

# **CAPSICUM & EGGPLANT NEWSLETTER**



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DI.VA.P.R.A.  
Plant Breeding And Seed Production**

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## **NEWSLETTER**

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Plant Breeding and Seed Production  
Via P. Giuris, 15 – 10126 Turin Italy



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## FORWARD

The seventeenth issue of "Capsicum and Eggplant Newsletter" includes an invited paper written by F.J.B. Reifschneider, C.S.C. Ribeiro and C.A. Lopez from Brasilia. It deals with pepper production and breeding in Brazil. Thank you very much to these Authors, for their kind willingness to co-operate with us. As usual, we would like to remind you that any suggestions on the topics and/or authors to be considered for the invited papers of the following issues of "Capsicum and Eggplant Newsletter" would be appreciated.

As in the past, the accepted contributions have not been modified, and have been printed as received. So, the authors are responsible for both the scientific content and the form of their reports.

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

Please, remember that this Newsletter is highly dependent on the financial support of the recipients. Therefore, a subscription fee is appreciated. The fees are the same as the previous year: 30 U.S.\$ for normal and 150 U.S.\$ for supporter subscribers. Also in order to make the payment less time-consuming and to reduce the bank costs, we have introduced the possibility of a 3-year subscription. As you know, it is possible (and suggested!) to order your own copy to quicken its delivery. Just fill in the order form on page 101 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 103. Because of the lower banking costs, credit card payment is most welcomed.

We regret to report that several papers were rejected because they fell outside the scope of the journal. Please remember that "Capsicum and eggplant Newsletter" deals only with the subjects of genetics and breeding, and therefore papers on other subjects (pathology, physiology, etc.) should not be submitted. Lastly, it is absolutely necessary to pay attention to the instructions given. It is imperative that you follow these instructions very carefully. Otherwise we will not accept the contributions and will have to return them.

The deadline for the submission of articles to be included in the next issue of the Newsletter (No. 18, 1999) is February 28, 1999. Please note that it is also possible to submit the paper on diskette or through Email (the address is: [plantbre@pop.fileita.it](mailto:plantbre@pop.fileita.it)). Details can be found on the enclosed sample sheet.

Turin, 15th June 1998

P;dro Belletti and Luciana Quagliotti

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## **PEPPER PRODUCTION AND BREEDING IN BRAZIL, AND A WORD ON EGGPLANTS**

### **PRESENT SITUATION AND PROSPECTS**

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#### **Pepper Production - an Overview**

World production of sweet and hot peppers in 1996 occupied *circa* 1300 thousand hectares according to FAG Production Yearbook; for that same year, it was estimated that some 10-12 thousand hectares were planted to *Capsicum* species cultivated in Brazil, although precise figures are not available for many states. The states of Sao Paulo and Minas Gerais generally account for roughly 40-50% of the total area planted to hot and sweet peppers in Brazil, significantly contributing to rank peppers among the 10 most important vegetable crops in the country. In 1996, Sao Paulo planted 2860 ha to sweet peppers and Minas Gerais 2179 ha; average yields were 22.2 t/ha in Sao Paulo and 26.1 t/ha in Minas Gerais, and total production for the two states was *circa* 120000 t. Although planted areas are similar to those reported by Galmarini (Galmarini, 1997) in Argentina, yields differ markedly,

The most common sweet peppers are either conical or block shaped and marketed green. The state of Rio Grande do Sul and the northeastern states have traditionally accepted block shaped and light green colored fruits, similar to 'Yolo Wonder' and its related cultivars.

Although colored hybrids have recently paved their way through an ever increasingly demanding cosmetically-oriented consumer, specially in medium-to-large urban centers, their consumption is still of little significance. Peppers are mostly planted in open fields, although the last decade has seen an increase in protected pepper cultivation, i.e. in plastic houses; the high cost of protected cultivation makes it specially suited for off-season plantings in

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southern, cooler and/or rainier parts of the country as well as for high-production cost colored-fruit hybrids.

Paprikas are also produced mostly in the states of Minas Gerais and Pernambuco, where they occupy *circa* 1000 ha. Some 70% of the paprika is exported, mostly to Europe.

### Seed Production and Cultivars

Several multinational and Brazilian companies produce and market sweet pepper seeds in Brazil, namely Hortec, Isla, Agroflora, Agrocerec and Asgrow. Most of the seed is locally produced, but colored hybrids are imported. As for hot peppers, five species account for almost 100% of the production: 'Malagueta' (*C. frutescens*), 'Dedo-de-moc;a' (*C. baccatum*), 'Cumari' (*C. praetermissum*), 'De-cheiro' and 'Bode' (*C. chinense*) and Agronomico 11 (*C. annuum*) (Souza & Casali, 1984). The major paprika cultivar has been derived from 'Mallorca'.

Agriflora and Agrocerec together have the lion's share of the market and, in 1997, roughly 10000 kilos were used for sweet and .ct peppers and paprika production in the country. Private sector estimates suggest total peppers seed market in Brazil to be US\$1.s million.

The table below indicates some of the major cultivars marketed by key companies in Brazil:

Company	Major Cultivars
Agroflora	Magsa, Ikeda, Calwonder 500, Marta, Amanda, Magali and Magali R
Agrocerec	Cascadura Itaipu, Cascadura Ikeda, yolo Wonder, Hercules AG-672, Agronomico 10-G, Apolo AG-511, Atenas AG-322, Safari and Nacional AG—511
Hortec	Agronomico 10-G, Cascadura Ikeda, All Bog, Zarco, Matador and Magnata
Isla	All Big, Cascadura Ikeda, Yolo Wonder and Itapoa 401
Topseed	All Big, Cascadura Ikeda, pibeta and Yolo Wonder
Asgro	Melody, Domino and Marengo

### Major and Potential Problems

Diseases and a few insects, mostly virus vectors, as well as abiotic stresses such as low soil pH and P content, can be considered the key problems faced by pepper growers in Brazil; most of the breeding efforts have been targeted towards the development of

agronomically-suitable disease resistant genotypes. Major diseases include: phytophthora blight, caused by *Phytophthora capsici*

(Reifschneider et al., 1986); bacterial spot (pathotypes of *Xanthomonas campestris* pv. vesicatoria - Poulos et al., 1991); bacterial wilt (*Ralstonia solanacearum*); spotted wilt (caused by TSWV); and mosaic (caused by PVY - Boiteux et al., 1996).

The recent increase in drip-irrigated pepper cultivation under plastic greenhouses has also indicated the need to better manage a new threat - powdery mildew. Similarly, geminiviruses have become a major problem in tomato crops in Brazil and are expected to significantly affect peppers soon. Major breeding efforts by public and private sectors alike will be continuously required to tackle these emerging problems.

### **Pepper Breeding - Public and Private Sectors**

Peppers, mainly sweet, have been the focus of breeding efforts in Brazil for several decades. Undoubtedly a historically important and key pepper research program has been that lead by Dr. Hiroshi Nagai, at the Instituto Agronomico de Campinas (IAC), in Sao Paulo. Over ten cultivars - known as the 'Agronomico' series - have been released by IAC in the last decades, most of them possessing high levels of resistance to specific PVY strains. Breeding has also been done by researchers at the Universidade Federal de Vicosa (UFV) and the Universidade Federal Rural do Rio de Janeiro (UFRRJ). Today, Embrapa's National Research Center for Vegetable Crops (Centro Nacional de Pesquisa de Hortaliças - CNPH), in the public sector, and Agroflora (sakata) as well as groceres (recently purchased by Monsanto) in the private sector, are considered to have the largest breeding programs; CNPH's program has been concentrated on breeding for multiple disease resistance for the past 18 years. Major cultivars developed in the country include the previously mentioned 'Agronomico' series, with emphasis on 'Agronomico 10-G', 'Magda', and the Cascadura group. It is also worth pointing out the contribution of individual farmers to pepper breeding efforts in Brazil.

With the approval of intellectual property rights and plant variety protection legislation in the country, the public sector is expected to be concentrating its breeding efforts, in due time, in the development of populations with superior characteristics, such as disease resistance, which could be transferred to the private sector for the derivation of inbred lines for hybrids and, eventually, open pollinated genotypes. For smaller and specific-niche markets, where there is no, or very limited, private sector interest, public sector will continue to have a major role in breeding fully-finished cultivars.

The private sector, specially large agroprocessing companies, are increasingly contracting out the public sector for specific breeding projects, indicating perhaps the lack of flexibility by the seed companies in Brazil. Contract breeding is exemplified by the recent development of high SHU 'Jalapeno' - like cultivars by CNPH for a major agroprocessing company in Brazil.

Public sector work will continue to address key questions linked to the identification of disease and insect resistant genes, germplasm conservation, enhancement and characterization, basic biological and molecular work, as well as the use of *Capsicum* biodiversity for the benefit of the Brazilian population. Genotypes developed in Brazil, such as *Xanthomonas campestris* pv. vesicatoria (XCV)- resistant line CNPH 703, are being internationally used as sources of resistance to all XCV pathotypes; this genotype has been included in the International Chili Pepper Nursery, promoted by AVRDC ( AVRDC, 1997), and is also resistant to ToMV and TMV. CNPH 703 has been named PBC 137 by AVRDC.

### **Tapping the Brazilian *Capsicum* Biodiversity**

UFV's and CNPH's are the major *Capsicum* collections in Brazil; CNPH's has over 750 accessions mostly in *C. annuum*, *C. chinense* and *C. frutescens*. Additionally, between 10 and 20 wild *Capsicum* species can be found in the remains of the Atlantic forest in Brazil (Bianchetti, personal communication, 1998); the latter germplasm has been the object of a relatively recent collection for enhancing Embrapa's collection. All this variability has not been put to use, with a few exceptions. Presently, there is a major classic and molecular breeding effort being organized by a group of *Capsicum* researchers in Brazil, which would begin to explore all this untapped *Capsicum* biodiversity, improving the knowledge of our collections and its genotypes. An exciting period seems to be developing for pepper breeding in Brazil.

### **A word on Eggplants - a traditional crop with a vast potential**

There is little verifiable data concerning eggplant production in Brazil, except for a few states. Total area planted to eggplants varies from 1000 to 1500 ha, the state of Sao Paulo leading the statistics with circa 1000 ha and an average yield of 45t/ha, , although several of the available hybrids easily reach 80 t/ha. The Brazilian market demands mostly dark purple, shiny, elongated fruit weighing an average of 200 g.

Main open pollinated and hybrid cultivars marketed in Brazil are:

Company	OP	Hybrids
Agroflora	Embu	F-100, S-F100, Napoli
Hortec	Embu	Cica, F-1000, Diamante Negro
Isla	Forida Mrket, Perta Comprida	
Topseed	Roxa Comprida	Onix F-100
Takii		Kokuyo
Rogers		Rima

Breeding programs have been conducted mostly by the public sector, responsible for the generation of 'F-100', 'Cica' and other genotypes. Breeding efforts have been made towards the development of disease-resistant genotypes: as an example, 'Cica', developed by CNPH is resistant to both *Phomopsis* and anthracnose. Efforts towards the development of *Verticillium* wilt and bacterial wilt (*Ralstonia solanacearum*)-resistant genotypes are underway. Recently, CNPH has finalized the characterization of over 150 genotypes using 19 IPGRI descriptors of interest to breeders, with the support of the Brazilian National Research and Development Council (CNPq). The collection's being constantly enriched and there are over 200 accessions.

### Acknowledgements

The first and third authors are thankful to support received from the the National Research and Development Council, CNPq.

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## **NEW HOT PEPPER VARIETIES PERFORMING WELL IN THE WESTERN PARTS OF ETHIOPIA**

Girma Abera: and Dagne wagaryi  
*LBako* Research Center, P.O. Box 3, Bako, Ethiopia

It is not well known when hot pepper was introduced to Ethiopia, but it was sufficiently long ago for a wide range of types have been developed with in the country. Research on hot pepper has been going on since 1968 in the country with the objectives of identifying and developing improved varieties and applicable agronomic practices. But the success is not as of its research age and expected.

Nowadays, pepper is produced in almost all parts of the country, however, the major production areas concentrate between 1400- 1900 masl(Hafnagel, 1961). It is the fourth in areas of production next to maize, tef, and noug and second in importance in the Bako area (Lege sse et. al., 1987).

For Ethiopians, the food is flat or test less without the addition of hot pepper, it is an important food item consumed in almost every day diet as vegetable and spice. Beside this, about 5% of the dry pod goes to processing plant for oleo-resin extraction. This crop is also a cash source for t]le farmers and the country. Hot pepper can also be exported easily and safely as dry pods or processed products.

As Bako is a potential area for pepper production, substantial contribution in evaluation of local collections and introductions have been made by Bako Research Center to improve the crop. The two varieties under production, 'Bako local' and 'Marekofana', at the time of their release were "" relatively better in their yield potential, tolerant to diseases and insect pests. But, currently they become very susceptible to most of the diseases and insect pests and as a result of which yields are decreasing.

Therefore, in order to replace them by other variety which can resist or tolerate diseases and insect pests and have desirable horticultural characteristics, collection of ' germplasm from local and/or external sources has been, underway. Accordingly, three lines (' Isolation No.5', Isolation No.7' and 'Isolation } out of the lines were attained the above requirements. Subsequently, for sustainable supply of high yielding and adaptable varieties with disease and insect pest reaction, the selected lines were evaluated with Bako local and Marekofana for two years at Bako and Didessa (Table 1).

In 1983, the cultivars had showed statistically significant differences in number of pod/plant, number of main branches, length of main branch, plant canopy, stand count at harvest, pod weight and length ( $p = 0.01$ ) and marketable - pod yield ( $p = 0.05$ ) and non-significant difference in total pod yield at Bako. Similarly significant differences were observed in plant height, canopy length, pod weight and length. Marketable and total pod yield ( $p = 0.01$ ), whereas, non-significant differences were observed in stand count at harvest, number of main branches, number of pod/plant and length of main branch at Didessa.

In 1984, statistically significant differences were found among the cultivars in total and marketable pod yield, plant height, pod length and weight ( $p = 0.01$ ) and stand count at harvest, number of main branches, number of pod/plant and plant canopy ( $p = 0.05$ ) at Bako. Also significant differences were observed among cultivars in number of pod/plant, pod weight and pod length ( $p = 0.01$ ) and plant height and plant canopy ( $p = 0.05$ ) and non-significant differences were observed in total and marketable pod yield, stand count at harvest, number and length of main branches at Didessa.

The cultivars with red pod color, Bako local and Isolation No.7, were promising and high yielder at Bako area and those with dark red pod color, Marekofana and Isolation No.15 were good and high yielder at Didessa area. This may be due to the direct effect of environmental and soil condition on the cultivars in both sites. The cumulative result over the two years indicated that Isolation No.7 out-smarted the other varieties at Bako, whereas, Marekofana outperformed all the varieties under study at Didessa followed by Isolation No.15. However, Isolation No.15 has higher oleo-resin (oil and resin) content all varieties under studied both locations.

Therefore, Isolation No.7 is recommended for Bako area for its high yield and good agronomic characteristics, whereas, Isolation No.15 can be recommended for Didessa area for its moderate yield and high oleo-resin content. Besides their high yield, these cultivars can be used for future breeding works in that they contain desirable horticultural characteristics.

Table 1. Pod yield (g/ha) of hot pepper varieties adjusted at 10% moisture level at Bako and Didessa in 1983 and 1984 cropping season.

Treatment	Bako				Didessa			
	83		84		83		84	
	Mark	Tot	Mark	Tot	Mark	Tot	Mark	Tot
Bakolocal	33.18	43.14	13.31	19.34	9.20	8.28	11.78	15.40
Marekofana	23.79	39.76	10.64	15.50	9.58	14.35	11.35	14.75
Isolation No.5	26.00	35.51	10.66	16.28	3.01	5.56	9.02	10.83
Isolation No. 7	31.72	46.23	15.42	15.42	20.05	3.55	7.21	9.03
Isolation No. 15	17.32	37.66	12.92	16.28	9.20	16.23	10.99	12.14
Mean	26.24	40.47	12.59	17.49	5.88	10.32	10.33	17.34
SE	3.30	5.08	0.72	0.64	1.17	1.37	1.20	1.13
CV(%)	25.15	25.14	11.53	7.43	39.79	26.65	23.33	17.34

Key: Tot = Total pod yield, mark = Marketable pod yield

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Legesse Dadi, Gemechu Gedeno, Tesfaye Kumsa and Getahum Degu. 1987. Diagnostic survey report no.1 Bako mixed farming zone wellga and Shewa region. IAR, Addis Abeba, Ethiopia

## THE CONTENTS OF CAPSAICINOIDS AND THEIR PHENOLIC INTERMEDIATES IN THE VARIOUS TISSUES OF THE PLANTS OF *Capsicum annuum* L.

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### Abstract

We determined the contents of capsaicinoids and their phenolic intermediates (trans-cinnamic acid, trans-coumaric acid, *trails-caffeic* acid, *tralls-ferulic* acid and vanillylamine) of the placenta, peduncles, leaves, stems and *roots* of *Capsicum annuum* L.cv. 'Jalapeno', which was grown ill a green house for ten months. In placenta, ca. 99 % of capsaicinoids, ca. 58 cof vanillylamine and ca. 49 % of phenolic intermediates were accumulated. 130th the amounts of phenolic intermediates and vanillylamine in placellta were less than several % of those of capsaicinoids in placenta, and were not correlated with the prodllection of capsaicinoidso

**Key words:** capsaicinoids, Capsicum. phenolic intermediates, vanillylamine

### Introduction

Capsaicinoids are the major pungent principle of Capsicum fruit. Tracer experiment using rn.-pheny |- [3-14C] alanine or lvaline- [U-14C] showed that the main formation and accumulation sites of capsaicinoids were the placenta (Iwai et al. 1979). The enzymatic formation of capsaicinoids from vanillylamine and C9 to C11 branched- chain fatty acids occurred using cell free extract of placenta of the fruits (Fujiwake et at. 1980). The pathway leading to the capsaicill has two distinct arms, one of which contributes the fatty acid moiety from valille are leucille through a -keto-isovalerate or *a* -keto-isocaproate (Suzuki et al. 1981). The other contributes the aromatic component from phenylalanine through cillnamic acid. coumaric acid, caffeic acid, ferulic acid, vanillin and vanillylamine (Fujiwake et al. 1982). Recently, the glycosylated forms of phenolic intermediates were proposed to be the stored forms of intermediates for the biosynthesis of capsaicinoids (Sukrasno and Yeoman 1993).

In our previous report, we reported the amounts of the compounds of the latter arms in the placenta of *Capsicum annuum* L.cv. 'Jalapeno' and cv.'Shimofusa (Sakamoto et al. 1994). The former was highly pungent, while the latter had no pungency. Although substantial amounts of free phenolics were detected in both cultivars, they were not correlated with the production of capsaicinoids in either cultivar. In this reports, we detected the amounts of these intermediates in various tissues of the plants in order to make clear whether there is a tissue accumulating and supplying these intermediates.

## Materials and Methods

The seed of *Capsicum a/lilt/urn* L. cv. 'Jalapeno' was sown at May 1993 and grown in a greenhouse for 10 months. The height of the plant was about 2 m and various stages of the fruits were grown on the plant.

The placenta tissues were taken from two mature fruits. The peduncles were taken from a mature and a young fruit (about 10 days after flowering). Two developed leaves were used. Two parts of the stems and the three parts of the roots were taken from the plant. About 20 to 200 mg of these samples were extracted with 80 % ethanol. The extract was dried and dissolved in 1 ml of dimethyl sulfoxide. Twenty. u I of each were applied to HPLC analysis. HPLC conditions were described in our previous report (Sakamoto et al. 1994).

## Results and Discussion

The end-products, capsaicinoids (capsaicin and dihydrocapsaicin), were specially accumulated in the placenta (Fig. I). The average contents of capsaicin and dihydrocapsaicin of placenta were ca. 33 and 38 JJ. mol/g, respectively. The amount of capsaicinoids of other tissues was as small as ca. 0.5 JJ. mol/g. which was only 0.7% of the amount of placenta.

The amounts of each intermediates were quite small and it is difficult to discuss them separately, although our analytical technic using HPLC were sensitive. And also cinnamic acid, coumaric acid, caffeic acid and ferulic acid are ubiquitously distributed in the main stream of phenylpropanoid metabolism. So their contents were summed and are presented as the amount of phenylpropanoids (Fig.I), as we did in previous paper (Sakamoto et al. 1994).

Phenylpropanoids contents of the placenta were 1.97 JJ. mol/g, and 2.8 % of total amount of capsaicinoids. Phenylpropanoids contents of the total of other tissues were 2.02 JJ. mol/g. Because the average amount of phenylpropanoids of other tissues was 0.54 :1:0.1 (SE) JJ. mol/g, the amount of phenylpropanoids in placenta was obviously higher, than other tissues. In the case of vanillylamine, its content in the placenta (0.90 JJ. mol/g) was 1.3 % of the total amount of capsaicinoids, but it was also higher than those in other tissues (0.14 :1:0.03 (SE)JJ.mol/g),

Our result showed that the amounts of these phenolic intermediates and vanillylamine in placenta and also other tissues were not correlated with the production of capsaicinoids. Sukrasno and Yeoman (1993) reported that the free phenolic intermediates from cinnamic acid to vanillylamine were not detected at any stage of the fruits of *Capsicum Frutescense*. They proposed the glycosylated forms of phenolic intermediates were the stored forms of intermediate for capsaicin biosynthesis, They showed the contents of cinnamoyl glycosides and vanillic acid-glycoside were 1300 and 100 nmol / g fr. wt., respectively. So, even the amounts of glycoside intermediates were less than 5 % of the amounts of the end products. It is expected that when the intermediates were common with other metabolisms, those components were not necessarily accumulated within special tissues. Another report also showed that capsaicin synthesis is not controlled via the activity of the enzymes phenylalanine ammonia-lyase and cinnamate 4-hydroxylase, which may determine the rate of entry of metabolites into phenylpropanoid pathway (Hall and Yeoman 1991). Precursor

biotransformation in immobilized placenta tissues of *Capsicum frutescens* showed that the feeding of coumaric acid, not phenylalanine or L-valine, was the best for capsaicinoids formation (Johnson and Ravishankar 1996). It was suggested that the key steps of capsaicin synthesis was near the last of the reaction pathway.

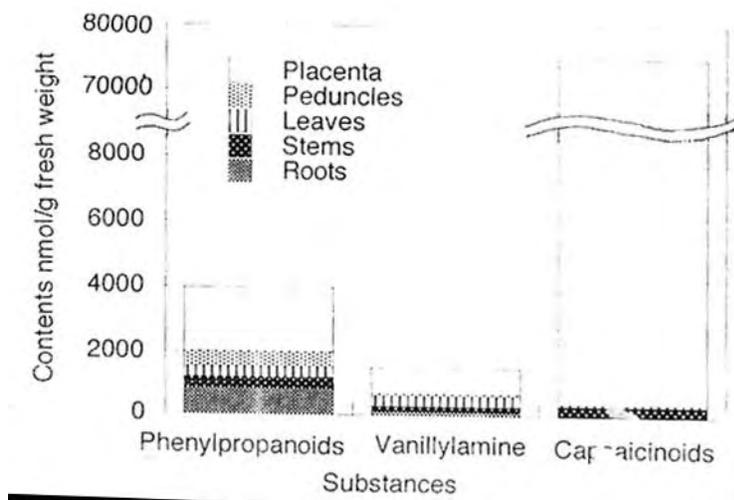


Fig. 1 The contents of capsaicinoids and their phenolic intermediates in the various tissues of the plants of *Cap. annuum* L. cv. 'Jalapeno'.

The amounts of capsaicinoids were the sum of capsaicin and dihydrocapsaicin.

The amounts of phenylpropanoids are the sum of cinnamic, coumaric, caffeic, and ferulic acid. The placenta tissues were taken from two mature fruits. The peduncles were taken from a mature and a young fruit (about 10 days after flowering). Two developed leaves were used. Two parts of the stems and the three parts of the roots were taken from the plant.

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## UNUSUAL CAPSAICINOID PROFILES FOUND IN *Capsicum pubescens*

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### Abstract

A novel unique capsaicinoid profile was observed in *Capsicum pubescens*. An isomer of dihydrocapsaicin was found to be the predominant capsaicinoid. No reports exist where the isomer of dihydrocapsaicin is the predominant capsaicinoid.

Pungency, one of the important quality attributes of chile (*Capsicum* spp.) is due to the presence of alkaloid compounds known as "capsaicinoids" in the fruit. The presence of five pungent compounds in chile fruits has been reported. The common profile for the capsaicinoids in chile is capsaicin found in the largest quantity followed by dihydrocapsaicin. These two compounds account for 69 and 22 % of the total capsaicinoids, respectively (Bennett and Kirby, 1968). Because of their abundance, capsaicin and dihydrocapsaicin are considered major capsaicinoids, while the others are considered minor capsaicinoids. This type of capsaicinoid profile is common in *Capsicum annuum*, *C. chinense*, *L. baccatum* and *C. frutescens*. After evaluation of a large array of *Capsicum* germplasm, Collins and Bosland (1994) reported capsaicinoid profiles where dihydrocapsaicin and nordihydrocapsaicin were predominant. In this paper we report on an unusual capsaicinoid profile observed in *C. pubescens*.

### Materials and Methods

*Plant material:* Five *C. pubescens* accessions, 'PI 585277', 'NMCA 80049', 'NMCA 80058', 'NMCA 80062', and 'NMCA 80065' were studied.

*Sample preparation:* Red matured fruits were used for the analysis. The methods described by Collins et al. (1995) for sample preparation and extractions of capsaicinoids were followed. Capsaicinoids were separated, and quantified using high-performance liquid chromatography (HPLC) following the 'long run' method. Confirmation of the capsaicinoids was done using gas chromatography with mass spectrometry (GC-MS). The procedures described by Torabi (1997) for GC-MS of underivatized samples were followed. Ion chromatogram of m/z 137 was recorded, which is the most abundant peak in mass spectra of capsaicinoids. Identification of each capsaicinoid was done based on the molecular weight and retention time as

compared with the standard.

## Results and Discussion

The distributions of the capsaicinoids for the accessions are shown in Table 1. The HPLC and the GC-MS chromatogram for 'PI 585277' are shown in Figure 1A and B, respectively. The capsaicinoid peaks detected by HPLC were similar to those detected by GC-MS. In the accession 'NMCA 80065', capsaicin was a predominant capsaicinoid. This profile is common in other species but rare in *C. pubescens*.

In accessions 'NMCA 80049' and 'NMCA 80062', dihydrocapsaicin was a predominant capsaicinoid followed by capsaicin and nordihydrocapsaicin, respectively. These types of capsaicinoid profiles were reported by Collins and Bosland (1994). The presence of large quantities of dihydrocapsaicin is the most common profile found in *C. pubescens*. ~ In accessions 'PI 585277' and 'NMCA 80058', an isomer of dihydrocapsaicin was a predominant capsaicinoid. It accounted for 36 % and 42 % of the total capsaicinoids for 'PI 585277' and 'NMCA 80058', respectively. The presence of such a profile has not been reported. An isomer of dihydrocapsaicin has the same molecular weight, 307 g/mol, as dihydrocapsaicin. Dihydrocapsaicin appears earlier than the isomer of dihydrocapsaicin in the chromatogram.

The results indicate that *C. pubescens* has a varied capsaicinoid profile that is generally different from the other *Capsicum* species. The variation will allow for a more complete study on the inheritance of individual capsaicinoids.

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Table 1. Capsaicinoids in *C. pubescens* accessions, in part per millions (ppm)

Accessions	NND <sup>1</sup>	ND	CAP	DC	ISO	HD
NMCA 80058	51	128	116	174	356	160
PI 585277	46	138	157	93	450	178
NMCA 80049	163	1228	1691	2743	85	54
NMCA 88062	303	1040	748	1906	124	46
NMCA 80065	508	323	664	507	40	42

NND<sup>1</sup> = Nornordihydrocapsaicin; ND = Nordihydrocapsaicin; CAP = Capsaicin; DC = Dihydrocapsaicin; ISO Isomer of dihydrocapsaicin; and HD Homodihydrocapsaicin

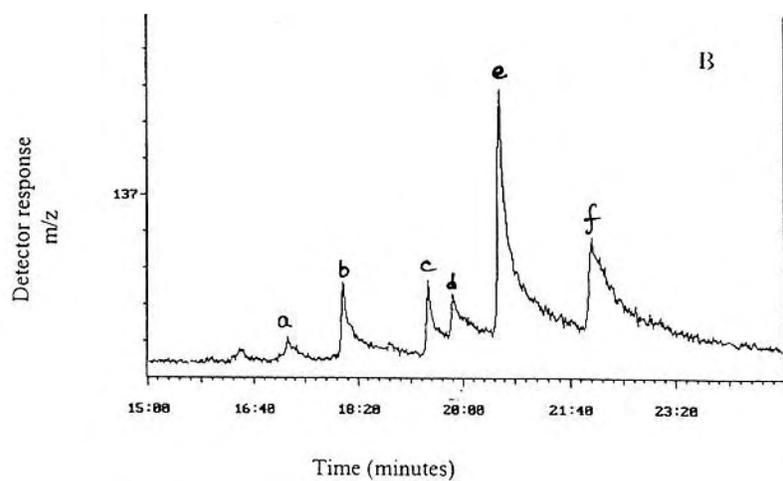
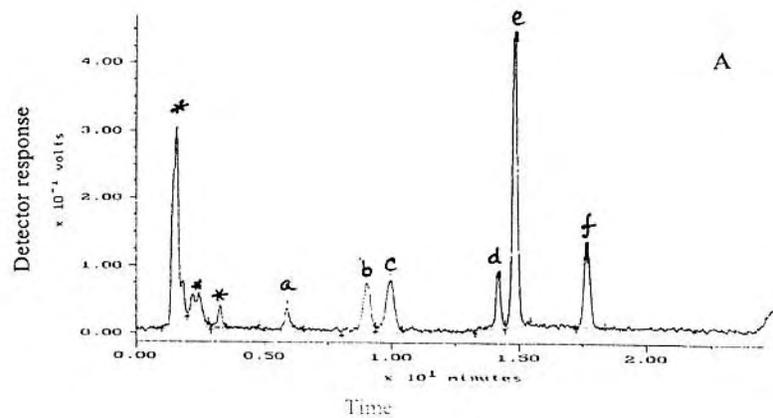


Fig. 1A and B HPLC and GC-MS chromatogram of accession 'PI 585277a' respectively. \*pigments; a=normordihydrocapsaicin; b=nordihydrocapsaicin; c=capsaicin; d=digydrocapsaicin; e=isomer of dihydrocapsaicin; and f= homodihydrocapsaicin

## INHERITANCE OF THE FRUIT SHAPE AT THE APEX AND THE PEDUNCLE ATTACHMENT OF PEPPER

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### Abstract

In the population of *Capsicum annuum* L. cv. 'Oh-natsume x *C. chinense* Jacq. PI 159236, the pointed shape of the apex and the acute shape of the peduncle attachment were inherited independently by dominant genes. We proposed a gene symbol *AP* for the pointed shape of the apex, and *P/:'O* for the acute shape of the peduncle attachment.

### Key words

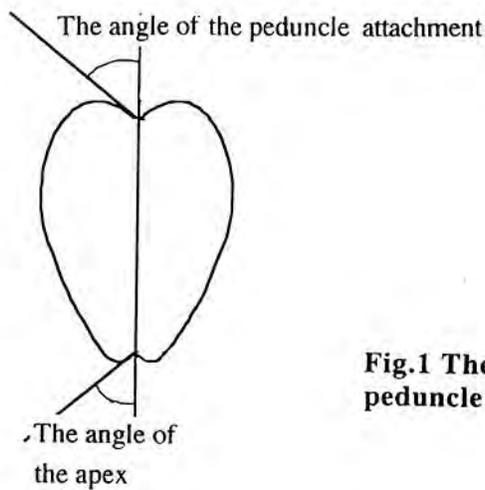
Apex, bell type, *Capsicum* elongate, peduncle

### Introduction

DNA markers, constructed by RFLP and PCR analysis, have become common in tile linkage analysis of useful traits in breeding programs (Prince et al. 1992). Besides the progress of these intensive lab work, identification and genetic analysis of tile traits by field work has become more necessary.

In this report we analyzed tile inheritance or a important trait in pepper; the one character of the fruit shape (the pointed or depressed shape of the x and the acute (bulged) or nonbulged shape of the peduncle attachment). As the characters of the fruit shape. gene 0 \was proposed using *Capsicum annuum*. (Peterson, 1958). They proposed that a major gene 0 (*round*) is completely dominant to its recessive allele (*o*) (*elongate*). The round fruit shape is designated 00 or 00 and the elongate as oo.

In order to analyze the fruit shape, we used *capsicum annuum* cv. 'Oh-natsume' and *C.chinense* Jacq. Plant Introduction (PI) 159236. *Capsicum annuum* L. cv.'Oh-natsume'is a bell type non-pungent pepper and has been used as a parental line io order to make the fruit blockey. This blockey shape of the fruit is dominant to most elongated pepper, but we did not yet investigated whether this cultivar has 0 or not. The center of the apex is sunken and tile peduncle attachment is bulged. *C.chinense* Jacq. PI 159236 is an elongated type, hot pepper. The fruit of this line has a pointed apex and an acute peduncle attachment. This line has contributed the introduction of TMV resistance to a bell type pepper. These two species



**Fig.1 The angles of the fruit apex and the peduncle attachment of pepper.**

Table 1. Chi-square test for 1-gene model for the fruit shape in the cross *C. annuum* L. – natsume’ (ON) x *C. chinense* Jacq.PI 159326 (PI).

(1) Apex

Population	Total Plants	Observed		Ratio test	$\chi^2$	p
		$\leq 90^\circ$	$>90^\circ$			
ON	5	5	0	---	---	---
PI	3	0	3	---	---	---
F1	3	0	3	---	---	---
BC	47	23	24	1:1	0	>0.900

(2) Peduncle Attachment

Population	Total Plants	Observed		Ratio test	$\chi^2$	p
		$\leq 90^\circ$	$>90^\circ$			
ON	5	5	0	---	---	---
PI	3	0	3	---	---	---
F1	3	0	3	---	---	---
BC	47	25	22	1:1	0.85	0.90-0.75

commercial hybrid varieties and their parents of *C. annuum* L., doubled-haploid progenies of *C. annuum* L: have been used in characterizing traits and linkage analysis (Lefebvre et al. 1995, Livneh et al. 1992, Shuh and Fontenot 1990, Tanksley and Iglesias-olivas, 1984).

*C.annuum* L. cv. 'Oh-natsume' and *C.chinense* Jacq. PI 159236 were crossed and BC generation were obtained. Using these populations. this paper showed that the pointed shape of the apex and the acute shape of the peduncle attachment were inherited independently by dominant single genes.

## Materials and Methods

The plants of *Capsicum annuum* L. cv. 'Oh-natsume' (5 plants, bell type pepper, hereafter described as ON), *C. chinense* J acq. Plant Introduction (PI) 159236 (3 plants, elongated type pepper, hereafter described as PI). F1 hybrid plants (3 plants) and the backcross populations to ON (47 plants, hereafter described as BC) were grown in the greenhouse for 1 year. Their heights were about 1 m and the fruits in various stages were grown by open pollination.

One to three fruits from each plant were measured. Mature, red fruits were measured. The fruits were cut in halves longitudinally and photocopied. The angles were measured as shown in Fig. 1

## Results and discussion

The center of the apex and the peduncle attachment of the fruit of ON was depressed. In this case, the angles of the apex and peduncle parts were 90° or less than 90°. On the other hand, PI had a pointed apex and the acute peduncle. The angles of the apex and peduncle parts were more than 90°. The angles of the apex and peduncle attachment of F1 were both more than 90° (Table I). In BC, the fruit shape at apex and the peduncle attachment was divided into two groups (Table I). For the apex, half of the population was depressed. Also, for the peduncle attachment, half of the population was bulged. The plant numbers of depressed apex, mild bulged base, depressed apex and nonbulged base, pointed apex and bulged base, and pointed apex and nonbulged base were 14, 10, 9 and 14, respectively. The shape at apex and peduncle was inherited independently. Although we are not familiar with the fruit having the acute peduncle and the depressed apex, for example, the fruits *C. chillense* 'chinchu-uchu' has this shape (Andrews 1995). The fruit having the bulged peduncle and pointed apex are quite common. Our results showed that the pointed shape of the apex was dominant to the depressed shape and the acute shape of the peduncle attachment was dominant to the bulged shape, and that these two traits inherited independently by single genes. As our results were the first report for describing the fruit shape at the apex and the peduncle attachment, we proposed the gene symbol *AP* for the pointed apex and *PED* for the acute peduncle attachment.

In the fruits with depressed apex, the angles of 50 % of them were ranged from 50 ° to 80°. For the fruits with pointed apex, the angles of 80 % of the fruits were ranged from 120° - to 140". For the peduncle, the angles of 90 % of the fruits were ranged from 50" to 90°, and the angles of 70 % were ranged from 120" to 160 ".

Fruit morphology was the principal means employed by early taxonomists to classify the domesticated capsicums, but now, fruit shape, color and position are of little taxonomic value because of parallel variation that occurs with 111C different species (Andrew 1995). The fruit shape was described as elongated, oblate, round, conical, campanulate and bell. Our results revealed that the inheritance of 111e shape of the base and the apex. We would like to investigate that *AP* and *PED* are applied to various domesticated capsicums with various fruit shapes.

### **Acknowledgments**

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Combining ability analysis for green fruit yield & yield components in chilli (*Capsicum annuum* L.).

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## INTRODUCTION

Success of any crop improvement programme is mainly dependent upon the selection of parents together with the information regarding nature and magnitude of gene effect controlling quantitative traits of economic importance. The knowledge of gene effect and combining ability not only provides information on inheritance of characters but also helps in selection of suitable parents for hybridization and development of promising hybrids for further exploitation. The present investigation carried out with aims to analyze combining ability of yield and other characters in chile (*Capsicum a/mum* L.).

## MATERIALS AND METHODS

The experimental material consisted of II parents (3 lines V iz. ,J wala,S-49 and G-4 and 8 testers Viz.,Jagudan-IO3,Gujarat chili-I,Resham Patti,Kumathi,S.G.-5,Anand Chilli-I,DPS-120 and ACS-92- I) and their 24F I hybrids were evaluated in a randomized block design with three replication at Plant Breeding Farm under Vegetable Research Unit,GAU, Anand during year 1996-97. A single row plot of twelve plants spaced at 60X60 cm for each entry was experimental unit. The observations on green fruit yield and its eight important components (Table-i) were recorded from five randomly selected competitive plants from each treatment. The data were subjected to statistical analysis (LXT analysis) as per Kempthorne (1957). The average degree of dominance was computed as  $(62sca/262gca) 1/2$ .

## RESULTS AND DISCUSSION

The ANOVA along with estimates of gca and sca variances and their ratio for different characters are presented in table-1 Significant differences were detected amongst genotypes, parents and hybrids for all the characters under study. The hybrids vs parents comparisons were significant for all the traits except plant height and fruit weight, indicating the expression of heterosis effects for the remaining traits. The partitioning of hybrid sum of squares revealed that variance due to female were highly significant for all the characters barring days to flowering, no.'s of primary branches and fruit yield per plant. Significant variance for males was observed for fruit girth only. The interaction of females with males was significant for all the characters except fruit girth. Apparently additive and non-additive gene effects were at work for the expression of all the fruits except fruit girth. However, lower average degree of dominance indicated that plant height, no of fruit per plant, fruit length, fruit girth, fruit I weight days to ripening and green fruit yield per plant predominantly controlled by additive gene effect. These finding are in agreement with those of Lippert (1975), Gopalkrishnan et al. (1987) and Singh and Singh (1978). Since there was preponderance of additive gene effect for all above traits, significant advancement could be achieved in the segregating generation through conventional breeding methods such as pedigree and bulk selection method. High average degree of dominance revealed preponderance of non-additive type of gene effect for days to flowering and no.'s of primary branches, this results are in accordance with findings of Gopalkrishnan et al.(1987), Pandey et al.(1981) and Singh and Singh (1978). Recurrent selection could be useful for the improvement of these characters.

*Capsicum* and Eggplant Newsletter, 17 (1998): 34-37.

The estimates of general combining ability effects along with per se performance for various characters are presented in Table-2. General combining ability effects revealed that none of the parents was a good general combiner for all the characters. The parents, Jwala, Kumathi and ACS-92-1 were good general combiners for earliness. For fruit length, fruit girth, fruit weight and fruit yield, the female parent S-49 had significant positive gca effects. The pollen parent DPS 20 was found to be a good general combiner for fruit yield along with numbers of fruits and early maturity whereas the line S-49 was a good general combiner for fruit yield as well as fruit size (fruit length and fruit girth). The significant gca estimates of parents G-4, Jagudan -103 and Rasham Patti indicated that they were good for contributing genes for increased plant height. Though the parents, Jwala, Kumathi and S.G-5 were poor general combiners for green fruit yield. They were good general combiners for more numbers of primary branches.

The crosses having desired significant specific combining ability effects are listed in Table-3, along with their per se performance and heterosis over better parent. For most of the characters, the best cross combination usually did not involve the respective best male and female parents. The F<sub>1</sub> Jwala-XS.G.-5 and G-4XAnand C-1 depicted significant positive sca effect and high degree of heterosis over better parent for fruit yield. The cross JwalaXKumathi displayed significant negative sca effects desired for nearness. For fruit length the hybrid JwalaxResham patti and JwalaxACS-92-1 exhibited significant positive sca effects as well as heterobeltiosis. The cross combination S-49X Anand C-1 recorded significant sca effect desired for increased fruit girth. For fruit weight, the crosses S-49XJagudan-I03 and S-49xKumathi had significant positive sca effect and heterobeltiosis as well. It was noted that parents with HxH, HxL, and LxL gca effects could produce desirable transgressive segregants of additive genetic system present in the good combiner and complementary epistatic effects in F<sub>1</sub> act in the same direction to maximize the desirable plant attributes.

The estimation of genetic variation influence breeding methodologies. The simple progeny selection in the pedigree method of breeding could exploit additive genetic variation. For a fuller utilization of both additive and non-additive gene effects some form of population improvement programme such as diallel selective mating or intense mating would be more useful. Majority hybrids depicted significant positive sca effects with high heterobeltiotic effect would favour heterosis breeding. It has now become possible to develop hybrids using male sterile lines available in this crop (Gill *et al.* 1973; and Lippert, 1975) or by converting one of the parents of promising hybrids into male sterile line, thus avoiding tedious and time consuming operations like emasculation and hand pollination.

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Table - 1 : Analysis of variance and estimate of combining ability variance for nine character in chilli

Source	d.f	Mean Squares								
		Days to flowering	Plant height (cm)	No.'s of branches	No.'s of fruit per plant	Fruit length (cm)	Fruit girth (cm)	Fruit weight (gm)	Days to fruit ripening	Fruit yield per plant
Replication	2	1.98	19.87	1.90	85.72	0.33	0.09	0.512	20.35	2726.66
Genotype	34	29.34**	204.31**	1.46**	4479.26**	5.47**	1.43**	3.56**	27.66**	20977.98**
Parents	10	24.85**	184.20**	1.07**	8424.41**	5.41**	3.21**	3.59**	38.87**	30109.69**
Parents Vs Hybrids	01	109.84**	0.0012	1.19**	9298.81**	4.05**	4.70**	0.189	74.28**	181137.79**
Hybrids	23	27.80**	221.94**	1.65**	2554.43**	5.55**	0.51**	3.70**	20.76**	10044.20**
Female	02	10.60	1109.65**	0.78	14132.18**	31.39**	2.17**	30.95**	62.34**	20962.50
Male	07	24.25	178.58	1.66	2425.71	1.45	0.69	0.87	23.19	12615.08
Female X Male	14	32.03**	116.81**	1.76**	964.83**	3.91**	0.17	1.22**	13.62**	7199.01*
Error	68	0.78	12.61	0.22	61.82	0.31	0.11	0.08	3.99	3277.64
$\sigma^2_{gca}$	10	- 0.885	31.96	- 0.033	443.279	0.758	0.076	0.89	1.76	581.19
$\sigma^2_{sca}$	23	10.343**	33.89**	0.504**	301.215**	1.188**	0.033	0.38**	2.94**	1138.73**
Average degree of dominance ( $\sigma^2_{sca}/2 \sigma^2_{gca}$ ) <sup>1/2</sup>		2.417	0.728	2.757	0.583	0.885	0.466	0.46	0.912	0.989

Table - 2 : General combining ability effects and *per se* performance of chilli parents

Parents	GCA effect and <i>per se</i> performance								
	Days to flowering	Plant height (cm)	No.'s at primary branches	No.'s of fruit per plant	Fruit length (cm)	Fruit girth (cm)	Fruit weight (gm)	Days to fruit ripening	Fruit yield per plant
<b>Lines</b>									
Jwala	- 0.444* (29.6)	- 7.022** (57.4)	0.206* (5.2)	12.569** (134.4)	0.193 (8.6)	- 0.256** (3.3)	- 0.433** (3.1)	0.033 (108.6)	3.750 (416.3)
S-49	- 0.319 (29.8)	0.469 (64.9)	- 0.128 (4.9)	- 27.972** (93.9)	1.035** (9.4)	0.332** (3.9)	1.288 (4.9)	2.871 (108.5)	27.500 (440.0)
G-4	0.766** (30.8)	6.553** (70.8)	- 0.078 (5.0)	15.403** (137.3)	- 1.228* (7.2)	- 0.076 (3.4)	- 0.855** (2.7)	2.292** (105.3)	- 31.250 (381.3)
<b>Testers</b>									
Jagudan-103	0.708* (30.8)	3.642** (68.0)	0.008 (5.0)	- 5.514** (113.3)	- 0.198 (8.2)	- 0.238 (3.3)	0.324** (3.9)	1.458* (108.2)	38.056 (374.4)
Guj. Chilli-1	1.486** (31.6)	1.464 (65.8)	- 0.147 (4.9)	18.708** (140.6)	0.342 (8.7)	- 0.038 (3.5)	- 0.236* (3.3)	1.236 (108.0)	38.611 (451.1)
Reshan Patti	0.486 (30.6)	7.664** (72.0)	- 0.081 (5.0)	- 0.847 (113.0)	0.127 (8.5)	0.517** (4.0)	0.094 (3.7)	1.458 (108.2)	- 2.5 (410.0)
Kumathi	- 1.069* (29.0)	- 1.203 (60.0)	0.408 (5.4)	- 31.736** (90.11)	0.178 (8.6)	0.284 (3.8)	0.571** (4.1)	0.681 (107.4)	- 361.389** (403.3)
S.G.-5	0.708 (30.8)	- 4.381** (64.3)	0.786** (5.8)	3.597 (125.4)	0.218 (8.6)	- 0.112 (3.4)	- 0.176 (3.4)	- 1.208 (105.6)	- 8.167 (403.3)
Anand Chilli-1	- 0.181 (29.9)	- 0.092 (64.3)	- 0.258 (4.8)	0.042 (121.9)	- 0.900** (7.5)	0.073 (3.6)	- 0.119 (3.4)	- 1.097 (105.7)	- 4.722 (407.8)
DPS-120	1.375** (31.4)	- 0.469 (63.9)	- 0.636** (4.4)	15.375** (137.2)	0.269 (8.7)	- 0.269 (3.3)	- 0.109 (3.5)	2.986** (103.8)	40.833 (453.3)
ACS-92-1	- 3.514** (28.6)	- 6.625** (57.8)	0.081 (5.0)	11.375** (133.2)	- 0.035 (8.4)	- 0.216* (3.3)	- 0.349** (3.2)	0.458 (107.2)	36.389 (448.9)
S.E. Lines	0.2039	0.7942	0.1028	1.5967	0.1210	0.0548	0.0592	0.4664	12.5545
Testers	0.3329	1.2969	0.1679	2.6074	0.1977	0.0894	0.0967	0.7289	20.5015

Table - 3 : Crosses showing desired significant SCA effect and their per se performance

Sr. No.	Character	Crosses	SCA effects	Mean value	Heterobeltiosis	S.E. ±
1	Days to Flowering	Jwala X Kumathi	- 4.89**	23.67	- 19.32**	0.5767
		Jwala X Anand Chilli-1	- 3.111**	26.33	- 10.23**	
		S-49 X Resham Patti	- 1.236*	29.00	2.35	
		S-49 X DPS-120	- 1.458**	29.67	- 11.88**	
		S-49 X ACS-92-1	- 4.563**	21.67	- 35.64**	
		G-4 X Jugudan 103	- 2.208**	29.33	3.53	
		G-4 X Resham Patti	- 1.986**	29.33	3.53	
2	Plant Height (cm)	Jwala X Kumathi	5.444**	61.60	- 10.98*	2.2463
		Jwala X ACS-92-1	8.933**	59.67	1.59	
		S-49 X DPS-120	7.753**	72.13	10.18**	
		G-4 X Anand Chilli-1	9.158**	80.00	16.73**	
3	No.'s of primary Branches	Jwala X S.G-5	1.039**	7.07	34.18**	0.2908
		Jwala X Anand Chilli-1	0.750**	5.73	8.86	
		Jwala X DPS-120	0.794**	5.40	2.53	
		S-49 X Kumathi	1.017**	6.33	25.00**	
		G-4 X Guj. Chill-1	0.789**	5.60	- 4.55	
		G-4 X Resham Patti	0.856**	5.73	- 2.27	
4	No.'s of Fruit per plant	Jwala X Jugudan-103	14.431**	140.33	- 27.41**	4.5161
		Jwala X Guj. Chilli-1	24.542**	177.66	- 8.10	
		Jwala X S.G.-5	11.319**	149.33	- 22.70*	
		S-49 X Resham Patti	18.639**	103.67	- 21.46**	
		S-49 X Anand Chilli-1	10.417**	104.33	- 20.96**	
		G-4 X Anand Chilli-1	20.042**	157.33	- 15.71	
5	Fruit length (cm)	Jwala X Resham Patti	1.26**	9.97	23.23**	0.3424
		Jwala X Anand Chilli-1	1.30**	8.99	11.04	
		Jwala X ACS-92-1	1.30**	9.85	21.10**	
		S-49 X Guj. Chilli-1	1.01**	10.77	7.45	
6	Fruit girth (cm)	S-49 X Anand Chilli-1	0.451**	4.373	- 21.25**	0.1549
7	Fruit weight (gm)	Jwala X Anand Chilli-1	0.363**	3.37	- 23.53**	0.1675
		Jwala X ACS-92-1	0.703**	3.48	- 20.91**	
		S-49 X Jugudan 103	1.338**	6.51	100.93**	
		S-49 X Kumathi	0.362**	5.78	32.16**	
		G-4 X Guj. Chilli-1	0.612**	3.08	- 3.14	
		G-4 X Resham Patti	0.352*	3.15	- 21.05**	
8	Days to fruit ripenign	Jwala X Kumathi	- 2.597**	104.67	- 4.27*	1.2625
		S-49 X Jugudan-103	- 3.917**	106.00	- 7.02**	
		G-4 X S.G.-5	- 2.708*	101.33	- 10.59**	
9	Fruit yield per plant	Jwala X S.G.-5	62.917*	470.00	39.60*	30.5096
		G-4 X Anand Chilli-1	66.806*	443.33	202.27**	

LINE X TESTER ANALYSIS FOR THE STUDY OF COMBINING ABILITY IN  
HOT PEPPER (*Capsicum annuum* L.)

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## INTRODUCTION

In development of high yielding varieties and hybrids of crop plants, the breeder often faces with the problem of selecting parents and crosses. Common approach of selecting parents on the basis of *per se* always does not lead to fruitful results (Allard, 1960). The selection of parents has thus to be based on complete genetic information and knowledge of combining ability of potential parents. Combining ability analysis in this respect is considered to be an efficient technique not only for selection of desirable parents and crosses but it also characterizes nature and magnitude of gene action in the expression of a trait. Such studies under temperate regions of India are very meagre. In the present investigation, therefore, line x tester analysis was employed to determine the extent of general and specific combining ability effects and gene action of important characters of hot pepper.

## MATERIALS AND METHODS

The experimental material comprised 3 females (testers) each crossed to 8 males (lines) to develop 24 Fls. The female parents, viz; Jawahar-218 (J-218), SC-I07 and Arka Lohit (AL) are the promising hot pepper lines. The 8 male parents viz; SC-I08, SC-502, SC-405, SC-304, SC-I01, SC- 61, BC- 212 and SC-460 represented different genetic backgrounds. The 24 hybrids developed following line x tester method were evaluated together with 11 parents in a randomized block design 3 replications during 1996 at Vegetable Farm, SKUAST, Srinagar. In every replication a single row of ten plants each of parents and F IS were planted at a spacing 60 cm between rows and 45 cm between plants. Data was recorded for 11 characters (Table 1). Combining ability effects were estimated by the method outlined by Kempthorne (1957). The additive (62) and non-additive (62D) genetic variances were estimated from the mean squares for general combining ability (gca), specific combining ability (sca) and error. Average degree of dominance (ADD) was calculated as  $\{O^2/O_A^2\}^{0.5}$

## RESULTS AND DISCUSSION

The variances due to gca and sca were significant for all the characters except for days to first fruit ripening where variance due to gca was non-significant (Table 1). This indicated that both additive and non-additive gene effects were involved in the genetic control, but the magnitude of non-additive (~D) gene action was invariably larger than the additive component (62 ~ for traits like days to first fruit ripening, plant height, plant spread branch number, fruit length, seed number and fruit yield Average degree of dominance which was found to be more than 1 for these characters (Table 1) confirmed the results of the above findings and also suggested that dominance or over-dominance influenced the manifestation of these characters. Similar results were also reported by Ignatov and Popova (1977), Singh *et al.* (1992) and Ahmed *et al.* (1994). For fruit girth, pericarp thickness, fruit number and average fruit weight the estimated additive genetic variances were higher suggesting predominance of additive gene action which was further confirmed by higher 62 A/62D values (> 1) and by the degree of dominance whose value was found below unity.

The estimates of gca effects of eleven parents presented in Table 2 revealed that none of the parents either among testers or among lines is a good combiner for all the character. However, among testers, the parent SC-I07 showed significant desirable gca effects and proved it to be good combiner for days to first fruit ripening, plant height, fruit girth, pericarp thickness and average fruit weight and a average combiner for fruit yield. J-218 was a high combiner fl-r fruit length. average fruit weight, seed number and fruit yield. AL showed highly significant gca effects for branch number and fruit number while for rest of the characters it was poor combiner. Among the lines, BC-212 exhibited significant gca effects for plant height, plant spread, branch number, fruit number and fruit yield and an average combiner for pericarp thickness and average fruit weight. SC-304 was the best combiner for fruit length, fruit girth, pericarp thickness and average fruit weight and an average combiner for fruit yield SC-502 had highly significant gca effects and was a best combiner for plant height, plant

spread, branch number, fruit length and average fruit weight whereas SC-IO I was a good combiner for fl11it number and fl11it yield and an average combiner for fruit length. SC-IO8 was a good combiner only for seed number.

- Although none of the parent was good combiner for all the traits however, the parents such as SC-IO7, SC-304, SC-IOI, SC-502, BC-212 and J-218 in general showed high gca effects for most of the omic traits like plant height, plant spread, branch number, fruit length, fruit girth, pericarp thickness, fruit number and fruit yield and thus identified as good general combiners. These parents could therefore be considered simultaneously while formulating a breeding programme for improving fruit yield and yield contributing characters as these parents having diverse genetic background, once crossed are expected to increase the frequency of pleiotropic genes and chromosome blocks of favourable linked genes.

The comparison of means and gca effects revealed that for plant spread, fruit length, fruit girth, pericarp thickness, fruit number, average fruit height and fruit yield, the parents that were found good general combiners also possess high mean values whereas in days to first fruit ripening, plant height, branch number and seed number parents with good gca did not exhibit good *per se* performance revealing that the combining ability of parents cannot always be judged accurately only by their *per se* performance. The selection of parents thus, should be based on both *per se* performance and gca effects.

A critical observation of the results with respect to sca effects revealed that none of the cross combination exhibited significant sca effects for all the characters. However, the number of crosses having significant sca effects in desirable direction were two for days to first fruit ripening, eight each for plant height, plant spread, fruit length, fruit girth and pericarp thickness, five each for branch number and seed number, seven for fruit number, nine for average fruit weight and ten for fruit yield. The *per se* performance of best five crosses and highly significant sca effects of five desirable crosses are given in Table 3. It was interesting to note that although none of the cross was good for all the characters, however, among desirable crosses five crosses, viz; SC-IO I x AL, BC-212 x SC-IO7, SC-304 x J-218, SC-IOI x J-218 and SC-502 x AL were identified as most superior cross combinations for fruit yield and most of the economic characters. The hybrid SC-IO I x AL besides having highest sca effects for fruit yield also exhibited high sca effects as well as superior *per se* performance for important characters like fruit length, pericarp thickness, fruit number, and average fruit weight. Similarly the cross BC-212 x SC-IO7 exhibited high sca effects for fruit yield, seed number, fruit length, fruit girth, pericarp thickness, plant height and average fruit weight, the cross SC-304 x J-218 for plant spread, pericarp thickness, fruit number, average fruit weight and fruit yield, SC-502 x AL for plant spread, fruit length, fruit girth average fruit weight and fruit yield and SC-IO I x J-218 for fruit girth, fruit number, branch number, plant height, plant spread and fruit yield.

It was also evident that in most of the characters the hybrids which showed superior *per se* performances also recorded desirable significant sca effects. This indicated that *per se* performance of these hybrids were reflected in their respective sca effect. Such correspondence between *per se* performance and sca effects may be useful in identifying hybrid combination for breeding programme. Further a comparison of sca effect of the crosses and gca effects of the parents involved revealed that gca effect mostly reflected the sca effects of the crosses. In majority of the crosses which showed best sca effects, the parental lines involved were at least one of the six outstanding parental lines namely SC-IO7, SC-304, SC-IOI, SC-502, BC-212 and J-218 which also had high gca effects for one or more characters contributing towards yield. This indicated that there is a strong tendency of transmitting the higher gain from parents to offspring.

The result obtained from the present investigation suggested the importance of heterosis breeding for effective utilization of non-additive genetic variance, which had predominant role for the improvement of days to first fruit ripening, plant height, plant spread, branch number, fruit length, seed number and the fruit yield. The characters namely fruit girth, pericarp thickness fruit number and average fruit weight where additive gene action was predominant, can be improved through simple selection. The crosses viz SC-IOI x AL, BC-212 x SC-IO7, SC-304 x J-218 and SC-502 x AL which possessed highest significant sca effects for yield and most of the characters also recorded maximum fruit yield, fruit number, fruit size, pericarp thickness and average fruit weight and thus proved as potential hybrids and could be considered for commercial exploitation of hybrid vigour in hot pepper.

**Table 1:** Estimates of components of variance for combining ability, genetic components of variance and average degree of dominance for yield and related traits in hot pepper

Source	Days to first fruit ripening	Plant height (cm)	Plant spread (cm)	Branch number	Fruit length (cm)	Fruit girth (cm)	Pericarp thickness (mm)	Fruit number	Average fruit weight(g)	Seed number	Fruit yield (g)
$\sigma^2_{gen}$	8.927	33.393***	4.778***	0.475***	0.524***	0.18***	81.68***	52.44***	1.41**	111.17***	1072.90**
$\sigma^2_{eca}$	21.360***	60.579***	28.135***	0.990***	2.036***	0.09***	87.24***	57.77***	0.77**	382.39***	7118.52***
$\sigma^2_A$	17.855	60.579	9.556	0.951	1.048	0.36	163.36	104.88	2.83	222.35	2145.81
$\sigma^2_D$	21.361	66.789	28.135	0.990	2.036	0.09	87.24	57.77	0.77	382.39	7118.52
$\sigma^2_{A/D}$	0.835	1.102	0.339	0.959	0.514	3.84	1.87	1.81	3.68	0.58	0.30
ADD	1.093	1.102	1.715	1.020	1.393	0.51	0.73	0.74	0.52	1.31	1.82

**Table 2.** General combining ability effects of parents for yield and yield components in hot pepper

Parents	Days to first fruit ripening	Plant height (cm)	Plant spread (cm)	Branch number	Fruit length (cm)	Fruit girth (cm)	Pericarp thickness (mm)	Fruit number	Average fruit weight(g)	Seed number	Fruit yield (g)
<b>A. Lines</b>											
SC-108	3.358	-2.935**	-1.388**	-0.017	-0.719**	0.259**	5.303**	-1.934	-0.230**	15.819**	-21.034
SC-502	3.231	11.928**	2.273**	2.516**	2.359**	-0.136**	0.625	-2.989**	0.413**	-5.338**	2.954
SC-405	0.354	-7.183	-4.396**	-1.504**	-0.648**	0.289**	1.825	-3.685**	-0.347**	6.760**	-35.820**
SC-304	-0.722	1.409*	2.071**	-0.143	0.437**	0.320**	13.069**	-0.331	0.774**	-1.644	38.512**
SC-101	-1.679	-3.850**	0.026	-0.119	0.481**	-0.436**	-8.419**	8.536**	-0.065	0.333	55.844**
SC-61	-3.141	-4.924**	-6.068**	-1.302**	-0.896**	0.111**	-11.364**	-8.403**	-0.124	-4.731**	-66.751**
BC-212	-3.210	8.354*	3.463**	0.380**	-0.019	-0.137**	3.703**	11.052**	0.400**	-7.727**	86.627**
SC-460	1.808	-2.798*	4.019**	0.190**	-0.996**	-0.269**	-4.742**	-2.245*	-0.821**	-3.472	-60.311**
SE.gi	1.795	-0.524	0.502	0.089	0.103	0.048	1.053	0.880	0.077	2.312	5.583
SE.gi-gi	2.329	0.741	0.711	0.127	0.146	0.068	1.490	1.245	0.109	3.270	7.896
<b>B. Testers</b>											
J-218	-0.433	-0.484**	-1.156**	-0.076	0.465**	0.125**	2.887**	-1.715**	0.459**	10.836**	11.163**
SC-107	-3.240**	5.700**	-0.216	-0.267**	0.083	0.397**	7.688**	-6.530**	1.074**	-12.219**	9.586**
Arka Lohit	3.673**	-0.716*	1.373**	0.343**	-0.548**	-0.522**	-10.575**	8.245**	-1.533**	1.383	-20.749**
S.E. gi	1.099	0.321	0.307	0.055	0.063	0.297	0.465	0.539	0.047	1.416	3.419
S.E. gi-gi	1.555	0.454	0.435	0.077	0.089	0.042	0.912	0.762	0.067	2.003	4.835

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

**Table 3:** Best 5 crosses each in respect of *per se* performance and significant desirable sca effects for eleven characters in hot pepper

Character	Crosses with <i>per se</i> performance	Crosses with significant sca effects
Days to first fruit ripening	3x10(150.26), 5x9(155.03), 7x9(155.05), 6x10(156.23), 7x10(157.59)	3x10(-10.32), 5x9(-6.32)
Plant height	7x10(76.33), 2x9(70.66), 4x10(58.00), 2x11(57.66), 1x10(57.50)	2x9(14.18), 7x10(12.74), 1x10(5.20), 3x9(4.96), 8x10(3.99)
Plant spread	2x11(61.21), 8x11(58.22), 4x9(54.00), 1x10(53.00), 7x10(52.25)	2x11(9.71), 1x10(6.75), 4x9(5.23), 8x11(4.97), 5x9(4.84)
Branch number	2x10(10.01), 2x9(8.80), 8x11(7.65), 5x9(7.44), 4x11(7.13)	2x10(1.67), 5x9(1.55), 8x11(1.03), 4x11(0.85), 1x11(0.67)
Fruit length	2x11(15.10), 2x9(13.63), 1x9(13.20), 5x9(12.43), 7x10(12.33)	1x9(2.23), 2x11(2.07), 8x10(1.26), 7x10(1.05), 6x11(0.96)
Fruit girth	4x10(5.13), 3x10(4.66), 6x9(4.46), 6x10(4.46), 1x10(4.31)	1x11(0.47), 4x10(0.46), 8x11(0.37), 6x9(0.27), 2x10(0.23)
Pericarp thickness	4x9(1.66), 7x10(1.54), 4x10(1.50), 1x10(1.47), 2x10(1.47)	4x9(14.16), 6x9(9.96), 5x11(9.71), 1x11(9.66), 8x10(7.11)
Fruit number	5x11(76.41), 7x11(70.48), 5x9(59.20), 7x10(57.00), 8x11(56.13)	5x11(11.02), 4x9(8.42), 3x10(6.72), 1x10(5.85), 8x9(4.29)
Average fruit weight	7x11(9.13), 4x10(8.96), 4x9(8.37), 6x10(7.85), 3x10(7.78)	5x11(1.23), 2x11(1.23), 7x10(0.94), 1x9(0.82), 6x9(0.65)
Seed number	1x9(144.44), 2x9(107.84), 5x9(106.21), 3x9(105.77), 1x11(104.33)	1x9(29.63), 4x10(28.13), 7x10(14.52), 2x9(14.18), 4x11(10.64)
Fruit yield	7x10(521.87), 5x11(483.34), 4x9(459.99), 5x9(414.39), 2x11(382.14)	5x11(127.88), 7x10(105.30), 4x9(89.95), 4x11(86.86), 2x11(79.60)

1. SC-108, 2. SC-502, 3. SC-405, 4. SC-304, 5. SC-101, 6. SC-61, 7. BC-212.  
8. SC-460, 9. J.218, 10. SC-107, 11. Arka Lohit.

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MICROPROPAGATION STUDIES ON A MALE STERILE LINE OF  
*Capsicum Annumm* L. AT NAGARJUNA UNIVERSITY

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**ABSTRACT**

Attempts were made to micropropagate an useful genetic male sterile mutant with very good combining ability and marker traits by using apical and auxiliary buds as explants. The explants were cultured in MS medium supplemented with various concentrations of auxins and cytokinins. Maximum percentage of shooting for both explants was observed in MS medium supplemented with 3mg BAP + 1mg IAA/lit. Maximum percentage of rooting was observed in medium having 2mg NAA+ 0.5 mg Kn/lit.

**INTRODUCTION**

Male sterile lines have immense importance in plant breeding programmes since they provide a means of emasculating plants genetically- In commercial crop like chilli they will have a greater use in the production of hybrid seed on a large scale. A robust, vigorous, genetic male sterile mutant with marker traits and good combining ability was located in 'G2' variety of *Capsicum annumm* by Murthy and Lakshmi (1979). The mutant was propagated in to a line. This line proved to be of immense use in exploitation of heterosis with both indogenous and exotic pollinator parents. The maintenance of male sterility through backcrossing is a tedious and time consuming process, in order to overcome this difficulty an in-vitro technique was devised by using terminal and axillary buds as explants.

**MATERIALS AND METHODS**

The backcrossed seeds were germinated in pots. The three week old seedlings were identified and collected with the help of marker traits. The seedlings were transferred to laminar flow and surface sterilized with 0.1% HgCl<sub>2</sub> The

shoot tips and axillary buds were excised and transferred on , to paper bridges made up of Watman No.1 filter paper mounted in liquid medium. After four weeks the young shoots were transferred to solid medium of same composition. MS medium was employed throughout the study. Hormones BAP, Kn, IAA and NAA were supplemented at different concentrations to MS medium. Sucrose 3% was used as carbon source. Bacto Agar 1% was used as gelling agent. Throughout the study 16 h light/8h dark photoperiod and  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  temperature was maintained.

## RESULTS AND DISCUSSION

The response' of shoot tip and axillary buds to different phytohormone combinations was variable. As the explants were initially cultured on paper bridges in liquid medium, there was no or little callus formation at the base of young shoots, but when regenerated shoots were transferred to solid medium of same composition, there was elongation of shoots, development of callus and also multiple shoot formation was observed. Both the axillary buds as well as shoot tips showed similar response in this aspect, but the response to liquid medium in which the explants were initially cultured was different. The shoot tips showed good response when compared to auxiliary buds (Table-1) . So far there were no reports of following paper bridge technique to culture the meristems. Maximum percentage of shooting was observed in medium supplemented with 3mg BAP + 1mg IAA/lit. The earlier workers Sadhana Agarwal et al (1988) Thenjin and Ming Wang (1990), proposed r 5mg BAP + 1mg IAA/lit and 2.5 mg BAP + 1mg IAA/lit respectively as best shooting supplements. Bahetee et al t (1994) worked with 4 varieties of *C. annuum* and suggested 4 different media supplements, 4mg BAP + 0.5 mg IAA/lit for variety "Ag.ni", 6mg Kn + 0.5 rag IAA/lit for variety "Jwala", 5mg BAP+0.5 mg IAA/lit for variety "Tej" and 6rrlg BAP+0.5 mg IAA/lit for varj.ety "G4".

The regenerated shoots both from axillary buds as well as terminal buds showed similar response in rooting medium. The response of young shoots directly transferred from paper bridges into shooting medium and those transferred after subculturing in solid medium was same. Profused rooting was observed in medium supplemented with 2rog NAA+1mg Kn (Table- 2), Sadhana

Agarwal et al (1988) observed highest percentage of rooting in MS medium supplemented with 1 mg NAA/lit. Bahetee et al (1994) proposed 0.5 mg IAA/lit or 0.5 mg IBA/lit as best supplements for root induction.

Table 1. Shooting response of meristems

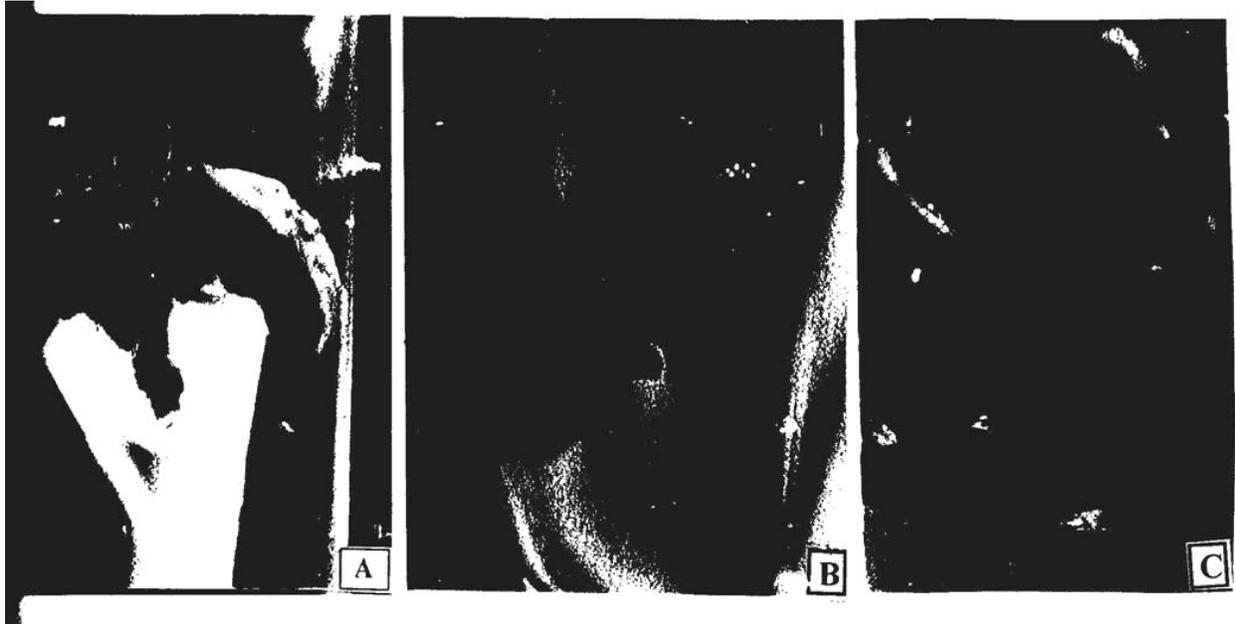
	Kn mg/lit						BAP mg/lit					
	1		2		3		1		2		3	
	T	A	T	A	T	A	T	A	T	A	T	A
	NR	NR	S (18%)	NR	S (31%)	S (18%)	NR	NR	S (31%)	NR (73%)	S (42%)	S
IAA 0.5 mg/lit	NR	NR	S (23%)	NR	S (38%)	S (21%)	NR	NR	S (42%)	S (28%)	S (82%)	S (48%)
IAA 1mg/lit	NR	NR	S (26%)	NR	S (36%)	S (28%)	NR	NR	S (48%)	S (31%)	S (85%)	S (49%)

NR = No Response                      T = Terminal bud  
S = Shoot                                      A = Axillary bud  
Percentage of response in brackets

Table 2. Rooting response of regenerated shoots

	IAA mg/lit			NAA mg/lit		
	1	2	3	1	2	3
		C	R (42%)	R (58%)	R (62%)	R (78%)
KIN 0.5 mg/lit	C	R (38%)	R (63%)	R (72%)	R (87%)	R (91%)
KIN 1mg/lit	C	R (45%)	R (54%)	R (78%)	R (94%)	R (92%)

R = Root                                      C = Callus



- A. Young shoot on paper bridge
- B. Multiple shoots on solid medium
- C. Root initiation of regenerated shoot

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Capsicum and Eggplant Newsletter, 17 (1998): 46-49.

## RESISTANCE OF *CAPSICUM* SPP. GENOTYPES TO PEPPER T. TITTLE POTYVIRUS ISOLATES FROM THE WESTERN HEMISPHERE

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Pepper mottle potyvirus (PepMoV) was first identified from pepper (*Capsicum* spp.) in Florida (19) and Arizona (12) in 1972. It causes pepper yield reductions in southern parts of the United States and in Mexico and Central America (3)

At the present time, the most effective way to control PepMoV is through host resistance. Resistance in *Capsicum* spp. to PepMoV is often genetically linked with resistance to two other potato Y (potyvirus) group members, tobacco etch potyvirus (TEV) and potato Y potyvirus (PVY) (8).

The purpose of this study was to determine the reactions of pepper genotypes with reported PepMoV resistance (6,7,9,15,16,17,18,19) when challenged with 18 PepMoV isolates from different countries in the Western Hemisphere. In addition, selected *Capsicum* genotypes with reported resistance to TEV and/or PVY (1,2,8,11) were challenged with the PepMoV isolates.

### MATERIALS AND METHODS

All screening experiments were conducted in a greenhouse during 1995 and 1996 at temperatures ranging from 21-35 C during the summer and 15-29 C during the winter.

Sources of seed (*C. annuum* unless otherwise indicated) were the following: PetoSeed Co. ('Agronomic 10C5', 'ELS 2-1', 'FLBG-1', 'King Arthur', 'VR-2', 'VR-4', 'Yolo Wonder', 'Yolo V'); Rogers NK Seed Co. ('Agronomic 10', 'Bomby', 'Elisa',

'Magda', 'Marquis', 'Reinger'); Asian Vegetable Research and Development Center (AMA 12', (*C. chinense*), 'COQ943' (*C. chinense*), 'C 01664', 'IAC UbatubaCambuci' (*C. baccatum*), 'Ikeda' (PBC 457A)]; M. Lhenny Company (all *C. frutescens*)

'Tabasco', 'Greenleaf Tabasco', 'Tabasco- Type Mexico 881; A.A. Cook, University of Florida; -- ay Bell', 'PI 264281', (P11), 'PI 152225' (*C. chinense*), 'PI 159236' (*C. chinense*); B. Villalon, Texas A&M University (Jaloro', 'Tambel2', 'Tam Veracruz); M K-e, Cornell University ('Avelar'); LL. Black, Louisiana State University (Durkee Ca- nne 9045', 'LP-1' (*C. frutescens*)). : : : Experiment 1. Eighteen isolates of PepMoV that had been identified by serology and host range were used. The

number of isolates from each location were as follows: Colombia 3, Ecuador 1, Honduras 5, Mexico 1, Nicaragua 5, United States 2, Venezuela 1. All APHIS-PPO permits were obtained for importation of isolates. Stock cultures of isolates were maintained at 4C in dehydrated host plant tissues. Selected virus isolates were activated by inoculation into *Nicotiana tabacum* cv 'NC95' plants. Ten pepper genotypes with reported potyvirus resistance (plus two susceptible controls, 'Yolo Wonder' and 'Tabasco') were selected to evaluate differences in virulence among the 18 isolates and to test the efficacy of their resistance against these virus isolates of different geographic origin. Seeds of test genotypes were planted in black plastic 64 cavity seedling flats (Jiffy Products, Batavia, IL) using Jiffy Mx Plus planting medium (Jiffy Products).

Inoculum sources consisted of infected plants of 'NC95' tobacco or susceptible pepper genotypes ('Yolo Wonder' or 'Tabasco'). Inoculum was prepared by grinding infected leaf tissue in cold 0.025 M potassium phosphate buffer, pH 7.1, with cold sterilized mortars and pestles. Eight plants of each genotype were inoculated for each PepMoV isolate-pepper line combination. Plants were inoculated at a young stage, generally between the cotyledonary and the two leaf stage. Eight uninoculated plants of each pepper genotype maintained in separate flats were kept as negative controls.

Symptom evaluation. Symptoms were evaluated about 3 wk after inoculation. Disease reactions were scored using the following designations: NS=no symptoms, MM=mild mosaic, M=mosaic, SM=severe mosaic, SMD=severe mosaic and leaf distortion, SN=systemic necrosis, ST=stunt.

Enzyme-linked immunosorbent assay (ELISA). Presence of PepMoV antigen in selected inoculated genotypes with mild or no symptoms was tested for by indirect ELISA using a direct antigen coating method (10). Samples consisted of the youngest fully expanded leaf from each of the eight plants collected 3 wk after inoculation. Additional testing of isolates for verification of PepMoV identity was conducted using commercial direct double antibody sandwich ELISA (4) kits (Agdia, Inc., Elkhart, IN) for TEV and PVY.

Experiment 2. Thirty pepper genotypes reported to be resistant to some potyvirus isolates were evaluated for their reactions to eight PepMoV isolates: C93-3 (Colombia), H92-35 and H92-32 (Honduras), N92-2 and N92-5 (Nicaragua), V92-3

(Venezuela), Tex82-1 (USA), and E94-C (Ecuador). Isolates were selected because they had representative or unusual patterns and/or levels of virulence and different geographic origins. The thirty pepper genotypes were selected to test the reactions of the virus isolates against a larger group of pepper genotypes, and to compare their resistance.

Experiment 3. Sixteen pepper genotypes were inoculated with the eight PepMoV isolates listed previously. Twelve of the 16 genotypes ('LP-1', 'PI 152225', 'IAC Ubatuba Cambuci', 'Delray Bell', 'Agronomic 10C5', 'VR-4', 'Jaloro', 'Greenleaf

'Tabasco', 'Tabasco- Type Mexico 88', 'PI 159236', 'Magda', and 'A-lar) were chosen as among the more resistant genotypes in Experiments 1 and 2, with resistance to a relatively large percentage of PepMoV isolates used. 'Yolo Y' and 'VR-2' were chosen due to their usefulness in distinguishing several of the isolates. 'Yolo Wonder' and 'Tabasco' were susceptible controls.

### RESULTS AND DISCUSSION

Experiment 1. Pepper genotypes varied greatly in their reactions to the PepMoV isolates. These included categories of no symptoms, reactions in which symptoms were visible in lower leaves only, and systemic foliar mosaic (Table 1).

Genotypes in the "no symptoms" and "foliar mosaic in lower leaves only" categories were combined to form a total "restricted symptom development" category in Table 1. 'Delray Bell', 'IAC Ubatuba Cambuci' (*C. baccatum*), and 'PI 152225' (*C. chinense*) were the only genotypes able to restrict symptom development of all 18 isolates of this virus, although none of the three genotypes was symptomless to all 18 isolates. Other genotypes showed low levels of resistance. 'Yolo Y' was symptomless when inoculated with three isolates (Table 1), two of which, C93-3 and H92-32, were selected along with six other isolates for use in Experiments 2 and 3.

Experiments 2 and 3. Data for Experiments 2 and 3 are combined in Table 2, for 30 genotypes with reported potyvirus resistance plus two susceptible controls, 'Yolo Wonder' and 'Tabasco'. For genotypes used in both experiments, ranges of results are presented. 'LP-1', 'PI 152225', 'Delray Bell', 'VR-4', 'Jaloro', 'Agronomic 10C5', and 'IAC Ubatuba Cambuci' all restricted symptom development of at least six of the eight isolates.

Table 3 shows symptom reactions in each virus isolate-pepper genotype combination in Experiment 3. 'Yolo Y' restricted symptom development of two isolates, C93-3 and H92-32 (Table 3). ELISA testing of symptomless 'Yolo Y' plants inoculated with these two isolates was negative (data not shown). Isolates C93-3 and H92-32 induced symptoms in few resistant genotypes. The inability of potyvirus isolates to infect 'Yolo Y' was more characteristic of PVY isolates (5). However, these two isolates were ELISA-negative when tested with PVY antisera using direct double-antibody sandwich commercial kits (data not shown). Paradoxically, isolate C93-3 induced symptoms in 'LP-1', one of the more resistant genotypes (Table 3)

Isolates N92-2 and N92-5 were each able to induce symptoms in many of the resistant genotypes. However, N92-2 was able to induce symptoms in the *C. chinense* line 'PI 152225', *C. frutescens* cv. 'Greenleaf Tabasco' (with potyvirus resistance derived from 'PI 152225' and 'PI 159236') and in line 'Tabasco- Type Mexico 88' (Table 3). In contrast N92-5 was unable to induce symptoms in the above *C. chinense* and *C. frutescens* genotypes, but induced systemic symptoms in the *C. annuum* genotypes 'Avelar', 'Magda', 'VR-4', 'Agronomico 10G5', and 'Delray Bell' in which N92-2 was less aggressive (Table 3).

Similar results were obtained for the two isolates in Experiment 2 (data not shown). Ariyaratne et al. (1) described two TEV isolates with contrasting affinities for infection of *C. chinense* genotypes vs. *C. annuum* genotypes.

Isolate E94-G had the broadest pattern of virulence of the eight isolates, inducing systemic symptoms or symptoms in lower leaves of all test genotypes except 'LP-t' (Table 3). The symptom phenomenon described here whereby symptoms were observed in old but not young leaves was reported in 'Avelar' by Subramanya (14).

Some differences occurred in virus isolate-pepper line interactions between Experiments 1, 2, and 3. This may be due to environmental effects on resistance as reported by Shiffriss and Cohen (13) in their work with PVY resistance in pepper. Experiments 1, 2, and 3 were carried out in the summer, fall, and winter, respectively, and greenhouse temperature and light conditions differed during these periods.

The pepper genotypes 'LP-t', 'Delray Bell', 'Agronomico 10G5', 'VR-4', 'PI 152225', and 'Jaloro', which restricted symptom development of many PepMV isolates in this research, were also shown to be resistant to many isolates of TEV by

Anyaratne et al. (1). These genotypes therefore appear to be good sources of resistance to both viruses. The *C. baccatum* cv. 'IAC Ubatuba Cambuci', which performs well in PepMoV screening, has been found in prior research in this laboratory to be highly susceptible to TEV isolates. If this line were used in breeding programs as a source of resistance to PepMV, a separate source of TEV resistance would be required.

#### ACKNOWLEDGMENTS

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**Table 1.** Reactions of 12 pepper genotypes to 18 pepper mottle potyvirus isolates in Experiment 1.

Pepper Genotype	Number of virus isolates which induced:			
	Systemic foliar mosaic	No symptoms	Foliar mosaic in lower leaves only	Restricted symptom* development
Delray Bell	0	15	3	18
IAC Ubatuba Cambuci	0	14	4	18
PI152225	0	14	4	18
Jaloro	2	16	0	16
PI159236	2	15	1	16
Avelar	2	12	4	16
Greenleaf Tabasco	5	13	0	13
Durkee Cayenne #9045	6	8	4	12
VR-2	10	6	2	8
Yolo Y	15	3	0	3
Yolo Wonder	18	0	0	0
Tabasco	18	0	0	0

\*Total number of isolates from the "no symptoms" and "foliar mosaic in lower leaves only" categories.

**Table 3.** Foliar symptoms induced by eight pepper mottle potyvirus isolates in 16 pepper genotypes in Experiment 3.

Pepper Lines	Virus Isolates <sup>a</sup> and Symptoms <sup>b</sup>							
	C93-3	H92-35	H92-32	N92-2	N92-5	V92-3	Tex82-1	E94-C
LP-1	M	NS	NS	NS	NS	NS	NS	NS
Delray Bell	NS	NS	NS	NS/M	M	NS	NS	NS/M
Agronomico 10C5	NS	NS	NS	NS/M	M	NS	NS	NS/M
VR-4	NS	NS	NS	NS/M	M	NS	NS	NS/M
PI152225	NS	NS	NS	M	NS	NS	NS	M
IAC Ubatuba Cambuci	NS	NS	NS	NS	M	NS	NS	M
Jaloro	NS	NS	NS	ST,M	M	NS	NS	NS/M
PI159236	NS	NS/M	NS	M	M	NS/M	NS	SM,SMD
Greenleaf Tabasco	NS	NS	NS	ST,M	NS	M	NS	ST,M
Tabasco-Type Mexico 88	NS	NS	NS	ST,M	NS	M	NS	ST,M
Magda	NS	M	NS	NS	M	NS	NS	SM,SMD
Avelar	NS	SM	NS/M	NS/M	M	NS	M	M,SM
VR-2	NS	NS	NS	M	SMD	M	M	M
Yolo Y	NS	SMD	NS	M	SMD	SMD	SMD	SMD
Yolo Wonder	SMD	SMD	SM	SM	SMD	SMD	SMD	SMD
Tabasco	ST	M,ST	M,ST	M,ST	M,ST	M,ST	SN	ST,SN

<sup>a</sup>Origin of virus isolates: Colombia (C93-3), Honduras (H92-35 and H92-32), Nicaragua (N92-2 and N92-5), Venezuela (V92-3), USA (Tex82-1), and Ecuador (E94-C).

<sup>b</sup>M=mosaic, NS=no symptoms, NS/M=no symptoms upper leaves, mosaic lower leaves, SM=severe mosaic, SMD=severe mosaic and leaf distortion, ST=stunt, SN=systemic necrosis.

**Table 2.** Reactions of 32 pepper genotypes to eight pepper mottle potyvirus isolates in Experiments 2 and 3.

Genotype	Average number of isolates which induced:			
	Systemic foliar mosaic	No symptoms	Foliar mosaic in lower leaves only	Restricted symptom development**
LP-1*	0.5	7.5	0	7.5
PI 152225*	1	6	1	7
Delray Bell*	1	5	2	7
VR-4*	1	5	2	7
Jaloro*	1.5	5.5	1	6.5
Agronomico 10C5*	1.5	5	1.5	6.5
IAC Ubatuba Cambuci*	2	6	0	6
Agronomico 10	3	5	0	5
Greenleaf Tabasco*	3	5	0	5
Magda*	3	5	0	5
Tabasco Type Mexico 88*	3	5	0	5
King Arthur	3	4	1	5
PI 159236*	3	4	1	5
PI 264281	3	4	1	5
Tambel 2	3	4	1	5
AMA 12	4	4	0	4
C 00943	4	4	0	4
FLBG-1	4	4	0	4
Ikeda	4	4	0	4
San Veracruz	4	4	0	4
Avelar*	4	3	1	4
Elisa	4	3	1	4
TSCH2	4	3	1	4
Marquis	4	2	2	4
VR-2*	4.5	3	0.5	3.5
Bomby	5	3	0	3
C 01644	5	2	1	3
Reinger	5	2	1	3
Yolo Y*	6	2	0	2
ELS 2-1	7	1	0	1
Yolo Wonder*	8	0	0	0
Tabasco*	8	0	0	0

\*Genotypes for which data are averages of results for Experiments 2 and 3. Data for remainder of genotypes are for Experiment 2 only.

\*\*Combined totals for 'no symptoms' and 'foliar mosaic in lower leaves only' categories.

## EVALUATION OF PEPPER CULTIVARS FOR RESISTANCE TO PEPPER VEINAL MOTTLE VIRUS IN NORTHERN NIGERIA

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### INTRODUCTION

The most important wet season disease limiting pepper production in Nigeria is pepper veinal mottle virus (PVMV) (Alegbejo, 1978; Atiri & Dele, 1985). This study was conducted between 1985 to 1987 to find a non-chemical control means of containing the disease.

### MATERIALS AND METHOD

Seedlings of sixteen pepper cultivars were raised in an insect-proof glasshouse and transplanted into the field at six weeks after sowing using a randomized complete blocks design. There were three replicates. Each plot consisted of two 6m - long ridges 0.6m wide with a total of twenty plants. One infector row of ten plants also infested with aphids were transplanted in between every two cultivar to serve as source of inoculum.

### RESULTS AND DISCUSSION

Only one of the cultivars (TCA14) was resistant, nine (Kimba, L3874, 12164, 12025, Caloro, Sakrho, Dantsiga, California mild and Danmeyere) were moderately resistant while the rest (12289, 12191, EX-Hunkuyi, 12190, & Ingarian yellow wax and Anaheim) were highly susceptible. It is advocated that TCA 14 be used in conjunction with other control measures to minimise the chances of the breakdown of its resistance.

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Table 1. Pepper cultivars screened for resistance to pepper veinial mottle virus under field conditions at Samaru in the 1985 wet season.

Pepper cultivar	PVMV - infected plants (%)	Disease severity (1-7)	Fruit yield (tonnes/ha)
Tca 14(C)	0.00	1.00	2.17
Kimba(C)	16.67	3.50	1.54
L3874(C)	16.67	3.61	1.72
L2164(C)	20.00	4.00	1.90
L2025(C)	21.67	4.10	1.64
Sakarho(C)	21.67	4.20	1.74
Darmeyere(C)	25.00	4.30	1.91
L2289(C)	28.33	4.10	2.43
L2191(C)	41.67	5.50	1.84
Ex-Hunkuyi(C)	41.67	5.51	1.81
L2190(C)	46.67	6.21	1.72
Caloro(S.P.)	21.67	4.50	1.95
Dantsiga(S.P.)	23.33	4.23	1.58
California mild(S.P.)	25.00	4.40	2.34
Hungarian yellow wax(S.P.)	35.00	5.32	2.32
Anaheim (S.P.)	45.00	6.34	2.31
S.E.D. (P=0.05)	3.83	1.05	0.31
C = Chilli	S.P. = Sweet pepper		

**Table 2. Pepper cultivars screened for resistance to pepper veinal mottle virus under field conditions at Samaru in the 1986 wet season.**

Pepper cultivar	PVMV - infected plants (%)	Disease severity (1-7)	Fruit yield (tonnes/ha)
Tca 14	0.00	1.00	2.21
Kimba	16.33	3.40	1.59
L3874	23.33	4.10	1.75
L2164	23.33	4.10	1.91
L2025	21.67	4.00	1.62
Sakarho	23.33	4.2	1.79
Danmeyere	23.33	4.15	1.94
L2289	31.67	5.00	2.48
L2191	33.33	5.40	1.83
Ex-Hunkuyi	41.67	5.50	1.84
L2190	41.67	5.60	1.68
Caloro	21.67	4.10	1.98
Dantsiga	21.67	4.10	1.61
California mild	21.67	4.15	2.29
Hungarian yellow wa	31.67	5.10	2.41
Anaheim	41.67	5.45	2.46
S.E.D. (P=0.05)	3.19	1.04	0.33

**Table 3. Pepper cultivars screened for esistance to pepper veinal mottle virus (PVMV) under field conditions at Samaru in the 1987 wet season.**

Pepper cultivar	PVMV - infected plants (%)	Disease severity (1-7)	Fruit yield (tonnes/ha)
Tca 14	0.00	1.00	2.22
Kimba	20.00	3.60	1.51
L3874	28.33	4.40	2.00
L2164	26.67	4.20	1.91
L2025	26.67	4.25	1.64
Sakarho	28.33	4.50	1.89
Danmeyere	20.00	3.70	1.94
L2289	36.67	5.50	2.59
L2191	45.00	6.35	1.89
Ex-Hunkuyi	46.67	6.40	1.94
L2190	45.00	6.32	1.64
Caloro	28.33	4.46	2.11
Dantsiga	25.00	4.15	1.67
California mild	26.67	4.21	2.67
Hungarian yellow wax	46.67	6.40	2.67
S.E.D. (P=0.05)	3.05	1.06	0.36

## MIXED INFECTION AMONG TMV, PVY, AND CMV I: YIELD OF THREE HOT PEPPER (*Capsicum annuum* L.) CULTIVARS

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### INTRODUCTION

Yield of hot pepper in Indonesia is relatively low as compared to one in other Southeast Asian countries. According to Duriat *et al.* (1994), average yield in the farmers' field is only 3.5 ton/ha. This yield is much lower than the potential yield of hot pepper (12 - 20 ton/ha). One constraint hampering hot pepper production in Indonesia and in other countries is disease due to viruses (A VRDC, 1995). Under certain condition in Indonesia, viruses may infect more than 90% of the hot pepper population in the field (Duriat, 1989).

At least 10 different viruses are found to infect hot pepper population in Indonesia. However, TMV, PVY, and CMV are the most commonly found (Duriat, 1989). Infections of CMV on hot pepper have been shown to reduce yield (Sulyo, 1984, Sulyo, 1988). In the field condition, however, mixed infection among viruses is often observed. In this report we present effects of mixed infection among TMV, PVY, and CMV on yield of three hot pepper cultivars (Hot Beauty, Keriting, and Jatilaba).

### MATERIALS AND METHODS

Hot pepper seedlings (15 days old) were transplanted onto polybag containing 10 kg of sterile potting soil mix (top soil: organic soil, 2: 1). One seedling was grown on each polybag. Seedlings were inoculated with the respective virus treatments at 15 days after transplant.

Virus treatments consisted of single virus inoculation (only CMV, PVY, or TMV), double inoculation (CMV+PVY, CMV+TMV, or PVY+TMV), or triple inoculation (CMV+PVY+TMV). Virus inoculation was conducted mechanically by rubbing single leaf. That has been dusted with Carborundum (400 mesh), with inoculum of the respective viruses. In this experiment, the experimental unit was consisted of five plants and each treatment was repeated three times.

Types of symptoms occurring among the inoculated plants were recorded during the first month after inoculation. In addition, plant height, number and weight of total fruits yielded by each plant, respectively, were also recorded. Subsequently, the data were analyzed statistically to determine the effects of virus treatments on vegetative growth and yield of hot pepper.

## RESULTS AND DISCUSSION

The results showed symptoms of virus infection due to TMV alone or in combination with PVY or CMV occurred at seven days after inoculation. In all three cultivars (Hot Beauty, Keriting, and Jatilaba), the symptom due to infection of TMV with or without PVY or CMV was severe mosaic. Inoculation of the three cultivars with PVY, CMV, or double inoculation of PVY and CMV induced either no symptom or mild vein clearing or yellowing.

Infection of CMV, PVY, or CMV+PVY did not cause reduction of plant height. On the other hand, infection of TMV, either alone or in combination with PVY or CMV, reduced height of the infected plants as compared to that of the control one (Table I). In general, inoculation of CMV, PVY, and TMV alone or in combination resulted in less biomass per plant than that of the control (Table I).

Virus infection on hot pepper affected the time required for harvesting the first fruit. Infection of CMV+TMV on hot pepper cv. Keriting resulted in longer time for the first harvest than that required by the control plants. For Hot Beauty and Tit L Super, however, the time needed to harvest the first fruit was not affected by the virus treatment

Fruit yield, measured as total number and total weight of fruit per plant, was affected by virus treatment or by cultivar. However, the interaction effect between virus treatment and cultivar was not significant. Yield of Hot Beauty was the least (36.0 fruits, 159.3 g per plant) while Tit L Super was the best (43.5 fruits, 173.2 g per plant). Yield of Keriting was between Hot Beauty and Tit L Super (Table 2). On the other hand, Hot Beauty has larger average fruit weight (4.5 g per fruit) than that of Keriting (2.0 g per fruit), or Tit L Super (4.2 g per fruit),

Infection of CMV, PVY, and TMV, either alone or in combination reduced yield of hot pepper. Plant that was inoculated with CMV+PVY+TMV yielded the least number of fruit per plant. On the other hand, one that was inoculated with TMV+CMV yielded the least fruit weight per plant (Table 2).

In this report we showed effects of mixed infection among TMV, PVY, and CMV on yield of hot pepper. Based on the data collected, yield reduction due to CMV infection was 22% (weight of fruit per plant) and 26% (number of fruit per plant). Reduction of yield due to PVY was 33% (number of fruit per plant) and 35% (weight of fruit per plant), while for CMV was 35% (weight of fruit per plant) and 40% (number of fruit per plant). More yield reduction was observed when the plant was infected with a mixture of either TMV +CMV (43% for number of fruit and 46% for weight of fruit per plant) or TMV+PVY+CMV (46% for number of fruit and 48% for weight of fruit per plant). Infection of other virus combination did not cause more yield reduction than that of single infection. Although CMV or PVY infection only induce mild symptoms on hot pepper leaves, their effects on yield was significant. In this experiment, all hot pepper cultivars tested showed similar trend of response to mixed infection of TMV, PVY, and CMV since there is no interaction effects between virus treatment and hot pepper cultivars.

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Table 1. Effects of mixed infection among TMV, PVY, and CMV on vegetative growth of hot pepper.

<b>Virus treatment</b>	<b>Plant height (cm)</b>	<b>Biomass (g/plant)</b>
CMV	76.5 b*)	33.1 ab
TMV	56.6 a	22.6 a
PVY	72.6 b	29.9 ab
CMV + TMV	49.0 a	22.9 a
CMV + PVY	73.9 b	29.4 ab
PVY + TMV	53.1 a	27.2 a
CMV + PVY + TMV	53.7 a	25.6 a
Control	72.1 b	39.8 b

NOTE: \*) The same letter on a column indicated not significantly different (DMRT, 5%)

Table 2. Effects of cultivars or mixed infection among TMV, PVY, and CMV on yield of hot pepper.

Factors tested	Total number of fruit per plant	Total weight of fruit (g/plant)	Average weight of fruit (g/fruit)
<i>Virus Treatment:</i>			
CMV	43.1 c*)	158.4 d	3.6 bcd
TMV	39.1 bc	122.0 abc	3.3 a
PVY	38.6 abc	132.2 bc	3.6 abc
CMV + TMV	33.2 ab	107.0 a	3.3 a
CMV + PVY	41.6 bc	142.1 cd	3.7 cd
PVY + TMV	36.4 abc	125.3 abc	3.4 abc
CMV + PVY + TMV	30.2 a	111.0 ab	3.9 d
Control	58.3 d	204.1 e	3.6 abc
<i>Cultivar:</i>			
Hot Beauty	36.0 a*)	159.3 b	4.5 c
Keriting	40.8 ab	80.8 a	2.0 a
Tit L. Super	43.5 b	173.2 b	4.2 b

NOTE: \*) The same letter on a column indicated not significantly different (DMRT, 5%)

## RESPONSE OF TEN HOT PEPPER LINES TO INFECTION OF CMV OR PVY

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### INTRODUCTION

Two of the most important viruses infecting hot pepper in Indonesia are CMV and PVY (Duriat, 1989). Therefore, hot pepper breeding for resistance to CMV and PVY is needed as alternative control strategy for these viruses.

To facilitate the development of hot pepper cultivar with resistance to CMV and PVY, lines having host gene for resistance to the viruses are required. Therefore, identification of hot pepper lines carrying the gene for CMV and PVY resistance is necessary. Since the cultivars will be grown in Indonesia, the resistance should be against Indonesian - isolates of CMV and PVY. In this report we determined the response of 10 hot pepper lines originated from various region against Indonesian isolates of CMV and PVY.

### MATERIALS AND METHODS

Hot pepper seedlings (15 days old) were Transplanted onto polybag containing 10 kg of sterile potting soil mix (top soil:organic soil, 2: 1). One seedling was grown on each polybag. Seedlings were then inoculated with Indonesian isolates of either CMV or PVY. Virus inoculation was conducted mechanically by rubbing single leaf, that has been dusted with Carborundum (400 mesh), with inoculum of the respective viruses. In this experiment, the experimental unit was consisted of one plants and each treatment was repeated eight times (total eight plants for each virus).

The occurrence of symptoms among inoculated plants were recorded during the first month after inoculation. For the plants showing symptoms, the identity of the virus causing such symptoms was checked using ELISA. For plants showing no symptoms, bioassay and ELISA was conducted to determine the presence of alive virus within the inoculated plants. In addition to the presence of symptoms, fruit yield (number and weight per plant) was also recorded. The yield data were analyzed statistically to determine the effects of virus treatments on each hot pepper lines. Once analyzed, all data were used to determine the response of each line against infection of Indonesian isolates of CMV and PVY. The response was grouped into highly resistance, resistance to systemic spread, tolerance, latency, and susceptible, according to Green (1991).

### RESULTS AND DISCUSSION

Results of the experiments indicated inoculation of CMV resulted in mild mosaic or yellowing on leaves of Matikas, Prapadaeng, M 1-1, LV 1592, Jatilaba, and Keriting. On other lines, infection of CMV induced no symptoms. Results of ELISA and bioassay test for

plants showing no symptoms of CMY infection indicated the absence of alive virus. Infection of CMY reduced number and weight of fruit yielded by each plant of Matikas, Prapadaeng, LV 1592, and Keriting, only reduced weight of fruit of Hot Beauty, and only reduced number of fruit of Kunya (Table 1 and Table 2). Infection of CMY did not reduce yield of BGI, Tumpang, M1-1, or Jatilaba. Summary of responses of the ten hot pepper lines against infection of CMY was presented on Table 3.

Inoculation of PVY resulted in vein clearing on leaves of BG 1, Prapadaeng, and M 1-1. On other lines, infection of PVY induced no symptoms. Among lines showing no symptoms of PVY infection, the virus is present in LV 1592 and Jatilaba. This indicated, although they showed no symptoms of PVY infection, the virus is alive within the plants. Infection of PVY reduced number and weight of fruit yielded by each plant of Prapadaeng, only reduced weight of fruit of Tumpang, Jatilaba, and Hot Beauty, and only reduced number of fruit of BGI, LV 1592 (Table 1 and Table 2). Infection of PVY did not reduce yield of Matikas, M 1-1, Kunya, or Keriting. Summary of responses of the ten hot pepper lines against infection of PVY was presented on Table 4.

Based on their response against CMY infection, BG 1 and Tumpang were highly resistant, while Matikas, Prapadaeng, LV 1592, and Keriting were susceptible to CMY. Kunya and Hot Beauty were resistant to systemic infection of CMY, while M1-1 and Jatilaba were tolerant to CMY. Similarly, based on their response against PVY infection, Matikas, Kunya, and Keriting were highly resistant, while Prapadaeng was susceptible to PVY. Tumpang and Hot Beauty were resistant to systemic spread while BG 1 and M 1-1 were tolerant to PVY.

The hot pepper lines showing resistance either to CMY or PVY were originated from different countries. It is possible each of these lines carry different genes for virus resistance. However, complementation studies among the resistance lines is required to determine whether such lines carry different resistance genes. If that is the case, the resistance genes can be combined in one line to obtain more durable resistance. Previously, the ten lines have been tested against PVY and CMY by AVRDC (AVRDC, 1995). In general, results of this test confirmed ones already reported. Therefore, BG 1 and Tumpang were a good candidate for sources of CMV resistance, while Matikas, Kunya, and Keriting were for sources of PVY resistance genes.

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Tabel 1. Effects of Indonesian isolates of CMV or PVY infection on number of fruits yielded per plant.

Kultivar Cabai	Kontrol	CMV	PVY
BG 1	26.6 a*	17.8 ab	16.4 b
Tumpang	10.4 a	8.5 a	6.1 a
Matikas	12.6 a	4.8 b	10.4 ab
Prapadaeng	27.4 a	5.1 b	4.3 b
M 1-1	8.9 a	2.9 a	6.5 a
LV 1592	18.4 a	10.4 b	7.1 b
Jatilaba	15.3 a	12.4 a	9.0 a
Kunya	20.1 a	15.5 b	21.8 ab
Keriting	22.1 a	10.3 b	18.1 ab
Hot Beauty	23.8 a	18.0 a	20.1 a

Note: \*) Data were obtained by averaging the yield of eight plants. The same letter on a row indicated not significant based on DMRT (5%).

Table 2. Effects of Indonesian isolate of CMV or PVY infection on weight (gram) of fruits yielded per plant.

Kultivar Cabai	Kontrol	CMV	PVY
BG 1	34.3 a*	23.3 a	24.3 a
Tumpang	106.0 a	96.6 a	65.5 b
Matikas	63.3 a	20.1 b	70.3 a
Prapadaeng	41.8 a	7.4 b	9.7 b
M 1-1	25.4 a	6.1 a	20.1 a
LV 1592	41.2 a	16.5 b	20.2 ab
Jatilaba	104.9 a	87.9 ab	65.7 b
Kunya	80.8 a	67.1 a	79.2 a
Keriting	101.3 a	40.5 b	62.8 ab
Hot Beauty	169.7 a	97.5 b	132.1 b

Note: \*) Data were obtained by averaging the yield of eight plants. The same letter on a row indicated not significant based on DMRT (5%).

Tabel 3. Response of 10 hot pepper lines to infection of Indonesian isolate of CMV.

Hot pepper lines	Virus replication	Virus spread	Symptoms of infection	Yield reduction	Type of response *)
BG 1	-	-	-	-	highly resistance
Tumpang	-	-	-	-	highly resistance
Matikas	+	+	+	++	susceptible
Prapadaeng	+	+	+	++	susceptible
M 1-1	+	+	+	-	tolerance
LV 1592	+	+	+	++	susceptible
Jatilaba	+	+	+	-	tolerance
Kunya	+/-	-	-	+/-	resistance to systemic spread
Keriting	+	+	+	++	susceptible
Hot Beauty	+/-	-	-	+	resistance to systemic spread

Note: \*) The response was assigned based on characters as described by Green (1991).

Tabel 4. Response of 10 hot pepper lines to infection of Indonesian isolate of PVY.

Hot pepper lines	Virus replication	Virus spread	Symptoms of infection	Yield reduction	Type of response*)
BG 1	+	+	+	+/-	tolerance
Tumpang	+/-	-	-	+ -	resistance to systemic spread
Matikas	-	-	-	-	highly resistance
Prapadaeng	+	+	+	++	susceptible
M 1-1	+	+	+	-	tolerance
LV 1592	+	+	-	+/-	latency
Jatilaba	+	+/-	-	+	latency
Kunya	-	-	-	-	highly resistance
Keriting	-	-	-	-	highly resistance
Hot Beauty	+/-	-	-	+	resistance to systemic spread

Note: \*) The response was assigned based on characters as described by Green (1991).

A leaf curl virus-like symptom on pepper at Samaru, Nigeria caused by the cold harmattan

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### **Abstract**

Leaves of most of the plants that developed leaf curl-like symptoms during the cold harmattan period at Samaru, Nigeria. were curled inwards and thread-like. Leaves of pepper plants with true leaf curl symptoms were curled inwards but were not thread-like.

### **Introduction**

The most important constraint to pepper production during the dry season in northern Nigeria is pepper leaf curl virus (PLCV). It is characterized by curling and distortion of leaves and the margins are curled inwards (Smith, 1972). Veins and veinlets are thickened and wrinkled (Singh, 1973).

During the cold harmattan of December/January of 1990/91, a leaf curl-like symptoms appeared on outdoor peppers at Samaru. The plants were stunted leaves were curled inwards and some were thread-like. A study was therefore conducted to find out what was responsible.

### **Materials and Methods**

Surveys were conducted in the 1990/91, 1991/92 and 1992/93 dry seasons at the outskirts of the Department of Crop Protection, Institute for Agricultural Research (IAR) irrigation site, Bomo village and the University Farm, all at Samaru.

Three hundred plants were surveyed at each site. The survey was conducted two times (January and March) each dry season. Four quadrats of 75 plants were set up in each field. Plants showing leaf curl-like symptoms were counted and tagged. In March, all plants still showing leaf curl-like symptoms were noted. A two-way analysis of variance was performed on all data and differences between means were compared using the Duncan's Multiple Range Test (DMRT).

### **Results**

Results of the three years are indicated in Table 1. There was a significant difference ( $P=0.05$ ) in the number of plants with leaf curl-like symptoms in the different locations. A significant difference was also observed in the percentage of recovered plants as well as percentage of plants with true leaf curl symptoms in March.

Leaf curl due to the cold harmattan made the leaves to curl inwards and the appeared thread-like, while leaf curl due to PLCV elicited inward curling and distortion of leaves, but they were not II thread-like.

### **Discussion**

The decrease in the incidence of leaf curl-like symptoms on pepper from December to March was probably due to the fact that some of the observed symptoms were due to the cold harmattan. Leaf curl is systemic in pepper and recovery has not been observed in plants inoculated in the glasshouse at Samaru during the hot season.

Table 1. Incidence of leaf curl-like symptoms on pepper at Samaru during the cold dry season (January) of 1991 - 1993 and the incidence of recovery in March of the same year.

Location	Number of plants with leaf curl-like symptoms out of 300 in January			Percentage of recovered plants in March			Percentage of plants with true leaf curl symptoms in March		
	1991	1992	1993	1991	1992	1993	1991	1992	1993
Outskirts of Dept. of Crop Protection	250.0a	233.0a	240.0a	48.0a	50.2b	47.9c	52.0a	49.8a	52.1a
I.A.R. Irrigation site	225.0a	218.0b	235.0a	46.8b	50.1b	50.1b	53.3a	49.5a	49.9b
Bomo Village	236.0a	227.0a	231.0a	51.7a	55.0a	56.3a	48.3b	44.9b	43.7c
University Farm	207.0b	198.0c	205.0b	47.3b	51.5b	46.3c	52.7a	48.5a	53.7a
S.E.D. (P = 0.05)	15.2	12.1	13.5	2.3	2.5	2.3	2.4	2.3	2.4

In each column, numerals followed by the same alphabet are not significantly different from each other at  $P = 0.05$  using the Duncan Multiple Range Test (DMRT)

## **RESISTANCE TO PHYTOPHTHORA BLIGHT IN *HOT* PEPPER GERMPLASM**

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**SUMMARY.** 1\_075 *Capsicum annuum* varieties and lines were inoculated with zoospores suspension of *Phytophthora capsici* at seedling stage in greenhouse to assess their resistance to *Phytophthora* blight. There were respectively 60 varieties resistant- 292 tolerant- 650 susceptible and 77 highly susceptible. There were significant resistance differences (57. significant level) among various cultivars- such as Zhoupi- , Chaotian- sweet pepper, Niujiào- Yangjiào- Xi an- Jian and Shizi. There were also significant resistance differences among the Niujiào peppers from different origins.

**Key words:** *Capsicum annuum*- *Phytophthora capsici*- resistance.

### **INTRODUCTION**

*Phytophthora* blight, one of the most important diseases of pepper is widespread in China and usually causes heavy loss of pepper yield. It is managed mainly by farming practices and spraying chemicals at present- but the results are always unsatisfactory. Therefore the emphasis of research is oriented to developing resistant pepper varieties. The aim of this research is to evaluate the resistance of pepper germplasm to *Phytophthora capsici* and to find resistance sources of pepper.

### **MATERIALS AND METHODS**

1,079 accessions of *Capsicum annuum* collected from all parts of China or abroad were evaluated in Hunan, Jiangsu and Shaanxi for their resistance to *Phytophthora capsici*. Isolates of *P. capsici* I used in the experiment were originally isolated from diseased tissues of pepper which were also collected from Hunan, Jiangsu and Shaanxi . The isolates were cultured and maintained on the oat agar medium. Pepper seeds were sown in sterilized soil after disinfection (5% TCCA solution for 5 min.) to raise seedlings

for resistance assessment. Pepper seedlings at 6-leaf stage were inoculated with zoospores suspension of *P. capsici* (1,000 CFU/ml, 3 ml/plant) into the soil near their stems. They were then incubated in greenhouse at 24-26 C for the disease development. After 7 days of incubation, reaction of plants was scored on a 0-5 scale for disease severity (0 = no symptom; 5 = wilt to death). Then disease incidence and disease index were calculated for each accession tested. The disease indexes were finally transformed into indexes of relative resistance (IRR) following Zheng' s method.

## **RESULTS AND DISCUSSION**

The results of the experiment showed that there were 60 varieties resistant, 292 tolerant, 650 susceptible and 77 highly susceptible to *P. capsici* among 1,079 accessions of pepper germplasm. There were significant resistance differences (5 significant level) among various varieties, such as Zhoupi pepper (surface wrinkled), Chaotian pepper (upright), sweet pepper, Niujiiao pepper (oxhorn), Yangjiao pepper (goat-horn), Xian pepper (long thin), Jian pepper (small, pointed) and Shizi pepper (heart). They were just in the resistance order from resistant to susceptible. There were also significant resistance differences in Niujiiao peppers from different regions, but no significant resistance differences existed in other peppers.

## LACK OF EVIDENCE FOR TRANSLOCATION OF RESISTANCE FACTORS BETWEEN ROOTS AND FOLIAGE OF *CAPSICUM ANNUUM* INFECTED BY *PHYTOPHTHORA CAPSICI*

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Translocation of compounds between the foliage and roots has been investigated in various plant physiological processes by reciprocal grafts. Disease resistance is one of the processes in which translocation of compounds has been studied. For example, it has been shown with reciprocal grafts that salicylic acid is not the translocated signal that induces systemic acquired resistance (Vemooij et al., 1994). The present study was conducted to determine if translocation of compounds between the roots and foliage may play a role in pepper resistance to *Phytophthora capsici* in the Mexican pepper line CM-334.

**Reciprocal grafts.** Seeds of the susceptible commercial cultivar New Mexico 6-4 (NM 6-4) and the resistant pepper line CM-334 were sown in pots with peat moss- vermiculite mix. Peters Professional general-purpose solution (20-20-20) was used to fertilize the plants. Plants were kept on a greenhouse bench, where the air temperature was 28 t 1° C during the day and 15 t 1° C at night. After 8 weeks CM-334 young vegetative buds were used as scion to graft onto NM 6-4 rootstock by side cleft grafting. Parafilm was utilized to hold the scion and stock together. Few leaves were kept on the stock until the graft was successful. At this time all the leaves from the stock were removed. The reciprocal graft was also performed. The plants were covered with transparent plastic bags after grafting and kept inside the bags for 1 week, to maintain high relative humidity. No water was added during this period. Before removing the bags they were perforated to let the plants adapt to the surrounding environment.

**Inoculation and disease assessment.** The roots were inoculated with 500,000 zoospores per plant five weeks after being grafted. Non-grafted plants of CM-334 and NM 6-4 were used as controls. To evaluate if resistance to *P. capsici* was confined to the roots the inoculum was applied to the soil with a syringe and kept under greenhouse conditions. The scale proposed by Bosland and Lindsey (1991) was used to score infected plants. 0 = no response, vigorous, healthy (as uninoculated control); 1 = slight root darkening, vigorous, healthy; 3 = brown roots, slight stunting, very small lesions on stems; 5 = brown roots, small lesions on stems, lower leaves wilted, stunted plants; 7 = brown roots, large lesions on stems, girdling, whole plant wilted, and stunted; and 9 = death.

To evaluate if resistance to foliar blight was affected by the roots, the protocol by Alcantara and Bosland (1994), was followed with modifications. Three young leaves were inoculated per plant in grafted and control plants, with approximately 30 III per leaf of a zoospore suspension of 250,000 zoospores/ml. The plants were kept in a

plastic tunnel with a humidifier inside to maintain high relative humidity (80-100%). Symptoms were assessed after five days.

## Results

The root rot experiment showed that when CM-334 was used as a scion and NM - 6-4 as a rootstock the plants died 5-7 days after inoculation similar to the NM 6-4 non-grafted controls. The disease index was 9 (Table 1). When CM-334 was used as a rootstock and NM 6-4 as a scion a 100% survival was observed. The disease index was 1, because only slight necrosis of some roots was observed with no above-ground symptoms like in the CM-334 controls (Table 3). The grafted plants (NM 6-4 scion/CM-334 rootstock) that survived developed normally producing the typical long green pepper pods of NM 6-4. Statistical analysis was not conducted because all the grafted plants NM 6-4 scion/CM-334 rootstock had the same score of 1, and all the plants with the inverse grafting CM-334 scion/NM 6- rootstock had a disease index of 9. In the foliar blight experiment when NM 6-4 was used as a scion and CM-334 as a rootstock NM 6-4 'Jves were susceptible and 5 days after inoculation dark green lesions water-soaked and irregularly shaped were observed, as in NM 6-4 non-grafted controls. When CM-334 was used as a scion and NM 6-4 as rootstock small necrotic lesions typical of a hypersensitive response were observed under the microscope on CM-334 leaves. The same response was observed in CM-334 non- grafted controls (Table 2).

Table 1. Disease severity of grafted and non-grafted plants 7 days after root inoculation with *P. capsici* zoospores.

Host	Disease index
CM scion/NM 6-4 rootstock	9
NM 6-4 controls	9
NM 6-4 scion/CM rootstock	1
controls	1

Table 2. Symptoms observed on leaves of grafted and non-grafted plants 5 days after foliar inoculation with *P. capsici* zoospores.

Host	Foliar Symptoms
CM scion/NM 6-4 rootstock (HR)	Small necrotic lesions
NM 6-4 controls	Dark green lesions
NM 6-4 scion/CM rootstock	Dark green lesions
CM controls (HR)	Small necrotic lesions

\*HR= hypersensitive response-

## Discussion

The root rot experiment showed that translocation of compounds from the foliage to the roots in CM-334 is not required for resistance to *Phytophthora capsici* causing Phytophthora root rot (PRR) in CM-334. Similarly, translocation of compounds from the roots of CM-334 to the foliage is not necessary for resistance to *P. capsici* causing foliar blight in CM-334. According to Walker (1997) the same genes that control resistance to foliar blight do not control resistance in CM-334 to PRR. Therefore, two different mechanisms appear to confer resistance to PRR and to foliar blight in CM-334. Our results also suggest that they are independent.

In countries where grafting vegetables is a Common practice (Lee, 1994) and where *P. capsici* is a problem on peppers, CM-334 represents an alternative as a tolerant rootstock to control PRR.

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## A NOVEL SEED GERMINATION TEST FOR PEPPER (*CAPSICUM* SPP.)

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### Introduction

Pepper researchers and seed companies commonly do seed germination testing. Seed germination testing under field conditions has proven unsatisfactory because the results cannot be repeated with reliability (Anonymous, 1985). Laboratory methods have been found to give reliable, repeatable germination data. The paper towel or the Petri dish method may be used for pepper seeds (Anonymous, 1985), but the Petri dish method is the most commonly used method (Osman and George, 1988; Bradford et. al., 1990). Both methods have drawbacks, including their labor requirement and potential for growth of saprophytic fungi. Recently, we developed a reproducible, easy method to test pepper seed germination utilizing sterile vermiculite that requires relatively little labor and has few problems with saprophytic fungi (A VRDC, 1997). However, no information was available comparing its performance with the two methods sanctioned by the ISTA. Therefore, we conducted a preliminary experiment at the A VRDC to compare the seed germination rate of pepper seeds determined by three different methods: Petri dish (PETRI), paper towel (PAPER), and sterile vermiculite (VERM).

### Materials and Methods

Ten pepper lines (Table 1), were sown on February 3, 1997 using the three methods, germination testing methods. For each line-method combination, four replicates of 25 seeds each were tested. All seeds were treated with a solution of 0.1 % Benlate (50% WP) immediately before sowing to prevent growth of saprophytic fungi. For PETRI, the seeds were placed on a moistened, sterile filter paper in an 11.5 cm diameter Petri dish. The dishes were placed inside a sealed clear plastic bag to maintain a high level of humidity. For PAPER, the seeds were placed on a moistened, sterile 23.5 x 23.5 cm brown paper towel, rolled up into a cylinder, and placed into a plastic cup with one cm of water in the bottom. For VERM, the seeds were placed one cm deep in sterile vermiculite in a five cm diameter plastic pot with drainage holes in the bottom. The germination test was carried out under controlled conditions in a growth chamber at a constant temperature of 24 C and photoperiod of 16/8 h light/ dark. The filter paper inside the Petri dish, the paper towel, and the sterile vermiculite were kept moist with sterile distilled water as needed throughout the experiment. Data was recorded up to 26 days on seed germination. Two values were obtained: days to 50% germination (GERM50), defined as the day when 50% of the seeds germinated (in this case, the 13th day), and % germination. A combined analysis of variance (ANCOVA) was computed over lines and treatments treating both as fixed effects to determine if there were significant differences ( $p \leq 0.05$ ) among treatments for GERM50. Duncan's Multiple Range Test

DMR1) was used to separate the means for each treatment within each line. The data were analyzed using SAS (SAS,1987).

## **Results and Discussion**

In the 10 lines tested, the mean days to GERM50 ranged from 9.3-18.3 d (Table 1), and the % germination ranged from 41-98% (Table 2) across the three methods. For six of the 10 lines, no significant variation was observed for % germination of a given line among the three methods (i.e., PBC 1075, with values of 82%,80%, and 84% for PETRI, PAPER, and VERM, respectively). For the other four lines, significant ( $p<0.05$ ) variation was observed (i.e. PBC 1358, with values of 54%,83%, and 64% for PETRI, PAPER, and VERM, respectively). Among the three methods, the mean % germination rate was intermediate, and the mean days to GERM50 was less, for VERM compared to PETRI and PAPER (Tables 1 and 2). The initial germination rate was faster for VERM compared to PETRI and PAPER; the average % germination at nine days after sowing was 17% for VERM, whereas it was 0% and 2% for PETRI and PAPER, respectively (data not shown).

The results showed that the VERM method was intermediate in determining the % germination rate, giving a higher value than the PETRI method but a lower value than the PAPER method. The initial emergence was also quicker in the VERM method compared to the PETRI and PAPER methods. The VERM method requires 1 labor and gives similar or better results compared to the other two seed testing methods, thus it can be used by anyone interested in determining days to GERM50 or % germination in pepper seeds.

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Table 1. Days to GERM50 for 10 lines determined using three germination methods.

Entry	PETRI	PAPER	VERM
PBC 8	12.5b*	12.0b	9.3a
PBC 74	12.3a	11.8a	10.8a
PBC 105	12.0b	11.3b	9.8a
PBC 142	13.3b	12.3a	13.8b
PBC 372	16.8b	15.8a	18.0b
PBC 407	17.0a	18.0a	16.8a
PBC 696	NA*	16.8a	18.3b
PBC1043	14.3b	11.0a	10.0a
PBC1075	16.0b	18.2c	13.2a
PBC1358	17.0a	14.8a	12.5a
<i>Mean</i>	<i>14.6c</i>	<i>14.2b</i>	<i>13.2a</i>

\* Duncan's Multiple Range Test; values within a row are not significantly different from each other if they have the same letter.

\* Not available because <50% of seeds germinated.

Table 2. Percent germination for 10 lines determined using three germination methods.

Entry	PETRI	PAPER	VERM
PBC 8	88.0a*	97.0b	87.0a
PBC 74	90.0a	94.4a	90.0a
PBC 105	94.0a	88.0a	90.0a
PBC 142	90.0a	88.0a	88.0a
PBC 372	90.0a	86.0a	90.0a
PBC 407	84.0a	81.0a	80.0a
PBC 696	41.0a	73.0b	61.0b
PBC1043	63.0a	98.0b	95.0b
PBC1075	82.0a	80.0a	84.0a
PBC1358	54.0a	83.0b	64.0a
<i>Mean</i>	<i>77.6a</i>	<i>87.0c</i>	<i>82.9b</i>

\* Duncan's Multiple Range Test; values within a row are not significantly different from each other if they have the same letter.

## LACK OF PRIMARY SEED DORMANCY IN PEPPER (*CAPSICUM* SPP.)

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### Introduction

Uneven emergence of pepper seedlings is frequently observed. This may be due to fruit immaturity (Cochran, 1934), seedcoat-bound seedlings (Baker, 1948), primary seed dormancy (PSD) (Randle and Honma, 1981; Edwards and Sundstrom, 1987; Illgham et al., 1993), or location of the plant (Gikalo, 1966) general, pepper seeds do not show dormancy and the seeds germinate immediately after extraction from the mature fruit (Odland, 1938). Poor germination and uneven emergence of freshly extracted pepper seeds has been occasionally observed in breeding lines at the A VRDC, Shanhua, Taiwan. This causes poor stand establishment and uneven early growth and maturity. Therefore, the objective of this experiment was to determine the extent of PSD present in a diverse set of pepper accessions and to determine the optimum length of warm, dry treatment (if any) required to break PSD.

### Materials and methods

Seeds of 48 pepper accessions representing three species (*C. annuum*, *C. frutescens* and *C. baccatum*) were used (Table 1). The 48 accessions were grown at the A VRDC experimental farm near Shanhua, Taiwan. The seeds were sown in May, the seedlings were transplanted in June, and the ripe fruits were harvested on 18 October 1996. Seeds were collected from fully matured open-pollinated fruits. The seeds were hand-extracted from the ripe fruit immediately after harvest, air dried for 48 hours at 20 C and 40% RH, and stored at 24.1 C and 60-70% RH. The seeds were sown in two replicates of 20 seeds replicate-1 from each accession. They were sown at seven day intervals for a period of 28 days beginning on 22 October, giving a total of five after-ripening treatments (0, 1, 2, 3, and 4 weeks). Seeds were sown one cm deep in plastic pots of five cm diameter filled with sterile vermiculite and placed in two temperature-controlled chambers, one replicate per chamber. The temperature was a constant 24 C and the photoperiod was 16/8 h light/ dark. The pots were watered once every two days and kept moist without drying out. A seedling was considered to be emerged when the cotyledon cleared the vermiculite surface. Emerged seedlings were removed from the pots. The days to 50% germination (GERM50) was recorded when 50% of the seedlings emerged in each pot (in this case, the 10th seedling).

A combined analysis of variance (ANOVA) was computed over accessions and weeks treating both as fixed effects to determine if there were significant differences ( $p \sim 0.05$ ) among genotypes for GERM50. An ANOVA was computed for each accession over weeks to determine if there were significant differences between weeks zero and four for GERM50, and Duncan's Multiple Range Test (DMRT) was used to separate the means for each week within each accession.

## Results and Discussion

The combined ANOVA indicated that there were highly significant ( $p \sim 0.01$ ) differences in mean GERM50 among accessions and among weeks. The mean GERM50 averaged across accessions decreased the first week and then was unchanged (Table 1). For individual accessions, the mean GERM50 was highest in the accession PBC137 (22.1 days) and lowest in the accession PBC404 (8.6 days).

Five accessions (PBC151, PBC368, PBC495, PBC586, and PBC679) had significantly lower GERM50 at four weeks compared to week zero, and two more accessions (PBC481 and PBC643), showed a trend towards lower GERM50 ( $p = 0.07$ ). This indicates that warm, dry storage broke any PSD existing in the seeds. Only one accession, PBC151, which is *C. baccamm*, required more than one week to break PSD. Most accessions showed no significant variation in GERM50 from week zero to week four (Table 1).

On a species basis, PSD existed in 4/44 *C. annuum* accessions and 1/2 *C. baccamm* accessions, but not in the two *C. frutescens* accessions, indicating that it is not species-specific. Previous researchers (Ingham et al., 1993) reported PSD occurred in Tabasco (*C. frutescens*), but our two accessions of Tabasco (PBC556 and PBC559) showed no PSD. In conclusion, PSD occurred to a limited extent in the 48 pepper accessions we tested, and one week of warm, dry treatment was sufficient to break PSD in most cases. Thus it should not be a factor in uneven seedling emergence.

## Acknowledgments

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PBC 634	<i>C. annuum</i>	Sri Lanka	13.5a	10.5a	11.0a	16.0a	10.0a
PBC 643	<i>C. annuum</i>	Kenya	17.5a	14.5a	17.0a	18.0a	9.0a
PBC 651	<i>C. annuum</i>	India	12.0a	8.5a	11.0a	9.5a	11.0a
PBC 679	<i>C. annuum</i>	U. S. A.	15.0a	8.0b	9.0b	8.0b	10.5b
PBC 719	<i>C. annuum</i>	India	10.0a	9.0a	10.0a	9.5a	10.0a
PBC 731	<i>C. annuum</i>	Korea	10.0a	8.0a	9.5a	9.5a	9.0a

Table 1. Mean days to 50% germination (GERM50) for seeds of 48 pepper accessions from three species after-ripened at 24 C for 0, 1, 2, 3, or 4 weeks.

Accession	Species	Origin	After-ripening (No. of weeks)				
			0	1	2	3	4
PBC 67	<i>C. annuum</i>	Malaysia	11.5a <sup>+</sup>	9.0a	9.5a	9.5a	10.5a
PBC 137	<i>C. annuum</i>	India	19.5a	26.5a	22.0a	20.0a	22.5a
PBC 148	<i>C. annuum</i>	India	10.5a	12.0a	12.0a	9.5a	14.0a
PBC 151	<i>C. baccatum</i>	Brazil	17.0a	15.0a	12.0ab	9.5b	10.0b
PBC 155	<i>C. annuum</i>	Thailand	10.0a	11.0a	10.0a	8.5a	9.0a
PBC 156	<i>C. annuum</i>	Thailand	12.0a	15.5a	14.0a	13.0a	14.0a
PBC 157	<i>C. annuum</i>	Thailand	13.5a	9.5a	12.5a	11.0a	15.0a
PBC 161	<i>C. annuum</i>	Sri Lanka	10.5a	9.5a	9.5a	9.0a	9.0a
PBC 197	<i>C. annuum</i>	U. S. A.	10.5a	8.5a	9.0a	8.5a	10.5a
PBC 198	<i>C. annuum</i>	Thailand	10.0a	10.0a	10.0a	8.5a	9.0a
PBC 365	<i>C. annuum</i>	Italy	11.5a	12.0a	14.0a	12.0a	11.5a
PBC 367	<i>C. annuum</i>	Sri Lanka	10.0a	8.0a	9.0a	8.0a	12.0a
PBC 368	<i>C. annuum</i>	Indonesia	13.0a	8.5b	9.5b	8.5b	9.0b
PBC 369	<i>C. annuum</i>	Indonesia	11.5a	9.0a	10.0a	8.0a	12.5a
PBC 370	<i>C. annuum</i>	Thailand	10.0a	8.5a	8.5a	7.5a	9.0a
PBC 371	<i>C. annuum</i>	Thailand	10.0a	8.0a	9.5a	9.5a	9.0a
PBC 382	<i>C. annuum</i>	Taiwan	11.5a	8.5a	14.0a	8.5a	12.0a
PBC 386	<i>C. annuum</i>	Malaysia	11.5a	9.0a	9.5a	10.0a	11.5a
PBC 400	<i>C. annuum</i>	Sri Lanka	10.0a	9.0a	9.5a	10.5a	9.0a
PBC 404	<i>C. annuum</i>	Nigeria	10.0a	8.5a	8.5a	7.0a	9.0a
PBC 480	<i>C. annuum</i>	Sri Lanka	10.0a	9.0a	8.5a	10.0a	10.0a
PBC 481	<i>C. annuum</i>	Sri Lanka	15.5a	15.0a	14.5a	10.0a	11.0a
PBC 485	<i>C. annuum</i>	Sri Lanka	11.0a	9.0a	8.5a	7.5a	9.0a
PBC 486	<i>C. annuum</i>	India	12.0a	15.0a	14.0a	12.0a	9.0a
PBC 495	<i>C. annuum</i>	France	13.5a	9.0b	10.0b	9.5b	9.0b
PBC 539	<i>C. annuum</i>	AVRDC	12.0a	8.0a	8.5a	9.0a	10.0a
PBC 549	<i>C. annuum</i>	Indonesia	12.0a	11.0a	8.0a	9.0a	11.0a
PBC 556	<i>C. frutescens</i>	U. S. A.	13.5a	16.5a	14.0a	10.0a	13.0a
PBC 559	<i>C. frutescens</i>	U. S. A.	14.5a	16.5a	16.0a	12.0a	18.0a
PBC 585	<i>C. annuum</i>	Thailand	13.0a	9.5a	10.5a	10.5a	10.0a
PBC 586	<i>C. annuum</i>	Thailand	13.5a	10.5b	11.0b	8.5b	10.0b
PBC 596	<i>C. annuum</i>	Thailand	10.5a	8.0a	10.0a	8.5a	9.0a
PBC 612	<i>C. annuum</i>	Thailand	13.5a	9.5a	10.5a	10.0a	10.0a
PBC 613	<i>C. annuum</i>	Thailand	11.5a	7.5a	8.5a	8.0a	9.0a
PBC 622	<i>C. annuum</i>	Taiwan	10.0a	8.5a	8.5a	9.0a	10.0a
PBC 629	<i>C. annuum</i>	India	10.5a	7.5a	7.0a	11.0a	10.0a
PBC 634	<i>C. annuum</i>	Sri Lanka	13.5a	10.5a	11.0a	16.0a	10.0a
PBC 643	<i>C. annuum</i>	Kenya	17.5a	14.5a	17.0a	18.0a	9.0a
PBC 651	<i>C. annuum</i>	India	12.0a	8.5a	11.0a	9.5a	11.0a
PBC 679	<i>C. annuum</i>	U. S. A.	15.0a	8.0b	9.0b	8.0b	10.5b
PBC 719	<i>C. annuum</i>	India	10.0a	9.0a	10.0a	9.5a	10.0a
PBC 731	<i>C. annuum</i>	Korea	10.0a	8.0a	9.5a	9.5a	9.0a

Table 1. Cont'd.

Accession	Species	Origin	After-ripening (No. of weeks)				
			0	1	2	3	4
PBC 743	<i>C. annuum</i>	Thailand	11.5a	13.5a	11.0a	11.0a	11.5a
PBC 770	<i>C. chinense</i>	Grenada	12.0a	8.5a	9.5a	8.5a	10.0a
PBC 972	<i>C. annuum</i>	Malaysia	12.5a	8.5a	10.0a	8.5a	11.0a
PBC1327	<i>C. annuum</i>	Taiwan	13.5a	14.5a	12.0a	15.5a	15.5a
PBC1329	<i>C. annuum</i>	Taiwan	10.0a	7.5a	10.0a	8.5a	10.0a
PBC1351	<i>C. baccatum</i>	France	12.5a	8.0a	9.5a	10.5a	14.5a
Mean			12.1a	10.5b	10.8b	10.1b	11.0b

\* Duncan's Multiple Range Test; GERM50 values within a row are significantly different from each other at the  $p \leq 0.05$  level if they are followed by a different letter.

## HETEROSIS STUDIES IN EGG PLANT (*Solanum melongena* L.)

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### INTRODUCTION

Eggplant having its center of origin in India and being an often cross pollinated crop possess considerable diversity for plant type, fruit yield and yield attributing traits and thus offers an opportunity to exploit the genetic diversity for development of hybrid varieties. Studies conducted elsewhere revealed manifestation of hybrid vigor in egg plant for earliness, yield and several other characters (Chadha *et al.* 1990; Viswanathan 1973 and Vijay and Prem Nath, 1975) and suggested its commercial utilization either through manual hybridization or by use of some self emasculating system. Similar studies on hybrid vigor are almost nil in this temperate region of India. Considering the importance of eggplant as one of the extensively used vegetable, the present study was therefore initiated for the first time with a view to study the extent of heterosis and to develop promising F<sub>1</sub> hybrids in egg plant involving three testers and five diverse lines.

MATERIALS AND METHODS Line x tester, set of crosses were attempted, involving five diverse lines viz; Arka Shirish (AS), Selection-4 (Sel-4), H- 7, JC-1 and Punjab Bahar (PB) and three testers viz; Local Long (LL), Pusa Purple Long (PPL) and Jamuni Gola (JG). Each tester was crossed to each line and developed 15 crosses. The 15 F<sub>1</sub> crosses along with 8 parents were grown during summer 1996 in randomized block design with 3 replications at Vegetable Farm, SKUAST, Srinagar. The parents and F<sub>1</sub> hybrids were planted in single row having 10 plants each. The rows were spaced at 60 cm apart and plants at 45 cm within the row. Observations were recorded on 11 characters (Table 1). Heterosis was measured as the proportion of deviation of F<sub>1</sub> values from the better parent (BP) and standard parent (SP). The better parental lines were established individually for the different characters based on their superior mean performances and the commercial cultivar Pusa Purple Long (PPL) was used as standard parent.

### RESULTS AND DISCUSSION

The analysis of variance revealed that testers, lines and hybrids resulting from line x testers mating exhibited significant differences for all the characters. The mean values of parents and best five F<sub>1</sub> hybrids are presented in Table 1. The F<sub>1</sub> mean value for all the characters except days to first fruit set was higher than the parental means indicating superiority of F<sub>1</sub> hybrids over parents. For days to first fruit set the F<sub>1</sub> mean value was lower suggesting early fruiting of hybrids over parents and is a more desirable attribute as it contributes to early yield. The parent LL recorded high mean value in respect of plant height and seed number and was also earliest than other parents whereas parent PB was best for plant spread, fruit girth, average fruit weight and fruit yield. For branch number and fruit yield parent AS was the best. Parent H- 7 for fruit length, JG for fruit girth and average fruit weight, Sel-4 for fruit number and JC-1 for drymatter were found superior as they recorded highest mean values.

Heterosis range, number of F<sub>1</sub> hybrids with desirable heterosis and four best hybrids heterotic over BP and SP for each character are presented in Table 2. For days to first fruit set where negative heterosis was preferred heterosis ranged from -5.43% to 2.32% and -3.66% to 0.98% over BP and SP respectively. Five hybrids showed significant and negative heterosis over BP whereas over SP only one hybrid, namely AS x LL showed significant negative heterosis and was also found earliest (106.14 days). The range of heterosis varied from -38.02% to 50.62% and -14.02% to 64.18% over BP and SP respectively for plant height. The hybrids Sel-4 x LL, Sel-4 x PPL, AS x JG and AS x PPL were taller and showed significant positive heterosis both over BP and SP. For plant spread 8 crosses over BP and 14 crosses over SP recorded significant desirable heterosis and it was as high as 79.77% and 85.02% over BP and SP respectively. Among crosses, the hybrid combinations PB x JG, Sel-4 x PPL, AS x PPL and JC-1 x LL had maximum spread and recorded highest positive heterosis both over BP and SP. In case of branch number, heterosis ranged from 0.0 to 31.82% over BP and 3.02 to 37.15% over SP with 8 and 11 crosses showing significant heterosis over BP and SP respectively. The hybrids AS x PPL, AS x JG, Sel-4 x PPL over SP and JC-1 x JG and Sel-4 x PPL over BP expressed

maximum heterosis as well as highest number of branches per plant.

Out of 15 crosses only four crosses over BP and one cross over SP manifested heterosis for fruit length. The heterosis was maximum to an extent of 23.49% over BP and to an extent of only 7.20% over SP. Only one cross AS x PPL recorded higher fruit length (18.19 cm) than parents and other hybrid combinations. For fruit girth, heterosis ranged from -32.23 to 17.06% and .278% to 74.79% over BP and the SP respectively. The crosses, PB x JG, PB x LL, PB x PPL, JC-1 x PPL exhibited significant positive heterosis over SP and Sel-4 x PPL, JC-1 x PPL and PB x JG over BP. Viswanathan (1973) and Vijay and Prem Nath (1975) also observed heterosis for fruit girth. Fruit number is the main component which ultimately results in increased fruit yield. Heterosis was as high as 69.74% and 93.57% over BP and SP respectively. For this trait, most of the crosses showed significant positive heterosis over both the BP and the SP. Maximum heterosis of 93.57% over SP was manifested coupled with highest fruit number of 26.78 by sel-4 x LL followed by Sel-4 x PPL (63.86%), AS xLL (58.64%) and AS xJG(53.41%). Manifestation of heterosis over both BP and SP was also observed for average fruit weight which ranged from -21.16 to 38.68% and 7.56 to 49.75% over BP and SP respectively. The crosses PB x JG, JC-1 x PPL, PB x LL, and Sel-4 x PPL exhibited high heterosis over both BP and the SP and also recorded greatest average fruit weight. For fruit yield, all the 15 hybrids out yielded the standard parent as well as the better parent with heterotic values ranging from 39.63% to 118.76% and 39.63% to 127.45% respectively. The most promising crosses showing superiority for yield and exhibiting maximum heterosis both over BP and SP were Sel-4 x PPL, Sel-4 xLL, AS xJG, and PB x JG. These crosses exhibited heterosis of 84.25 to 118.76% over BP and 91.80 to 127.45% over SP with an average fruit yield of 2.46, 2.32, 2.08 and 2.07 kg respectively. Vijay and Prem Nath (1975) and Chadha *et al.* (1990) also reported high magnitude of heterosis for fruit yield in egg plant. With regard to seed number heterosis ranged from -15.28 to 136.80/0 and 15.28 to 106.70% over BP and the SP respectively. Among 9 heterotic crosses, the cross AS x LL, Sel-4 x LL, PB x LL, and H- 7 x LL exhibited maximum heterosis over SP and also recorded high mean seed number. For drymatter content, heterosis ranged from -17.40 to 16.23% and -12.48 to 24.20% over BP and SP respectively with two crosses, namely H- 7 x JG and PB x LL exhibiting significant heterosis as well as high drymatter content.

Among the heterotic crosses, the hybrids namely Sel-4 x PPL, Sel-4 x LL, AS x JG, PB x JG and PB x LL recorded substantial increase in fruit yield over BP and SP and were the highest yielding cross combinations with significant standard heterosis as high as 127.45%. High fruit yield and heterosis observed in the crosses is probably resulted due to combined heterosis of its component characters such as average fruit weight, fruit size, fruit number, branch number, plant height and plant spread as these hybrids also recorded significant positive heterosis for most of these yield contributing traits. Vijay and Nath (1975) and Chadha *et al.* (1990) also made similar observations in egg plant. Further, it was evident from the results that almost in all the hybrids which showed the best heterotic effects, the parental lines involved had at least one of the most outstanding parental line, namely AS, Sel-4, and PB which in general showed high mean values for one or more of the characters contributing towards yield. From economic point of view, the results suggested that it is necessary to utilize the best performing parental line having one or more characters associated with yield such as number of fruits per plant, fruit size, fruit weight, branch number and plant habit in order to achieve higher gain in the F I hybrid.

The present study also revealed that the crosses which showed high heterosis for yield also recorded high seed number. It is, therefore, worthwhile to exploit heterosis of the best crosses Sel-4 x PPL, Sel-4 x LL, PB x JG, AS x JG and PB x LL for fruit yield and yield attributing traits through heterosis breeding which is quite feasible in this crop due to its low seed rate per hectare and considerably high number of seeds can be produced from a single pollination.

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**Table 1: Mean performance of parents and best five F<sub>1</sub> hybrids for different characters in egg plant**

Source	Days to first fruit ripening	Plant height (cm)	Plant spread (cm)	Branch number	Fruit length (cm)	Fruit girth (cm)	Pericarp thickness (mm)	Fruit number	Average fruit weight(g)	Seed number	Fruit yield (g)
<b>Limes</b>											
1. AS	116.75	52.83	54.55	4.08	17.21	8.08	16.53	68.14	1.13	394.3	7.26
2. Sel-4	119.00	50.17	38.00	3.67	12.30	7.07	22.84	49.19	1.12	249.7	7.18
3. H-7	117.00	41.17	46.00	3.67	17.70	8.55	11.08	70.60	0.78	386.3	9.15
4. JC-1	115.33	43.28	43.11	3.15	16.80	12.08	11.75	87.31	1.08	581.0	9.51
5. PB	113.39	68.94	77.11	3.50	13.36	15.51	11.28	96.88	1.12	568.7	8.58
<b>Testers</b>											
6. LL	107.72	81.39	69.44	3.78	16.14	12.53	8.05	87.42	0.70	914.0	9.08
7. PPL	110.17	53.67	42.83	3.67	16.97	10.82	13.83	78.25	1.08	584.7	8.57
8. JG	113.50	43.33	58.92	3.75	9.17	17.45	9.21	106.81	0.98	345.0	7.49
<b>Mean of parents</b>	<b>114.11</b>	<b>54.35</b>	<b>53.74</b>	<b>3.66</b>	<b>14.96</b>	<b>11.51</b>	<b>13.10</b>	<b>80.57</b>	<b>1.00</b>	<b>502.9</b>	<b>8.35</b>
<b>Mean of crosses</b>	<b>108.80</b>	<b>65.10</b>	<b>66.34</b>	<b>4.30</b>	<b>16.22</b>	<b>13.38</b>	<b>19.64</b>	<b>97.51</b>	<b>1.91</b>	<b>843.4</b>	<b>8.63</b>
<b>Five best crosses (Means)</b>											
a)	1x6 (106.14)	2x6 (38.11)	5x6 (79.25)	1x7 (5.03)	1x7 (18.19)	5x8 (18.91)	2x6 (26.78)	5x8 (116.71)	2x7 (2.46)	1x6 (1208.5)	3x8 (10.64)
b)	4x7 (107.33)	2x7 (80.83)	2x7 (77.00)	1x8 (4.86)	4x7 (17.42)	5x6 (16.00)	2x7 (22.67)	4x7 (111.82)	2x6 (2.32)	2x6 (1108.2)	5x6 (10.34)
c)	4x6 (107.50)	5x6 (80.58)	4x6 (75.67)	2x7 (4.83)	4x6 (17.12)	5x7 (15.75)	1x6 (21.94)	5x6 (109.52)	5x8 (2.08)	5x6 (1092.3)	4x6 (9.74)
d)	2x7 (107.67)	2x8 (72.83)	1x7 (75.33)	4x8 (4.83)	2x6 (16.99)	4x8 (14.28)	1x8 (21.22)	2x7 (108.52)	1x8 (2.07)	3x6 (1057.3)	4x8 (9.00)
e)	-	4x6 (70.83)	5x7 (73.92)	5x7 (4.65)	4x8 (16.61)	1x8 (13.80)	1x7 (21.17)	5x7 (105.24)	5x6 (2.02)	4x6 (998.3)	1x7 (8.86)
C.D at 5%	3.20	4.68	6.11	0.51	0.60	0.43	1.53	4.57	0.15	110.8	0.33
C.D at 1%	4.28	6.27	8.18	0.76	0.81	0.58	2.05	6.11	0.20	148.2	0.44

**Table 2:** Heterosis range, number of F<sub>1</sub>'s heterotic over BP and SP and four best hybrids in egg plant

Characters	Range of heterosis (%) over		Number of F <sub>1</sub> s with significant desirable heterosis over		Four best hybrids heterotic over	
	BP	SP	BP	SP	BP (heterosis %)	SP (heterosis %)
1. Days to first fruit set	-5.43 to 2.32	-3.66 to 0.98	5	1	3x8(-5.43), 5x8(-4.47), 1x8(-4.22), 4x8(-4.14)	1x6(-3.66)
2. Plant height (cm)	-38.20 to 50.62	-14.02 to 64.18	4	5	2x7(50.62), 1x8(30.6), 1x7(28.05), 2x6(8.26)	2x6(64.18), 2x7(50.62), 1x8(28.63), 1x7(28.05)
3. Plant spread (cm)	-19.80 to 79.77	-19.33 to 85.02	8	14	2x7(79.77), 4x7(60.57), 1x7(38.09), 1x8(23.51)	5x8(85.02), 2x7(79.77), 4x6(76.65), 1x7(75.87)
4. Branch number	0.00 to 31.82	3.02 to 37.15	8	11	2x7(31.82), 4x8(28.89), 5x7(26.82), 3x8(23.58)	1x7(37.15), 1x8(32.60), 2x7(31.82), 4x8(31.82)
5. Fruit length (cm)	-12.57 to 23.49	-17.56 to 7.20	4	1	2x8(23.49), 1x7(5.73), 2x6(5.24), 5x8(4.72)	1x7(7.20)
6. Fruit girth (cm)	-32.23 to 17.06	-2.78 to 74.79	5	13	2x7(17.06), 4x7(14.02), 5x8(8.39), 4x6(5.24)	5x8(74.79), 5x6(47.87), 5x7(45.59), 4x8(31.97)
7. Fruit number	-22.66 to 69.74	25.94 to 93.57	13	15	4x6(69.74), 3x8(63.61), 5x6(63.52), 3x6(58.29)	2x6(93.57), 2x7(63.86), 1x6(58.64), 1x8(53.41)
8. Average fruit weight (g)	-21.16 to 38.68	7.56 to 49.15	8	14	2x7(38.68), 4x7(28.07), 4x6(14.23), 5x6(13.08)	5x8(49.15), 4x7(42.90), 5x6(39.97), 2x7(38.68)
9. Fruit yield/plant (g)	39.63 to 118.76	39.63 to 127.45	15	15	2x7(118.76), 2x6(106.54), 5x8(85.05), 1x8(84.25)	2x7(127.45), 2x6(114.75), 5x8(92.29), 1x8(91.89)
10. Seed number	-15.28 to 136.80	-15.28 to 106.70	8	9	3x8(136.80), 1x8(127.39), 2x8(75.75), 1x7(60.21)	1x6(106.70), 2x6(89.54), 5x6(86.83), 3x6(80.84)
11. Drymatter content (%)	-17.43 to 16.23	-12.51 to 24.21	2	3	3x8(16.24), 5x6(13.96)	3x8(24.20), 5x6(20.74), 4x6(13.68)

INOCULUM CONCENTRATION, SEEDLING AGE AND B. W. SEVERITY TO  
STANDARDIZE SCREENING IN EGGPLANT

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INTRODUCTION

Bacterial wilt caused by Pseudomonas solanacearum is one of the most important and wide spread bacterial disease of crops in tropical environments. Among the solanaceous crops, potato, tobacco and tomato are greatly affected by this disease, while under certain tropical conditions pepper and egg plant is also severely affected. There is a little information, however, about the extent of damage caused to eggplant by bacterial wilt.

The technique for BWR screening for eggplant at AVRDC was derived from the one for tomato. The inoculum concentration of

8 x 10<sup>8</sup> cells/ml is used in tomato and pepper at AVRDC. The response of eggplants to this concentration was fine in the preceding screening trials. However, the effect of inoculum concentration on disease development in eggplant is unknown. On the other hand, resistance to diseases is sometimes increased when the age of plants increases in some crops. Currently the 30-day-old seedlings were used for screening and the following 30 days were conserved as symptom observation. To investigate the effect of inoculum concentration and seedling age on the BW severity of resistant and susceptible eggplant varieties, three experiments were conducted during 1994.

MATERIALS AND METHODS

For this investigation, three varieties, TS56B (a resistant variety), Bonne (a susceptible variety) and Pingtung Long (a moderately resistant variety) were selected for first trial. For the second trial TS56(B) (a resistant variety) Bonne (a susceptible variety) and Black Gnome (moderately resistant variety) and for the third trial TS56B, Pingtung Long and Bonne were selected. In the first experiment seeds of three varieties, were sown at 10 days interval on 3rd June, 13 June and 23 June 1994, respectively. The seedlings were inoculated with two

6 x 10<sup>8</sup> concentrations 10 and 10 cells/ml on 13 July 1994 when they were at 20, 30 and 40 days after sowing.

In the second and third experiments, seeds of the above said varieties were sown on 5 days interval. In the second trial the seeds were sown on 12 Aug, 17 Aug and 22 Aug. On the third experiment the above mentioned varieties were sown on Nov 7, Nov 12, and 17 Nov 1994. The seedlings were inoculated on Sept 9 and 17 Oct 1994, respectively. The seedlings were inoculated at 20, 25 and 30 days after sowing in 2 concentrations, 10 and 10 cells/ml. The inoculum of P. solanacearum was prepared by the Pathology Department. The trials includes 18 treatment were

arranged in RCBD with three replications. Each replication contained 15 plants. The method of inoculation and disease reading were same viz., soil drenching with supplement root cutting.

## RESULTS AND DISCUSSION

In the first experiment among the three varieties the disease severity was highest in Bonne under both 10 and 100 cells/ml inoculum concentration. The soil drenching with root severity produced fairly good differentiation between Bonne and the resistant varieties. A decrease in the inoculum concentration from 100 to 10 cells/ml of inoculum did not affect the disease severity on 20-40 days old seedlings of Bonne and 40 days old TS56B and Pingtung Long. But however the severity was decreased in 20-30 days old TS56B and Pingtung Long seedlings. The susceptibility to BW decreased as the seedling age increased from 20-40 days in all the three varieties but however the differences were not significant in susceptible Bonne variety for this seedling ages. The disease indices of all the three varieties were not significantly different when the seedlings were 20-30 days old (Table 1).

Since the mature plant resistance was observed on the resistant and moderately resistant varieties and as there were less severity at 10 cells/ml concentration the second experiment was redesigned.

A decrease in the inoculum concentration from 100 to 10 cells/ml inoculum in general did not affect the disease severity relatively in Bonne Black Genome and Pingtung Long variety. The Disease index were not different significantly in Bonne and Black Genome varieties as the age of seedling increased from 20 to 30

days. However, in moderate resistant variety Pingtung Long there were decrease in severity of disease both at 10 cells and 100

a cells/ml concentrations as the seedling age increased from 20-30 days. Among the three varieties Pingtung Long differ significantly over other two varieties at all three seedling ages, and two concentrations (Table 2).

In the third experiment in the susceptible variety Bonne and resistant variety TS56B reduction in inoculum concentration to

10 did not affect the disease severity but in moderately resistant variety, Pingtung Long the severity is high in the 100 cells/ml inoculum on 20 days old seedlings. Generally even 30 days old seedlings of Bonne, Pingtung Long at both inoculum

100 and 10 cells/ml concentrations, inoculum concentration the disease severity is less when compared

100 to 10 cells/ml concentrations on 20 days old seedlings and the mature plant resistance was observed. However, the disease severity at 25 days old seedlings

were higher than 20 days old seedlings of all the three varieties at 10 cells/ml inoculum concentrations but the differences were not significant only in TS56B. Among the three varieties two were not significantly different for the disease severity at both levels of inoculum concentration (Table 3).

The result of the above three sets of experiments leads to the following conclusions. Among the different levels of inoculum concentrations the disease severity was not much different in mature plants. But the severity of younger seedlings

was comparably less at 10 cells/ml concentrations. Among the four varieties involved TS56B exhibited significantly lesser severity of disease over Bonne the susceptible variety. The moderately resistant variety Black Genome turned to be a more susceptible variety. The Pingtung Long the moderately resistant variety the severity of a disease was moderate.

Regarding the age of seedlings 40 days old seedlings the susceptibility to BW decreases both in resistant and moderately resistant varieties and the mature plant resistance was observed. In between 20-30 days old seedlings the average severity was higher when 20 days old seedlings were inoculated with both

7 inoculum levels. But with 10 cells/ml the disease index was higher in 25 days old seedling in third experiment it may be due to the change of environmental conditions since the experiment was conducted in heated green house. The influence of the environmental factors on disease development was also reported by Rambavan (1990) in tomato.

The data revealed that small young plants of resistant and moderately resistant lines were highly susceptible. Similar results were also reported by Winstead and Kelman (1954) in tomato. Inoculation of 30 days old seedlings with a

concentration of 10 cells/ml gave good estimate of relative levels of resistance. However, for BWR mass screening of segregating populations a younger seedlings age of 20 days with

10 cells/ml inoculum concentration is suggested.

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Table 1 The disease indices as affected by inoculum concentration and seedling ages in eggplant after inoculated with *Pseudomonas solanacearum* PSS 97 for 30 days<sup>a</sup>

Variety	10 <sup>8</sup> cells/ml			Mean	L.S.D. (5%)	C. V.	10 <sup>6</sup> cells/ml			Mean	L.S.D. (5%)	C. V.
	20 DAS	30 DAS	40 DAS				20 DAS	30 DAS	40 DAS			
Bonne	100 <sup>b</sup>	97	95	97.3	10.5	4.8	100	100	96.9	99.0	4.4	2.0
Pingtung Long	40	40	4	28.0	34.8	54.9	22	15	4	13.6	26.6	86.1
TS56B	17	7	0	8.0	15.8	87.2	4	2	0	1.9	5.2	
Mean	52.2	48.3	32.9				41.9	39.0	33.6			
L.S.D. (5%)	19.6	29.5	13.2				13.5	24.0	11.8			
C. V.	16.6	26.9	17.7				14.2	27.1	15.4			

<sup>a</sup> Planting dates: sowing, first 3 June 1994; second 14 June 1994; third 23 June 1994; inoculating, 13 July 1994.

<sup>b</sup> Disease index % =  $(\sum n_i \times i) / (N \times i_{max})$ , where  $i = 0 - 5$ ,  $i_{max} = 5$ ,  $n =$  no. of plants;  $N =$  total plants.

Cul. 5.03 var. 6.7; Lt age 13-16\* cul x var. 4.18

cul x age 2.42 var. x age 2.47\* cul x var x age 1.19

Table 2

The disease indices as affected by inoculum concentration and seedling ages in eggplant after inoculated with *Pseudomonas solanacearum* PSS 97 for 30 days<sup>a</sup>

Variety	10 <sup>8</sup> cells/ml			Mean	L.S.D. (5%)	C. V.	10 <sup>7</sup> cells/ml			Mean	L.S.D. (5%)	C. V.
	20 DAS	25 DAS	30 DAS				20 DAS	25 DAS	30 DAS			
Bonne	97.78 <sup>b</sup>	100	98.22	98.67	10.6	10.74	100	98.93	100	99.64	4.9	4.92
Pingtung Long	44.85	26.22	24.44	31.85	21.2	66.56	51.11	18.67	24.89	31.55	17.21	54.57
TS56B	98.22	96.00	98.22	97.63	12.6	12.90	98.22	97.78	100.0	98.67	1.17	1.19
Mean	80.29	74.07	73.62				83.11	71.79	63.11			
L.S.D. (5%)	28.2	31.4	42.59				27.2	48.6	13.9			
C. V.	35.12	42.39	57.86				32.72	67.6	16.72			

<sup>a</sup> Planting dates: sowing, first 12 Aug 1994; second 17 Aug 1994; third 22 Aug 1994; inoculating, 12 September 1994.

<sup>b</sup> Disease index % =  $(\sum n_i \times i) / (N \times i_{max})$ , where  $i = 0 - 5$ ,  $i_{max} = 5$ ,  $n =$  no. of plants;  $N =$  total plants.

cul 7.82 var. 728.62 age 13.46 cul x var x 7.28

cul x age x 2.78 var x age 4.79 cul x var x age 2.41\*

Table 3

The disease indices as affected by inoculum concentration and seedling ages in eggplant after inoculated with *Pseudomonas solanacearum* PSS 97 for 30 days<sup>a</sup>

Variety	10 <sup>8</sup> cells/ml			Mean	L.S.D. (5%)	C. V.	10 <sup>7</sup> cells/ml			Mean	L.S.D. (5%)	C. V.
	20 DAS	30 DAS	40 DAS				20 DAS	30 DAS	40 DAS			
Bonne	100 <sup>b</sup>	100	93.67	97.89	11.2	52	94.86	96.6	88.93	93.47	4.1	2.6
Pingtung Long	53.97	33.2	32.1	39.75	32.3	61.0	26.57	33.2	18.2	25.99	27.8	89.4
TS56B	17.4	11.07	18.2	15.56	16.4	68.2	16.46	25.57	0.0	14.01	6.9	1.92
Mean	57.12	48.09	47.99				45.96	51.79	35.71			
L.S.D. (5%)	21.0	31.0	14.6				14.2	26.2	11.4			
C. V.	17.2	27.9	17.92				17.9	27.9	16.2			

<sup>a</sup> Planting dates: sowing, first 7 Nov 1994; second 12 Nov 1994; third 1994; inoculating, 17 Dec 1994.

<sup>b</sup> Disease index % =  $(\sum n_i \times i) / (N \times i_{max})$ , where  $i = 0 - 5$ ,  $i_{max} = 5$ ,  $n =$  no. of plants;  $N =$  total plants.

cul 6.01 var. 711.2\* age 14.21 cul x age 2.62

var x age 4.98\* cul x var. 5.27 cul x var x age 2.10\*

CORRELATION AND PATH ANALYSIS FOR YIELD, FRUIT BORER INFESTATION, LITTLE LEAF INCIDENCE AND QUALITY TRAITS IN BRINJAL (*SOLANUM MELONGENA* L.)

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## INTRODUCTION

Knowledge of correlation and path analysis help plant breeder to ascertain the real components of yield and provide an effective basis of selection. However, in case of vegetable crop like brinjal, infestation of diseases and insects as well as quality traits are also considered to be important. If these characters can be identified as contributing significantly to yield then they could be useful as alternate selection criteria in yield improvement programme. Therefore, the present study was planned to study the correlation and path analysis using 41 E genotypes of brinjal.

## MATERIALS AND METHODS

The experiment comprised of 41 diverse genotypes of brinjal was laid out in randomised block design with four replications at Plant Breeding Farm, Gujarat Agricultural University, Anand during 1995-96. Each plot had two rows and each row consisted of 10 plants. The inter and intra row spacing was 90 and 60 cm, respectively. Five random plants were selected from each plot for recording the observations on fruit yield per plant (g), fruit borer infested fruits (%) and little leaf incidence (%). While, uniformly ripen fruits were collected at random from each plot to determine the chemical composition. The fruits were cut and dry matter was obtained by drying to a constant weight at 105 for 6 hours. The quality determining traits like polyphenoloxidase activity (Taneja and Sachar, 1974), total phenols (Malik and Singh, 1990), glycoalkaloid content (Currie and Kuc, 1975) and total soluble as well as reducing sugars (Sadasivam and Manickam, 1992) were estimated by using the standard procedures given by various scientists. Anthocyanin content was determined from the peel of the fresh fruit (Ranganna, 1976). Correlation and path analysis was worked out according to the method suggested by Wright (1921 and 1934).

## RESULTS AND DISCUSSION

### CORRELATION

The data presented in Table 1 revealed that the fruit yield per plant had significant positive correlation with total soluble sugars and reducing sugars at both genotypic and phenotypic levels, and with total phenols at genotypic level only. While,

the correlation between fruit yield and dry matter was found negative and significant. Similar associations were also found by Sidhu et al. (1981) and Kapadia (1995). The fruit borer infested fruits were positively and significantly correlated with anthocyanin content, total soluble sugars and reducing sugars at both the levels. Whereas, its association with polyphenoloxidase activity, total phenols and glycoalkaloid content was found negative and significant. Little leaf disease which is considered to be serious damaging to the crop was significantly and positively correlated with anthocyanin content. However, the association of this trait with glycoalkaloid content and total phenols was observed to be negative and significant. Kapadia (1995) had also reported similar associations in both the biotic stress traits. Total soluble sugars and reducing sugars had negative significant association with total phenols and glycoalkaloid content. While the association between these two traits was found to be significant and positive. The results of the present investigation suggested that fruit yield per plant can be improved by selecting genotypes having higher total soluble and reducing sugars, total phenols and lower dry matter. It also suggested that selection of genotypes having higher glycoalkaloid content, total phenols and high polyphenoloxidase activity; moderate soluble and reducing sugars; and low anthocyanin content may help in improving resistance against fruit borer infestation and little leaf incidence without compromising fruit yield potential in brinjal.

#### PATH ANALYSIS

The direct and indirect effects on yield of other traits are presented in Table 2. Total phenols had the highest positive direct effect on yield and its indirect effect via polyphenoloxidase activity and total soluble sugars were negative and high. Reducing sugars had also a high positive direct effect on yield and its indirect effect through total soluble sugars was negative and high but via total phenols its indirect effect was high and positive. High negative direct effect on yield was observed for, total soluble sugars but its indirect effect via fruit borer infested fruits reducing sugars, total phenols and anthocyanin content was high and positive which not only counterbalanced the negative direct effect but resulted into a positive correlation with fruit yield per plant. Positive direct effect on Yield was observed for dry matter but its indirect effects via remaining traits except total i.e. negative, which changed the direction and magnitudes of relationship between these traits i.e. negative significant correlation with fruit Yield per plant.

Correlation and path analysis revealed that total phenols and reducing sugars were directly associated with fruit yield in brinjal, because of its positive significant associations and positive direct effects on fruit yield.

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Table 1 : The estimates of genotypic (lower left diagonal) and phenotypic (upper right diagonal) correlation between fruit yield per plant and other quality characters as well as among quality characters

Characters	Fruit yield per plant (%)	Fruit borer infestation (%)	Little leaf incidence (%)	Dry matter (%)	Anthocyanin content (mg/100g)	Polyphe-nol oxidase activity (U/g fresh wt.)	Total phenols (mg/100mg dry wt.)	Glycoal-kaloid content (mg/100g)	Total soluble sugars (mg/100mg dry wt.)	Reducing sugars (mg/100mg dry wt.)
Fruit yield/plant		0.2556	0.1755	-0.3083*	-0.0578	-0.0403	0.2597	-0.0764	0.3486*	0.4132**
Fruit borer infestation	0.2774		0.1801	-0.2865	0.3362*	-0.3240*	-0.4372**	-0.3392*	0.7189**	0.3144*
Little leaf incidence	0.1867	0.1843		-0.2199	0.3985*	-0.2630	-0.3201*	-0.3660*	0.2133	0.0367
Dry matter	-0.3271*	-0.2102	-0.2259		-0.0679	0.1620	-0.1374	0.1397	-0.1989	-0.2981
Anthocyanin content	-0.0614	0.3426*	0.3973**	-0.0580		0.2746	-0.0860	-0.1870	0.4076**	-0.1287
Polyphenol oxidase activity	-0.0436	-0.3261*	-0.2733	0.1590	0.2748		0.0046	0.2471	0.1662	0.1005
Total phenols	0.3409*	-0.6239**	-0.3805*	-0.1967	-0.1173	0.0043		0.1395	-0.3600*	-0.4263**
Glycoalkaloid content	-0.0831	-0.3472*	-0.3623*	0.1404	-0.1872	0.2689	0.1896		-0.6224**	-0.3412*
Total soluble sugars	0.3751*	0.7348**	0.2383	-0.1987	0.4080**	0.1867	-0.3468**	-0.6230**		0.5549**
Reducing sugars	0.4403**	0.3202*	0.0385	-0.2991	-0.1288	0.1007	-0.5911**	-0.3421**	0.5558**	

Table 2 : Path coefficient analysis showing direct (underlined) and indirect effects of nine quality traits on fruit yield in brinjal

Characters	Fruit borer infestation (%)	Little leaf incidence (%)	Dry matter (%)	Anthocyanin content (mg/100g)	Polyphe-nol oxidase activity (U/g fresh wt.)	Total phenols (mg/100mg dry wt.)	Glycoal-kaloid content (mg/100g)	Total soluble sugars (mg/100mg dry wt.)	Reducing sugars (mg/100mg dry wt.)	Genotypic correlation with fruit yield
Fruit borer infestation	<u>1.3861</u>	0.0501	-0.2288	0.5164	-0.0869	-0.2917	0.1969	-1.7024	0.4378	0.2774
Little leaf incidence	0.2555	<u>-0.2721</u>	-0.2459	0.5989	-0.4918	0.0914	0.2054	-0.5514	0.0526	0.1867
Dry matter	-0.2914	-0.0615	<u>1.0885</u>	-0.5066	-0.0646	-0.4631	-0.0796	0.4604	-0.4089	-0.3271*
Anthocyanin content	0.4749	0.1081	-0.3653	<u>1.5074</u>	-0.4945	-0.2762	0.1061	-0.9453	-0.1761	-0.0614
Polyphenol oxidase activity	0.0669	0.0744	0.0391	0.4142	<u>-1.7995</u>	1.4228	0.0334	-0.4326	0.1377	-0.0436
Total phenols	-0.1717	0.0106	-0.2141	-0.1768	-1.0875	<u>2.3545</u>	-0.1075	-0.6645	0.3980	0.3409*
Glycoalkaloid content	-0.4813	-0.0986	0.1528	-0.2822	0.1060	0.4464	<u>-0.5670</u>	0.4529	0.1877	-0.0831
Total soluble sugars	1.0185	0.0648	-0.2163	0.6150	-0.3360	0.6756	0.1108	<u>-2.3169</u>	0.7598	0.3751*
Reducing sugars	0.4438	0.0105	-0.3256	-0.1942	-0.1812	0.6854	-0.0778	-1.2877	<u>1.2671</u>	0.4403*

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

**EFFECTS OF EFFECTIVE MICROORGANISMS (EM) AND CALCIUM NITRATE (Ca(NO<sub>3</sub>)<sub>2</sub>) ON BACTERIAL WILT ESTABLISHMENT IN EGGPLANT (*SOLANUM MELONGENA* L.).**

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Abstract

An experiment was conducted to study the effect of Effective Microorganisms (EM), calcium nitrate (Ca(NO<sub>3</sub>)<sub>2</sub>) fertilizer and EM in combination with Ca(NO<sub>3</sub>)<sub>2</sub> on the incidence of bacterial wilt in eggplant grown in the field. The results indicated that the ; development of bacterial wilt in the eggplants grown in plot treated with combination I, EM and Ca(NO<sub>3</sub>)<sub>2</sub> were significantly reduced compared with those of the other ii" treatments. In such plot, the initial lag phase of the disease development was extended if 1 from two to four weeks after transplanting. At seven weeks after transplanting, where the endemic began to decline, the percentage of wilt incidence in the plot treated with combination EM and Ca(NO<sub>3</sub>)<sub>2</sub> was 65%, whereas those of untreated and treated with either EM or Ca(NO<sub>3</sub>)<sub>2</sub> were 92%, 92.1 % and 88.6% respectively.

Keywords: control, bacterial wilt, eggplant, Effective Microorganisms (EM).

Introduction

Bacterial wilt caused by *Burkholderia*, (*P, s~udomona~*) *solanacearum* E.F. Smith is the Iii' most important disease and, has been a limiting factor in eggplant (*Solanum me~ongen,a* L.) production In the tropics (Singh, 1991). The major strategy for controlling this disease is breeding for disease resistant cultivars. Nevertheless, fully resistant cultivars I of eggplant have not always been developed (Ano, *et al*, 1991).

The use of Effective Microorganisms (EM) has been suggested as an alternative li means for controlling the disease in Solanaceous crops (Jonglaekha, *et al*, 1992). EM is a group of beneficial microorganism, of which, through fermentation in the soil would be able to produce organic acids, plant hormones, vitamins and antibiotics that can benefit the growing plant, such as protecting the plant from soil-borne pathogens. Mixed cultures of EM were reported to be effective in establishing a favourable microbiological equilibrium in the plant rhizosphere, and thus help to control the disease infestation (Higa, 1994).

A field study was conducted to determine the effect of EM, with and without Ca(NO<sub>3</sub>)<sub>2</sub> fertilizer addition, on bacterial wilt infestation ill eggplant, and to see the possibility of using such method in controlling the disease.

## Materials and Methods

The experiment was conducted in the field at Farm 2, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia. The field with sandy loam soil types was infested with *B. solanacearum*, having previously been cropped with bacterial wilt susceptible eggplant cultivar, in which more than 85% bacterial wilt incidence was observed. To ensure uniformity of pathogen distribution in the soil, the blocks were located in the field where the previous crop had been uniformly affected. The treatments were as follows,

1. without use of neither EM nor Ca(NO<sub>3</sub>)<sub>2</sub>, as control
2. treated with EM only
3. treated with Ca(NO<sub>3</sub>)<sub>2</sub> only
4. treated with both EM and Ca(NO<sub>3</sub>)<sub>2</sub>

The plot size was 5 x 5 m and each plot was surrounded by a drainage ditch (30 cm wide and deep). Twenty-five healthy six week-old seedlings of moderately susceptible accession of eggplant were planted at a spacing of 1 x 1 m in each plot. The treatments were arranged in a Randomised Complete Block Design, with five replicates.

Suspension of stock cultures of EM designated EM4 were diluted 1: 100 with distilled water and applied at a rate of 2,000 ml of diluted suspension per hectare by spraying onto the soil and plants one day after transferring. Ca(NO<sub>3</sub>)<sub>2</sub> fertilizer at the rate of 30 kg per hectare were incorporated into the soil one day before transplanting. The entire plots were overlaid with white polythene sheets as a means for controlling weeds and maintaining soil moisture. The plots were daily watered by using sprinkler system.

The incidence (% affected plants) of bacterial wilt in each plot were monitored at one week intervals over two months. The presence of *B. solanacearum* in the affected plants were confirmed through the observation of bacterial ooze released from the cutting stem after being immersed in distilled water. At eight weeks after transplanting, marketable fruit yields per treatment were determined from the survived plants.

The data were subjected to the statistical analysis and pair comparison of weekly disease incidence between treatments were made by using the least significant difference (LSD) test.

## Results and Discussions

Data showing weekly statistical significance of bacterial wilt incidence between treatments is shown in Table 1, while graph illustrating the disease epidemic development in each treatment is shown in Figure 1. The result indicated that there was a progressive increase in the incidence of the disease within population of eggplant over seven weeks of monitoring. The disease growth pattern is sigmoid with three phases of the curves, an initial lag, exponential increase phase and a final decline (Figure 1).

Wilt symptoms began to develop at about five days after transplanting. Within two weeks after transplanting, the incidence of wilting did not differ significantly among the treatments. At 3 - 4 weeks after transplanting the incidence in EM and Ca(NO<sub>3</sub>)<sub>2</sub>-treated and control plots began to increase sharply, whereas those in plot treated with combination of EM and Ca(NO<sub>3</sub>)<sub>2</sub> remained significantly lower. This may be attributed to the unfavourable soil environment for the pathogen development in EM+Ca(NO<sub>3</sub>)<sub>2</sub>

treated plot. At seven weeks after transplanting, where the endemic began to decline the percentage of wilt incidence in plot treated with a combination of EM and Ca(NO<sub>3</sub>)<sub>2</sub>, either EM or Ca(NO<sub>3</sub>)<sub>2</sub> alone, and without treatment were 65%, 92.1 %, 88.6% and 92.0% respectively. Average fruit weight per plot for the plants treated with a combination of EM and Ca(NO<sub>3</sub>)<sub>2</sub>, either EM or Ca(NO<sub>3</sub>)<sub>2</sub> alone, and without treatment were 1,615g, 565g, 392g and 388g respectively.

The result clearly showed that the initial lag phase of disease development in EM and Ca(NO<sub>3</sub>)<sub>2</sub> combination-treated plot was extended from two weeks to four weeks after transplanting (Figure 1). Thus, the phase of exponential increase in such plot was delayed, and yields would profitably be produced before wilting reaches a critical level.

### **Conclusion**

This study revealed the ability of EM to control bacterial wilt of eggplants. The application of EM alone is not sufficient to reduce the disease incidence to a control level. However, EM utilized in a combination with Ca(NO<sub>3</sub>)<sub>2</sub> fertilizer could maintain pathogens pressure below a critical level and minimize stress on the crop production. Further studies will need to be conducted in order to quantify the usefulness of EM in large scale production of the crop.

### **Acknowledgements**

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Table 1: Percentage of bacterial wilt incidence in the eggplant population grown in the field treated with effective Microorganism (EM) and Calcium Nitrate ( $\text{Ca}(\text{NO}_3)_2$ ).

Treatments	Weeks after transplanting						
	1	2	3	4	5	6	7
Control	10.2 <sup>a</sup>	12.5 <sup>a</sup>	31.8 <sup>a</sup>	66.8 <sup>a</sup>	78.4 <sup>a</sup>	88.7 <sup>a</sup>	92.0 <sup>a</sup>
EM	7.9 <sup>a</sup>	7.9 <sup>a</sup>	27.3 <sup>ab</sup>	58.0 <sup>a</sup>	72.3 <sup>a</sup>	86.4 <sup>a</sup>	92.1 <sup>a</sup>
$\text{Ca}(\text{NO}_3)_2$	6.8 <sup>a</sup>	6.8 <sup>a</sup>	23.0 <sup>ab</sup>	55.7 <sup>a</sup>	75.0 <sup>a</sup>	84.1 <sup>a</sup>	88.6 <sup>a</sup>
EM + $\text{Ca}(\text{NO}_3)_2$	5.7 <sup>a</sup>	5.7 <sup>a</sup>	13.9 <sup>b</sup>	26.4 <sup>b</sup>	40.1 <sup>b</sup>	56.1 <sup>b</sup>	65.0 <sup>b</sup>
LSD at 5% level	1.69	1.77	3.19	3.02	2.6 <sup>c</sup>	2.16	1.96

In column, means followed by a common letter are not significantly different at the 5% level by DMRT.

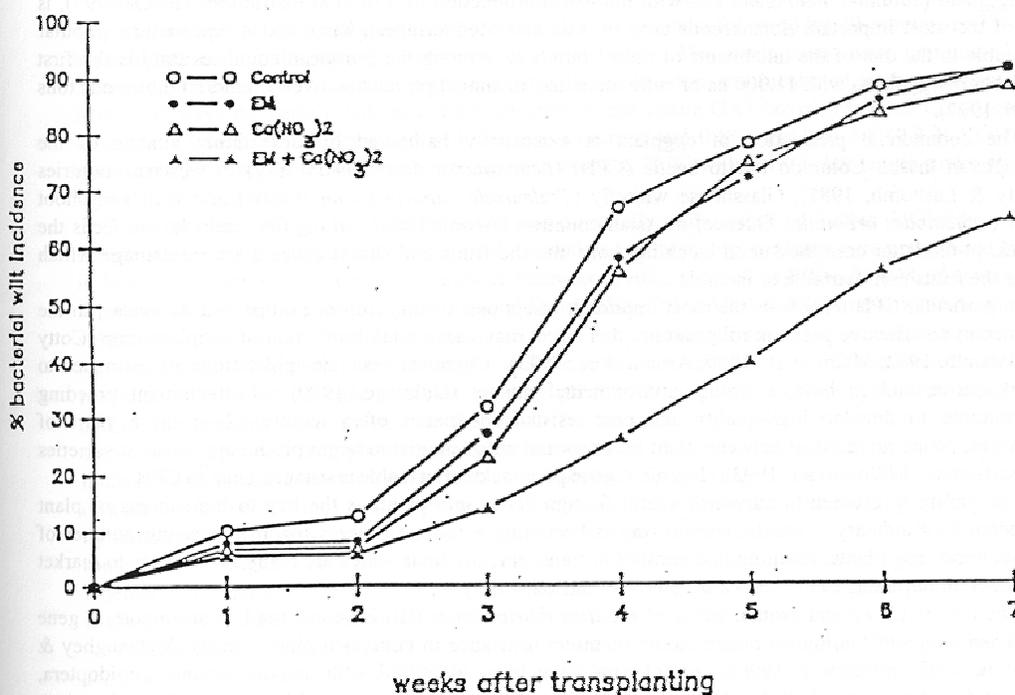


Figure 1: Effect of effective microorganisms (EM) and Calcium Nitrate ( $\text{Ca}(\text{NO}_3)_2$ ) on the incidence of bacterial wilt (% affected plants) following transplanting of eggplant (*Solanum melongena*) to treated plots.

## REGENERATION OF TRANSGENIC EGGPLANTS (*SOLANUM MELONGENA* L.) FOR A CYSTEINE PROTEINASE INHIBITOR

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### ABSTRACT

Transgenic eggplants (*Solanum melongena* L.) were recovered as a result of *Agrobacterium tumefaciens* mediated transformation performed with ERA101 strain carrying the binary plasmid pCYS. Plasmid pCYS contains a cysteine proteinase inhibitor gene derived from soybean (*Glycine max* L.). The effect of growth regulators and antibiotics on eggplant transformation was also studied and optimised. A total of 25 independent transgenic lines were obtained from 300 co-cultivated explants of five eggplant lines. Specific PCR analysis of the putative transformants demonstrates the presence of fragment corresponding to the kanamycin selective marker NPTII. Protease inhibitors are an important element of plant defence response to insect re-dation. The role of protease inhibitor genes is discussed in the context of multigene resistance to develop an effective strategy to achieve and maintain an higher and durable level of pest resistance when combined with Bt gene.

**INTRODUCTION** Eggplant (*Solanum melongena* L.), with the world production of 9 million metric tons (FAO, 1995), is one of the most important Solanaceous crop in Asia and Mediterranean basin and it represents a popular vegetable in the diet of the inhabitants of these countries. Among the European countries Italy is the first producer of eggplants with 11.000 ha of cultivation and an annual production over 3 hundred thousands tons (Setti, 1997).

The commercial production of eggplant is extensively hampered by devastating attacks of the coleopteran insect, Colorado Potato beetle (CPB) (*Leptinotarsa decemlineata* Say) in western countries (Cotty & Lashomb, 1981), Glasshouse whitefly (*Traleurode vaporariorum* Westw) and fruit and shoot borer (*Leucinodes orbonalis*, Guenee) in Asian countries (Atwal, 1986). In the first cases larvae feeds the leaves, in the latter case they upon hatching, bore into the fruits and shoots causing severe damage which make the fruits non-marketable or inedible.

In particular CPB represents the most important insect pest of this crop in Europe and America. In the absence of an effective pest control program, this insect may cause total destruction of eggplant crop (Cotty & Lashomb, 1982; Maini *et al.*, 1990; Arpaia *et al.*, 1995). Chemical pesticide applications are estimated to be expensive and to have a strong environmental impact (Dulmage, 1980). Conventional breeding programmes to develop high-quality and pest resistance varieties often require 12 or more years of sustained, cooperative effort between plant breeders and entomologist to be in producing resistant varieties (Roberts *et al.*, 1988; Stoner, 1992). Eggplant gene pool lacks of valuable resistance gene to CPB.

The ability to efficiently introduce useful foreign genes into plants is the key to the success of plant biotechnology industry. Genetic engineering is becoming a routine process for an increasing number of horticultural crop plants, resulting in a number of transgenic products which are ready for or close to market introduction (Grumet, 1995; Mol *et al.*, 1995; Woodson, 1997).

The insecticide crystal protein genes of *Bacillus thuringiensis* (Bt) have emerged as an important gene family in the biotechnological manipulation of insect resistance in cultivated plant species (McGaughey & Whalon, 1992; Flischhoff, 1996). Endotoxins have been identified with activity against Lepidoptera, Diptera, Coleoptera, and both Lepidoptera and Diptera (Peferoen *et al.*, 1990; Gill *et al.*, 1992), and various strains and formulations of *B. thuringiensis* are currently used as microbial pesticides against insects in these three taxonomic orders.

The development of plant regeneration (Gleddie *et al.*, 1983; Rotino *et al.*, 1987) and transformation technique in eggplant (Rotino & Gleddie, 1990) provides the opportunity to transfer new specific traits of interest (i.e. those for insect pest resistance) into valuable genotypes. Moreover, transgenic eggplants resistant to Colorado Potato Beetle by means of *B. thuringiensis* endotoxin have been successfully obtained (Arpaia *et al.*, 1997; Iannaccone *et al.*, 1997). The other type of gene for plant resistance that has been field tested codes a protein inhibiting proteases - in animal digestive systems (Ryan, 1990). The proteases are essential enzymes mediator for the digestion of plant proteins by herbivores.

Several inhibitor protease genes have been tested. They are identified as serine, cysteine, aspartic or metallo-proteases based on the active amino acid in the reaction centre (Ryan, 1990). Insects mainly use one or a combination of serine, cysteine and aspartic proteases as major digestive proteolytic enzymes (Ryan *et al.*, 1981). Plant cysteine protease inhibitors are typified by the phytocystatins, which inhibit proteases of the papain superfamily. In some Coleopteran and Hemipteran insects, major digestive proteolytic activities are apparently the result of papain-like cysteine proteases that are susceptible to inhibition by plant cysteine protease inhibitors. Transgenic plants expressing cysteine protease inhibitors show enhanced resistance to predation by pests, indicating the useful function of these inhibitors (Johnson *et al.*, 1990; Urwin *et al.*, 1995; Bolter & Latoszekgreen, 1997).

In the present study we transformed eggplant using cysteine inhibitor from Soya bean gene to confer pest resistance in this Solanaceous crop. Our final aim is to combine in the same line such resistance with the already obtained *B. thuringiensis* endotoxin resistance in eggplant in order to produce a synergistic effect to maintain a higher and durable level of resistance to CPB.

## **MATERIAL AND METHODS**

The lines Tina, Tal 8-1, Tall-I, SM5-44, and DR2, were employed. Seeds for *in vitro*-grown plants were surface-sterilised by dipping in 70% ethanol for 30 seconds, followed by 20 minutes in 7% calcium hypochlorite and finally rinsing three times in sterile water. The sterile seeds were germinated in petri dishes (25-30 per plate) on a filter paper soaked with sterile water and incubated in the darkness at 28°C. After 7-10 days the germinated seeds were transferred into sterile GA7 boxes (Magenta Corp.) containing 40 ml of V3 medium supplemented with 2% (w/v) sucrose and 0.6% agar, pH 5,8 (0,2 N KOH prior to autoclaving). Plants were maintained in a growth room at 25°C with a 16 h day-length under fluorescent I light (50 uEm<sup>-2</sup> sec<sup>-1</sup>).

The procedure for eggplant transformation was essentially as described by Rotino & Gleddie (1990) and Rotino *et al.* (1992) with modifications. Leaf, cotyledon and hypocotyl explants were precultured for 2 days in MS macro- and micro-nutrients, Gamborg vitamins (1968), 0,5 gl<sup>-1</sup> of MES, 20 μM of acetosyringone supplemented with the growth regulators (mg<sup>-1</sup>l) 0,5 ZEA, 0,3 BAP, 0,2 KIN and 0,1 NAA; media were solidified with 2 gl<sup>-1</sup> of Phytigel (Sigma), pH 5,8. For explant infection, an overnight liquid culture of *Agrobacterium tumefaciens* was centrifuged and the pellet re-suspended at 0,1 OD<sub>600</sub> density in MS basal medium, 2% glucose, 200 μM of acetosyringone, pH 5,5. The cut edges of the hypocotyls were cut again and all the explants were infected by dipping in the bacterial suspension for 5 minutes, blotted dry onto sterile filter paper and then placed back in the same plates. After 48 h of co-cultivation the explants were transferred to selective medium (described above) without acetosyringone and supplemented with 50 mg<sup>-1</sup>l of kanamycin and 500 mg<sup>-1</sup>l of cefotaxime. Shoot-buds differentiation and shoot elongation was achieved by transferring calli with compact green nodules to the same selective medium without NAA. Shoots were rooted and propagated in V3 medium (Arpaia *et al.*, 1997) without antibiotics. Regenerated plants were labelled according to the original callus (first no.) and shoot (second no.). Transgenic plantlets were grown in the greenhouse and flower buds were covered with paper bags for self-pollination.

Alternatively, a second protocol was compared with the above described one. This protocol was set up for "Hibush" eggplant cv. by Billings *et al.* (1997) and it differs from the first one for the culture regeneration culture medium which contains 0,1 μM thidiazuron (TDZ) combined with 10 to 20 μM N-6- (isopentyl) adenine (2iP). Augmentin at 300 mg<sup>-1</sup>l was used after co-cultivation to eliminate *A. tumefaciens* instead of cefotaxime. For each of these two protocols 300 explants were used.

Leaf-discs from putative transformants were cultured on regeneration medium containing 30 mg l<sup>-1</sup> of kanamycin to verify their ability to produce callus. Expression of NPTII marker gene was also monitored just after plantlet acclimatisation by spraying with a 300 mg l<sup>-1</sup> kanamycin solution according to Sunseri *et al.* (1993).

Plant DNA was isolated from young leaves according to Doyle & Doyle (1990). PCR analysis was performed using the primers which amplified a 839-bp fragment of the NPTII coding region (Arpaia *et al.*, 1997). PCR reaction was performed using 400 ng of template DNA in 50 µl of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 0.001% (w/v) gelatine, 200 µM dNTPs, 50 pM of each primer and 1 U *AmpliTaq* polymerase (Perkin Elmer).

Amplification was carried out in a thermocycler (Perkin Elmer)- programmed for: one cycle of 5 min at 95°C; 35 cycles of 15 sec at 95°C, 1 min at 60°C, 3 min at 72°C; and one final cycle of 10 min at 72°C. PCR products were subjected to electrophoresis in a 1% (w/v) agarose gel containing 0.1 µg ml<sup>-1</sup> of ethidium bromide and analysed under UV light.

## RESULTS AND DISCUSSION

With the first treatment explants following co-cultivation formed callus and shoots on selective media. White, friable callus was visible along the cut edges of the explants within 3-4 weeks from the infection. No shoot or bud differentiation was observed at this stage. After three or more subcultures on selective medium in constant presence of kanamycin (50 mg l<sup>-1</sup>), despite the colour of the explants was yellowish, some regions of the calli turned green and compact green nodules were formed. Thirty-seven resistant calli were selected and shoot primordia differentiated from most of the nodules.

Twenty-five putative transgenic plant lines were obtained from 300 co-cultivated leaf explants with a transformation frequency of 8.3%. No callus formation or shoot organogenesis was observed in control explants cultured on selective regeneration medium. This result indicates that kanamycin at 50 mg l<sup>-1</sup> was an efficient level of selection. After co-cultivation, the explants subjected to the Billings treatment enlarged, showed a very brilliant green colour and formed abundant mass of callus. But they suddenly necrotised and died after two subculture. The different genotypes employed may explain the contrasting results obtained.

The DNA amplification (PCR analysis) clearly showed the presence of strong fragment NPTII marker gene for all the transformant events. The presence of an active NPTII gene was also confirmed by the absence of chlorosis after *in vivo* spray of kanamycin solution. The rooted transformed plants are now growing in the greenhouse to perform molecular and biochemical analyses and insect bioassays.

The ability of insect populations to overcome plant resistance has been a continuing problem for pest resistance breeding. This has been true for conventionally bred genotypes resistant to insects (Gracen, 1985), and it is expected to happen for genetically engineered resistance, too. Other species of caterpillar have evolved populations resistant to *B. thuringiensis* in place where it was used intensively as a spray (McGaughey, 1985; Shelton *et al.*, 1993).

Transgenic eggplants containing cysteine protease inhibitor gene from dicot plant, soybean, were regenerated and after a further experimental field trial, to determine the real resistance level, it can be used to cross with lines transformed for *B. thuringiensis* endotoxin with the final aim to accomplish the two different genes into a multigenic resistance line with a possible synergistic effect. An appropriate strategy of resistant varieties deployment should be also evaluated to preserve the resistance traits.

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## ANNOUNCEMENT

### FOURTEENTH BIENNIAL NATIONAL PEPPER CONFERENCE

San Antonio, October 13-15, 1998

The 14th biennial National Pepper Conference will be held at the San Antonio Marriott Rivercenter, October 13-15, 1998.

Registration will begin at 4.00 p.m. Tuesday, October 13 with a welcome reception to follow at 7.00 p.m.

An all day tour of the San Antonio area pepper trials and processing plants is scheduled for Wednesday, October 14. Formal conference sessions (oral and poster) will begin at 8.00 a.m. Thursday, October 15. The conference will conclude with a San Antonio Style banquet Thursday evening.

Information: Ben Villalon or Lynn Brandenberger, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596 - USA Tel.: +1 956 968-5585, Fax: +1 956968-0641  
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### NEW BOOKS

F. Nuez, R. Gil Ortega and J. Costa, 1996. *El cultivo de pimientos, chiles y ajies*. Ediciones Mundi-Prensa, Madrid-Mexico. ISBN 84-7114-609-6.

The culture of peppers is one of the most extended anywhere in the world and particularly in Spain and America. Indeed because this book goes oriented so much to the Spanish reader as to the Latin-American, the title of the book includes tVvQ terms next to the voice peppers, as they are 'chiles', current denomination I. I Mexico and next countries, and 'ajres', particularly used voice in South America.

The great technological development that is experiencing the culture of peppers, requires the access to knowledge on the crop that are dispersed or partially treated. Besides, with peppers can be developed a great variety of commercial uses that usually have important implications in the distinct management of the crop.

This is the first book in Spanish language that considers the whole culture of peppers. It will introduce to you in the origins of the species, the development of its culture, from the most primitive forms still today used, to the most modern techniques of greenhouse growing so and now they are mc.de in the Mediterranean area. Tr.e book does not lack an analysis of the possibilities of the crop mechanization. The description of the more used varieties, is completed with the study of the diverse fungal and virus diseases, accidents and pests. As it is tried that all this information has an eminent practical trend, the book does not lack a chapter on the subjects of production costs, handling post-harvests and marketing.

The three authors of the book, beyond being true specialists in the matter, complement themselves in their knowledge on the crop contributing to obtain a complete and realistic toil.

''' ''

## RECIPES

*Here there are some new recipes, in which pepper or its derivatives are used. They have been sent by Terry Berke (A VRDC, Taiwan).*

Hot and Sweet Nuts (from the Peppers 1995 calender, by Susan Belsinger)

1/4 cup water . 1/4 cup sugar  
2 cups peanuts, almonds, or pecans  
2 Tb. chilli powder .  
1/2 tsp. salt

*Combine ingredients in a small skillet over medium heat. Stir for a few minutes, until all the water evaporates. Put the nuts onto a plate to cool. Heat level c, 'be adjusted by varying the amount of chilli powder.*

\*\*\*\*\*

Jalapeno-Apple Coleslaw (from the December 1997 issue of Chile Pepper magazine)

6 cups shredded cabbage 1/4 cup chopped onions  
3 jalapeno peppers, chopped  
2 apples, cored and chopped . 1 red bell pepper, chopped . 1/4 cup vinegar  
2 Tb. apple juice . 1 tsp. salt.

*Combine ingredients, cover, and refrigerate overnight. Serve chilled.*

Chilli Fish Sauce (from N.S. Talekar, AVRDC, chilli connoisseur)

2 Tb. Fish sauce  
2 Tsp. Lemon juice  
4 fresh chillies, seeds removed and chopped

*Puree ingredients and serve with chips (if you just happen to be fresh out of fish sauce, open a can of anchovies and put them in the sun for a day or two, then puree with a little ;!;*

**Jamaican Hot Tomato Run Down Sauce** (from "The Hot Sauce Bible" by Dave DeWitt and Chuck Evans)

2 cups coconut milk  
1 onion, chopped  
3 cloves garlic  
4 tomatoes, chopped  
2 Habaneros, chopped  
1/8 tsp allspice  
1 Tb. Thyme  
1 Tb. Vinegar  
1/4 tsp. Black pepper  
2 cups water

*Boil coconut milk, reduce heat, add onion and garlic, and simmer until soft. Add other ingredients and simmer for 30 minutes. Puree in a blender and strain. If you are feeling run down, this will pick you up in no time.*

**Tomato Serrano Juice** (from "Jump Up and Kiss Me" by Jennifer Thompson) . 3 cups tomato juice

2 serranos, stems and seeds removed  
9-10 fresh cilantro leaves  
1 Tb. lemon juice  
1/2 tsp. salt  
dash black pepper  
3 Tb. horseradish

*Blend all ingredients in a blender. Chill and drink. This juice doesn't kiss you, it bites, so watch out!*

**WHAMMO! Hot Sauce** (by Terry Berke)

1 qt. canned tomatoes  
1 1/2 cups chopped chilli peppers  
1/2 onion  
2 cups vinegar  
1 C1/2p sugar  
1 Tb. mixed pickling spices (dill, celery, mustard, bay)

Tie spices inside a cheesecloth bag. Put vegetables, spices, and vinegar in a kettle and cook until soft. Press through a sieve or strainer. Add sugar and cook until desired thickness is reached, stirring frequently to prevent burning. Pour into sterilized jars and refrigerate until use. At first, the spices make you think you are eating a pickle, and then WHAMMO! the heat from the chillies kicks in.

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