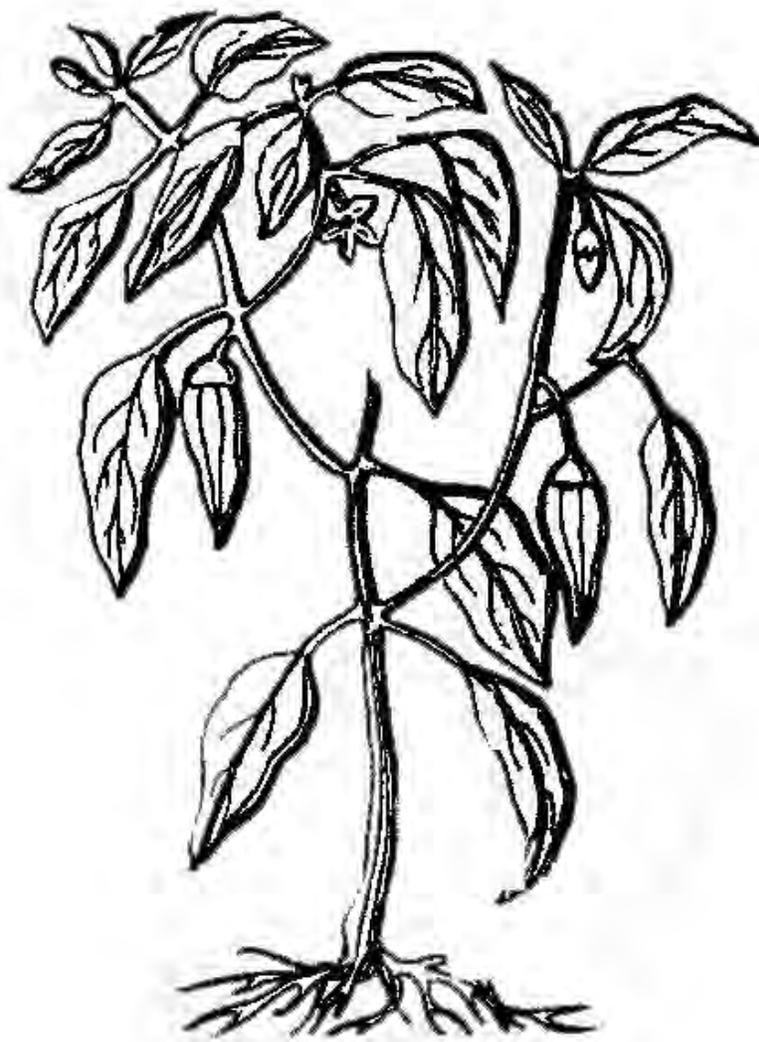


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# **CAPSICUM & EGGPLANT**

## **NEWSLETTER**

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**University of Turin  
DI.VA.P.R.A.  
Plant Breeding And Seed Production  
Italy**



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# FOREWORD

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First of all we would like to welcome Paul Bosland, who has recently joined our Scientific Committee. Thank you, Paul, for your kind availability and good work!

The fifteenth issue of "Capsicum and Eggplant Newsletter" includes two invited papers: the first one, written by P. Pasko, R. Gil Ortega and M. Luis Arteaga, deals with resistance to PVY in pepper, while the second is a review! by P. Nowaczyk and R. Andrzejewski, on the situation of pepper breeding in Poland. Thank you very much to the above mentioned Authors, for their kind willingness to increase the scientific value of our publication.

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

The co-operation between the Newsletter and the Food and Agriculture Organisation (FAO) has been renewed also for this year. In this way we are able to distribute the Newsletter to Institutions in about 140 countries all over the world. For more detailed information on distribution of the Newsletter, see the Table in the following page.

Please, remember that this Newsletter highly depends on the financial support of the recipients. Therefore, a subscription fee will be much appreciated. Due to the rise of printing and mailing costs, we have been forced to increase the fees with respect to those of the previous years. In the meantime, to make the payment less time-consuming and to reduce the bank costs, we have introduced the possibility of a 3-year subscription. Remember also that it is possible (and suggested!) to book your own copy, so quickening its delivery. Just fill in the order form on page 79 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 81. Because of the lower banking costs, credit card payment is definitely welcome.

The deadline for the submission of articles to be included in the next issue of the Newsletter (No. 16, 1997) is February 28, 1997. Please note that it is also possible to submit the paper on diskette. Details can be found in the enclosed sample sheet.

Piero Belletti and Luciana Quagliotti

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Capsicum and Eggplant Newsletter, 16 (1996): 11-27. Invited paper.

## RESISTANCE TO POT A TO VIRUS Y IN PEPPERS

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### SUMMARY

The *Capsicum-potato* virus Y (PVY) interaction is a complicated one. The PVY worldwide, extension, the existence of many strains, their mutation, generally to more virulent ones, - the lack of an accurate characterization using standard *Capsicum* varieties, etc., seem to have increased study difficulties. Besides, it is not yet possible to establish a clear-cut distinction in the relationship between *Capsicum*, potato, tobacco and tomato PVY strains.

Sometimes different denominations are given to the same resistance genes; Other times these genes appear as 'strain-dependent I and another times as I non strain-dependent'. Despite the existence of two main patterns on the genetics of resistance to PVY on *Capsicum*, the monogenic recessive instead of oligogenic theory is more accepted and used in PVY studies and in breeding resistant pepper varieties.

### 1. INTRODUCTION.

Detected and described first by Smith in 1931 in potato (cited by De Bokx and Huttinga, 1981) potato virus Y (PVY) is widespread allover the world. It affects mainly the Solanaceous species, including important crops as potato, peppers, tomato and tobacco, but also other species of *Amarantaceae*, *Chenopodiaceae*, *Compositae* and *Leguminosae* families. Since the 50-ies, it causes real losses in *Capsicum*, alone and especially when associated with other viruses attacking peppers.

PVY is naturally transmitted by at least 25 species of aphids in the non-persistent manner and in the presence of a virus-coded helper component protein factor which assists transmission of the viral code (Brunt, 1988). *Myzus persicae* is considered the most important and efficient vector. Experimentally, PVY is easily transmitted by mechanical sap inoculation (De Bokx and Huttinga, 1981).

#### 1.1. Serologic propenies

The PVY group is considered moderately immunogenic and with serologic relationships W among many members (Francki *et al.*, 1991). PVY has a strong immunological reaction, which enables the obtention of high titer antisera, and by using enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977) it is possible to detect it in very low concentrations. Immunodiffusion can be applied using degraded virus as antigen (De Bokx and Huttinga, 1981).

considered serologically distant or not related with other potyviruses as tobacco etch virus (TEV), pepper veinal mottle virus (PVMV), bean yellow mosaic virus (BYMV), henbane mosaic virus (HMV), potato virus A (PVA) and passion-fruit woodiness virus (PWV) by De Bokx and Huttinga (1981), Gebre Selassie *et al.* (1985), Kim *et al.* (1987), Buchen-Osmond (1990), Abdel Salam *et al.* (1989) and Moghal and Franck (1976). On the contrary, serologic relationship was found between PVY and TEV (Nagai, 1984), or between PVY and water melon mosaic virus 2 (WMV-2) (Abdelsalam *et al.*, 1989). According to Caranta and Palloix (1996), at least five serologic groups have been reported in pepper potyviruses: PVY, TEV, pepper mottle virus (PeMV), chilli veinal mottle virus (CVMV) and PVMV.

Shukla *et al.* (1989) noted that polyclonal antibodies (Pab) are more useful than monoclonal antibodies (Mab) in potyvirus detection; on the other hand, the use of Mab-s has increased the sensitivity of ELISA method in PVY detection (Rose and Hubbard, 1986; Boonekamp *et al.*, 1991), and gives the possibility for PVY strain distinction in potato (Von Weidemann, 1991) but not in peppers (Gebre Selassie *et al.*, 1985; Soto *et al.*, 1994).

Using the electro-blot immunoassay on reactivities of Pab-s for some potyviruses (PVY, TEV, soybean mosaic virus N (SMV-N), SMV-V, PWV, BYMV, clover yellow vein virus; (CYVV), maize dwarf mosaic virus A (MDMV-A), MDMV-B, MDMV-O and Johnson grass mosaic virus (JGMV) Shukla *et al.* (1989) reiterate the conclusions of Moghal and Franck (1976) that, unless considerable caution is used in the interpretation of serologic data, serology may be a misleading approach for tracing relationships among potyviruses, and coat protein sequence data analysis are recommended.

### 1.2. Molecular properties

After Shukla *et al.* (1989) the coat protein sequence homology between distinct potyviruses was 38 to 71 percent, whereas for strains of individual potyviruses it was greater than 90 percent. Dougherty and Carrington (1988) reported that the amino-acid sequence of the capsid protein showed 60-80 percent similarity in TEV, tobacco vein mottle virus (TVMV), PVY and PeMV, which corresponds with the data of Van Der Vlugt *et al.* (1989), who found 51-63 per cent homology between PVY-N and the coat protein of TEV, TVMV, plum pox virus (PPV) and SMV and 91 per cent between PVY-N and PeMV.

### 1.3. PVY symptoms on peppers.

The typical symptoms of PVY on peppers, observed by most of the authors, are '**vein clearing**' which usually progresses into '**mosaic**' or '**mottling**', and generally in '**veinbanding**' (Cook, 1963; Gebre Selassie *et al.*, 1985; Luis Arteaga and Gil Ortega, 1983). Vein and leaf necrosis are frequent, sometimes followed by **stem necrosis**, defoliation and/or even death of the plants (Simmonds and Harrison, 1959; Rana *et al.*, 1971; Ragozzino *et al.*, 1972; Marchoux *et al.*, 1976; Gracia and Feldman, 1977). Symptoms are not always evident on fruits (Lovisolio and Conti, 1976). Necrotic spots and fruit mosaic are observed in some pepper varieties (Ragozzino *et al.*, 1972; Luis

Arteaga and Gil Ortega, 1983) but particular symptoms are not always obtained on fruits. **Chlorotic and necrotic spots, mosaic, deformation and size reduction** are the most frequent fruit symptoms observed (Simons, 1956; Ragozzino *et al.*, 1972; Lockhart and Fischer, 1974).

Other different symptoms are reported on naturally PVY infected pepper plants, **as stunting, severe mosaic, leaf deformation and flower abortion** (Simons, 1956; Rana *et al.*, 1971; Ragozzino *et al.*, 1972; Lockhart and Fischer, 1974; Marchoux *et al.*, 1976; Gracia and Feldman, 1977; Luis Arteaga and Gil Ortega, 1983).

**Atypical symptoms** are noted on various pepper varieties, as chlorotic or necrotic local lesions (Makkouk and Gumpf, 1976; Nelson and Wheeler, 1981; Gebre Selassie *et al.*, 1983 and 1985; Marte *et al.*, 1991), systemic speckling mottle (Zitter, 1972) and chlorotic spots (Erkan, 1986).

Symptoms on pepper plants can vary depending on strain and cultivar, while the symptoms on indicator plants are generally stable and used as a safe method for PVY diagnosis or assay. This permits to distinguish PVY from other possible viruses (De Bokx and Huttinga, 1981). The severity of symptoms depends on the plant age, the young ones being more susceptible (Zitter, 1972). The symptoms are strengthened by cold (Von Der Pablen and Nagai, 1973).

## 2. PVY STRAINS AND PATHOTYPES.

Due to the worldwide expansion of PVY, a large and even upsetting number of strains and pathotypes were reported by many authors. The classification of pepper PVY isolates into pathotypes is based on the differential resistance response presented to them by some particular *Capsicum* cvs. In pepper-PVY, the situation shows to be confused. Various authors have used different standard series of pepper varieties for PVY isolate classification. Furthermore, (i) the use of different methods in classifying pepper varieties as susceptible or resistant, e.g., the systems and genetic patterns used by Von Der Pablen and Nagai (1973) or by Singh and Chenulu (1985), are very different from those used by Cook (1961 and 1963) and by Gebre Selassie *et al.* (1985) and (ii) the large number of mutations reported on PVY strains (Cook, 1961; Zitter, 1972; Von Der Pablen and Nagai, 1973; Gebre Selassie *et al.*, 1985; Thomas *et al.*, 1989) increase the identification and classification difficulties.

In the attempt to distinguish and classify pepper PVY strains, much work on pepper PVY has been made in Brazil and Argentina (Nagai and Costa, 1972; Von Der Pablen and Nagai, 1973), where at least six PVY strains were noted. In vast research led in California on 18 pepper PVY isolates, Makkouk and Gumpf (1974) could distinguish up to nine PVY strains in peppers using eight pepper varieties. After continuous and systematical investigation led in INRA-Montfavet (France) by Marchoux *et al.* (1974), Pochard (1977) and Gebre Selassie *et al.* (1985), the PVY isolates were classified first into two and later into three pathotypes (PVY-O, PVY-1 and PVY-1-2) (Table 1). In these studies mainly

pepper varieties proposed by Cook (1962 and 1963), by Nagai and Smith (1968) and by Smith (1974) were used. Using the same pepper differential series proposed by Gebre Selassie *et al.* (1985), a similar PVY pathotype hierarchy was found in Spain (Luis Arteaga and Gil Ortega, 1983 and 1986, Luis Arteaga *et al.*, 1993), in Australia (Thomas *et al.*, 1989) in Italy (Marte *et al.*, 1991) and in Turkey (Palloix *et al.*, 1994). The applicability in breeding makes the system proposed by Gebre Selassie *et al.* (1985) the more commonly used and accepted now for pepper PVY strain classification into pathotypes.

Some pepper differential varieties recommended by Gebre Selassie *et al.* (1985) were occasionally used in other PVY studies, enabling us to make some relative comparisons of PVY strains originating from various geographical areas (Table 2).

Anyway, the *Capsicum*

- PVY pathotype relationship in these studies either does not correspond totally with that found in France, e.g., the different resistance level of 'Puerto Rico Wonder', 'Moura' and 'Ikeda' verified in Spain (Luis Arteaga and Gil Ortega, 1986, Pasko *et al.*, 1995) or often all the differential pepper varieties were not used. These cases do not enable us to identify all the French pathotypes with those found in Spain, Italy, Australia, or elsewhere, but only to find similarities between them.

Table 1. Classification of PVY isolates into pathotypes (Gebre Selassie *et al.*, 1985; Marchoux and Gebre Selassie, 1989).

Pepper varieties	Resistance Genes	Pathotypes		
		PVY-0	PVY-1	PVY-1-2
Bastidon	Pvr2+(=y+=vy)	SN	SN/SM	SN/SM
Yolo Wonder	Pvr2+(=y+=vy+)	SM	SM	SM
Yolo Y	Pvr2+(=ya=vy1)	R	SM	SM
Flordia VR-2	Pvr22(+eta=vy2)	R	R	SM
Serrano Veracruz	Vy2s???	R	R	R

S=susceptible, R= Resistant, N= necrosis, M= mosaic. After Palloix and Kyle (1995) and Palloix *et al* (1996)

In the present situation remains yet actual the objection put by Makkouk and Gumpf (1974), 'development of a standard series of virus indicator pepper cultivars . . . should be continued', 'such series will both ease the strain identification of new isolates and aid the search for new sources of resistance '. A careful control of seed origin and their multiplication is also necessary, due to the relatively high allogamy rate reported in peppers mostly in presence of resistance of recessive nature as it is the case of most known PVY resistances.

### **2.1. Atypical strains**

In California, an atypical strain called PVY-S (which causes 'speckling' on 'Early Calwonder' pepper cv.) was described by Zitter (1972). In Taiwan, a pepper PVY strain causing not only chlorotic spot lesions, but also systemic chlorotic spots and veinal- spreading lesions on *Chenopodium amaranticolor* and *C. quinoa*, is reported by Kim *et al.* (1987). PVYo-sbp, which causes severe mosaic in bell peppers in India, is also considered as an atypical strain of PVY (Sharma *et al.*, 1989).

### **2.2. Influence of origin plant on host range of PVY isolate**

Most of the PVY isolates infecting peppers originate from pepper plants, but there are some strains originating from other solanaceous crops, as potato, tomato and tobacco, or from non-solanaceous weeds.

Gebre Selassie *et al.* (1985), after studies led in France presented a situation where the PVY pathotypes PVYO and PVyN from potato, did not infect peppers by mechanical inoculation. Nevertheless, other strains of those groups could be transmitted by vectors (Feres *et al.*, 1993). In USA, peppers could be infected with potato PVY isolates as, e.g., PVY-P (Cook and Anderson, 1959), or in Italy with the potato isolate Um9 (Marte *et al.*, 1991). Besides, in Italy, the same authors consider potato crops as the main source for PVY infection on peppers in summer.

Other PVY strains, e.g., from tomato (Cook and Anderson, 1959, 1960; Gebre Selassie *et al.*, 1985; Luis Arteaga and Gil Ortega, 1986), tobacco (Cook and Anderson, 1959; Marte *et al.*, 1991), *Solanum nigrum* L. and *Portulaca oleracea* L. (Gebre Selassie *et al.*, 1985), do infect peppers. According to MacDonald and Kristjansson (1993), tobacco PVY strains PVY-NN and PVY-MN can infect peppers with very strong symptom expression. On the other hand, in France, PVY isolates from peppers, tomato, *S. nigrum* and *P. oleracea*, were shown to be unable to infect potato (Gebre Selassie *et al.*, 1985), which is confirmed 'for the majority of PVY isolates' from tomato, tobacco and peppers in Australia (Thomas *et al.*, 1989).

### **2.3. Necrotic reactions**

Similarly to PVY -N (necrotic) pathotype in potato and tobacco (piccirillo, 1988), PVY isolates causing local or systemic necrosis in certain *Capsicum* cvs. are described by v various authors too (Cook, 1963; Yon Der Pablen and Nagai, 1973; Makkouk and Gumpf, 1976; Nelson and Wheeler, 1981, Gebre Selassie *et al.*, 1985; Luis Arteaga and Gil Ortega, 1986; Horvath, 1986a; Marte *et al.*, 1991).

Early in the 60-ies, Cook (1963) described the following situation: None of the tested pepper varieties in study presented



TABLE 2. CAPSICUM - PVY INTERACTIONS

Capsicum varieties	Cook, 1961; Greenleaf, 1986					Gehrs Selassie et al., 1985; Marchoux and Oebre Selassie, 1989					Luis Arteaga and Gil Ortega, 1986; Paako, 1993					Nagai and Costa, 1972; Von Der Pahlen and Nagai, 1973				
	Resistance Genes <sup>1</sup>		PVY, TEV and PeMV strains		TEV-S	Resistance Genes <sup>1</sup>		PVY pathotypes		1-2	Resistance Genes <sup>1</sup>		PVY pathotypes		1-2	Resistance Genes <sup>1</sup>		PVY strains		PVY <sup>d</sup>
	PVY-C PVY-N	TEV-C PVY-NTR	PeMV	0		1	0	1	0		1	0	1	PVY <sup>a</sup>		PVY <sup>b</sup>	PVY <sup>c</sup>	PVY <sup>d</sup>		
Yolo Wonder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yolo Y(=YRP10)	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+
PI 264281 (P 11)	-	-	+	+	+	(vy <sup>2</sup> )	+	+	+	+	-	-	-	-	-	-	-	-	-	-
SC 46252 (P34)	-	-	+	+	+	vy <sup>2</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Florida VR2	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Avelar	-	-	-	-	+T	(vy <sup>1</sup> )	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Delray Bell	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
PI 159236	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
PI 152225	-	-	+T	-	-	+	+	+	+	+	HR	-	-	-	-	-	-	-	-	-
Serrano Veracruz SCM 334	-	-	-	-	-	vy <sup>2</sup> Pvr4	-	-	-	-	(Pvr4)	-	-	-	-	-	-	-	-	-
Puerto Rico Wonder	-	-	-	-	-	(vy <sup>1</sup> )	+	+	+	+	(vy <sup>2</sup> )	-	-	-	-	-	-	-	-	-
Casca Grossa	-	-	-	-	-	(vy <sup>1</sup> )	+	+	+	+	(vy <sup>2</sup> )	-	-	-	-	-	-	-	-	-
Moura	-	-	-	-	-	(vy <sup>1</sup> )	+	+	+	+	(vy <sup>2</sup> )	-	-	-	-	-	-	-	-	-
Ikeda	-	-	-	-	-	(vy <sup>1</sup> )	+	+	+	+	(vy <sup>2</sup> )	-	-	-	-	-	-	-	-	-
Agronomico 4	-	-	-	-	-	(vy <sup>2</sup> )	-	-	-	-	+	HR	+	HR	+	-	-	-	-	-
Agronomico 8	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Agronomico 10	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: + susceptible; -, resistant; T, tolerant; HR heterogenous response.

<sup>1</sup> For new nomenclature see Table 1.

had served as parental, showed necrosis when inoculated with PVY -PR strain. Nevertheless, the observed rate did not allow the author to draw any conclusion on the genetic determination of systemic necrosis. Pochard (1977) described another situation where the F1 plants of the crosses between 'A velar' (showing light mosaic symptoms) and 'Agronomico 8' (resistant, symptomless) on one side, and 'Yolo Wonder' (showing mosaic) on the other, were totally characterized by necrotic veins. Our own results confirm these situations. A cytoplasmic influence in necrotic symptom expression was also postulated (Pasko, 1993).

In conclusion it could be confirmed the characterization made by Marchoux *et al.* (1987) that 'the necrotic reaction depends on variety, but necrotic aptitude depends on virus strain independently on its virulence'.

#### **2.4. Pathotype expansion and evolution**

The more mentioned and expanded PVY strains in different pepper cropping areas, are noted as common strains, classified as PVY -0 pathotype. After the introduction of the resistant varieties 'Yolo Y', 'Florida VR2' or 'Agronomico', more virulent PVY strains emerged on peppers (Tables 1 and 2).

After Cook (1962) strains do not only mutate into more virulent but into less virulent pathotypes too. He showed that the mutated pathotype PVY-NYR that can infect 'Yolo Y', and obtained after repeated inoculations of PVY -Non the resistant cv. 'Yolo Y', turned back into a less virulent pathotype after being repeatedly inoculated on tobacco.

Pochard (1977) and Pasko (1993) have also pointed out the strong reaction caused by PVY- 1-2 pathotype on 'Yolo Wonder'. So the most virulent pathotype PVY -1-2 did not obey the general rule of Vanderplank (1968) that most virulent strains would be less aggressive.

### **3. SOURCES OF GENETIC RESISTANCE**

The most used resistance to PVY comes from *C. annuum*. The mutation detected by Cook in the bell shaped variety 'Yolo Wonder', named initially 'YRP10' (from which 'Yolo Y' was bred), is a unique case. Its resistance level was surpassed by more virulent pathotypes in the same area (Cook, 1961). 'P11', 'Mogi das Cruzes', 'Casca Grossa' and 'Avelar' are the most used varieties as resistance source both in USA and Brazil and, based on the breeding lines produced, many commercial varieties and hybrids were derived from them (Nagai and Costa, 1972; Greenleaf, 1986). Although less used, another resistance source is the Indian pepper line 'Perennial' (Sharma *et al.*, 1989; Caranta and Palloix, 1996). The Mexican 'Serrano' group which belongs to *C. annuum* too, is also distinguished for its high resistance level to different PVY pathotypes in various areas. The most recent description was on *C. annuum* Serrano Criollo de Morelos-334 (SCM-334) (palloix, 1992; Pasko *et al.*, 1992; Boiteux *et al.*, 1996; Dogimont *et al.*, 1996) (Table 2).

Good resistance to PVY was also reported in *C. chinense*, *C. frutescens*, *C. baccatum* var. *pendulum*, *C. eximium*, *C. flexuosum* and *C. pubescens*, but these species are not used very much in breeding programmes, probably because of crossing barriers, relative low resistance level, low fruit quality or other drawbacks in breeding work.

Tolerance to PVY -0 and PVY -1 has been reported on variety 'Luesia' (Gil Ortega *et al.*, 1986) but the inheritance of tolerance expression had not been reported.

Due to (i) the large differences in virulence checked on the PVY strains in different areas, (ii) the lack of classification of PVY strains used during the genotype screening, (iii) the fact that the studies on PVY deal with local strains, (iv) the lack of information on pedigree or resistance level of new varieties, which tend to be F1 hybrids, and finally, (v) the impossibility to assure the needed information on this subject, neither could we establish a complete list of the possible resistant sources to PVY, nor could we draw definite conclusions about the existence of any totally resistant source to all PVY pathotypes and on the best source combination. Nevertheless, the contribution of the known sources in breeding resistant varieties is considerable and it was shown indispensably for successful pepper growing in the severely attacked areas in the last twenty years in USA, Brazil, Mediterranean basin, etc.

#### **4. GENETICS OF RESISTANCE**

After a large scale test of more than 200 pepper accessions for resistance to PVY and TEV led in U.S.A. in the 70-ties and where 'nothing was found which was completely symptom free when tested against the most virulent pathotype', Smith (1974) notes also that 'the large number of strains of both PVY and TEV appears to be matched by a surprising number of genes for resistance in peppers', which fits well to the present situation too.

##### **4.1. Main patterns**

Two are the main patterns on the genetics of resistance found in *Capsicum* to PVY strains (Table 2). The oldest, the most commented and accepted, is the monogenic recessive pattern with allelic gene series proposed in Florida by Cook (1960, 1961, 1962 and 1963) and by Cook and Anderson (1960). The second one is the oligogenic pattern proposed in Brazil by Nagai and Costa (1972). After this pattern recessive and dominant gene combinations can give different resistance levels.

The first pattern is defended and largely used in breeding not only in U.S.A., but also in France, Italy, Spain and Australia. The second one is used only in South America: in Brazil and Argentina (V on Der Pahlen and Nagai, 1973). Applying both patterns, first in U.S.A, then in Brazil and Europe, valuable resistant pepper breeding lines, cultivars and FI hybrids are bred, which though obtained in different geographic areas, have contributed to the control of PVY epidemics all over the world. Nevertheless, the second pattern seems to have attracted more criticism than acceptance. In spite of valuable pepper varieties obtained in Brazil, which were resistant to PVY strains in U.S.A. and Europe,

this theory is sometimes criticized even based on results obtained from trials led with different pepper varieties and PVY strains (Sharma *et al.*, 1989).

The monogenic recessive theory is based in screening and allelic tests made by Cook (1960, 1961, 1963) and Cook and Anderson (1960). In USA a single gene *ya* was supposed - to be responsible for PVY -C and PVY -N resistance in 'YRP10' (Table 2) (Cook and Anderson, 1960). This gene proved to be allelic with *er* that had been previously described by Greenleaf (1956) as giving resistance also to TEV(C). Later, the allele *eta* was labeled as *yea* by Cook (1960). Anyhow, 'YRP10' was susceptible to TEV(C). More over, with the arrival of a more virulent PVY pathotype (pVY-NYR) on 'Yolo Y', it was noted that the gene *er* gave resistance also to PVY -NYR. Genes *ya* and *er* were labeled *vyl* and *vr* in France (Gebre Selassie *et al.*, 1985).

In a presentation of pepper resistance gene hierarchy to PVY, TEV and PeMV, Greenleaf (1986), who mentions also that 'Avelar' is only tolerant to PeMV (Table 2), suggests the following scheme. The symbol < used by this author means 'not dominant'.

$$y+ \ y- \ < \ \text{eta} \ < \ \text{etaV} \ < \ \text{etcl} \ < \ \text{etc2}$$

PVY -C    TEV -C    PeMV    TEV-S

PVY-N    PVY-NYR

< < is put by us to show the threshold between the susceptible and resistant genotypes)

Based in the same theory and screening different *Capsicum* sp. genotypes against various PVY isolates, classified into three pathotypes, the French group of INRA-Montfavet (pochard, 1977; Gebre Selassie *et al.*, 1985; Marchoux and Gebre Selassie, 1989), assigned a new nomenclature and placed them in the following hierarchical order:

$$vy+ \ < \ < \ vy^1 \ < \ vy^2 \ < \ (vy:z. ???*)$$

**PVY-O    PVY-I    PVY-I-2**

(\*  $vy^{2s}$  was never shown to be allelic to the *vy* series)

Due to similar results obtained using the same differential pepper cvs. carrying their respective resistance genes, this system is found practical not only for classification of PVY strains into pathotypes, but also in screening and breeding in Spain (Luis Arteaga and Gil ;, Ortega, 1986), in Italy (Marte *et al.*, 1991) and Australia (Thomas *et al.*, 1989).

Very recently, Palloix and Kyle (1995) and Palloix *et al.* (1996), after a revision of gene nomenclature for potyvirus resistance genes in *Capsicum*, have proposed the symbols Pvr2+, pvr21 and pvr22 instead of *vy+*, *vyl* and *vy2*. These authors have also reviewed the present knowledge on monogenic inheritance of pepper resistance to different potyviruses,

showing some complex interactions between the resistances to different potyviruses and underlying that many allelism tests remain to be made.

We tried to construct a similar drawing for the combinations of the three independent genes, one dominant and two recessive, after the oligogenic pattern (Nagai and Costa, 1972; Yon Der Pablen and Nagai, 1973) (Table 2), but we couldn't fix any hierarchical order. The oligogenic pattern seems more a polygenic one, if the presence of resistant genotypes in F2 and F3 generations from susceptible and high susceptible parents is considered, as reported by Yon Der Pablen and Nagai (1973). These authors concluded that "based on the additivity of genes any level of resistance is obtainable". This is reinforced also by the report of Nagai (1984), when he says that in Brazil 'frequently farmers find resistant plants in their farms', which gives one the impression of transgressive plants in a typical F2 segregation. Anyhow, this theory shows problems of interpretation.

It is to be noted that the monogenic theory is not able to explain cases of systemic necrosis appeared in F2 and BCs between parents not showing this symptom (Cook 1963), or even in F1 (Pochard, 1977; Pasko, 1993).

It is accepted that the use of different PVY isolates in different areas does not allow the identification of different resistances found in the same genotype, probably because they belong to different pathotypes (Cook and Anderson, 1959). Yon Der Pablen and Nagai (1973) give an example where the variety 'Avelar', resistant in Florida, was shown to be susceptible in N.E. Argentina; similarly, accessions 2207 and 2120, respectively resistant in Argentina and California, were shown to be susceptible in Florida. Other alike cases are, e.g., 'Puerto Rico Wonder' which was shown to be susceptible in Trinidad, resistant in Puerto Rico, tolerant to only one PVY strain (PVY<sup>W</sup>) in Brazil (Nagai and Costa, 1972), resistant to the commonest pathotype (PVY -0) in France (Gebre Selassie *et al.*, 1985) and to three pathotypes (PVY -0, PVY -1 and PVY -1-2) in Spain (Pasko *et al.*, 1995) (Table 2); 'PI 159236' was shown to be highly resistant in California, resistant to PVY -0 in France, to PVY-O and PVY-1 in Spain and to PVY-I-2 in Brazil, while 'PI 152225', susceptible to PVY -0 and PVY -1 in France, was resistant to PVY -0 and PVY -1 in Spain and highly resistant in California (Marchoux *et al.*, 1974; Greenleaf, 1986; Pasko, 1993, Boiteux *et al.*, 1996) (Table 2).

#### **4.2. Other patterns**

Except these two patterns, there are other reports on resistance expression to PVY and on a- genetic determination as:

- **one dominant** gene for resistance to three PVY pathotypes (pVY -0, PVY -1 and PVY -1- " 2) was recently reported in 'Serrano Criollo de Morelos 334' (Palloix, 1992; Boiteux *et al.*, 1996). Allelism tests confirmed independent segregation with pvr21 and pvr22. The symbol Pvr4 was proposed for this locus (Palloix and Kyle, 1995; Dogimont *et al.*, 1996; Palloix *et al.*, 1996).
- **complementary recessive combination of two loci** (Simmonds and Harrison, 1959);

heterogeneity within virus inocula was supposed to account for such results by Cook (1963);

- **two independent recessive genes** were reported by Smith (1974) in 'Serrano' group accessions 2207 and 1534;

- **partial dominance of resistance** was reported in cv. 'Florida VR2' by Shifriss and Marco (1980) based in phenotypic distinction of heterozygous plants (y8+y8) from the homozygous ones and in the 1 :2: 1 rate obtained in *F2*. The virus concentration tested by ELISA was used as criterion. '

- **oligogenic resistance** (controlled by two recessive independent genes that need the ; presence of modifier genes for complete resistance) was shown in the Indian cv. 'Perennial' to 'PVY 1-2' pathotype, the most virulent one in France (Pochard *et al.*, 1983). More recent data showed that the resistance to PVY-1-2 was partial and poligenically controlled and that Perennial also possesses an oligogenic complete resistance to PVY-O (Caranta and Palloix, 1996). This was further confirmed by molecular mapping of the genes (Caranta *et al.*, 1995). Sharma *et al.* (1989) showed that Perennial carries a recessive gene imparting resistance to PVY<sup>0-sbp</sup>, an atypical strain described in India.

- two recessive genes were reported to control respectively 'resistance' and 'medium resistance' during an allelic test where some accessions of *Capsicum* spp. as *C. annum*, *C. angulosum*, *C. pubescens*, *C. fasciculatum*, *C. praetennissum*, and *C. microcarpum* were included (Singh and Chenulu, 1985).

In some reports, two ways of interpretation of intermediate resistance (or susceptibility) in *F1* are to be noted: one by distinguishing heterozygous allele combination (Shifriss and Marco, 1980) and the other supposing two different genes controlling 'resistance' and 'medium resistance' (Singh and Chenulu, 1985).

After Pochard (1977), Palloix *et al.* (1990) and, Palloix (1992), considering together the pepper reactions to various potyviruses, it is obvious that in the PVY case, maybe due to larger pathotype diversity, all the types of reaction and genetic control found in other potyviruses are met, which could partially explain the conflictive data and the difficulties in interpreting them.

## 5. OTHER TRENDS IN PVY RESISTANCE AND PVY INTERACTIONS.

- **Resistance to PVY transmission** by *Myzus persicae* and *Aphis craccivora* was found on pepper cvs. 'Ikeda and 'Moura' in Israel. It worked also for other non-persistent viruses as cucumber mosaic virus (CMV) and alfalfa mosaic virus (AMV) (Cohen, 1982, cited by Jones, 1987), but no genetic determination was given.

- **Inhibitory substances**, which were first considered not to affect the virus but the aphids; were reported in the pepper variety 'Italian EI' (Simons and Moss, 1963). Later, Simons (1966), after checking susceptibility differences between varieties 'Italian EI' and 'California Wonder', supposed that the inhibitors in 'Italian EI' plants "could conceivably cause a slower rate of virus multiplication. . . probably making through an effect on the host plant cell rather than on a virus direction". After Pasko *et al.* (1992), 'Italian EI' variety carries the gene *pr2*<sup>1</sup> or any other allelic to it.

- Some **weed inhibitors** from *Aeonium haworthia*, *A. arboreum*, *Mesembryanthemum caproletum* and *Agave americana* were found till 100 per cent effective against juice inoculation of PVY, but not against aphid transmission (Simons *et al.*, 1963).
- A known protein from pokeweed (*Phytolacca americana*) called PAP (pokeweed **antiviral protein**) is reported to fully protect the plants from various virus infections, including PVY, and transgenic tobacco plants resistant to PVY are being produced (Chen *et al.*, 1991).
- Coat protein-mediated (C-P) resistance producing immunity against PVY, is obtained ,in transgenic potato plants resistant to PVY and tobacco plants resistant to both PVY and TEV, so against heterologous viruses, are successfully obtained, but, as the author puts it, extensive field trials are needed for a full evaluation of this kind of resistance (Beachy *et al.*, 1990).
- **Interactions between PVY and *Phytophthora capsici* L.**, which resulted in the inhibition of the resistance to *P. capsici*, were found in two pepper cvs. Previously PVY infected (pochard *et al.*, 1981). In a similar test, Cristinzio *et al.* (1988) noted increased susceptibility to *Phytophthora* in one pepper cv., but decreased in two others.
- **A synergism between tobacco mosaic virus (TMV) and PVY**, resulting in severe symptoms was checked in 'Bahamian' hot chile, but not in two other pepper cvs. (Sherwood *et al.*, 1988), so it is not generalized as the PVY -PYX synergism in potato.
- **Cross protection** between different pepper PVY strains resulting in reduction of the local lesions caused on *N. tabacum* 'Havana 425' has been reported in California by Makkouk and Gumpf (1976).

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## DEVELOPMENT OF PEPPER BREEDING IN POLAND

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### **Abstract**

Poland is now the most North-East salient area of pepper production in Europe. Pepper breeding in Poland began about 30 years ago. At first, for several years, we had only one, then two pepper cultivars. Now we can observe an increasing interest in pepper breeding in Poland; six breeding centres deal with pepper and there are 16 registered pepper cultivars on fresh market. Up to now the main breeding method has been the selection of hybrid material, which is necessary to provide the producers with cheap seeds of the settled cultivars. At present we are getting more and more interested in heterosis breeding. One hybrid cultivar is in production now, next cultivars are being tested respecting their registration. Yielding of cultivars increased from 5-6 kg/m<sup>2</sup> to 8-9 kg/m<sup>2</sup> in unheated plastic greenhouse, which is the main place of pepper production for fresh market because it guarantees good yielding, and good fruit quality in Polish climatic conditions.

### 1. Introduction

The data and results presented in this paper refer to the cultivars of sweet pepper because only these cultivars are produced for fresh market. Breeding of hot, medium-hot and outdoor-grown cultivars has not been taken into consideration. Breeding of Polish cultivars made the producers get interested in pepper, caused a development of cultivars' variety (Nowaczyk, 1988) and resulted in an increase of pepper-growing area and pepper consumption. It is worth mentioning that Polish market is very conservative and introducing a new species of vegetable is rather difficult. Polish producers got interested in pepper culture only after new, Polish cultivars of high fruitfulness had been registered. Heterosis breeding gives the best effects (Nowaczyk, 1981) but breeding of very fruitful settled cultivars was necessary to quickly develop pepper-growing because of - very low price of the settled cultivars' seeds. Interest in pepper on fresh market was gradually increasing. At present pepper is a vegetable in demand though its - price is high. Pepper on fresh market comes mainly from production in unheated plastic greenhouse. Such a way of production is not an energy-consuming method and it gives good yielding and good fruit quality.

### 2. Material and methods

All the data presented in this paper refer only to the registered cultivars of sweet pepper which are grown in unheated plastic greenhouses. The results of the

experiments are based on this kind of pepper culture. Growing of pepper in plastic greenhouses in Poland lasts from mid-May till the end of September.

The first part of result presents the information concerning the number and the dates of registrations of new Polish cultivars. The information has been based on literature about cultivar investigation and registration, and on the author's own data. The information on the methods used in Polish pepper-breeding has been prepared on the same basis. Five of the registered cultivars have been bred by the first of the two authors of this paper.

Cultivar yielding has been determined on the basis of the results of experiments carried out under plastic cover. Only market yield has been taken into consideration, in the presented data. Data concerning the period 1984 - 1993 come from experiments carried out by Research Centre of Cultivars Testing every year in several experiment stations situated all over the country. The yield results of the most and the least fruitful cultivars have been presented with regard to each year of experiments.

### 3. Results,

The first pepper cultivar was registered in 1972 (figure 1). It was the cultivar 'Poznanska Slodka' and its registration was the beginning of Polish breeding of sweet pepper. The second cultivar, 'Remi', was registered in 1978. Ten years later both of them were withdrawn from plastic greenhouse production because of introducing new cultivars.

Next cultivars, 'Jantar' and 'Kujawianka', coming from newly established breeding centres were introduced into production in 1986. The third of the registered cultivars, 'Stano', was bred by the author of this paper and up to now has been the most fruitful settled cultivar since the beginning of registration testing. The end of the 80-ties was an especially favourable period in pepper breeding. In 1987 the next three new cultivars 'Zefir', 'Ino' and 'Kano' were registered. Two cultivars were registered in each of the two following years. In 1988 the cultivars 'Bryza'" and 'Passat' were introduced into production. In 1989 the cultivar'Monsun' and the first Polish yellow cultivar 'Sono' bred by the 'author of this paper, began to be produced.

At the beginning of the 90-ties three new cultivars 'Buran', 'Mira' and 'Zorza' began to be produced. Last year the next cultivar bred by the author of this paper was registered; it was 'Stanola F I' - the first Polish heterosis hybrid cultivar. In respect of quality, a new stage in Polish breeding of pepper was begun.

Up to now the selection of hybrid material has been the most effective method used for improving cultivars (table 1). First of all original hybrids were used, and Bulgarian, Hungarian, Italian and Dutch cultivars were the materials to make them. One of the parent materials for new cultivars was the first registered cultivar 'Poznanska Slodka' which at the same time was a source of mutants: two mutants appeared to have such economic value that they were registered as original cultivars. Mutation changes were mainly in the shape and size of fruit. Only one of Polish cultivars was the result of selection of offspring of foreign

heterosis hybrid. One cultivar, recently registered, was obtained as a result of heterosis breeding. Comparing yielding of the registered cultivars seems to be most interesting (figure 2). It should be remembered that only market yield, not total yield, has been presented in this paper. Fruits, which are not products for fresh market, have not been taken into consideration. Maximum and minimum yields from among all the estimated cultivars have been presented for each year of the last decade. Yields of the first Polish cultivar, estimated in 1976 in the author's own experiments, have been shown, too.

The cultivar 'Stano' proved to be the most fruitful in each of the years of experiments. The moment the hybrid cultivar 'Stanola F I' began to be tested, it proved to be the most fruitful one. Differences between yields in particular years of investigations are worth considering; they were especially significant between the years 1985 and 1986.

#### 4. Discussion

Polish pepper breeding began about 30 years ago while pepper production for fresh market started only in the middle of the last decade, which was possible due to introducing new, fruitful cultivars. The question is if the number of cultivars designed for culture under plastic cover and produced for fresh market is sufficient. Polish pepper breeding seems to be satisfactory in this respect and what is more, several new cultivars are now being tested respecting their registration. The situation concerning cultivar variety seems to be worse. There is only one yellow cultivar in the register though this is not the real problem. There is a very little variation of cultivars in respect of the shape of fruits. Nearly all fruits are cone-shaped while those, which are trapezium-shaped, are in greater demand on fresh market. The hybrid 'Stanola F I' meets these requirements in some extent.

Using heterosis is a new element in breeding Polish cultivars of pepper. This method makes it possible to increase the variety of cultivars and to improve quality characteristics. It is worth mentioning that 'Stanola F I' is characterized by great vitamin C content, also when in rock wool culture (Konys, 1994). Additionally, this cultivar can be grown in different regions of the country, including Northern Poland (Nowaczyk and Michalak, 1994).

It can be expected that heterosis hybrids will soon remove the settled cultivars from production for fresh market. This conclusion seems to be even more justified when we consider the fact that next hybrids are being tested with respect to their registration. Their economic value, estimated by Research Centre of Cultivars Testing, will decide about their being registered and introduced into production. Research Centre of Cultivars Testing is the Polish Centre dealing with testing cultivars and making decisions about their registration. Testing of cultivars lasts 2 - 3 years and a decision about their registration is made when the cultivar is original, even and solid. Each of the registered cultivars must meet all these requirements.

The registered cultivars designed for breeding under unheated plastic cover come from three centres. At present, breeding is conducted in six centres, mainly in central regions of the country. An increasing number of breeding centres shows a great interest in pepper. There were only two Polish cultivars at the end of the 70-ties and at the beginning of the 80-ties. Their yields ranged from 5 - 6 kg/m<sup>2</sup> and this level of yielding remained characteristic for less fruitful cultivars. Breeding progress can now be observed very clearly. The most fruitful cultivars give the yields of 8 - 9 kg/m<sup>2</sup> and this level of yielding can be regarded as very satisfactory in Polish climatic conditions. With such fruitfulness of cultivars the production for fresh market is very profitable. The results of other experiments (Nowaczyk and Nowaczyk, 1990) show that obtaining higher yields is difficult.

The number of registered cultivars does not give complete information about the importance of pepper as a kind of vegetable and about the scale of production. It is difficult to estimate the pepper growing area of both outdoor culture and plastic cover culture. Information about seed production could be of some help but again, we do not have complete data in this field. Breeding and trade centres are unwilling to present this kind of data. Information gained through personal contacts is of estimated value; seed production can be estimated as amounting to several hundred kilograms per year.

Stable though less intensive increase of seed production can be expected because the pepper-growing area is getting bigger. Four new hybrid cultivars are being tested respecting their registration. It can be expected that they will meet the market requirements in a greater extent. Only cultivars of good quality can win the competition on fresh vegetable market. At present, improving pepper quality is the main task of pepper breeding in Poland.

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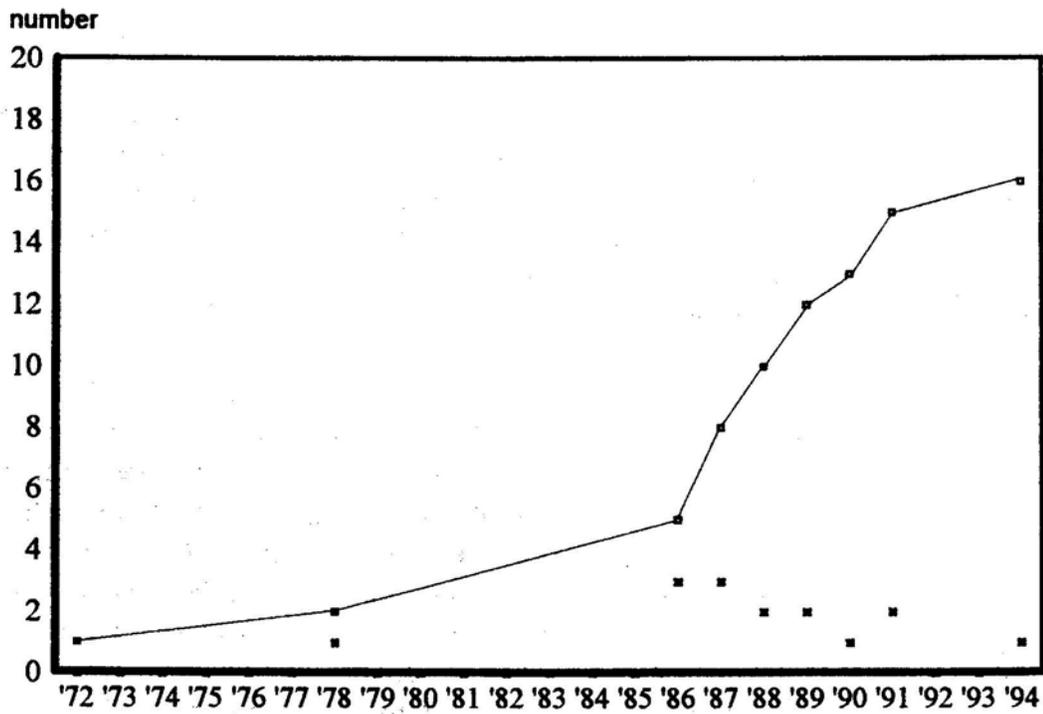
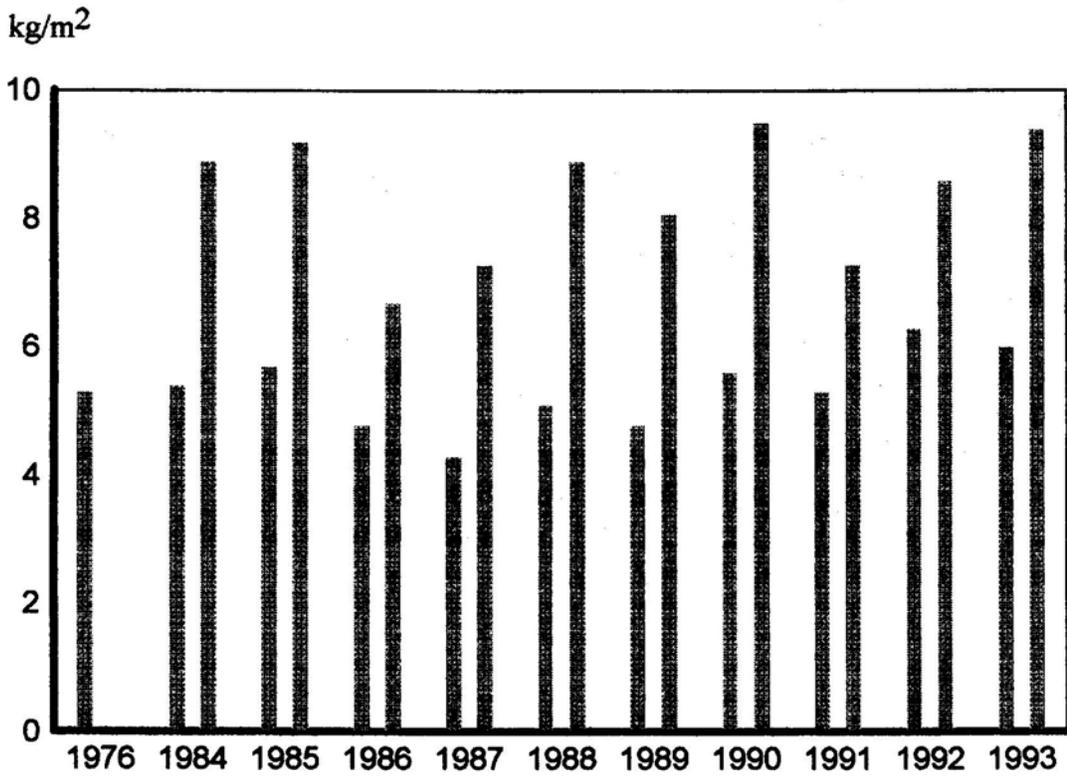


Figure 1 - The number of registered cultivars in years 1972 - 1994

Table 1 - The breeding methods and number of registered cultivars.

Total	Mutations	Selection of hybrids	Heterosis
16	2	13	1



## CELL CYCLE SYNCHRONIZATION IN ROOT -TIP MERISTEMS OF *CAPSICUM ANNUUM*

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### Introduction

The availability of isolated and intact plant chromosomes is of large interest in many research areas, ranging from chromatin structure study to the detection of low copy sequences by *in situ* hybridization (Dolezel *et al.*, 1994). Furthermore, suspensions of chromosomes make it possible to analyse and sort single chromosome types by flow cytometry, which can be used for the construction of chromosome-specific gene libraries and for gene mapping (Gray and Cram, 1990).

In plants, it is rather difficult to obtain high-quality suspensions of chromosomes suitable for further analysis, mainly due to the low degree of mitotic synchrony in plant tissues and the tendency of chromosomes to stick and clump after treatment with metaphase-blocking 'agents'. Notwithstanding this, the isolation of plant chromosomes has already been reported in several species, among which tomato (Arumuganathan *et al.*, 1991). In most cases the chromosomes were obtained from *in vitro* cultured cells, through protoplast isolation and cell wall lysis. Since chromosome instability of cultured cells is frequent (Lee and Phillips, 1988) and protocols for protoplast obtention are not available for many species, the use of vegetative material appears to be more advantageous.

In this paper we describe a procedure for the obtention of large quantities of intact mitotic chromosomes from root-tips of *Capsicum annuum*, in view of subsequent isolation and sorting. To synchronize the cell cycle, we used hydroxyurea (HU), which reversibly inhibits the enzyme ribonucleotide reductase and therefore the production of deoxyribonucleotides, so preventing DNA synthesis (Kornberg, 1980). Following the removing of HU, the cells progress more or less synchronously through subsequent mitosis phases.

### Material and Methods

Seeds of *Capsicum annuum* cv 'Corno di Toro' (Semencoop, Cesena, Italy) were germinated at 25 °C in the dark. At radicle protrusion, the seeds were transferred into Petri dishes containing perlite imbibed with a 2.5 mM hydroxyurea (HU, Sigma H8627) solution for 18 hours at 25 °C. Concentration and time length of imbibitions were adopted on the basis of preliminary tests (data not reported). Seeds were then rinsed in distilled water and transferred in a HU-free medium. Samples of root tips were collected at one-hour intervals up to 10 hours and analysed for mitotic activity.

To further accumulate mitotic cells in metaphase, germinating seeds after 3, 4, 5 and 6 hours of incubation in HU-free medium were transferred into Petri dishes containing perlite imbibed for 4 hours at room temperature with a saturated solution of 1,4-Dichlor-benzol (PDCB, Fluka 35370), a tubulin polymerization inhibitor. This treatment was found to be optimal for metaphase block (data not reported). Mitotic activity and metaphase frequency were analysed on squash preparation: samples of root tips were fixed overnight at 5 °C in Carnoy (ethanol-acid acetic 3: 1 in volume) and then stained according to the standard Feulgen method (lanteri, 1991). On each slide, at least 500 cells were recorded. Five meristem per treatment were analysed and the whole experiment was repeated four times.

For flow cytometric analysis, root meristems were homogenized in icecold nuclear extraction buffer (Saxena and King, 1989) and filtered through a 251.µm mesh nylon filter. The intact released nuclei were stained with propidium iodide and treated with DNase-free RNase (Sigma R5000). Fluorescence was measured using a FACScan flow cytometer (Becton and Dickinson, USA) equipped with a 488 nm light source (argon laser). Two filters were used to collect the red fluorescence due to PI staining the DNA, one transmitting at 585 nm and the other above 620. The flow rate was set at about 100 nuclei/sec and at least 5,000 nuclei were analysed for each sample. Data were recorded in a Hewlett-Packard computer (HP 9000, model 300) using CellFit software (Becton and Dickinson).

## Results

Mitotic activity of meristem cells in untreated root tips varied among replicates, the mitotic index ranging from 3.00 to 7.59%, with an average of 4.98%. A possible explanation is the different vigor, which is peculiar for each seed. Furthermore, the size of the meristem taken for analysis could have also influenced the mitotic index, being the mitotic activity limited in a very small portion of the apex. Flow-cytometric analysis of nuclear DNA content showed that most of the cells were in G2 phase (50.3%), being the frequency of cells in G1 and S phase, respectively, 30.4 and 19.3%

The treatment with HU resulted in a considerable block of DNA. Synthesis: after 18 hours treatment, a very low number of cells were found in mitosis. The flow cytometric analysis proved that most of the cells were accumulated at the G1/S interface and that the frequency of cells in G2 phase was very low. After the release from HU, meristem cells started synthesising DNA and entered the S phase: the frequency of cells in mitosis gradually increased and reached the maximum 5 hours after block releasing, when the frequency of cells in mitosis was, on average, about four times that of the control (Fig. 1). Subsequently, the frequency of mitotic cells decreased and reached, after about 9 hours, the value of the control.

The frequency of cell blocked in mitosis was furtherly increased by the PDCB treatment (Tab. 1): moreover, in this case, most of the cells were in metaphase, which is the optimal stage for further chromosome analysis.

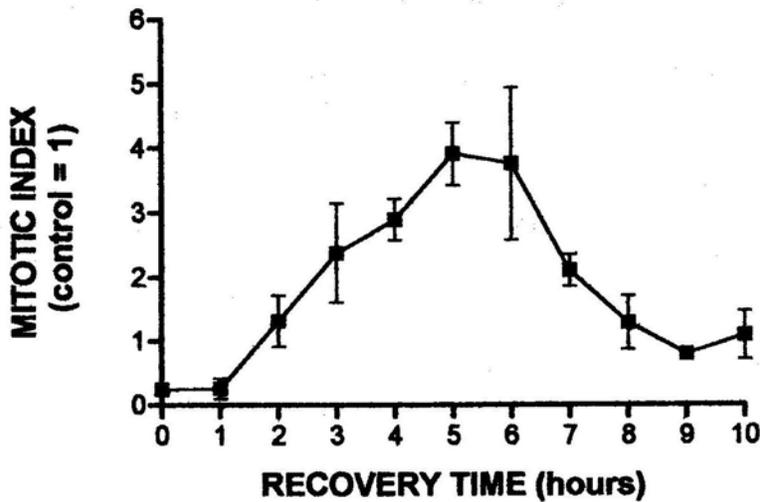


Fig. 1 - Mitotic indices in *Capsicum annuum* root tips (mean  $\pm$  standard error) during the recovery from the Hydroxyurea block.

RECOVERY TIME (h)	A	B
3	2.39	2.69
4	2.91	4.81
5	3.92	4.39
6	3.78	4.27
<b>LSD (5% level)</b>	<b>0.29</b>	<b>0.46</b>

Tab. 1 - Frequency of cells in mitosis (expressed as reference to the control) following HU treatment for 18 hours, recovered from HU for different times and submitted (B) or not (A) to a pre-treatment with 1,4-Dichlor-benzol for 4 hours.

## Discussion

The results of our study show the possibility to obtain a relatively high frequency of metaphase cells by a combined treatment with a DNA-synthesis inhibitor and a tubulin-polymerization inhibitor. In other species, as *Vicia faba*, it was possible to reach higher values of mitotic index than ours (Dolezel *et al.*, 1992): this is probably due to differences in physiological behaviour among species. In fact, in *Vicia faba*, the highest frequency of cells in mitosis was reached 8 hours after the release from the DNA-synthesis block, while in our study this occurred only after 5 hours. Moreover, Dolezel *et al.* worked on

material characterized by a higher mitotic index of untreated tissue: 9.1 *versus* 4.98% in our study. It should be emphasized the need for an accurate definition of the experimental procedures and the great importance of the physiological stage of donor material: possibly this explains the large variability among replicates observed in our study and makes it very difficult to compare results obtained in different experiments.

In order to define a complete procedure for chromosome isolation, the next step of our work will be to perform research on chromosome isolation, purification and sorting. Protocols are available for other species, as *Vicia faba* (Lucretti *et al.*, 1993), where it was possible to sort, through flow cytometric - techniques, more than 25,000 chromosome of a single type (corresponding to 0.2 J.tg of DNA) with a purity of more than 90% during a working day. A difficulty, which will be probably encountered when working on pepper, will be the high similarity of chromosomes.

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## RAPD FINGERPRINTING OF PEPPER (*Capsicum annuum*.) BREEDING LINES I

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### INTRODUCTION

Knowledge of genetic similarity between genotypes is useful in a breeding program because it facilitates efficient use of inbreds and helps the design of crosses. The breeder can use genetic similarity information to make informed decisions regarding the choice of genotypes to cross for development of new segregating populations, or to facilitate the identification of diverse parents to cross in hybrid combinations in order to maximise the expression of heterosis.

Estimates of genetic similarity are based in the detection of polymorphism at the DNA level. The most widely used technique is restriction fragment length polymorphism analysis (RFLP). Base substitution in a restriction end nuclease site or insertions or deletions between sites result in detectable differences in the fragment length of restriction enzyme-digested DNA. These polymorphisms already have been shown to be consistent with expectations based on known breeding behaviour and pedigrees in numerous crops (Smith et al. 1990, Nienhuis et al, 1992). More recently, the random amplified polymorphic DNA (RAPD) technique (Williams et al. 1990, Welsh et al. 1990), based on the polymerase chain reaction (PCR), has resulted in a potentially useful tool for cultivar discrimination. RAPD involves the amplification of DNA segments using random sequence primers, generally of ten bases, to find polymorphic regions within the genome defined by the primer sequence. The products formed and separated by agarose-gel electrophoresis reveal sequence variation in the form of variable numbers of bands of variable length, which may be characteristic of species and/or cultivars within species. RAPD requires no previous sequence information for the fingerprinting of cultivator genomes.

The Purpose of this work is to make use of DNA polymorphisms to study genome relationships among different pepper (*Capsicum annuum* L.) inbred lines and to use this information to aid in plant breeding decisions in pepper. A variety of computer programs have also become available for generating phylogenetic trees based on DNA information from different individuals, making the task of studying genome relationships more efficient and accurate. The ability to extract and analyse DNA from plant is an essential aspect of plant molecular biology. The isolation of plant DNA must ensure that significant amounts of DNA are not trapped in the cell debris, and that the DNA is completely dissociated from proteins and other contaminants that might copurify and interfere with subsequent analyses. The preparation of high quality DNA from polyphenolic-containing plants such as pepper is difficult, because of DNA degradation mediated by secondary plant products such as phenolic terpenoids and tannins, which may bind to DNA and/or RNA after cell lysis (John 1992). The method described here is based on modified protocols of John (1992), Murray et al. (1980) and Pich et al. (1993). It combines the complexation of polyphenolic compounds by polyvinylpyrrolidone (PVP) and complex carbohydrates by cetyltrimethylammonium bromide (CTAB), following cell lysis and selective precipitation for removal of PVP and CTAB complexes and DNA recovery.

**Table 1. List of peppers lines with type designation.**

Line	original cross	cross code	type inbreed	
1	yellow 'california' x yellow 'california.	A	yellow 'california'	F7
2	yellow 'california. x yellow 'california'	A	yellow 'california'	F7
3	yellow 'california. x yellow 'california.	A	yellow 'california'	F7
4	orange 'california. x orange 'california.	B	orange 'california'	F7
5	red 'california. x red 'california'	C	red 'california'	F9
6	red 'california' x red 'california'	D	red 'california'	F9
7	red lamuyo. x yellow 'california.	E	yellow lamuyo.	F7
8	red 'lamuyo' x red 'california'	F	red rocky	F9
9	red lamuyo. x red lamuyo.	G	red lamuyo.	F6
10	red lamuyo. x red lamuyo.	H	red lamuyo'	F6
11	chilli cultivar 'Serrano criollo de Morelos		(population)	

## MATERIALS AND METHODS

### *Growth of plants*

All lines were field grown at the Pioneer's Agricultural Research Station in Almeria. Eleven genotypes of pepper (*Capsicum annuum* L.) were chosen for this study (table I), A set of eight 'california' type inbred lines, two 'lamuyo' type inbred lines and one chilli cultivar, 'Serrano Criollo de Morelos', were used to ascertain their degree of similarity

### *DNA isolation*

Young leaf tissue samples were used immediately after collection for DNA extraction or were stored " at -80°C prior to DNA extraction. Fresh or frozen tissue (3 g) was ground in liquid nitrogen with a mortar and pestle. The powder was transferred to a 35 mL-centrifuge tube and 10 mL of extraction buffer (100 mM Tris-HCl pH 8, 50mM EDT A pH 8, 500 mM NaCl 2% CT AB, 1 % PVP-40, 2% SDS and 10 mM DTT) was: added. After 10 min at 65°C 4 mL of 5M potassium acetate was added. After 10 min at 0°C, the sample was centrifuged for 20 min at 10.000 rpm. The supernatant was filtered through 2 layers of miraclore and transferred to a new 35 mL tube. The DNA was precipitated by the addition of 8 mL of isopropanol and recovered by centrifugation for 15 min at 10.000 rpm after incubation on ice for 30 min. The pellet was dried and redissolved in 0.7 mL of TE (10 mM Tris-HCl pH 7.4, 1 mM EDT A). After the sample was transferred to a eppendorf tube and centrifuged at 10.000 rpm during 10 min to remove the insoluble things. The supernatant was transferred to a new eppendorf tube and 75, uL of 3M sodium acetate and 0.5 mL of isopropanol were added. The pellet was washed with 70% ethanol after centrifugation for 30 sec at 10.000 rpm. After the pellet was dried and resuspended in 0.3 mL of TE. Contaminating RNA was removed by , digestion with 30 ,ug Ribonuclease A for 20 min at 37°C. The DNA was purified by extracting once with an equal volume of phenol followed by extraction with an equal volume of chloroform/isoamyl alcohol (24: 1). The DNA was precipitated by the addition of NaCl to a final concentration of 0.2M and 2 volumes of cold ethanol. After 10 min at DoC, the sample was centrifuged at 10.000 rpm for 15 min. The final pellet was washed with 70% ethanol, dried and dissolved in 0.3 ml of TE. DNA was quantified by fluorometry with a Hoefer TKO 100 mini fluometer and following procedures supplied by the manufacturer. All samples were diluted to a DNA concentration of 0.2 ng/uL.

### *Amplification of DNA*

42 random decamer oligonucleotide primers obtained from Operon Technologies, Inc, Alameda, CA, USA, were used as single primers for PCR. DNA amplification was based on the method described by Williams et al. (1990) with minor modifications. The reaction mix was carried out in 15 ILL reactions containing 30 ng template DNA; 10 mM Tris-HCl pH 9.0, 50 mM ClK, 2.5 mM MgCl<sub>2</sub>; 0.2 mM each of dATP, dCTP, dGTP and dTTP; 4 pM primer; 1 U Taq DNA polymerase ( Pharmacia Biotech). Amplification was performed in a Techne PCH-3 termociclador programmed as followed: 1 min 94°C for denaturing, 1 min at 34°C for annealing, 1 min at 72°C to synthesis, repeat for 45 cycles. Amplified samples were kept at 4°C until further use. Amplified products were separated electroforetically in 1.4% agarose gels in TBE buffer (0.09 M Tris-borate, 0.002 M EDTA pH 8 ) at 135 V constant voltage for 3 h, stained with ethidium bromide and photographed under transilluminated UV light.

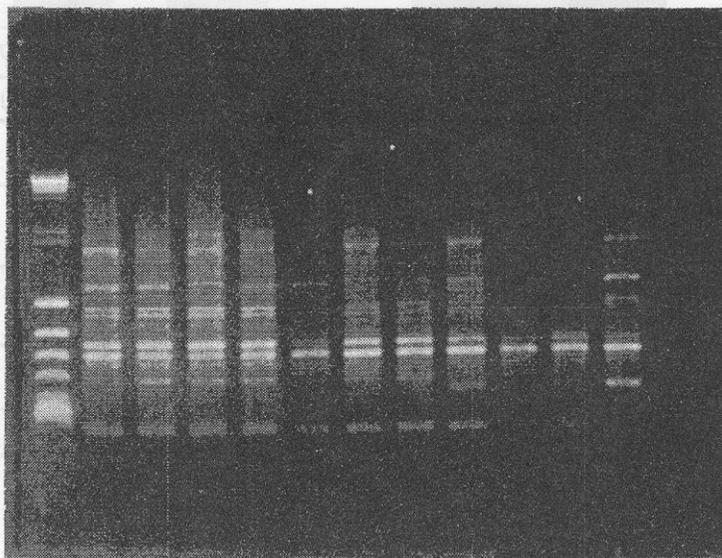
### *Statistical Analysis*

Data from PCR amplification of the 11 lines with 42 different oligonucleotide primers were analysed as follows. A number was assigned to each scorable polymorphism and the presence or absence of a band was coded by 0, or 1 respectively. Similarity coefficients were calculated using the equation (Nei and Li, 1979):

$$S = \frac{2n_{xy}}{n_x + n_y}$$

where  $n_x$  and  $n_y$  are the numbers of markers observed in individuals  $x$  and  $y$ , respectively, and  $2n_{xy}$  is the number of markers shared by the two individuals. The data were entered into Microsoft Excel v 5.0 for Windows. The data matrix was read by NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System for Personal Computer v.1 80, Exeter Software, Seautek, N.Y.). A dendrogram was constructed using UPGMA (unweighted pairgroup method with arithmetic averages) with the SAHN (sequential, agglomerative, hierarchical and nested clustering) routine.

**Figure 1.-** Random amplified polymorphic DNA analysis with primer T04 (5'-CACAGAGGG A-3'). DNA was fractionated on a 1.4 % agarose gel and stained with ethidium bromide. Lane 1 DNA marker (lambda DNA-Hind III/ $\Phi$ X-174 DNA- Hinc II digest. Pharmacia Biotech). Lanes 2 to 9 are 'california' type inbred lines of peppers; lanes 10 and 11 are 'lamuyo' type inbred lines of peppers; lane 12 is the chilli cultivar 'Serrano Criollo de Morelos'; and lane 13, negative control .



## RESULTS AND DISCUSSION

Fifty primers of arbitrary sequences were tested on 11 lines of *capsicum annum L.* Of these, 42 showed bands in all lines. The other 8 primers failed to amplify. A total of 219 fragments were visualised across all lines investigated, and each primer produced approximately five to six fragments from each line (figure 1). Among 219 fragments, 144 (65,75 %) were *monomorphic* (all lines had the band), 52 (23.74 %) of the bands were *polymorphic* (bands were absent from at least one line), and 22 (10.04 %) of the total bands were *unique* to a single line. The cultivar chilli inclusion in bands variations data obtained with different primers, table 1, diminishes the percentage of monomorphics bands from 76,24% to 65,75 %, which shows the higher distances of this population from the rest of the studied genotypes. The number of bands generated by specific primer varied from 1 to 10. These values indicate that a very large number of RAPDs can be generated from a small number of reactions, reducing the cost of using RAPD in marker assisted selection in a breeding program.

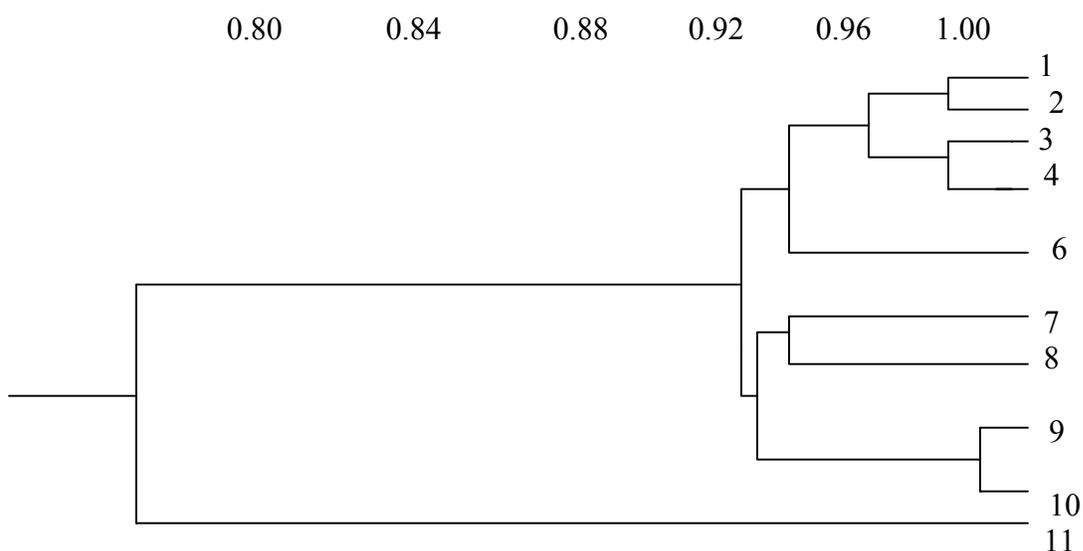
Only 3 of the 42 primers amplified only one band (*unique*) to a single line. Twenty-four of the total primers failed to amplify only one band. The percentage of *unique* band to a single line was very low, and at the same time the percentage of common bands to all the lines (*monomorphic* bands) was greater than the 50 % of the total bands. This suggest that the genetic base of the lines is very narrow.

**Table 2.-** Matrix of similarity coefficients among 11 lines of pepper.

Genotype	1	2	3	4	5	6	7	8	9	10	11
1	-										
2	0,98016	-									
3	0,96111	0,96935	-								
4	0,94915	0,98016	0,97222	-							
5	0,93593	0,95530	0,95342	0,95821	-						
6	0,94586	0,95428	0,94677	0,95726	0,94943	-					
7	0,91573	0,95774	0,95027	0,96067	0,94736	0,93484	-				
8	0,94413	0,95798	0,95054	0,94972	0,96418	0,92957	0,95555	-			
9	0,93333	0,94707	0,93442	0,94444	0,95890	0,92997	0,95027	0,94505	-		
10	0,93370	0,94182	0,93478	0,93922	0,95367	0,92479	0,94505	0,94535	0,98913	-	
11	0,87292	0,87534	0,86956	0,86740	0,88828	0,85793	0,88461	0,88524	0,88043	0,87567	-

A similarity matrix for all lines was calculated for their RAPD bands (Table 2). The mean similarity index value from all of these comparisons was 0.9423. The lowest similarity index value, 0,8579, was observed between the cultivar 11 and line 6. The genotype 11 showed low similarity index values (mean value is 0,8770) when compared to all of the other 'california' and 'lamuyo' type lines (mean value is 0.9520.) and appeared as the most divergent line. The highest similarity index value was 0,9891 and was observed between 'lamuyo' type lines 9 and 10, which are from the same origin.

Figurs 2. Pepper dendrogram generated using UPGMA clustering



Based on the rectangular matrix dates showed in the table 2, a cluster analysis was realised using the computer program above mentioned (NTSYS-pc)? Then dendrogram built for the genotypes using UPGMA cluster is showed in figure 2. It is observed a high genetic diversity in the chilli cultivar in this dendrogram. The rest genotypes are grouped in two subgroups ('california' and 'lamuyo' types). The root of which has a similarity index of 0.9458. The composition of these subgroups is highly homogeneous, with the discrepancy of the line 5 of 'california' type. This result is supported by the crossing studies between 5 and 3 that produce a hybrid with a high vigour. The lines 2, 3 and I form a high genetic similarity cluster due to their common origin, in which the line 4 is also included. Crosses between the last one and tile other components of the cluster produce no specially vigorous hybrids, which is in agreement with the genetic similarity predicted by RAPD analysis. The 'lamuyo' cluster consists on two clearly differentiated subgroups. One of them formed by peppers of pure 'lamuyo' type (lines 9 and 10) and the other consisting on peppers proceeding from a crossing between 'california' and 'lamuyo' parental types, subsequently selected with the 'california' pattern (7 and 8). These subgroups have a similarity rate of 0.95. As it is showed in the dendrogram the pure, lamuyo' genotypes are found genetically further from the pure 'california' genotype than the components of the other subgroup (lines 7 and 8).

#### ACKNOWLEDGEMENTS.

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## EVALUATION OF FRUIT EDIBLE RATE OF HOT PEPPER GERMPLASM

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### Introduction

*Capsicum* varieties are widely cultivated in China. There are three distinct groups of varieties, i. e. hot pepper used for dried chile powder, hot pepper suitable for the fresh market, sweet pepper. The goals of the *Capsicum* breeding in China are as follows, 1) superior fruit quality, 2) resistance to virus (TMV and CMV) and *Phytophthora capsici*, 3) high yield (including earliness yield). Each kind of *Capsicum* requires different quality. Quality means different things to different people (Bosland, 1993). Hence different aspects of fruit quality standard vary according to uses by growers, shippers, sellers, and consumers. As *Capsicum* fruits move through market channels, the fruit quality parameters demanded by the consumers should be considered. For hot pepper for fresh market and sweet pepper, the consumers judge fruit quality not only in fruit size, colour, nutrition, but also in fruit edible rate, which is becoming more and more emphasized as time goes on.

Fruit wall (or fruit flesh) is the edible component of hot and sweet pepper fruits. About 25 % of the raw fruit of Truhart Perfection Pimiento variety was waste (Cochran, 1963). According to our investigation (Wang, 1993), fruit edible rates of the cultivars for fresh market, newly bred in China, were not too high (80 % - 85 %). Breeding hot pepper with high edible rate (more than 90 %) will have been concerned in the near future breeding programmes. This preliminary study investigated fruit edible rates of hot pepper germplasm, aiming to provide information associated with breeding for high fruit edible rates.

### Materials and Methods

Twenty-two hot pepper accessions were planted in completely randomized design with one replication in the field, twenty plants per accession. At green maturity, ten fruits were randomly chosen from five plants per accession. For the individual fruit, the measurement of fruit weight, fruit flesh weight, placenta weight, fruit stem weight, and seed weight were undertaken. The character means were used for analysis. Then fruit edibles rates (FERs) (ratio of fruit flesh weight to fruit weight) were determined. Fruit edible characteristics of the germ plasm were graded as extremely high, high, intermediate, low, extremely low with more than 90 %, 80 - 90 %, 70 - 80 %, 60 - 70 %, less than 60 % fruit edible rate (FER), respectively.

### Results and Discussion

These germ plasm showed wide spectrum of variation in fruit edible rate (Table 1). Following the above grouping criteria, the accessions fell into three groups, i. e. high FER Group, intermediate FER Group and low FER Group, and the number of the accessions in these three groups was 12, 9, and 1, respectively. In high FER Group, the variety 'WP 199-1-2' had the highest fruit edible rate (86.04%), followed by R841 (84.18%) and R408 (83.76%). The accession '93-19' had the lowest. Fruit edible rate (62.85%). For most of the germplasm tested, fruit edible rates range from 77 % to 86 %. None of the accessions with the edible rate more than 90 % was found in our study, suggesting that the elite germplasm with extremely high fruit edible rate was in the minority, and needed to be widely collected and explored. It could also be seen that, ratio of placenta weight to fruit weight was higher than that of fruit stem weight to fruit weight, usually higher than that of seed weight to fruit weight. This indicated that

Placenta was a major component of fruit non-edible parts. Hence, the strategy of breeding hot pepper for extremely high fruit edible rate (>90%) should be focused on 1) increasing the fruit flesh weight and 2) reducing the non-edible part weight, especially placenta weight.

Table 1 Fruit edible rate of hot pepper germplasm

Accessions	Fruit	Fruit	Placenta	Ratio Of	seed
	Weight	Edible		Stem	
	(g)	Rate	Weight to fruit weight		
WP 199-1-2	53.0	86.04%	6.81%	2.59%	4.56%
R841	25.5	84.18	4.17	3.97	7.14
R408	52.8	83.76	7.95	3.61	4.68
WP 119-1-12	43.3	82.78	8.42	3.19	5.61
E22	18.0	82.42	6.62	5.38	5.58
R401	14.9	81.87	7.03	3.67	7.43
6C-63	16.7	81.60	6.72	4.30	7.38
E18	14.6	81.54	7.68	3.32	7.56
R168	22.1	81.41	7.80	4.31	6.48
R610	34.0	81.13	7.37	3.54	7.96
6C-376	17.0	81.10	6.67	6.07	6.16
E6	33.4	80.88	8.12	4.89	6.11
6C-37	28.2	79.78	7.96	4.16	8.10
E3	45.8	78.66	9.68	7.70	3.96
R850	17.4	78.59	7.90	4.86	8.65
R33	34.6	78.28	10.52	4.00	7.20
F21-1	17.0	77.51	8.70	3.65	10.14
E18	17.5	76.37	9.79	3.42	10.45
R85036	22.1	75.85	11.51	4.38	8.26
Yiliniujiao	39.9	74.18	13.44	5.82	6.56
6C-1278	22.6	72.62	12.40	4.84	10.14
93-19	22.4	62.85	16.08	8.75	11.32

Ratio of placenta weight to fruit weight was extremely significant, significant positive correlated with that of stem weight to

## **EFFECT OF pH ON PEPPER SEEDLING GROWTH**

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### **Introduction**

Soils in Galicia (NW of Spain) usually have an acid pH (Macias, 1993). The "Padron" pepper (*Capsicum annuum* L. var. *annuum*) is a local variety with great commercial value, which is grown in various parts of Galicia. There is no general rule about optimum pH for growing peppers, but values from 6 to 8 are usually recommended (Andrews, 1995; DeWitt & Bosland, 1993; Zapata et al., 1991.). However there is little information on the influence of rhizosphere pH on pepper seedling growth and development (Stoffella et al., 1991). The purpose of this research is to determine the effects of pH on pepper seedling growth.

### **Materials and methods**

Two days old seedlings were grown for a week in perlite soaked with nutritive solutions composed of 6mM KNO<sub>3</sub>, 4 mM Ca (NO<sub>3</sub>)<sub>2</sub>, 2 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 1 mM MgSO<sub>4</sub>, 50 11M KCl, 25 11M H<sub>3</sub>BO<sub>3</sub>, 2 11M MnSO<sub>4</sub>, 2 11M ZnSO<sub>4</sub>, 0.5 11M CUSO<sub>4</sub>, 0.5..JIM H<sub>2</sub>MoO<sub>4</sub>, 20 11M EDTA and 20 11M Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>). Solutions were adjusted to desired pH adding appropriate quantities of 10 mM PO<sub>4</sub>HK<sub>2</sub> and 10 mM PO<sub>4</sub>H<sub>2</sub>K. Experiments were carried out using solutions buffered at pH 5.1, 6.6 and 7.3, which were renewed every day. After a week roots, hypocotyl, cotyledons and leaves were collected, weighed and extracted using 0.1M potassium phosphate buffer pH 7.4 containing 0.5 mM DTT, 2 mM cysteine, 2 mM EDTA and 8 mM 2-Mercaptoethanol. In addition 1 mg PVPP per. each 20 mg of fresh weight was added to the sample. Crude extracts were centrifuged and supernatants were analyzed. Soluble proteins were determined according to Sedmak & Grossberg (1977), total chlorophyll as per Arnon (1949), and shikimate dehydrogenase as previously described (Diaz et al., 1994).

### **Results and discussion**

Most of the organs of the seedlings grown at pH 7.3 had a higher fresh weight than those of plants grown at pH 6.6 and 5.1 (Fig. 1). These differences are similar in all the organs because they represented a similar percentage of the total fresh weight of seedling in all the treatments (Fig. 2). As previously stated by Stoffella et al. (1991), our results suggest that all of the organs of seedlings were affected in the same degree by the different pH treatments. However our data showed that seedlings reached the highest fresh weight at pH 7.3, but Stoffella et al. (1991) found that pH 5.9 was a more favourable pH for seedling growth. Differences between the two studies could be due to the fact

That different ways used to buffer the solution. Another difference was the starting stages: Stoffella et al (1991) applied the pH treatment from seed germination, whereas we used two days old seedling.

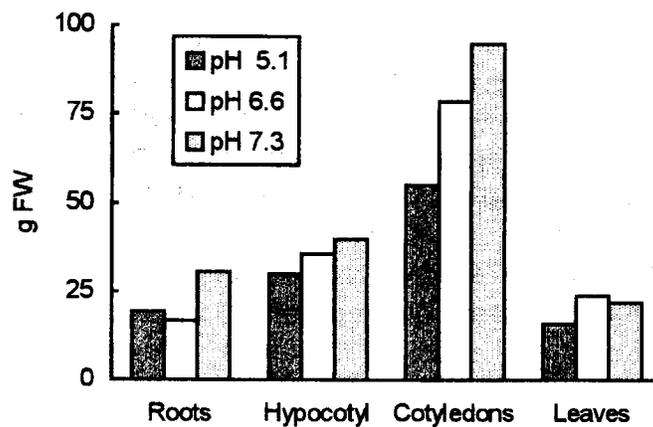


Fig 1 Fresh weight (FW) of seedling organs after different pH treatment

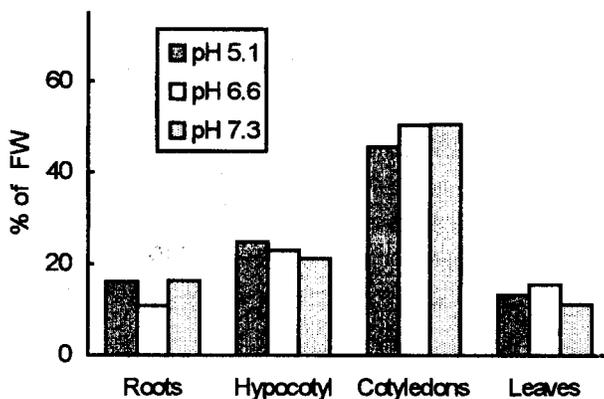


Fig Distribution of fresh weight of seedling after different pH treatments

Protein and chlorophyll content was determined in order to obtain additional information about the status of seedling. In addition, shikimate dehydrogenase activity was measured because it had been previously observed that the enzyme level is affected by different kinds of abiotic stresses.

(Diaz & Merino, unpublished results). Table 1 shows that the more acid the pH was, the more protein, chlorophyll and shikimate dehydrogenase levels per gram of fresh weight occurred in most organs. However there are not as many differences between treatments if data per organ are considered (Table 1). The latter results suggest that differences in fresh weight are due to differences in- water content in the plant. Therefore plants at pH 7.3 contained more water than the others. An explanation of this phenomenon could be that some nutrients are less available at neutral pH than at an acid one, and plants have to absorb more water to maintain their needs. Further research will be carried out in order to clarify this effect and examine the effect of different pH on plants in the breeding stage.

Table 1. Protein and chlorophyll content, and shikimate dehydrogenase activity related to pH treatments.

Organ	pH	Protein		Chlorophyll		Shikimate	dehydrogenase
		mg/g FW	ma/organ	mg/g FW	mg/organ	nkat/g FW	nkaUoraan
Roots	5.1	0.78	15.1	nd	nd	1.18	23
	6.6	0.57	9.6	nd	nd	0.70	12
	7.3	0.56	17.1	nd	nd	1.49	46
Hypocotyl	5.1	0.48	14.5	nd	nd	0.43	13
	6.6	0.40	14.5	nd	nd	0.28	10
	7.3	0.24	9.7	nd	nd	0.12	4.8
Cotyledons	5.1	2.35	129.4	91.4	5.03	3.63	295
	6.6	1.56	122.6	54.7	4.29	2.71	315
	7.3	1.67	158.5	62.2	5.89	2.46	273
Leaves	5.1	4.80	76.8	90.6	1.45	18.45	200
	6.6	2.93	70.4	64.6	1.55	13.12	212
	7.3	2.95	64.8	77.1	1.70	12.43	233

nd= not determined. FW= fresh weight.

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## **EFFECTS OF DIFFERENT FRUIT MATURITY STAGES AND STORAGE CONDITIONS ON CHEMICAL COMPOSITION AND MARKET ACCEPTABILITY OF FRUIT IN DIFFERENT VARIETIES OF SWEET PEPPER**

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### **INTRODUCTION**

Importance of sweet pepper has gradually increased not only by nutrition conscious people of India but has gained popularity among vegetable growers because of its high export value. Keeping in view its export importance and nutritional qualities, in the present study an attempt was made to know the effect of different fruit maturity stages and storage conditions on chemical composition and market acceptability of fruits. Secondly to assess and identify cultivars which are rich in nutrients and can store well for longer period with minimum loss of nutrients and market acceptability.

### **MATERIALS and METHODS**

The experiment with 15 cultivars (cv.) was conducted at Vegetable Experimental Farm, S.K.U.A.S.T., Srinagar during kharif 1993 following RBD with 3 replications. Seedlings were planted in 3.1.1 x 2.4 in plots at spacing 50cm x 30cm and a healthy crop was raised. Fruits of different maturity stages (green edible, matured green, breaker and ripe/yellow) from 8 cvs. and green edible fruits from all the 15 cvs were harvested separately, washed and used in triplicate for estimation of total chlorophyll (TC), total soluble solids (TSS), ascorbic acid (AA) dry matter content (DM) and market acceptability (MA). TC mg/g was estimated as per Mackinney (1941). TSS matter determined from fruit juice using hand refractometer and expressed as °B. AA was determined using 2,6-dichlorophenol indophenol dye method (A.O.A.C. 1975). DM was determined by drying the fruit samples at 60±2°C to a constant weight. MA was determined considering & colour, shape, size, texture and overall appearance and graded as excellent (5), good (4), fair (3), poor (2) very poor (1) unacceptable (0). For storage studies, edible green marketable fruits from 8 cvs were stored under ordinary room temperature (20-28°C, 70-72% RH) and at controlled condition (8°C, 85-90% RH). Fruits from two storage conditions were analyzed for TSS, AA, cumulative weight loss (CWL) and MA at weekly intervals and changes in these chemicals and MA were noted.

## Results and Discussion

Significant difference exceeded among cultivars in respect to chemical composition (table1). DM varied from 7.17 to 14.67 percent and TSS from 2.83 to 5.08. DM and TSS were significantly high in cv. Oskash and KSP-3 followed by KSPS-461. AA content varied from 56.43 to 114.73mg/100g fresh fruit. CV oskash recorded significantly high AA (114.73 mg;100g) followed by KSPS 3 (106.50) and KSPS-201 (91.42). AA was minimum in Arka Gaurav and hybrid KT-1. The total chlorophyll which indicates green fruit colour was highest in cv. World beater (8.97mg/g) followed by KSPS-2, HC 201 and KSPS 401 and were dark green. The other hand hybrid MA 1 in general was excellent except hybrid KT1 Oskash and KSPS- 461. These cvs owing to their poor colour and among different cultivar Oshash and KSPS-3 by having superior nutritional qualities could be considered as the potential genotypes for future exploitation. Varietal variation for DM and AA in Chilli was also observed by Bajaj et al (1980)

Table 1 Chemical composition and market acceptability of some sweet pepper cultivars

Cultivars	DM%	TSSoB	TC mg/g	AA (mg/100g)	MA
Cal. Wonder	8.94	3.83	4.42	66.53	5.0
KT-1 fl	8.85	2.83	0.84	58.20	4.0
HC-201	10.20	2.91	6.48	67.29	5.0
Oskash	13.21	5.08	1.72	114.73	4.0
Arka Gaurav	8.83	3.33	5.02	56.43	5.0
Bull nose	7.45	4.58	6.24	68.39	5.0
World Beater	9.82	3.50	8.97	66.66	5.0
Chinese Giant	8.57	2.19	6.24	61.37	5.0
KSPS-1	8.70	3.66	5.66	61.37	5.0
KSPA2	9.82	3.83	6.70	74.03	5.0
KSPS3	14.67	4.66	4.57	106.50	5.0
KSPS 401	7.78	5.00	6.44	67.53	5.0
KSPS 206	9.54	3.41	6.37	82.39	5.0
KSPS461	10.63	4.58	4.18	84.60	4.0
KSPS201	7.17	3.14	4.40	91.42	5.0
CD at 5%	.53	.25	.29	6.35	

Table 2. Chemical composition and market acceptability of some cultivars of sweet pepper as affected by different fruit maturity stages (average of triplicate estimations).

	Maturity stages																	
	Green edible				Matured green				Breaker stage				Red ripe/yellow					
	TC	DM	ISS	AA	MA	DM	TSS	AA	MA	TC	DM	TSS	AA	MA	DM	TSS	AA	MA
C.Wonder	4.4	8.9	3.8	66.5	5.0	9.3	4.1	62.8	4.0	3.3	9.5	4.8	58.0	2.0	9.8	5.3	50.7	0.0
KT-1(F1)	0.8	8.8	2.9	58.2	4.0	9.2	3.3	57.0	3.7	0.6	9.0	4.6	50.9	2.3	9.5	4.8	49.3	0.6
HC-201	6.5	10.2	2.8	67.3	5.0	10.5	3.3	71.5	4.0	4.9	10.4	6.1	64.6	2.0	10.8	6.0	60.0	0.0
Oskash	1.7	13.2	4.8	114.7	3.7	14.6	4.9	100.8	3.0	1.0	13.9	6.0	91.0	2.3	14.5	6.0	71.0	0.0
Arka Guarav	5.0	8.8	3.6	57.4	5.0	9.5	4.0	55.3	4.3	3.7	9.4	4.6	51.0	2.0	10.4	5.0	50.3	0.0
KSPS-1	5.7	8.7	4.0	83.7	5.0	9.9	4.6	78.3	4.0	4.2	10.5	5.1	69.7	2.0	10.9	5.1	64.2	0.0
KSPS-2	6.7	9.8	4.1	74.0	5.0	10.7	5.4	20.2	4.3	5.0	10.9	5.5	69.3	2.0	11.5	5.8	64.0	0.0
KSPS-3	4.6	14.7	4.7	106.4	4.0	15.3	5.1	89.0	3.0	3.5	14.9	5.1	83.2	2.0	15.5	5.9	68.7	0.0
Mean	4.4	10.4	3.8	78.4	4.6	11.1	4.3	23.1	3.8	3.3	11.1	5.2	67.2	2.1	11.7	5.5	59.8	0.1
% change over green edible	-	-	-	-	-	7.1	13.0	-6.7	-17.4	-25.4	6.5	36.3	-14.3	-54.8	12.5	43.1	-23.8	-98.2

Note: TC was estimated only at green edible stage and breaker stage.

Table 3. Chemical composition and market acceptability of some cultivars of sweet pepper as affected by storage conditions (average of triplicate estimates).

	Storage conditions															
	Ordinary room temperature(20.8°C,70-72%RH)						Controlled conditions (8°C 85-90% RH)									
	After one week			After two week			After two week			After four week						
TSS	AA	CWL	MA	TSS	AA	CWL	MA	TSS	AA	CWL	MA	TSS	AA	CWL	MA	
C.Wonder	4.6	40.8	14.5	2.0	5.1	33.1	26.6	1.0	3.9	61.8	5.1	5.0	3.7	57.4	8.0	4.0
KT-1(F1)	3.2	34.8	20.8	2.0	3.8	29.5	36.9	1.0	2.9	57.9	3.9	4.0	3.1	61.0	5.4	3.0
HC-201	3.3	45.3	10.9	2.0	3.8	33.4	19.0	1.0	3.2	58.8	3.2	5.0	3.3	60.4	5.6	4.0
Oskash	5.4	74.5	19.5	2.0	5.8	61.2	31.4	1.0	4.8	105.5	3.0	4.0	5.0	101.4	4.7	3.0
Arka Gaurav	3.6	41.6	11.4	2.0	3.9	30.7	18.8	1.0	3.5	62.9	2.8	5.0	3.6	57.7	5.5	4.0
KSPS-1	4.1	64.7	10.5	2.0	4.9	41.3	17.0	1.0	3.7	84.0	2.7	5.0	3.9	79.9	4.3	4.0
KSPS-2	4.5	52.0	14.6	2.0	5.4	52.1	24.3	1.0	4.0	67.3	4.9	5.0	4.0	63.4	7.4	4.0
KSPS-3	5.1	75.0	9.4	2.0	5.9	64.3	16.9	1.0	4.7	107.8	2.3	4.0	5.0	99.7	4.9	3.0
Mean	4.2	53.5	13.9	2.0	4.8	43.2	23.8	1.0	3.8	75.7	3.4	4.6	3.9	72.6	5.7	3.6
% change over 12.2 fresh harvested green edible	-31.6	13.9	-56.7	28.2	-44.9	23.9	-78.3	2.1	-3.4	3.5	0.0	5.0	-7.4	5.7	-21.6	

Edible stage but thereafter both increased gradually and were maximum at ripe stages in all the cultivars. Changes in chemical composition of fruits under storage condition is given in Table 3. The results indicated that fruits stored under controlled conditions irrespective of cultivars showed no appreciable change in chemical composition old MA. After four weeks of storage the TSS slightly increased by 5.0% while AA decreased to an extent of 7.4%. MA of fruits was excellent Among different cvs., the minimum weight loss was in 'KSPS-1' and 'HC-201'. On the contrary, fruits stored at ordinary room temperature showed drastic changes in chemical composition and fruits were unacceptable after one to two weeks of storage. The CWL was as high as 36.98% in 'KT-1' after two weeks of storage (followed by 'Oskash' (31.40%), where as it was minimum in cv.'KSPS-3' followed by 'KSPS-1' (17.0%) 'HC-201' (18.97%) and 'Arka Gaurav' (18.76%). TSS in all the cultivars gradually increased from one week to two weeks of ordinary storage where as it was reverse with AA which decreased gradually and rapidly to an extent of 44.9% with no MA after two weeks at ordinary storage. Decrease of AA level by 75% after 18 days of storage at *chilli*. was also reported in Brinjal (Esteban 1989).

Among two storage conditions, the controlled storage condition (8°C, 85-90% RH) was considered best as fruits could be stored for four weeks with minimum effect on chemical composition and MA. Among cultivars, 'KSPS-1' and 'HC-201' under both the storage conditions showed minimum loss in weight and ascorbic acid content along with good market acceptability. These cultivars can therefore be best utilized for storage purposes.

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## **A SIMPLE METHOD FOR DETERMINING THE DEGREE OF PUNGENCY OF PEPPERS**

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### **Introduction**

Plant breeders and seed growers who work with hot peppers often encounter difficulty in determining the pungency of various lines and individual plant selections. For this, tasting has generally been employed. However, direct tasting of fruit has proved unsatisfactory because taste ability is quickly "destroyed", and non-pungent fruits cannot then be identified. Attempts to determine pungency by tasting diluted fruit extracts have also proved unreliable. On the other hand, various instrumental methods, including spectrophotometry (Bajaj, 1980; Mori, 1976), gas-liquid chromatography (Todd, 1977) and high-performance liquid chromatography (Weaver, 1986) have been employed for the determining content of capsaicin. However, since these methods involve complicated in sample preparation and the instruments necessary are expensive, they are not suitable for determining the pungency of a large number of plants. A simple chemical test for pungency in peppers using vanadium salts as an indicator has been reported (Ting, 1942). However, a dried sample must be prepared, and so fresh fruits cannot be tested directly. Therefore, to determine the degree of pungency of peppers, a simple method allowing direct testing of fresh fruits and rapid sample preparation was developed.

### **Materials and Methods**

(1) A piece of fresh pepper fruit containing the placenta was treated with diethyl ether to extract the pungency agent, capsaicin, and the extract was mixed with 0.5 N sodium hydroxide solution. Then a mixture of 1% ferric chloride solution, 1% potassium ferricyanide solution and concentrated hydrochloric acid was added to the solution as an indicator. The degree of pungency of the pepper fruits was roughly determined by comparing the green color intensity of the lower layer of the solution with that of a standard solution containing reagent-grade capsaicin with the naked eye. The absorbance of the lower layer was measured at 750 nm with a spectrophotometer, and was then compared with the capsaicin content determined by the colorimetric method (Mori, 1976) to investigate the correlation between color intensity and capsaicin content.

grade capsaicin with the naked eye, and the  
1g. The tasting panel included seven persons.

a faint greenish- yellow color reaction,  
he content of capsaicin. The coefficient of

the lower layer. From these results, a method  
1 in Scheme 1. In this method, fresh fruits can  
is the degree of pungency of the pepper fruits  
tion with the naked eye, the method is a  
ion are also shown in Scheme 1.

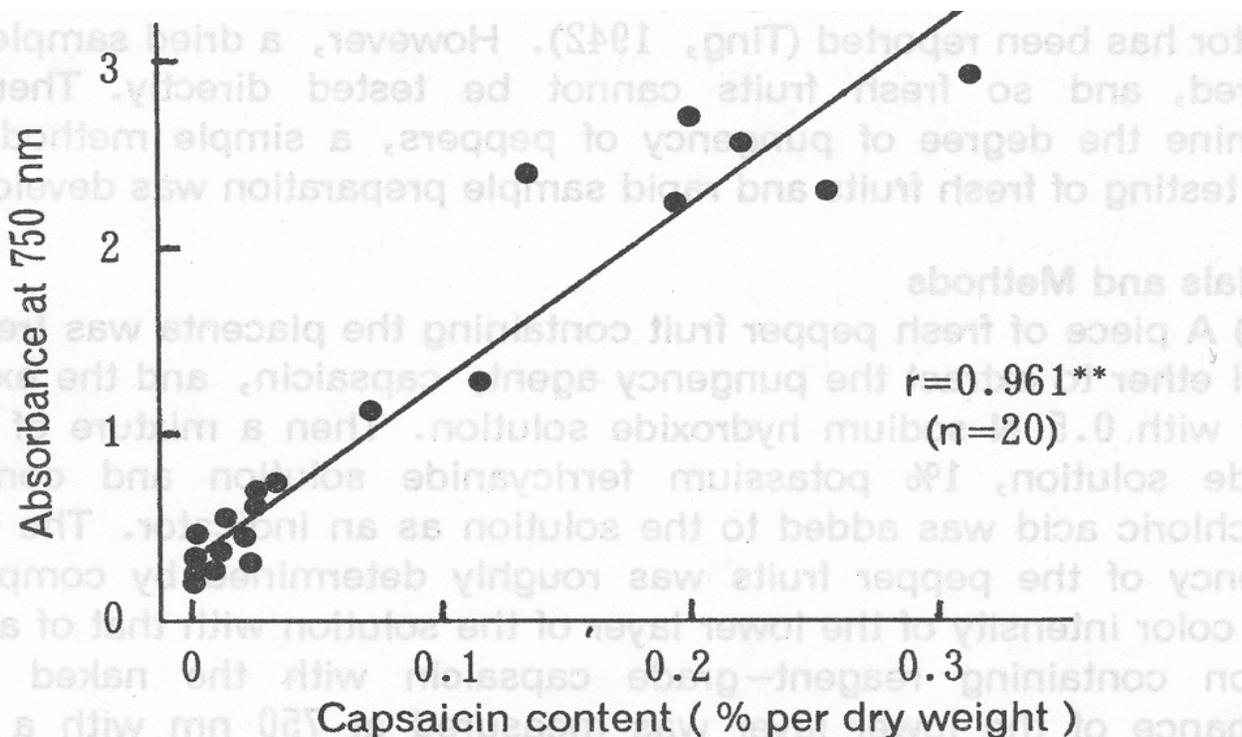


Figure 1. Correlation between absorbance and capsaicin content.  
The solution was prepared by the method shown in Scheme 1

Place a section of fresh fruit including the placenta (about 1 g) into a test tube.

After adding 5 ml of diethyl ether, mix for about 10 s with a lab. mixer.

- Transfer 1 ml of diethyl ether extract solution to another test tube.

After adding 1 ml of reagent A, mix for about 10 s using a lab. mixer.

After adding 3 ml of reagent B, mix for about 10 s using a lab. mixer.

Evaluate the degree of pungency by comparing the intensity of the green color of the lower layer with that of the standard solution with the naked eye. ' Scheme 1. A simple method for determining the degree of pungency of peppers.

Reagent A : Dissolve 2 g of sodium hydroxide and 2 g of sodium chloride in 100 ml of water.

Reagent B : Solution 1 (dissolve 1 g of ferric chloride in 100 ml of water), solution 2 (dissolve 1 g of potassium ferricyanide in 100 ml of water) and concentrated hydrochloric acid are mixed equivalently. The indicator is relatively unstable and it is undoubtedly best to make it up fresh just before the test.

Standard solution: One milliliter of 0.001% capsaicin in reagent A and 3 ml of reagent B are mixed. The solution is relatively unstable and it is undoubtedly best to make it up fresh just before the test.

(2) The green color intensity prepared by the method shown in Scheme 1 and the degree of pungency determined by tasting for the F 2 population are shown in Table 1. The green color of non- pungent plants was lighter than that of the standard solution, whereas the color of highly pungent plants was darker. These results indicate that this method can be applied for selecting sweet plants from segregating populations between sweet and hot peppers.

Table 1. Relation between the degree of pungency and the intensity of the color of the lower layer in two F z populations. .

Degree of pungency	Number of plants			
	Test 1		Test2	
	Lighter	Darker	Lighter	Darker
None	14	0	34	0
Very low or faint	26	23	16	28
Moderate	4	34	1	25
High	0	12	0	9

. Sample solutions were prepared by the method shown in Scheme 1. The tasting panel included seven persons.

Y 113 plants of the Fz population between' Shin-sakigake 2' (sweet, pepper) and' CB17 - 2- 2- 1' (hot pepper) were used for Test 1, and 113 plants of the F z population between' Shin-sakigake 2' (sweet pep per) and' CH 4- 4- 3 ' (hot pepper) were used for Test 2.

x The intensity of the green color of the lower layer was lighter than that of the standard solution.

W The intensity of the green color of the lower layer was darker than that of the standard solution.

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## SCREENING OF CHILLI CULTIVARS AND ACCESSIONS FOR RESISTANCE TO CUCUMBER MOSAIC VIRUS AND POTATO VIRUS Y

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Key Words: Screening, accessions, Chilli, mosaic

### Introduction

Chilli (*Capsicum annum* L.) is a common vegetable crop in India. Its cultivation is severely affected by many viruses including cucumber mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus and leaf curl (1,3,4,5) which have potential to cause heavy yield losses. It was therefore, decided to screen out accessions/cultivars resistant to CMV and PVY.

### Materials and Methods

#### a) Screening under natural conditions

Forty-six accessions were transplanted on ridges in the month of July each year 1992-94. Two 5 m rows of each accession (3 replicates) were transplanted 60 cm apart with a distance of 45 cm between plants. For high inoculum build up, a row of highly susceptible cultivar Pusa Jwala' was planted between every two rows of the test material and around the edges of the trial. Observations on disease incidence were recorded regularly at 10 days interval until crop maturity.

#### b) Screening under artificial conditions

Two batches of 25 seedlings each of the nineteen accessions rated as highly resistant and moderately resistant on the basis of field response were transplanted in pots at 2-leaf stage. The pots were kept in the green house, under insect proof conditions and inoculated mechanically, after the plants had established well. One batch was inoculated with CMV and the other with PVY using carborundum (600 mesh) as abrasive. The supernatants (centrifuged 6000 rpm, 15 mins) of the saps prepared in 0.2 M phosphate buffer (pH 7.0), using young leaves of chilli plants showing bright symptoms of CMV and PVY individually were used as inoculum.

#### c) Serological testing

OAC-ELISA using CMV and PVY antisera was done (2) to confirm the presence of CMV/PVY in the artificially inoculated plants. Sampling was done by taking one top leaf per plant from each of 25 plants comprising one batch. The observations were based on visual colour changes.

#### d) Reaction category

On the basis of percent disease incidence, the cultivars/accessions were given the following grading:

<u>Reaction</u>	<u>Disease incidence (%)</u>
<b>Highly resistant</b>	<b>0.0</b>
Moderately resistant	Up to 10
Moderately susceptible	11-20
Susceptible	21-50
Highly susceptible	51 and above

## Results

On the basis of per cent disease incidence in field trials (1992-94), eight genotype viz., I HC-1-1', 'HC-151', 'HC-22', 'HC-28', 'HC-691', 'HC-226', 'Pusa Sadabahar' and 'Virus Free-11 were found to be highly resistant both under field conditions and artificial inoculation with CMV and PVY (Table). These accessions/cultivars were negative for the presence of CMV or PVY by ELISA testing. Out of the eleven accessions which were categorized moderately resistant under natural conditions, for remained so to CMV and PVY after artificial inoculation.

## Discussion

Studies pertaining to reaction of different genotypes of *Capsicum annuum* L. to CMV, TMV and PVY have been carried out by many workers (4, 6, 7). During the present studies, seven accessions/cultivars found highly resistant have the potential of being used in resistance breeding programme. Two accessions viz., I HC-28' and 'HC-44' possessing multiple disease resistance and good agronomic traits with a yield potential of 17.9 and 18.7 tonnes ha<sup>-1</sup> respectively, have been released by Haryana Agricultural University, Hisar as varieties 'Hisar Vijay' and 'Hisar Shakti' for cultivation.

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Table. Reaction of Capsicum annum. L. germplasm to mosaic disease.

Disease Reaction	Germplasm		
	Natural Field condition	Artificial Inoculation Conditions	
		CMV	PVY
Highly resistant	HC1-1, HC-15, HC-22, HC28 HC-69, HC226, Virus Free 1, Pusa Sadabhar	HC1-1, HC-15, HC-22, HC28 HC-69, HC226, Virus Free 1, Pusa Sadabhar	HC1-1, HC-15, HC-22, HC28 HC-69, HC226, Virus Free 1, Pusa Sadabhar
Moderately resistant	H- 13, HC-44 ,HC-46-2, HC-47, HC-58, HC-71, HC-74-1, HC-102-1, HC-109-2, HC-250, HC-174-1	HC-44 HC-58, HC-109-2, HC-174-1, HC-250,	HC-44, HC-58, HC-109-2, HC-250 , HC-46-2
Moderately susceptible	HC-4, HC-6, HC-12, HC-42-3, HC-46, HC-48, HC-51, HC-79-2, HC-164, P-47	HC-47, HC-71, HC-74-1, HC-102-1	HC-47, HC-71, HC-74-1, HC-102-1, HC174-1
Susceptible	HC-20-5, HC-29, HC,102, HC-259, P27		
Highly susceptible	HC-8-2, HC-17-1, HC-17-3, HC-27, HC-27-1, HC-50-1, HC-70, P-14, P-16, P20,P52		

Data based on average of three years (1992-94)

Reaction based on visual symptoms

Reaction based on ELISA test.

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## **SCREENING OF *CAPSICUM CHACOENSE* ACCESSIONS FOR TSWV RESISTANCE BY MECHANICAL INOCULATION**

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Genetic resistance to TSWV has been reported in several species of *Capsicum*: *C. chinense* (Black *et al.*, 1991; Boiteux *et al.*, 1993), *C. frutescens* (Diez *et al.*, 1993), *C. baccatum* (Boiteux *et al.*, 1993; Gil, 1993), *C. pubescens* (Diez *et al.*, 1993; Nuez *et al.*, 1994). Nevertheless, the authors have not found reports on TSWV resistance in *C. chacoense*.

This paper shows the response of eight *C. chacoense* accessions to mechanical inoculation with TSWV. The trials were carried out in a climatic room at 28/18 °c, (day/night) temperature, 70-90% of relative humidity, 65-80 ~mol m<sup>-2</sup>s<sup>-1</sup> of irradiance and 14 hours of photoperiod. The inoculum L-93940 (Jorda *et al.*, 1994) was used. The plants were inoculated at the 4-6-leaf stage and a second inoculation was repeated a week afterwards. *C. annuum* cv. 'Negral' was used as susceptible control. The plants were maintained in the climatic room for 60 days after inoculation. Symptoms were noted and samples for ELISA analysis were taken each 10-15 days. Later, the resistant plants were transplanted to greenhouse and the evolution was followed for 20 days. Plants with evident TSWV symptoms and/or ELISA positive were considered as infected.

The first symptoms in control plants appeared as necrotic lesions, these plants became wilt later, dying 20 days after inoculation. Accessions C-152 and C-279 were highly susceptible, all of the plants dying 15 days after first inoculation. Local lesions appeared after inoculation on the other accessions. From 2 to 4 plants of accessions C-151, C-154, C-175 and C-176 became infected, showing a delay in symptoms appearance and a heterogeneous behaviour (Table 1). Accessions C-153 and C-280 showed a higher level of resistance. Necrotic lesions and apical death appeared in one single plant of C-153 after inoculation, but it regrew and developed a new bud, which showed no symptoms and was found to be ELISA negative. Only one plant of accession C-280 showed TSWV symptoms and died before the end of the trial.

The previous results suggest that the resistance found in *C. chacoense* C-153 can be interesting for its introgression in *C. annuum*. The response of these accessions to TSWV inoculation by *Frankliniella occidentalis* Perg. is currently in course.

Table 1. Number of infected plants and symptoms observed in eight accessions of *C. chacoense* mechanically inoculated with TSWV.

Accession	infected plants/total plants	Symptoms
C-151	4/7	NA, NS, NL, W
C-152	7/7	NA, NS, W
C-153	0/9	NA-+Regr
C-154	4/8	NA-+Regr-+W
C-175	4/7	Nt, NA-+Regr
C-176	2/8	NA, NA-+Regr
C-279	8/8	NA, NS -+W
C-280	1/7	NS-+W

**NA:** Necrotic lesions on the apex.

**NL:** Necrotic lesions on the leaves. **NS:** Necrotic lesions on the stem. **W:** Wilt.

**Regr:** Regrew.

**-+:** Evolution of symptoms.

#### ACKNOWLEDGEMENTS

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## **INHERITANCE OF RESISTANCE TO PHYTOPHTHORA BLIGHT IN HOT PEPPER**

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**Abstract** Genetic analysis of resistance to *Phytophthora capsici* in hot pepper (*Capsicum annuum* L.) with 4 X 4 half-diallel cross revealed the importance of both additive and dominant component in the inheritance of the disease index character. However, dominant component was higher than additive one. The mean degree of dominance indicated overdominance for the disease index character. To exploit both the additive and dominant genetic component, the use of biparental mating in early generation among the selected lines and use of heterosis breeding method are possible in breeding for resistance to *Phytophthora capsici*.

### **Introduction**

Disease is a major factor limiting the production of hot pepper in China, and Phytophthora blight caused by *Phytophthora capsici* is one of the most serious problems. It is common all over the country and severely causes pepper yield loss. Presently, the disease is controlled by routine fungicide applications that have limited success. Primary infections also can be reduced by using well-drained soil, providing good weed control, and following crop rotations that exclude susceptible plants. Currently, no Phytophthora blight-resistant pepper cultivars are available in China. However, some researchers have screened pepper germplasm for sources of Phytophthora blight resistance in China. The inheritance of Phytophthora blight resistance was reported to be genetic diversity (Wang *et al*, 1995). Our research was designed to study the inheritance of resistance to Phytophthora blight in hot pepper breeding lines.

### **Materials and Methods**

Four homozygous hot pepper genotypes were selected, representing different levels of resistance to *Phytophthora capsici*. Given in order of increasing resistance, the following three lines and one cultivar were selected: 408, E9, F21 - 1 and 8212. All possible crosses were made among the 4 genotypes, excluding reciprocals. All the 6 F<sub>1</sub>s along with 4 parents were raised in greenhouse in completely randomized block design with two replications; each plot consisted of 6 culture pans, 2 plants per culture pan.

Screening for resistance to *Phytophthora capsici* was conducted in the greenhouse at 25 to 28°C. When the sixth true leaf were expanded. The plants were inoculated by soil-drench method with inoculation suspension of 2,000 zoospores per milliliter, one milliliter per plant.

Reaction of plants was scored on a 0 to 9 scale (Wang *et al*, 1995) for disease severity seven days after inoculation. Then the disease index was determined. The genetic analysis of disease index was carried out using the method of Singh *et al* (1981) and Virk *et al* (1983).

### **Results and Discussion**

The results of analysis of variance for disease index revealed significant difference among parents and hybrids. The *f* estimate to test the uniformity of the  $W_r$ ,  $V_r$  values were not significant, and the regression coefficient of  $W_r$  to  $V_r$  was 0.9245.

Compared with 1, and is not significant. Indicating fulfillment of diallel assumptions. The estimates of genetic parameters for disease index character was given in Table 1, which confirmed the importance of additive as well as dominant gene action.

**Table 1 Estimates of genetic parameters of the disease index character**

Genetic parameters	Estimates	Genetic parameters	Estimates
D	75.56	(H <sub>1</sub> /D)T	1.678
F	24.18	H <sub>2</sub> /4H <sub>1</sub>	0.231
H <sub>1</sub>	212.91	K=h <sup>2</sup> /H <sub>2</sub>	0.532
H <sup>2</sup>	196.32	[(4DH <sub>1</sub> )T+F]/[(4DHI)T-F]	1.211
h <sub>2</sub>	104.52	r. (W <sub>r</sub> + V <sub>r</sub> )Y <sub>r</sub>	0.7917
t <sub>2</sub>	3.06	Broad heritability (%)	73.18
		Narrow heritability (%)	29.94

Analysis of genetic components showed that dominance component was larger than additive component. H<sub>1</sub> greater than D inferred that manifestation of disease index character is mainly governed by dominant gene action. The value of (H<sub>1</sub>/D) t was bigger than 1. Indicating the operation of over dominance. The positive correlation between the mean values of the parents Y<sub>r</sub> and the order of dominance Y(W<sub>r</sub>+V<sub>r</sub>) suggested that the dominant genes were associated with low mean expression.

The H<sub>z</sub>

Component was smaller than H<sub>1</sub>. Indicating the unequal proportion of positive and negative alleles in the loci governing the character. The asymmetrical distribution of genes in the parents was evidenced by the value of H<sub>z</sub>/4H<sub>1</sub>, which was less than 0.25. F value was positive, indicating that dominant alleles were more frequent than recessive ones in the parents. This was also corroborated by the value of [(4DH<sub>1</sub>) T+F]/[(4DH<sub>1</sub>) T- F]. The number of blocks of genes influencing the disease index character was one as revealed by the h<sub>z</sub> I H<sub>z</sub> value. Heritability in broad, narrow sense was 73.18%, 29.94%, respectively. H/H<sub>i</sub> value was 409, which clearly showed that additive gene effect accounted for 40.9% of total genetic effect.

In the present study, since both additive and dominant genetic components were important in governing the disease index character, biparental mating in early generation among the selected lines can be adopted in breeding programmes for the improvement of resistance to *Phytophthora capsici*. Thinking about the value and direction of dominant gene effect in gene system of the disease index character, it is suggested that breeding for resistance to *Phytophthora capsici* should be carried out by heterosis breeding method.

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## **REACTION OF ASIAN GERMPLASM OF PEPPER (*CAPSICUM ANNUUM* L.) TO ITS ASIAN POPULATIONS OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)**

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**SUMMARY.** The reaction of accession of *Capsicum annuum* to four Italian populations of *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* were evaluated in a glass-house at 27 :t2°C. Groups of seven plants of each line were transplanted in plastic trays with 10 dln3 of steam-sterilized sandy soil artificially infested with 10,000 eggs and juveniles of each nematode population per plant. All accessions from Indonesia, Malaysia, Philippines, Taiwan, Thailand and three accessions from France were resistant to *M. incognita*, *M. javanica* and *M. arenaria*. The accessions C 00602 "Black Cluster" and CO1171 "PM 687" were resistant to *M. incognita* and *M. javanica* but susceptible to *M. arenaria*. All accessions tested were susceptible to *M. hapla*.

The root-knot nematodes *Meloidogyne incognita* (Kofoid et White) Chitwood, *M. javanica* (Treub) Chitw., *M. arenaria* (Neal) Chitw. and *M. hapla* Chitw., cause severe damage to pepper (*Capsicum annuum* L.) in greenhouses and outdoors in sandy soil (Di Vito et al., 1985). The control of these nematodes on pepper is based mainly on the use of nematicides, but this control is expensive and may cause pollution. Few pepper varieties are known to be resistant to only *M. javanica* (Di Vito et al., 1991). Therefore, an I; experiment was undertaken for evaluating the reaction of some accessions of mostly an

Asian germplasm collection of pepper, to Italian populations of *M eloidogyne* spp.

## **MATERIALS AND METHODS**

Nineteen accessions of *C. annuum* (Tab. 1), from the genoplasm collection of the Asian Vegetable Research and Development Center of Taiwan (A VRDC), were sown in plastic trays containing steam sterilized sandy soil. These accessions had previously been evaluated as resistant to a Taiwanese population of *M. incognita* by the AVRDC (1989). At the two-leaf stage, four sets of seven seedlings of each accession were transplanted in other trays with the same substrate. A week later the plants were inoculated with 10,000 eggs and juveniles per plant of one Italian each per set. The populations used as inoculum were: *M. incognita* host race 1 (Di Vito and Cianciotta, 1991) from Castellaneta (Apulia), *M. javanica* from Torchiarolo (Apulia), *M. arenaria* host race 2 from Verona (Veneto) and *M. hapla* from Ferrara (Emilia Romagna). All nematode populations had been rearely extracted from infested roots by using the NaOCI method (Hussey and Barker, 1973). Seven seedlings of pepper "Como di Toro Rosso", for each nematode population, were used as a susceptible control. All plastic trays were randomly arranged on benches in a glass-house maintained at 27:t2°C. Forty days after inoculation the plants were uprooted, the roots gently washed and galls and egg masses counted after staining by dipping the roots in a 0.015% Phloxin B solution for 15 min (Dickson and Ben Struble, 1965). Gall (GI) and egg mass index (EI) was then assessed according to a 0-5 scale, where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 =

more than 100 galls or egg masses (Taylor and Sasser, 1978). The data were then statistically analyzed by ANOVA and LSD's calculated. A pepper plant was considered resistant when the GI and EI was  $\leq 2$ .

## RESULTS AND DISCUSSION

All accessions from Indonesia, Malaysia, Philippines, Taiwan, Thailand and the 10 accessions "Serrano VC (pM 164)", "Criollo de Morelos 334 (pM 702)" and "PI 201234" from INRA, France were resistant to *AI. incognita*, *M. javanica* and *M. arenaria* (Tab. 1- 2). The accessions "Black Cluster" (Hungary) and "PM 687" (France) were resistant to *M. incognita* and *M. javanica* but susceptible to *AI. arenaria*. All germplasm tested, including the control "Como di Toro Rosso", was susceptible to *M. hapla*. The results obtained with this experiment are interesting because we have confirmed new sources of resistance to *M. incognita*, *M. javanica* and *M. arenaria*, which can be useful for future breeding programmes to introduce resistance to root-knot nematodes in pepper. The inheritance of these sources of resistance is not known. More studies are needed, therefore, to understand the genetics of this character; and further screening is necessary to find sources of resistance to *M. hapla*.

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TABLE 1. Gall index and reaction type of 19 accessions of *Capsicum annuum* inoculated with four species of root-knot nematodes (*Meloidogyne* spp.) in glass-house.

Accession and cultivar	Name	Origin	Gall index (GI)(0 - 5) and reaction type (RT)									
			<i>M. incognita</i>		<i>M. javanica</i>		<i>M. arenaria</i>		<i>M. hapla</i>			
			GI*	RT**	GI	RT	GI	RT	GI	RT		
C 00573	Pangalegan-1	Indonesia	0	R	0	R	0	R	0	R	4.4	S
C 00104-1	MC 4	Malaysia	0	R	0	R	0	R	0	R	3.6	S
C 00543	Unnamed	Philippines	0	R	0	R	0	R	0	R	4.4	S
C 00490	Hot Pepper	Taiwan	0	R	0	R	0	R	0	R	3.3	S
C 00466	L.P. 2	Thailand	0.4	R	0	R	0	R	0	R	3.6	S
C 00474	L.P. 1	"	0	R	0	R	0	R	0	R	4.6	S
C 00476	Kralee Kao	"	0	R	0	R	0	R	0	R	3.5	S
C 00550	Unknown 5	"	0	R	0	R	0	R	0	R	4.5	S
C 00551	Unknown 6	"	0	R	0	R	0	R	0	R	4.8	S
C 00561	Unknown 16	"	0	R	0	R	0	R	0	R	3.4	S
C 00563	Unknown 18	"	0	R	0	R	0	R	0	R	4	S
C 00584	Unknown 30	"	0	R	0	R	0	R	0	R	4.6	S
C 00585	Unknown 31	"	0.9	R	0	R	0	R	0	R	3.1	S
C 00590	Unknown 36	"	0	R	0	R	0	R	0	R	5	S
C 00602	Black Cluster	Hungary	0.3	R	0	R	0	R	4.1	S	4.9	S
C 01171	PM 687	France-INRA	0.1	R	0	R	0	R	3.6	S	4.1	S
C 01173	PM 164	"	0	R	0	R	0	R	0.6	R	4	S
C 01175	PM 702	"	0	R	0	R	0	R	1.5	R	4	S
C 01176-A	PI 201234	"	0	R	0	R	0	R	0	R	4	S
Corno di Toro Rosso		(Check) Italy	4.8	S	4.4	S	4.7	S	4.7	S	5	S
LSD			0.42		0.11		0.36		0.57			
P < 0.01			0.56		0.14		0.47		0.76			

\* 0 = 0 gall; 1=1-2 galls; 2=3-10; 3=11-30; 4=31-100; and 5=more than 100 galls.

\*\* R = Resistant, gall index ≤ 2; and S = Susceptible, gall index > 2.

TABLE 2. Egg mass index and reaction type of 19 accessions of *Capsicum annuum* inoculated with four species of root-knot nematodes (*Meloidogyne* spp.) in glass-house.

Accession and cultivar	Name	Origin	Egg mass index (EI)(0 - 5) and reaction type (RT)											
			<i>M. incognita</i>		<i>M. javanica</i>		<i>M. arenaria</i>		<i>M. hapla</i>					
			EI*	RT**	EI	RT	EI	RT	EI	RT	EI	RT		
C 00573	Pangalegan-1	Indonesia	0.4	R	0.1	R	0.4	R	0.4	R	4.6	S		
C 00104-1	MC 4	Malaysia	0	R	0	R	0.2	R	0.2	R	4.4	S		
C 00543	Unnamed	Philippines	0	R	0.3	R	0.4	R	0.4	R	4.6	S		
C 00490	Hot Pepper	Taiwan	0.5	R	0.3	R	0.6	R	0.6	R	4.3	S		
C 00466	L.P. 2	Thailand	0.4	R	0.4	R	0	R	0	R	4	S		
C 00474	L.P. 1	"	0.3	R	0.3	R	0.3	R	0.3	R	4.9	S		
C 00476	Kralee Kao	"	0.4	R	0.2	R	0	R	0	R	4	S		
C 00550	Unknown 5	"	0.4	R	0.2	R	0.4	R	0.4	R	4.7	S		
C 00551	Unknown 6	"	0.3	R	0.2	R	0.2	R	0.2	R	4.8	S		
C 00561	Unknown 16	"	0	R	0.3	R	0	R	0	R	4.6	S		
C 00563	Unknown 18	"	0	R	0.3	R	0.3	R	0.3	R	4.6	S		
C 00584	Unknown 30	"	0	R	0	R	0.1	R	0.1	R	4.9	S		
C 00585	Unknown 31	"	1	R	0	R	0	R	0	R	4.7	S		
C 00590	Unknown 36	"	0.1	R	0	R	0.3	R	0.3	R	4.9	S		
C 00602	Black Cluster	Hungary	0.7	R	0	R	4.6	S	4.6	S	4.9	S		
C 01171	PM 687	France-INRA	0.6	R	0.4	R	4.3	S	4.3	S	4.7	S		
C 01173	PM 164	"	0	R	0.4	R	0.9	R	0.9	R	4.6	S		
C 01175	PM 702	"	0.3	R	0.7	R	1.6	R	1.6	R	4.6	S		
C 01176-A	PI 201234	"	0.2	R	0.6	R	0.2	R	0.2	R	4	S		
Como di Toro Rosso		(Check) Italy	5	S	4.8	S	5	S	5	S	5	S		
SD	P ≤ 0.05		0.54		0.43		0.51		0.51		0.49			
	P ≤ 0.01		0.71		0.57		0.88		0.88		0.64			

\* 0 = 0 egg mass; 1=1-2 egg masses; 2=3-10; 3=11-30; 4=31-100; and 5 = more than 100 egg masses.  
 \*\* R = Resistant, egg mass index ≤ 2; and S = Susceptible, egg mass index > 2.

FIELD EVALUATION OF BRINJAL VARIETIES AGAINST BACTERIAL WILT ( Pseudomonas solanacearum E. F. smith.) N.K.Pathania, Yudhvir Singh, P.Kalia and A.Khar Deptt. of Vegetable Science and Floriculture, HPKV, Palampur-176 062 (INDIA)

The bacterial wilt caused by Pseudomonas solanacearum smith. limits brinjal cultivation in humid tropical, subtropical and warm temperate areas of the world (Kelman, 1953). In India this disease has become a serious problem (Gowda et al. 1974). Of late, it has become a major bottleneck in successful cultivation of brinjal during Kharif in humid areas of Himachal Pradesh. Since the bacterium is soil borne, its chemical control through soil treatment is both cumbersome and uneconomical (Madalageri et al., 1983). That is why, breeding varieties for bacterial wilt resistance combined with high yields and acceptable quality is the present day need. Therefore, a study on all the bacterial wilt resistant varieties of brinjal developed in India was undertaken at HPKV, Palampur so as to identify a stable genotype possessing bacterial wilt resistance and other desirable horticultural attributes.

#### MATERIAL AND METHODS

Fourteen varieties of brinjal including 'Pusa Purple Long' (susceptible check) were grown in randomized block design with 3 replications. The disease intensity was recorded under natural epidemic field conditions. The wilting of susceptible check indicated the presence of virulent inoculum. The disease rating was done as per scale suggested by Mew and Ho (1976).

Resistant (R) : <20% wilting  
Moderately Resistant (MR) : 20 to 40% wilting  
Moderately Susceptible (MS) : 41 to 60% wilting  
Susceptible (S) : > 60 % wilting

Data on plant survival was recorded after 30, 60 and 90 days of transplanting. Observations were also recorded on number of fruits per plant and yield.

#### RESULTS AND DISCUSSION

Highly significant differences were observed among the different varieties for bacterial wilt incidence, number of fruits per plant and yield (Table 1&2).

Table 1: Bacterial wilt incidence (%) in brinjal varieties after 30, 60 and 90 days of transplanting

Variety	Bacterial wilt incidence (%) after		
	30 days	60 days	90 days
Arka Neelkanth	0.00	0.00	0.00
SM 6-7	0.00	8.33	8.33
Pant Rituraj	26.67	100.00	100.00
Arka Nidhi	1.67	1.67	1.67
Pant Samrat	28.33	98.33	100.00
Arka Keshav	0.00	0.00	0.00
HOE 444	38.33	96.67	100.00
SM 6-6	0.00	8.33	8.33
APAU Sel.1	53.33	100.00	100.00
H-8	5.00	30.00	30.00
Pusa Purple Long	81.67	100.00	100.00
Pusa Purple Cluster	1.83	10.17	10.17
BB-7	0.00	56.67	59.31
BB-44	0.00	13.33	16.67
S.E.	5.90	7.67	8.58
C.D.* (0.05)	12.13	15.77	13.54

\*C.D. was calculated for angular transformed data

Table 2: Mean performance and disease reaction of brinjal varieties under humid subtemperate conditions

Variety	Number of fruits per plant	Yield (q/ha)	Disease Reaction
Arka Neelkanth	12.63	136.41	R
SM 6-7	9.31	137.65	R
Pant Rituraj*	-	-	S
Arka Nidhi	19.85	176.38	R
Pant Samrat*	-	-	S
Arka Keshav	8.10	66.82	R
HOE 444*	-	-	S
SM 6-6	18.71	147.06	R
APAU Sel. 1*	-	-	S
H-8	4.75	120.83	MR
Pusa Purple Long*	-	-	S
Pusa Purple Cluster	25.31	170.52	R
BB-7	2.14	11.88	MS
BB-44	10.45	147.99	R
S.E.	1.51	1.37	
C.D. (0.05)	3.19	2.90	

Perusal of Table 1 indicates that 'Arka Neelkanth' and 'Arka Keshav' showed 100 per cent resistance to bacterial wilt whereas 'Pant Rituraj', 'Pant Samrat', 'HOE 444', 'APAU Sel.I' and 'Pusa Purple Long' (susceptible check) recorded 100 percent susceptibility. There was marginal increase in wilt incidence after 60 days of transplanting. High incidence of this disease after 60 days of transplanting could be due to the prevalence of higher temperature, humidity and rainfall during July. Bacterial wilt resistant genotypes viz. 'HOE 444' and 'BB-7' recorded 100 and 59.31 per cent wilt incidence, respectively, indicating the prevalence of different races. Seven varieties were observed to be resistant and five as susceptible. 'H-8' and 'BB-7' were observed to be moderately resistant and moderately susceptible, respectively.

Perusal of Table-2 indicates that 'Arka Nidhi' recorded the highest yield (176.38 q/ha) followed by 'Pusa Purple Cluster' (170.52 q/ha). 'Pusa Purple Cluster' (25.31) recorded highest number of marketable fruits per plant followed by 'Arka Nidhi' (19.85) and 'SM 6-6' (18.71). Among round types, 'H-8' has been found to be promising genotype having desirable horticultural traits along with moderate resistance to bacterial wilt. 'SM 6-7', a collection from Kerala Agricultural University was also observed to be potential genotype by virtue of its high resistance to bacterial wilt and reddish purple oblong fruits. The highly resistant varieties viz. 'Arka Neelkanth' and 'Arka Keshav' were found to be poor yielder. These can be utilised in hybridisation programme for transferring resistance and other desirable horticultural traits (like dark purple colour) in otherwise recommended susceptible varieties.

Keeping in view the bacterial wilt resistance, fruit colour and other desirable traits, 'Arka Nidhi' and 'Pusa Purple Cluster' among long types, 'H-8' and 'SM 6-7' among round and oblong types, respectively appear to be promising for varietal improvement and for cultivation in wilt prone areas of western Himalayas.

#### SUMMARY

Fourteen varieties of brinjal including 'Pusa Purple Long' (susceptible check) were grown in randomised block design with 3 replications at a spacing of 60cm \* 45cm in uniformly wilt sick soil. Highly significant differences were observed among the varieties for bacterial wilt incidence, number of fruits per plant and yield. Seven varieties were observed to be resistant and five as susceptible. 'H-8'

and 'BB-7' were observed to be moderately resistant and moderately susceptible, respectively. 'Arka Nidhi' recorded the highest yield (176.38 quintals per hectare) followed by 'Pusa Purple Cluster' (170.52 quintals per hectare). Maximum number of fruits per plant were recorded in 'Pusa Purple Cluster' (25.31) followed by 'Arka Nidhi' (19.85) and 'SM 6-6' (18.71). Among long varieties, 'Pusa Purple Cluster' and 'Arka Nidhi' (purple fruited) seem to be promising for cultivation in wilt prone areas of western Himalayas. 'H-8' (round fruited) and 'SM 6-7' (oblong fruited) " also offer a promise.

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## EVALUATION OF RESISTANCE TO BACTERIAL, FUSARIUM AND VERTICILLIUM WILT IN EGGPLANT AND EGGPLANT-RELATED SPECIES COLLECTED IN GHANA

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Nine accessions of eggplant, *S. melongena* and 62 accessions of eggplant-related species, *s. gila*, *S. aethiopicum*, *s. integrifolium*, *S. macrocarpon* and *s. anguifolium* collected in Ghana were used for the evaluation of resistance to bacterial, fusarium and verticillium wilt. Inoculations were conducted by dipping roots of seedlings into bacterial or spore suspensions of the diseases. Seedlings were then planted to a bed whose temperature was maintained at 30°C for bacterial wilt, 28°C for fusarium wilt and 22°C for verticillium wilt. Disease severity of each plant was evaluated using a symptom index of 0 (no symptom)-4 (death) scale at about one month after inoculation. Ten plants were used for bacterial wilt, 20 plants for fusarium and verticillium wilt.

Disease indices and percentage of diseased plants of eggplant and eggplant-related species accessions inoculated with bacterial wilt were 2.60-4.00 and 75.0-100.0%, respectively. The disease index of 'GJ93/042' of *S. macrocarpon* was the lowest, but finally all plants of the accession died of the disease. There were no bacterial wilt-resistant accessions in eggplant and eggplant-related species.

All eggplant accessions inoculated with fusarium wilt showed a high disease index and high percent of diseased plants, and were susceptible to fusarium wilt, whereas there were no diseased plants in the accessions of eggplant-related species showing high resistance to fusarium wilt.

Percentage of diseased plants of all eggplant accessions inoculated with verticillium wilt were 100.0% and disease indices were 3.70-4.00, and none of the eggplant accessions showed resistance to verticillium wilt. Disease indices and percentage of diseased plants of eggplant-related species accessions inoculated with verticillium wilt were 2.55-4.00 and 90.0-100.0%, respectively. Disease indices of 'GJ93/040' and 'GJ93/090' in *S. gilo* and 'GJ/094' in *S. aethiopicum* were lower than those of the other accessions. This suggests that these accessions may have some degree of ~ resistance, but the disease indices of the accessions were higher than the resistant control cultivars, 'Meet' or 'Taiby VF', which are used practically as rootstocks in Japan.

Table 1. Reaction of eggplant and eggplant-related species to bacterial, fusarium and verticillium wilt.

Accession	Bacterial wilt		Fusarium wilt		Verticillium wilt		Accession	Bacterial wilt		Fusarium wilt		Verticillium wilt	
	DI <sup>y</sup>	PD <sup>a</sup>	DI <sup>y</sup>	PD <sup>a</sup>	DI <sup>y</sup>	PD <sup>a</sup>		DI <sup>y</sup>	PD <sup>a</sup>	DI <sup>y</sup>	PD <sup>a</sup>	DI <sup>y</sup>	PD <sup>a</sup>
<i>S. melongena</i>													
	%		%		%			%		%		%	
1 GJ93/033	3.20	80.0	3.95	100.0	4.00	100.0	41 GJ93/173	3.50	90.0	0.00	0.0	3.75	100.0
2 GJ93/068	3.30	90.0	4.00	100.0	3.70	100.0	42 GJ93/175	4.00	100.0	0.00	0.0	3.75	100.0
3 GJ93/091	3.60	100.0	3.75	100.0	4.00	100.0	43 GJ93/186	4.00	100.0	0.00	0.0	3.90	100.0
4 GJ93/092	3.70	100.0	4.00	100.0	4.00	100.0	44 GJ93/189			0.00	0.0		
5 GJ93/108	3.30	90.0	4.00	100.0	4.00	100.0	45 GJ93/191	4.00	100.0	0.00	0.0	3.78	100.0
6 GJ93/121	4.00	100.0	3.62	90.5	4.00	100.0	46 GJ93/198	4.00	100.0	0.00	0.0	3.80	100.0
7 GJ93/137	3.40	100.0	4.00	100.0	4.00	100.0	47 GJ93/206	4.00	100.0	0.00	0.0	3.60	100.0
8 GJ93/226	3.90	100.0	4.00	100.0	4.00	100.0	48 GJ93/210	4.00	100.0	0.00	0.0	3.95	100.0
9 GJ93/261	3.60	90.0	4.00	100.0	3.90	100.0	49 GJ93/220	4.00	100.0	0.00	0.0	4.00	100.0
<i>S. gilo</i>							50 GJ93/221	4.00	100.0	0.00	0.0	3.90	100.0
10 GJ93/007	3.70	100.0	0.00	0.0	3.70	100.0	51 GJ93/241	4.00	100.0	0.00	0.0	3.90	100.0
11 GJ93/024	4.00	100.0	0.00	0.0	3.75	100.0	52 GJ93/305	4.00	100.0	0.00	0.0	3.29	92.9
12 GJ93/040	3.60	90.0	0.00	0.0	2.75	95.0	<i>S. aethiopicum</i>						
13 GJ93/049	3.60	90.0	0.00	0.0	3.85	100.0	53 GJ93/058)	4.00	100.0	0.00	0.0	3.46	100.0
14 GJ93/057	4.00	100.0	0.00	0.0	2.80	95.0	54 GJ93/062	3.80	100.0	0.00	0.0	3.19	100.0
15 GJ93/063	4.00	100.0	0.00	0.0	3.48	100.0	55 GJ93/077	4.00	100.0	0.00	0.0	3.80	100.0
16 GJ93/071	4.00	100.0	0.00	0.0	3.65	100.0	56 GJ93/094	4.00	100.0	0.00	0.0	2.55	100.0
17 GJ93/072	4.00	100.0	0.00	0.0	3.30	100.0	57 GJ93/124	4.00	100.0	0.00	0.0	3.95	100.0
18 GJ93/085	4.00	100.0	0.00	0.0	3.70	100.0	58 GJ93/131	4.00	100.0	0.00	0.0	3.70	95.0
19 GJ93/086	4.00	100.0	0.00	0.0	3.96	100.0	59 GJ93/153	4.00	100.0	0.00	0.0	4.00	100.0
20 GJ93/089			0.00	0.0	4.00	100.0	60 GJ93/154	4.00	100.0	0.00	0.0	3.85	100.0
21 GJ93/093	4.00	100.0	0.00	0.0	2.75	90.0	61 GJ93/155	4.00	100.0	0.00	0.0	3.80	100.0
22 GJ93/095	4.00	100.0			3.75	100.0	<i>S. integrifolium</i>						
23 GJ93/118	4.00	100.0	0.00	0.0	3.40	100.0	62 GJ93/286	4.00	100.0	0.00	0.0	3.35	100.0
24 GJ93/119	4.00	100.0	0.00	0.0	3.10	92.9	<i>S. macrocarpon</i>						
25 GJ93/122	4.00	100.0	0.00	0.0	3.50	100.0	63 GJ93/042	2.60	80.0	0.00	0.0	3.80	100.0
26 GJ93/123	4.00	100.0	0.00	0.0	3.55	100.0	64 GJ93/298	3.30	90.0	0.00	0.0	3.25	100.0
27 GJ93/129	4.00	100.0	0.00	0.0	3.40	95.0	<i>S. anguivi</i>						
28 GJ93/130	4.00	100.0	0.00	0.0	3.12	94.4	65 GJ93/044	4.00	100.0	0.00	0.0	3.30	95.0
29 GJ93/132	4.00	100.0	0.00	0.0	3.45	95.0	66 GJ93/135	4.00	100.0	0.00	0.0		
30 GJ93/136	4.00	100.0	0.00	0.0			67 GJ93/139	4.00	100.0	0.00	0.0		
31 GJ93/145	4.00	100.0	0.00	0.0	3.75	95.0	68 GJ93/147	4.00	100.0	0.00	0.0	4.00	100.0
32 GJ93/146	4.00	100.0	0.00	0.0	3.84	100.0	69 GJ93/148	4.00	100.0	0.00	0.0		
33 GJ93/151	3.60	90.0	0.00	0.0	4.00	100.0	70 GJ93/150	3.00	75.0	0.00	0.0		
34 GJ93/156	4.00	100.0	0.00	0.0	3.60	90.0	71 GJ93/211			0.00	0.0		
35 GJ93/157	4.00	100.0	0.00	0.0	3.84	100.0	-----						
36 GJ93/158	4.00	100.0	0.20	5.0	3.85	100.0	72 LS1934*	0.40	10.0	0.00	0.0	3.70	100.0
37 GJ93/159	3.90	100.0	0.00	0.0	3.95	100.0	73 Meet <sup>m</sup>			0.00	0.0	1.75	85.0
38 GJ93/166	4.00	100.0	0.00	0.0	4.00	100.0	74 Taibyo VF <sup>m</sup>			0.00	0.0	1.80	90.0
39 GJ93/169	4.00	100.0	0.00	0.0	3.70	100.0	75 LS2436 <sup>m</sup>			0.00	0.0	2.45	95.0
40 GJ93/170	4.00	100.0	0.60	16.3	3.75	100.0	76 Kitsuta <sup>y</sup>	4.00	100.0	3.95	100.0	3.95	100.0

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### **1996 NATIONAL PEPPER CONFERENCE (Naples, Florida, USA - 8-11 December 1996)**

The Conference will be held on the beach of the Naples Beach Hotel & Golf Club, 851 Gulf Shore Blvd. North, Naples, Florida 33940. Deadline for room reservation is November 8, 1996. The cost for the Conference is \$ 150 (participants) and \$ 125 (accompanying persons).

The aim of the Conference is to bring together those with interest in the current status of research, extension and technology in *Capsicum* spp. Topics of interest include:

- . Germplasm evaluation and utilization
- . Crop physiology and ecology
- . Production methods and cultural systems
- . Alternative and sustainable approaches to production
- . Quality standards and post harvest physiology and technology.
- . Economics, marketing and trade.
- . Pest management

All conference papers will be published in a bound proceeding book to be distributed at the Conference. To assist in planning the program, paper titles, author names and affiliation as well as the format of presentation (oral, poster or printed) must be received by June 15, 1996. Completed manuscripts are due by September 20, 1996.

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