P-11-85 | P-12-85 | P-8-88 | P-9-88 | P-3-90 |
| Chenopodium amaranticolor | lln/o | lln/o | lln/o | llcn/o | llcn/o | llcn/o | llcn/o | llcn/o | llcn/o |
| Chenopodium quinoa | lln/o | lln/o | lln/o | llcn/o | llcn/o | llcn/o | llcn/o | llcn/o | llcn/o |
| Datura stramonium | LLn/o | LLn/o | LLn/o | lln/o | lln/o | lln/o | lln/o | lln/o | lln/o |
| Gomphrena globosa | --- | --- | --- | --- | --- | --- | o/o(-) | --- | /o(-) |
| Lycopersicon esculentum 'Montserrat' | o/Mo | --- | --- | o/o(-) | --- | --- | --- | --- | o/o(-) |
| Nicotiana clevelandii | o/MoSt | o/MoSt | o/MoSt | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo |
| N. glutinosa, N. tabacum 'Xanthi nc' | LLn/o | LLn/o | LLn/o | LLn/o | lln/o | lln/o | lln/o | lln/o | lln/o |
| N. tabacum 'Paraguay' | LLn/o | LLn/o | LLn/o | lln/o | lln/o | lln/o | lln/o | lln/o | lln/o |
| N. tabacum 'Samsun' | o/Mo | o/Mo | o/Mo | o/o(+) | o/o(+) | o/o | o/o | o/o | o/o(-) |
| N. rustica | --- | --- | --- | lln/o(-) | --- | --- | --- | --- | lln/o(-) |
| Petunia hybrida 'Rose du Ciel' | LLn/o(-) | LLn/o | LLn/o | o/o | --- | --- | --- | --- | o/Mo |
| Physalis floridana | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo |

Legend: Local reaction/Systemic reaction
a = Abscission
c = Chlorotic
cn= Chloronecrotic
LL= Big local lesion (2-3 mm)
ll= Small local lesion (1 mm)
Mo= Mosaic
n = necrotic
St = Stunting
Tn = Top Necrosis
o = No reaction
---= Non-inoculated species
(+)= Positive infection by backinoculation
(-)= Negative infection by backinoculation
T = Tomato
P = Pepper

Table 2. Response of Capsicum spp. genotypes to nine tobamovirus isolates.

| Test species | Virus Isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | T-1-75 | T-5-84 | P-3-85 | P-10-85 | P-11-85 | P-12-85 | P-8-88 | P-9-88 | P-3-90 |
| Capsicum annuum 'Doux des Landes' | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo |
| C. annuum 'Yolo Wonder' | LLna/o | LLna/o | a/o | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo |
| C. frutescens 'Tabasco' | LLna/TN | LLna/TN | a/TN | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo | o/Mo |
| C. chinense P.I. 159236 | lln/o | lln/o | lln/o | lln/o | lln/o | lln/o | lln/o | lln/o(-) | o/Mo(+) |
| C. chaconse P.I. 260429 | lln/o | --- | --- | a/o | --- | --- | a/o | --- | lln/o |

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# DEVELOPMENT OF A SCREENING TECHNIQUE FOR EVALUATION OF RESISTANCE TO PSEUDOMONAS SOlANACEARUM IN PEPPER

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Bacterial wilt caused by Pseudomonas solanacearum is an important disease of pepper (Capsicums spp.), especially sweet pepper, under certain tropical conditions. There is little information about the extent of damage caused by this disease. Resistant varieties offer an effective means for its control. Development of a standardized technique in evaluating pepper germ plasm is necessary, as uniform field epidemics are often difficult to create for pepper crops. Three inoculation techniques toothpick stabbing with a bacterial culture, soil drenching with root wounding and soil drenching without root wounding - were tested using 20- and 35- day old seedlings with two isolates (PS 71 and PS 4) of f solanacearum. Soil drenching was applied with a 107 ceil/mi inoculum suspension of the bacterium. The susceptible variety 'Giant Bell (F1)' was used. Toothpick stabbing and soil drenching with root wounding gave a similarly high percentage of wilt (Fig. 1). The root wounding technique, however, was relatively easy to apply and resembled natural inoculation most. The percentage wilt was not significantly affected by the age at which the plants were inoculated. Although there was no significant difference in percentage wilt caused by the two strains, the pepper isolate PS 71 was slightly more aggressive than the tomato isolate PS 4 (Fig. 2). Other studies to confirm resistant genotypes using the same inoculation techniques are in progress.

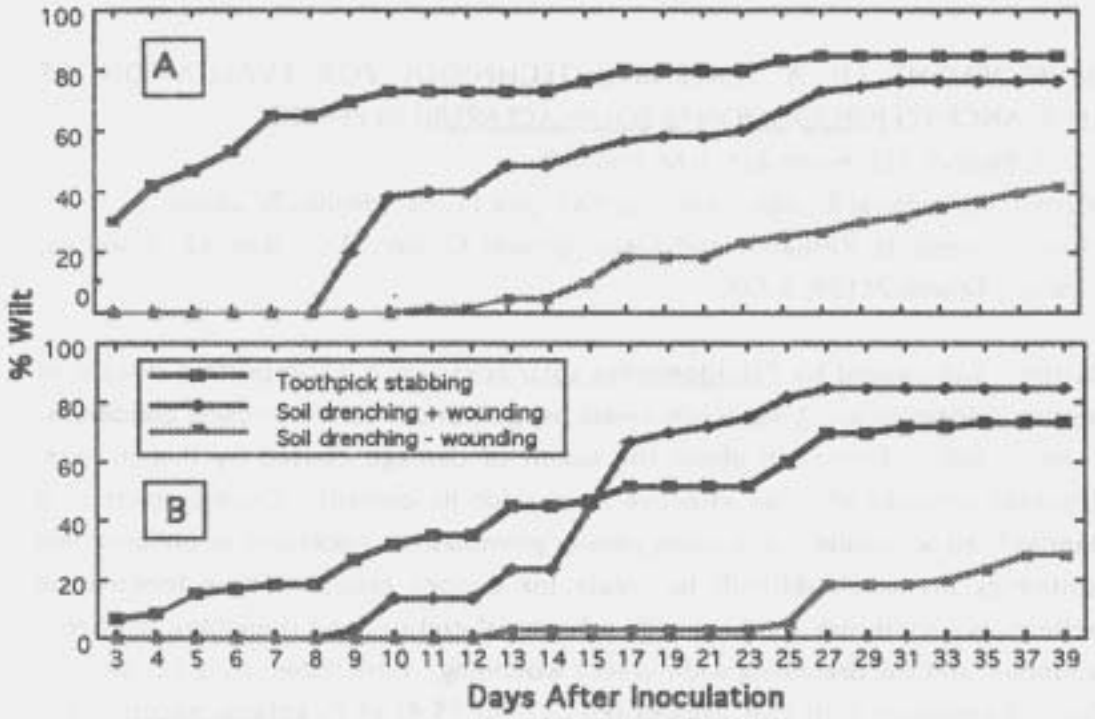


Fig. 1. Bacterial wilt on 20- (A) and 35-day old peppers (B) inoculated with three inoculation techniques.

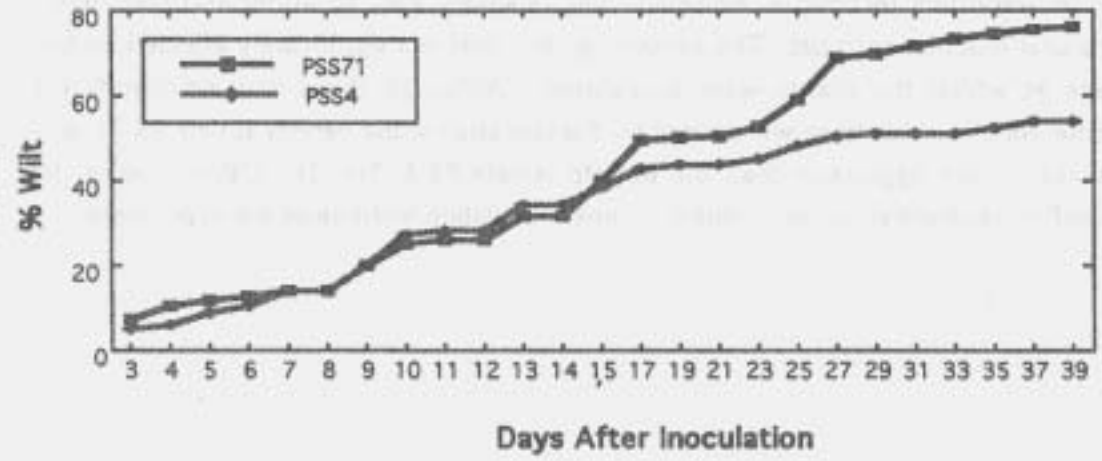


Fig. 2. Percentage bacterial wilt combined over 20- and 35-days and over inoculation techniques for two strains of Pseudomonas solanacearum.

Capsicum Newsletter 11 (1992). 33-34.

# SOURCES OF RESISTANCE AMONG CAPSICUM SPP. TO FUSARIUM WILT OF PEPPER

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Fusarium oxysporum f. sp. capsici (FOC) was recently described as the causal agent of a vascular wilt of pepper. The pathogen was isolated from wilted 'Tabasco' Capsicum Frutescence plants and from soil from production fields at Avery Island, Louisiana. Occurrence of the disease is associated with periods of high rainfall, and is more prevalent in poorly drained areas of the fields. Disease symptoms in the field include flagging of the leaves, slight yellowing of the foliage, vascular discoloration of the roots and crown, and a permanent wilt of the plant isolates were obtained by plating surface sterilized tissue on acidified PDA, or air dried field soil on Komada'a selective medium. Plant inoculation studies were conducted to confirm pathogenicity of isolates. Inoculum consisted of two 7-day-old cultures propagated by streaking conidia on PDA in 10cm petri plates and incubating at room temperature under continuous fluorescent light. Contents of both plates were blended for about 10 seconds in a Warning blender with 250ml of distilled H2O to form a slurry consisting of conidia, mycelium, and agar bits. Two to 3-wk-old seedlings of two susceptible cultivars, 'Yolo Wonder' ,and 'Tabasco', and a resistant accession, PI 188803, representing ,C. annuum, .c. frutescens, and,C. baccatum, respectively, were root dip inoculated (3.5minl in the slurry. The plants were immediately transplanted to planter flats with 5-cm cells, Todd Planter Flats, Model 200 (Speedling, Inc., Sun City, FI), containing a combination of peatlite soil mix and pasteurized sand 3:2 (v/v). Plants inoculated with different isolates were kept separate on greenhouse benches to avoid cross contamination from water splash. Greenhouse temperatures were maintained at 28:t 4C. The same inoculation procedure was used to assess the response of Capsicum accessions to a highly virulent isolate, PS-1 a, of f.Q..C;. Assays to identify wilt resistance among accessions of several species of Capsicum showed that high levels of resistance occur in baccatum. Of the baccatum accessions assayed, 16 of 27, were homogeneous in their response to inoculation and showed no wilt and little or no evidence of root damage. Among accessions of -C.annuum, -C chacoense, c. frutescens, .c. microcarpum, and ,C. pubescens tested, single accessions of C. annuum and .c. chacoense were found to be highly resistant. Plants of most other accessions tested among these species were highly susceptible, but plants of a few accessions survived inoculation while showing stunt and root damage, indicating an intermediate type reaction. All accessions of .c. frutescens tested were highly susceptible. Using nitrate nonutilizing mutants (nit), all pathogenic isolates of FOC were found to be in the same vegetative compatibility group. No compatibility was observed when nits of nonpathogenic isolates from tissue and soil were paired with the pathogenic nit testers. Isolates of EQ.C (A TCC 66420/66421) may be obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

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Table: Capsicum accessions resistant to Fusarium oxysporum f. sp. Capsici isolate PS-1a (ATCC 66420)

Capsicum annuum (62 accessions tested)

C00758

Capsicum chacoense (8 accessions tested)

C01553

Capsicum baccatum

Pl 188803

PBC 151

C00302

C00313

C00313-1

C00475

C00754

C00755

C00774

C00803

C00941

C01218

C01300

C01526

C01527

C01543

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Capsicum Newsletter, 1 (1992). 35-36.

# TEST FOR RESISTANCE TO VERTICILLIUM DAHLIAE KLEB. IN CAPSICUM ANNUUM L.

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In 1991 a test for resistance to V. dahliae on Capsicumannuum was made. Ten genotypes reported-by several authors as resistant to that parasite were included. 'Yolo Wonder' and the Spanish pimiento type culti.var 'R,iguel' were used as susceptible controls (Table 1).

The roots of 9-week-old seedlings were washed and then dipped into a suspension of 3.2 x 10E6 propagules/ml for 3 minutes (KENDRICK and MIDDLETON, 1959). Fifteen to thirty seedlings per variety were transplanted into pots containing a pasteurized mixture of 1 Humin-Substrat, 1 peat, 1 perlite, 1 sand, 1 clay-loam soil (by volume). Pots were placed on greenhouse benches where average of minima and maxima air temperatures were 13°C and 30°C.

Plants were evaluated for Verticillium resistance by weighing their aerial parts three months and a half after inoculation. In order to establish a index which could be regarded as an estimation of resistance (IR), each plant weight was referred to the average weight of six to ten non-inoculated plants of the same variety. Data was treated by analysis of variance.

It was difficult to establish statistical differences between genotypes, with the exception of 'Yolo Wonder' which behave as the most susceptible one (Table 1). Within the rest of genotypes 'Riguel' and 'Buketen' as the more susceptible ones and 'T1-1' and 'New Mexico Accesion' as the more resistant can be pointed out.

These results obtained by artificial inoculation on greenhouse do not exactly agree with the response of same genotypes grown in V. dahliae highly infected fields. Classification of varieties according to their IR is in some way influenced by the inoculation method used and by the climate where the assay is made (PALLOIX et al., 1990).

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Recurrent selection for resistance to verticillium dahliae in pepper. EuphytJ.ca 47: 79-89.

Table 1. Genotypes of *Capsicum annuum* ordered by resistance (IR) to *Verticillium dahliae*.

| Genotype | IR (0-100) | | Source and/or Reference |
|---|---|---|---|
| Yolo Wonder | 26.9 | a | 'Bell' type Cultivar (USA) |
| Riguel | 59.6 | b | Pimiento type cultivar (Spain) |
| Buketen | 69.1 | bc | Christov and Popova (Bulgaria) |
| Podarok Moldavii | 76.8 | bcd | Lobb (USSR) |
| Luesia | 82.3 | bcd | Gil Ortega (Spain) |
| Serrano C.M. 334 | 85.3 | bcd | Guerrero (México)<br>Palloix *et al*. (France) |
| C-169 (*C. minimum*) | 88.5 | bcd | IVT (The Netherlands) |
| Pallagi (WYR4618) | 92.6 | cd | Inst. Vavilov (USSR) |
| L-25 | 93.0 | cd | Marinkovic *et al*. (Yugoslavia) |
| Line 724 | 93.1 | cd | Aleksic *et al*. (Yugoslavia) |
| New Mexico Accession | 96.0 | d | Bosland (USA) |
| T1-1 | 97.3 | d | Marinkovic *et al*. (Yugoslavia) |

Means separations according to Newman-Keuls test, level 5%.

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# CHARACTERIZATION OF VERTICILLIUM DAHLIAE KLEB. ISOLATES ON A SET OF Capsicum SPP GENOTYPES.

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The pathogen behaviour of nineteen verticillium dahliae Kleb. isolates on five Gapsicum genotypes during two years has been evaluated. The 19 isolates were evaluated in 1989 while only the 5 most representative were considered in 1990. The isolates had been obtained from diseased plants of pepper. To carry out the assays two-pepper varieties, which are susceptible to V. dahliae ('Yolo Wonder' and 'Riquel') and three partially rest Stan genotypes ('Podarok Moldovy', 'Luesia' and C. minimmun 'C169') have been used.

The roots of 9-week-old seedlings were washed and dipped into a suspension of 3.2 x 10E6 propagules ml for 3 minutes (KENDRICK and MIDDLETON, 1959). Five seedlings per variety in 1989, and 8 seedlings in 1990 were transplanted to pots containing a pasteurized mixture of 1 Humin-Substrat, 1 peat, 1 perlite, 1 sand, 1 clay-loam soil (b I volume). Pots were placed on greenhouse benches where average a r temperatures ranged from 17°C to 22°C in 1989 and 18°C to 26°C in 1990.

Plants were evaluated for Verticillium resistance by weighting their aerial parts two months after inoculation in 1989, and four months in 1990. In order to establish a percentage which could be regarded as an index of resistance, each plant weight was referred to the average weight of five non- inoculated plants of the same variety in 1989, and eight in 1990~ The data was treated by analysis of variance.

1989 and 1990 trials showed that only six isolates were able to differentiate between pepper genotypes which generally speaking, were classified by resistance as expected. Nevertheless, some of the interactions found in 1989 could not be confirmed in 1990. The non-existence of a clear host-parasite cx interaction joined to the slight importance of some specific c- reactions did not permit the definition of vertical pathotypes. Nevertheless, isolates could be ranked by order of aggressiveness. Data from 1990 experiment are shown in Table 1.

## REFERENCES

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Table 1. Average percentage of fresh weight of V. dahliae inoculated pepper plants referred to controls

| | | Pepper Genotypes | | | |
|---|---|---|---|---|---|
| V. dahiae Isolated | Yolo W. | Riguel | Podarok | Luesia | C-169 |
| VD08 | 16,2a | 12,2a | 37,3a | 25,1a | 67,8a |
| VD 13 | 8,9a | 13,0a | 36,7a | 29,1a | 77,0ab |
| VD05 | 13,6a | 14,5a | 31,0a | 32,5a | 72,8ab |
| VD12 | 54,5ab | 48,6ab | 62,9a | 67,7b | 111,1b |
| VD03 | 68,3b | 71,2b | 74,4a | 103,6b | 42,3a |

Mean separation by Newman-Keuls test, 1% level (for the graphic expression of such separation, letters in the vertical direction and straight lines in the horizontal direction were used).

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# REULTS OF SEED TESTS. XIV. - ON GERMINATION PARAMETERS REGISTERED FOR SEED SAMPLFS OF CAPSICUM ANNUUM L.

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Paprika /Capsicum annuum L./ is a traditional vegetable in Hungary, and numerous old varieties, and races etc. are collected in the gene bank of Research Center for Agro botany /formerly the National Institute of Agrobotany/. Shmidt /1959/ and /1974/ reported the results of agro botanical investigations made on some stocks and Szabo /1968/ investigated the cardinal points of germination temperature. Szaboand VimaYi /1970/ tested seeds stored in unconditioned environment, and the seeds loss their germ inability rapidly. However we have not data about connections of germination parameters registered in these tests.

In course 0 f our trials 134 - 134 seed samples stored for 1 and 7 years were investigated respectively. The seeds were stored in conditioned environment at 0-4 °c in hermetically sealed containers' with 7-8 % initial moisture content. The germination tests were' carried out according to . /1985/ suggestions. Data of percentage 0 f germination and of rates of seed molding and seedling abnormality were registered by samples, and correlation matrices of these parameters were computed. .

Significant differences were not stated for means of variables' registered in tests of seeds samples stored for 1 or 7 years. The mean value of germ inability was 92.13 .t 3.17 after 7 years. The correlations computed for the two seed populations /Table 1. 2. / show significant connections and similar tendencies at seed samples stored for different time periods. The computed correlations are signaling negative connections in pairs of germination-molding and germination-abnormality, and positive one in pair of molding-abnormality showing the possible tonic effect of the fungi colonizing the seeds. The co connections were similar to those that were reported in case of lupins and pea earlier maY and HortvAth, 1990; 1991/.

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Table 1: Correlations of germination parameters after 1 year storage

| | Germinating | Molding | Abnormality |
|---|---|---|---|
| /G/ | 1 | -.6791+++ | -03062+++ |
| /M/ | | 1 | .2598++ |
| /A/ | | | 1 |

---

++; P=0.01,+++; P=0.001

Tables 2: Correlations of germination parameter after 7 year storage

| | Germinating | Molding | Abnormality |
|---|---|---|---|
| /G/ | 1 | -.6081++ | -.2741++ |
| /M/ | 1 | 1 | .2319 |
| /A/ | | | 1 |

+ P= 0.05, ++ P=0.001

Capsicum Newsletter, 11 (1992) 41-42.

# Screening of Germplasm For Yield and Quality Egg Plant in The Punjab.

Dr. Altaf hussain, M. Magir A. Chaudary, Mansoor Ahmad, Vegetable Research Institute, Faisalabed- Pakistan.

Egg plant is a very important vegetable crop in the Punjab province of Pakistan. Three crops are grown in this part of the globe during the year. Evaluation of available germplasm to screen out high yielding varieties was conducted Trial No. 1 March –July, 1989 and Trial 2 August 1989 – January, 1990

In the first trial 9 variteties were included in the studies. Nursery was sown in Feb under plastic tunnel, to protect it from the frost, where as the transplanting was done in the 2nd week of March, RCBD system of layout with four replications was followed. Net Plot size was (8x3) m2, row spacing 1.5m and with in rows 0.5 m. was maintained.

In second trial, in addition to the varieties included in the first, one more variety Local Selection was Asses. In and with in rows 0.5 m. was kept

Nursery was sown in the 2nd week of January and transplanting was done during the 2nd week of March, 1989 for second experiment the nursery was sown in last week of August, 1989, yield data recorded in both the cases are given in the table-1.

The data collected on yield of edible fruit in both the cases revealed that the variety “Neelum” produced included in the trials. Even all the varieties included in the trial. Even the standard variety Multan Selection could not compete it. Multant selection got the 2nd position on merit list in the first experiment, and fourth in the 2nd. The variety Black King produced significantly the lowest yield. In addition to the seasonal effect the plant population also affected the yield significantly. In first trial the density of the plant population was leaser whereas in the second experiment it was more i.e. row spacing 1.5m and 1. Respectively. The yield recorded was such higher inn the 2nd experiment as compared with that of 1st experiment except in case of No. 7.

Table: Snowing The Fruit Field of Different Varieties During Spring and Autumn, 1989-90

| S.No. Name of the variety | Yield in tomes per hectare | |
|---|---|---|
| | Trial No. 1 March-July, 89. | Trial No. 2 Aug-Jan 1990 |
| 1. Nealum | 12.59a | 18.31a |
| 2. Multan Gelection | 11.53b | 16.16b |
| 3. Qaisar | 11.4b | 17.02ab |
| 4. White Egg. | 8.22c | 12.06 cd |
| 5. Sarhandi Long. | 7.85c | 11.40cd |
| 6. Choraium Purple | 7.76c | 13.83c |
| 7.Shah Jamal | 7.72c | 7.21e |
| 8. Pusa Purple Long | 7.66c | 1322cd |
| 9. Black King. | 4.57d | 6.83e |
| 10. Local selection | | 17.68ab |
| Cd1 | 0.882 | 2.031 |
| Cd2 | 1.196 | 2.742 |

Capsicum Newsletter, 11, 43-44

# EFFECT OF GELLING AGENTS ABD ACTIVATED CHARCOAL ON SOLAN18 DLONGENA PLANT REGENERATION

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Research Institute for vegetable Crops, P.O.BOX 48 - ~ Pontecagnano
(SA), Italy.

In S.melongenaseveral previous studies showed that both genotypes and growth regulators influenced plant regeneration through ; organogenesis. The aim of this study was to evaluate the effect of agar and gerlite as gelling agents and the influence of activated charcoal on the morphogenetic response of two eggplant genotypes (Giant of China and WIR 769).

Murashige and Skoog medium supplemented with 2 mg/l glycine was used as basal medium. Cytokinins 6-benzylaminopurine (BAP) and zeatin (ZEA) were tested singly at 0.5,. 1,. 1.5, 5 and 10 mg/l or in combination using 0.5/1. 0.5/5, 1/1 and 1/5 mg/l of BAP/ZEA. All these media were solidified with 0.8% purified Agar-agar (MERK) or 0.25% gerlite (KELCO). Besides, agar-containing media were also tested with addition of 0.5% activated charcoal (MERK). The pH of all media was adjusted to 5.8 prior to autoclaving at 120°C for 20 min. All the cultures were kept in a 2 -1 controlled environment chamber at 25 + 2°C under 50 uEm s for a 16h day cycle.

For plant regeneration, leaf disks (1 cm ) were .prepared from young fully expands leaves *of* in vitro grown-plants. Leaf-disks were placed with the lower side in contact with the medium in 100 x 15 mm petri dish. A total *of* 21 explants (7/plate) were used for each genotype and treatment. After 4 weeks the number *of* shoots and shoot buds per explant was collected under a dissecting microscope.

For both genotypes the gelling agent had a strong effect on the frequency *of* the shoot regeneration process. Agar was superior to gerlite in all the media tested (tab. 1 and 2). The positive effect *of* agar was more dramatic in BAP-containing media and for the cv. Giant *of* China where it allowed up to a twenty fold increment in the regeneration frequency (tab. 1). Surprisingly activated charcoal completely inhibited shoot regeneration in both genotypes. At the concentrations tested. ZEA was more effective than BAP to achieve shoots and shoot-buds differentiation. On average,' WIR 769 showed an higher morphogenetic response than Giant *of* China. These results might be utilized in genetic transformation experiments to introduce,. Arsrobacterium tumefaciens, useful foreign genes in the eggplant genetic pool.

Tab. 1 - Number of shoots and shoot-buds per leaf-disk (mean ± SE) of cv Giant of China as influenced by growth regulators, gelling agents and activated chorcoal.

| Growth regretators (mg/l) | | Gerlite | Agar | Agar + activated charcoal |
|---|---|---|---|---|
| ZEA | BAP | | | |
| 0.5 | - | 6.9 ± 4.4 | 19.0 ± 2.6 | 0 |
| 1 | - | 2.3 ± 2.7 | 15.5 ± 4.0 | 0 |
| 1,5 | - | 2.0 ± 2.6 | 18.0 ± 2.4 | 0 |
| - | 1 | 6.3 ± 5.7 | 15.2 ± 1.1 | 0 |
| - | 5 | 0.8 ± 1.1 | 14.4 ± 1.3 | 0 |
| - | 10 | 0.6 ± 0.9 | 13.6 ± 2.9 | 0 |
| 0.5 | 1 | -(a) | 23.3 ± 2.1 | 0 |
| 0.5 | 5 | - | 21.6 ± 4.5 | 0 |
| 1 | 1 | - | 16.9 ± 5.0 | 0 |
| 1 | 5 | - | 19.8 ± 2.8 | 0 |

a, - = media not tested

Tab. 2 - Number of shoots and shoot-buds per leaf-disk (mean ± SE) of WIR 769 genotype as influenced by growth regulators, gelling agents and activated charcoal.

| Growth regulators (mg/l) | | Gerlite | Agar | Agar + activated charcoal |
|---|---|---|---|---|
| ZEA | BAP | | | |
| 0.5 | - | 19.0 ± 9.9 | 30.3 ± 4.5 | 0 |
| 1 | - | 5.2 ± 4.1 | 25.0 ± 5.8 | 0 |
| 1.5 | - | 21.2 ± 4.9 | 24.3 ± 7.0 | 0 |
| - | 1 | 6.6 ± 4.0 | 12.3 ± 6.7 | 0 |
| - | 5 | 8.2 ± 6.1 | 23.1 ± 9.9 | 0 |
| - | 10 | 1.6 ± 1.2 | 7.4 ± 5.1 | 0 |
| 0.5 | 1 | - (a) | 23.5 ± 9.9 | 0 |
| 0.5 | 5 | - | 21.8 ± 10.6 | 0 |
| 1 | 1 | - | 26.7 ± 7.9 | 0 |
| 1 | 5 | - | 28.2 ± 7.5 | 0 |

a, - = media not tested

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# INHERITANCE OF NPTII GENE AND SCREENING FOR KANAMYCIN RESISTANCE IN TRANSENIC SOLANUM MELONGENA PLANT

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The aim of this study *is* to carry out genetic and molecular analysis of NPTII gene segregation in progenies of transgenic eggplant cv. 'Picentia' previously obtained (Rotino and Gleddie. 1990). Besides an easy and not-destructive screening method was also set up to select for functional NPTII gene as proposed by Weide et al., (1989) for tomato.

Seven RO plants derived from independent transformation events were used screening was carried out using three R1 progenies of T1, T14, T20 transgenic plants and un transformed control. Sterile seeds and plantlets were grown on selective MS basal medium containing 100-mg/l kanamycin. Plantlets exhibiting normal growth were considered to be kanamycin resistant. Two plants showed a 3 km :1 km ratio as expected in the case of one NPTII gene insertion, plant T14 gave a segregation ratio (2 kmR:1 kmS) that fit with the hypothesis of a lethal mutation, acting at the homozygous state caused by T-DNA insertion (Tab. 1). Resistant plants were randomly selected and selfed to obtain R2 seeds assay was performed on six R1 progenies two R2 progenies and untrasformed control. To this end, greenhouse-grown plantlets at the fourth

true leaf were sprayed twice a day (at 8 hours interval) for three consecutive days with a solution of 300 mg/l kanamycin dissolved in water. After 3 weeks plants were scored as resistant/sensitive to kanamycin on the basis of absence/presence of bleaching sectors in the sprayed leaves. The in vino assay worked equally well as the in vino screening. In fact, the R1 progenies of plant T1 and plant T20 gave the same segregation ratio in both in vino and in vino assay. All the R1 progenies segregated as one active copy of NPTII gene was inserted in the genome (Tab. 2). The T1-R2 progenies did not segregate because, probably, the selfed resistant T1-R1 plan was homozygous for NPTII gene; while the T20-R2 progenies showed a 3 kmR:1km ratio, this suggests that it derived from an heterozygous plant for NPTII gene.

All the plants gave segregation ratios that indicated inheritance of the * Km phenotype in a Mendelian fashion. The transgene was stably expressed through two sexual generations. Work is, now in progress to characterize the transgenic progenies. at molecular an enzymatic level. Rotino G.L. and Gleddie S., 1999 - Transformation of eggplant (S. melongena 1.) using a binary agrobacterium tumefaciens vector. Plant Cell Rep., 9: 26-29.

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Tab. 1 - In vitro assay for kanamycin resistance segregation in $R1_R$ progenies derived from three indipendent transgenic plant ($km^R$=kanamycin resistant; $km^S$=kanamicyn sensitive).

| Transgenic plant | Total No. of seeds | Germination (%) | No. of seedlings tested | Phenotype $km^R$ | Phenotype $km^S$ | $\chi^2$ value (P=0.05) | Segregation $km^R$:$km^S$ |
|---|---|---|---|---|---|---|---|
| T1-R1 | 153 | 100 | 153 | 116 | 37 | 0.054 | 3:1 |
| T14-R1 | 219 | 100 | 219 | 147 | 72 | 0.020 | 2:1 |
| T20-R1 | 123 | 60 | 88 | 73 | 15 | 2.970 | 3:1 |
| Control | 140 | 97 | 136 | 0 | 136 | - | - |

Tab. 2 - In vivo assay for kanamycin resistance segregation in six R1 and two R2 $_R$ progenies derived from six indipendent transgenic plant ($km^R$=kanamycin resistant; $km^S$=kanamycin sensitive).

| Transgenic plant | Germination (%) | No. of seedlings tested | Phenotype $km^R$ | Phenotype $km^S$ | $\chi^2$ value (P=0.05) | Segregation $km^R$:$km^S$ |
|---|---|---|---|---|---|---|
| T1-R1 | 100 | 145 | 114 | 31 | 1.013 | 3:1 |
| T1-R2 | 88 | 135 | 135 | 0 | - | - |
| T10-R1 | 46 | 66 | 53 | 13 | 0.990 | 3:1 |
| T12-R1 | 91 | 120 | 92 | 28 | 0.178 | 3:1 |
| T16-R1 | 85 | 118 | 96 | 22 | 2.542 | 3:1 |
| T20-R1 | 95 | 102 | 84 | 18 | 2.941 | 3:1 |
| T20-R2 | 98 | 141 | 110 | 31 | 0.683 | 3:1 |
| T22-R1 | 67 | 100 | 81 | 19 | 1.920 | 3:1 |
| Control | 90 | 135 | 0 | 135 | - | - |

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