

CAPSICUM & EGGPLANT NEWSLETTER



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

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FOREWORD

There is some important news about *Capsicum and Eggplant Newsletter*. The first one concerns the change of address: the Agricultural Faculty of the Turin University has recently moved into the new location of Grugliasco, about 10 km from the centre of Turin. So, the new postal address for the Newsletter is:

Capsicum and Eggplant Newsletter
University of Turin
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Please note that the name of the Department who is in charge of the Newsletter has changed: from "Plant Breeding and Seed Production" to "Agricultural Genetics". To the contrary, the fax number is the same as in the past: +39 11 6502754. When it is changed, we will inform our readers and collaborators.

Capsicum and Eggplant Newsletter now has its own Email, to which it is possible to send messages as well as submit contributions for publication. The address is **capsicum@agraria.unito.it**. Lastly, we have activated a Home Page on the Internet, where it is possible to find all information about the Newsletter. The URL is: **<http://www.agraria.unito.it/dip/divaprlgenetilceni>**.

At the recent EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant (Avignon, September 7-11, 1998) it was decided to add three more members to the Scientific Committee: M.C. Daunay (Vegetable Breeding Station, INRA, Montfavet, France), R. Lester (University of Birmingham, United Kingdom) and A. Moor (Vegetable Crops Research Institute, Budapest, Hungary). Welcome Marie-Christine, Richard and Andrea and good work! The participants in the Meeting at Avignon also decided that the *Capsicum and Eggplant Newsletter* would be the official reference for the nomenclature and mapping of pepper and eggplant genes.

The eighteenth issue of *Capsicum and Eggplant Newsletter* includes two invited papers. One is written by Benjamin Steinitz, Dalia Wolf, Tania Matzevitch-Josef and Aaron Zelcer from The Volcani Center, Israel. It deals with genetic transformation and with specific reference to the perspectives offered by biotechnology in improving pepper production. The other paper has been prepared by Tomas Depestre from Havana, and gives us information on pepper breeding in Cuba. As usual, we thank these Authors very much for their efforts and for their kind willingness to increase the scientific value of our publication. In addition, we would like to remind you that any suggestions on the topics and/or authors to be considered for invited papers in future issues of *Capsicum and Eggplant Newsletter* would be appreciated.

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

Please, remember that this Newsletter is dependent on the financial support of the recipients. Therefore, a subscription fee is appreciated. The subscription fee is the same as last year: 30 U.S.\$ for normal and 150 U.S.\$ for supporter subscribers. Remember that to make the payment less time-consuming and to reduce the bank costs, we have introduced the possibility of a 3-year subscription. It is possible (and suggested!) to book your own copy to quicken its delivery. Just fill in the order form on page 103 and send it to us, together with a copy of the payment order, which must always be made out to Eucarpia. In case you decide to pay by credit card, please use the voucher on page 105. Because of the lower banking costs, credit card payment is definitely welcomed.

The deadline for submission of articles to be included in the next issue of the Newsletter (No. 19, 2000) is February 28, 2000. Please note that it is also possible (and suggested) to submit the paper on diskette or through Email. Details can be found on the enclosed sample sheet.

We regret to report that several papers had to be rejected because of the lack of attention paid to the instructions. It is imperative that you follow these instructions very carefully. Otherwise we will not accept the contributions and will have to send them back to you. Beginning from the next issue, a stricter policy will be in force!

Piero Belletti and Luciana Quagliotti

Turin, 31 st May 1999

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REGENERATION *IN VITRO* AND GENETIC TRANSFORMATION OF PEPPER (*Capsicum* spp.): THE CURRENT STATE OF THE ART

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Abstract

The review presents an update on pepper biotechnology of the recent years. The progress and difficulties of the following topics are discussed: regeneration via organogenesis and somatic embryogenesis, vegetative micropropagation, culture of anthers and isolated microspores, and genetic transformation as a tool for transgenic breeding of *Capsicum annuum* L.

Introduction

About three decades ago tobacco and petunia became models for research of certain aspect of modern biotechnology and molecular biology. At the same time potato turned to be a prominent archetype for clonal micropropagation and virus elimination by meristem culture. Transgenic cultivars of these solanaceae, as well as of tomato and eggplant cultivars, have been released to the market, or are being at different stages of field trials. By contrast, pepper (*Capsicum annuum* L.) lags behind, and is only at the entrance to the era of advanced biotechnology and of transgenic breeding.

This review is not a comprehensive literature survey. Rather, it presents an update on pepper *in vitro* technologies and genetic transformation, based on information accumulated in recent years. Further details can be found in previous reviews (Fari & Andrasfalvy 1994, Regner 1996). The objectives of our presentation are: first, to display contemporary progress on regeneration *in vitro* and genetic transformation related to pepper breeding and horticulture; second, to highlight unresolved problems that merit further research and development.

Regeneration

Regeneration via organogenesis

Effective pepper regeneration systems *in vitro* serve three main purposes: (a) Micropropagation of special value elite plants (e.g. male sterile plants, or F1 plants displaying heterosis (i.e. Gupta et al. 1998)). (b) The production of transgenic plants. (c) The generation of microspore- or pollen-derived dihaploid plants (Renger 1996).

The last years have seen numerous studies on regeneration via shoot and root organogenesis induced on explants prepared from seedlings at different times after sowing. In rare cases spontaneous shoot regeneration, from explants cultured on a medium devoid of growth regulators, has been observed (Ezura et al. 1993, Binzel et al. 1996a). Usually, as in all papers cited hereafter, regeneration had to be induced and accomplished by exogenous growth regulator supplements to the medium. The explants used were: "half seed explants" (Binzel et al., 1996a), cotyledons (Szasz et al. 1995), hypocotyls and decapitated hypocotyls (Szasz et al. 1995, Ramirez-Malagon & Ochoa-Alejo 1996, Ramage & Leung 1996), leaf tissue (Christopher & Rajam 1996, Zhu et al. 1996), or protoplasts derived from leaves taken from *in vitro* shoots (Prakash et al. 1997). Commonly, explants were placed onto an agar-solidified shoot induction medium supplemented with a cytokinin (benzyl adenine (BA), kinetin, zeatin or thidiazuron

(TDZ), and often also an auxin (1M, IBA or NM). Subsequently, shoot elongation may, in some cases, take place after transplantation of small shoots *ex vitro* (Ebida & Hu 1993). However, in most instances shoot - or shoot bud - clusters were transferred to a shoot elongation medium *in vitro*, because shoot elongation has repeatedly been found as a major obstacle in obtaining normal pepper plants. Gibberellin acid (GA3) is usually the plant growth - regulator added to elongation medium. Franck-Duchenne et al. (1998) attempted increasing stimulation of stem elongation by planting shoot buds on a medium including 24-epi-brassinolide. Although this growth regulator did improve the rate of plantlet recovery, it did not provide a general alleviation of stem elongation difficulties. Adventitious shoots root either spontaneously on a hormone-free medium, or consequently to planting shoots onto a medium supplemented with a low auxin concentration.

The number of plants regenerated per explant stands usually in the range of 1-10. In some papers even lower regeneration rates have been indicated. We assume that there are genotypes for which serious attempts to develop regeneration procedures failed, and obviously these failures have not been published. Our group was also involved in developing regeneration and transformation technology for the last four years, and we gained similar experience: A screening for genotypes with regeneration capabilities was conducted. Explants prepared from young seedling organs were cultured according to numerous protocols published. Out of more than 40 genotypes tested, obtained from the breeding collection available at the Volcani Center and from a seed company, only 4 genotypes were finally identified to have consistent repeatable regeneration capabilities. Using our best genotypes and the choice protocols, we were able to generate an average of about 5 fully functional plants per (cotyledon or hypocotyl) explant (Wolf et al. 1998). Other genotypes had either lower regeneration rates or did not display any normal regeneration. Several characteristics emerge from the studies reported by different laboratories: (a) The hormonal composition and the sequence of application of growth regulators has to be adapted to the genotype and to explant type. (b) Cytokinin, whether applied alone or in combination with an auxin, is the critical regulator for adventitious shoot induction. These two rules have been found to be valid in different *Capsicum* species (e.i. Wang et al. 1991, Christopher & Rajam 1994, Fari & Andrasfalvy 1994), and they are not unique to pepper species. (c) Invariably of pepper genotype or explant type, profuse bud formation is visible within 2-3 weeks from culture initiation. Unfortunately, a majority of these buds develop either leafy structures or stunted and otherwise aberrant shoots. Only after transfer of cultures from a shoot induction to a stem elongation medium, a minority of buds eventually develops into normal shoots. The major problems during shoot elongation are less severe in hot pepper (Ochoa-Alejo & Irenta-Moreno, (1990; Valera-Montero & Ochoa-Alejo, 1992) and in some experiments in *C. frutescens* (Wang et al., 1991) than in sweet pepper varieties. Defects in shoot meristem differentiation or primordia organization cause the recurrent low incidence of normal plant recovery. Conditions that permit abundant normal shoot development have generally not been detected.

From the viewpoint of micropropagation via adventitious shoot organogenesis, many pepper genotypes are still considered recalcitrant. In spite of the difficulties described, we find a few encouraging cases of success. For example, Ma et al. (1991) reported an annual production of 4.7 million plants by micropropagation based on a multiplication rate of 9: 1. This propagation scenario, being laborious and performed manually, can be economically viable provided the culture-derived plants are cheaper than the seedlings.

Regeneration via somatic embryogenesis

Direct somatic embryogenesis was first described in chilli pepper by Harini & Sita (1993) and in sweet pepper by Binzel et al. (1996b). In both studies, immature zygotic embryos were inoculated on a medium including 2,4-D, kinetin or TDZ, coconut water and 6-10% sucrose.

Somatic embryos formed directly on the immature zygotic embryo without the formation of an intermediate phase of embryogenic callus. The entire process, from embryogenesis induction to somatic embryo maturation, was accomplished on the initial medium without subculture. Noteworthy, somatic embryogenesis occurred on 10-85% (Binzel et al. 1996b) or even on all (100%) zygotic embryos explanted (Harini & Sita 1993). The multiplication rate (somatic embryos:zygotic embryo) was 13 (Harini & Sita 1993), or up to 8 (Binzel et al. 1996b).

Somatic embryos were obtained also from mature zygotic embryos, however, through an intermediate stage of embryogenic callus. The callus was generated on a medium with 2,4-D, and then transferred through a sequence of subcultures in different media (Buykalaca & Mavituna 1996). Moreover, a recurrent somatic embryogenesis process in liquid media was developed: all stages of embryogenesis, from growth of the embryogenic suspension cultures to embryo maturation, were performed in a bioreactor as a series of drain-and-fill batches, keeping the embryos in the bioreactor all the time (Mavituna & Buykalaca 1996). The multiplication rates obtained by recurrent somatic embryogenesis in a bioreactor were significantly higher than the common multiplication rates reported for caulogenesis. The production of artificial seeds, consisting of somatic *C. annuum* embryos encapsulated in calcium alginate gel beads, has also been achieved (Buykalaca et al. 1995). Although it is conceivable that some details of the culture conditions will require genotype-dependent adaptations, propagation in automated computer-controlled bioreactors could become the way to profitable large-scale micropropagation of elite material. However, since (somaclonal?) variations amongst culture-derived pepper plants were detected (Shen et al. 1994), future research and development of mass propagation technologies should include genetic stability and a follow-up in horticultural fidelity of the propagules.

Anther and microspore culture

Immature pepper pollen or microspores can switch from a gametophytic to a sporophytic developmental pathway. Microspore embryogenesis is usually triggered by the exposure of freshly excised anthers to a few days of elevated temperature stress of about 35°C in the dark, followed by incubation at 25°C in the light. Anthers are inoculated onto a solidified medium supplemented with sucrose, 2,4-D and kinetin (Dumas de Vaulx et al. 1981). The extent of androgenic response is clearly source genotype-dependent (Kristiansen & Andersen 1993, Mityk et al. 1995, Dolcetsanjuan et al. 1997), and is influenced by the growth conditions of the plants from which anthers are harvested (Kristiansen & Andersen 1993). Recently, Dolcetsanjuan et al. (1997) considerably modified the protocol of Dumas de Vaulx et al. (1981): 35°C induction treatment was substituted by a 7°C treatment; sucrose in the medium was replaced by maltose; the culture vessel atmosphere was periodically ventilated with CO₂-enriched air; finally, anthers were cultured on a growth regulator-less medium. The new procedures increased, in some genotypes, the incidence of embryogenesis induction to such an extent that the limiting factor in the number of plants eventually regenerated resided not in embryo formation but in embryo maturation and conversion to plants (Dolcetsanjuan et al. 1997).

Embryogenesis induction in cultures of isolated microspores rather than in anther cultures is another approach that could lead to an increase in the yield of microspore-derived embryos. Research in this direction was initiated several years ago by Gonzalez-Melendi et al. (1995), Testillano et al. (1995), and Gonzalez-Melendi et al. (1996). Multicellular, initial stages of embryo-like and callus-like structures were successfully induced in isolated microspore cultures,

but the formation of fully developed embryos has not yet been achieved.

The regeneration potential of microspore embryos in anther culture, and the recovery of the homozygous trait through chromosome doubling, has been utilized for breeding of new cultivars as well as for genetic studies by research laboratories and seed companies (see Vagera 1990,

Regner 1996 and references therein). Hence, while the application of vegetative micropropagation in horticultural practice is still limited, androgenesis has already contributed to the establishment of commercial products worldwide.

Transformation and transgenic breeding

The recent literature contains reports describing the generation of transgenic pepper plants. We discuss mostly full articles and refrain from expanding on short meeting reports that, unfortunately, lack experimental details. Transformed shoot buds were reported by Liu et al. (1990), but no functional transgenic plants could be secured. Since the early 90's, communications from laboratories in China have indicated successful transformation of *Capsicum frutescens* (Wang et al., 1991) and *Capsicum annuum* (Dong et al., 1992; Zhang et al., 1994; Zhu et al., 1996). In this later species, the creation of transgenic resistance to CMV, either by the use of cDNA from a viral satellite or the viral coat protein. was the main goal of the work. A similar work was reported by a Korean group, which described the transformation of hot pepper (*C. annuum*) with a CMV satellite construct (Lee et al., 1993; Kim et al., 1997). Partial attenuation of symptoms and a decrease of virus titer were observed. A recent report from India described the generation and characterization of transgenic hot chilli (*C. annuum*) (Manoharan et al., 1998).

In spite of this promising progress, tremendous efforts invested worldwide in the direction of transgenic pepper breeding has not yielded other successful results accompanied by scientific documentation. A critical evaluation of the published papers discloses: (a) A very low transformation efficiencies (Szasz et al. 1995, Yu-Xian et al. 1996, Manoharan et al. 1998, Mihalka et al. 1998, Wolf et al. 1998). (b) The use of similar strategies by the different successful groups: *Agrobacterium* mediated transformation was exclusively used, cotyledons were generally preferred as the target explants, and NPT II (coding for resistance to kanamycin) was the selective tool in all cases. In most cases, each group concentrated on a specific *C. annuum* cultivar, and we therefore cannot exclude a strong genotype dependency for the described protocols.

The genetic transformation of pepper is also actively pursued in laboratories from the private sector. A patent on "genetically transformed pepper plants and methods for their production" was granted to DNA Plant Technology Co. (US 5262316) in 1993. The APHIS-USDA database for Environmental Releases (<http://www.aphis.usda.gov/bbep/bpD>) includes 9 individual field trials with transgenic peppers that were performed in US to the present (table 1).

Table 1: Genetically engineered and field tested pepper produced by the biotechnology industry in the US.

Company	Year	Introduces Transgene	Selectable gene	Attempted modification
Seminis	1998	?	NPTII	Resistance to CMV
DNAP	1997	Hemicellulase	ALS	Prolonged shelflife
Seminis	1997	CMV and TEV coat protein	?	Resistance to CMV and TEV
Seminis	1997	CMV coat protein	NPTII	Resistance to CMV
Peto Seed	1996	CMV coat protein	NPTII	Resistance to CMV
Seminis	1996	CMV coat protein	NPTII	Resistance to CMV
DNAP	1996	?	ALS	Altered fruit ripening
DNAP	1995	B- 1,3- glucanase antisense	ALS	Altered fruit ripening
DNAP	1994	b-1,4- endoglucanase antisense	ALS	B1,4 endoglucanase

Only partial information is provided on the composition of the transgenic material and no details are available on the genotypes utilized, the efficiency of the methodology and the induced phenotypes. However, some general statements can be offered: 1) NPT II and ALS (acetolactate synthase) are the preferred selectable genes. 2) Resistance to viruses and modification of the *fruit* ripening process seem to be major targets for transgenic improvement. It was mentioned elsewhere that sense suppression of endo-(1-4)- β -D-glucanase in some of these transgenic lines resulted in modifications of the glycan cell wall fraction of ripening fruits, with a concomitant decrease of water loss and an improved shelflife (see Bedbrook, in Carpita et al. 1996). Hopefully, full reports will eventually shed light on these promising findings.

Conclusions and future prospects

The repetitive search in laboratories worldwide - along decades - after the basics in pepper regeneration methods, reflects a persisting dissatisfaction from the state of the art amongst scientists, breeders and growers. The observation common to regeneration studies from somatic cells and from microspores, is the marked source-genotype dependency of the developmental process to a given set of culture conditions. This may indicate that an expectation to find protocols of validity and reproducibility across a wide range of *Capsicum* genotypes is, perhaps, unrealistic. Consequently, the regeneration hurdle will have to be overcome by adjustment of culture conditions for individual cases where transgenic breeding is being planned. Proliferation of somatic embryos in computer-controlled bioreactors will probably become the technology of preference for future mass micropropagation. Regarding androgenesis, the interesting new findings by Dolcetsanjuan et al. (1997) on improved rates of microspore embryogenesis indicates, on the one hand, that there is ample space for improvements of recovery of haploid and doubled haploid plants in anther culture. On the other hand, the isolated microspore culture technology can be described as still being in its "early embryonic" stage. Finally, the genetic transformation discipline is only at its infancy. Therefore, we have all the reasons to anticipate the field of pepper biotechnology to be very interesting and dynamic in the 21st century.

Acknowledgments

Because of space constraints many references could not be included, and we apologize. This paper is a contribution from the Agricultural Research Organization, The Volcani Center, Institute of Field and Garden Crops, Bet Dagan, Israel, No 7/99. We gratefully acknowledge the funding of the transgenic pepper biotechnology project 261-0262-1997/8 by the Chief Scientist of the Ministry of Agriculture.

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AN APPROACH TO PEPPER BREEDING IN CUBA

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Pepper is an important commercial crop in Cuba. It occupies an area of 4 000 hectares from which about 5 000 tons each year are exported. These values should be increased in a next future because this culture is a priority to improve people's nutritional quality and diversity as well as national exports, since pepper has been a traditional one.

Among the five domesticated pepper species *Capsicum annuum* is the most cultivated in Cuba. People demand mainly bell or rectangular- shapeQ. fruits for stuff or for salads and heart- shaped fruits for canned products. They also claim the cone- shaped types and the little ones or spice non hot peppers which are used daily in the preparation of different foods. The 'Cachucha' types belonging to *C. chinense* are widespread in the country, they are requested to add flavor and odor to some cuban traditional meals like black beans. Some short fruited sweet varieties called ..all the year round'. from *C. baccatum* are also widespread in backyards, they fruit set well even in the hot and humid season; people use them also as spice.

It is interesting to highlight that people in Cuba, on the contrary to other countries of the region, prefer better sweet peppers than hot peppers, but in all houses you can find a pungent sauce made from the little *C. frutescens* types, ready to add to some meals.

Pepper production is done mainly in five provinces under open field conditions and at sea level: Pinar del *Rio*, La Habana, Villa Clara, Ciego de Avila and Gramma. In all of them furrow irrigation is used. Main sweet pepper varieties used in Cuba are shown in Table 1. Nowadays some pepper production begins to be developed under sheltered conditions and mainly F1 hybrids are used.

Growing season begins in October and ends in December so production takes place during the winter months. During this period temperatures are nearer to pepper biological requirements than in any other time; it is also dry. As this period is very short, climatic adaptation is necessary in new genotypes in order to open it and get higher yearly pepper production.

Name	Origin	Type	Use
California Wonder	USA	Square	Fresh Consumption and export
Califorina Wonder 300	--	--	--
Tropical CW3	Cuba	--	--
Espanol Liliana	--	Recatangular	Fresh consumption and canned
Truw heart		heart	canned
Regalo de Modavia	Moldavia	Cone	Fresh consumption
Verano 1	Cuba	Cone	--
Medalla de oro	Bulgaria	Long cone	Fresh consumption and canned
SC81	Cuba	short cone	Spice
Chay	--	--	--

Main pepper constraint in Cuba are lack of climatic adaptation in Foreign varieties used during the growing season which promotes low yields lack of varieties for growing off season and damage caused by diseases as follow:

Soil Fungus	Importance
Fusarium oxysporum	2
Phytophthora capsici	2
LEAF Fungus	
Cercospora Capsici	3
Bacteria	
Xanthomonas campestris pv vesicatoria	1
Virus	
Tobacco etch virus (TEV)	1
Potato virus Y (PVY)	1
Tobacco mosaic virus (TMV)	1
Cucumber mosaic virus (CMV)	1
Pests	
Polyphagotarsonemus latus	1

A first step of a Cuban pepper breeding work began in the early sixties in order to find out better varieties than traditional ones. More than 500 foreign varieties, mainly from USA, France, Spain, Hungary, Bulgaria and Moldavia were studied, This way didn't offer too much results since only two varieties: 'Medalla de Oro' and 'Regalo de Moldavia' performed well in the country showing yield stability in tropical conditions, both are nowadays well used by growers.

The great opportunity to breed pepper began in 1975 through the national project .Tropical tubers and vegetable crops.. sponsored by the Ministry of Science, Technology and Environment who finances important pepper research in the country, coordinated by the .Liliana Dimitrova.. Horticultural Research Institute (IIHLD), in cooperation with the ..Alejandro de Humboldt" Fundamental Research Institute on Tropical Agriculture (INIFAT) both located in Havana and belonging to the Ministry of Agriculture (MINAG),

..

The main goals of the breeding program are to get varieties suitable for:

- . Fresh market or export - for Cuban consumption large or medium bright green or yellow fruits which turn red when ripen, with sweet taste, square, rectangular or cone- shaped; for the foreign market, large square green fruits with a thick pericarp.
- . Canned food industry - pleasant flavor fruits suitable for processing different canned products.
- . Off season crop - plants that may fruit set well at open field under high temperature and humid conditions.
- . Spice - short sweet fruits suitable to add taste and odor to meals.
- . Protected culture - big fruited and high yielding plants, well adapted to different seasons and shelter technologies used in the country; since there's a great interest in growing pepper even in the summer season, in order to fulfill the increasing tourism demand.

In general, it is wanted to get resistant genotypes to the most widespread diseases with high yield and good quality fruits well adapted to different production systems in Cuban tropical conditions.

Consequently, several national genetic resource expeditions were undertaken during 1978 - 1980. One of them got outstanding results because of the great variability found in the *Capsicum* genus at the Island of Youth in southern Havana. 42 collected entries from a Spanish type segregant population were studied in this opportunity at IIHLD, it means from a local variety where spontaneous crosses had been made through the time mainly due to *Exoma/opsis pulchella*, Cresson, very active in Cuban conditions; natural cross pollination in pepper is high in Cuba due to this reason mainly.

These entries were studied through six self-pollinated generations (So - S5). Advance in selection was possible according to high variation coefficients found in the studied traits: plant height, fruit shape, fruit width and length, pericarp thickness, fruit weight, number of fruits per plant and yield.

Data from evaluations made in each generation were used to get a correlation matrix; a path coefficient analysis was also used in order to know the effects of different traits on yield, Phenotypic correlations were positive and highly significant between yield and its components, number of fruits per plant and fruit weight and between pericarp thickness and fruit weight but negative and highly significant between pericarp thickness and number of fruits per plant.

The convenience of using both yield components in pepper selection at a same ponderated level was evidenced since they showed a similar direct contribution on it. The possibility of getting numerous and large fruited genotypes through breeding at

Narrow sense calculated heritability based in the parent-descent regression was high for all traits but yield where it was intermediate. Three outstanding lines resulted from this work which were tested in different environmental conditions during the winter for three years. In this trial, variation sources due to genotype and years were highly significant for yield and its components showing the importance of varietal differences as well as growing season on pepper productivity. Since weather conditions may change very much during the Cuban winter months from one year to another, turning difficult high pepper production in some cases where even during this season temperature keeps high.

Some F₂ hybrids were made from these lines, all F₂ combinations made showed a great heterosis effect on yield and number of fruits per plant under tropical conditions.

Efforts were made to develop and standardize efficient and reliable laboratory tests against the main pepper diseases in order to aid selection. The *in vitro* androgenesis technique has been tried on Cuban pepper cultivars. Anther response was influenced by genotype but also induction factors had a strong influence on the androgenetic process of each genotype.

Evidence was given that through selection it is possible to get good pepper varieties as: 'Espanol Liliana', 'Jovito' and 'SC 81' from variability present in natural populations. Productivity in new varieties was higher than in wide grown varieties of the same types because of their climatic adaptation and disease resistances. For these reasons they have been spread and now replaced them. Selection in pepper has been also exploited through the INIFAT breeding program, some prominent varieties like: Tropical CW 3', True Heart 28' and 'Verano I' have resulted from it. Research institutes supply elite seed from sponsored pepper varieties and a state enterprise is in charge of supplying other seed classes to growers.

These important results led us to a superior step of the program. In 1991 a cooperative work on pepper breeding began between the "Station d' Amelioration des Plantes Maraicheres", INRA- Montfavet, France and the "Liliana Dimitrova" Horticultural Research Institute, MINAG-Cuba. Its main goals are:

- . Creation of new broad action virus resistance sources.
- . Stability evaluation of these entries in natural infection conditions,
- . Selection of square or rectangular large fruited sweet pepper lines, well adapted to different environmental conditions,

Four viruses were selected to work: TMV, PVY, CMV and TEV, the last one because of its importance in America and its aggressivity in Cuba. The purpose was to build up a rich genetic source population named 'LIRA' because of Liliana and INRA. Different subpopulations were made according to virus resistance searched. Resistant genitors with agronomic value from France and Cuba were chosen in order to create multiresistant systems more useful.

These were:

- . IIRAT. TMV resistant at high temperatures, from 'SC 81', 'Espanol Liliana' and 'Vania',
- . IIRAP - PVY (1,2) resistant from 'SC 81', 'PM 949', 'Samka' and 'HD 801'.
- . LIRAC - CMV resistant from 'Vania', 'Flambeau', 'SC 81', 'Samka' and 'ORIENT' population,
- . URAE - TEV resistant from ('SC 81' x 'Vania')F₂ and 'Avelar'.
- . IIRAV - PVMV resistant from 'HD 801', 'PM 1100' and (PM 1100'x 'Vania').

Other pathogens like *Xanthomonas campestris* pv *vesicatoria* and *Pseudomonas solanacearum* were included in the program.

Best plants from each subpopulation were selected, tested and selfed but also pollen mixtures were made using them. Two selection cycles were carried out each year one in France for testing and one in Cuba for open field selection. After the first self pollination cycle, TMV resistant plants

were found in 100 % of families from LIRA T. After the second self pollination cycle, CMV resistant plants were found in 96 % of families from LIRAC; PVY resistant plants were found in 100 % of families from LIRAP and TEV resistant plants were found in 100 % of families from LIRAE.

Several cycles of recurrent selection have to be performed in order to increase the frequency of desired alleles in the population but first inbred lines have already been fixed from each subpopulation.

Collaboration with other institutions in the intertropical countries will aid to the purpose of breeding for multiresistant bell pepper cultivars adapted to intertropical regions.

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**CHARACTERISTICS OF CHILLI PEPPER CULTIVARS RELEASED BY
THE INSTITUTE FOR AGRICULTURAL RESEARCH SAMARU NIGERIA**

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INTRODUCTION

Pepper is a major vegetable crop in Nigeria. Annual production in 1991, 1992, 1993 were 850,000,880,000 and 900,000 metric tons (Anonymous, 1993). The low yield obtained by farmers is due to lack of improved cultivars, and virus diseases especially pepper veinal mottle virus (PVMV) (Alegbejo, 1918). Consequently, this trial was conducted to evaluate six pepper cultivars for good agronomic characteristics (e.g. high yield) and PVMV resistance before being released for use by farmers.

MATERIALS AND METHODS

Seedlings of the six pepper cultivars were raised in an insect- free glasshouse and transplanted into the field at six weeks after sowing (late June 1993 and 1994) using a randomized complete block design. There were three replicates. Each plot consisted of two 6m- long ridges, 0.15m wide with a total of twenty plants. The field was allowed to be naturally infected by PVMV due to the high incidence of the disease at the time the trial was conducted. All recommended practices for pepper production (except pesticide application) were observed. The following agronomic characteristics, fruit length, plant height, maturity days, dried fruit yield and resistance to PVMV were taken. Data were analysed using the two-way analysis of variance.

RESULTS AND DISCUSSION

Fruit length ranged from 3.5f to 4.40cm in 1993, and 3.5 to 4.50cm in 1994. Plant height varied from 28.50 to 40.20cm in 1993 and 40.00 to 41.00cm in 1994 (Tables 1 & 2). Maturity days varied from 135 to 155 (Tables 1 & 2). Dried fruit yield of 0.85 to 1.2 t/ha was obtained (Tables 1 & 2). SAMPEP 3 & 4 were moderately resistant to PVMV, SAMPEP 1,2 and 6 were moderately susceptible while SAMPEP 5 was highly susceptible (Tables 1 & 2). In terms of overall performance SAMPEP 4 was the best followed in descending order by SAMPEP 1, 6, 3, 2 and 5.

These cultivars have been sent to the Varietal release committee of the national Centre for Genetic Resources and Bio technology, Ibadan for National release to Nigerian farmers. Effort is being made to Institutes for the about characteristics

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Table 1. Performance of six chilli pepper cultivars at Samaru in the 1993 wet season

Original name	IAR accession number	Fruit length (cm)	Plant height (cm)	Maturity days	Dried fruit yield (t/ha)	PVMV-infected plants (%)
PL - 38475	SAMPEP-1	3.51	40.20	145.00	1.15	30.20
PL - 2289	SAMPEP-2	3.85	35.20	145.00	1.01	29.03
P - Sakarho	SAMPEP-3	4.10	28.50	135.00	1.02	22.00
U - Kimba	SAMPEP-4	4.40	40.00	155.00	1.05	16.50
UL - 2190	SAMPEP-5	3.75	38.00	145.00	0.85	47.00
UL - 3878	SAMPEP-6	4.40	42.1	145.00	1.03	31.00
S.E.D. (P=0.05)		0.52	6.20	7.30	0.22	3.80

Table 2. Performance of six chilli pepper cultivars at Samaru in the 1994 wet season

IAR accession number	Fruit length (cm)	Plant height (cm)	Maturity days	Dried fruit yield (t/ha)	PVMV-infected plants (%)
SAMPEP-1	3.90	46.00	140.00	1.00	28.33
SAMPEP-2	3.50	47.00	145.00	1.20	29.01
SAMPEP-3	4.0	45.00	130.00	1.20	21.67
SAMPEP-4	4.50	40.00	140.00	0.96	16.67
SAMPEP-5	3.80	55.00	137.00	1.00	46.67
SAMPEP-6	4.50	45.00	135.00	1.30	30.12
S.E.D. (P=0.05)	0.60	7.10	7.01	0.25	3.71

TOTAL PIGMENT CONTENT IN RED PEPPER CULTIVAR FOR GRINDING TODOROVA V., PEVICHAROVA G., TODOROV Y.

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ABSTRACT

The content and the quality of total pigments in red pepper fruits and ground paprika are of a great importance. That is why they were studied in five different cultivars from Bulgaria, Spain and Hungary, grown under the same conditions during 1996 - 1997.

Spanish cultivars, especially Negral Can not mature well and realize their bigger possibilities under the conditions of Plovdiv - Bulgaria.

The average two-year data of extractable colour in AST A units among the tested cultivars in the following downgrade order:

Buketan 50 (262) > Kalocsai 801 (212.5) > Belrubi (211.5) > Gorogled 6 (201.5) > Negral (177)

The results of the average colour ratio "dye" between first and second picks for yellow red pigments respectively show that Kalo,sai 801 has the most favorable ratio (0.972 -0.986). Belrubi and Buketen 50 are high quality cultivars too,

INTRODUCTION

The quantity and the quality of total pigments in red pepper fruits and ground paprika are of a very great importance that is why they are subject of many investigations (Kappeler, Markus, 1983; Costa et al. 1989; Todorov, 1987; Levy et al. 1995 and others).

Our previous research shows that the basic essential character of red pepper - the content of pigments is genetically determined by a complicated biochemic path-way stipulated almost equally &om additive, dominant and environmental factors (Todorov, 1991; Todorov and

Manuelyan, 1989), The object of the present study is to investigate the content and the quality of total pigments in red pepper fruits from different paprika cultivars, grown in the conditions of Plovdiv.

MATERIALS AND METHODS

At the Maritsa vegetable Crops Research Institute - Plovdiv the next cultivars were analyzed: Gorogled 6. Buketen 50 from Bulgaria, Negral, Belrubi from Spain and Kalocsai 801 from Hungary.

The plants of the chosen cultivars were grown in the same condition during 1996 - 1997 in plastic greenhouses - isolators on a furrow surface 70/15 cm by seedling produced in advance.

Twenty samples of the ripen fruit twenty plants, chosen at random of the mentioned above cultivars were harvested in the end of October. Immediately after harvesting their fruits were dried at 45-55°C with active ventilation. Only the pericarp of the fruits was analyzed individually for content of total pigments by ASTA method 19 (1960). The analyzes of dried fruits of 1996 crop were made after a six months storage at usual room conditions. We get carination data for content of total pigment in ASTA units and color ratio dye between the extensions of the first and second picks at λ . 450nm and 470nm for the maximum of absorption of yellow and red pigments respectively. The obtained data were subjected to statistical analyses and the significance of differences was estimated by Duncan's multiple range test (1955).

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RESULTS AND DISCUSSION

The average values in Table 1 show that during 1996 vegetation period Buketen 50 accumulate comparatively the biggest content of pigments - 269 ASTA. After Buketen 50 the other cultivars are set in the following order: Gorogled 6, Kalocsai 801, Belrubi and Negral. The Spanish cultivars, especially Negra! C3rul0t realize their bigger possibilities that they have in the conditions of Murcia according to Costa et al (1989). Under our climate conditions the total carotenoid pigments of Negral are below its maximum and when the fruits are harvested they contain a certain quantity of chlorophyll that reflects on tile value of the first pick at A. 450run. Besides that the Spanish cultivars have lower percent of retained colour after a six month storage, These results explain partially the relatively higher values of their total pigments in 1997 crop, when tile fruits were analysed comparatively soon after halve8ting and drying without any storage. In addition 1997 was extremely unflavourable with respect to meteorological conditions. 11le sintesis of carotenoids in the foreign cultivars, especially in Negral was more accelerated and at the moment of harvesting the fruits had less quantity of chlorophyll in comparison with 1996.

Table 1

CONTENT AND QUALITY OF TOTAL PIGMENTS

Variety	1996		1997	
	ASTS	Ratio I/I	ASTA	Ratio I/II
Gorogled 6	218 c**	1.001c	185a	1.012bc
Buketen 50	269d	1.004bc	255c	1.019c
Negral	164a	1.042d	190a	1.017
Belrubi	193b	1.001b	230b	1.004b
Kalocsai 801	206bc	0.972a	219b	0.986a

** Means not sharing a letter in common differ significantly ($P < 5\%$) according to Duncan's multiple range test.

During 1997 trial Buketen 50 was again in the first place Belrubi and Kalocsai 801 were at the second place while Negrral and gorogled 6 were the third in accumulation of total carotonoid pigments. The average two year data of extractable colour in ASTA units arrange the tested cultivars in the following downgrade order:

Buketen 50(262) Kalocsai 801 (212.5) Belrubi (211.5) Corogled 6 (201.5) Negral (177)

The results of the average colour ratio dye between frist and second pick for ywllow and red pigments respectively are shown also in Table1

Kalocsai 801 has the most favourable ratio (0,972 - 0.986) and it is mathematically proved at $P > 5\%$ 'Yo, Belrubi is characterized a." a high quality cultivar and it is the second in "dye" ratio (1.001 - 1,004). Buketen 50 follows these cultivars and in 1996 does not differ significantly ($P < 5\%$) from Belrubi and Gorogled 6. Perhaps the more favourable conditions of 1996 for growing and development of the plants stipulate the better quality composition of carotenoids and tllat is why the ratio between the first and second picks shows the better values lower values of all cultivars with the exception of Negral . That can be explained also with the available chlorophyll in the fruits of this cultivars The availability of the chlorophyll in the

extracts of paprika reflects considerably on the value of I pick at A 450nm and therefore in "dye" ratio II n pick.

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THE EFFECT OF SEASONAL CHANGES ON THE PUNGENCY LEVEL OF PADRON I PEPPER FRUITS)

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Abstract

Seasonal changes (from June to October 1997) had no significant effect on the evolution of the fresh or dry weight in the development of Padron pepper fruits. The pattern of capsaicinoid accumulation was the same during the different months, but we did find a considerable increase in capsaicinoid levels in the final months in all the stages studied.

Introduction

The pungency of the pepper is an important organoleptic characteristic which is determined by capsaicinoids, a group of pungent phenolics derived from the phenylpropanoid pathway (Andrews, 1995). The capsaicinoid content is genetically controlled, but it is also subject to environmental variables such as temperature, light, soil moisture and fertilization level (Suzuki & Iwai, 1984).

In a previous paper, we reported that the accumulation of capsaicinoids in Padron pepper fruits (the most commercially important cultivar in Galicia, NW Spain) is associated with the fruit age (Estrada et al., 1997). We have also identified mineral supplementation as a factor that may affect capsaicinoid content during the whole process of fruit maturation (Estrada et al, 1998) and water supply (Estrada et al., 1999)

Considering that seasonal changes may also affect the capsaicinoid content, we examined the evolution of this compound in Padron pepper fruits during the ripening process over a five month period in 1997

Material and methods

Plant Material

Pepper seeds (*Capsicum annum* L var *annuum* L cv Padron) were soaked overnight in tap water before being sown in a mixture of perlite and compost They were then placed in chambers under controlled conditions with 16h of light (using fluorescent light, 49IJ.mol m⁻² sol provided by Sylvania Standard fluorescent tubes F36\!\!15~) 8h dark cycle, 70% relative humidity and :25°/18°C day/night. Sixty days later, the seedlings \!were transplanted to pots filled with the same amount of soil moisture and placed in the greenhouse from April to October 1997 '

Extraction and quantification of capsaicinoids by HPLC

Pepper fruits were harvested every 7 days from 14 to 42 days after flowering. They were then weighed, oven dried at 60°C for 2-5 days and stored, Whole or sliced fruits were ground to a fine powder using a Kelnor laboratory mill. Pungency was quantified using an HPLC analysis of capsaicinoids (Collins et al 1995) with a Spherisorb 00S2 C18 column and a Photo diode array detector Waters 996 reading at 280 nm.

Results and discussion

Changes in fresh weight dry weight and water content at different maturation levels over five months.

We studied the evolution of the fresh weight, dry weight and water content of Padron pepper fruits monthly, over a five month period, (from June to October 1997) in order to study the effect of the seasonal changes on fruit development.

In general, we found an increment in the fresh and dry weight with the development of the fruit, evident on the 35th day after flowering, resulting in the highest values regardless of the month studied (Table 1 A, B). This pattern of growth was accompanied by a loss of water (Table 1 C). From August to October, we detected a substantial decrease in the water content of the fruits during the final stages of development (35 and 42 days after flowering).

A	Days after flowering				
	14	21	28	35	42
June	3.78(1.3)	7.60(1.8)	11.98(1.1)	18.58(5.3)	-
July	3.47(1.2)	7.91(2.0)	14.42(1.8)	23.36(6.3)	8.71(4.5)
August	3.10(1.4)	7.06(1.5)	12.24(1.5)	24.91(6.5)	18.64(5.2)
September	2.12(0.9)	8.80(2.1)	12.64(1.3)	22.39(5.7)	19.10(6.0)
October	3.78(1.2)	8.52(1.9)	13.41(1.6)	25.70(4.8)	19.63(5.8)

B	Days after flowering				
	14	21	28	35	42
June	0.287(0.07)	0.567(0.10)	0.884(0.13)	1.314(0.13)	-
July	0.280(0.09)	0.690(0.11)	1.201(0.14)	1.771(0.15)	1.297(0.12)
August	0.242(0.08)	0.600(0.10)	1.085(0.11)	2.800(0.14)	2.810(0.20)
September	0.212(0.06)	0.688(0.12)	1.205(0.12)	2.467(0.15)	2.890(0.21)
October	0.300(0.12)	0.728(0.11)	1.161(0.15)	2.930(0.15)	2.900(0.20)

C	Days after flowering				
	14	21	28	35	42
June	92.40	92.53	92.62	92.92	-
July	91.93	91.27	91.67	92.41	85.10
August	92.19	91.50	91.13	88.75	84.92
September	90.00	92.18	90.46	88.98	84.86
October	92.06	91.45	91.34	88.59	85.22

Table 1.- Changes in fresh weight (gr) (A), dry weight (gr) (B) and water content (%) (C) of Padron pepper fruits at different maturation stages during a five month period in 1997. Standard errors are shown in brackets.

Changes in Capsaicinoid content at different maturation levels and months

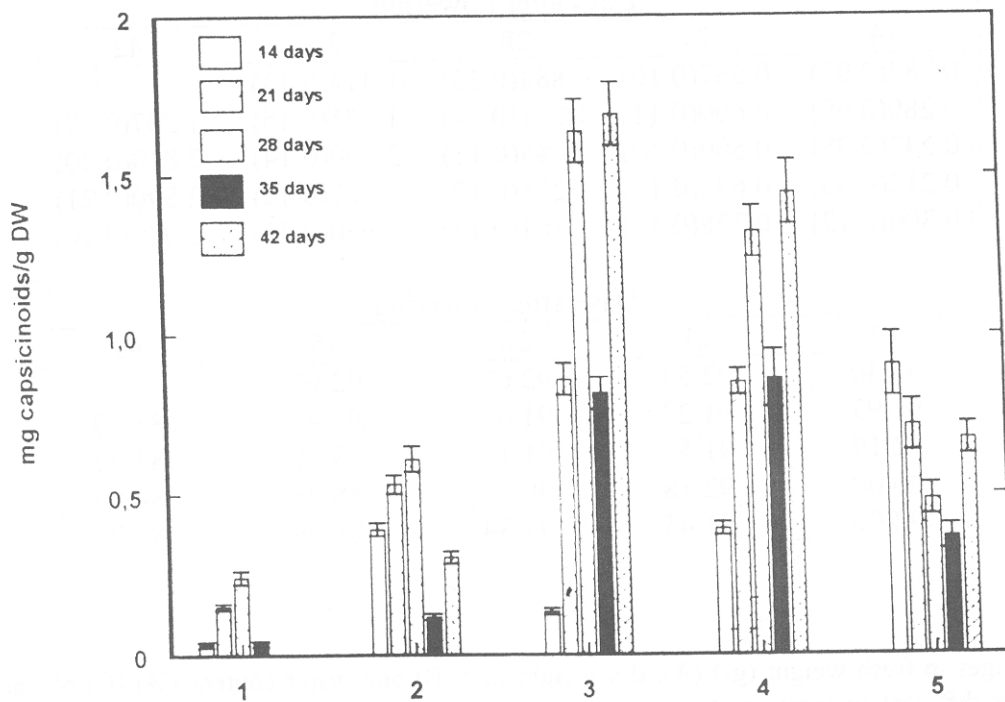
Padron pepper fruits were analyzed for their capsaicinoid content (capsaicin and dihydrocapsaicin) (Figure 1).

Seasonal change had no significant effect on the pattern of capsaicinoid accumulation - in the period from June to September. These results are in keeping with our report on the moment in time when capsaicinoids can be detected and when they reach maximum values (Estrada et al., 1997).

However, the fruits from October showed a different pattern: the highest values were found 14 days after flowering and gradually decreased, reaching minimal values at around the 35th day. On the 42nd day after flowering the capsaicinoid content had increased, but at a lower level than the values at 14 days.

These changes in the pattern of capsaicinoid accumulation, may be related to the change in the sink-source relations in the plant with the senescence process.

After determining the capsaicinoid content from June to July, we observed low levels in all the stages studied. In August and September, however, there was a considerable increase the levels of capsaicinoids, especially 42 days after flowering. In October, the capsaicinoid content began to decrease.



These results agree with the finding that capsaicinoid content is slightly higher in the fruits of summer plants than in the autumn ones (Balba et al., 1968). This may be linked to the relationship between pungency, temperature and light. Otha (1960) found that high night temperatures brought about higher capsaicinoid content and Iwai et al. (1977) reported that capsaicinoid was produced in *Capsicum annuum* var. *grossum* during post-harvest ripening under continuous light for 4 to 7 days.

It is not known which of these factors is the most important. However, in terms of seasonal changes, it may be concluded that capsaicinoid accumulation in Padron pepper fruits is highly sensitive to environmental conditions.

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Differential Parthenocarpy Ability on Selected Local Varieties of Pepper Grown in Unheated Greenhouse

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Introduction In Tunisia, low temperatures are prevailing during winter and represent a major limiting factor to increased pepper production when grown in unheated greenhouse. Low night temperatures were found to affect negatively not only the number of pollen grains, but their viability as well in most species of *Solanaceae* (Polowick and Sawhney, 1985; Fernandez-Munoz *et al.*, 1995) and indirectly affect yielding ability. The fruit produced under these circumstances are usually seedless and characterized by poor quality (Rylski, 1986)

Using genetic parthenocarpy could be an alternative to alleviate the effect of low temperatures and other potential limiting growing conditions on pepper production when grown in unheated greenhouse. However, several authors reported that parthenocarpic fruits of pepper have a limited size and are malformed (Rylski and Spigelman; 1982; Polowick and Sawhney, 1985), genetic variability of parthenocarpic fruits with acceptable size should be available in pepper germplasm. Previous investigations indicated that useful genetic variability of large parthenocarpic fruits were found in tomato (Philouze and Maisonneuve, 1978; Lin *et al.*, 1984; Corella *et al.*, 1986). In pepper, these studies are scarce

This investigation attempts to evaluate the parthenocarpy in a selected local pepper varieties and its effect on fruit quality and quality related traits.

Materials and methods Seven sweet and hot local pepper varieties ('Baklouti', 'D'hirat', 'Baker', 'Semmane', 'Meski', 'Marconi' and 'Beldi') and three breeding lines from NRAT (Hh, Hi and Hj) were used in this experiment. Seeds were germinated in 'jiffy pot-7"', seedlings with 7.,8 true leaves were transplanted during the first week of November 1997 in plastic-covered greenhouse The soil in the greenhouse was sandy loam. The fertigation and the irrigation were applied at recommended rates. Temperature evolution. was recorded using thermo hygrometer during the whole cycle of crop

Three methods of pollination were applied: natural pollination (T1), emasculation and hand pollination with pollen collected in the same variety (T.) emasculation without pollination (T 1) as reported by Rylski

(1973); Ali and Kelly (1993); Polowick and Sawhney (1985); Baker (1989). The treatment combination (Variety x method of pollination) was layout as randomized bloc design with three replications and analyzed i as a factorial experiment. A sample of twenty fruits were picked green ripe from each treatment combination ' \at two periods (February and April) in order to appreciate the relative parthenocarpic fruit quality to those developed normally. The fruits were weighed, their length was measured, and the seeds from each fruit were counted separately. Percent reduction of weight and length in parthenocarpic fruits and the ratio of weight to seed number ill natural pollination were used to evaluate the parthenocarpy ability of the different varieties.

I Results and discussion

IReduced fruit weight and length were associated with emasculation (Fig 1a and 1b) This effect was

more pronounced with emasculation without pollination (T3) than with hand pollination (T2) Differential variety response to the three treatments was noted 'I3lconi', 'Hi' and 'Hj' were found to be less sensitive to emasculation than 'Beldi' and 'Hh'. Lack of fruit set was noted in 'Baklouti' and 'D'hirat' suggesting that these varieties are recalcitrant to emasculation. This result could be partly attributed to a disruption of hormone synthesis and damaged ovary tissues in emasculated fruits (Polowick and Sawhney, 1985). Although, emasculation without pollination (T3) significantly reduced seed number per fruit, no significant difference was observed between hand and natural pollination for this trait (Fig.1c). These results were not in agreement with those reported by Rylski (1973) and Pressman *et al* (1998) and would result from a poor quantity of pollen. Previous investigations indicated that the reduction of fruit weight and length in *Solanaceae* species is attributed to a limited seed number per fruit (Maisonneuve, 1978; Rylski, 1973).

To appreciate parthenocarpy ability of the different pepper varieties, the percent reduction of fruit weight and length, and the ratio of weight to seed number per fruit were used. Using this approach, two groups

were identified. The first group include 'Semmane', 'Meski', 'Marconi', 'Hi' and 'Hj' while 'Baklouti', 'D'hirat', 'Beker' and 'Beldi' are included in the second group.

Seedless fruits from group one have a comparable weight and length as those of seeded ones (Fig. 2) whereas, an important reduction for these traits is observed in group two. However, seedless fruits obtained in the first group were always deformed. This could be attributed to low temperature as reported by Polowick and Sawhney (1985); and Baker (1989). The use of growth regulators could be an alternative to alleviate the effect of low temperature in parthenocarpic fruits as have been found in tomato (Abad and Guardiola, 1986; Corella *et al.*, 1986). In sweet pepper, gibberellin A3 was found to be effective in improving fruit quality - (Jankiewicz *et al.*, 1991~ EI Asdoudi, 1993). The ratio of weight to seed number per fruit for the ten varieties is illustrated in Figure 3. Greater ratio is observed in 'Hj', 'Hi', 'Meski', 'Marconi' and 'Semmane' of the group one, whereas, 'Baklouti', 'D'hirat' and 'Beker' of group two had the lowest ratio. The naturally parthenocarpic might be useful to develop pepper varieties adapted to a wider range of growing conditions and particularly to low temperature. In order to understand the genetic control of parthenocarpic expression and fruit related traits, an inheritance study using the selected varieties of the first group will be carried out.

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Fig.1: Effect of natural pollination (T1), hand pollination (T2) and emasculating without pollination (T3) on fruit weight (a) ; fruit length (b) and seed number per fruit (c) in ten local pepper varieties.

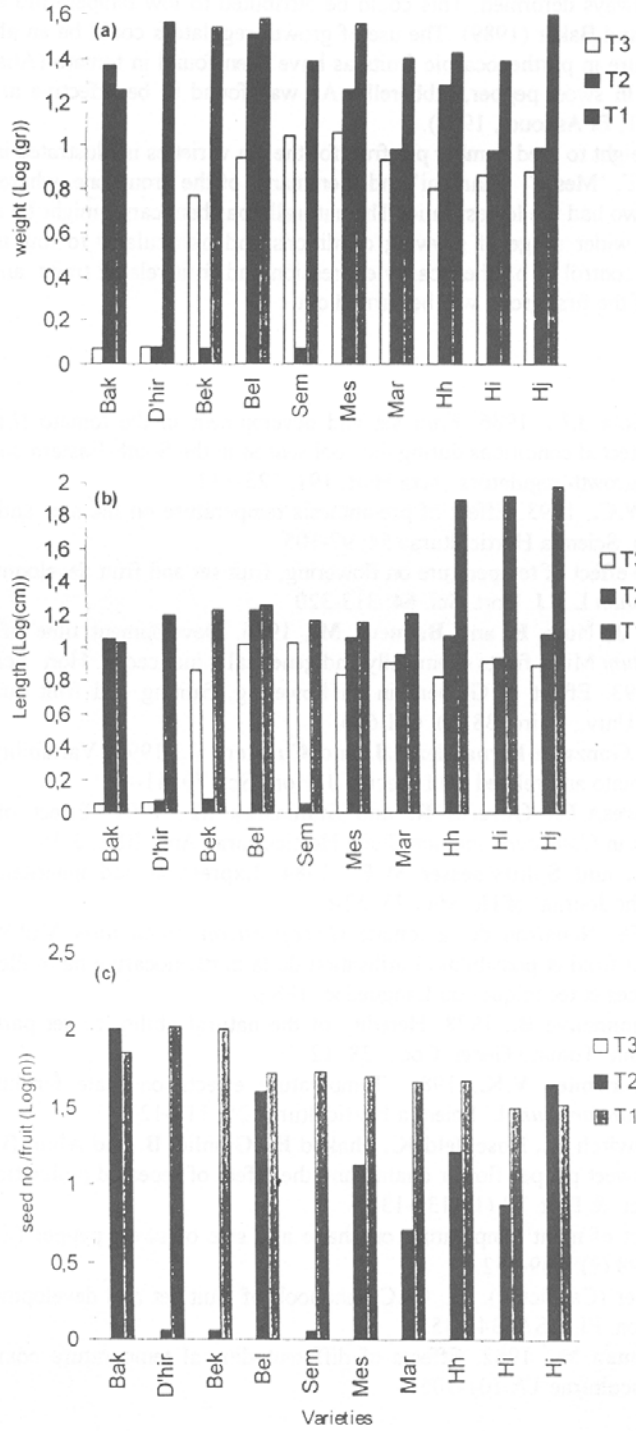


Fig.2: Reduction rate of fruit weight and length in ten local pepper varieties

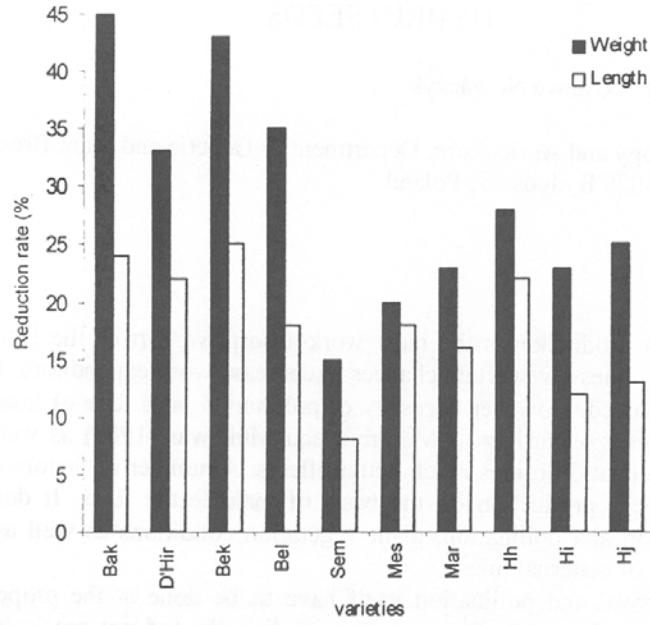
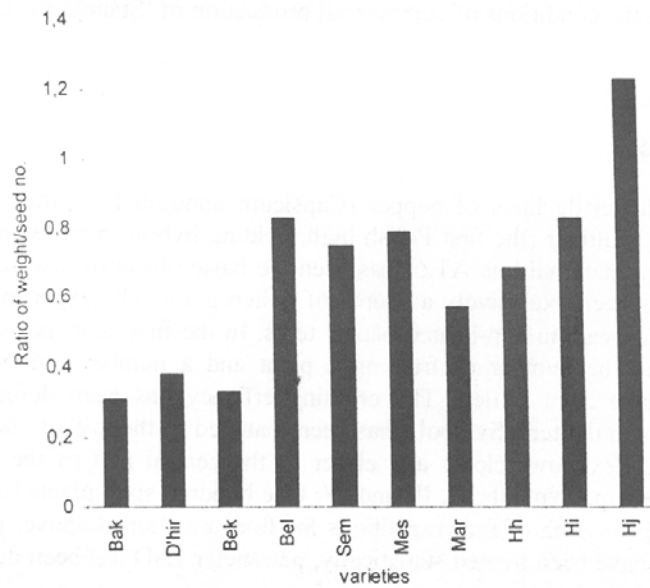


Fig.3: Ratio of fruit weight to seed number per fruit in ten local pepper varieties.



THE CROSSING EFFECTIVENESS IN THE PRODUCTION OF PEPPER .J HYBRID SEEDS Pawel Nowaczyk and Luboslawa Nowaczyk

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Introduction

A hybrid seeds production is the most work-absorptive part of the heterosis pepper breeding. Male sterility lines give certain chances to decrease work expenditure. Emasculation is possible to be eliminated. However necessity of pollination lasts. Use of insects gives not satisfactory effectiveness. According to Meshram and Mukewar (1985) as well as Gill and Gill (1995) manual pollination brings much better effects. A number of factors decide on the effectiveness of F I seeds production on the basis of male fertile lines. It depends on the method of emasculation and pollination, plant vegetation conditions as well as physiologic and genetic characters of maternal lines.

An anther removal and pollination itself have to be done at the proper phase of a flower bud development. Emasculation carried on negligently and not precisely affects in a flower dropping. Besides that, in the natural conditions also only a part of flowers turn into fruit. Crossing manipulation, being undoubtedly a stressogene factor, deepens the flower dropping inclination. This elaboration is a trial to estimate the crossing effectiveness in a production of F I pepper seeds on the basis of a male fertile maternal line. It's main purpose was to define a crossing efficacy and relation between a number of fruit as well as a mean weight of fruit and a yield of F I seeds in the conditions of commercial production of 'Stanola F I' cultivar sowing material.

Material and methods

The two male fertile lines of pepper (*Capsicum annuum* L.), which were parental forms of "Stanola F " cultivar (the first Polish high yielding hybrid) have been a material for research. Obviously a maternal line has been the basic object of research: a large-fruit male line A TM I has been exclusively a source of pollen grain. The experiments have been carried on within two years in non-heated plastic tents. In the first year the yielding efficacy and relation between the number of fruit on a plant and a number and weight of seeds included in them have been settled. The crossing efficacy has been defined for various localization of plants in the tent. Symbol I has been matched to the row of plants just against the wall of the tent, next rows closer and closer to the central part of the tent have been indicated by the following symbols: II, III and IV. One hundred sixty plants have taken a part in the experiment, in each of four repetitions for four, mentioned above, places of plants cultivation. Results have been treated statistically, parameter LSD has been defined using the Tukey test at $P=95\%$

In the next year the relation between a mean weight of fruit obtained in the crossing process and a yield of F I seeds included in them has been described. The fruit have been classified to the groups according to their weight. Eleven groups with the unit weight from 40-50g to 140-150g have been created. Later in this work, the fertility of fruit will mean a number of seeds included in them.

Results and discussion

The least number of flowers has been pollinated in the row number I (Table I). In the statistical sense it was indeed clearly lesser than for plants from the rest three rows. The similar comparison of results has been confirmed for the number of set hybrid fruit. Also the crossing pollination efficacy has been lesser for I-st row of plants.

Table I. Crossing efficacy

Row number	Number of Pollinated flowers	Set fruit	Pollination efficacy (%)
I	7,91 a	4,18 a	52,6 a
II	12,93 c	7,70 b	60,5 ab
III	12,50 c	7,80 b	63,9 b
IV	10,82 b	7,98 b	75,3 c
Mean	11,04	6,92	63,0
LSD	1,46	1,52	9,4

Results, presented above show that about two third of the total number of pollinated flowers have set fruit. It is worth to ask, is such efficacy satisfactory in the F I seeds production? The answer could be - yes, it is. However the distinct differences concerned the location of plants should be taken under consideration. It has been confirmed that much better effects have been obtained for the plants situated in the central part of the tent. Simply, there was more stable temperature, especially in the night. Microclimatic conditions in the close neighborhood of the tent wall are less favourable and, as results of the experiment show, the hybrid seeds production efficacy is much worse there.

The crossing efficacy is an important element in the process of estimation of usefulness of maternal line in a production of hybrid seeds. The yield of hybrid seeds obtained from one plant is obviously an assessing indicator. It depends on the number of fruit as well as the weight of seeds included in them. The analysis of results shows a certain difference in the weight of hybrid seeds from one fruit between groups of plants represented by different number of set fruit (Table2). '

Fruit from the plants with more than ten set fruits characterized very similar and stable weight of hybrid seeds. The number of hybrid seeds from one fruit was directly proportional to their mean weight ($r=0,818$). Groups of plants differentiated to each other with regard to the weight of 1000 seeds. The highest value of this feature has been observed for those plants with the least set fruit that means five, six or seven, with the weight of 1000 seeds over 109. The greater number of fruit on the plant the lesser weight of hybrid seeds - such general

tendency has been observed. Similar dependencies have been characteristic for the number of seeds in a fruit and the number of fruits from one plant.

Table 2. Dependencies and correlation between the number of fruit on the plant and the weight and number of seeds from one fruit as well as the weight of 1000 seeds.

Mean number of fruit on one plant Total weight of seeds in one fruit [g] Total number of seeds in one fruit Weight of seeds of seeds of on one lant in one fruit in one fruit 1000

Mean number of fruit on one plant	Total weight of seeds in one fruit [g]	Total number of seeds in one fruit	Weight of 1000 seeds [g]
5	1,61	154	10,45
6	1,45	148	10,16
7	1,50	150	10,00
8	1,45	154	9,41
9	1,51	163	9,32
10	1,33	143	9,30
11	1,35	136	9,93
12	1,31	136	9,63
13	1,35	141	9,57
correlation (r)	-0,849	-0,639	-0,606

Estimation of data concerning the yield of hybrid seeds at dependence on the fruit weight seems to be easy (Table 3). Both features, the weight of seeds in one fruit and number of them were directly proportional to the weight of a fruit. The number of seeds in one fruit increased together with the increase of a mean weight of a fruit. Although the increase was faster, up to the level of 100g of a mean weight of fruit. Within such range of changes of the mean weight of fruit also the weight of 1000 seeds increased proportionally. This increase was limited to the fruit with a mean weight below 120g.

Table 3. Dependence and correlation between a mean weight of a fruit and a number of seeds from one fruit as well as a weight of 1000 seeds.

Mean weight of a fruit [g]	Weight of seeds in one fruit [g]	Number of seeds in one fruit	Weight of 1000 seeds [g]
40-50	0.19	25	7.56
50-60	0.27	35	7.75
60-70	0.51	64	7.99
70-80	0.65	78	8.30
80-90	0.76	88	8.67
90-100	0.70	78	8.92
100-110	0.80	84	9.52
110-120	0.88	90	10.00
120-130	0.94	97	9.62
130-140	1.02	114	8.94
140-150	1.00	112	8.92
correlation (r)	0,955	0,931	0,792

Analysis of dependencies between factors deciding about the yield of hybrid seeds obtained in the process of crossing indicates that increasing number of fruit on one plant can be disadvantageous for the weight and number of hybrid seeds. The number of ten fruit on one plant was the limit, above which further decreasing of the seeds yield hasn't been observed for the line being the subject of research. Simultaneously an advantageous conjunction has been confirmed between the increasing fruit weight and the yield of seeds.

The weight of 1000 seeds studied in this experiment could be recognized as an auxiliary criterion for estimation of their quality. For a hybrid seeds producer it is obviously a secondary feature because due to a form of the seeds sale, important are either weight of seeds or number of them. The recapitulation of presented research should include some practical conclusions concerning the production of hybrid seeds. Research results by earlier mentioned authors (Meshram and Mukewar, 1985) who for male sterility lines obtained 68-100 F₁ seeds by manual pollination and 10-12 seeds from one fruit when the pollination was carried on by insects indicate that the line A TZ 1 is enough good to be used in a production. It is worth to underline that hereditary features of different genotypes (Nowaczyk and Nowaczyk, 1990) linked to their fertility can cause their uselessness in a hybrid seeds production.

The most interesting practical conclusion is doubtless an advice not to use plants situated in the close neighborhood of plastic tent walls. Low crossing efficacy for such located plants means total low efficiency of hybrid seeds.

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PCR-ANALYSIS OF F1 HYBRID DERIVED DH PEPPER LINES Gyulai G*, J.A. Gemesne**, Zs. Sagi**, L. Zatyko**, L. Heszy*, G. Venczel** *Department of Genetics and Plant Breeding, Agricultural University, Godollo, H-2103. **Vegetable Crops Research Institute, Budapest, Hungary, H-1775.

ABSTRACT. Spontaneous doubled haploid (DH-RI) plants were developed from F1-hybrids of 'blocky type' pepper (*Capsicum annuum* L. var. *annuum*, $2n=24$) in anther culture, and grown in a greenhouse for seed production to R2-generation (DH-R2) prior to flow cytometer analysis, which revealed plants with haploid (n), and diploid (2n) genome sizes. DNA samples of DH-R2 individuals were pooled in nine groups and subjected to molecular studies of RAPD-, SSR-, and ISSR-PCR analysis. All of the nine pools of DH-R2 plants were discriminated at least by one primer. In total, the 26 primers generated 54 scorable PCR bands. Of these, 19 (35.2 %) were polymorphic.

KEY WORDS. anther culture; androgenesis; DH, doubled haploid plants; PCR, polymerase chain reaction; ISSR, inter simple sequence repeats; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeats

INTRODUCTION. Plant tissue culture techniques such as mutant isolation, protoclone- and somaclone selection, etc. have supplied new genetic sources for pepper breeders, especially in resistance breeding (Rao et al. 1997). Of these, DH-techniques such as androgenic pollen-, anther- and gynogenic ovule culture are the most advanced techniques (Fari 1986). Their significance in breeding is, first, the ability to develop haploid plants with one set of chromosomes, second, to produce homozygous, doubled haploid (DH) pure lines, and third, in the case of F1-hybrids as anther donor plants, to reveal meiotic recombinants with unique genome constitutions. The aim of the study presented was to select new meiotic recombinants from F1 hybrid of blocky type pepper of DH origin for a further release of new cultivars, and to prove genetic diversity of DH plants by molecular techniques of RAPD- (Williams et al. 1990); SSR- (Gupta et al. 1994); and ISSR-PCR (Zietkiewicz et al. 1994).

MATERIAL and METHODS. *Anther culture.* F1 hybrid of intercultural crosses as anther-donor plants were grown in greenhouses. Flower buds were collected at uninucleate microspore stage. Anthers (fifteen anthers per petridish, 6 cm) were excised and laid onto a nutritive medium of Dumas de Vault et al. (1981), supplemented with 0.01 mg/12,4-dichlorophenoxyacetic acid (2,4-D) and kinetin, respectively. Sugar sources and concentrations were changed according to the modifications of Gemesne Juhasz et al. (1998). Cultures were incubated for 8 days at 35 °C in the dark, then transferred to 25 °C with a 12h photoperiod. Anthers were transplanted onto 2,4-D-free plant regeneration medium supplemented with 0.1mg/l kinetin (Dumas de Vault et al. 1981). Plantlets developed from embryos were rooted on hormone-free medium. Regenerated (R₁) plants were potted and grown in greenhouses either for flow cytometry, or R₂ - seed production. *Chromosome doubling.* For the development of induced dihaploids, the axillary buds of haploid regenerants were removed and 1% w/w colchicine was applied in gelling material (for a further analysis). *Flow cytometry.* The determination of ploidy levels was carried out by a PARTEC Ploidy Analyser. PA-I (Partec Munster, 1 Germany) equipped with a high-pressure-lamp (Osram HBO-100W/2) according to the method of Dolezel et al. (1989) prior to samples preparation. DH-R₁ pieces of leaves were chopped and 1 macerated in LBO1 (1 ml) analysis buffer to release intact nuclei. The supernatant was filtered through a Whatman 1 filter from type nylon filter with a pore size of 30 µm to eliminate cell debris. The cell nuclei were labeled with 4',6'-diamino-2-phenylidole, 14 IJ.M, (DAPI, Sigma) contained LBO1 (1ml) buffer. Each sample was measured in three replications in the control of R₀ diploid plants prior to chromosome counting ($2n=24$) of root tips in acetocannine preparation. *PCR study. DNA-isolation.* DH-RI seeds were germinated in petridishes for molecular analysis. Pooled genomic DNA samples were extracted from individuals of nine DH-RI plants, and from the control R₀ anther donor plants, respectively.

Seedlings were subjected to leaf squeezing (Ravenel Spec. Inc.) using 750 IJ.I of CTAB-buffer, followed by the method used by Dweikat et al. (1994), utilizing the modifications of Gyulai et al. (1997). DNA samples were treated with RNase, 5 I, for 30 min at 37°C. *PCR analysis.* Amplification

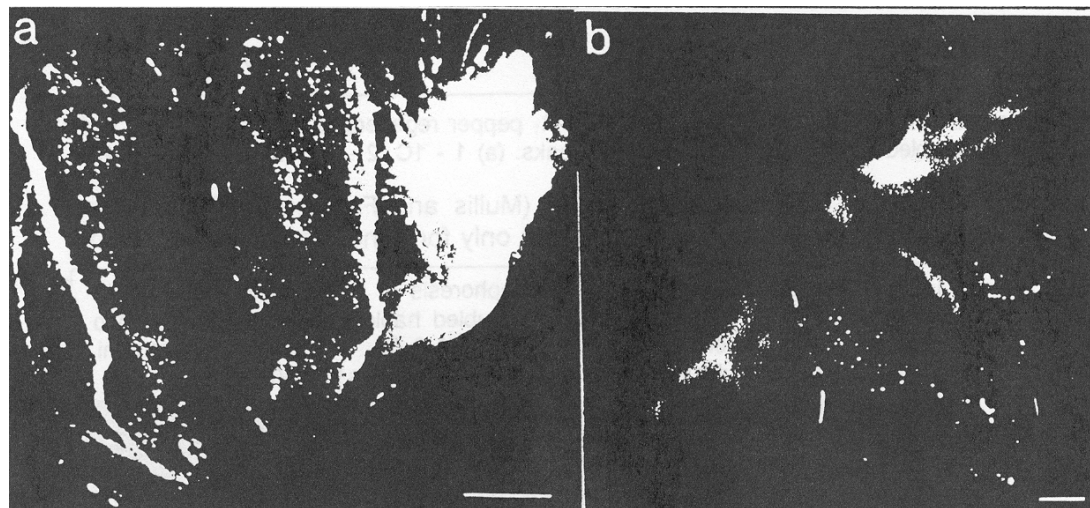
reactions were run in a volume of 25 μ l by a CoyTempCyder, 110 SM. The reaction mixture contained 15.625 μ M of each deoxynucleotide; 0.05 mM MgCl₂; 1.5 U Taq-polymerase (either Sigma, Promega, or WestTeam); 24 pM of primers (either SSSR/ISSR or RAPD/Operon Technologies); 2.5 μ l of 10 x thermophylic buffer; and 20 ng template DNA. DNA content was measured by UV-spectrophotometer (Jenway 6105) at OD260 nm. *PCR-primers*. The following primers were applied, OP/A 1-20 for RAPD analysis; (ACTG)₄, (GACA)₄ for SSR-analysis; and CA(GACA)₄, AC(GACA)₄, (GACA)-C, (GACA)₄CA for ISSR analysis. The reaction mix was overlaid with a drop of mineral oil (Sigma). For PCR amplification, 40 cycles were run with the following steps: (1) 20 sec at 94 °C for DNA denaturation prior to a preheating of samples at 84 °C for 5 min; (2) 20 sec at 40 °C for primer annealing; (3) 90 min at 72 °C for DNA polymerase reaction; (4) a final extension at 72 °C for 5 min, and a final soak at 4 °C until the samples were unloaded. A minimum of three independent DNA preparations of each line as used. Each successful reaction with scorable bands was repeated at least twice. Amplifications were assayed by agarose (1.8 %, SeaKem LE, FMC) gel electrophoresis (Owl system), stained with ethidium bromide (1 μ g/ μ l) at 80 V in 1 X TAE buffer and photographed on a UV Tran illuminator (Pharmacia) by Polaroid camera. *Fragment analysis*. Sharp PCR fragments were scored for the presence versus absence of profiles. PCR fragments at low intensities were only scored as present when they were reproducible in repeated experiments. A negative control which contained all the necessary PCR components except template DNA was included in PCR runs.

RESULTS AND DISCUSSIONS. *Anther culture*. The responsive anthers on culture media

Showed direct embryo development by the fourth to eighth week of incubation time. (table 1) The frequency of plant development, 5.06 % (152 regenerant) can be considered as a medium responsive reaction, according to the classification of Mityko et al. (1995). Of the 152 regenerants 53 (34.9% of the regenerants) were proved to be spontaneous doubled haploid by flow cytometer analysis.

Table 1. The frequencies of different responses of pepper anther culture.

N ^o of excised anthers	N ^o of responsive anthers	N ^o of regenerants	N ^o of DH-R ₁ plants
3.000	529	152	53



Flow cytometry. In the case of adult plants, it is easy to differentiate haploids from diploids based on their morphological character. However, flow cytometry has provided a rapid and precise tool to detect the ploidy level of a plant, since the first application in plant genome study (Heller 1973). It is also very important to distinguish the regenerants in the juvenile stage to subject the haploids to colchicine treatment. In the present study, flow cytometer analysis revealed 53 plants (34.9 % of the regenerants) with dihaploid (2n = 2C), and 99 plants (65.1 %) with different ploidy levels of n, 4n, and aneuploids (Fig.2). Obviously, in the

Case of haploids no further change of chromosome number was revealed, while in the case of diploids, a spontaneous chromosome doubling occurred resulting in doubled haploid DHR1 regenerants which is common

in pepper anther culture (Vagera 1990). DH-R1 plants were self pollinated for seed production for genetic diversity analysis of DH-R1 plants were self pollinated seed production for genetic diversity analysis of DH-R2 generation.

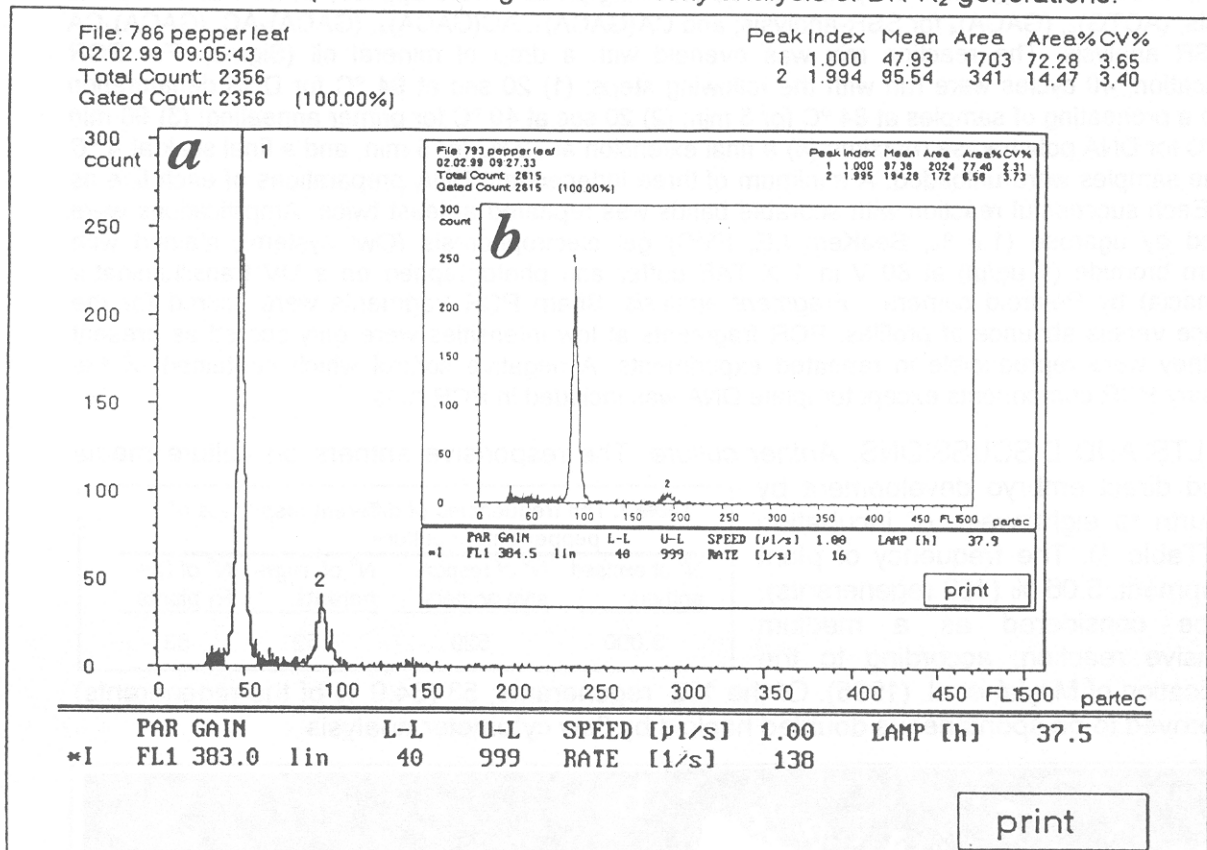
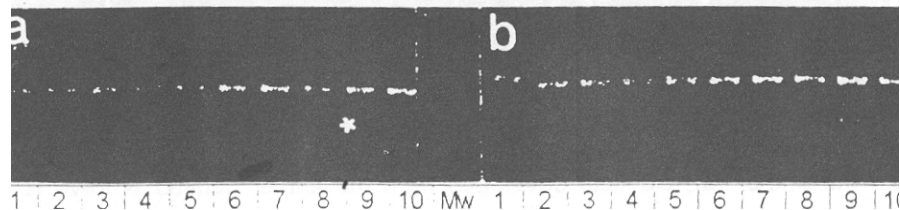


Fig2. Flow cytometer analysis of the anther derived DHR1 pepper regenerants with haploid (a) and spontaneous doubled haploid (b) genome size Peaks(a) 1-1C-2-2C (b) 1-2C,2-4C

PCR analysis. The use of PCR based techniques (Multis and Falona 1978) in pepper breeding has also become increasingly important not only for genetic analysis but also for the cultivar discrimination of plants of tissue culture origin. In our study the DNA samples of 53 anther derived DHR2 plants grouped in 9 pools showed genetic diversity analysed by RAPD SSR and ISSR-PCR in comparison to the control of Ro anther donor plants. A total of 55 loci were scored. Of these 19 (35.2%) showed polymorphism. In SSR (Gupta et al 1994) and ISSR (Zietkiewicz et al. 1994) analysis using six different primers only the (ACG) primer revealed a single polymorphic band in the 4th DH-R2 pool (Table 2) In RAPD analysis using primer of Operon set a (OP/A/1-20) 16 primer produced scorable bands of these 6 generated polymorphic bands (table) Among the polymorphic bands the primers OP/A 12, 5- TCGGCGATAG-3 amplified a uniquely discriminative band in the 8th DH-R line at 1000 bp, molecular weight marker 1000 bp increments to 1000 bp.



about a 300 bp length. (Fig.3). This marker band, *OP/A* 12-300, was named for the primer used and the products size in bp (Yang and Quiros 1993). All of the polymorphic bands generated in the experiments could originate from a locus-specific sequence rearrangement in the genome as result of the meiotic recombination

occurring during male gametogenesis, which resulted in a new RAPD- or SSR-primer binding site in the genome. In similar experiments studying the highly

heterozygous *Solanaceae* species of *S. phureja*, RAPD analysis also indicated a high level of genetic diversity among DH-lines. The 26 anther derived monoploids analyzed by 13 decamer primers revealed 54 polymorphic loci (Teparkum, Veilleux 1998).

In conclusion, spontaneous doubled haploid fertile pepper plants (DH-R1) were developed and distinguished by flow cytometer analysis. PCR analysis revealed a high level of genetic diversity among DH-R2 plants. The results proved the usefulness of androgenesis in the production of DH pepper lines, and the application of PCR marker selection in the recognition and selection of meiotic recombinants with new genome constitutions with a final aim of new cultivar release.

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Table. 2. The N° of polymorphic bands of PCR profiles in the DH-R₂ pepper plants (1st to 9th). C, control of Ro plants.

primers	N° of polymorphic bands									
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	C
(ACTG) ₄	-	-	-	1	-	-	-	-	-	-
OP/A-3	-	-	-	-	-	-	-	-	1	-
OP/A-9	-	-	-	-	-	-	1	-	-	-
OP/A-12	-	-	-	-	-	-	-	1	-	-
OP/A-16	-	1	-	1	-	-	-	-	-	-
OP/A-18	-	-	-	-	-	1	-	-	-	-
OP/A-19	1	2	3	1	2	1	1	1	1	-

INHERITANCE OF DSRNAs IN *CAPSICUM ANNUUM* GENOTYPES

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Double stranded ribonucleic acid (dsRNA) is not normally found in plants unless they are infected with an RNA virus (Morris & Dodds, 1979). Although, during the past few years, there have been reports of dsRNAs isolated from symptomless plants (Dodds et al., 1988) including pepper, (Valverde & Fontenot, 1991). Some of the dsRNAs found in pepper are the genome of Cryptic viruses (Arancibia & Valverde, 1995). Other dsRNAs do not appear to be of viral origin (Valverde et al., 1990). DsRNAs found in pepper are transmitted at high rates through the seed but not through grafting. The present research was aimed at determining how these dsRNAs are transmitted in crosses of pepper cultivars containing different dsRNAs.

MATERIALS AND METHODS

Capsicum annuum genotypes 'Yolo Wonder', 'Jalapeno M', 'Hungarian Wax', and 'Mexican Chili' were used for the crosses. With the exception of 'Mexican Chili' (dsRNA free) these genotypes contained unique dsRNA patterns (determined by polyacrylamide gel electrophoresis). DsRNA patterns were designated according to the number of dsRNA bands [0 = no bands ('Mexican Chili'), 1 = one band ('Yolo Wonder'), 2a = two close bands ('Hungarian Wax'), and 2b = two distant bands ('Jalapeno M')] (Fig. 1). All crosses were conducted in the greenhouse. Seeds resulting from crosses were planted and 12 plants selected for dsRNA analysis. DsRNA was analyzed from 3.5g of foliar tissue. Samples were extracted with phenol and dsRNA purified by cellulose chromatography (Valverde et al., 1990). DsRNAs were analyzed on 6% polyacrylamide gels. Selected plants from the F1 progeny were allowed to self pollinate in order to determine dsRNA transmission rates.

RESULTS AND DISCUSSION

DsRNA profiles of the different pepper genotypes, including dsRNA mixtures are shown in Figure 1. Schematic representation of crosses among different pepper genotypes are shown in Figure 2. Pepper dsRNAs were transmitted 100% maternally. With the exception of 'Hungarian Wax' pepper the percentage of dsRNA transmission by pollen ranged between 20 and 75%, suggesting that dsRNAs from these pepper genotypes are localized in the cytoplasm. Transmission rates of dsRNA by pollen for 'Hungarian Wax' pepper were 100%, suggesting that this dsRNA may be localized in the nucleus. DsRNA type 2b ('Jalapeno M') was reported to be the genome of a cryptic virus (Arancibia & Valverde, 1995). Virus-like particles have been found in 'Hungarian Wax' as well (Valverde, unpublished). DsRNA analysis of seedlings from self-pollinated plants from the F1 progeny resulted in 100% transmission. These results suggest that once a plant "acquires" a particular dsRNA it will pass it to the progeny as long as it is self-pollinated. The role of these dsRNAs in pepper plants is unknown.

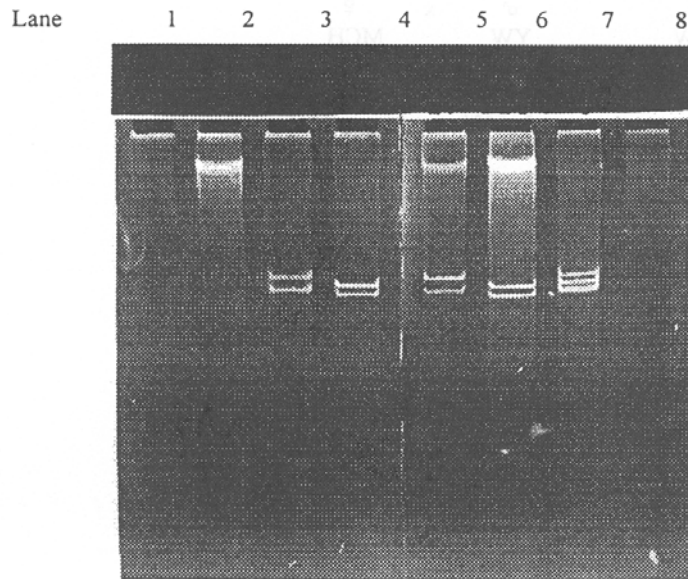


Fig. 1. Polyacrylamide gel electrophoresis with dsRNAs extracted from pepper plants. Lane 1 and 8, 'Mexican Chili' (0); lane 2, 'Yolo Wonder' (1); lane 3, 'Jalapeño M' (2b); lane 4, 'Hungarian Wax' (2a); lane 5, 'Yolo Wonder' and 'Jalapeño M' (1+2b); lane 6, 'Yolo Wonder' and 'Hungarian Wax' (1+2a); lane 7, 'Hungarian Wax' and 'Jalapeño M' (2a+2b); and lane 8, 'Mexican Chili'.

MCH	YW	YW	MCH
(0)	(1)	(1)	(0)
1=100%		1=20%	
0=?		0=80%	

MCH	HWX	HWX	MCH
(0)	(2a)	(2a)	(0)
2a=100%		2a=100%	
O=?		O=?	

YW	HWX	HWX	YW
(1)	(2a)	(2a)	(1)
2a=50%		2a=50%	
1+2a=50%		1+2a=50%	

YW	JAM	JAM	YW
(1)	(2b)	(2b)	(1)
2b=40%		2b=25%	
1+2b=60%		1+2b=75%	

<i>JAM</i>	HWX	HWX'	<i>JAM</i>
(2b)	(2a)	(2a)	(2b)
2a=90%		2a= 5 0%	
2a+2b=10%		2a+2b=500..	

Fig. 2. Crosses among different pepper genotypes containing different dsRNAs and their inheritance. MCH='Mexican Chili', YW='Yolo Wonder', JALM='Jalapeno M', and HWX='Hungarian Wax'.

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Analysis of seed proteins by polyacrylamide gel electrophoresis (PAGE) in diploids, tetraploids and tetraploid hybrids of *Capsicum*

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Proteins are attractive for direct genetic study because they are primary products of structural genes. Any change in the coding sequence of gene generally reflect corresponding change in the primary structure of its protein. Analysis of proteins and isozymes by electrophoresis is an additional tool for supplementing the evidence obtained by comparative morphology, breeding experiments and cytological analysis. They are also used in evolutionary studies in establishing origin and phylogenetic relationships in several crop plants. In chilli, work pertaining to the electrophoretic analysis of proteins and isozymes was restricted to species and varietal differentiation, but comparative work on diploids and tetraploids was initiated for the first time in our laboratory. In the present study, seed proteins were analysed by electrophoresis involving diploids, tetraploids and tetraploid hybrids.

Materials and methods

The capsicum varieties employed in the present investigation include 7 stabilized autotetraploid varieties viz., x180, x206, Santaka, Jawahar, Tc1 Sel, Lec21 of *C. annuum* and one of *C. chinense*: their corresponding diploid varieties and tetraploid hybrids developed through breeding programme. Soluble seed proteins were extracted by SDS - polyacrylamide gel electrophoresis (PAGE) technique of Weber and Osborn (1969) with slight modifications. The degree of electrophoretic similarity of the materials was determined by calculating the similarity index 's' for each variety (Vaughan and Denford, 1968).

Results and Discussion:

The electrophoretic patterns of soluble seed proteins in diploids, tetraploids and tetraploid hybrids are presented in electrophorograms (Fig. 1). The results, in general, showed that variation in number and intensity of protein bands within the parental varieties was relatively narrow compared with that of tetraploids and tetraploid hybrids. In diploids, the number of bands ranged from 7 (x180, Santaka, Tc1 and Sell) - 9 (Jawahar and Lec21) and they are in the mobility range of 0.03 - 0.7. In tetraploids, the number of bands ranged from, 7 (Lec21) - 9 (x180, x206, Jawahar and Tc1) and they are in the mobility range of 0.03 - 0.70. In tetraploid hybrids, the number of bands ranged from 7 (x180 X x206, Jawahar X x206, and Jawahar X Santaka) - 10 (Jawahar X *C. chinense* and sel IX Santaka) and they are in the mobility range of 0.03 - 0.75. In all tetraploids, a

common band was observed at Rf value 0.25 while in diploids it was observed in 5 varieties only. In x180, Santaka and Sell it was not present. In all tetraploid hybrids also, a common band was observed at Rf value 0.25 except in Santaka X x180 and Jawahar X Tc1.

The amount of variation in the protein pattern of the hybrid sample is directly related to its parental variety, as indicated by percentage of Similarity Index (Table 1). The homology varied from 7.9 - 75.00% among diploids, 7.69 - 55.55% among the tetraploids and

5.55 - 100.00% among tetraploid hybrids. When tetraploids are compared with diploids, the Similarity Index varied from 6.67 75.00%.

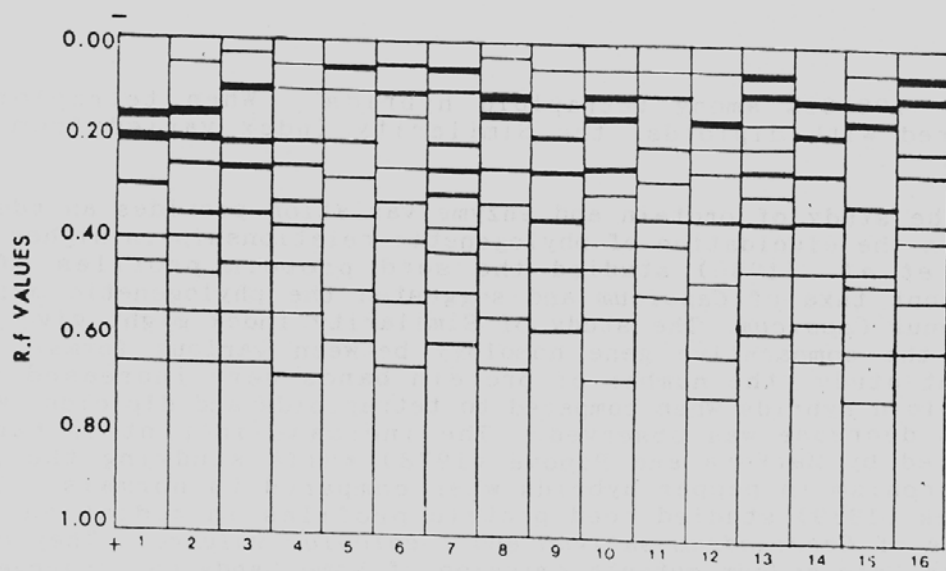
The study of protein and enzyme variation provides an additional clue in the elucidation of phylogenetic relationship in higher plants. Panda *et al.* (1986) studied the seed protein profiles of eight different taxa of *Capsicum* and suggested the phylogenetic origin for the genus *Capsicum*. The study of Similarity Index might give an idea about the comparative gene homology between various forms. In the present study, the number of protein bands were increased in some tetraploid hybrids when compared to tetraploids and diploids, while in some a decrease was observed. The increase in protein bands was observed by Markova and Popova (1978) while studying the protein polymorphism in pepper hybrids when compared to normals. Das and Mallick (1989) studied seed protein profiles in radiation induced mutants of *Corriandrum sativum* and *Foeniculum vulgare*. They observed that in *Corriandrum* mutants omission of some bands was observed while in *Foeniculum* mutants addition of some bands was observed when compared to the normal plants and they inferred that it may be due to stable gene mutation induced through gamma-irradiation or due to stability of beneficial gene alteration through point mutation at low dosages of gamma rays.

In the present study, the absence of some parental bands in the hybrids can be attributed to the suppression of the action of certain genes. In some tetraploids and tetraploid hybrids addition of some protein bands was observed which may be due to the addition of genes due to hybridization.

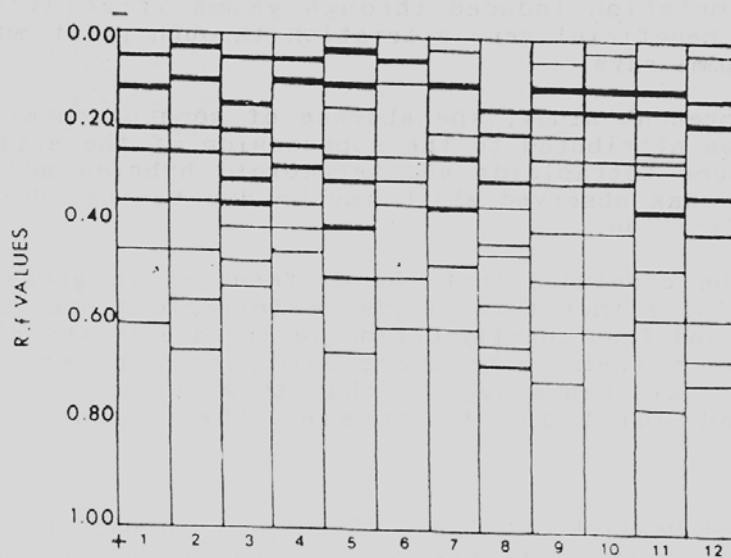
It can be concluded that the differences in gross fractions of the proteins of either tetraploids or tetraploid hybrids, though not structurally and functionally definable are due to the altered genetic constitution in them. The close similarity between parents and hybrids in certain bands suggest that these are closely related, and addition and deletion of certain other bands reflect genic differences.

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A



B

Electrophoretograms of soluble seed proteins in

(A) Diploids and tetraploids of *Caesalpinia*

1,2 : x180 diploid and tetraploid

3,4 : x206 diploid and tetraploid

5,6 : Santaka diploid and tetraploid

7,8 : Jawahar diploid and tetraploid

9,10: Tc_1 diploid and tetraploid

11,12: Sel_1 diploid and tetraploid

13,14: Lec_{2H} diploid and tetraploid

15,16: *C. chinense* diploid and tetraploid

(B) Tetraploid hybrids

(1) x180 X x206

(2) x180 X Santaka

(3) x206 X Jawahar

(4) Santaka X x180

(5) Santaka X Sel_1

(6) Jawahar X x206

(7) Jawahar X Santaka

(8) Jawahar X Tc_1

(9) Jawahar X *chinense*

(10) Sel_1 X x206

(11) Sel_1 X Santaka

(12) *chinense* X x206

ECO-FRIENDLY MANAGEMENT OF VIRAL DISEASES OF CHILLI BY NON-HOST BARRIER TRAP CROPS P. Dhawan and N. Rishi

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Key words: Barrier Crops, Management, Chilli, Viral diseases. Introduction:

Chilli (*Capsicum annum* L.) suffers a heavy yield loss due to a large number of viral diseases (Anjaneyulu and Apparao, 1967; Rishi and Dhawan, 1989). Breeding resistant chilli cultivars is a time taking process (Rishi and Dhawan, 1988; Dhawan *et al*, 1996; Arora *et al*, 1996; Pandita *et al*, 1995). Use of insecticide has not been helpful because of non-persistently transmitted viruses. An attempt was therefore made to study the use of non-host barrier trap crops in the management of viral diseases of chilli.

Material and Methods:

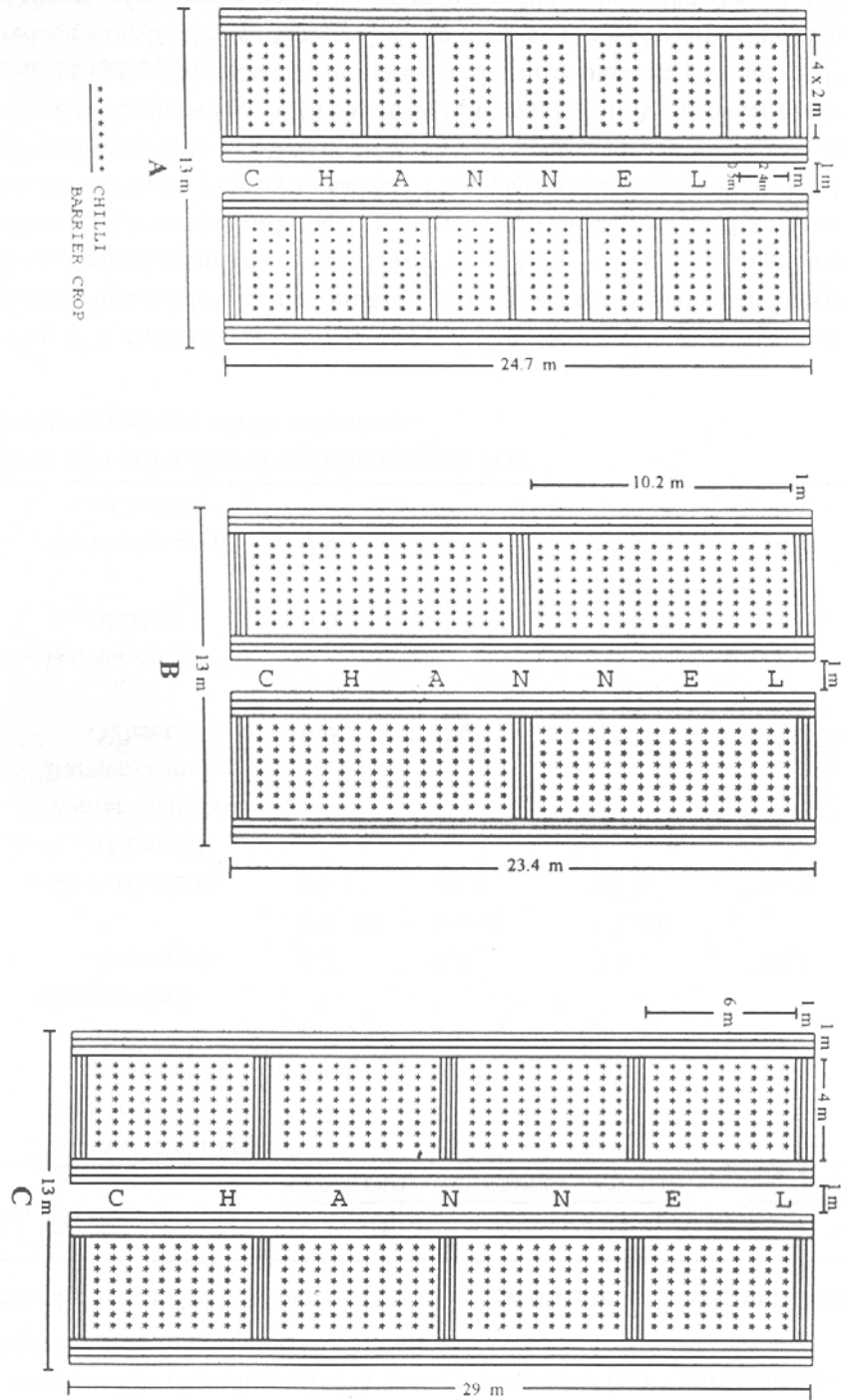
Field plan, barrier crops and observations –

Three field plans A,B and C were tried in the first year pilot experiment as shown in Figure. Field plan C was used for all further studies, Barriers of pearl millet and sesamum for summer crop and wheat and barley for winter crop were planted 20 days earlier than the transplantation of the main chilli crop. Data on disease incidence was recorded at 15 days interval. Yield was calculated on the basis of 4 pickings of green mature fruits at interval,

Results and Discussion:

Viral diseases can be reduced by altering Vector efficiencies through crop management strategies such as use of non-host barrier crops, In the present study field plan C was found to be the most suitable as there was a problem of lodging and shadowing by barrier crops in plan A and ineffective avoidance of vectors in plan B. Sesamum as barrier for summer crop and wheat as barrier for winter crop were found more effective in viral disease management and enhancement of yield of chilli fruits (Table). Use of narrow strips of 3 rows one ft. apart of barley as barrier crop for cauliflower seed beds reduced virus incidence

FIG. BARRIER CROPS IN CONTROLLING VIRAL DISEASES OF CHILLI-FIELD PLAN



to

about one-fifth (Broadbent, 1964). In the present study barley gave a marked reduction in mosaic disease redemption (70%) over control.

Table: Effect of non-host barrier crops on viral disease incidence and yield in chilli*

S.N.	Treatment	Disease Incidence (%)			Yeild	
		Leaf curl	Bunching	Mosaic	Kg/plot	Q/ha
1.	Summer chilli crop Barrier crop pearlmillet	10.0 (59.19)	13.5 (46.0)	5.2 (48.0)	27.0	112.99
	Barrier crop Seasamum	5.2 (78.78)	8.4 (66.4)	2.5 (76.0)	32.4	134.10
	No barrier crop (Control)	24.5	25.0	10.0	22.3	92.99
2.	Winter Chilli crop Barrier crop wheat	0.0	0.0	6.0 (90.8)	25.2	105.08
	Barrier crop Barley	0.0	0.0	15.0	21.6	90.07
	No barrier crop (control)	0.0	0.0	65.0	14.4	60.64

* Mean of 4 years data of replications each year Disease redemption within parenthesis.

Sowing of 2 rows of pearlmillet 3 weeks before seeding pea crop followed by use of Disulfoton insecticide to barrier resulted in a significant disease reduction (cowpea mosaic) and yield enhancement in cowpea (Gay *et al*, 1973) A disease redemption of 59.19%, 46 0% and 48.0% in leaf curl, bunching and mosaic with pearl millet as barrier was achieved in the present study. Primary spread ofPYV in pepper reduced to 50% by bordering plots with sunflower, 70% by planting 50 ft.,swath of beans outside sunflowers and 85% by spraying the beans weekly with parathion (Simons, 1957). A one meter barrier of maize around bell pepper was found to be more effective than soyabean, amaranth and frenchbean in reducing the incidence of mosaic disease complex caused by viruses belonging to poty and cucumo virus groups with 50% increase in yield (Chowfla and Sharma, 1990). Efforts for the selection of proper barrier crop to avoid insect population and manage the disease

incidence with enhancement in yield should be encouraged to overcome the problems related to use of insecticides.

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PERFORMANCE OF SOME PEPPER SELECTIONS SCREENED FOR RESISTANCE TO THE POTYVIRUSES THAT COMMONLY INFECT PEPPERS

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Introduction

The most important virus diseases of peppers worldwide belong to the potato virus Y group of viruses. These include pepper veinal mottle virus (PVMV), pepper mottle virus (PeMV), potato virus Y (PVY) and tobacco etch virus (TEV) (Alegbejo, 1983). The study was conducted to screen indigineous as well as exotic pepper cultivars against the common potyviruses that infect peppers.

Materials and Methods

Standard extracts of PVMV-ss, PeMv-AZD, PVY(NC-57), and TEV(PV-69) were prepared separately by triturating infected leaves with 0.1 M phosphate buffer (pH 7.0) in a ratio 1:l(W/V). These were then strained through two layers of cheesecloth. Ten seedlings of each pepper cultivar at four leaf stage were separately inoculated with each virus on carbo- rundo (600 mesh) dusted leaves. Two healthy plants of each cultivar inoculated with sap from healthy plants served as controls. The experiment was repeated two times. The trial was conducted in the cool-dry months of January to February and the warm humid months of August to September 1987. Severity of Symptoms on each cultivar was scored using a scale of 1-7, where: 1 = No visible symptoms; 3 = Mild leaf mottling but no leaf distortion; 5 = Moderate leaf mottling distortion and slight stunting of the plant; 7 = Very severe leaf mottling, distortion and severe stung of the Plant. Resistance was determined using the scale below:

<u>Rating</u> <u>severity</u>	<u>Percentage infection</u>	<u>Disease</u>
Resistant	1.0 - 15.9	1.0 - 2.9
Moderately resistant	16.0 - 25.9	3.0 - 4.9
Moderately susceptible	26.0 - 36.9	5.0 - 6.9
Highly susceptible	37 and above	7.0

Results and Discussion

Only Tca 14 was resistant to PVMV-ss during the cool-dry months of January to February (Table I), while L2289, California mild, Kimba and OY2 were moderately resistant. All other cultivars were either moderately susceptible or highly susceptible. The cultivars were either moderately resistant, moderately susceptible or highly susceptible to PeMV, PVY and TEV.

The pepper cultivars were more susceptible to PVMV-ss during the warm humid months of August to September. TCa 14 was resistant (Table 2) while only 12289 was moderately resistant. The cultivars were either moderately resistant, moderately susceptible or highly susceptible to the other viruses. The obvious differences in the performance of the pepper cultivars during the two periods indicate that environmental conditions could affect the reaction of peppers to infection by viruses under greenhouse conditions. Should out- break of PVMV, PeMV, PVY and TEV occur in Nigeria, there are resistant or tolerant cultivars to the viruses.

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Table 1. Performance of thirty one pepper cultivars screened for resistance to four potyviruses in the cool-dry months of January and February 1987

Virus	Resistant cultivars	Moderately resistant cultivars	Moderately susceptible cultivars	Highly susceptible cultivars
Pepper veinal mottle virus (Samaru strain)	TCal4	L2289, California mild, Kimba, OY2	Dantscga, Sakarho, Danmeyere, L2164, OY1, OY3, S01, S02, S03, KD1 KD2, KD3, KD4, KN1, KN3	L2190, L2191, Caloro, Anaheim, Hungarian yellow wax, Sakarho, Ex-Hunkuyi, L3874, OY4, S04, KN2
Pepper mottle virus (Arizona-Datura strain)	-	California mild, Kimba, Dantsiga L2289, L3874, L2164, L2025, L2191, OY1, OY2 & OY3	Anaheim, Dameyere, TCal4, OY4, S02, S03, S04, KDI,	L2190, Sakarho, Ex-Hunkuyi, Caloro, Hungarian yellow wax, S01, KDI, KD3, KD4, KN1, KN2, KN3,
Potato virus Y (North Carolina strain 57)	-	L2025, L2164, TCal4 California mild, Dantsiga, L3874 L2191, Hungarian yellow wax, OY3 & OY4	L2289, Kimba, L2190, Danmeyere, Caloro, OY1, S01, S02, S04 KN1, KN2, EX-Hunkuyi	Sakarho, Anaheim, OY2, S03, KDI, KD2, KD3, KD4, KN3
Tobacco etch virus (strain PV-69)	-	L2164	Dantsiga, L3874, L2191, TCal4, OY3, KN1, KN3, Dameyere, Ex-Hunkuyi, Sakarho,	L2289, California mild Kimba, L2190, L2025. Caloro, Anaheim, Hungarian yellow wax OY1, OY2, OY4, S01, S02, S03, S04, KDI, KD2, KD3, KD4, KN2.

Table 2. Performance of thirty one pepper cultivars screened for resistance to four potyviruses in the warm-humid months of August and September 1987.

Virus	Resistant cultivar	Moderately resistant cultivars	Moderately susceptible cultivars	Highly susceptible cultivars
Pepper veinal mottle virus (Samaru strain)	Tca 14	L2289	California mild, Kimba Danmeyere, L2164, OY1, OY2, OY3, S02, S03, KD2, KD4, KNI, KN3	L21690, L2025, Dantsiga Sakarho, L3874, L2191, Caloro, Anaheim, OY4, S01, S04, KD1, KD3, KD2. Hungarian yellow wax, Ex-Hunkuyi
Pepper mottle virus (Arizona-Datura strain)	-	Dantsiga, California mild	L2289, L2025, L3874, L2191, L2164, Ahaheim, TCal4, OY1, OY2, OY3, OY4, S04, KNI	Kimba, L2190, Sakarho, Ex-Hunkuyi, Danmeyere, caloro, Hungarian yellow wax, S01, S02, S03, KD1, KD2, KD3, KD4, KN2, KN3
Potato virus Y (North Carolina strain 57)	-	L2025, L2164, TCal4, OY4	L2289, California mild, Kimba, Dantsiga, Ex-Hunkuyi, Danmeyere, L3874, caloro, L2191, OY1, S02, OY3, KNI, KN2 Hungarian yellow wax,	OY2, L2190, Sakarho, Anaheim, S01, S03, S04, KD1, KD2, KD3, KD4, KN3
Tobacco etch virus (strain PV-69)	-	-	Sakarho, Ex-Hunkuyi, L3874, L2191, L2164, OY3, KN3, Sakarho.	L2289, California mild, Kimba, L2190, L2025, Dantsiga, Danmeyere, caloro, Anaheim, Hungarian yellow wax, OY1, OY2, OY4, S01, S02, S03, S04, KD1, KD2, KD3, KD4, KNI, KN2

EFFECT OF SINGLE AND MIXED INFECTION OF PEPPER
VEINAL MOTTLE POTYVIRUS AND/OR PEPPER LEAF CURL GEMINIVIRUS ON THE
YIELD OF CHILLI AND SWEET PEPPERS IN NORTHERN NIGERIA

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Introduction

Pepper veinal mottle potyvirus (PVMV) and pepper leaf curl Gemini-virus (PLCV) sometimes occur in mixed infection both in the dry and wet seasons in pepper and in blacknight shade (*Solanum nigrum* L.) (Alegbejo, 1994) in northern Nigeria. The two viruses thrive all the year round in "Fadama" areas (Alegbejo, 1978; Ricketts, 1982). PLCV and PVMV are transmitted by *Bemisia tabaci* Gen. and *Persicae Sulzer* respectively (Alegbejo, 1986 & 1994). This study was therefore conducted to investigate the effect of single and mixed infection of these viruses on pepper yield.

Materials and Methods

Seedlings of Tattasai pepper cv. L5962-2 and chilli pepper cv. Sakarho were raised in a glasshouse, and transplanted into the field in mid-January (dry season trial) in 1994. The experiment was laid out as a split-plot design using one pepper cultivar per sub-plot. There were ten rows and one hundred plants per sub-plot. Treatments were replicated five times.

Two sets of five plants, one infected with PVMV and the second set infected with PLCV were transplanted into each of the four sides of each sub-plot to serve as source of inoculum. Each set of infected plant was also infested with the respective vector of the virus it was carrying. PVMV and PLCV-infected plants in each sub-plot were monitored weekly. Four weeks later, ten plants each infected with PVMV, PLCV and a mixed infection of PVMV and PLCV as well as ten healthy plants in each sub-plot were tagged. Their respective yields (ripe fruits) were taken and yield/ha estimated.

Results and Discussion

Yield loss under field conditions due to PLCV on *Capsicum annum* L. cv. L5962-2 (Tattasai) in the 1994 dry season (Table 1) was 1.10 t/ha while on *C. Frutescens* L. cv. Sakarho (chilli) it was 0.95 t/ha. Losses due to PVMV on pepper cv. L5962-2 was 0.39 t/ha while on Sakarho it was

0.30 t/ha. Losses due to mixed infection of PVMV and PLCV on L5962-2 was 0.94 t/ha while on Sakarho it was 0.64 t/ha. In the 1994 wet season, losses of 1.59 and 1.44 t/ha occurred on L5962-2 and Sakarho respectively due to PVMV while losses of 0.32 and 0.31 t/ha respectively occurred due to PLCV (Table 1). A mixed infection of PVMV and PLCV caused losses of 0.8 and 0.7 t/ha respectively on L5962-2 and Sakarho.

These results indicate that there was an interference between PVMV and PLCV. PVMV caused more yield loss during the wet season while PLCV caused more yield loss during the dry season probably because of the abundance of the aphid and whitefly vectors respectively during these periods. In both seasons, yield losses caused in both cultivars by a mixed infection of both viruses were significantly higher than the losses caused solely by PLCV, however, this was not the case for sole infection of PVMV. The losses were significantly higher than in the mixed infections. A mixed infection of both viruses during the wet and dry seasons therefore poses a threat to the effective production of both tatasai and chilli peppers in northern Nigeria.

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Table 1. Yield loss due to infection of pepper by pepper vein mottle Potyvirus (PVMV) and/or Pepper leaf curl Geminivirus (PLCV) in the field at Samaru during the 1994 dry (January - April) and wet (June - October) seasons.

Cultivar	Yield (tons per hectare)			PVMV + PLCV infected	Mean	S.E.D.(P=0.05)
	Healthy	PVMV infected	PLCV infected			
Dry Season						
Tattasai	2.60	2.21	1.50	1.66	1.99	0.40
Sakarho	2.53	2.23	1.65	1.89	2.08	0.35
Mean	2.58	2.22	1.58	1.75	2.03	0.37
S.E.D. (P=0.05)	0.51	0.30	0.25	0.35	0.30	
Wet Season						
Tattasai	2.65	1.06	2.33	1.85	1.97	0.39
Sakarho	2.61	1.17	2.30	1.87	1.99	0.36
Mean	2.63	1.12	2.32	1.86	1.98	0.38
S.E.D. (P=0.05)	0.50	0.25	0.30	0.20	0.23	

RESISTANCE TO PepMoV AND PVY-O FROM AVELAR ARE CONTROLLED BY DISTINCT RECESSIVE GENES AND EVWENCE FOR INDEPENDENCE BETWEEN *pvr3* AND *pvr5*

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Introduction

Among the five well-described potyviruses infecting pepper, the potato virus Y (PVY) occurs world-wide but is most prevalent in warmer areas (Green and Kim, 1991). The classification of the pepper PVY isolates led to the definition of three pathotypes -0, -1 and -1,2 according to their virulence on the *pvr2* resistance system (Gebre-Selassie et al., 1985).

The development of pepper cultivars with genetic resistance to potyviruses is one of the most practical, economical and environmentally secure strategies for reducing important losses caused by this group of viruses. Three major resistance genes effective against different PVY pathotypes were already described in different pepper accessions (for review see Kyle and Palloix, 1997) : the recessive genes *pvr2*/ (from *C. annuum* 'Yolo Y') and *pvr5* (from *C. annuum* 'Criollo de Morelos 334') are effective only against PVY-O, *pvr~* (from *C. annuum* 'Florida VR2') is effective against both PVY-O and PVY-1 and until now, the dominant gene *Pvr4* (from 'Criollo de Morelos 334') was never overcome by all tested PVY isolates, including PVY -1,2. In addition to these simply inherited factors, several quantitative trait loci (QTLs) for PVY resistance have been mapped and one major QTL was detected in the vicinity of the *pvr2* locus in Perennial suggesting a possible allelism relationship between major genes and QTLs (Caranta et al., 1997). One of the characteristics of these resistance factors is that some of them like *pvr5* are pathotype-specific while others are involved in the resistance to several potyviruses ; *pvr 2* controls both PVY and common strains of tobacco etch virus (TEV) and *Pvr.J* controls PVY and pepper mottle virus (PepMo V).

In 1973, Litter and Cook reported an extreme resistance to PVY pathotype 0 and TEV in the *C. annuum* line 'Avelar', in addition to a monogenic recessive and partial resistance to PepMoV. Partial resistance to PepMoV was attributed to the *pvr3* locus and *pvr3* was shown to have no discernible effect on TEV (Blauth et al., 1998). At this moment, the genetic determinism of resistance to PVY from this source and the relationship between resistance to PVY and PepMo V remained unclear.

The first objective of this work was to test the allelism between *pvr 5* and *pvr 3*. This necessitates to elucidate the genetic determinism of resistance to PVY -0 from 'Avelar' and to determine whether *pvr 3* is involved in this resistance.

Material and methods

The PVY -0 resistant line 'Avelar' (pI 342940) was supplied by M. Vandenberg (Peto Seeds, USA). 'Volo Wonder' was used as PVY susceptible parent. For allelism tests, 'Avelar' was crossed with the doubled-haploid line 'C69' issued from the F 1 hybrid between 'Criollo de Morelos 334' and 'Yolo Wonder'. This doubled-haploid line was chosen because it contains only the *pvr5* locus involved in complete resistance to PVY -0.

PVY resistance tests were performed using the T 0- 72 strain that corresponds to the pathotype O. In each resistance test, a differential host-range to distinguish PVY pathotypes (with the genotypes 'Yolo V', 'Florida VR2' and 'Criollo de Morelos 334') was included to be sure we were working with the good pathotype.

PepMoV resistance test was performed using a strain originating from Texas (USA). Inoculum and inoculation procedure were as described previously (Caranta and Palloix,- 1996). Plantlets were mechanically inoculated at the I -leaf stage and transferred into a growth- chamber after inoculation. Plants were scored for symptoms 4 to 5 weeks after inoculation and plants without obvious symptoms were evaluated for presence/absence of the virus by the DAS-ELISA method. Segregation data were compared to theoretical segregation ratios by a chi-square goodness-of-fit tests.

Results and Discussion

Inheritance of resistance to PVY pathotype 0 in *C. annuum* 'Avelar'

Four weeks after inoculation, the PVY -0 strain To- 72 induced systemic vein clearing and mosaics on 'Yolo Wonder' leaves. The line 'Avelar' never showed any symptoms after mechanical inoculation with PVY -0. The F I hybrid (Avelar X Yolo Wonder) developed a systemic necrotic reaction when inoculated with PVY -0 indicating the resistance is recessive. Among the 225 backcross progenies [(Avelar X Yolo Wonder) X Avelar] inoculated with PVY - 0, 120 developed mosaic symptoms and/or necrosis on the upper leaves and 105 remain symptoms free. All these visual evaluations were confirmed by DAS-ELISA serological tests using the PVY -10E3 monoclonal antibody from Ingenasa (Madrid, Spain) Observed segregation for resistance to the pathotype 0 of PVY was consistent with a 1 resistant: 1 susceptible ratio (32=1, P=31.7%), suggesting that one recessive gene is involved in 'Avelar' resistance to PVY -0.

Is PVY 3 controlling resistance to PVY -0 ...

To determine whether the recessive gene involved in ' Avelar' resistance to PVY -0 is *pvr 3*, the 105 backcross progenies [(Avelar X Yolo Wonder) X A velar] resistant to PVY-O were inoculated with PepMoV. Inoculation was performed on the two upper leaves. Twenty six days after Pep\10 V inoculation, each backcross (BC) plant ~'as tested for presence/absence of PVY using the PVY -I OE3 monoclonal antibody and for presence/absence of PepMo V using the alkaline phosphatase conjugated antibody for potyvirus group from Agdia (IN, USA) Among the 105 BC plants tested, none was positive using the P\Y-IOE3 monoclonal antibody and 57 were positive using the antibody 'for potyvirus group. These results indicated that among the 105 BC plant resistance to PVY-O, 57 were susceptible to PepMoV and 48 were resistant. This observed ratio for PepMo V resistance corresponds to the segregation of the *pvr3* resistance gene (32=0.77, P=38%). According to this data, we can conclude that a recessive gene independent from *pvr 3* controls resistance to PVY -0 in the *C. annuum* line , Avelar'.

Allelism test between 'Avelar' and 'C69' for PVY -0 resistance

We tested the allelic relationship between the PVY -0 resistance recessive gene from 'Avelar' and the *pvr5* gene also involved in PVY -0 resistance from the doubled-haploid line 'C69'. No symptoms were observed on 'Avelar', 'C69' and 20 F1 (Avelar X C69) plants inoculated with PVY -0 and no viral coat protein was detected by ELISA test suggesting that PVY -0 resistance in these accessions was allelic.

To confirm these results, 217 backcross progenies [(Avelar X C69) X C69] and 294 reciprocal backcross progenies [(Avelar X C69) X Avelar] were tested for resistance to PVY-O. Among the 511 plants tested, 7 (5 from the first BC and 2 from the second BC) presented typical symptoms of PVY (i.e., 1.36% of susceptible plants). These visual observations were confirmed by ELISA tests.

Two main hypotheses can explain these results: (i) an allele of *pvr 5* is present in the line 'Avelar' and is involved in PVY -0 resistance but in the present resistance test, overcoming of the resistance occurred in few plants (1.36%), (ii) a recessive gene distinct but linked to *pvr5* is involved in 'Avelar' resistance to PVY -0, and these genes are able to complement partially an heterozygous state (total complementation in 20 F 1 hybrids but partial complementation in 511 BC progenies).

Conclusion

As a consequence of these results, screening progenies from Avelar for the resistance to PepMo V will not allow to drag PVY resistance and reciprocally. Moreover, transitivity between resistance loci (*pvr5* allelic or tightly linked to PVY -0 resistance from 'Avelar' but independent from *pvr 3*) indicates that *pvr 5* is distinct from *pvr 3*. The genetic mapping of *pvr 5* is underway and its map location should confirm the independence between these two loci.

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The pepper line "Perennial" is susceptible to potato virus Y pathotype 1-2.

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The resistance to several potyviruses present in the Indian pepper line "Perennial" was studied by Caranta and Palloix (1996). According to their studies the line "Perennial" carries a complete resistance against the pathotype 0 (PVY 0) of potato virus Y (PVY) and potyvirus E, where genes with partial effects play a role, while a more complex genetic basis could explain a partial resistance against PVY pathotype 1-2 (PVY 1-2). These same authors studied also the resistance in "Perennial" to chili vein mottle virus (CVMV) and they suggested a model where two unlinked major genes confer an absolute and dominant resistance.

While the pepper line "Perennial" carries several complex resistances against potyviruses, the Mexican line "Serrano Criollo de Morelos-334" (SCM-334) carries some monogenic resistances. Thus, the *Pvr4* allele present in SCM-334 confers a dominant resistance to all known PVY pathotypes and also against pepper mottle virus (PepMoV) (Dogimont *et al.*, 1996). SCM-334 also carries the *pvr5* allele which confers a recessive and monogenic resistance only against PVY 0 (Dogimont *et al.*, 1996). Nevertheless, these two genes are not enough to explain the response observed on different lines derived from SCM-334 (Arnedo Andres *et al.*, 1998).

In order to study the response of "Perennial" to PVY 1-2, we inoculated that line with the isolate P-22-88 belonging to PVY 1-2 and all the plants tested were susceptible showing vein banding mosaic and vein necrosis. Plants were also

tested by ELISA test and PVY was detected in non- inoculated leaves of all the plants. Previous studies (Caranta and Palloix, 1996) reported a partial resistance to PVY 1-2 present in "Perennial" and, although they never observed symptoms after inoculation with PVY 1-2, PVY was weakly detected by ELISA test in the upper' leaves. In our case ~ did not find lower absorbances values in "Perennial" than those from "Yolo Wonder", the susceptible control. Consequently, the inoculation results together with the ELISA data suggest that the "Perennial" line we have used and some others lines ("S20-1" and "S-118") we got from Singh and Thakur (1977), do not have resistance against PVY 1-2. Moreover, recently we found a RAPD marker (UBC 191420) linked in repulsion to the *locus Pvr4* in the variety "SCM-334". The marker is present in other pepper lines including 'Perennial' and absent only in 'SCM-334' carrying the *Pvr 4* allele. The presence of that marker does not definitely indicate that the allele *Pvr4* is not present in "Perennial" but it indicates that if a resistance allele is present in that *locus* in "Perennial", it 'NOuld have a different origin than the resistance *Pvr 4* allele present in the line "SCM-334".

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SCREENING OF PEPPER CULTIVARS FOR RESISTANCE TO PEPPER LEAF CURL VIRUS

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Introduction

A high percentage of peppers grown in Nigeria come from the Guinea and Sudan savanna zones of Nigeria where pepper leaf curl virus (PLCV), a geminivirus, is usually severe and endemic on irrigated peppers (Alegbejo, 1994). The virus is transmitted persistently by whiteflies Bemisia tabaci Gen. Dhanraj & Seth, 1968. This study was conducted to identify pepper cultivars with resistance or tolerance to PLCV.

Materials and Methods

The study was conducted at Samaru, Northern Guinea Savanna zone of Nigeria (Latitude 11°11'N, Longitude 07°38'E, Attitude 686m) during the 1992/93 dry season. Seedlings of the thirty four pepper cultivars were raised in an insect-proof screenhouse and transplanted after seven weeks into the field in the first week of January on two ridges, 4.5 x 1.2m. An infector row infested with whiteflies was transplanted in between every two cultivar to serve as source of inoculum. Disease severity on each plant was rated as shown below on a scale of 1-7, where:

1 = No visible symptoms; 3 = Top leaves curled and slight stunting of plant; 5 = All leaves curled and slight stunting of plant; 7 = severe curling of leaves, stunting of plants and proliferation of axillary shoots.

Resistance level was determined using the scale outlined below.

Rating	Percentage infection	Disease severity
Resistance	1.0 - 15.9	1.0 - 2.9
Moderately Resistant	16.0 - 25.9	3.0 - 4.9
Moderately Susceptible	26.0 - 36.9	5.0 - 6.9
Highly susceptible	37 and above	7.0

Twelve seedlings each of the thirty four pepper cultivars were transplanted into 15.50cm diameter plastic pots containing sterilized soil. Twenty viruliferous whiteflies were aspirated and released onto each caged test plant for two days inoculation access feeding period (IAFP). Ten test plants (six weeks old) of each cultivar were used. Two other healthy test plants were used as control. Inoculated plants

were sprayed with Rogor (Dimethoate) at the rate of 1.35g a.i./litre to kill the whiteflies. The data were analysed using analysis of variance after which the Duncan's Multiple Range Test (DMRT) was used to separate means based on F values at 5 percent level of significance.

Results and Discussion

The 1992/93 field results (Table 1) show that one cultivar, PBC 067 was moderately resistant, twenty six were moderately susceptible, while seven were highly susceptible. Results of the greenhouse screening were similar (Table 2). Significant differences ($P = 0.05$) were observed in the reaction of the cultivars to PLCV (Tables 1 & 2). The moderately resistant cultivar will be used with other control measures to reduce the devastation caused by the disease. Efforts will be made to search for more resistant/tolerant cultivars.

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Table 1. Reaction of thirty four pepper cultivars screened for resistance to PLCV at Samaru in the field during the 1992/93 dry season.

Pepper Cultivar	PLCV-infected plants (%)	Disease severity(1-7)	Yield (t/ha)
PBC 066	27.00mno	3.00klm	2.90abcdef
" 067*	25.15o	3.03jklm	3.10abcd
" 076	27.15lmno	3.12jklm	2.40fghijkl
" 140	31.15ghijklm	3.93bcde	2.30hijklmn
" 146	31.85fghijk	3.27hijklm	2.17klmn
" 147	31.35fghijkl	3.23hijklm	2.27hijklmn
" 155	30.35hijklmn	3.87bcde	2.63efghijk
" 156	34.65defg	3.67cdefghi	2.40fghijkl
" 157	32.85defghi	4.0bcde	2.07lmn
" 186	34.35defgh	3.97bcde	2.23ijklmn
" 207	36.85d	3.73cdefgh	2.20jklmn
" 210	31.00hijklmn	3.47efghijkl	2.93abcde
" 223	29.35ijklmno	3.70cdefgh	2.93abcde
" 270	33.85defgh	3.47efghijkl	2.77bcdefgh
" 373	45.85abcd	4.13abc	2.23ijklmn
" 374	42.00c	4.07bcd	2.73cdefghu
" 375	42.00c	4.30ab	2.47efghijkl
" 376	48.50a	4.30ab	3.2abc
" 377	36.15de	3.80bcdefg	2.33ghijkl
" 378	35.50def	3.70cdefgh	2.27hijklmn
" 151	28.15jklmno	3.23hijklm	2.37ghijkl
" 398	42.52bc	3.10jklm	2.70cdefghij
PBC 401	46.15ab	3.73cdefgh	2.63efghijk
" 402	27.85klm	2.83m	1.83mn
" 405	27.15lmno	2.97lm	2.53efghijkl
" 511	32.35efghij	3.30fghijklm	2.83abcdefg
COO 104 3	33.65defgh	3.87bcde	2.83abcdefg
" 2230	49.65a	4.60a	1.80fgh
" 533	33.00defghi	3.83bcdef	2.37ghijkl
" 2284	32.65defghi	3.13ijklm	2.43efghijkl
" 1052	35.00defg	3.83bcdef	2.47efghijkl
CO 1175	27.00mno	3.53defghijk	2.50efghijkl
" 376	27.66lmno	3.57defghi	3.33a
" 352	27.35lmno	3.27hijklm	3.27ab

Table 2. Reaction of thirty four pepper cultivars screened for resistance to PLCV at Samaru in the glasshouse during the 1992/93 dry season

Pepper cultivar	PLCV-infected plants(%)	Disease Severity(1-5)
PBC 066	28.40s	4.17i
" 067*	25.52t	3.18k
" 076	28.72gr	4.45ghij
" 140	35.30k	4.27ij
" 146	35.27k	4.27ij
" 147	33.31m	4.20j
" 155	34.28l	4.27ij
" 156	38.21h	4.65fg
" 157	36.51j	4.42ghij
" 186	39.53g	4.45ghij
" 207	33.40m	4.36hij
" 210	31.56op	4.24j
" 223	32.87mm	4.57gh
" 270	36.57j	5.03cde
" 373	47.10e	5.14cde
" 374	47.00e	5.14cde
" 375	48.97d	5.31bc
" 376	60.10a	5.80a
" 377	40.80f	4.30hij
" 378	48.98d	5.27bcd
" 151	32.17no	4.44ghij
" 398	49.38d	5.31bc
" 401	51.24c	5.44b
" 402	30.53q	4.37hij
" 405	29.49r	4.17j
" 511	37.01ij	5.01de
COO 1043	38.10h	5.10cde
" 2230	54.64b	5.43b
" 533	38.40h	4.90ef
" 2284	37.58hi	4.65fg
" 1052	40.68f	4.55gh
CO 1175	30.64pq	4.30hij
" 376	31.53op	4.34hij
" 352	31.50op	4.30hij

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

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Introduction

Pepper veinal mottle virus is a major limiting factor to pepper production in the wet season in Nigeria (Alegbejo, 1978). Several control measures have been attempted for controlling the disease. The use of host-plant resistance has been the most effective.

Materials and Methods

The experiment was conducted at Samaru, northern guinea savanna zone of Nigeria in the 1993 and 1994 wet seasons using thirty-four pepper cultivars obtained from the Asian Vegetable Research and Development Centre, Taiwan. Seedlings were raised on heat sterilized soil in a screenhouse. Seedlings were transplanted (45cm apart) into the field seven weeks after sowing. Each plot (4.5 x 1.2m) was made up of two rows and there were three replicates. An infector row infested with *persicae* Sulzer was transplanted in between every four cultivar to serve source of inoculum. Disease severity on individual plants was rated using a scale of 1 - 7 where: 1 = No visible symptoms; 3 = Mild leaf mottling but no leaf distortion and stunting of plant; 5 = Moderate leaf mottling distortion and slight stunting of the plant; 7 = Very severe leaf mottling, distortion and severe stunting of the plant.

Resistance level was determined using the scale outlined below:

Rating	Percentage <u>infection</u>	Disease <u>severity</u>
Resistant	1.0 - 15.9	1.0 - 2.9
Moderately resistant	16.0 - 25.9	3.0 - 4.9
Moderately susceptible	26.0 - 36.9	5.0 - 6.9
Highly susceptible	37 and above	7.0

Serological tests were carried out each year between the test virus and antiserum to PVMV, tobacco mosaic virus (TMV), and cucumber mosaic virus (CMV) using the microprecipitin test as described by Nordam (1973).

Random and non-random symptomatic leaf samples of infected leaves were collected from the top, middle, and bottom portion of each plant sampled. The season for collecting non-random symptomatic samples was to identify other viruses that might be present in the field.

Results and Discussion

In the 1993 trial (Table 1), three cultivars PBC 067, PBC 076 and CO 352 were moderately resistant, nineteen were moderately susceptible while twelve were highly susceptible. In 1994 (Table 2), the same cultivars maintained their moderate resistance while eighteen were moderately susceptible and thirteen were highly susceptible. Significant differences ($P = 0.05$) were observed in the reaction of the pepper cultivars to PVMV. All the leaf samples reacted strongly to PVMV antiserum. The moderately resistant cultivars will be used in combination with other control measures to combat the menace caused by the disease in Nigeria.

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Table 1 Reaction of thirty four pepper cultivars screened for resistance to pepper veinal mottle virus (PVMV) at Samaru in the 1993 Wet Season.

Pepper Cultivar	PVMV-infected Plants (%)	Disease Severity(1 - 7)	Yield (t/ha)
PBC 066	26.67k	3.07ghijk	2.80ab
" 067**	25.88kl	2.67klmn	2.73abcd
" 076**	24.83l	2.53lmn	2.77abc
" 140	31.17i	3.37bcdefghi	2.43bcdefghij
" 146	34.05gh	3.40bcdefgh	2.40bcdefghij
" 147	31.17i	2.90hijkl	2.23ghijkl
" 155	29.88ij	3.33cdefghi	2.5bcdefgh
" 156	34.50gh	3.37bcdefghi	2.07ijkl
" 157	34.38gh	3.37bcdefghi	2.03jkl
" 186	35.45g	3.33cdefghi	2.30efghijk
" 207	40.05e	3.27defghi	2.20hijkl
" 210	34.72gh	2.90hijkl	2.7abcd
" 223	28.67j	3.43bcdefgh	2.43bcdefghij
" 270	35.33gh	3.37bcdefghi	2.77abc
" 373	46.28b	3.70bcd	2.50bcdefgh
" 374	44.12c	3.83ab	2.73abcd
" 375	42.45d	3.57bcdef	2.67bcdef
" 376	46.45b	3.83ab	2.73abcd
" 377	38.05f	3.77abc	2.63bcdefg
" 378	43.12cd	3.20efghij	2.43bcdefghij
" 151	30.95i	3.13fghijk	1.97kl
" 398	44.17c	3.23defghij	2.47bcdefghi
" 401	49.28a	3.67bcde	2.50bcdefgh
" 420	26.22kl	2.40n	1.43m
" 405	26.50k	2.47mn	2.47bcdefghi
" 511	45.78b	3.13fghijk	2.60bcdefgh
COO 143	34.55gh	3.33cdefghi	2.60bcdefgh
COO 2230	48.83a	4.17a	1.83l
" 533	34.83gh	3.30cdefghi	2.33defghijk
" 2284	33.78h	2.77jklmn	2.27fghijk
" 1052	38.45f	3.5bcdefg	2.37cdefghij
CO 1175	26.05kl	3.30cdefghi	2.23ghijk
CO 376	27.22k	3.07hijk	3.10a
CO 352**	25.95kl	2.97hijkl	3.10a

Numbers followed by different letters are significant from one another at P = 0.05 using the Duncan's Multiple Range Test (DMRT)

** = Moderately resistant

Table 2. Reaction of thirty four pepper cultivars screened for resistance to PVMV at Samaru in the 1994 Wet Season.

Pepper Cultivar	PVMV-infected Plants (%)	Disease Severity(1 - 7)	Yield (t/ha)
PBC 066	26.55lm	3.07ghij	2.87ab
" 067**	25.88lm	2.87ijk	2.73abcde
" 076**	25.00m	2.77jkl	2.77abcd
" 140	31.00k	3.47cdefg	2.37bcdefg
" 146	34.12ij	3.57bcdefg	2.40bcdefg
" 147	31.38k	3.13ghij	2.20efgh
" 155	30.50k	3.37efghi	2.47bcdefg
" 156	34.72ij	3.50cdefg	2.23fgh
" 157	34.50ij	3.43defg	2.17fgh
" 186	35.95hij	3.57bcdefg	2.47bcdefg
" 207	40.78f	3.43defg	2.33cdefg
" 210	34.95ij	3.13ghij	2.77abcd
" 223	29.45k	3.57bcdefg	2.83abc
" 270	36.17hi	3.43defg	2.87ab
" 373	48.12ab	3.90abcd	2.47bcdefg
" 374	43.83d	3.90abcd	2.67abcdef
" 375	42.17def	4.03ab	2.33cdefg
" 376	46.05c	3.97abc	2.67defgh
" 377	38.17g	3.77bcde	2.27defgh
" 378	41.83ef	3.40defgh	2.33cdefg
" 151	31.00k	3.17ghij	2.20fgh
" 398	43.67de	3.13ghij	2.53bcdefg
" 401	49.28a	3.77bcde	2.53bcdefg
" 402	27.12l	2.47kl	1.67i
" 405	26.78lm	2.40l	2.53bcdefgh
" 511	46.45bc	3.30efghi	2.67abcdef
COO 14-3	34.50ij	3.53bcdefg	2.73abcde
" 2230	48.72a	4.33a	1.83hi
" 533	34.05j	3.47cdefg	2.43bcdefg
" 2284	34.28ij	2.90hijk	2.37bcdefg
" 1052	37.72gh	3.70bcdef	2.30defgh
CO 1175	26.50lm	3.50cdefg	2.13gh
" 376	26.50l	3.20fghij	3.13a
" 352**	25.72lm	3.07ghij	3.13a

Numerals followed by different letters are significant from one another at P = 0.05 using the Duncan's Multiple Range Test (DMRT)

** = Moderately resistant

SCREENING OF PEPPER CULTIVARS FOR RESISTANCE TO PEPPER VEINAL MOTTLE VIRUS IN NIGERIA

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Introduction

Pepper veinal mottle virus (PVMV) is a major limiting factor to pepper production in the wet season in Nigeria (Alegbejo, 1996). It belongs to the potyvirus group and is transmitted non-persistently by several species of aphids (Brunt & Kenten, 1971; Alegbejo 1986). One of the most effective means of controlling the disease is the use of resistant cultivars. Consequently, this trial was conducted to identify sources of resistance to PVMV.

Materials and Methods

The trial was conducted at Samaru in the 1997 wet season. The fourty two pepper cultivars used were obtained from National Research Institutes and State Agricultural Development Projects (ADPs). Seedlings were raised in the screenhouse on heat - sterilized soil. Seven week old seedlings were transplanted into the field in the first week of July at 45cm apart on two rows, 4.5 x 1. 2m, making up a plot. Ten pepper seedlings artificially inoculated with PVMV and infested with aphids, persicae Sulzer were used as infector row in-between two adjacent cultivars. Disease severity was rated using a visual scale of 1 - 7 where, 1 = No visible symptoms; 3 = Mild leaf mottling but no leaf distortion and stunting of the plant; 5 = Moderate leaf mottling, distortion and slight stunting of the plant; 7 = Very severe leaf mottling, distortion and severe stunting of the plant.

Resistance was determined using the scale outlined below:

Rating	Percentage Disease	<u>infection severity</u>
Resistant	1.0 - 15.9	1.0 - 2.9
Moderately resistant	16.0 - 25.9	3.0 - 4.9
Moderately susceptible	26.0 - 36.9	5.0 - 6.9
Highly susceptible	37 and above	7.0

Random and non-random symptomatic leaf samples were collected from the top, middle, and bottom portion of four infected plants from each plot. These were subjected to antigen coated plate (ACP) enzyme linked immunosorbent assay (ELISA) using monoclonal antibodies to PVMV (4AI0 & 7CI0) obtained from Dr. Huguenot of IDRC Vancouver, Canada. Data were analysed using the two-way analysis of variance.

Results and Discussion

All the leaf samples acted positively to the PVMV monoclonal antibodies. One cultivar TCa 14 (check) was resistant while two Kenba and Kashin burgu were moderately resistant. Thirty four cultivars were moderately susceptible while five cultivars were highly susceptible. The resistant and moderately resistant cultivars were also high yielding (Table 1). Significant differences ($P = 0.05$) were observed in the reaction of the pepper cultivars to PVMV. The trial will be conducted for at least one more year after which the resistant and moderately resistant cultivars will be used in an integrated management programme to bring the disease below economic injury level.

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Table 1. Reaction of pepper cultivars screened for resistance to pepper veinal mottle virus (PVHV) at Samaru in the 1977

wet season.

Pepper cultivar	PVMV infected plants (%)	Disease severity(1-7)	Fruit yield (t/ha)
FNHVID	27.00jkl	4.90abcd	2.40cd
FNHVIC	28.00ghijkl	4.90abcd	2.27cdef
FHVIB	29.10fhijkl	4.70abcdef	2.10defgh
FHVIE	32.43cdefghij	4.80abcde	2.20cdefg
FNHVIF	27.60hijkl	5.10ab	2.10efgh
FNHVIA	27.53ijkl	4.61abcdef	2.17defg
FNHVIG	30.10defghijk	4.70abcdef	2.20cdefg
RODO(DT95/307)	33.17bcdefghij	4.60bcdef	1.90ghijk
SOMBO(DT95/297)	35.60abcde	4.63abcdef	1.80hijk
NH94/343	36.83abc	4.80abcde	1.87ghijk
DAN DAMASAK1	36.47abcd	4.80abcd	1.87ghijk
DAN DAMASAK2	36.50abcd	4.90abcd	1.73ijk
DAN DAMASAK3	36.03abcde	5.03abc	1.73ijk

Table 1. cont'd.

Pepper cultivar	PVMV-infected plants (%)	Disease severity(1-7)	Fruit yield (t/ha)
EX-WANDI DASS	37.60abc	5.10ab	1.80hijk
EX-MINGI	39.00ab	5.20ab	1.70jk
EX-T/WASS DASS	32.33cdefghij	4.63abcdef	1.93fghijk
EX-DARAZO	30.03defghijkl	4.70abcdef	1.90ghijk
ZUGANDE	33.03bcdefghij	4.60bcdef	1.90ghijk
TSIDUHU	34.10abcdegh	4.70abcdef	1.92ghijk
GAMA PADA	36.00abcde	4.80abcde	1.90ghijk
TATTASAI-M	35.10abcdef	4.70abcdef	1.90ghijk
TSICHFI	37.30abc	3.80gh	1.80hijk
ATARUGU-M	25.73kl	3.90fgh	1.80hijk
TC914**(check)	0.00m	1.00j	2.97a
TUGONDE-HS	39.20ab	5.10ab	1.63k
HANCHIN DUNYA	40.37a	5.43a	1.60k
ATARUGU-DG	36.17abcd	5.10ab	1.60k
TASSHI-KD	35.20abcdef	4.00fgh	1.87ghijk
DAN DAMASA-B	33.40bcdefghij	3.90fgh	1.90ghijk
HANCIN-SA	27.60hijkl	3.70h	2.00efghij
KENBA*	25.03kl	2.67i	2.73ab
BORTHONO-DM	29.60efghijkl	4.03efggh	2.33cde
ATARUGU-B	29.57efghijkl	5.00abc	2.07defghi
DAN MUNCHR	30.03defghijkl	4.80abcde	2.00efghij
TATTASAI-KT	31.27cdefghijk	4.50bcdefg	2.20cdefg
DAN DAMASO	28.13ghuhjk	4.60bcdef	2.03efghij
HANCINSA	29.03fghijkl	4.70abcdef	2.20cdefg
KASHIN BURGU*	24.50l	3.90fgh	2.5bc
CHILLI HOT	28.70fghijkl	4.10defgh	2.03efghij
ATARUGU-KD	33.60bcdefghi	4.27cdefgh	2.10defgh
TATASAI-KD	34.19abcdefg	4.10defgh	2.00efghij
CHILLI-KD	36.13abcd	4.80abcde	1.90ghijk

** = Resistant

* = Moderately resistant

QUALITATIVE LOSSES OF CHILLI FRUITS DUE TO INFECTION BY

Colletotrichum capsici (Syd) Butler and Bisby

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INTRODUCTION

Chilli (*Capsicum annum* L.) an important cash crop being affected by many fungal, bacterial and viral diseases. Of which fruit rot incited by C.capsici (Syd) Butler and Bisby causes considerable damages in recent years, inflicting severe qualitative and quantitative losses. As we know "chilli fruits are directly valued for its pungency, colour and aroma in culinary purpose and indirectly by their products in pharmaceutical and cosmetic industries. The biotic and abiotic stresses are known to alter the chemical constituents of the fruit. During infection and rotting of plant tissues by various pathogens, the metabolic changes were found to be distinct and different compared to the healthy tissues. In the present investigation, attempts were made to assess the loss of chemical constituents of chilli fruits due to C.capsici infection.

MATERIALS AND METHODS

The major constituents of chilli fruits such as sugars (Reducing, Non reducing and Total sugars), ascorbic acid, proteins, capsaicin, total phenolics, oleoresin and surface wax were assessed both in healthy and diseased fruits of cv.K-2. Fresh ripe fruits were used for estimating sugars (Somogyi, 1952), ascorbic acid (Mahadevan and Sridhar, 1982), Protein (Lowery et al., 1981) and total phenolics (Spies, 1955). For the estimation of Oleoresin (Mathew et al., 1971) and capsaicin (Theymoli et al., 1982) dry chilli-fruits were used and for the estimation of surface wax (Martin and Batt, 1958) dry fruit skin was used. Each analysis was repeated thrice and the results are furnished in Table 1.

RESULTS AND DISCUSSION

The diseased fruits contained 50 per cent depleted quantities of Capsaicin (67.40 mg), oleoresin (6.10 mg), the pungency principle for which the condiment is valued. Azad, (1991) reported that capsaicin content was maximum in resistant variety while minimum in susceptible variety. The

surface wax imparting characteristic lusture to the chilli fruit was also observed to be depleted in diseased fruits which affects the marketability of the produce. The ascorbic acid and protein content were found to be reduced in diseased fruits than healthy fruits. This was in agreement with the earlier findings of Sujathabai (1992).

Table 1. Changes in chemical constituents of chilli fruits during infection with *C. capsici*

Constituents	Per 100g weight	
	Healthy fruit	Diseased fruit
Reducing sugars	1.37 g	0.53 g
Non reducing sugars	0.73 g	0.29 g
Total sugars	2.00 g	0.82 g
Ascorbic acid	125.20 mg	106.64 mg
Proteins	8.87 g	2.33 g
Total phenolics	10.91 mg	34.00 mg
Oleoresin	11.13 mg	6.10 mg
Capsaicin	118.10 mg	67.34 mg
Surface wax	312.00 mg	197.00 mg

* Mean of three replications

The phenolics and the sugar contents were higher in the diseased fruits. The increased content of total phenolics in the diseased fruits might be either due to the triggered metabolism of their synthesis via Shikimic acid pathway or by the transportation of phenolics from the neighbouring healthy tissues to the infection site as a host defence against the pathogenic infection as reported by Madhukar and Reddy (1991). Subbaraja (1981) reported that sugars were important for the synthesis of phenolics which were associated with disease resistance. The results obtained in the present investigation were in confirmatory with the earlier reports cited.

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AGGRESSIVENESS OF *LEVEILLULA TAURICA* ON PEPPER

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Introduction

Leveillula taurica (Lev.) Am. is the main causal agent of powdery mildew on pepper plants, *Capsicum annuum* L. (Braun, 1980). This fungus is present in most Mediterranean countries (Tramier, 1963; Daubeze *et al.*, 1995a) and in tropical regions (Hirata, 1968) including Sudan (Tarr, 1955; Nour, 1958). In Sudan, the disease was reported to cause serious damage to pepper during winter cultivation from October to March (Orner, 1984; Ahmed *et al.*, 1998). This species can also infect numerous other crops including tomato (Correll *et al.*, 1987) and other *Solanaceae* (Palti, 1988), Artichoke, cucumber (Molot and Lecoq, 1986), Okra, Cotton, Faba bean (Nour, 1958) and many weed plants (Palti 1988).

Under natural conditions, several pepper varieties exhibited good levels of field resistance (Daubeze *et al.*, 1995a; Ahmed *et al.*, 1998). But the level of resistance of some varieties appeared to be different when tested in different geographical locations. For instance, the variety 'PM 687' appeared to be only partially resistant in open field under natural infection conditions in southern France and in Sicily (Daubeze *et al.*, 1995a) while it behaved as a highly resistant variety under Sudan conditions (Ahmed *et al.*, 1998). Such differences may be attributed to differences in environmental parameters that could influence the epidemiology of the disease. They could also be related to variability in the aggressiveness of isolates in natural populations of *L. taurica*.

The present study was initiated to test the aggressiveness of several isolates of *L. taurica* coming from different locations, including France, Sicily and Sudan, on susceptible and partially resistant pepper varieties under controlled conditions,

Materials and Methods

The two pepper genotypes 'Vania' and 'PM687' were tested in this study. Vania is a bell pepper inbred line obtained by INRA from recurrent selection for resistance to cucumber mosaic virus. It was found to be highly susceptible to *L. taurica* (Daubeze *et al.*, 1995a). PM 687 is partially resistant to *L. taurica*. This variety was fixed through selfing from 'PI 322719' (Daubeze *et al.*, 1995a). Seeds of these two varieties were provided by INRA (Unite de Genetique et d'Amelioration des Fruits et Legumes, Montfavet, France). Plants were raised in a greenhouse in pots of 12cm diameter. The plants were five weeks old when inoculated. They were decapitated above the second leaf 241 hours before inoculation with the fungus as suggested by Daubeze *et al.* (1995b),

Six isolates of *L. taurica* were used in this study (Table 1). These isolates were obtained from diseased peppers in open field plots and single-spored according to the method of Bertrand (1991). The fungus was grown under controlled conditions according to the method derived from Molot *et al.* (1990) and Daubeze *et al.* (1995b).

Conidia of each isolate were separately brushed into sterile distilled water and all suspensions were adjusted to 105conidia/mJ. For each plant, the lower face of the two first leaves was sprayed with the spore suspension. The time between the preparation of spore

suspensions and the application on plants never exceeded 15 minutes. For each variety, 5 plants were tested per isolate. Each plant received 2ml using a gas sprayer (Ecospray, Labo Chimie France). Immediately after spraying, the plants were placed in a dew chamber at 100% relative humidity (RH) for 48 hours in darkness. For the rest of the experiment, conditions were adjusted to 14h of light at 23°C and 60% RH and 10h of darkness at 16°C and 80% RH as suggested by Daubeze *et al.* (1995). Symptoms were assessed on the lower face of inoculated leaves according to a scale from 0 (no visible sporulation) to 5 (whole leaf area covered with conidia) as described by Daubeze *et al.* (1995b). Evaluations were done five times at weekly intervals from the 7th day after inoculation. For variety 'PM 687', the evaluation of symptoms was continued until 49 days after inoculation. Analysis of variance was done using Statistica software (StatSoft Inc., Tulsa, USA). The Least Significant test was performed to determine whether differences between isolates were significant

Results and discussion

Successful infection of pepper with *I. taurica* was obtained under controlled conditions with artificial inoculation: all isolates were virulent to the susceptible line 'Vania'. Fourteen days after inoculation, mild symptoms were observed with all 6 isolates (Figure 1). After 35 days, the average disease index was greater than 1.5 for all 6 isolates. On the partially resistant variety 'PM 687' no symptoms were observed for isolates P32 and P38 throughout the experiment, while very weak symptoms were observed with other isolates. The highest average disease index (0.4) was recorded for isolate P45, 28 days after inoculation but symptoms disappeared after 42 days. For other isolates (P31, P46, P49) the average disease index never exceeded 0.1, indicating the detection of very weak sporulation, usually on only one plant out of five.

The assessment of aggressiveness was done on 'Vania' using the semiquantitative scale from 0 to 5. Different levels of aggressiveness were observed. Thirty five days after inoculation, the average disease index was only 0.5 for isolate P49, indicating that sporulation covered about 20% of the inoculated leaves. It was 3.8 for P45, indicating that sporulation covered about 60% of the inoculated leaves (Table 1). Statistical analysis indicated that P45 was the most aggressive while P49 was the least aggressive isolate. Others displayed intermediate levels of aggressiveness (Table 1).

This study demonstrates that different levels of aggressiveness can be detected for *I. taurica* on susceptible pepper variety 'Vania'. Furthermore, aggressiveness may not be related to the geographical origin of isolates as both the most and the least aggressive isolates examined in this study (P45 and P49) were collected in Sudan (table 1). Based on this preliminary work, which is currently being repeated, it may also be suggested that differences reported earlier on the field behaviour of variety 'Pvf687' in different geographic locations may not be related to the absence of aggressive strains of *I. taurica* in Sudan.

Figure 1 . Evolution of Powdery Mildew symptoms on pepper plants cv Vania inoculated with 6 isolates of *Leveillula Taurica*

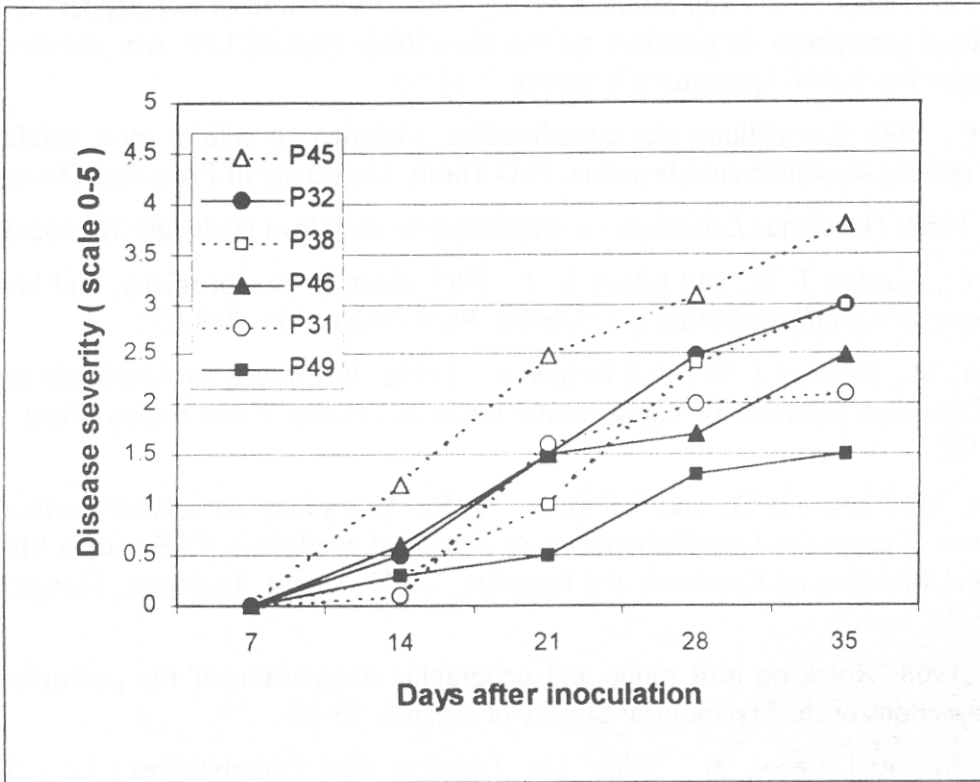


Table Compared aggressiveness of six *Leveillula taurica* isolates on pepper cv.Vania

Isolate	Origin	Average disease index 35 days after inoculation	AUDPC
P45	Sudan	3.8 a	10.6 a
P32	France	3.0 b	7.9 bc
P38	Sicily, Italy	3.0 b	7.5 bc
P46	Sudan	2.5 bc	6.1 bc
P31	France	2.1 cd	5.8 cd
P49	Sudan	1.5 d	3.9 d

Pants (5per isolate) were rated on a scale 0-5, numbers followed by different letters are significantly different ($p < 0.05$) based on the least Significant difference test

Area under the disease progress curve, number followed by different letters are significantly different ($p < 0.05$) based on the least Significant Difference test

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RELATIONSHIP BETWEEN β TUBULIN ACCUMULATION AND NUCLEAR REPLICATION IN OSMOPRIMED *Capsicum annuum* L. SEEDS.

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INTRODUCTION

Priming treatments in osmotic solutions (osmoconditioning) can considerably improve seed quality by enhancing rates and uniformity of germination. They consist of the incubation of seeds in an osmotic solution dissolved in water, for a specific period of time at a certain temperature. After priming seeds are re-desiccated to allow storage and handling. Improved seed germination performance after priming has been explained by completion of DNA repair (Osborne, 1983), and by a more favourable metabolic balance of primed seeds at the start of germination in water (Dell'Aquila *et al.* 1978). Using flow cytometry, we previously studied nuclear replication activity in *Capsicum annuum* seeds during germination and after priming in PEG (polyethylene glycol) solutions. In this species the quiescent embryo arrests nuclear division at the pre-synthetic G1 phase, showing a DNA content conventionally indicated as 2C (Lanteri *et al.*, 1993, 1994, 1996). Priming can induce DNA synthesis in the embryo root tips, depending on the osmotic potential of the solution used and the length of treatment. After seed desiccation the amount of nuclei induced to enter the synthetic phase is stably arrested in G2 (4C DNA content) and correlates with the improvement in seed germination performance (Lanteri *et al.* 1994, 1996).

Cell-cycle activity during germination was also followed by analysing the expression of β -tubulin, which, being a constitutive protein of microtubules (Goddard *et al.*, 1994), is present at all stages of cell cycle (De Castro *et al.* 1995, 1998). During seed maturation, a sharp β -tubulin decrease was observed in embryonic root meristems, just before the acquisition of complete seed desiccation tolerance (Portis *et al.*, 1999).

In this paper the expression of β -tubulin after priming of unaged and controlled deteriorated pepper seeds has been analysed and correlated with nuclear replication activity. Our aim was to gain a better understanding of the molecular processes activated during priming treatments.

MATERIALS AND METHODS

Seeds of pepper (*Capsicum annuum* L. cv. "Quadrato di Carmagnola"), from a commercial lot, were subjected to controlled deterioration at 40°C for 6 d at 75% RH.

Deteriorated seeds, as well as unaged seeds, were osmoprimed in PEG 6000 solutions at (20°C in darkness) for 6 or 12 d at -1.5 MPa and 6 d at -1.1 MPa. After priming, seeds were washed in running tap water to remove the osmotic agent and dried back to the initial seed moisture content (8.5% on a dry/weight basis)

Germination tests were carried out according to ISTA guidelines (1993), the germination of primed and unprimed pepper seeds was scored by counting daily the number of germinating seeds. The mean germination time (MG1) was evaluated according to the formula Ind/N (n =number of germinated seeds on each day; d =number of days from the beginning of the test; N =total number of germinated seeds). Data on germination percentage (GP) and MGT were analysed by Tukey's HSD test.

Samples of nuclei for flow cytometry were obtained by chopping the material with a razor blade in a nucleus isolation buffer as reported by Lanteri *et al.* (1993). To detect DNA, 10 mg/l of propidium iodide was added (Saxena and King, 1989). Each sample consisted of 10 embryo root tips, every determination was made in duplicate. Fluorescence was measured using a FACScan flow cytometer (Becton & Dickinson, Mountain View, CA USA) equipped with a 488 nm light source (argon laser). Data were recorded in Hewlett Packard computer (HP 9000,

model 300) using CellFit software (Becton & Dickinson) All analyses were performed using peak height detection (Bino *et al.* 1993) The DNA amount, proportional to the fluorescent signal, was expressed as arbitrary C values (1 C corresponds to the DNA content of the unreplicated haploid chromosome complement).

Total proteins were extracted from excised embryo root tips as described by de Castro *et al.* (1995) Ten embryonic root tips were frozen in liquid nitrogen and subsequently ground to a powder with quartz sand. 20 l of extraction buffer (de Castro *et al.*, 1998) were added to the powder and samples were vigorously mixed for 1 minute before boiling for 5 minutes. Supernatants were recovered after centrifuging 5 minutes at 17000g at room temperature, while pellets were washed once with 20 μ l of extraction buffer Protein concentration of pooled supernatants was measured spectrophotometrically following micro-protein assay procedure and using bovine serum albumin, fraction V (BSA, Sigma), as a standard. 40 μ g of total proteins were separated on a 15% SDS-polyacrylamide gel run at 100 V for 90 minutes at room temperature Two different concentrations of pure bovine brain tubulin (Sigma), 1 and 10 ng, were used as a reference samples. Molecular weight prestained standards (Sigma) was also routinely loaded. After PAGE, proteins were electrotransferred from the gel to a Hybond-polyvinylidene difluoride (PVDF) membranes (0.2 μ m, Bio-Rad), for 90 minutes at 4°C, 50 V, using a Mini trans-blot electrophoretic transfer cell (Bio-Rad). Transfer buffer was as reported by De Castro *et al.* (1995)

All immunodetection steps were carried out at room temperature with gentle agitation in a roller incubator. After blotting, the membrane was briefly washed in distilled water. Blocking (2h) and incubation in anti- α -tubulin monoclonal antibody (Boehringer Mannheim) (2h) were followed by double washing in TBST -5 and 0.5 blocking solution for 5 min 50 mU/ml of peroxidase- conjugated secondary antibody (Boehringer, Mannheim) were diluted in 0.5% blocking solution (Boehringer, Mannheim) and membranes were incubated for 1 hour Reactions were detected using a Chemiluminescence Blotting Substrate kit (Boehringer, Mannheim) following manufacturer's instructions and exposing films for 10 to 30 seconds

RESULTS AND DISCUSSION

The percentage of normal seedling of untreated seeds was 98.1 and the MGT 5,2d The controlled deterioration treatment did not induce a significant decrease in GP, but increased the MGT by 3d In unaged, as well as controlled deteriorated seeds priming treatments were always effective in decreasing MGT without affecting GP The most effective was the one performed at the lower osmotic potential (-1.1 MPa) which induced a decrease In MGT of about 2.5 and 5 d in unaged and controlled deteriorated seeds respectively (Tab 1)

Flow-cytometric profiles of embryo root tips from dry pepper seeds exclusively showed nuclei with a pre-synthetic DNA content (2C) Among the tested priming conditions only the most effective in Improving seed germination (-1.1 MPa) was found to induce nuclear replication activity In the embryo root tips of both unaged and controlled deteriorated seeds In the latter the amount of nuclei showing a 4C DNA content was lower as well as less marked the effectiveness of the treatment As previously observed (Bino *et al.* 1993 Lanter *et al.*, 1994, 1996 Saracco *et al.* 1995), under the same osmotic condition the amount of priming-induced nuclear replication IS strongly Influenced by the Initial seed vigor and correlates to priming efficiency However a discrimination is not possible among treatments which do not induce DNA replication

Interestingly the longest (12d) treatment at -1.5 MPa on unaged seeds did not induce DNA replication but was as effective in reducing seed MGT as the one performed on aged seeds at -1.1 MPa for 6d (about 12% of nuclei in G2 observed). The induction of nuclear replication, besides initial seed vigour, seems to be strongly influenced by the applied osmotic conditions. Possibly during priming other processes, not necessarily leading to DNA synthesis, may also play an important role in improving seed germination performance; these processes could be activated at lower osmotic potential than those required for DNA replication.

A more accurate evaluation of the metabolic progress induced by osmoconditioning seems possible by analyzing data on [β -tubulin accumulation. In our seed lots no [β -tubulin signal could be detected in sample from excised root tips of dry seeds (Fig 1, lane 1). Possibly the level of [β -tubulin was below the immunodetection discriminating power (1 to 10 ng of pure brain tubulin) or losses of the rather low level of the constitutive protein occurred as a consequence of protease activity. However a clear [β -tubulin signal could be always detected after priming of both unaged and controlled deteriorated seeds (Fig 1). DeCastro *et al.* (1995) reported that replication of DNA and [β -tubulin accumulation are both induced during germination and delayed in aged tomato seeds. From our data it appears that during priming [β -tubulin synthesis possibly anticipates DNA replication or, in any case, is activated at lower osmotic potentials.

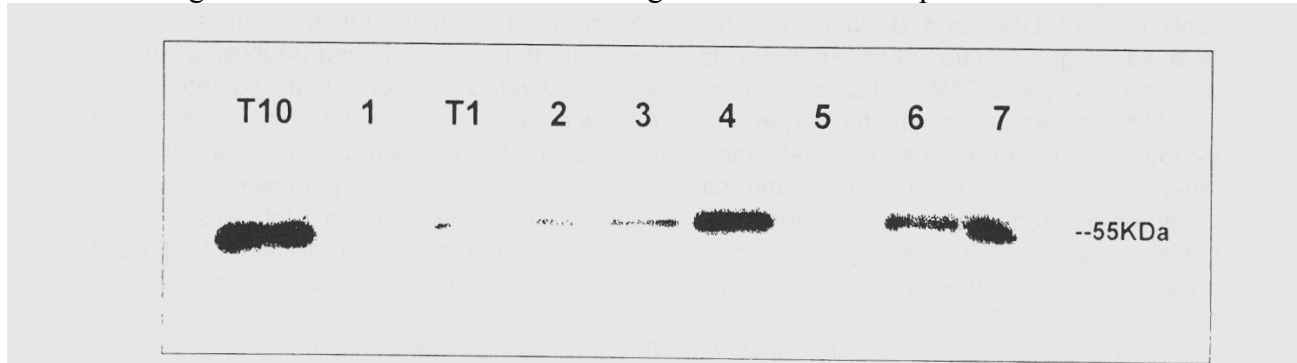
Although, at present, the technique does not allow an accurate quantitative measurement of (β -tubulin levels, our data show a detectable difference in total (β -tubulin accumulation following different priming treatments (Fig 1) and that its amount well correlates to priming effectiveness in improving seed germination performance. The amount of priming induced [β -tubulin synthesis, therefore, appears a more accurate molecular marker than nuclear replication to predict progress in germinative events. Induced by priming treatments and for estimating, a priori, their effectiveness.

In a previous work we also observed that priming treatments which induce nuclear replication make seeds more vulnerable to deteriorative effects imposed during subsequent controlled deterioration treatments. This was hypothesised to be a consequence of their higher DNA content, which is a more vulnerable target for mutation-inducing factor as well as their more advanced progress in germinative events which make seeds less resistant to deteriorative factors imposed during storage (Saracco *et al.*, 1995). The next step of our investigation will be to test if monitoring priming efficiency on the basis of [β -tubulin synthesis can better allow to single out effective priming treatments which do not affect seed storability.

Table 1: Effect of controlled deterioration (40' for 6 d) and osmoconditioning in PEG solutions of unaged and controlled deteriorated seeds on germination percentage (GP), mean germination time (MGT), percentage of 4C nuclei in the embryo root tips. Within a column, means with the same letter are not significantly different (P<0.01 Tukey's HSD test)

Treatment	GP (%)	MGT (d)	4C (%)
Control	98.1 ± 2.7 a	5.2 ± 0.3 d	0
PEG -1.5 MPa 6d	97.0 ± 2.0 a	4.2 ± 0.2 c	0
PEG-1.5MPa 12d	99.5 ± 2.2 a	3.0 ± 0.3 b	0
PEG-1.1MPa6d	98.0 ± 1.8 a	2.5 ± 0.1 a	20.8 ± 3.2 a
Aged 6d	94.1 ± 2.1 a	8.2 ± 0.4 e	0
Aged 6d + PEG -1.5 MPa 6d	90.1 ± 1.8 a	5.3 ± 0.3 d	0
Aged 6d + PEG -1.5 MPa 12d	98.0 ± 1.5 a	3.7 ± 0.5 cb	0
Aged 6d + PEG -1.1 MPa 6d	97.0 ± 1.9 a	3.0 ± 0.2 b	11.6 ± 2.7 b

Figure 1 Chemiluminescent immunodetection of β tubulin after Western blotting of total protein extracted from embryo root tips of dry of dry seeds (lane1) seed osmoprimered for 6 or 12 d at 1.5 Mpa (lanes 2-3) or 12d at 11 Mpa (lane4 controlled deteriorated seeds osmoprimered for 6 or 12 at -15 Mpa lanes 5-6 and 12 days at -11 Mpa lane 7 10 and 1 ng of pure bovine brain tubulin (Sigma) were used as a reference samples (T10 and T1) the position of the band correlated with a molecular weight of 55 KDa as indicated on the right. The film was exposed for 15 seconds



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Studies on bacterial wilt resistance of selected egg plant accessions inoculated with *P. solanacearum* PSS 97 for 30 days under growth room conditions at Asian Vegetable Research and Development Center.

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Introduction

Bacterial wilt is one of the most important limiting factor in egg plant cultivation in tropics. This is caused by *Pseudomonas Solanacearum* (Davidson, 1935). The use of resistant variety is one of the efficient methods of combating bacterial wilt and hence attempts were made to evolve resistant lines. For this purpose the 15 proven resistant lines under green house conditions were tested again under growth chambers.

Materials and Methods

The preliminary screening for resistance in 95 accessions collected from India was done in three batches. The 3 sowing dates were 26th April, 14th June, and 12th August, 1994 and the 3 inoculating dates were 24th May, 13th July, and 13th September, 1994 respectively. The two check varieties TS56B as resistant check and Bonne as susceptible check were also included in each batch. The resistant varieties were screened in pathology growth room along with checks. The sowing dates were 27th September, 28th October, and inoculating dates were 27th October and 28th November, 1994. Seeds were sown in 3 inch plastic pots in the growth chamber. The experiment design was RCBD with three replications. Each replication contained 15 plants (pots). Bacterial wilt inoculum PSS97 was obtained from the plant pathology unit. BW screening was done by soil drenching method to supplement root cutting. When seedlings were 30 days old, each pot drenched with 30 ml inoculum adjusted to a concentration of 10⁸ bacteria cells/ ml right after the root wounding. After inoculation, soil moisture was maintained at a high level and temperature was maintained at 25-32° C in the greenhouse. The room temperature was maintained at 28° C.

The inoculated plants were observed daily, all records were kept of the date of appearance of wilt symptom and plant death. Disease reading, based on the scale of 0 - 5 (0 = no symptoms, 1 = one leaf wilted, 2 = two or three leaves wilted, 3 = all leaves wilted except top two or three, 4 = all leaves wilted, 5 = dead) was taken at 7 days intervals and converted to disease indices. The experiment terminated 30 days after inoculation (DAI) and data were analysed statistically

Table 1. Bacterial wilt resistance of selected egg plant accessions inoculated with *P. solanacearum* PSS 97 for 30 days under growth room condition

Acc. No.	Variety	AUDPC	Disease index ^b %	% of non symptomed plants	Bacterial wilt reaction ^c
2	Arka Keshav	16.88	2.89	97.0	R
3	Arka Need Kantha	81.94	4.36	95.5	R
6	BB-1	126.76	10.19	89.9	M.R.
13	Annamalai	166.45	9.20	90.8	R.
41	EP 143	309.50	20.00	80.0	M.R.
68	EP 98	189.25	20.78	79.09	M.S.
141	Surya	0	0	0	F.
	TS56B R.C.	270.00	19.60	81.4	M.R.
	Bonne S.C.	1403.90	100.00	0	S
	Mean	284.96	9.70	81.3	
	L.S.D.	432.34	21.42	21.42	
	CV	51.72	45.28	26.34	

Planting dates: Sowing, 27 September, 1994; inoculating: 27 October, 1994.

Disease index % = $(\sum n_i \times i) / (N \times i_{max})$, where $i = 0 - 5$, $i_{max} = 5$, n = number of plants; N = total plants

R = less than 10% plants wilted; MR = 10 to 20% plants wilted; MS = 20 to 40% plants wilted; S = more than 40% plants wilted

Table 2. Disease indices and percentage of non symptomed egg plant (*Solanum melongene*) after inoculated with *Pseudomonas solanacearum* PSS97 for 30 days^a

Acc. No.	Variety	AUDPC	Disease index ^b %	% of non symptomed plants	Bacterial wilt reaction ^c
1	Arka Nidhi	45.10	4.80	95.20	R
7	BB-2	194.9	12.60	87.40	MR
8	BB-7	435.1	19.40	79.60	MR
10	BB13-1	146.2	10.75	89.25	MR
11	BB44	339.55	4.33	95.63	R
8	BB49	33.32	4.33	95.63	R
23	K.Local 1	24.61	2.66	97.30	R
21	SM6-6	5.32	1.33	98.70	R
RC	TS56B	142.30	10.62	89.30	MR
SC	Bonne	1452.70	97.60	2.40	S
	Mean	278.91	16.74	83.26	
	L.S.D.	436.24	7.20	27.21	
	CV	56.41	43.00	32.54	

Planting dates: Sowing, 28 October, 1994; inoculating: 28 November, 1994.

Disease index % = $(\sum n_i \times i) / (N \times i_{max})$, where $i = 0 - 5$, $i_{max} = 5$, n = number of plants; N = total plants

R = less than 10% plants wilted; MR = 10 to 20% plants wilted; MS = 20 to 40%

Results and Discussions

Generally the disease indices of the accessions *were* slightly higher than the green house screenings. That may be due to the photoperiod as suggested by Mondal *et al.* (1991). Based on growth room studies the accessions Arka Keshav, Arka Neelkantha, Armamalai, Arka Nidhi, BB44, BB49, Kerala Local 1, SM6-6 were resistant with DI ranged 1.33 in SM6-6 to 9.2 in Arulamalai. The only accessions Surya appeared to have highest resistance of 0 per cent DI at 30 DAI. The other six accessions viz. BB1, BB7, EP 143, BB13-1 and BB-2 and the resistant check TS56B were moderately resistant. There were significant difference among the accessions both for DI and area under disease progress curve. The AUDPC ranged between 0 in Surya to 19.55 in BB44. In the resistance check it was 112.3 and in susceptible check it was 1452.7 (Table 1 and 2).

Conclusion

The study reveals that the accession viz., Surya proves immune to Bacterial wilt in egg plant caused by *Pseudomonas solanacearum* Smith.

Reference

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- Mondal, S.N., M.A. Khalil, Tauhidur Rahman, M.A. Rashid, and S. Nahar. 1991. Reaction of egg plant cultivars and wild species of solanum to bacterial wilt. *Ann. Bangladesh Agric.*, 1(2): 65-68..

NEW SOURCES OF RESISTANCE TO BACTERIAL WILT

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INTRODUCTION -

One of the important factors limiting brings cultivation in the tropics is the incidence of bacterial wilt caused by *Pseudomonas Solanaccarum* (1935). It is difficult to control by chemical or cultured methods. The use of resistant varieties is the cheapest and most effective method of combating bacterial wilt. Bacterial wilt resistant lines have been reported from Japan, South Africa, Indonesia, Ceylon and Puerto Rico (Davidson, 1935). A few lines have been reported as resistant from India also (Sadasluva *et al.*, 1994). The first requirement of any programme of breeding for resistance is to find out a suitable source of resistance from existing or old varieties wild forms of the same species and closely related species. The objective of the present study is to screen eggplant accessions for resistance to *P solanacearum*

MATERIALS AND METHODS

The screening for resistance in 95 accessions was done in three batches. The three sowing dates were 26th April, 14th June and 12th August, 1994; and the inoculation dates were 24th May / 13th July and 13th September, 1994, respectively. The two check varieties TS56B as resistant check and Bonne as susceptible check were also included in each batch. Seeds were sown in 3-inch plastic pots in the greenhouse. Each entry composed of 15 plants in a tray and arranged in RCBD with three replication. Bacterial wilt inoculum PSS 97 was obtained from the plant pathology unit of AVRDC, Taiwan. BW screening was done by soil drenching method to supplement root cutting. When seedlings were 30 days old, each pot drenched with 30 ml inoculum adjusted to a concentration of 10⁸ bacteria cells/ml right after the root wounding, soil moisture was maintained at a high level and temperature was maintained 25-32° C in the green house.

The disease index and percentage of non symptomatic plants (*Solanum Melongea*) after inoculated with *Pseudomonas solanacearum* PSS97

Acc. No.	Variety	Disease index %	% of nonsymptomatic plants	Bacterial wilt reaction
1	Arka Nidhi	0	100	R
2	Arka Keshav	0	100	R
3	Arka Shirish	35	65	MS
4	Arka Neelkantha	0	100	R
5	Arka Kusumakar	76	24	S
6	BB1	0	100	R
7	BB7	18	82	MR
8	BB49	0	100	R

9.	EP 47-Annamalai	0	100	R
10.	EP 51-PPL	85	15	S
11.	EP 58	29	71	MR
12.	EP 62	87	13	S
13.	EP 65-PLR.1	47	53	S
14.	EP 95	100	0	S
15.	Green Long	90	10	S
16.	Local-1	1	99	R
17.	Local-2	63	37	S
18.	Pant Rituraj	44	56	S
19.	<i>S. integrifolium</i>	71	29	S
20.	<i>S. macrocarpon</i>	53	47	S
21.	SM6-6	2	98	R
S120	Bonne (sus.ck)	100	0	S
TS56B	TS56B (res.ck)	1	99	R
	Mean	39.20	-	-
	LSD	37.93	-	-
	C.V.(%)	96.72	-	-

a Planting dates: sowing, 26th April, 1994; inoculating, 24th May, 1994.

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

c R = less than 10% plants wilted; MR = 10 to 20% plants wilted;
MS = 20 to 40% plants wilted; S = more than 40% plants wilted.

Table 2. The disease indices and percentage of non-symptomed plants (*Solanum melongena*) after inoculated with *Pseudomonas solanacearum* PSS97 for 30 days^a

Acc. No.	Variety	Disease index ^b (%)	% of non-symptomed plants	Bacterial wilt reaction ^c
38	EP 96	93	8	S
39	EP56	73	7	S
41	EP 143	18	92	MR
43	EP 69	81	19	S
44	EP 103	86	14	S
48	EP 100	71	29	S
51	EP 55	57	43	S
52	EP 86A	67	33	S
53	EP 36	78	22	S
54	EP 33	69	31	S
57	EP 93	73	27	S
58	EP 142	74	26	S
60	EP 89	62	38	S
61	EP 94	64	36	S
63	EP 78	77	23	S
67	EP 147	34	66	MS
68	EP 98	16	84	MR
69	EP 86B	57	43	S
70	EP 153	21	79	MS
71	EP 2	29	71	MS
72	EP 21	83	17	S
76	EP 60	55	45	S
77	EP 76	54	46	S
79	EP 54	88	12	S

80	EP 25	27	73	MS
81	EP 49	93	7	S
83	EP 44	58	42	S
84	EP 41	88	12	S
139	Punjab	74	26	S
140	Hissar	93	7	S
141	Surya	0	100	R
73	EP 92	97	3	S
78	EP 59	53	47	S
88	EP 68	88	12	S
S 120	Bonne (sus.ck)	96	4	S
TS56B	TS56B (res.ck)	7	93	R
	Mean	62.57	-	-
	LSD	25.79	-	-
	C.V.(%)	26.27	-	-

a Planting dates: sowing, 14th June, 1994; inoculating, 13th July, 1994.

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

c R = less than 10% plants wilted; MR = 10 to 20% plants wilted;
MS = 20 to 40% plants wilted; S = more than 40% plants wilted.

Table 3. The disease indices and percentage of non-symptomed plants of eggplants (*Solanum melongena*) after inoculated with *Pseudomonas solanacearum* PSS97 for 30 days^a

Acc. No.	Variety	Disease index ^b (%)	% of non-symptomed plants	Bacterial wilt reaction ^c
85	EP 87	86	14	S
87	EP 89	67	33	S
89	EP 39	90	10	S
90	EP73	60	40	S
91	EP 33	81	19	S
95	EP 9	60	40	S
97	EP 8	67	33	S
99	77 x 45/36	100	0	S
101	75 x 79/3	88	12	S
103	77 x 22/20	68	32	S
104	77 x 22/20/2	91	9	S
105	36 x 43/48	67	33	S
106	75 x 74/19	82	18	S
108	77 x 60/24	89	11	S
109	31 x 45/34	55	45	S
110	36 x 22/47	77	23	S
111	36 x 22/2	97	3	S
114	77 x 43/50	64	36	S
115	36 x 43/28	67	33	S
117	77 x 60/50	77	23	S
119	35 x 74/36	85	15	S
120	36 x 22/25-2	67	33	S
122	31 x 22/18-1	81	19	S
124	31 x 74/5-3	80	20	S
128	S.P.3	84	16	S

129	S.P.4	84	16	S
130	S.P.5	80	20	S
132	S.P.7	58	42	S
133	S.P.8	80	20	S
134	S.P.9	81	19	S
135	S.P.10	81	19	S
136	S.P.11	85	15	S
137	S.P.12	73	27	S
138	S.P.13	73	27	S
25	PPC	28	72	MS
11	BB 44	0	100	R
10	BB 13-1	10	90	R
7	BB 2	9	91	R
126	SP 1	57	43	S
131	SP 6	71	29	S
S 120	Bonne (sus.ck)	96	4	S
Ts56B	TS56B(res.ck)	4	96	R
	Mean	69.76	-	-
	LSD	24.76	-	-
	C.V.(%)	35.49	-	-

a Planting dates: sowing, 12th August 1994, inoculating 13th September 1994

b Disease index % plant wilt Mr = 10 to 20% plants wilted MS= 20 to 40% plants wilted S= more than 40% plant wilted

Among the 95 accessions screened under greenhouse conditions 12 accessions exhibited high level of resistance. With accessions showed no wilt symptoms. They were Arka Nidhi, Arka Keshav, Arka Neelkantha, BB1 BB44 BB 49 EP 149 and Surya. The remain for were BB 13-1 BB2 K Local and SM 6-6

There are some moderate resistant lines viz, BB7 Ep 58 EP 143 EP 98 The accessions viz EP 153 EP 2 14 7 EP 25 appeared to be moderate susceptible. The remaining entries were rated as susceptible

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- Davidson HF. 1985, bacterial wilt of Solanaceous crop Trop. Agriculture IXXXXV (4) 257-259
- Sadashiva, ATK Madhavi Reddy AA Deshpande and Roopali Singh. 1994 Performance of eggplant lines resistant to bacterial Wilt. Capsicum and Eggplant News Letter, 13:104-105

ANNOUNCEMENT

1st INTERNATIONAL CONFERENCE ON ALTERNATIVE AND TRADITIONAL USE OF PAPRIKA

Szeged, Hungary, 8-11 September 1999

The aim of the Conference is to bring together those with interest in the current status of research, extension and technology in paprika (*Capsicum*), to know the alternative and traditional use of paprika and its history since 1492.

Topics to be discussed during the Conference are as follows:

- . germ plasm evaluation and utilisation
- . production methods and cultural systems
- . postharvest physiology and technology
- . biochemical quality
- . biotechnology
- . pharmaceutical and cosmetic utilisation . food industry
- . transformation systems (drying, milling, etc.)
- . non-human utilisation
- . ornamental peppers in the garden art
- . traditional Hungarian peppers for fresh food

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FIFTH INTERNATIONAL SOLANACEAE CONFERENCE

Nijmegen, The Netherlands, 24-29 July 2000

The Conference will deal with the following topics:

- **Taxonomy:** classification, molecular impact, problems and progress related to both wild and cultivated species
- **Conservation:** diversity, *in situ* and *ex situ* conservation, landraces, etc., and databases for building an International Solanaceae Network (ISIN)
- **Biotechnology:** genetic engineering
- **Crop Science:** breeding tools, domestication, future developments and prospects of miscellaneous crop plants, e.g. potato, tomato, pepper, eggplant, tobacco.

An one-day post-conference excursion will be organised which may include visits to an open air museum and typical Dutch landscape. If there is sufficient interest for a program for accompanying persons, activities like visits to museums and other excursions may be organised during the Conference.

Conference address and info:

Faculty of Sciences, University of Nijmegen, Botanical Garden Toernooiveld, 1
NL-6525 ED NIJMEGEN The Netherlands

Tel. +31 24 3652751/36528883 Fax+31243653290

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RECIPES

Terry Berke (Asian Vegetable Research and Development Center, Tainan, Taiwan, Republic of China) goes on sending us recipes, in which pepper or its derivatives are used. We are pleased to share these recipes with our readers, hoping that you find them interesting.

Cajun Okra Soup(from Shut My Mouth! Maison Louisianne Creole Products)

- . 2 pints okra (or 50 counted) .
- 6 fresh tomatoes
- . 2 onions, chopped fine .
- 2 tablespoons butter .
- 3 sprigs parsley .
- 2 sprigs thyme
- . 1 bay leaf
- . 3 quarts hot water
- . salt, pepper to taste .
- Red pepper pods to taste, without the seeds

Wash and stem the okra, and then slice it very fine. Chop the tomatoes fine, being careful to preserve the juice. Chop the onions fine and fry them in the butter. Add the chopped thyme, bay leaf, parsley, and tomatoes, and the pepper pod. After letting them stew for about five minutes, add the okra. Stir almost constantly, as the okra bums quickly. When well browned, add the juice of the tomatoes. Then add the hot water, and let the soup simmer well for 1 1/2 hours. Season to taste with salt and pepper and serve hot with croutons.

Anti-allergy Pasta Sauce

Many of the foods we regularly eat are medicines-in-waiting. Onions, garlic and chili peppers are all foods that therapeutically soothe hay fever symptoms. Try cooking the following spicy sauce and see if it clears up your stuffy head and nose.

- . 5 medium onions. diced .
- 5 cloves of garlic, diced
- . 1 tsp dried chili peppers (more if you can stand it) .
- 2 15 oz. cans of tomato sauce
- . 1 15 oz. can of tomatoes, or 2 fresh tomatoes. cut up, or 1 10 oz. can of .
- Rotel tomatoes (tomatoes and green chilies diced) . 1 tsp. oregano .
- 1 tsp. basil
- . 1 tsp. thyme
- . 1 tsp. allspice (optional)
- . dash of vinegar (optional) . 1 tbsp. olive oil

Gently saute diced garlic in olive oil in a large pot over low to medium heat for one minute. Add onions. Saute onions until they're translucent, stirring frequently. Add rest of ingredients. Stir thoroughly. Cover with lid. Turn head down to low and let sauce simmer

all day. (Use a slow cooker if you have one). When sauce is done, serve over your favorite pasta with a fresh green salad and garlic bread covered with plenty of fresh garlic. Have a cup of green tea after dinner, a wonderful anti-asthmatic beverage.

Jalapeno Garlic Sauce (from the May 1992 issue of Gourmet magazine)

- . 1 cup bottled mayonnaise
- . 3 whole pickled jalapeno chillies, stems discarded, plus 1/2 jalapeno for
- . garnish
- . 2 tablespoons tomato paste
- . 1 garlic clove, chopped
- . 1 teaspoon red-wine vinegar
- . 1/2 teaspoon Worcestershire sauce

In a blender blend together the mayonnaise, the whole jalapenos, the tomato paste, the garlic, the vinegar, and the Worcestershire sauce until the mixture is smooth, transfer the sauce to a bowl, and garnish it with the jalapeno half. Serve the sauce with meat or chicken.

Roasted Tomato Salsa (from the February 1998 issue of Chile Pepper magazine)

- . 12 tomatoes, cored 1 large onion, sliced .
- 2 bunches cilantro 2 jalapeno peppers .
- 6 garlic cloves 2 teaspoons salt
- *Roast tomatoes, onion slices, peppers, and garlic under broiler until well charred. Combine all ingredients in a blender or food processor and process to a chunky texture. Serve immediately.*
- Olive Salsa (from October 1996 Chile Pepper Magazine)
- . 1 cup pitted black olives;
- . 1 cup grated mozzarella cheese;
- . 1/4 cup evaporated milk; . 1/4 cup vegetable oil; . 6 Serrano peppers; . 1 cup mayonnaise.

Blend all ingredients except mayonnaise until smooth in a blender. Mix with mayonnaise and refrigerate until served.

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