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The picture in the cover is derived from the "Herbario nuovo di Castore Durante", Venetia, MDCXXXVI

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FOREWORD

The sixteenth issue of "Capsicum and Eggplant Newsletter" includes no less than three invited papers: the first one, written by Dale E. Marshall from Michigan State University, deals with mechanical harvesting of pepper and the contribution that breeding can do to the improve the situation. The second paper has been prepared by Claudio Galmarini from Mendoza, and gives us information on pepper breeding in Argentina, while the third one reports an updated genetic map of pepper and has been written by the INRA group from Montfavet. Thank you very much to these Authors, for their kind willingness to increase the scientific value of our publication. By the way, we would like to remind you that any suggestions on the topics and/or authors to be considered for the invited papers of the following issues of "Capsicum and Eggplant Newsletter" would be appreciated.

As 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.

The co-operation between the Newsletter and the Food and Agriculture Organisation (FAO) has been renewed also for this year. In this way we are able to send the Newsletter to researchers in more than 140 countries allover the world

Please, remember that this Newsletter is dependent on the financial support of the recipients. Therefore, a subscription fee would be appreciated. The fees are the same as the previous year: 30 U.S.$ for normal and 150 U.S.$ for supporter subscribers. Remember also that, in order to make the payment less time-consuming and to reduce the bank costs, we have introduced the possibility of a 3-year subscription. As you know, it is possible (and suggested!) to book your own copy, so quickening its delivery. Just fill in the order form on page 125 and send it to us, together with a copy of the payment order, which must always be made to Eucarpia. In case you decide to pay by credit card, please use the voucher on page 127. Because of the lower banking costs, credit card payment is definetively welcome.

The deadline for the submission of articles to be included in the next issue of the Newsletter (No. 17, 1998) is February 28, 1998. Please note that it is also possible to submit the paper on diskette. 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 we give. 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, 30th June 1997

LIST OF THE CONTRIBUTIONS

LIST OF THE AUTHORS

Capsicum and Eggplant Newsletter, 16 (1991): 15-21. Invited paper.

DESIGNING A PEPPER FOR MECHANICAL HARVESTING

Dale E. Marshall, Agricultural Engineer, USDA Agricultural Research Service, Agricultural Engineering Department, Michigan State University, East Lansing, MI 48824 USA.

INTRODUCTION

Considerable effort has been expended since 1965 to develop a mechanical harvester for many different types of peppers *(Capsicum* spp.). In 1965, Ernest Riggs, Las Cruces, NM, was the first known person to attempt mechanical harvest of *Capsicum* peppers in the United States, Fig. 1 (Riggs, 1971). Three years later after joining the chile processor Cal Compack, the design was used to build the inclined, counter-rotating brush or flap units shown in Fig. 2. It was designed and operated similarly to a cotton stripper. Also in New Mexico, Wondel Creager designed a vertically oriented, twin, single open-helix2 concept to harvest chiles (Creager, 1971) (Fig. 3). These designs have multi-harvest potential because they remove fruits throughout the plant and leave the plants attached to the ground.

By 1967, bell peppers were being harvested mechanically in California with modified tomato harvesters. During the rest of the 1960s and 1970s, various designs were tested by California dehydrators, yet less than ten harvesters were used commercially. These once- over bell pepper harvesters replaced the tomato shaker bed with an aggressive, fluted, counter-rotating, rubber roll cleaning bed. The principal pepper plant stems were cut at ground level and the plant was elevated to drop onto the cleaning bed. Two or three people' using long-handled hoes agitated and rolled the plants around, increasing plant foliage exposure to the aggressive rolls (Fig. 4).

In 1971, after earlier attempts at mechanization, the University of Georgia tried a harvesting concept with twin, single, double, and triple open-helixes2 on pimientos, settling on a double design (Fullilove and Futral, 1972) (Fig. 5). In the early 1970's the Agricultural Engineering Institute in Israel built a 2-row experimental harvester (Fig. 6) (Wolf and Alper, 1985). Each row's harvesting element consisted of inclined, twin, 65 mm diameter double open helix elements. About six mechanical chile harvesters were built in Israel to harvest peppers for dehydration, some by Shamoa, Ltd (Fig. 7).

In 1977, the US Department of Agriculture (East Lansing, MI), expanded research on Georgia's twin, double open helix concept. It was evaluated on 15 different pepper types including: long green and red chiles, banana, bell, yellow wax, small cayenne, cherry, jalapeno, Mississippi Sport, and pimiento. The twin, double, open-helix design was judged the most satisfactory for harvesting a wide range of peppers (Marshall, 1979, 1981). In 1978, Jim McClendon, Tulia, TX, built two trailer-model harvesters using the twin, double open-helix concept (Fig. 8) (McClendon, 1981). Self-propelled models followed the next year. At least 27 McClendon harvesters were built through 1996. Boese Equipment Co. has the newest harvester with the twin, double open-helix design (Fig. 9).

Any mechanical harvester that uses a concept that leaves the plant in the ground has potential for multiple harvesting. This can be accomplished with certain cultivars if there is a sufficiently long growing season, and irrigation is used to assist plant recovery. At the Texas

1 All programs and services of the U. S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap. Trade names or company names are used in this paper solely to provide specific information Mention of a product name does not constitute an endorsement of the product by the U.S. Department of Agriculture to the exclusion of others not mentioned.

2 Definitions: twin = two counter-rotating assemblies; single = one rotating assembly; single open-helix = helical assembly consisting of one rod wound around a central shaft; double open-helix = helical assembly consisting of two rods wound around a central shaft, with helixes being equally spaced along the shaft.

A & M Research and Extension station at Weslaco, Posselius and Valco (1985) successfully tested the concept of zone harvesting (harvesting horizontal layers in the lower, middle, and upper portion of a plant). Because peppers mature first at the bottom of the plant, a range of maturity was found to exist from the bottom of the plant to the top. They tested the use of shortened double open helixes (having helixes only at the lower end of the welded helix assembly). The first harvest or pass through the field harvested the lower portion of the plant. Subsequent harvests, days later, used medium helixes, and finally full-length helixes were used to harvest the rest of the plant

In the late 1980's, experimentation began using powered, oscillating, forced balance shakers (FBS) originally developed for harvesting tomatoes by Studer (1980). These FBS's are approximately 90 cm in diameter with 15 mm diameter soft-coated fingers. FMC (Hoopeston, IL), Pik Rite (Lewisburg, PA) (Fig. 10), Pacific Coast Harvesting (Bakersfield,CA), and Johnson Machinery (Woodland, CA) are experimenting with various modifications of the basic FBS concept. These once-over harvesting designs cut the plant at ground level.

In North Carolina, RJR Foods built rotary brush and finger stripper units with vacuum and pressure assist for very pungent, Bahamian peppers (Smith and Joyce, 1974). Presently, Bahamian peppers are harvested in Oklahoma with 4-row soybean heads on standard grain combines (with modifications to the cleaning screens). In Spain an inclined brush concept is being tested for harvesting peppers for dehydration (Fig. 11) (Palau and Torregrosa, 1995).

In 1980, the University of Arizona, University of Florida, Louisiana State University, New Mexico State University, Rutgers University, University of Tennessee, Texas A & M, USDA- ARS (California) and USDA-ARS (Michigan) experimented with pepper harvest mechanization. By 1990, there were no active projects for pepper mechanization.

In summary, over 225 concepts or harvesters are known to have been tested in Bulgaria, Canada, Hungary, Israel, Italy, Spain, the United States, and the former USSR. Approximately thirty principles have been evaluated. The open helix principle is used in more than 25% of the harvesters - more than any other principle.

With the continued worldwide need for labor-saving production methods, research on mechanical pepper harvesting by manufacturers and processors continues. I project that by the year 2000, interest and usage of mechanical harvesting will increase significantly.

IDEAL PLANT TYPE

Since at least 20 different types of peppers are grown commercially in the United States, it is rather difficult to describe an ideal plant type for mechanical harvesting because of the many variations between different types. This greatly complicates the challenge for a harvester designer. Some types have acceptable plant characteristics that are more desirable than others for optimum mechanical harvesting. For maximum success, plant breeders must work cooperatively with harvester design engineers to develop the plant for the harvester and to design the harvester for the plant.

Improved pepper architecture may be accomplished in at least two ways: 1) Breeding cultivars to fit a certain mechanical harvester, and 2) Modification of the plant environment, such as through in-row plant spacing and nutrition. However, the specifications for the "ideal" plant type might be generalized as follows:

1. The plant should have an upright principal stem, such as in most jalapenos, New Mexican serranos, plant introduction cherry-types (Fig. 12), and Spanish chile (Fig. 13). It is not desirable to have excessive base branching since a large number of angled lateral branches may entrap many of the crown-set fruit during harvesting. However to maintain a reasonable yield, a number of stems will likely be required.

2. The plants should not have a wide branch or crotch angle, such as cherry (Fig. 14) or bell pepper plants (Fig. 15), because they entrap and crush fruit and their branches may break off during harvesting. Depending on the harvesting concept, additional efforts will be required to remove the peppers from the severed branches.
3, The upright principal plant stems should be flexible and willowy such as in some jalapenos (Fig. 16) and serranos. Any harvesting concept that leaves the plant in the ground essentially has to move the plant laterals into a nearly vertical orientation as the harvester passes over the plant.
4. For the plants not once-over harvested, it is easier if the plant is at least 600 mm high so that the harvester conveyor can fit under the plant canopy.
5. The branches should not have large nodes, such as found on cherry-type plants (Fig. 14) or bell plants (Fig. 15) which frequently break off during harvesting. Again, additional efforts are required to remove peppers remaining on branches that are broken off the plant.
6. Crown set should be minimal. The first fruit set should be located more than 100 mm above the soil surface, such as in Figs. 12 and 13, and be dispersed throughout the entire plant canopy rather than low and concentrated.
7. Pendant fruit are preferred to erect fruit, especially for inclined harvesters that strip up through the plant. When the harvesting elements rotate upward, the outward rotation of the pepper pods breaks the pepper stems free from the plant.
8. Fruit detachment forces should be medium to low, such as those for jalapeno or sweet cherry. The serrano is an example of low (but acceptable) detachment force. Bell, hot cherry, and cascabell are examples of pepper types with high detachment force.
9. Many of the harvesting concepts now being evaluated lift up or rotate the pepper pod outward. Therefore, a pendant pod is more likely to break loose from the principal and lateral branches, breaking the pedicel fibers loose from the peduncle. Plants with an erect (solar) pod orientation require more aggressive stripping or shaking to detach the peppers which may result in greater damage. Orientation of pepper types with lower detachment are less of a concern.
10. Reduced detachment force in high-force cultivars are found by detailed observation, measurement, and selection, such as evaluated by Motsenbocher (1996) when high and low detachment force pedicels were detached from the pepper pods.
11. The root system must be well developed. For example, uprooting is a much greater problem with transplanted peppers, especially cherry and bell peppers, as compared to less-bushy jalapeno transplants. Jalapeno plants are less bushy, have more flexible laterals, with a more vertical architecture. Direct-seeded plants have fewer uprooting problems.

JALAPENO PEPPER DETACHMENT RESEARCH NEEDS

In the United States, many processors of jalapeno require that peppers be de-stemmed. They are typically used as either jalapeno rings or dices. To obtain de-stemmed peppers, the processors pay US$ 0.023 per kg for laborers to hand de-stem the jalapenos. Dillon (1981) tested concepts for de-stemming jalapeno peppers, but the principles have not been adopted by industry. Magrin manufactured a de-stemmer, but excessive mechanical wear and low throughput rates have stopped their manufacture and reduced their use. If either an improved design of de-stemmer or a reduced detachment force jalapeno pepper is

developed, it would reduce harvesting costs, significantly reduce production costs and expand the use of a mechanical harvester. CULTURAL ASPECTS
Certain pepper types have been grown at increased plant populations obtaining increased plant height, yield per hectare, and harvestability. In 1980, average yield/plant generally decreased with decreased plant in-row spacing and yieldi ha increased with decreased plant in-row spacing for hot and sweet banana (Table 1).

Banana	IN-ROW PLANT SPACING (mm)									
Pepper	25		75		150		300		450	
type	g/plt	t/ha	g/plt	t/ha	g/plt	t/ha	g/plt	t/ha	g/plt	t/ha
Hot	184	22.3	131	10.3	248	29.4	520	22.4	640	19.5
Sweet	79	10.3	192	17.9	310	19.1	429	17.5	328	11.0

Table 1. Average yield/plant and yield/ha for hot and sweet banana at five in row spacings, with 760 mm row spacings, Ohio, 1980 marshall, 1981).

For four pepper types, in every case, yield per hectare increased and yield per plant decreased for 200 mm in-row-spacings compared to 400 mm spacings (760 mm between rows): 2 percent and 89 percent for bell, 29 percent and 84 percent for hot cherry, 50 percent and 81 percent for Santa Fe Grande, and 75 percent and 78 percent for hot banana peppers, respectively (unpublished 1982 Michigan data by author). Plant bushiness (volume of laterals) decreased and plant height increased with decreased in-row plant spacing.

For some mechanical harvesters on non-bed cultures, a 100 to 150 mm high ridge has been found to be useful (Fig. 17). It can be formed by rolling fingered cultivators or cultivator sweeps, throwing up soil around the plant during 1 to 3 passes. This aids in weed control and provides physical support to the principal plant stem to withstand side winds which may cause the plants to tip, lean, and lodge. It also aids in reducing plant uprooting problems.
For the short term, selections made from existing pepper types and plant introduction lines and decreasing in-row-spacing hold promise to improve the ease of mechanical harvesting. Renewed pepper breeding efforts hold promise for the long term. Interest in mechanical - harvesting in the 1990's in the United States has resumed with the trend towards decreased labor availability and increased minimum wage for hand harvesting the crop. Therefore, it is desirable to have improved pepper types that will be more suitable for mechanical harvesting.
In general, doubling or tripling the normal plant population, will reduce a plant's bushiness, and make the plant easier to harvest mechanically with less plant breakage. Closer in-row spacings force the plants to grow taller, with fewer and more upright flexible branches, narrower crotch angles and higher fruit placement. The resulting reduction in yield per plant is made up by more plants per hectare. For the short term, selections made from existing pepper types and plant introduction lines and decreased in-row-spacing holds the greatest promise to improve the ease of mechanical harvesting. New interest in processing type pepper breeding holds promise for the long term.
Harvest mechanization experimentation has been going on for more than 32 years, yet mechanical harvesting is still in its infancy. Mechanical pepper harvesting will become a reality because of the renewed interest in harvester development and improved pepper

types that will be more suitable for mechanical harvesting. Worldwide interest and usage of mechanical harvesting is expected to continue to increase significantly in the near future.
A bibliography on mechanical harvesting of *Capsicum* peppers and related subjects has been compiled (Marshall, 1996). For a limited time it is also available directly from the author in MS DOS computer text processor floppy format.

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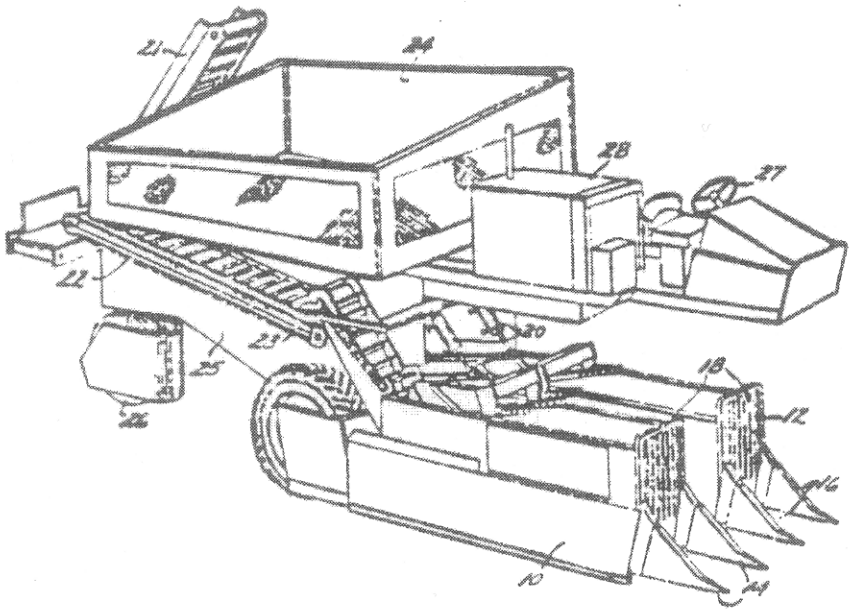


Figure 1. Experimental, inclined, counter-rotating brush chile harvester built by Ernest Riggs, Las Cruces, New Mexico, U.S. (Riggs, 1971).

Figure 2. Inclined, counter-rotating brush chile harvester built by Cal Compack, based on Riggs' 1971 patent.

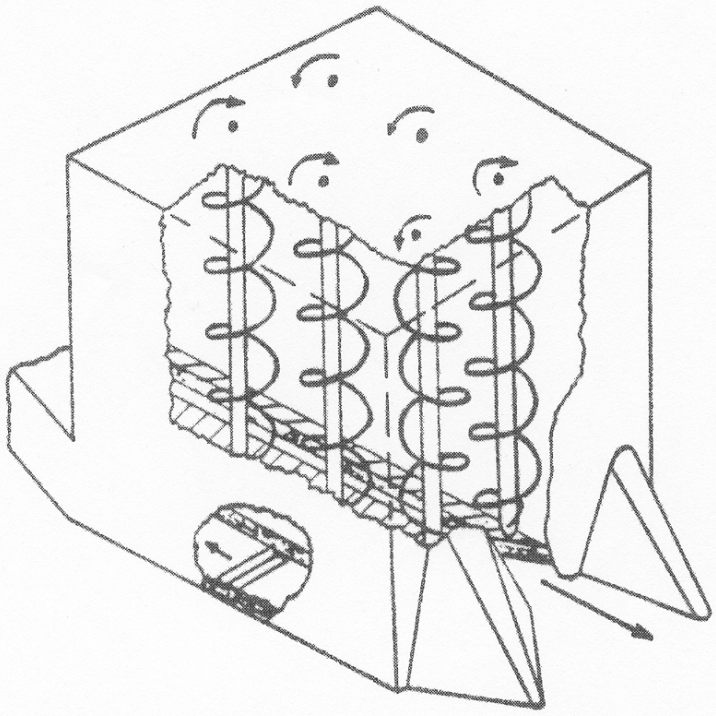


Figure 3. Vertically-oriented, twin, single open-helix chile harvester manufactured by Wondel Creager, Salem, New Mexico, U.S. (Creager 1971).

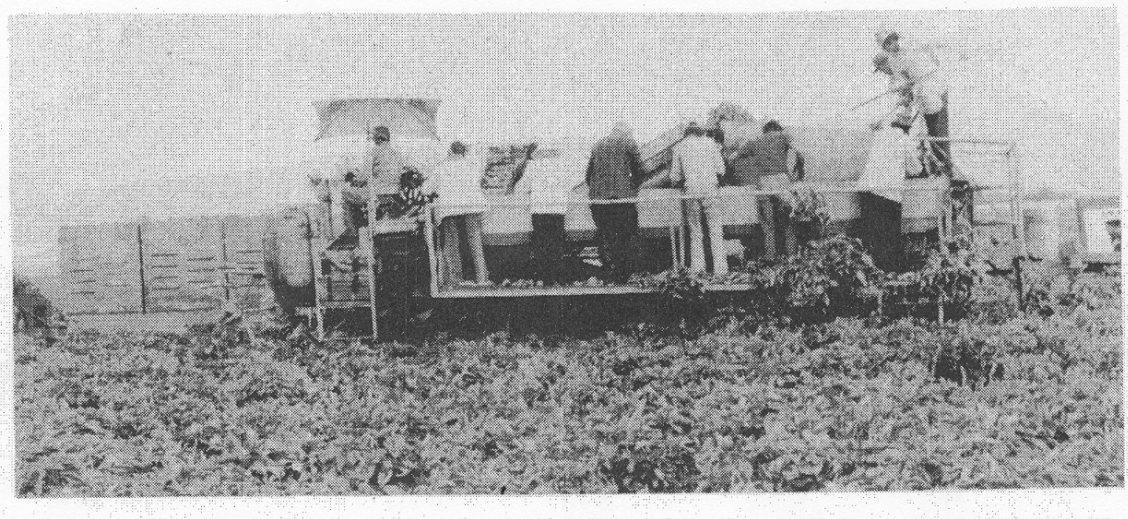


Figure 4. Bell peppers being once-over harvested mechanically with a modified self-propelled tomato harvester. Three people are shown at upper right using long-handled hoes to roll the plants over to increase foliage exposure to an aggressive, rubber roll cleaning bed which replaced the shaker, California, U.S., 1977.

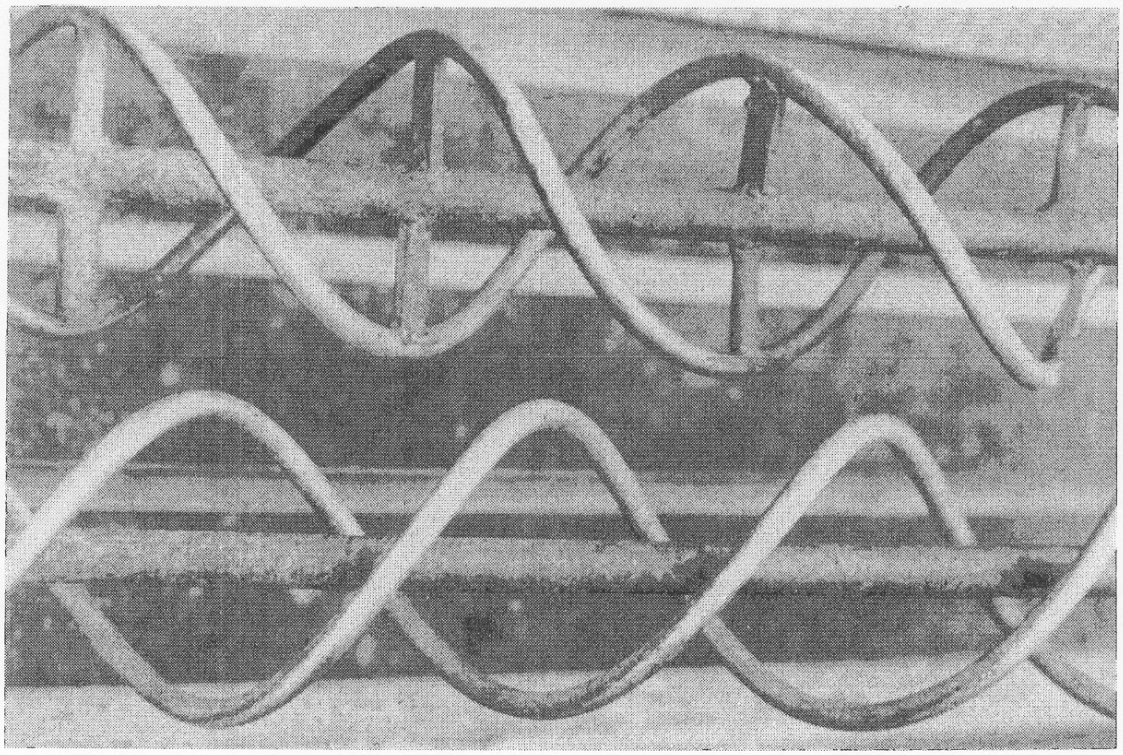


Figure 5. Experimental, twin, double open-helix concept (160 mm diameter shown) tested on pimiento peppers in Georgia, U.S. (Fullilove and Futral, 1972)

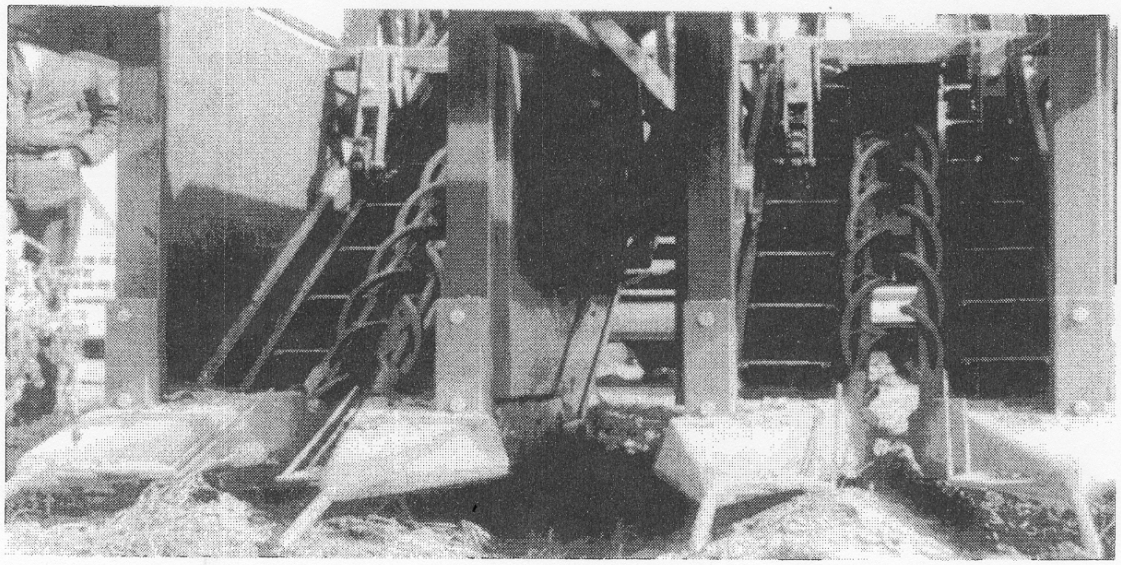


Figure 6. Experimental two-row, 160 mm diameter twin, double open helix self-propelled harvester for chiles for dehydration; built by the Agricultural Engineering Institute, Israel (Wolf and Alper, 1985).

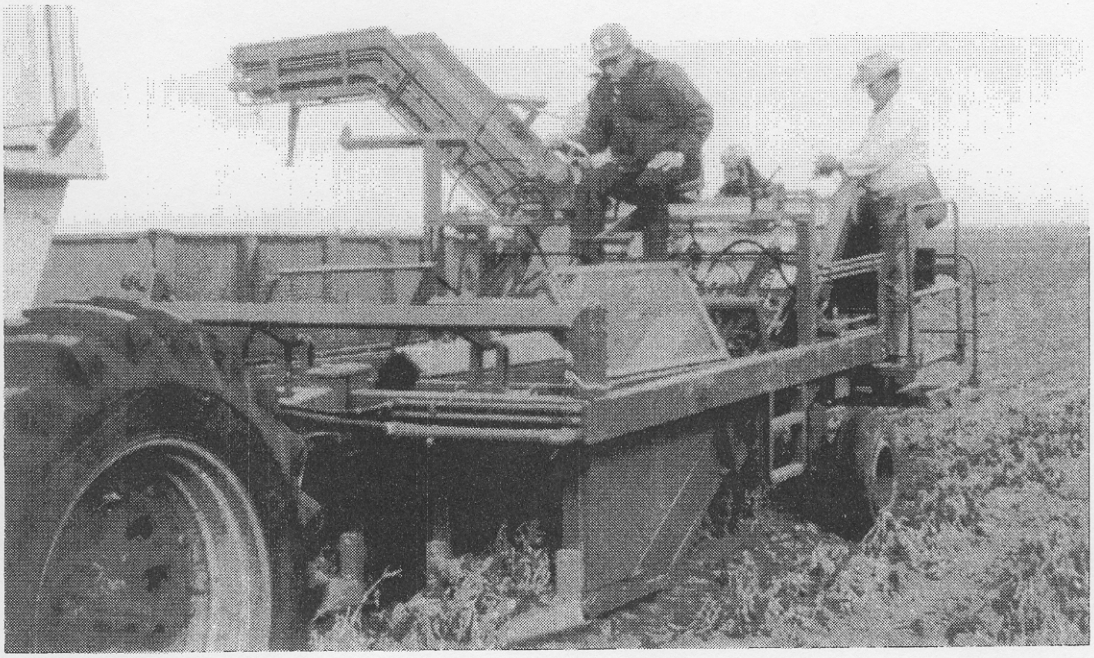


Figure 7. Commercial two-row, twin, double open helix trailer harvester for chiles for dehydration tested in Texas; manufactured by Sharnoa, Ltd., Israel.

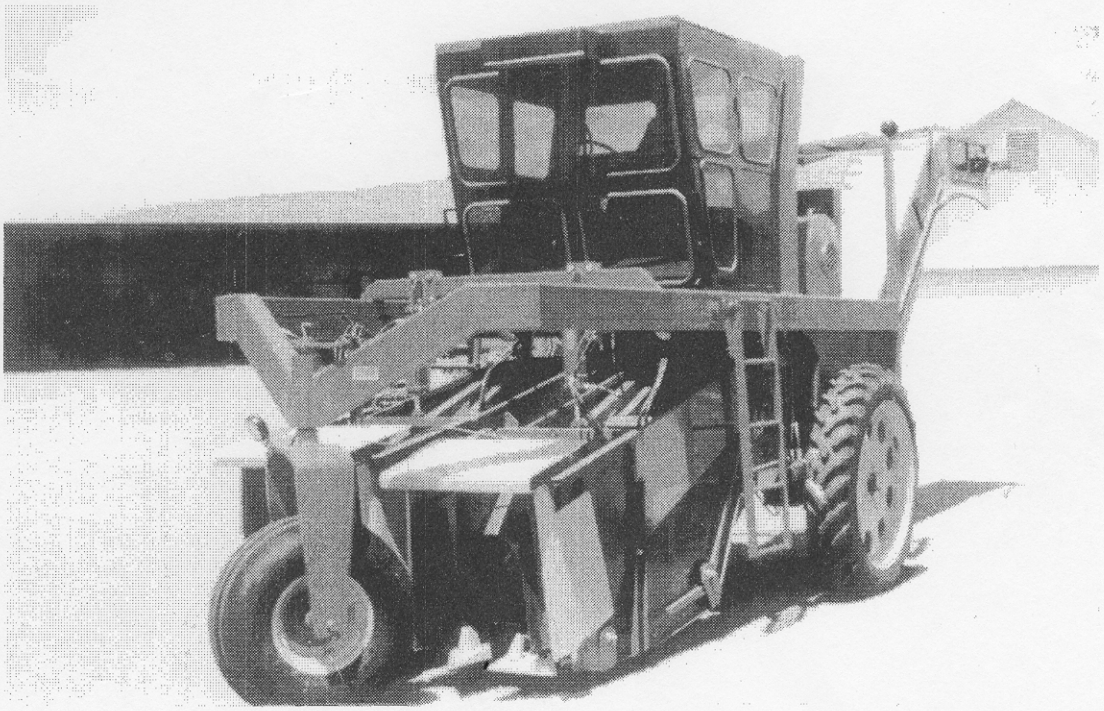


Figure 8. Commercial two-row, twin, double open-helix self-propelled, pepper harvester manufactured by McClendon Welding, Tulia, Texas, U.S..

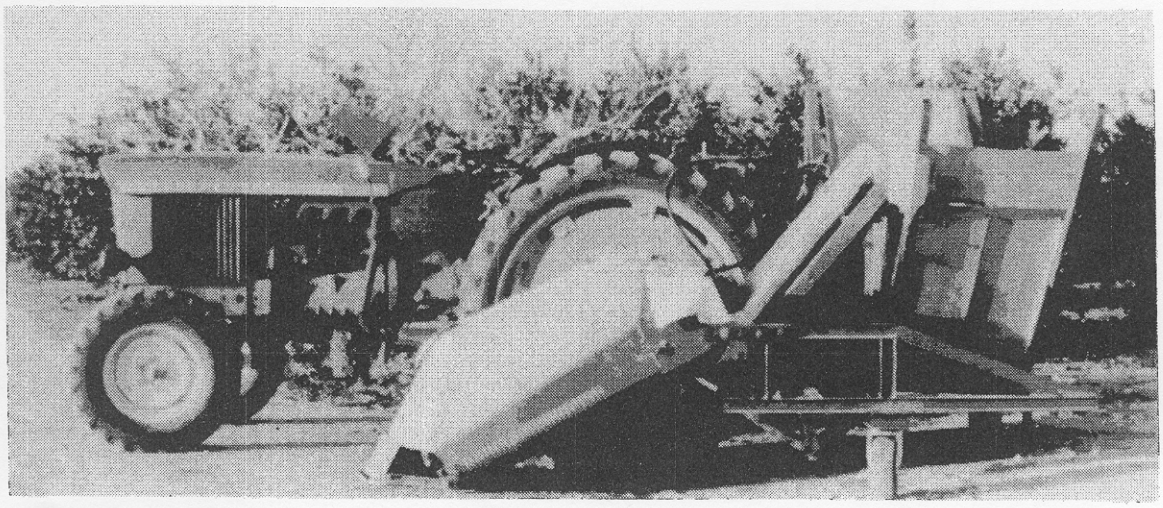


Figure 11 (Above). Commercial prototype one-row, inclined, counter-rotating, brush harvester tested in dehydration peppers; manufactured by Industrias David, Spain.

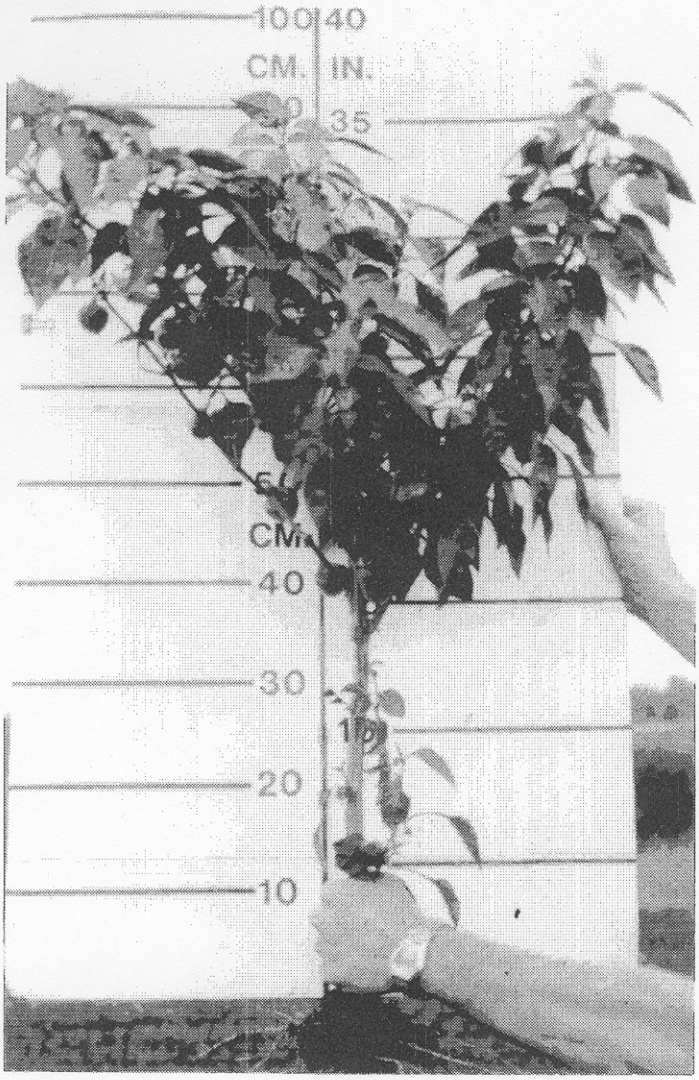


Figure 12 (Left). Plant Introduction-type cherry with upright principal stem.

Figure 9. Commercial two-row, twin, double open-helix, self-propelled, pepper harvester manufactured by Boese Equipment, Saginaw, MI, U.S.

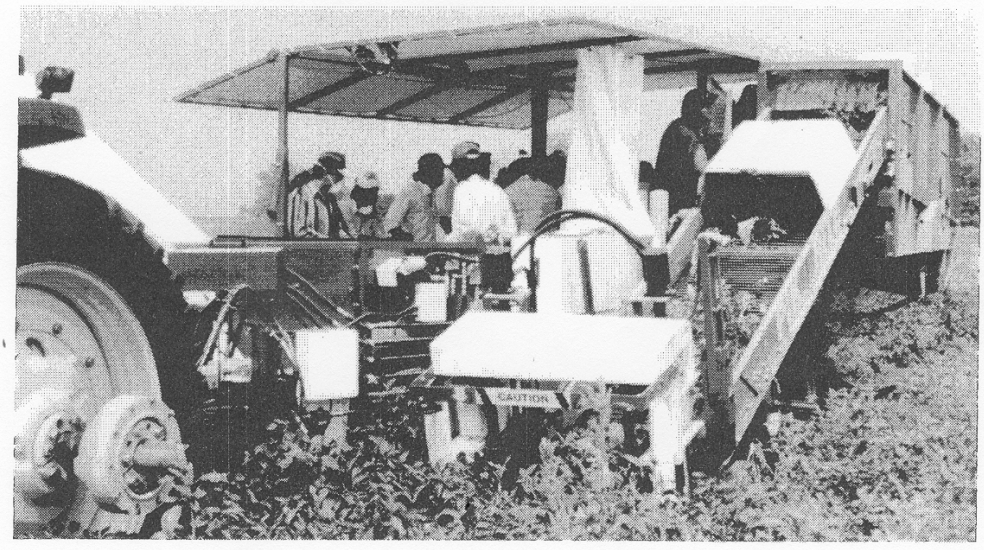


Figure 10. Commercial, powered, oscillating, forced balance shaker with soft-coated fingers, trailer pepper harvester, originally developed for harvesting tomatoes; manufactured by Pik Rite, Lewisburg, Pennsylvania, U.S.

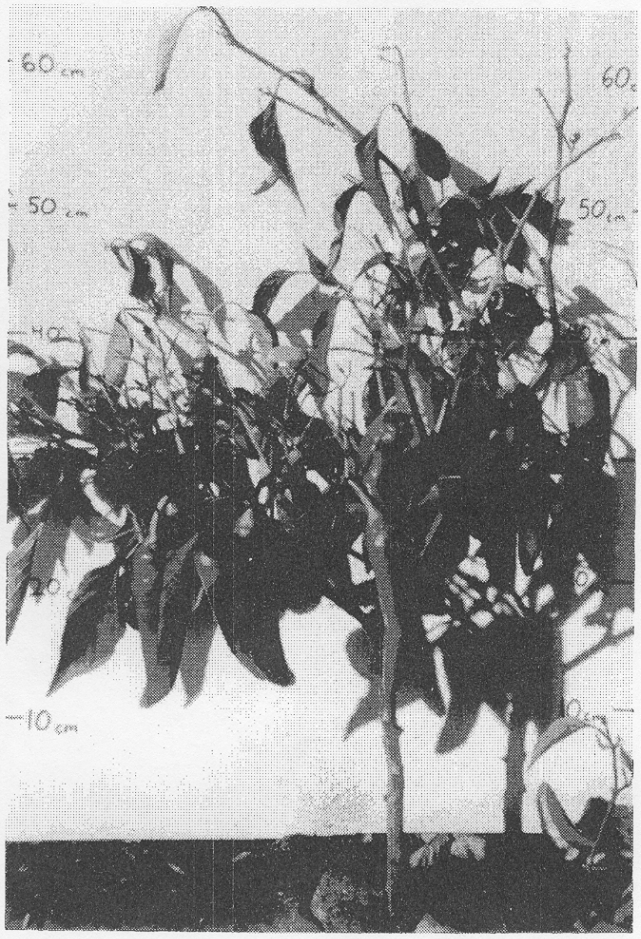
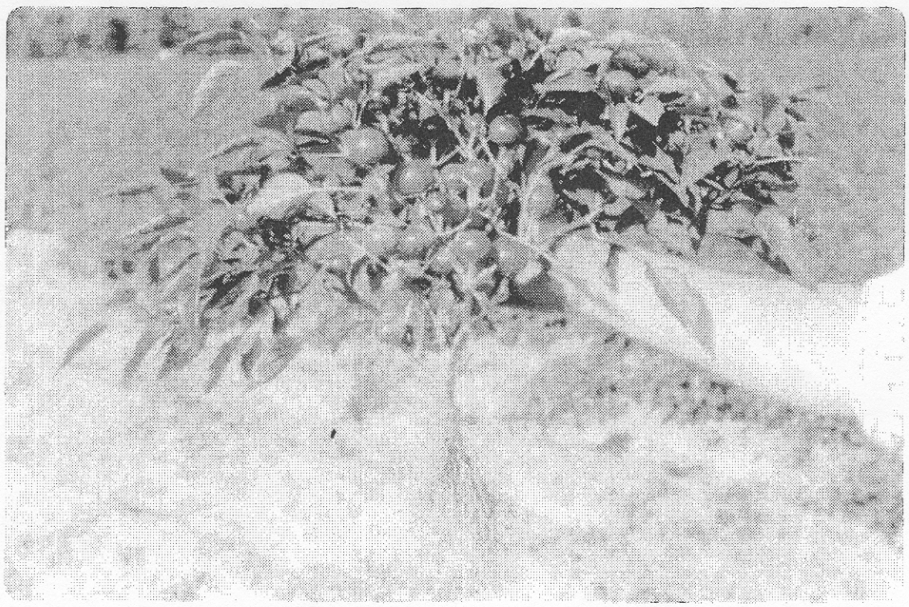


Figure 13 (Left). Spanish chile plant with desirable single upright principal stem.

Figure 14 (Below). Plants should **not** have a wide branch or crotch angle, such as this cherry-type pepper.



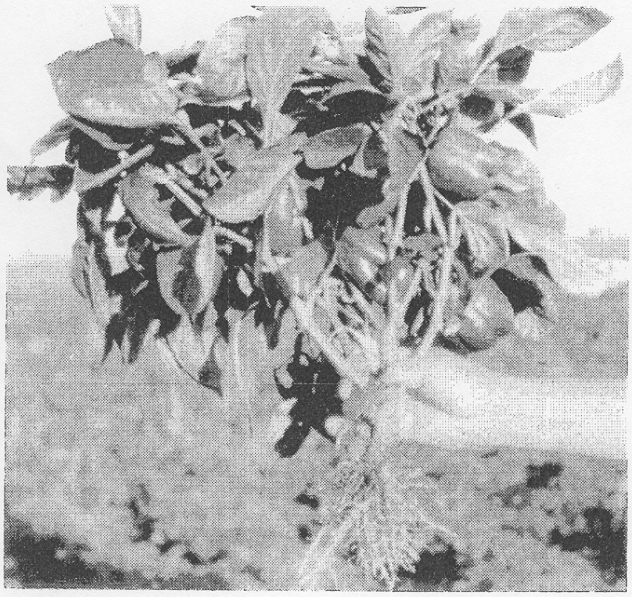


Figure 15 (Above left). Plants should <u>not</u> have a wide branch or crotch angle, such as this bell pepper.

Figure 16 (Above right). Principal plant stems should be flexible and willowy such as in serranos and some jalapeños (shown).

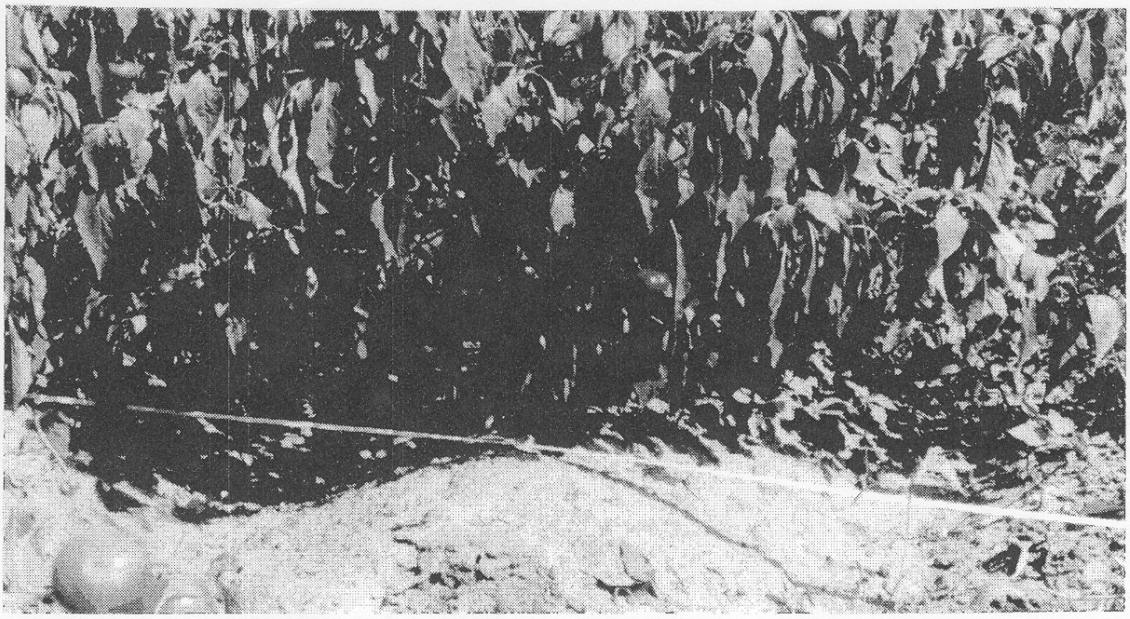


Figure 17. Beneficial 100 to 150 mm high soil ridge for some mechanical harvesters on non-bed cultures formed by rolling fingered cultivators or sweep shovels which throw soil around the plant base. This also provides physical support to the plant to withstand side winds and reduces plant uprooting problems.

Capsicum and Eggplant Newsletter. 16 (1997): 28-34. Invited paper.

Pepper Breeding in Argentina

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1. Introduction

The *Capsicum* species were originated in America and accepted almost immediately by African, Asiatic and European cultures. As a consequence the crop was spread allover the world. The consumption of fresh peppers (sweet and pungent) and the powders and sauces derived from them has increased in the last decades in many countries. Argentina has not been an exception to this pattern. The production area of peppers, particularly the one grown under protected cultivation, has increased quickly. The dehydration industry has a good market and demands cultivars adapted to its requirements. The pharmaceutical industry is interested in cultivars with high capsaicin content. Since the prohibition of synthetic colorants, the interest for *Capsicum* species as a source of natural colorants has also increased. All these reasons in addition to the incidence of diseases and pests that reduced dramatically the yields in certain regions, contribute to support pepper breeding efforts in our country. Despite of the importance of pepper few breeding programs have been supported; the most important is been held by the National Institute for Agricultural Technology (INTA) at La Consulta Experiment Station.

1. 1. Importance of the crop

In Argentina 13,000 ha are yearly grown with peppers. The average production is around 65,000 t (Table 1). Almost all the area is cropped with sweet cultivars. The national market consumes very low quantities of pungent peppers.

The main growing areas are: Salta and Jujuy for the production of paprika - and early sweet peppers; Mendoza and San Juan for the production of peppers destined to the canning and dehydration industry, Buenos Aires and the Litoral Region produces sweet peppers for the fresh market (Galmarini, C, 1993 a).

Nowadays a great part of the sweet pepper production is done under plastic greenhouses in the north of the country and mainly F1 hybrids are used. A high income covers the higher cost of F1 hybrid seeds.

The paprika production area is concentrated in the northwest in Andean irrigated valleys. Industries related with oleoresin extraction are interested in this particular area. Most of the cultivars used are OP and derived from Spanish cultivars, like: Trompa de Elefante, Nora, and Negral. Recently some cultivars like Papri King and Papri Queen have been introduced (Galmarini, C., 1993 b).

For the canning and dehydration industry mainly OP cultivars are used. Most of them developed by national breeding programs, like the cultivars Calafyuco INTA and Fyuco INTA. The most important producing areas are

Mendoza and San Juan, were around 1,800 hectares are yearly grown for this purpose. In both provinces irrigation is used.

Table* 1. *Pepper production in Argentina

	Area (ha)	Production (t)
Fresh market and industry		
Total	8033	80824
Salta	1030	14020
Jujuy	557	8160
Corrientes	276	3080
Tucuman	1540	18502
Mendoza	1378	8055
Buenos Aires	972	9170
Other provinces	2280	19830
Paprika (*)		
Total	2420	2638
Catamarca	1249	1307
Salta	996	1148
Other Provinces	175	183

* Paprika production is expressed as dry product.

Source: *Secretarfa de Agricultura, Ganaderfa* y *Pesca de la Naci6n* .

2. Breeding Programs

In pepper, as in many other vegetables, the main breeding programs have been developed by the public sector; there has been little effort by the private national seed industry in this area.

The strongest breeding program is carried on at La Consulta Experiment Station that belongs to INTA. This program was initiated in 1963 by Humberto Galmarini, then was continued by Alberto Senetiner, and nowadays by the author of this article. Besides this program there have been breeding activities in other Experiment Stations of INTA, at Salta working in the introduction of virus resistance in paprika cultivars, and at San Juan also developing paprika cultivars.

The main objectives of La Consulta's program are:

Introduction of disease resistance: The effort has been concentrated in the introduction of resistance to *Phytophthora capsici* Leonian and several viruses like PVY, PSMV and TMV.

Developing cultivars for the canning industry.

Developing cultivars suitable for the paprika industry.

Disease Resistance

One of the diseases that causes serious damage in irrigated areas is Phytophthora blight. Etiological studies by Palazon (1989) established three causes that can develop similar symptoms: *Phytophthora capsici* Leo., *Verticillium dahliae,* and root anaerobiosis. In Argentina the disease is caused mainly by *Phytophthora capsici,* although under certain conditions *Verticillium dahliae* has been found (Gal marini, C. 1990}. Using as sources of resistance PI 201234 and PI 201232 from the University of California; Davis, thecultivars FYUCO INTA, CALAFYUCO INTA and DON HUMBERTO INTA were developed (Galmarini H. and Senetiner, 1986; Galmarini C. *et a/.,1991,* and - Galmarini C. *et a/.,* 1996). All of them have field resistance to this disease. The resistance has not worked in other environments, like Zaragoza (Spain) (Ramiro Gil Ortega, personal communication), suggesting that there might be different races. The epidemiology of this disease has also been studied under our conditions (Piccolo and Galmarini, 1990).

Among the diseases caused by virus, the most important are TMV, CMV, PVY, PMMV and PMSV (Pahlen and Nagay, 1973, Feldman *et a/.* 1977). Also TSWV is becoming a problem in peppers grown in greenhouses. A great effort to identify and study this virus has been done by several virologists, especially Olga Gracia and Jose Feldman, who work together with the breeding program. As a result of this collaboration resistance to TMV and PVY have been introduced to Argentine cultivars (Galmarini, C *et a/.,* 1991).

CMV incites important damage especially in the northwest of Argentina. There is no resistant cultivar available. Efforts to introduce resistance using genetic engineering techniques are been done at INTA Castelar and INGEBI

Pests also cause problems, especially nematodes, like *Naccobus* and pepper moth. No breeding strategies have been directed towards these problems.

Canning and dehydration industry

The canning industry is well developed in the Cuyo region, mainly, in Mendoza. Perfection type peppers are used for this purpose, generally they are called "Calahorra" or "Morron". Resistance to *Phytophthora* and TMV have been introduced into cultivars destined to this purpose. Also several aspects like fruit shape suggested by other authors (Cochran, N., 1965), wall thickness, flavor and color have been targets of the breeding program.

The dehydration industry requires cultivars with high solids content. Efforts in this direction have been initiated.

Paprika Industry

This area is developed in the northwest of Argentina, and more recently in Cuyo. The activity is carried mainly by small farmers. The breeding efforts have been concentrated in the introduction of resistance to virus and fungus diseases and the improvement of fruit quality, mainly looking for high color and color stability during the drying process.

3. Released Cultivars

The breeding program of La Consulta Experiment Station has released several cultivars belonging to the "Bell Group" and "Pimiento Group" according to the classification proposed by Smith *et al.* (1987).

BELL GROUP

VYUCO INTA: Resistant to PVY. Derived from a backcross program, between California Wonder Types, Ambato (Pahlen, 1967) and a source of resistance provided by Dr. Cook (1966). It has quadrangular shaped fruits that CP change from green to red coloration at maturity (Galmarini, H. and Senetiner, 1986).

FYUCO INTA: Resistant to *Phytophthora capsici* Leonian. PI 20132 and PI 201234 from the University of California-Davis were used as sources of resistant. During the program California Wonder, Keystone Resistant Giant, Fidelio and Vyuco were used as recurrent parents (Galmarini, H. and Senetiner, 1986). The fruits have rectangular shape, are sweet, with thick walls, the coloration turns from green to red at maturity. It is widely used for fresh consumption and also by the dehydration industry in Argentina and Chile.

LUNGO INTA: Resistant to *Phytophthora capsici* Leonian. Originated from a selection out of a Fyuco population (Galmarini, C. and Lopez Frasca,1991); has long rectangular fruits (12 cm long x 5 cm wide). This cultivar was released recently and is destined for fresh market (Galmarini, C. *et al.* ,1995).

PIMIENTO GROUP

CALATAUCO INTA: Resistant to TMV. A Yolo type (Porter *et al.,1952,* Cook, 1966) was used as source of resistance and was backcrossed by a local Perfection selection. Has red, sweet, heart-shaped fruits at maturity, is used mainly by the canning industry (Galmarini, H. and Senetiner, 1986).

CALAFYUCO INTA: Resistant to TMV and *Phytophthora capsici* Leonian. Developed in 1990 (Galmarini, C. *et aI.,* 1991). The same source of resistance to *Phytophthora capsici* Leo. was used as for FYUCO INTA. Has red, sweet, heart-shaped fruits, with very thick walls at maturity. The fruits of this cultlvar ~ are smaller than Calatauco fruits. It is now widely used by the canning - industry**.**

DON HUMBERTO INTA: Resistant to *Phytophthora capsici* Leo., has yellow-orange, heart-shaped fruits at maturity. Was selected from a mutant shown in a Calafyuco INTA population and released in 1996 **(Galmarini, C *et al.,1996).***

4. Seed production

Pepper seed production is a traditional activity of the Cuyo region. At La Consulta Experiment Station research regarding seed production techniques and seed quality have been conducted. Plant distribution (Gaviola *et aI.,* 1990;

Gaviola, 1993; Del Monte *et al.,* 1996), irrigation, fertility, seed priming (Sales *et al.,1994),* seed pathology and quality are the main research areas.
At the seed quality laboratory a detailed study regarding the principal pathogens present on peppers seed has been conducted by Maria Makuch (1995).

5. Genetic Resources

The genetic erosion of the *Capsicum* germpl~sm in Latin America is a serious problem (Gonzalez and Bosland, 1991). In Argentina the active collection maintained at La Consulta includes 225 C. annuum cultivars and several wild species like: C. *chinense,* C. *baccatum,* and C. *frutescens* (Galmarini, C.,1993 a). More than 20 species have been identified in the - *Capsicum* genera; some of them that are present in Argentina have . importance as probable donors of useful genes e.g., C. *chacoense* A.T. Hunz. and C *eximium* A.T. Hunz . An excellent taxonomic approach has been carried out by Dr. Hunziker (1969).
The collection of old cultivars that were introduced by the Spanish conquerors and that are still maintained by some farmers are an important resource, because they are being quickly replaced by new cultivars and hybrids.

6. Final Remarks

This article summarize the efforts that have been done to breed pepper cultivars in Argentina. There is still a lot of work to be done. The establishment of collaborative programs between different countries, institutions and also the active participation of the seed industry is required to enhance future work.

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UPDATED INTRASPECIFIC MAPS OF PEPPER

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Introduction

A molecular linkage map is a prerequisite to genetic dissection of complex traits (Lefebvre & Chevre, 1995). Few data are available on the genetic structure of pepper. The members of the *Capsicum* genus are generally diploid with 24 chromosomes (2n=2x=24) (Pickersgill, 1977). C. *annuum* possesses 2 acrocentric pairs of chromosomes (Pickersgill, 1977). Translocations were described among the different species of *Capsicum* (Pickersgill, 1988). Pepper possesses a big genome, about 4-fold the size of tomato genome (2.76 pg/C in pepper *vs.* 0.74 pg/C in tomato) for the same number of chromosomes (Arumuganathan & Earle 1991). The 12 trisomics corresponding to the 12 chromosomes were obtained in C. *annuum* by Pochard (1970) and designated by a French colour name (violet, indigo, bleu, vert, jaune, orange, rouge, pourpre, noir, brun, bistre, gris). Three chromosomes can easily be identified with cytology: the I (violet) corresponding to the larger chromosome, the XI (jaune) corresponding to a small acrocentric chromosome, the XII (pourpre) corresponding to the smallest acrocentric chromosome bearing the nucleolus organisator.

Several partial linkage maps of pepper have already been published (Tanksley, 1984; Tanksley *et al.* 1988; Prince *et al.* 1993, Lefebvre *et al.* 1995). The first three were constructed from interspecific crosses between C. *annuum* and C. *chinense.* The last one was constructed from intraspecific crosses between C. *annuum* lines. Intraspecific maps present advantages over interspecific maps: (i) many breeding programs in pepper use the intraspecific variability of C. *annuum* and the use of intraspecific polymorphism will facilitate marker-based selection, (ii) the low fertility and low recombination rates or chromosomal aberrations (translocation, inversion, skewed segregation...) often found in interspecific crosses are expected to be reduced. Although the intraspecific polymorphism is lower than the interspecific polymorphism, the level of intraspecific polymorphism in C. *annuum* was previously judged sufficient for mapping initiation (Prince *et al.* 1992; Lefebvre *et a/.* 1993). Different kinds of genetic markers were used in pepper. Very few isozyme polymorphism was detected inside C. *annuum* (Conicella *et a/.* 1990). RFLPs were proved to be more informative in intraspecific studies than protein markers (Lefebvre *et al.* 1993). RAPDs were considered to be more polymorphic than RFLPs because several polymorphic loci may be revealed with one primer whereas a single polymorphic locus was generally revealed with one RFLP probe (Lefebvre *et al.* 1995).

In this paper, we report the construction of two updated molecular linkage maps of C. *annuum* generated with two doubled haploid (DH) populations obtained from intraspecific F1 hybrids. RFLPs, RAPDs, known function genes, an isozyme and phenotypic markers were used. Doubled haploids allow the continuous addition of markers to the map and simultaneous mapping of numerous monogenic and polygenic traits in the same progeny.

Material and methods

Two DH populations of 94 and 98 lines were derived using *in vitro* androgenesis (Dumas de Vaulx *et a/.* 1981) from F1 hybrids of the crosses 'Perennial' x 'Yolo Wonder' and 'H3' x 'Vania', respectively. Parents and segregating populations were described by Lefebvre *et 81.* (1993 & 1995) and Daubeze *et a/.* (1995).

Plant genomic DNA isolation, RFLP (Southern blot, probes preparation, hybridisations) and RAPD assays were carried out as described by Lefebvre *et a/.* (1993 & 1995). DNA probes of tomato (named TG for tomato random genomic DNA, CD

and CT for tomato random cDNA) and pepper (named PG for pepper random genomic DNA) were kindly supplied by S.D. Tanksley and M.M. Kyle, respectively (University of Cornell, N. Y, USA) Clones of known genes were used for RFLP assay. CAB-1 encoding the major chlorophyll *a/b* binding protein, r45S encoding the large 45S subunit rRNA and Rbcs3 encoding the small ribulose biphosphate subunit were kindly supplied by S.D. Tanksley (Bernatsky & Tanksley, 1986). The potato cDNA CB3 encoding a class I basic chitinase was kindly supplied by E Kombrink (Max Planck Institut, Germany) (Beerhues & Kombrink, 1994). The tobacco cDNA CA3 encoding a class III acid chitinase was kindly supplied by M. Legrand (Institut de Biologie Moleculaire des Plantes, France) (Stinlzi *et 81.* 1993). The tobacco cDNA PR4 encoding a pathogenesis-related protein of class 4 was kindly supplied by K.A. Lawton (Ciba-Geigy Corporation, N.C., USA). Oligonucleotide primers for RAPD procedure were purchased from Operon Technologies Inc. (Alameda, CA., USA). The isozyme marker Mnr-1 (first region of the menadione reductase: 1.6.992) segregating in 'Perennial' x 'Yolo Wonder' DH population was scored by P. Belletti (University of Turin, Italy) using previously described method (Belletti *et 81.* 1992). Four phenotypic markers were studied in the DH populations. They are described below.

The X21df goodness-of-fit value for unskewed haploid segregation was calculated for each marker in each progeny (Mather 1951, Lefebvre *et al.* 1995). The Mapmaker software (Version 3.0 b, Lander *et 81.* 1987) was used to construct the genetic linkage maps with a maximum recombination fraction of 0.3 and a minimum LOD score of 3.0 for 'Perennial' x 'Yolo Wonder' DH population and 5.0 for 'H3' x 'Vania' DH population. Some markers close to each other (5 cM) could not " be mapped in a single order. The most likely order is presented on the maps. Distances between markers were calculated using the Kosambi mapping function (Kosambi, 1944).

Results and discussion

Poiymorohism and searegation

To assess RFLPs, 332 and 146 DNA clones were tested on survey filters comprising parental DNA of 'Perennial' x 'Yolo Wonder' and 'H3' x 'Vania' crosses, respectively, digested with each of 5 enzymes *(Ora* I, *EcoR* I, *EcoR* V, *Hind* III, *Xba* I). A total of 125 (37.7%) and 58 (23.6%) clones detected RFLPs with at least one enzyme in 'Perennial' x 'Yolo Wonder' and 'H3' x 'Vania' crosses, respectively. To assess RAPDs, 248 and 290 random primers were tested on parental DNA of 'Perennial' x 'Yolo Wonder' and 'H3' x 'Vania' crosses, respectively. A total of 96 (38.7%) and 65 (22.4%) primers revealed RAPDs, respectively. These values confirm the percentages of polymorphism given by Lefebvre *et aI,* (1993 & 1995).

A total of 47 (24.2%) markers (13 RFLPs, 33 RAPDs and *up)* was skewed in the 'Perennial' x 'Yolo Wonder" DH population, and only 6 (6.8%) markers (4 RFLPs and 2 RAPDs) in the 'H3' x 'Vania' DH population, with a type I error of a=0.O1. Many skewed RAPDs markers were clustered, Thus, the number of biased markers of the 'Perennial' x 'Yolo Wonder' DH population has to be balanced by their relative position on the map. Anyway, it is lower than in observed interspecific crosses (Tanksley *et a/.* 1988, Prince *et ai,* 1992, M. Kyle pers. comm.) and than in intraspecific DH progenies involving 'Criollo de Morelos 334' (Lefebvre *et a/.* 1992), Heun & Helentjaris (1993) ascribed skewed segregation ratio to the low reproducibility of some RAPDs, primarily those with weak amplifications. They may also be due to the fact that two polymorphic fragments can comigrate.

The 'Perennial' x 'Yolo Wonder intrasoecific linkage map

Segregation data of 194 markers were used for linkage analysis. Among them, 189 markers (114 RAPDs, 71 RFLPs, 1 isozyme and 3 phenotypic markers) were mapped into 16 linkage groups (LG) (14 major and 2 minor) that span a map distance of 1515 cM (Fig. 1). Five markers remain unlinked. This map was estimated to cover 67 to 100 % of the pepper genome according to the previous estimates of the pepper genome length (Lefebvre *et* a{ 1995). Clusters of markers were observed on five linkage groups. They were mostly composed of RAPD markers.

The 'H3' x 'Vania' intrasoecific linkage map

Segregation data of 92 markers were used for linkage analysis. Among them, 83 markers (52 RAPQs, 29 RFLPS 'and 2 phenotypic markers) were mapped into 15 LGs (6 minor), spanning a map distance of 417 cM (Fig. 1). Nine markers remain unlinked. This map was

estimated to cover 19 to 31 % *of* the pepper genome. Clusters *of* markers were observed on four linkage groups.

- *Comparison of the two intraspecific map*

For both the maps, the number of linkage groups is higher than the number of chromosome (x=12) - and some markers remain unlinked, indicating an unsaturated map. Both the intraspecific maps cover a maximum length of 1582 cM with 227 different markers distributed on a total of 18 different LGs (45 markers common to the two maps). Ten LGs are shared by the two maps; 8 LGs are cross-specific. Orders of RFLP markers suited with previously published maps for the corresponding sets of markers, although some differences in distance can be found. RAPD markers with the same-sized polymorphic fragment in both the DH populations were notably mapped to the same location - indicating that some RAPD markers are locus-specific in pepper and that integration of RFLPs and RAPDs from different maps is possible.

- RAPD clusters

Clusters of RAPD markers were supposed to correspond to centromeres of pepper chromosomes. Only one cluster per linkage group was observed. RAPDs are known to detect polymorphisms in high-copy number DNA sequences (Paran & Michelmore, 1993; Giese *et a/.* 1994) like centromeres and telomeres (Grandillo & Tanksley, 1996). Clusters could result from a reduction of genetic recombination, generally observed around centromeres (Roberts, 1965) or telomeres (Lefevre, 1970). The use of tomato DNA probes linked to the putative centromeres of tomato (Tanksley *et al.* 1992, Presting *et al.* 1996) allowed us to assign pepper RAPD clusters to corresponding tomato centromeres. The group of markers TG66-CT141 and the marker CD8, linked to the cluster on pepper LG 1, flanked the putative centromeres of tomato chromosomes 3 and 9, respectively. The marker CD25 linked to the cluster on pepper LG 3 flanked the putative centromere of tomato chromosome 6. Markers TG55 and TG83 linked to the cluster on pepper LG 5 flanked the putative centromere of tomato chromosome 1. The groups of markers CD186- TG379 and TG1 04- TG4 7 -CT120, linked to the cluster on pepper chromosome Brun, flanked the putative centromeres of tomato chromosomes 5 and 11, respectively. The marker TG483 linked to the cluster on pepper chromosome Jaune and the marker CT259 linked to the cluster on pepper LG 4P flanked the putative centromere of tomato chromosome 4. Differentiation between pepper and tomato seems frequently involved chromosome breakage in *or* close to the centromeric regions.

- Assignment *of* linkage groups to chromosomes

Five linkage groups are now assigned to five different chromosomes. The phenotypic markers L (resistance to Tobacco Mosaic Virus and Pepper Mild Mottle Virus, Holmes, 1937, Palloix, 1992), C (pungent fruit, Deshpande, 1935), up (upright habit *of* fruit, Lippert *et al.* 1965) and *pvr2* (resistance to PVY(O) , PVY(1) and TEV, formerly named *vy* locus, Gebre-Selassie *et a/.* 1985, Palloix & Kyle, 1995) were used to assign linkage groups to pepper chromosomes Brun, Jaune, Noir and Orange, respectively, thanks to results from the trisomic analyses (Pochard 1977, Pochard & Dumas de Vaulx, 1982). The45S rRNA-encoding DNA marker *(r45S)* allows the assignment *of* one linkage group to the pourpre chromosome thanks to the combined results of enzyme allele dosage in trisomics (Tanksley, 1984), chromosome *in situ* hybridisation assay and mapping (Tanksley *et a/.* 1988). Land C loci segregated in both the DH populations. *up* and *r45S* loci segregated in the 'Perennial' x 'Yolo Wonder' DH population. The *pvi2* locus only segregated in the 'H3' x 'Vania' DH population. It was mapped in the vicinity *of* the RFLP TG132 and the RAPDs AC10_0.3 and R11_0.4, on the 'H3' x 'Vania' DH population. Although neither 'Perennial', nor 'Yolo Wonder', possesses the resistance allele *pvr2,* it was possible to assign one linkage group to the chromosome Orange in the 'Perennial' x 'Yolo Wonder' DH population thanks to comparative mapping.

- **Genes of agronomic interest**

These two maps have been used to map numerous genetic factors of economic importance:
- one fruit-morphology locus and one fruit-quality locus: *up* and C (lefebvre *et at.* 1995),
- major disease resistance loci: *L* (lefebvre *et at.* 1995), *pvr1* (M. Kyle, personal communication), *pvr2* and *pvr6* (Caranta *et at.* 1996; Caranta *et at.* submitted),
- polygenic disease resistance to *Phytophthora capsici* (lefebvre & Palloix, 1996), Cucumber Mosaic Virus (Caranta *et at.* 1997) and3 potyviruses (Caranta *et at.* Submitted).

These results provided evidence for colocalization of several resistance factors. Genes or QTLs of interest associated with linked molecular markers will serve in marker-based selection.

Conclusion and prospects

The two intraspecific maps of pepper spanned a total length of 1582 cM. Five linkage groups were assigned to five chromosomes. Six centromeres of pepper chromosomes were tentatively located. These maps are still in progress. In order to saturate them, we are developing AFlP marker (Zabeau, 1993; Vos *et at.* 1995), a novel PCR-based assay for DNA fingerprinting, and other mapping populations to complete our exploration of the pepper genome for genes of interest. A 300-F2 population from the 'Yolo Wonder' x 'Criollo de Morelos' cross will allow us to precise the order of clustered markers and to develop fine mapping programs.

The tagging of resistance genes to Tomato Spotted Wilt Virus, to *Xanthomonas campestris* pv. *vesicatoriae,* to *Metoidogyne* nematodes and QTL analysis for resistance to the powdery mildew caused by *Leveittuta taurica* and for different components of the resistance to Cucumber Mosaic Virus are also underway.

Mapping different progenies allows the exploration of a larger number of polymorphic regions and the integration of different traits on a single linkage map. Our objective is to concentrate all genetic informations on a pepper reference integrated map thanks to genetic markers linked to genes of interest. This aim will contribute to a better understanding of the organisation of genetic factors of agronomic interest on the pepper genome.

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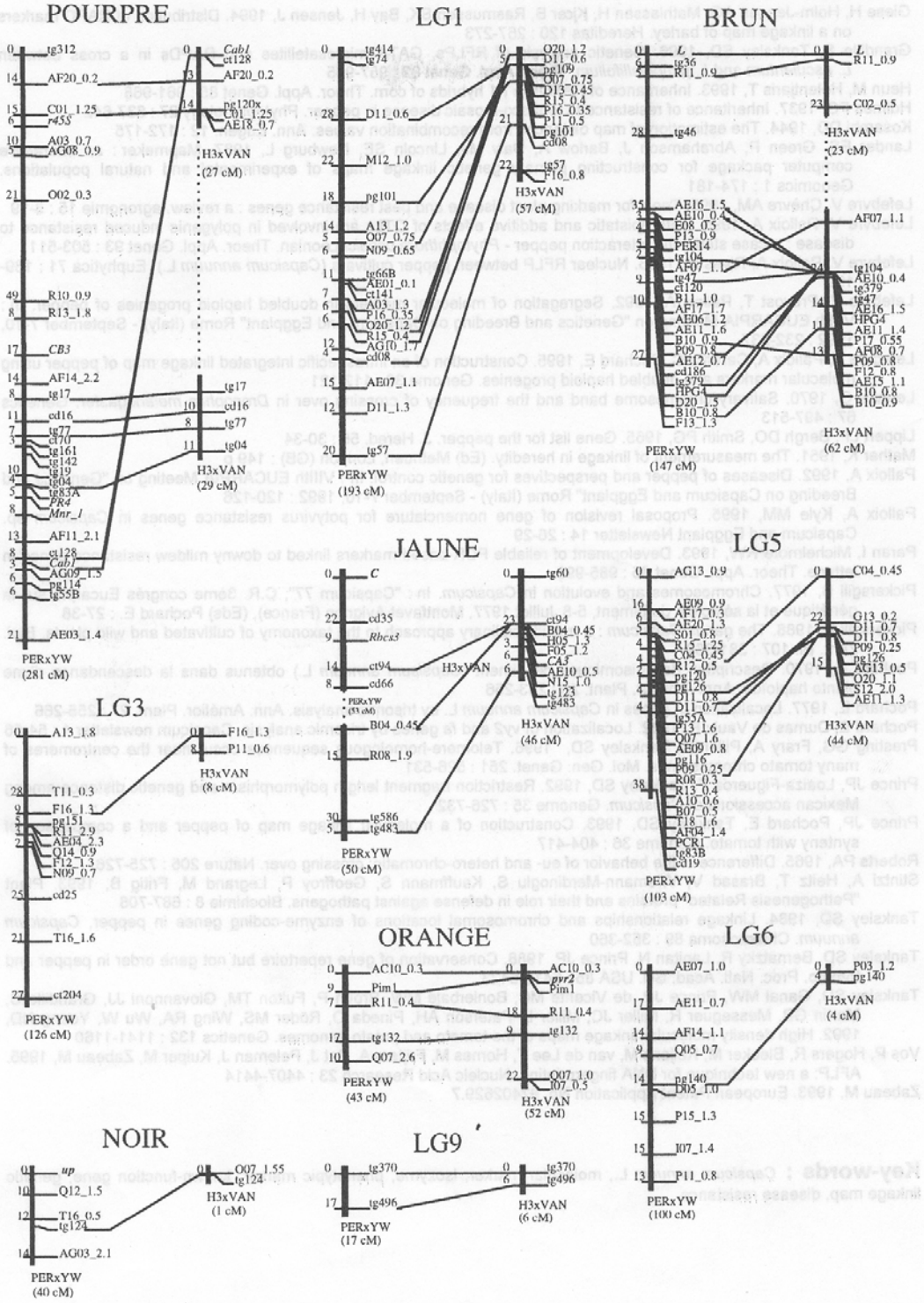
Key-words: *Capsicum annuum* L., molecular marker, isozyme, phenotypic marker, known-function gene, genetic linkage map, disease resistance


POURPRE
LG1
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NOIR
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PERxYW (281 cM)
H3xVAN (27 cM)
H3xVAN (29 cM)
PERxYW (193 cM)
H3xVAN (57 cM)
PERxYW (147 cM)
H3xVAN (23 cM)
H3xVAN (62 cM)
PERxYW (50 cM)
H3xVAN (53 cM)
H3xVAN (46 cM)
PERxYW (103 cM)
H3xVAN (44 cM)
PERxYW (126 cM)
H3xVAN (8 cM)
PERxYW (43 cM)
H3xVAN (52 cM)
PERxYW (100 cM)
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PERxYW (40 cM)
H3xVAN (1 cM)
PERxYW (17 cM)
H3xVAN (6 cM)

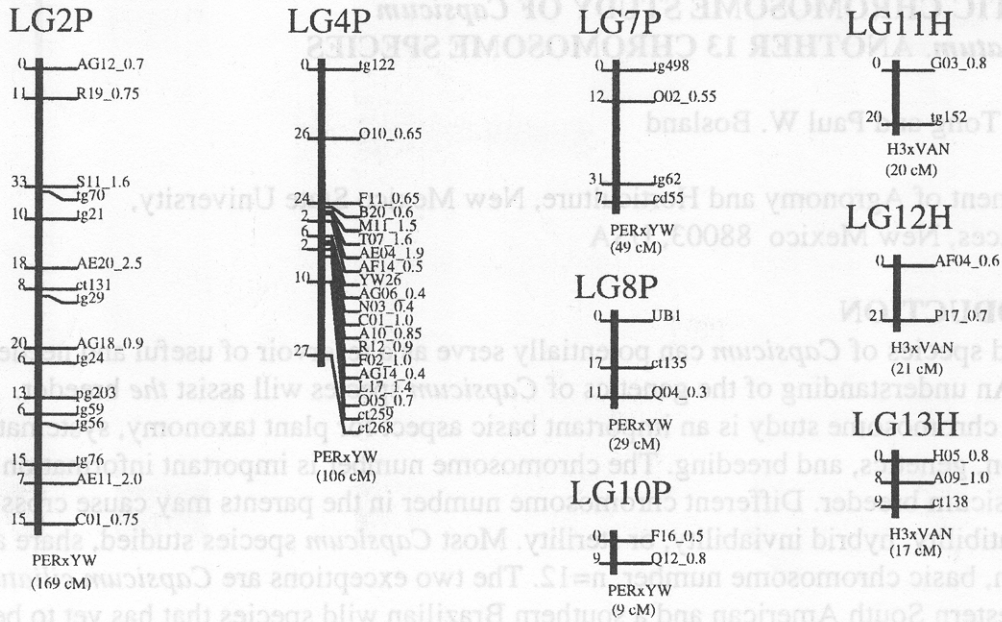

Fig. 1 (on 2 pages) : Intraspecific linkage maps of pepper (*C. annuum*) from the crosses 'Perennial' x 'Yolo Wonder' (PERxYW) and 'H3' x 'Vania' (H3xVAN). Linkage groups (LG) are schematically represented by vertical lines. LG numbers are given arbitrarily. LGs suffixed by P and by H correspond to LGs specific for 'Perennial' x 'Yolo Wonder' cross and for 'H3' x 'Vania' cross, respectively. The French colour names assigned to certain chromosomes refer to those given by Pochard (1970). Symbols for loci are shown on the right of the LG. Numbers on the left correspond to map distances between markers in cM (Kosambi function). RFLP markers are prefixed with 'tg', 'ct', 'cd', 'pc' for the loci detected with random DNA clones. The markers PCR1, Pim1, YW26, PER14, HPG4 and UB1 correspond to RAPD or RFLP markers obtained with primers or probes of our laboratory. Clones corresponding to known function genes are designated by their specific names. Some RFLP markers are suffixed with capital letter; this indicates that more than one locus is detected in pepper with the same probe. Nomenclature for RAPD loci indicates the Operon primer kit designation (a letter and a number) and the relative molecular weight, in Kb, of the visualised RAPD band. Maps were drawn using the MapDisplay software developed by Decoux & Causse (in preparation).

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MEIOTIC CHROMOSOME STUDY OF *Capsicum lanceolatum.* ANOTHER 13 CHROMOSOME SPECIES

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INTRODUCTION

Wild species of *Capsicum* can potentially serve as a reservoir of useful and needed genes. An understanding of the genetics of *Capsicum* species will assist *the* breeder. Meiotic chromosome study is an important basic aspect for plant taxonomy, systematics, evolution, genetics, and breeding. The chromosome number is important information for the Capsicum breeder. Different chromosome number in the parents may cause cross- incompatibility, hybrid inviability, or sterility. Most *Capsicum* species studied, share a common, basic chromosome number, n= 12. The two exceptions are *Capsicum ciliatum* from western South American and a southern Brazilian wild species that has yet to be identified with certainty. These two species have n = 13 chromosomes (Pickersgill. 1991).
Capsicum lanceo/atum (Greenm. ex J.D. Sm.) Morton and Standley, a wild species, was reported by Standley in 1939 to grow in "a wet, damp forest" (Standley and Steyennark.1940). Therefore, it may contain genes for resistance to water molds, bacterial diseases, and other moist environmental pathogens. It is a non-pungent species, and is found in Guatemala. The chromosomes of this species have not been documented. Meiotic chromosome investigation was performed to count basic chromosome number and to study chromosome configurations in meiotic cells of C. *lanceolatum.*

MATERIALS AND METHODS

The experimental plants were from Guatemala and were grown in the New Mexico State University Chile Breeding Program's greenhouse. For the meiotic chromosome investigation, flower buds from experimental plants were collected in the morning fixed in an ethanol-acetic acid (3: 1) mixture overnight, and stored in 70% ethanol for at least 24 hours. Anthers were taken from individual flower buds to make a chromosome sample.
Chromosome count and chromosome pairing were detected at diakinesis, metaphase-I and anaphase-I of pollen mother cells (PMCs) using the aceto-carmine normal squash method (Singh, 1993).

RESULTS

In a total of 50 PMCs analyzed cytologically, 26 chromosomes (n = 13) were observed at metaphase-I and anaphase-I. Chromosome pairing at diakinesis and metaphase-I of PMCs is listed in Table 1. The types of chromosome pairing in the PMCs were bivalent and quadrivalent. Chromosomes in 62.5% of the PMCs paired as 13 bivalents. In 31.3% of the PMCs. 11 bivalents and 1 quadrivalent were presented. And in 6.2% of the PMCs, other types of pairing including 9 bivalents and 2 quadriva1ents and 7 bivalents and 3 quadrivalents were observed. Meiotic chromosomes behaved as 13 bivalent or II bivalent and 1 quadrivalent in most PMCs (93.8%). In meiosis division, quadrivalents at

metaphase-I can disjunct normally into anaphase-I. In this study, no irregular behaviors of meiotic chromosomes (such as a chromosome lag) were found.

Table 1. Meiotic chromosome pairing *of* PMCs in C. *lanceolatum*

Chromosome pairing		
II	IV	No. of cells examined
13		20(62.5%)
11	1	10(31.3%)
9	2	1(3.1%)
7	3	1(3.1%)
		Total 32(100%)

Note: II and IV denote bivalent and quadrivalent respectively.

DISCUSSION

Pickersgill (1991) mentioned one southern Brazilian wild species that had chromosome number of 2n = 26. However, that species most likely is not C. *lanceolatum* because C. *lanceolarum* is not found in Brazil. Therefore, the results of this experiment indicate C. *lanceolarum* is the third *Capsicum* species that has been found to have a chromosome number of 26.

It is interesting to note that C. *lanceolarum* and C. *ciliatum* are both non-pungent wild species, and have the same basic chromosome number (2n = 26). It may be that wild, non-pungent *Capsicum* species have 26 chromosomes. To verify this assumption, more research covering non-pungent and wild species will need to be conducted.

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ESTABLISHMENT OF TETRAPLOID PLANTS OF *Capsicum annuum* L. BY COLCHICINE TREATMENT WITH THE ANALYSIS OF FLOW CYTOMETRY

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Abstract

Tetraploid plants were obtained efficiently by colchicine treatment of the seeds in the concentration of 0.5 or 1.0 % for 4 days. The polyploidy was determined by flow cytometry analysis of the leaves and the pollen. Both 2C and 4C peaks were obtained by the flow cytometric analysis of pollen isolated from the flower buds of tetraploid plants. These histograms were clearly distinguished from those obtained by the pollen isolated from the flower buds of diploid plants.

Introduction

Chromosomes are efficiently doubled by colchicine treatments, and double-haploid and tetraploid plants are willingly used in the breeding programs (Jensen, 1974). Tetraploid plants are also useful in the breeding of *Capsicum annuum* L. One usage of them is in the overcome of sterility in far-distance crossing. In the breeding of *Capsicum annuum,* many useful traits, such as disease resistances and fruit productivity, have been introduced from other species. But interspecific hybrids between some different species showed sterility and even the back *Gross* generations could not be obtained. It was reported that fertile amphidiploid plants were obtained in the hybrid between *Iris* (Yabuya,1985), and Cyclamen *persicum* Mill. and C. *hederifolium* Aiton (Ishizaka and Uematsu, 1994). Another usage of tetraploid plants is improvements of the resistances against various stress, enlargement of some organs, such as flower, leaves, fruits or tubers (Okada and Matsumoto, 1993) etc.
Even these usefulness, tetraploid plants of capsicum have not been used in breeding programs so far. Recently, it becomes more necessary to introduce new disease resistance genes from various species, to improve various stress resistance, and to improve fruit characters and increase the useful secondary metabolites. We expected that tetraploid plants would be a new clue in these breeding programs. In this report we showed the method for tetraploid plant production by colchicine treatment of the seeds and the determination of the polyploidy by flow cytometry.

Materials and Methods

The seeds of *Capsicum annuum* L. cv. 'New face' (Nihon Horticultural Production Institute) were surface sterilized with 1 % sodium hypochlorite solution for 15 min and rinsed 3 times in sterile distilled water. About 40 seeds were then soaked in 10 ml of the various concentrations of colchicine solution (0,, 0.5 and 1.0 %) in Petri dishes (9 cm in diameter), in which one sheet of filter paper was put. One , two and four days after soaking, the seeds were transferred onto Murashige and Skoogs I medium (1969) with 3% sucrose solidified with 0.2% Gellan Gum and cultured on 25 OC under continuous light condition.
Several weeks after culture, plantlets were acclimatized and transferred to pots

and cultured on the same environmental conditions. Ten weeks after colchicine treatments, DNA contents of leaves of these plantlets were analyzed by flow cytometry. The plantlets of this stage had 3 to several branches. One leaves from each branches were investigated. For flow cytometric analysis of these leaves, the leaves were chopped with a sharp razor blade in the buffer (buffer component; O.lM tris-HCI (pH7.5), 0.1 % Triton X-100, 2mM MgClz and 2 mg/l DAPI). After chopping, the suspension was filtrated with *20ttm* nylon mesh and immediately analyzed with flow cytometer (Partec Cell analyzer CAII, Germany).

After determination of polyploidy, 15 control (diploid, not colchicine treated) and 15 tetraploid plantlets were transplanted onto the soil in a greenhouse. DNA contents of the pollen were analyzed by now cytometry. The pollen was isolated from the flower buds which would bloom next day and dipped in the buffer, and immediately analyzed.

Results and Discussion

Capsicum anlluum L. cv. 'New face' is one of the popular cultivated variety in Japan. The fruits are bell type and sweet, and harvested at 3 weeks after flowering. Germination rates were 100 % in both control and colchicine treated condition.

Polyploidy were determined by the now cytometric analysis of the leaves at first and then pollens. Two C and 4 C peaks were obtained in the leaves from diploid (Fig. 1 *A»* and tetraploid plants (Fig. 1 B», respectively. The flow cytometric analysis were reported using whole seeds and isolated embryos in the standard methods (Lanteri et al., 1992).

Tetraploid plants were efficiently obtained by the colchicine treatment of the seeds. Twenty four % of the plants were tetraploid, by soaking of the seeds in the 0.5 ot 1.0%

Table 1. Tetraploid plants obtained by colchicine treatment of the seeds with different concentrations and periods.

Colchicine Treatment		
Concentrations (%)	Periods (days)	% of Tetraploid
0	1	0
	2	0
	4	0
0.5	1	0
	2	0
	4	24.0
1.0	1	4.0
	2	12.0
	4	24.0

Twenty five plants each were analyzed

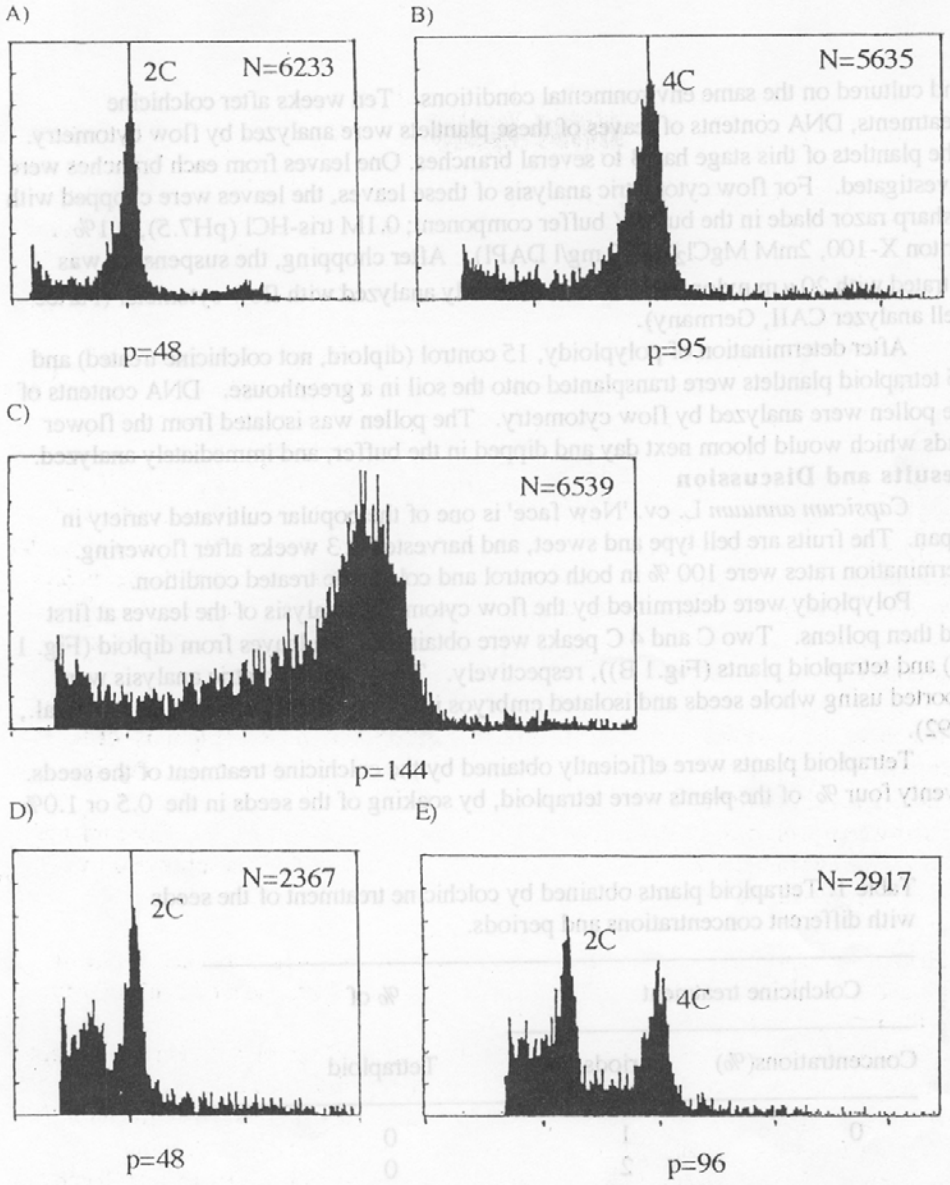

Fig. 1. Histograms of flow cytometric analysis of nuclei from the leaves and pollen. A) Leaf from a diploid plant. B) Leaf from a tetraploid plant obtained after colchicine treatment of the seed. C) Leaf from a high polyploidy plantlet obtained after colchicine treatment of the seed. D) Pollen from the flower bud of a diploid plant. E) Pollen from the flower bud of a tetraploid plant. X-axis:relative DNA amount per nucleus, Y-axis:number of nuclei. N=total number of counts. P=peak position,i.e.channel with highest number of counts in a peak.

colchicine solution for 4 days at 25 °c. We repeated this experiment and both experiments showed that the tetraploid plants were most efficiently obtained in this condition.
Octoploid plantlets were obtained by the colchicine treatments for more than 4 days or 5.0 % colchicine treatments (Fig. 1 C). These plantlets had short, thick roots and hypocotyls, and stopped to grow before development of the real leaves. Fifty days after colchicine treatments, 50.0, 76.5 and 100.0 % plantlets were abnormal by 1,2 and 4 days treatment of 5.0% colchicine, respectively.
After the flow cytometric determination of the polyploidy, 15 diploid and 15 tetraploid plants were transferred to a greenhouse, and grown further. The DNA contents of the pollen isolated from these plants were analyzed. Four and 2 C peaks were obtained in the pollen from a tetraploid plant (Fig. 1 E)). It is expected that vegetative and generative nucleus gave 4 and 2 C peaks, respectively. Although pollen from a diploid plant did not give clear IC peak (Fig. ID)), the histograms from diploid and tetraploid plants were clearly distinguished from each other.
Van Tuyl et al. reported the identification of 2n-pollen using flow cytometry (1989). They used mature pollen just after anthesis and dried then in as exsiccator. We used the pollen isolated from the flower buds one day before blooming in order to exclude contamination of the pollen from other flowers.
In this report we showed the method for tetraploid plant production by colchicine treatment of the seeds and the flow cytometric determination of the polyploidy of the leaves and pollen. Now we are investigating the tetraploid plants and applying this technique to other cultivars for breeding.
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Cytomorphological Studies of some polyploid hybrids of Capsicum

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Introduction:

Polyploid breeding is one of the important methods of breeding since it creates variation there by giving rise to new, novel and superior types than the existing ones. The realization of far reaching effects of polyploidy in producing new and superior types of plants to the existing ones naturally lead to the experiments on artificial production of polyploids and their utilization in breeding programmes. Chilli, an attractive red pepper belonging to the genus Capsicum of the family Solanaceae, is the major cash crop in India with wide range of applications in diverse fields. Although there were several reports on the production of polyploids in other crops, there was total lack of work on employing the polyploids obtained in further breeding programmes. In the present study it is planned to conduct hybridizations among 8 already existing autotetraploids of Capsicum and to evaluate the polyploid hybrids morphologically and cytologically.

Materials and methods:

The Capsicum varieties employed in the breeding programme include 8 stabilized autotetraploids viz., x180, x206, Santaka, Jawahar, TC1' Se II' Lec2;\ of C. annuum and C. chinense.

Results and discussion:

A total of 2,800 crosses were tnade in 56 different combinations involving seven tetraploid varieties of C. annuum and one of C. chinense. Results indicate that combinations involving C. chinense showed higl1er degree of success followed by Jawahar and Santaka. Of the 56 combinations attempted, only 28 combinations were successful. Fruit set was highest in Jawahar XTC1' and Sell X Santaka

It was observed that seedling emergence was on eighth day. Of the 28 combinations studied, germination was observed in 26 combinations only. In C. *chinense* X Sell and TC1 X 180, although seedlings emerged, they perished after two days. The crosses were not successful in all Lec21 combinations. The morphometrics of F1 hybrids are given in Table 1. In Fl hybrids minimum plant height was observed in C. *chinense* X TC1 (25.67 cm) and maximum plant height was observed in Sel1 X x206 (62.50 cm) followed by Santaka X Sel1 (60.00 cm). Plant spread ranged from 30.67 cm (5antaka X C. *chinense)* to 72.40 cm (Jawahar X C. *chinense).*

The number of branches per plant ranged from 15.00 (Santaka X C. *chinense)* to 81.00 (Jawahar X x206). In tetraploid hybrids thick, dark green foliage was observed as in tetraploids. All the tetraploid hybrids are quite healthy when compared to the tetraploids and diploids and they are apparently disease free. Maximum number of fruits per plant was observed in C. *chinense* X x206 (66.00) and minimum in x180 X TC1 (4.00). Fruit length was maximum in x206 X Jawahar (8.54 cm) and minimum in Santaka X x180 (4.19 cm). Maximum fruit girth was observed in 5antaka X Sell (3.71 cm) and minimum was observed in 5antaka X Jawahar (2.68 cm).

All the 7 varieties of C. *annuum* and one of C. *chinense* used as parents in the present breeding programme are autotetraploids and are showing cytological stability with respect to chromosome number. All of them are showing 2n=48 number with varying frequencies of quadrivalents and bivalents. In all the - tetraploid hybrids also, cytology was studied with respect to chromosome associations and chiasma frequencies. A detailed analysis of later stages of meiosis was made. Univalents ranging from 0-4 were observed in 4 combinations (0-2 in Sell X Santaka and Sell X TC1' 0-4 in x180 X C. *chinense* and Santaka X TC1)' Trivalents were observed in 5antaka X TCl hybrid combination

only. In all the remaining hybrids, quadrivalents were observed along with bivalents with varying frequencies. The number of chiasmata per cell varied from 28-40. High chiasma frequency *of* 33-40 was observed in Santaka X C. *chinense* and TCl X Jawahar followed by 32-40 in Sell X x180. Low chiasma frequency *of* 29-35 was found 'in C. *chinense* and x180 followed by 28-36 in x180 X C. *chinense.* Unequal separation and formation of lagging chromosomes were the major irregularities encountered in all the te'trap1oid hybrids.

In chilli, the earlier work on hybrid vigour has been confined only to the diploids and hybrids (Max 1987; Jarnail Singh, 1987; Prakash et al., 1991). Though there were reports on induction *of* polyploidy in chilli, instances *of* actual utilization *of* autopolyploids in plant breeding programmes are practically absent. In the present study, majority *of* the hybrids exhibited positive heterosis over their respective mid and better parents for most *of* the parameters. Of the 26 combinations *of* crosses obtained, significant heterosis for number *of* fruits per plant over mid parents was observed in 12 combinations viz., x180:' X x206, x180 X Santaka, x206 X Jawahar, Santaka X x180, Santaka x Sell' Jawahar X x206, Jawahar X Santaka, Jawahar X C. *chinense,* Jawahar X TC1' Sell X x206, Sell X Santaka and C. *chinense* X x206. In meiotic studies, no marked deviation was observed in the frequency *of* multivalents and bivalents between hybrids and autotetraploid parents.

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Table 1 : Morphometrics of F_1 tetraploid hybrids of *Capsicum*

S.No.	Name of hybrid	Plant height (cm)	Plant spread (cm)	No. of branches/ plant	No. of fruits/ plant	Fruit length (cm)	Fruit girth (cm)
1.	x180 X x206	51.00	66.00	68.00	18.00	7.36	3.28
2.	x180 X Santaka	36.40	45.20	52.00	27.00	8.14	3.57
3.	x180 X TC_1	50.50	41.50	42.00	4.00	6.70	2.97
4.	x180 X *C. chinense*	51.00	44.00	37.00	28.00	7.06	3.14
5.	x206 X Jawahar	44.40	47.00	43.00	18.00	8.54	3.38
6.	Santaka X x180	39.40	58.40	65.00	25.00	4.19	3.37
7.	Santaka X Jawahar	41.50	40.50	31.00	8.00	5.57	2.68
8.	Santaka X TC_1	45.00	62.00	57.00	16.00	5.24	2.96
9.	Santaka X Sel_1	60.00	65.80	62.00	57.00	7.82	3.71
10.	Santaka X *C. chinense*	30.33	30.67	15.00	8.00	5.48	3.45
11.	Jawahar X x206	41.00	57.60	81.00	41.00	7.56	3.63
12.	Jawahar X Santaka	39.60	59.60	58.00	24.00	8.16	3.45
13.	Jawahar X TC_1	38.75	53.25	61.00	24.00	7.84	2.81
14.	Jawahar X Sel_1	55.80	58.20	67.00	13.00	8.15	3.24
15.	Jawahar X *C. chinense*	48.00	72.40	67.00	38.00	7.34	3.32
16.	TC_1 X Santaka	39.50	39.00	35.00	11.00	6.42	3.19
17.	TC_1 X Jawahar	52.50	50.50	42.00	9.00	6.53	2.92
18.	Sel_1 X x180	39.80	54.40	54.00	14.00	7.86	3.18
19.	Sel_1 X x206	62.50	64.75	77.00	21.00	7.72	3.16
20.	Sel_1 X Santaka	53.00	63.20	55.00	23.00	7.07	3.42
21.	Sel_1 X TC_1	42.50	34.00	39.00	5.00	5.13	2.78
22.	*C. chinense* X x180	37.00	42.80	54.00	17.00	6.58	3.06
23.	*C. chinense* X x206	42.60	57.60	58.00	66.00	7.66	3.35
24.	*C. chinense* X Santaka	37.20	47.60	46.00	19.00	6.93	3.38
25.	*C. chinense* X Jawahar	52.50	50.00	67.00	13.00	6.73	3.32
26.	*C. chinense* X TC_1	25.67	36.33	20.00	8.00	5.91	3.31

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HIGH ASCORBIC ACID CONTENTS IN THE FRUITS OF A DEEP-GREEN CUL TIVAR OF *Capsicum annuum* THROUGHOUT THE FRUIT DEVELOPMENT

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Abstract:
The chlorophyll and ascorbic acid contents of the fruits of *Capsicum annuum* L.cv Sarara' throughout the fruit development were investigated. 'Sarara is a newly released cultivar characterized by deep-green fruits. Our results showed that 'Sarar' fruits contained 40% more chlorophyll and 22% more ascorbic acid compared with an ordinary cultivar Mihata. 'Sarara will be expected to be a good material for the further breeding of Capsicum with high ascorbic acid contents.

Key words: ascorbic acid, *Capsicum annuum,* chlorophyll

Introduction

In the breeding of vegetables, one of the recent aim is to increase ascorbic acid contents. But the analysis of ascorbic acid contents of hundreds plants within a limited time period is a hard work. The breeders have longed for easy selective markers, such as visible markers. There are some reports which shows colorful vegetables contains a lot of ascorbic acid, and expecting relationship between color and ascorbic acid contents (Mozafar 1994).
Recently, a cultivar of *Capsicum annuum* characterized by deep-green fruits, named Sarara is released. In this report, we showed the chlorophyll and ascorbic acid contents of the fruits of 'Sarara' throughout the fruit development, and revealed that this deep green cultivar contained high amounts of ascorbic acid.

Materials and methods

The seeds of *Capsicum annuwn* cv."Sarara and Mihat (Nihon Horticultural Production Institute, Japan) were sowed on March and grown in the green house. The fruits of various stages (from 2 to 12 weeks) were harvested on 22th August and measured the fresh weights, and the contents of chlorophyll and analyzed ascorbic acid.
Chlorophyll contents were determined by the method according to AOAC official methods of analysis using 5 g fresh weight of pericap tissues (Isaac 1990).
Determination of ascorbic acid contents was done by 2,4-dinitrophenylhydrazine methods (1989) using 10 g fresh weight of pericap tissues (Okamura and Arakawa 1989).

Results and Discussion

The fresh weights of the Sarara and Mihata fruits increased from 2 to 8 weeks after flowering and reached to the maximum weights at 8 weeks, and from 8 to 12 weeks after flowering the weight of both fruits were plateau (Fig. 1). Both 'Sarara and Mihata were sweet pepper and consumed as vegetables. Their fruits were usually harvested 3 weeks after flowering, at this time, both fruits were still in immature stage and weighed ca.30 g fresh weight which were just the recommended size in the Japanese markets. So in the breeding of Capsicum in Japan, the improvement of immature fruits are required**.** On the

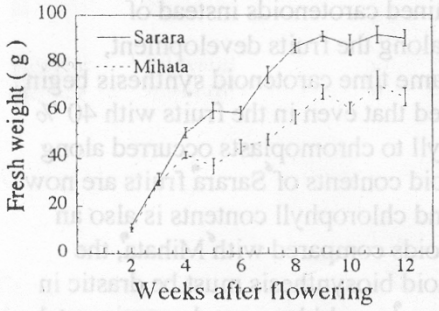

Fig.1. Fresh weights of the fruits of *Capsicum annuum* L.cv 'Sarara' and 'Mihata' throughout the fruit development.

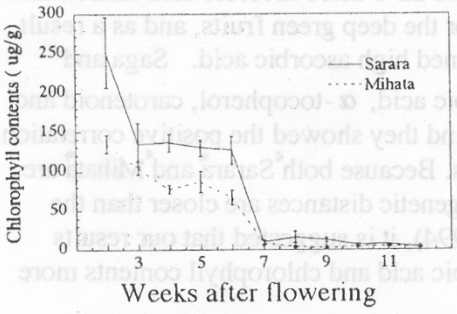

Fig.2. Chlorophyll contents of the pericarps of *Capsicum annuum* L.cv'Sarara' and 'Mihata' throughout the fruit development.

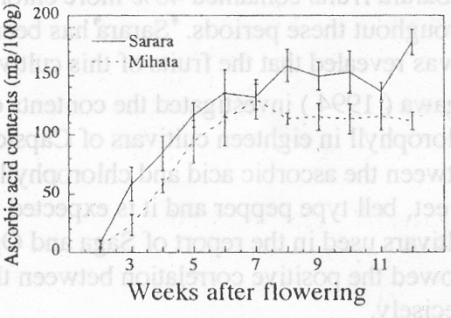

Fig. 3. Ascorbic acid contents of the pericaps of *Capsicum annuum* L.cv 'Sarara' and 'Mihata' throughout the fruit development.

other hand, mainly mature fruits are consumed in Europe, South America, or South Asia; For those countries the traits of mature fruits are important (Rahman et al. 1978). Because of this differences, the strategies of breeding in Japan and the other countries must be different.
Comararing with the weights of 'Mihata' and 'Sarara' after 4 weeks; the fresh weights of them became different. The "Sarara fruits from 4 to 12 week old weighed 40 % more than Mihata At mature stage, 'Sarar and 'Mihata fruits weighed about 90 and 65g fresh weight, respectively.
Chlorophyll contents of both 'Sarara and 'Mihata were investigated using fruits older than 2-week fruit. Chlorophyll contents were the highest at the 2-week fruit and then gradually decreased until 7 weeks (Fig.2). From 7 to 12 weeks after flowering the chlorophyll contents were almost zero. 'Sarara fruits from 2 to 6 week-old contained 40% more chlorophyll comparing with 'Mihata fruits.
The fruits of 7 to 12 weeks after flowering contained carotenoids instead of chlorophyll (data not shown). It was reported that along the fruits development, chloroplasts transform to chromoplasts, and at the same time carotenoid synthesis begin (Camera et aI. 1982, Saga 1993). Our results showed that even in the fruits with 40 % more chlorophyll, the transformation from chlorophyll to chromoplasts occurred along - with the fruit development. The analysis of carotenoid contents of Sarara fruits are now in progress. The relationship between carotenoid and chlorophyll contents is also an interesting subject. If' Sarara contains more carotenoids compared with 'Mihata', the conversion from chlorophyll biosynthesis to carotenoid biosynthesis must be drastic in 'Sarara fruits of 6 to 7 weeks after flowering, and 'Sarara would be a good experimental materials.
Ascorbic acid contents of both cultivars increased from 2 to 8 weeks after flowering, and became plateau from 8 to 12 weeks (Fig.3). As the same with chlorophyll contents, Sarara fruits contained higher amounts of ascorbic acid then 'Mihata. Throughout 2 to 12 weeks Sarara fruits contained ca.22% more ascorbic acid than 'Mihata fruits. The 3 week- old fruits of Sarara contained twice amount of ascorbic acid comparing with the 3 week- old fruits of Mihata. Sarara fruits contained 40% more chlorophyll and 22% more ascorbic acid than Mihata throughout these periods. Sarara has been bred for the deep green fruits, and as a result, it was revealed that the fruits 'of this cultivar contained high ascorbic acid. Saga and Ogawa (1994) investigated the contents of ascorbic acid, *a* -tocopherol, carotenoid and chlorophyll in eighteen cultivars of Capsicum. And they showed the positive correlation between the ascorbic acid and chlorophyll contents. Because both Sarara and Mihata are sweet, bell type pepper and it is expected that the genetic distances are closer than the cultivars used in the report of Saga and Ogawa (1994), it is suggested that our results showed the positive correlation between the ascorbic acid and chlorophyll contents more precisely.
Besides from the correlation with chlorophyll contents, another correlation could be discussed. Rahman et al. (1978) discussed in his report that chilies contained significantly more ascorbic acid than sweet peppers, at fully ripened fruits. Some chilies contained high ascorbic acid in mature green fruits. Saga and Ogawa (1994) also reported that Shishitou, Fushimi amanaga, Sapporo Wase and Hirosaki Zairai contained high ascorbic acid in

mature fruits. The fruits of Shishitou and Fushimi amanaga are both long shaped. From the shape of the fruits and the characters of the plants, it is suggested that the chillytype must be crossed in establishment of those two cultivars. In breeding of 'Sarara chilly type cultivar was crossed in order to introduce disease resistant characters. The Sarara's character of high ascorbic acid contents may come from the chilly type ascendant. It is expected that the genetic analysis of the chilly type and sweet pepper reveal the loci for high ascorbic acid contents.

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Capsicum and Eggplant Newsletter, 16 (1997): 56-59.

USE OF NEAR INFRARED REFLECTANCE TO MEASURE CAPSAICINOIDS.lN,PEPPER (CAPSICUM SPP).

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Abstract

Capsaicinoids are the principal components responsible for pungency in pepper fruits. Information on the composition of this component is important for hot pepper breeding programs and the processing industry. Several analytical methods have been used to measure capsaicinoids, but most of them require prior extraction of capsaicinoids, which is time, consuming. Near-infrared reflectance (NIR) spectroscopy has been used to develop a rapid analytical method of measuring capsaicinoids without extraction. The regression line developed for a NIRSystem 6500 scanning spectrophotometer has a slope of 1.03, y-intercept of 0.21, standard error of calibration (SEC) of 0.85, and coefficient of determination (R2) of 0.91. At higher levels of capsaicinoids (> 1.3 mg g-1) the accuracy of the NIR system is similar to HPLC analysis, but as the level of capsaicinoids approaches zero a more accurate analytical method may be desirable, depending on the objective of the analysis.

Introduction

Plant breeders who work with chilli peppers *(Capsicum* spp.) often have difficulty to accurately determine the level of pungency in the fruits. Direct tasting is not reliable, because the organoleptic ability of the mouth is quickly: saturated and then non-pungent fruits cannot be detected. For this reason, the Scoville test was developed (Scoville, 1912), which measures pungency as Scoville units in a given weight of fruit tissue. However, this method is subjective because it relies on panel of tasters, and individual tasters vary in their sensitivity. Various analytical methods have been proposed, including spectrophotometry (Bajaj, 1980; Mori et al., 1976), gas-liquid chromatography (GLC) (Todd et al., 1977), paper chromatography (Trejo-Gonzalez and Wild-AI Tamirano, 1973), capillary gas chromatography (capillary GC) (Hawer et al., 1994), and high-performance liquid chromatography (HPLC) (Weaver and Awde, 1986). These all require time-consuming sample preparation procedures. Methods for rapid sample preparation have been reported which utilize vanadium salts (Ting and " Barrons, 1942) or iron salts (Anan et al., 1996). However, they require solvent extraction, and daily mixing of fresh reagents. Near infra-red reflectance (NIR) was reported to provide reliable determination of capsaicinoids with rapid sample preparation (Iwamoto et al., 1984). Therefore the A VRDC developed an analytical method utilizing NIR with rapid sample preparation and without the need for solvents for sample preparation, which is described below.

Materials and Methods

Sample preparation

For each sample, 150 g of fresh pepper fruits were washed in tap water and then dried at 45 C for four days in a forced hot-air oven to -10% moisture. The dehydrated samples, including seeds, were then ground in a centrifuge mill with a 0.5 mm screen (A VRDC, 1990). The powdered samples were used for HPLC and NIR analyses.

HPLC analysis

A set of 78 representative samples with high, medium, and low pungency was chosen from a total of 436 samples to make the calibration set. Five different sets of 32 samples each, none of which were in the calibration set, were chosen to validate the calibration. Each sample was analyzed by HPLC to determine its capsaicinoid (capsaicin and dihydrocapsaicin) content. Capsaicin extracts were extracted from the powdered samples with acetone by placing on a shaker at room temperature for four hours. The extract was filtered through No. 41 Whatman filter paper and acetone was evaporated at 55 C. The residue was re-dissolved in ethanol and the volume adjusted to 10 ml for each sample. The HPLC assay was performed isocratically on a Merck Lichrosorb RP-18 column (5 *u)* under room temperature. The mobile phase was a mixture of methanol and water at a ratio of 35/65 (v/v). The flow rate was one ml min- I and detection was at 229 nm (A VRDC, 1990).

NIR calibration

The capsaicinoid value obtained by HPLC was used as the reference value in the NIR calibration. Each sample in the calibration set was scanned with a NIRSystem 6500 scanning spectrophotometer to obtain its NIR spectra from 400,-2500 nm. A modified partial least squares (PLS) regression was used to establish a calibration curve. Five independent sets of 32 samples each were used to validate the calibration equation (A VRDC, 1993).

Results and Discussion

The mean and range of capsaicinoid values in the calibration set were 4.75 and 0.02-9.32 mg gol, respectively. The regression line slope, y-intercept, standard error of calibration, and R2 values for the prediction ofcapsaicinoids in pepper fruit are shown ... in Table 1. In an ideal calibration, the slope= 1.0, the y-intercept=0, the SEC=0, and 2 the R^2=1.0. The standard error of prediction (SEP), R value, mean, and range for each validation set is given in Table 2.

It should be noted that NIR is a secondary analytical technique which relies on a reference to a primary manual analysis 'in this case, HPLC), thus any inaccuracies in the primary analysis are reflected in the NIR. At relatively low levels of capsaicinoids, a more accurate analytical method may be desirable, depending on the objective of the analysis.

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EVOLUTION OF CAPSAICINOIDS IN Capsicum annuum L, var. annuum cv. PADRON FRUIT AT DIFFERENT GROWTH STAGES.

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Introduction.

Pepper for fresh market is one of the most important crops in Spain. Capsicum annuum L. var. annuum cv. Padron is cultivated in the North of Spain (Galicia), and is highly appreciated when fried.
Pungency is the most outstanding property of hot peppers, resulting from the accumulation of capsaicin and other related compounds. The preference for particular types of pepper in different countries is based on the different degrees of pungency they stimulate (Govindarajan et al., 1987). Padron peppers, because they are used fresh, are harvested when they are approximately 5.5 cm long and 2.5 cm wide, green, undeveloped and with a low degree of pungency.
Although many studies have been carried out on the evolution of the capsaicinoid levels in relation to the fruit development of other peppers (Suzuki et al., *1980;* Salgado-Garciglia & Ochoa-Alejo, *1990;* Iwai et al., 1979), the special characteristics of cv. Padron prompted us to do this research. In this paper we report both the changes in fruit size, weight and color (chlorophyll and carotenoids), and the capsaicinoid accumulation during the ripening proccess of the Padron pepper fruit.

Material and methods.

Plant Material.

Plants of Capsicum annuum L. var. annuum cv. Padron were grown in a greenhouse from May to September 1996. Anthesis began ca 3 months after germination; individual flowers were numbered and the date of flowering recorded. Pepper fruits were harvested every 7 days from 14 to 42 days after flowering, weighed, oven-dried at 60°C for 2-5 days, and stored in sealed plastics bags at '" 20°C until processed.

Extraction and *quantification of capsaicinoids* by HPLC.

Capsaicinoids were extracted from pepper fruits using the technique described by Collins et al. (1995). Samples were analysed using a Waters LC616 System equipped with a Waters 717plu9 Autosampler, a Waters Temperature Control Module, a Waters 996 Photodiode Array Detector and Millennium Software for data processing. Reverse phase HPLC was carried out on a Spherisorb ODS2 C18 column (5µm particle size, 150mm x 46 mm). A precolumn guard cartridge Spherisorb ODS2 C18 column, was also used. HPLC operating conditions to determine capsaicinoids included 25°C, a flow rate of 1ml min-1, and

Table Slope, y intercept and SEC and R2 for the regression line computed using capsaicinoid data from 78 samples

Slope	y-intercept	SEC	R^2
1.03	.11	0.38	0.94

Table 2. Number of samples of (n), SEP, R^2 mean and range for five validation sets used to predict capsaicinoid content (mg g-1).

Batch	N	SEP	R2	Mean	Range
1	32	0.95	0.86	3.75	0.19-7.81
2	32	0.64	0.91	4.77	0.02-8.12
3	32	0.68	0.91	4.85	0.11-7.22
4	32	0.39	0.90	3.15	0.01-6.07
5	32	0.65	0.89	5.39	0.21-9.32

A 14 min run. The mobile phase was isocratic, with 50% solvent A (100% acetonitrile- HPLC grade) and 50% B (10% acetonitrile).

Days after flowering	Fresh weight (gr)	Dry weight (gr)	Sixe (cm)	Chlorophyll(mg/fruit)	Carotenoid (mg/fruit)
14	4.035	0.245	3.5	1.14	0.85
21	8.220	0.565	5.5	3.50	1.75
28	12.430	0.790	6.5	15.07	3.49
35	19.540	1.820	7.5	12.55	3.59
42	16.670	1.500	7.5	4.12	109.80

Table 1 shows the fluctuarions in weight, size and chlorophyll ans carotonoid content in Padron peppers with regard to the maturation stage. Padron pepper fruit from the frist harvest (14day) were small and undeveloped. Fruit length and weight increased gradually from 21 to 28 days, reaching a maximum at 35 days, when pepper were fully developed and mature- green. At 42 days, when peppers were red, fruit weight began to decrease.

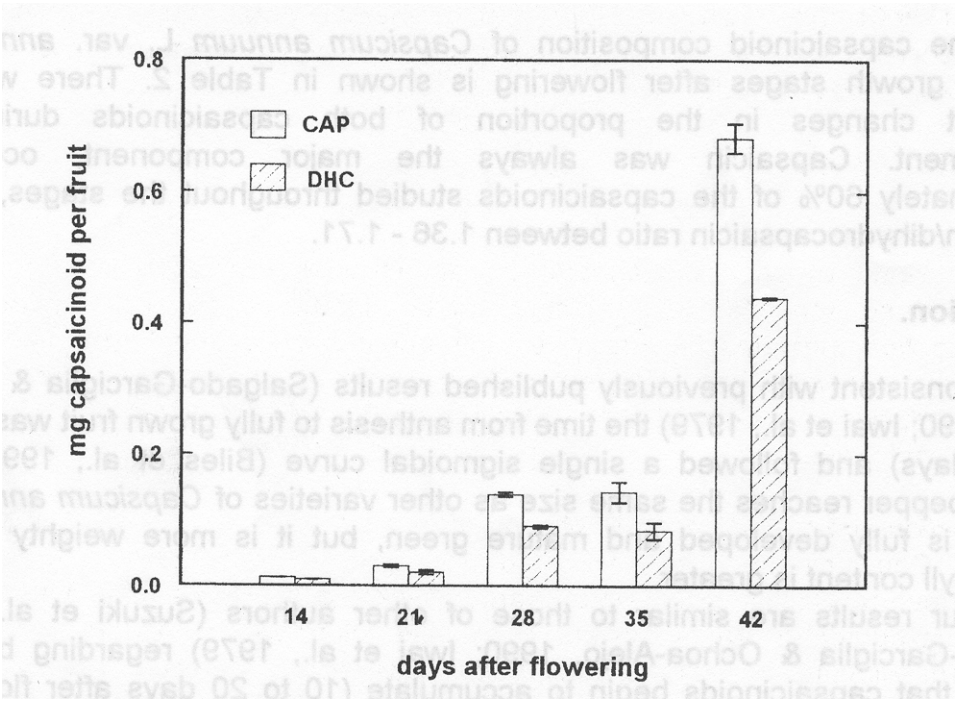

Fig 1. capsaicinoid levels at different growth stages. CAP-capsaicin; DHC dihydrocapsaicin

Chlorophyll content continued to increase until 28 days after flowering, then it began decreasing gradually. The content of carotenoids was tow until the 35th day, and suddenly began to increase 42 days after flowering.

Pepper fruits were analyzed for their capsaicinoid content. As shown in Fig. 1, capsaicin and dihydrocapsaicin were detected 14 days after flowering, but their levels were low for 21 days. 28 days after flowering, capsaicinoids increased moderately, accumulating progressively until .the 35th day. Finally, the Padron pepper showed a great increase in capsaicinoids levels at the end of development, with the highest values found 42 days after flowering.

Table 2

Days after flowering	Total capsicinoids (mgr/fruit)	%CAP	%DHC	CAP/DHC Ratio
14	0.022	57.6	42.3	1.36
21	0.050	59.9	40.1	1.51
28	0.228	60.6	39.3	1.54
35	0.226	63.0	37.0	1.71
42	1.120	60.8	39.2	1.55

The capsaicinoid composition of *Capsicum annuum* L. var. *annuum* at different growth stages after flowering is shown in Table 2. There were no important changes in the proportion of both capsaicinoids during the development. Capsaicin was always the major component, occupying approximately 60% of the capsaicinoids studied throughout the stages, with a capsaicin/dihydrocapsaicin ratio between 1.36 - 1.71.

Discussion.

Consistent with previously published results (Salgado-Garciglia & Ochoa-Alejo, 1990; Iwai et al., 1979) the time from anthesis to fully grown fruit was similar (30-40 days) and followed a single sigmoidal curve (Biles et al., 1993). The Padron pepper reaches the same size as other varieties of *Capsicum annuum* L. when it is fully developed and mature green, but it is more weighty and its chlorophyll content is greater.

Our results are similar to those of other authors (Suzuki et al., 1980; Salgado-Garciglia & Ochoa-Alejo, 1990; Iwai et al., 1979) regarding both the moment that capsaicinoids begin to accumulate (10 to 20 days after flowering) and the time they reach maximum values (30 to 40 days). However, the level of capsaicinoidsin cv. Padron was very smalr even in the last stage (1.12 mg/fruit)

The proportion of capsaicinoids studied in the Padron pepper *(ca* 60%'for capsaicin and 40% for dihydrocapsaicin), coincide with those found by Iwai et al.

(1979) in *Capsicum annuum* L. var. *annuum* cv. Karayatsubusa, with capsaicin being the major component. The ratio of capsaicin to dihydrocapsaicin has been considered a characteristic of the species. Govindarajan et al. (1987) states that, in the case of *Capsicum annuum* L., the ratio varies in a range from 0.64 to 1.94, which is in accordance with our results.
It is interesting to point out that cv. Padron shows a low capsaicinoid level at the commercial stage (21 days after flowering). However, this low level is greatly appreciated in Galicia by pepper consumers, who reject sweet or highly pungent peppers. Capsaicinoid accumulation is controlled by several factors: age of the plant, temperature, light, nutritional status and so on (Iwai et al., 1979). Therefore, the influence of different growing conditions is currently being researched in our laboratory.

ACKNOWLEDGEMENT

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ANALYSIS OF ISOZYME PATTERNS (PEROXIDASE) OF CAPSICUM HYBRIDS THROUGH P AGE

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Isozyme studies have tremendous impact on the fields of biochemistry, molecular biology, physiology, genetics and evolutionary studies in recent times as they are widely used tools for estimation of genetic diversity, cultivar identification, confirmation of hybridity, marking monogenic traits, analysis of polyploids, detection of point mutations, identification of clonal variation, construction of linkage maps and conduct of breeding programmes. The peroxidases are a group of isozymes with similar catalytic properties that act on a great variety of substrates.

In chilli, work partaining to electrophoretic analysis of isozymes was restricted only to species and varietal differentiation (Tanskly 1984), but qualitative analysis of isozymes on hybrids developed through 'ms' breeding was initiated for the first time in our laboratory. In the present investigation leaf peroxidases were analyzed by PAGE involving F1 hybrids developed through 'ms'breeding and corresponding pollinator parent. During ,electrophoresis these multiple molecular forms of peroxidases reach different regions of their isoelectric points to appear as separate bands.

MATERIALS AND METHODS:

Peroxidase isozymes were analysed from leaf extracts of F 1 hybrids and their pollinator parents by polyacrylamide gel electrophoresis teGhnique of Davis (1964) with slight modifications. The gels were stained in orthodiansidine. '

The 'Rf' values for different bands were calculated by using the formula

$$Rf = \frac{\text{Distance traveled by enzyme band}}{\text{Distance travelled by tracking dye}}$$

The degree of electrophoretic similarity among different samples was estimated by calculating the similarity index (SI). Similarity index values are calculated using the formula

$$SI = \frac{\text{Number of homologous bands}}{\text{Number of Non-homologous bands + Number of homologous bands}} \times 100$$

RESULTS AND DISCUSSION

The electrophoretic banding pattern of leaf peroxidases in 1 hybrids and their corresponding, parents have been represented as a zymogram. The results revealed that the variation in number as well as intensities of enzyme profiles within parental varieties was relatively *less* than that of F I hybrids. Among parents a maximum number of seven bands was observed in *C. annuum* var.x960. A minimum of only one band was observed in male sterile line which was employed as a pistillate parent. The 'Rf' values ranged between 0.022 to 0.366. In hybrids the number of bands ranged between 3 to 8. A minimum number of three bands were observed in *msx C .annuum* varLEC11 and the maximum number of bands were seen in msx *C. annuum* var.x206, msx *C. annuum* var.x235, msx *C. .annuum* var LEC. The 'Rf' values of hybrids ranged from 0.022 to 0455. The isozyme profiles revealed that there was appearance of new bands and disappearance of some bands in F I hybrids when compared to their pollinator parent.

Among parents similarity index values varied from 18.18% to 100%. The lowest similarity was noticed between *C.annuum* var.x960 and *C.annuum* var.LEC23' and the m~imum similarity index value was observed between *C.annuum* var.AVRDC pbc 156 and purple mutant. The similar- ity index values among most of the parents ranged between 22.22% to 50%.

The magnitude of isozyme similarity between different parents' and hybrids varies from a minimum of 13.33% between *C.annuum* var.x960 and msx *C.annuum* var.LEC21 and a maximum of 76.72% between *C.annuumvar.x180* and msx *C.annuum* var.xI80. Most of the hybrids exhibited highest similarity index values to their corresponding pollinator parents.

The study of isozyme profiles are useful to elucidate genetic variability between parents and their corresponding hybrids. In the present investigation species specific peroxidase patterns with different mobilities were observed. When enzyme profiles were relatively compared, hybrids showed new bands which were not present in their parents. Similar observations were made by Smith et at (1970) in *Nicotiana* amphidiploids and Iwara et al (1978) in *Raphano brassica.* Markova and Papova (1978) also observed an increase in number of bands while studying electrophoretic protein polymorphism in pepper hybrids. The appearance of new bands in genetically reconstituted hybrids can be attributed to the expression of structural genes which were not expressed previously or expression of new genes contributed by male sterile parent. The disappearence of some bands in hybrids when compared to their corresponding hybrids may be due to the negative regulation of gene action or suppression of previously active genes in reconstituted hybrid genome. Vaughan et al (1970) reported the same phenomenon in amphidiploids of *Brassica napus* which exhibited a reduction in number of proteins and isozyme bands from either of the parents viz., *B.compestris* and *B. Oleraceae* .

Based on the present investigation in conjunction with the earlier work from the laboratory (RaIna Chandra Rao, 1996), it may be concluded that different parents and their respective hybrids are not showing remarkable and well defined variations in isozyme patterns. However the variation among the parents and hybrids is sufficient enough to bring about significant morphological changes. A near 50% or more than 50% isozyme similarity between parents and hybrids suggests that they are having close phylogenetic affinities.

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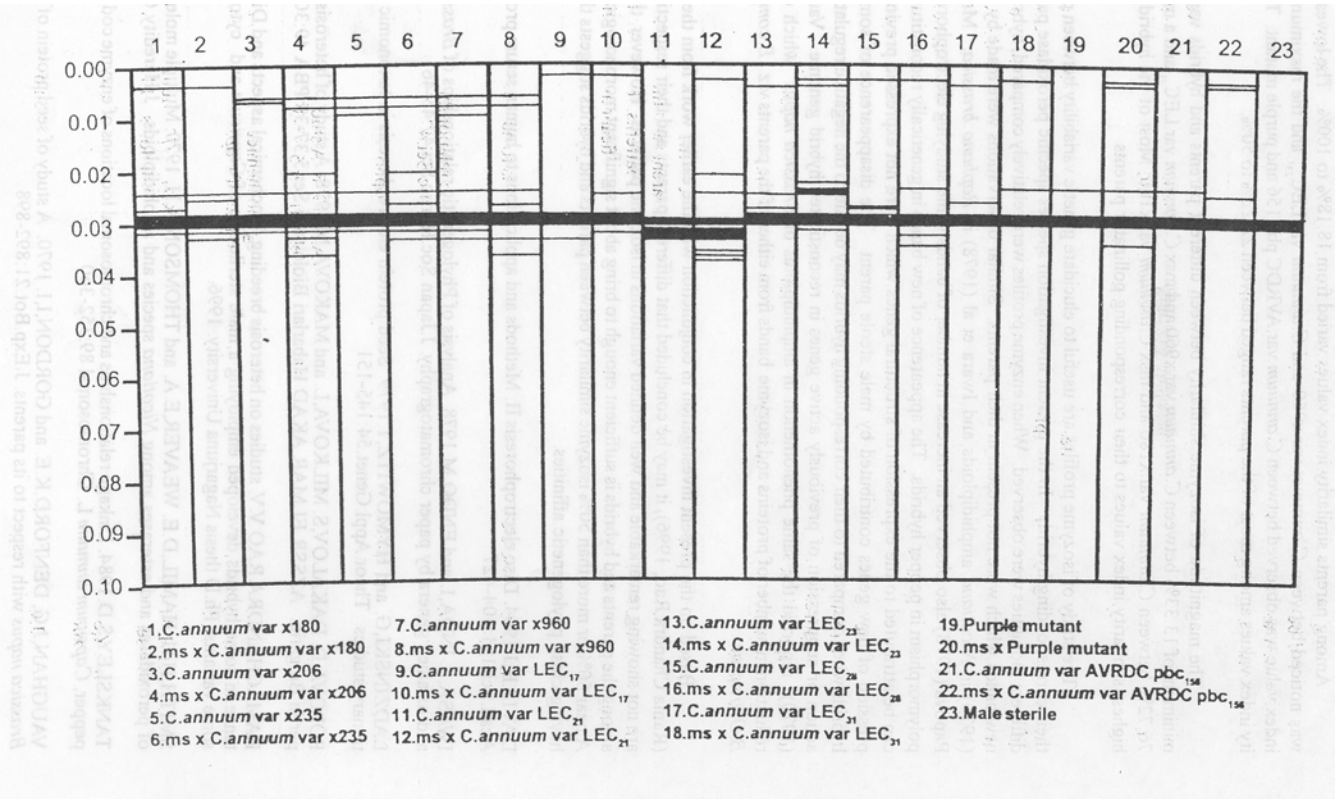
ZYMOGRAMS SHOWING ISOZYME PEROXIDASE PEOFILE IN PARENTS AND CORRESPONDING HYBRIDS



SIMILARITY INDEX VALUES OF PEROXIDASES AMONG PARENTS, HYBRIDS AND BETWEEN PARENTS AND HYBRIDS.

	C.annuum var.x180	C.annuum var.x206	C.annuum var.x235	C.annuum var.x960	C.annuum var.LEC17	C.annuum var.LEC21	C.annuum var.LEC23	C.annuum var.LEC28	C.annuum var.LEC31	purple mutant	AVRBC Pbc156	msxC.annuum var.x180	msxC.annuum var.x206	msxC.annuum var.x235	msxC.annuum var.x960	msxC.annuum var.LEC1	msxC.annuum var.LEC2	msxC.annuum var.LEC2	msxC.annuum var.LEC2	msxC.annuum var.LEC3	msxPurple mutant	msxAVRDC Pbc156	Male sterile
C.annuum var.x180		40.00	36.36	33.36	25.00	28.57	88.88	50.00	50.00	28.57	28.57	72.72	40.40	36.36	16.66	44.44	28.57	88.88	40.00	28.57	40.00	66.66	33.33
C.annuum var.x206			90.90	83.88	25.00	28.57	22.22	25.00	25.00	57.14	57.14	36.36	76.92	90.90	72.72	22.22	15.38	22.22	25.00	50.00	40.00	22.22	33.33
C.annuum var.x235				76.92	22.22	25.00	20.00	22.22	22.22	50.00	50.00	33.33	71.42	71.42	61.53	20.00	14.28	16.66	18.18	44.44	36.36	20.00	28.57
C.annuum var.x960					40.00	22.22	18.18	20.00	20.00	22.22	44.44	46.15	30.00	80.00	71.42	50.00	13.33	28.57	16.66	40.00	33.33	18.18	25.00
C.annuum var.LEC17						40.00	28.57	66.66	33.33	40.06	40.06	44.44	36.36	36.36	40.00	28.57	36.36	44.44	50.00	66.66	50.06	40.00	50.00
C.annuum var.LEC21							33.33	40.00	40.00	50.00	50.00	40.00	20.00	20.00	22.22	66.66	20.00	25.00	57.14	40.00	28.57	33.33	66.66
C.annuum var.LEC23								57.14	57.14	33.33	33.33	60.00	16.66	16.66	18.18	50.00	33.33	60.00	44.44	28.57	44.44	50.00	40.00
C.annuum var.LEC28									33.33	40.00	40.00	22.22	18.18	18.18	20.00	57.14	36.36	44.44	75.00	66.66	50.00	57.14	50.00
C.annuum var.LEC31										40.00	40.00	44.44	18.18	36.36	20.00	28.57	18.18	57.14	25.00	33.33	50.00	57.14	50.00
Purple mutant											100.00	25.00	40.00	40.00	44.44	53.33	20.00	25.00	28.57	50.00	57.14	33.33	66.66
AVRBC Pbc156												25.00	40.00	40.00	44.44	33.33	20.00	25.00	28.57	50.00	57.14	33.33	66.66
msxC.annuum var.x180													42.85	42.85	30.76	40.00	28.57	66.66	18.18	22.22	36.36	40.00	28.57
msxC.annuum var.x206														37.50	66.66	16.66	12.50	28.57	15.38	36.36	30.76	16.66	22.22
msxC.annuum var.x235															80.00	16.66	25.00	28.57	15.38	36.36	30.76	33.33	22.22
msxC.annuum var.x960																18.18	13.33	15.38	16.66	40.00	33.33	18.88	25.00
msxC.annuum var.LEC17																	66.66	60.00	66.66	57.14	44.44	50.00	40.00
msxC.annuum var.LEC21																		42.85	46.15	18.18	30.76	50.00	22.22
msxC.annuum var.LEC23																			36.36	44.44	54.54	80.00	28.57
msxC.annuum var.LEC28																				50.00	60.00	44.44	33.33
msxC.annuum var.LEC31																					75.00	57.14	50.00
msxPurple mutant																						66.66	33.33
msxAVRDC Pbc156																							40.00
Male sterile																							

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CHARACTER ASSOCIATION IN HOT PEPPER (*Capsicum annuum* L.)

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INTRODUCTION

Yield is a complex polygenic character which is directly or indirectly depended f on number of traits known as yield components. The knowledge of association of yield components with yield and among themselves will be helpful in the
improvement of a complex trait like Yield for which direct selection is not very effective. In India, a wide range of variability for hot pepper is available which provides great scope for improving fruit yield through a systemic and planned selection programme for one or more direct or indirect yield components. The purpose of this research is to determine the nature and degree of association among characters and their direct and indirect effects on yield.

MATERIAL AND METHODS

Seventy-one lines of hot pepper were raised in a randomised block design with three replications at Vegetable Farm, S.K.U. A.S.T, Srinagar during summer 1993. In each replication, each line was grown in two rows of 4.5 m length at a
spacing 60 cm. Standard cultural practices and plant protection measures were , followed to raise a healthy crop. Observations on ten characters (Table 1) were recorded on ten randomly selected plants of each line after eliminating border plants.

The correlations were worked out as per Aljibouri *et al* (1958) and path coefficients of various characters were calculated according to Dewey and Lu (1959).

RESULTS AND DISCUSSION

Correlation

Correlation coefficient establishes the extent of association between yield and its components. So that these yield components may form additional criteria for selection in breeding programme. In the present study, the genotypic correlation, coefficients were higher in magnitude than corresponding phenotypic correlation, coefficients, indicating inherent association between various characters. Fruit yield exhibited positive and highly significant correlation with number of fruits per plant, average fruit weight, plant height, plant spread and fruit length suggesting that these characters are the most important yield components and that effective improvement in fruit yield could be achieved through selection based on these component characters. Significant but positive correlation between fruit number and branch number per plant, plant height and plant spread, plant height and fruit size (length and thickness), plant spread and fru1t length, plant spread and average fruit weigh branch number and maturity, fruit length and fruit thickness, fruit length and average fruit weight, fruit thickness and pericarp thickness and fruit thickness and average ; fruit weight was also existed there by suggesting positive association between these

traits and thus, improvement aimed at anyone of the character will automatically lead to improvement in other traits such as selection for higher number of branches results in more number of fruits per plant which in turn lead to higher fruit yield. Dahiya *et al.* (1991) and Khurana *et al.* (1993) also observed similar association of components with yield and among themselves.

On the contrary days to maturity showed significant negative correlation with yield and yield components indicating its undesirable association (Table 1). Therefore any selection aimed for early maturity in general may not be useful either in improving yield or yield associated characters. Such significant negative association also existed between plant spread and pericarp thickness, branch number and fruit size, branch number and average fruit weight and fruit number and average fruit

weight indicating that these characters do not got together during selection programme. If selection is practiced for increased number of branches and fruits there will be a corresponding decrease in fruit size and average fruit weight. The presence of negative association among traits, put the plant breeder in a great risk. In such correlations, the breeder must assess further, which character to be given more preference based on interrelationship of these traits with rest of the yield components.

Path analysis

Since yield is influenced by many factors, selection based on simple correlations without taking into consideration the interactions between the component characters may some time prove misleading, The path coefficient analysis here provides on effective measure of untangling direct and indirect causes of association and permits a critical examination of specific causes acting to produce a given correlation and measures the relative importance of each factor. In the present study, the fruit thickness showed positive correlation with the yield, but the path coefficient analysis (Table 2) revealed that it has negative direct effect on the yield. This positive correlation with the yield was largely because of positive indirect contribution mainly through average fruit weight, where as days to maturity revealed negative direct effect on fruit yield as well as negative indirect effect via most of the characters giving ultimately negative correlation thereby further confirmed that selection for early maturity will be at the expense of fruit yield and yield components.

Among different yield components, the fruit number per plant exhibited highest direct positive effect on fruit yield and also showed indirect effect through branch number and plant height. Plant height which had positive correlation indicated positive direct effect on fruit yield and also showed positive indirect effect through average fruit weight and fruit number, thereby by suggesting a good scope for improvement of fruit yield by selecting for plant types bearing a large number of branches and fruits. Similar observatlions have also been made by Sundaram and Ranganathan (1978). Average fruit weight had a positive direct effect of desirable magnitude towards fruit yield but its indirect effect via number of fruits per plant was negative and of higher magnitude.

The direct effect of fruit length, plant spread, pericarp thickness and branch number towards fruit yield were of lower magnitude in comparison to fruit number and

average fruit weight but had very strong positive indirect effect via average fruit weight leading to positive correlation with fruit yield. Similarly number of branches per plant showed highly indirect positive effect via fruit number per plant towards fruit yield. The rest of the indirect associations were very meagre. The residual effect (0.0698) indicated that the components under study accounted for about 93 percent variability in the yield.

The present study in general revealed that number of fruits per plant and average fruit weight are the most important component traits which had positive and significant correlations and also had high direct effects on fruit yield indicating similar picture of the causal system of the factors governing fruit yield and major portion of the variability present in yield was contributed largely by number of fruits per plant and/or by average fruit weight. Therefore, it is suggested that selection based on any of these characters is expected to give desired response. In addition to fruit number and average fruit weight, the characters like number of branches per plant and plant height having positive correlation with fruit yield and by indirectly contributing to higher fruit number and plant spread, fruit length and pericarp thickness being positively associated with yield and by indirectly contributing towards higher average fruit weight should also be considered in the selection programme aimed at improving total fruit yield in hot pepper.

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Table 1 CORRELATION CO-EFFICIENT AMONG TEN CHARACTERS IN HOT PEPPER

Characters	Days to maturity	Plant height (cm)	Plant spread (cm)	Branch number/ plant	Fruit length (cm)	Fruit thickness (cm)	Pericarp thickness (mm)	Fruit number/ plant	Average fruit weight (g)	Fruit yield/ plant(g)
Days to maturity		-0.198*	-0.362**	0.278**	-0.276**	-0.215**	0.038	-0.265**	-0.193*	-0.341**
Plant height (cm)			0.453**	-0.446	0.219**	0.199*	0.066	0.096	0.148	0.304**
Plant spread (cm)				-0.135	0.261**	-0.047	-0.277**	0.035	0.275**	0.311**
Number of branches/plant					-0.213**	-0.241**	-0.069	0.408**	-0.419**	0.066
Fruit length (cm)						-2.83	0.138	-0.011	0.429**	0.354**
Fruit thickness (cm)							0.566**	-0.132	0.408**	0.158
Pericarp thickness (mm)								-0.080	0.320**	0.154
Fruit number per plant									-0.247**	0.643**
Average fruit weight (g)										0.519**

*, ** **Significant at 5% and 1% levels, respectively.**

Table 2 DIRECT AND INDIRECT EFFECTS OF DIFFERENT CHARACTERS ON FRUIT YIELD PER PLANT

Characters	Days to maturity	Plant height (cm)	Plant spread (cm)	Branch number/ plant	Fruit length (cm)	Fruit thick-ness(cm)	Pericarp thickness (mm)	Fruit number/ plant	Average fruit wt. (g)	Correlation coefficient with fruit yield
Days to maturity	-0.0207	-0.0208	-0.0190	0.0078	-0.0174	0.0111	0.0012	-0.2065	-0.1348	-0.341**
Plant height (cm)	0.0040	0.1056	0.0238	-0.0013	0.0138	-0.0104	0.0024	0.0745	0.1020	0.304**
Plant spread (cm)	0.0075	0.0479	0.0525	-0.0038	0.0164	0.0023	-0.0087	0.0276	0.1920	0.311**
Number of branches/plant	-0.0057	-0.0048	-0.0070	0.0282	-0.0134	0.0125	-0.0020	0.3181	-0.2927	0.066
Fruit length (cm)	0.0057	0.0230	0.0136	-0.0060	0.0631	-0.0147	0.0047	-0.0085	0.2996	0.354**
Fruit thickness (cm)	0.0044	0.0211	-0.0024	-0.0068	0.0178	-0.0521	0.0191	-0.1031	0.2853	0.158
Pericarp thickness (mm)	-0.0007	0.0073	-0.0131	-0.0016	0.0084	-0.0283	0.0351	-0.0805	0.2153	0.154
Fruit number/ plant	0.0054	0.0101	0.0018	0.0115	-0.0006	0.0068	-0.0036	0.7795	-0.1726	0.643**
Averag fruit weight (g)	0.0039	0.0155	0.0144	-0.0118	0.0270	-0.0212	0.0108	-0.1926	0.6984	0.519**

Underlined figures denote direct effects on fruit yield, Residual effect = 0.0698

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COMBINING ABILITY ANALYSIS FOR FRUIT YIELD AND ITS COMPONENT CHARACTERS IN SWEET PEPPER *(Capsicum annuum* L.)

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INTRODUCTION

Sweet pepper is an important vegetable in subtropical and temperate regions of India. - Its improvement by breeding has been mostly limited to the exploitation of varietal differences and selection from local variability, with very little genetic investigations. The knowledge of gene action and combining ability not only provides information on inheritance of characters but also serves in selection of suitable parents for hybridization and promising hybrids for further exploitation in breeding programmes. Such information is scantly for economic characters in sweet pepper. Hence the present investigation aims to collect this information from a 6 x 6 diallel cross.

MATERIALS AND METHODS

Six diverse cultivars of sweet pepper namely 'California Wonder' (CW), 'KSPS-3', KSPA- 2', 'Arka Gaurav'(AG), 'World Beater' (WB) and 'KSPS-1' were crossed in all possible combinations excluding reciprocals. The experimental material comprising of fifteen F1 s and six parental lines were planted in a randomized block design with three replications at Vegetable Farm, SKUAST, Srinagar. In each replication a single row of ten plants each of parents and F1s were planted at a spacing 60 cm between rows and 45 cm between plants within the row. The observations on ten different characters (Table 1) were recorded from five randomly selected plants from each treatment of every replication leaving the border plants at both ends. The combining ability analysis was carried out according to Model 1 Method 2 of Griffing (1956). The additive (G2D) and non-additive (G2A) genetic variances were estimated from the mean squares for general combining ability (gca), specific combining ability (sca) and error. Heritability in narrow sense (Hn) was determined employing estimated additive and non-additive genetic variances.

RESULTS AND DISCUSSION

The variances due to both gca and sca were highly significant for all the ten characters (Table 1) indicating importance of both additive and non-additive gene effects in the inheritance of all the traits. However the estimated additive genetic variances *(G2A)* were higher in magnitude as compared to the estimated non-additive variances (G2D) for days to first fruit set, fruit length, fruit diameter, flesh thickness, seed number, fruit number and average fruit weight revealing preponderant role of additive genetic component Considering the significance of additive genetic variance and simultaneous realization of high heritability estimates (Table 1), it is suggested that all these characters will respond favourably to direct selection. Joshi and Singh (1987) and Miranda *et a/.* 1988,also reported similar results. On the other hand non-additive genetic component played major role coupled with low heritability for plant height, plant spread and fruit yield per plant as the magnitude of non-additive variance was much higher than that of estimated additive variance. Under this situation, exploitation of heterosis appears to be of great value.

Estimates of gca effects showed that it was difficult to pick up a good combiner for all the characters together as the combining ability effects were not consistent for all the yield components (Table 2). It was possibly because of the negative association of the characters. However, the parents 'KSPS-3' and KSPA-2' were good general combiners for days to first fruit set, plant height, plant spread, fruit length, fruit number and fruit yield. Similarly, parent 'KSPS-1' showed high gca effects and proved good combiner for fruit diameter, seed number and fruit yield and average combiner for days to first fruit set, plant spread, flesh thickness and average fruit weight Hence these parents having favourable genes for earliness as well as for yield and yield components could effectively be used in the breeding of early and high yielding sweet pepper varieties. Parents 'CW', 'AG' and 'WB' were good to average combiners for flesh thickness, seed number, average fruit weight and fruit diameter and for rest of the characters they were poor combiners. The results also revealed that for most of the characters except fruit yield, the parents which exhibited high gca effects also recorded high mean performances indicating that the performance of parent *per* se was in line with their combining abilities, where as for fruit yield there was no such agreement suggesting that the combining ability of parents cannot always be judged accurately only by their *per* se performance especially for yield which is controlled by polygenes and highly influenced by environment. Therefore, both gca effects and mean performance should be considered for assessing true breeding potentiality of the parents (Suthanthirapandian and Shanmugavelu, 1992).

Best five crosses each in respect of mean performance and sca effects are presented in Table 3. The perusal of sca effects indicated that out of 15 F1 cross combinations for days to first fruit set which measures earliness the hybrids AG x KSPS-1, AG x WB and KSPS-3 x KSPA- 2 showed significant desirable negative sca effects and proved best cross combinations. The hybrids KSP5-3 x KSPA-2 and AG x WB for plant height, KSPA-2 x KSPS-1 for plant spread, CW x KSPA-2 and KSP5-3 xWB for fruit length, CW x KSPS-1 and CW x KSPA-2 for fruit diameter, KSPS-3 x KSPA-2 and CW x KSPA-2 for flesh thickness, WB x KSPS-1 and CW x KSPA-2 for seed number, CW x KSPS-1 and KSPA-2 x KSPS-1 for average fruit weight and CW x KSPA-2, KSP5-3 x KSPA-2 and KSPS-3 x KSPS-1 for fruit number showed highest desirable positive sca effects and thus proved to be the best hybrid combinations. For fruit yield, the cross combinations CW x KSPA-2, CW x KSP5-1 and KSPA-2 x KSPS-1 by having sca effects proved superior and were heterotic also.

The results given in Table 3 also revealed in general that *per* se performance of most of the cross combinations were in accordance with their sca effects. Such positive relationship is more useful in breeding programmes, however for selection of cross combinations both the *per* se performances and sca effects of the crosses should be considered as ranking of the
crosses based on *per* se performance and sca effect varies. Further, it was also evident that among different hybrids, CW x KSPA-2, CW x KSPA-1 and KSPA-2 x KSPS-1 which showed highest sca effects and maximum fruit yield also exhibited significant sca effects for fruit diameter, fruit length, flesh thicknes'5, seed number, fruit number and average fruit weight. The sca effect of these crosses was related to gca effect of its parents which involved one parent with high gca and the other parent was average or poor general combiner. Hence it appears that both additive and non-additive genetic components are playing an important role in the inheritance of fruit yield and yield components. In view of high mean value and sca effects the hybrids CW x KSPA-2, CW x KSPS-1 and KSPA-2 x KSPS-1 could be considered for commercial exploitation of hybrid vigour and/or could be utilized to create desirable recombinants following intermating amongst selects in biparental mating fashion in early generations or recurrent selection

Table 1 : Analysis of variance (Mean squares) for combining ability, estimates of additive (σ^2_A) and non-additive (σ^2_D) genetic variances and heritability in narrow sense for yield and yield components of 6 x 6 diallel cross in sweet pepper

Source	df	Days to first fruit set	Plant height (cm)	Plant spread (cm)	Fruit length (cm)	Fruit diameter (cm)	Flesh thickness (mm)	Seed number/ fruit	Fruit number/ plant	Average fruit wt. (g)	Fruit yield/ plant (g)
gca	5	43.93**	51.13**	66.29**	2.33**	2.98**	0.012**	12691.19**	128.45**	853,22**	19656.58**
sca	15	1.31**	16.00**	134.71**	0.38**	0.10**	0.002**	1788.42**	11.15**	72.22**	76289.56**
Error	40	0.48	0.27	0.41	0.01	0.01	0.0001	66.12	0.20	2.53	711.21
Estimation of genetic variance and heritability											
σ^2_A		10.86	12.71	16.47	0.58	0.74	0.003	3156.26	32.06	212.67	4736.34
σ^2_D		0.83	15.73	134.30	0.36	0.08	0.002	1722.30	10.95	69.68	75578.35
Hn (%)		89.30	44.28	10.89	60.82	88.61	59.17	63.83	74.19	74.65	5.84

** Significant at 0.01 probability level.

Table 2: General combining ability effects of parents for yield and yield components in sweet pepper

Parents	Days to first fruit set	Plant height (cm)	Plant spread (cm)	Fruit length (cm)	Fruit diameter (cm)	Flesh thickness (mm)	Seed number/ fruit	Fruit number/ plant	Average fruit wt. (g)	Fruit yield/ plant (g)
1.CW	1.91**	0.78	-0.46**	-0.29**	0.21**	0.04**	14.97**	-1.10**	2.56**	16.77
2.KSPS-3	-2.39**	1.60**	2.60**	0.99**	-1.17**	-0.07**	-78.99**	7.46**	-17.77**	56.01**
3.KSPA-2	-2.63**	2.33**	1.02**	0.14**	-0.16**	-0.01**	-0.72**	0.76**	-5.17**	2.25
4.AG	3.16**	-4.81**	-5.53**	-0.12**	0.30**	0.01**	15.68**	-4.14**	11.87**	-80.08**
5.WB	0.74**	-0.30	1.14**	-0.12**	0.40**	0.02**	21.48**	-2.28**	6.14**	-32.39**
6.KSPS-1	-0.79**	0.40	1.23**	-0.59**	0.41**	0.02**	27.59**	-0.69**	2.37**	37.44**

**Significant at 0.01 probability level.

Table 3: Best five crosses each in respect of mean per se performance and significant sca effect for ten characters in sweet pepper

Character	Five best crosses with mean per se performance	Five best crosses with significant sca effects
Days to first fruit set	2X3 76.00), 3X6(79.98), 2X6(80.33), 2X5(80.77),1X3(81.26)	4xb(-2.17), 4x5(-1.53),2x3(-1.44)
Plant height (cm)	2x3(61.94), 1x3(58.33), 3x6(57.49),1x5(54.37), 12x5(53.70)	2x3(6.69), 4x6(6.15), 1x3(3.91), 3x6(3.45), 2x4(3.37)
Plant spread (cm)	3x6(50.46), 2x5(49.05), 1x6(48.55), 2x4(46.38), 1x3(46.13)	3x6(5.78), 1x6(5.36), 4x5(3.64), 1x3(3.15)2x5(2.88)
Fruit Lengh	2x5(8.64), 2x3(8.43), 2x4(8.39), 1x3(8.22), 1x2(7.58)	1x3(1.35), 2x5(0.75), 1x6(0.69), 2x4(0.50), 3x6(0.35)
Fruit diameter (cm)	1x6(6.11), 4x5(5.75), 1x5(5.67), 4x6(5.60), 5x6(5.56)	1x6(0.41), 1x3(0.22)
Flesh thickness (cm)	1x6(0.42), 4x5(0.40), 1x3(0.39), 1x4(0.38), 4x6(0.38)	2x3(0.07), 1x3(0.05), 4x5(0.05), 1x6(0.04)3x6(0.04)
Seed number/ fruit	5x6(416.6), 4x5(353.5), 1x3(335.8), 1x5(332.3), 4x6(328.6)	5x6(88.14), 1x3(42.17), 2x3(37.04), 4x5(2.43), 3x6(1.98)
Fruit number/plant	2x3(30.11), 2x6(27.55), 2x5(25.22), 1x3(23.00), 2x4(22.63)	1x3(5.73), 2x3(4.27), 2x6(3.37), 2x5(2.43), 3x6(1.98)
Average Fruit weight(g)	1x6(87.00), 4x5(86.67), 4x6(83.14) 3x4(75.00), 1x4(71.31)	1x6(18.50), 3x6(7.81), 3x5(5.45), 4x6(5.33), 4x5(5.08)
Fruit yield plant(g)	1x3(1423.0) 2x6(1387.0), 1x6(1363.2), 3x6(1347.9), 2x5(1300.5)	1x3(354.9), 1x6(259.9), 3x6(259.2), 2x6(244.5), (2x5(227.8)

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CURRENT STATUS OF MAJOR *CAPSICUM* GERMPLASM COLLECTIONS WORLDWIDE

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The Asian Vegetable Research and Development Center has the world's largest collection of *Capsicum* germplasm with 6,844 accessions representing; eight species (AVRDC, 1997). Large (>1,000 accessions) collections have also been reported in Costa Rica, Germany, Mexico (2), the Netherlands, and the United States. Smaller collections are held at many different locations worldwide.

A summary of the status of *Capsicum* germplasm collections world wide was published in 1990 (Bettencourt and Konopka, 1990). However, some of the information is incorrect or outdated. Therefore we prepared a current summary of the status of major *Capsicum* germplasm collections worldwide. However, come germplasm banks did not respond to. our requests for information. If anyone knows about other large collections not included herein the authors would appreciate receiving that information.

The Asian Vegetable Research and development Center has the world's largest collection of *Capsicum* germplasm with 6,844 accessions from 95 countries representing eight species. It contains duplicates of both the CATIE and the USDA collections. A total of 2,722 accessions have been characterized at the A VRDC and another 800 have been characterized at the National Plant Genetic Resources Laboratory (NPGRL) in the Philippines and Kasetsart University in Bangkok, Thailand. Characterization is based on the International Plant Genetic Resources Institute (IPGRI) descriptors. The number of accessions species-l is shown in Table 1. The curator, Uwayway Englef can be reached by email at lmengle@netra.avrdc.org.tw.

Two large collections have been reported in Mexico. The largest one is " at the Instituto Nacional de Investigaciones Forestales y Agropecuarias (INW AP) in Celaya, Guanajuato, with 3,590 accessions. The number of accessions species-1, as reported previously (Bettencourt and Konopkaf 1990), is shown in Table 2. A second collection is housed at the Germplasm Bank in Mexico City, with 1,500 accessions. Repeated requests for current information on the status of these collections have not been answered. If anyone has current information about the collectio~ in Mexico, the authors wouJd appreciate receiving it.

The Genetic Resources unit at the: Centro Agronomico Tropical de Investigacion yEnsenanza (CATIE) in Turrialba, Costa Rica, maintains 1,580 accessions representing seven species. The number of accessions Species-l is shown in Table 3. Sixty-eight percent have been multiplied and characterized with the IPGRI descriptors (Rodolfo Sanchez, pers. comm. 1997). The main countries represented in their collection are Guatemala, Mexico Costa Rica,

Peru, Ethiopia, Ecuador, Honduras, and Panama. The curator, Rodolfo Sanchez, can be reached by email at amora@catie.ac.cr.

The Centre for Genetic Resources in the Netherlands (CGN) in Wageningen, The Netherlands Dlaintains 1,036 accessions representing 14 species (this is the old NT collection from the former Institute for Horticultural Plant Breeding, now merged into CPRO). Three hundred forty-nine accessions, including 308 *annuum,* nine *baccatum,1* 27 *chinense,* and five *frutescens,* have been regenerated and given a CGN accession number, while 687 still need regeneration. The accessions were donated mainly by CATlE, Costa Rica, T apioszele, Hungary, the University of Reading, UK, and the USDA and University of California, Davis, USA. Only small seed quantities can be requested. The curator, Ietje W. Boukema, can be reached by email at i. w .boukema@cpro.dlo.nl, and their home page is located at www.bib.wau.nl/cgn/ .

The USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, Georgia, maintains 3,815 accessions representing 11 species. The number of accessions species-l is shown in Table 4. The curator, Robert Jarret, can be reached by email at bjarret@gaes.griffin.peachnet.edu. Bettencourt and Konopka (1990) reported that the Northeast Regional Plant Introduction Station in Geneva, New York held a large *Capsicum* collection, but that is incorrect; there are no *Capsicum* accessions there Games McF~rson, pers. comm,,1 19%).

The Central Institute for Genetics and Germplasm in Gatersleben, Germany, maintains 1,359 accessions representing nine species. Most of them have been regenerated and characterized. The number of accessions species-l is shown in Table 5. The curator, Karl Hammer, can be reached by email at hammer@ipk-gatersleben.de.

Table 1, A VRDC germplasm collection (A VRDC,1 1997).

Species	No. of accessions
C.annuum	4,064
C.baccatum	356
C. Chacoense	30
C. chinense	380
C. eximium	4
C. frutescens	365
C.praetermissum	4
C.pubescens	30
C.sp	1,581
Total	6,844

Table 2. INIFAP germplasm collection (Bettencourt and Konopka, 1990)

Species	N. of accessions
C. annuum var annuum	2,787
C.annuum var. aviculare	219
C. baccatum	167
C. cardenasii	1
C. chacoense	220
C. chinense	220
C. eximium	2
C. frutescens	81
C. galapagoense	2
C. praetermissum	5
C. pubescens	50
C. tovari	1
C.sp.	40
Total	1580

Table 3. CATIE germplasm collection (Sanchez, pers.com, 1996)

Species	No. of accessions
C.annuum	488
C. baccatum	45
C. chinense	107
C. frutescens	449
C. galapagoense	1
C. longum	2
C. pubescens	32
C.sp.	456
Total	1580

Table 4. USDA germplasm collection (pcGRIN, 1997)

Species	No. of accessions
C.annuum	2,429
C.anomalum	1
C. baccatum	571
C. cardenasii	2
C. chacoense	18
C. chinense	468
C. eximium	4
C. frutescens	216
C. galapagoense	1
C. praetermissum	7
C. pubescens	73
C.sp.	248
Total	3,815

Table 5. IPK germplasm collection (Hammer, per.comm.. 1997)

Species	No. of accessions
C.annuum	1,036
C. baccatum	36
C. chacoense	5
C. chinense	40
C. eximium	4
C. frutescens	178
C.microcarpum	7
C.praetermissum	4
C. pubescens	20
C.sp.	29
Total	1,359

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CURRENT STATUS OF THE INTERNATIONAL CHILLI PEPPER NURSERY

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Summary
The Asian Vegetable Research and Development Center (A VRDC)estab\lished the International Chilli Pepper Nursery (ICPN), formerly called the International Network for Tropical Hot Pepper (INTHOPE), in 1989. Its goal is to mediate the eXChatlge of chilli pepper *(Capsicum* spp.)germplasm and information on disease resistance and other plant traits among researchers working on chilli pepper in the tropics. As of 1996, six seed sets have been assembled and distributed to researchers " in at least 48 countries around the world. The 7th ICPN was assembled in February 1997 and is currently available for distribution to researchers.

Introduction

The International Chilli Pepper Nursery, formerly called the International Network for Tropical Hot Pepper, was established in 1989 by the Asian Vegetable Research and Development Center (A VRDC, 1991). It was initiated in response to needs expressed by participants of the International Symposium on Integrated Management Practices for Tomato and Pepper Production in the Tropics (A VRDC, 1989). Its goals are to introduce better-adapted chilli pepper germplasm into tropical production regions, to monitor the performance of this germplasm in diverse environments, and to gather information on pathogens attacking chilli peppers.

Materials and Methods

The 7th ICPN trial contains 20 entries, including three checks. A local check should also be included in each environment. One gram of seed (-200 seeds) is sent per entry. Seeds are prepared for shipment every year in February. A simple, two-page feedback form is included with each seed set. The nursery is grown at the A VRDC during the hot, rainy season and evaluated for several plant and fruit traits, including days to anthesis, plant height, fruit weight, fruit length, and fruit capsaicinoid content. Entries are screened at the A VRDC for resistance to 10 different diseases, including anthracnose *(Colletotrichum* spp.), Phytophthora root rot *(Phytophthora capsici),* bacterial wilt *(Rolstonia solanacearum),* bacterial spot *(Xanthomonas campestris* pv. *campestris),* cucumber mosaic virus (CMV), chile veinal mottle virus (CVMV), potato virus Y (PVY), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), and pepper mild mottle virus (PMMV). Disease screening and other results obtained at the A VRDC are summarized and sent to ICPN recipients every year in November, along with an invitation to receive the next ICPN trial.

Discussion

Seed sets for the 6th ICPN trial were sent to more than 40 recipients in more than 29 countries in 1996. Feedback has been received from nine researchers as *of* February 1996; several lines have been selected for further testing in Vietnam and Indonesia. The 7th ICPN trial is currently available to any interested researcher. To receive the ICPN 7 trial, please contact the author as soon as possible. The countries of American Samoa, Antigua, Argentina, Australia, Bahamas, Bahrain, Barbuda, Bolivia, Brunei, Cameroon, Cook Islands, Dominica, Egypt, EI Salvador, Fiji, Grenada, Guatemala, India, Indonesia, Iran, Iraq, Kenya, Malawi, Malaysia, Malta, Nepal, New Caledonia, Nigeria, Philippines, St. Lucia, Singapore, Solomon Islands, South Africa, Sweden, Uganda, Uruguay, Wallis & Futuna, Western Samoa, Yemen, and Zimbabwe require an import permit to import chilli pepper seeds.

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PAPRIKA VARIETIES FOR COLD ZONES OF THE REGION DE MURCIA-SPAIN.

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The pepper for paprika has a great economic importance in the Region of Murcia. During the last years a series of problems has provoked a decrease of the surface devoted to this cultivation.
going from more than 4000 ha in the year 1989 to less of 2000ha in 1995 (CoSta, 1996) ~
Three are the principal problems that affect now to the cultivation: The Tomato Spotted Wilt Virus (TSWV), the increase in price of the labour and the hydric deficit The Tomato Spotted Wilt Virus (TSWY) made its appearance in Murcia around the end of year 1989 (Costa et al., 1996). Since then, its expansion and consolidation have run parallel to that of its vector the thrips Frankinella occidentalis. As far as labour, labours of transplant and harvest can arrive to suppose more than 500/0 of the total costs. The water lack motivated by the drought has been aggravated with a new consumption, produced by the installation of the new intensive export horticulture in the area.
With the propose of reducing the culture costs, the CIDA has developed new varieties of grouped ripeness, susceptible of mechanically harvested (Costa et al., 1995). These varieties of grouped ripeness present furthermore the particularity of the short cycle. Also, they have been developed varieties of half-grouped ripeness that allow lower harvest cost
A possible solution for the problem of the TSWV and for the hydric deficit would be the displacement of this cultivation to other areas with climates more colds and with more water. In these areas the incidence of the virus is smaller. The only problem to solve would be to adjust the cultivation cycles to avoid the low temperatures during spring and autwnn.
A series of varieties with different cultivation cycles was selected: Of traditional (echeloned) ri~ss with the long cycle cultivation: Datler, Negra}, ME - 15, ME - 321, ME -372 and ME- 17. The half-grouped ripeness with the middle cultivation cycle: SA-I, SA-2 and SA-3. The grouped ripeness lines with the short cultivation cycle: MAG-27, MAG-32, MAG-I45, MAG-12 and MAG-1 00. To accomplish the trials was chosen two localities of the Region of Murcia: Jumilla with colder climate and Lorca with a similar climate to the traditional growing areas, but with the smaller incidence of the virus of the TSWY (Lacasa et al., 1996). The means temperatures, corresponding to a period of 10 years, in the two localities of the trial are shown in the figure 1. In Jumilla the low temperatures during winter avoid the thrips proliferation. Z Furthermore, these areas enjoy of dry and warm summers and can arrive to be converted into alternative zones for the cultivation of the pepper for paprika.
The results of trial showed that the grouped ripen lines reached the commercial maturity three months of the transplant. The breeding line MAG-100 was the most early, must be harvested 15 days before that the other to avoid losses of production. The MAG-100 and MAG-27 varieties seem the most interesting. Of the rest of the varieties, the most adequate for these areas, seems be ME-IS since apart from its productivity and quality (table-1) has the advantage of be early. This variety achieve the good grouped ripeness when the irrigation is suppressed twenty days after the 50% of plants present some red fruit The variety Datler presents interest by its great production and quality. The intensity of the colour, expressed in degrees ASTA(AST- 1985), for

all of the genotypes studied (table 2), ttlffied out the 11,4% greater in the samples taken in Lorca that in those of Jumilla (F (1,43)=9,841). This fact is possibly due to the better climatic conditions in Lorca, what probably induces a greater pigment's concentration in the fruits.
With respect to the relationshipp between the red and yellow pigments were not found significantly differences between localities and only between genotypes (F(13,43)=2,397). The relationships between weight fresh fruit and dry weight fruit (table 2) is more favourable to Lorca conditions, probably due because in the moment of the harvest the fruits presented in this localit), a maturity stage more advanced.. Consequently the relationship fresh/dry weight was smaller for the set of the genotypes in Lorca, presenting a value of 4,67 front to 5,51 in Jumina (F(143) =14,898).

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Acknowlement..

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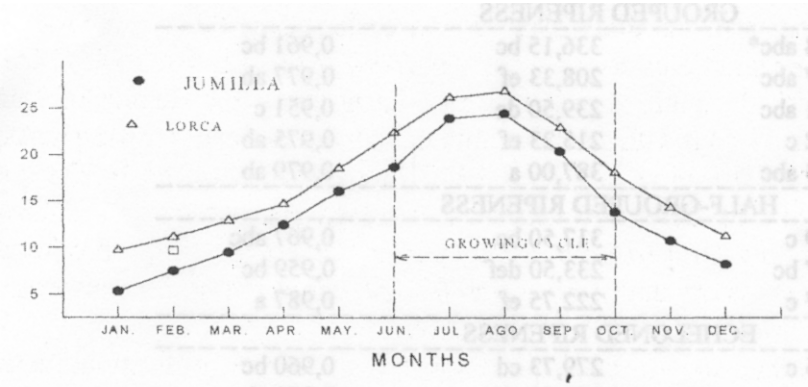

Figure 1 - Means temperatures for the last ten years in the two growing areas.

Table 1 Means for yield/plant (Kg.) number of fruits/plant and weight average (gr) in two areas and all the varieties.

VARIETY	YIELD/PLANT (kg)	N°.OF FRUITS/PLANT	MEAN WEIGHT FRUIT (gr)
		GROUPED RIPENESS	
MAG-100	0.1270 ab*	9.7732 bc	13.3781 a
MAG-145	0.1000 d	8.0576 c	12.3756 ab
MAG-32	0.1053 bc	10.2917 b	10.2891 bc
MAG-27	0.1500 a	13.9083 a	10.7389 bc
MAG-12	0.1210 bc	11.6937 a	10.1240 c
JUMILLA	0.1414 a	10.5390	13,4938 a
LORCA	0.1001 b	10.9497	9,2680 b
		HALF-GROUPED RIPENESS	
SA-1	0.1254	5.7988	21.8791
SA-2	0.1355	7.8713	17.4919
SA-3	0.1209	8.3022	14.8383
JUMILLA	0.1118	7.0374	21.8567
LORCA	0.1439	7.6109	14.2827
		ECHELONED RIPENESS	
NEGRAL	0.2873 bc	22.8947 c	12.9896 b
ME-15	0.3544 b	28.3137 b	11.4962 b
ME-321	0.2657 bcd	24.1280 b	11.0559 b
ME-17	0.2176 d	16.1219 c	13.3360 b
ME-372	0.3783 a	21.9503 c	17.3138 a
DATLER	0.4100 a	33.5141 a	12.7935 b
JUMILLA	0.3500	20.3344 b	15.6851 a
LORCA	0.3441	28.6408 a	10.6434 b

*Means followed by unlike letter(s) are significantly different at 5% significance level using Duncan test.

Tabla 2 . Relationships between weight fresh fruit and dry weight fruit (FW/DW), Extractables pigments ASTA and - relationship between the red and yellow pigments (DYE).

VARIETY	FW/DW	ASTA	DYE
		GROUPED RIPENESS	
MAG-100	4,98 abc*	336,15 bc	0,961 bc
MAG-145	5,57 abc	208,33 ef	0,977 ab
MAG-32	4,91 abc	239,50 de	0,951 c
MAG-27	4,52 c	215,23 ef	0,975 ab
MAG-12	5,03 abc	387,00 a	0,979 ab
		HALF-GROUPED RIPENESS	
SA-1	4,49 c	317,50 bc	0,967 abc
SA-2	4,77 bc	233,50 def	0,959 bc
SA-3	4,47 c	222,75 ef	0,987 a
		ECHELONED RIPENESS	
NEGRAL	4,65 c	279,73 cd	0,960 bc
ME-15	5,19 abc	373,50 ab	0,971 abc
ME-321	4,97 abc	199,25 ef	0,972 abc
ME-17	5,43 abc	180,88 f	0,967 abc
ME-372	6,23 a	328,00 bc	0,967 abc
DATLER	6,08 ab	320.75 bc	0,977 ab
JUMILLA	5,51 a	246,4657 b	0,967
LORCA	4,67 b	278,1706 a	0,972

*Means followed by unlike letter(s) are significantly different at 5% significance level using Duncan test.

Capsicum and Eggplant Newsletter. 16 (1997): 85-86.

NEW POTATO VIRUS Y PATHOTYPE IN PEPPER

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Based on the differential reaction on a set of peppers cultivars, potato virus Y (PVY) isolates infecting peppers in the Mediterranean area have been classified into three pathotypes (PVY-O, PVY-1 and PVY-1-2) (non shaded section of Table 1, Gebre ,. Selassle *et al.,* 1985; Marchoux and Gebre Selassie, 1989). The varieties used to distinguish among these pathotypes are 'Yolo Wonder'., susceptible to the three pathotypes, 'Yolo Y', resistant to PVY-O, 'Florida VR2', resistant to PVY-O and PVY-1, *p,* and 'Serrano Veracruz', resistant to the three pathotypes.

Table 1. Classification of PVY isolates into pathotypes.

Pepper varieties	Resistance genes	Pathotypes			
		PVY-0	PVY-1	PVY-PRW	PVY1-2
Doux Des Landes	Pvr2 (=y=vy=pr2)	SN	SM	SN	SN
Yolo Wonder	Pvr2(=y=vy=pr2)	SM	SM	SM	SM
Yolo Y	Pvr21(=ya=vy=pr2)	R	Sm	SM	Sm
Florida VR-2	Pvr22(=eta=vy2pr22)	R	R	R	SM
Puerto Rico Wonder	Pvr23???	R	R	SM	R
Serrano Veracruz	Vy2s???	R	R	R	R

S=susceptible; R=resistant; N=necrosis; M=mosaic. 'After Pailoix and Kyle (1995).

Most Spanish PVY isolates from pepper belong to PVY -0, but some PVY -1 and one PVY -1-2 isolates have also been found (Luis Arteaga *et al.,* 1993).

Pasko *et al.* (1995) reported the variety 'Puerto Rico Wonder' ('PRW) resistant to the three PVY pathotypes. Later, we found a 'PRW plant that showed systemic vein banding symptoms after inoculation with our PVY-1-2 isolate. From this plant we obtained an isolate that always produces infection on 'PRW. This isolate also infects cultivars 'Yolo Wonder' and 'Yolo Y' but cannot infect 'Florida VR-2' and 'Serrano Veracruz' (Table 1). According to these results this isolate could be classified within a new pathotype that provisionally is given the name of PVY-PRW. This isolate can not infect the variety 'Serrano Criollo de Morelos (SCM-334)' either.

According to Pasko *et al.* (1995), 'PWR' resistance to PVY-1-2 is recessive and very likely monogenic, although a complete genetic test has not been accomplished *yet.* Supposedly 'PRW carries a new allele on the locus pvr2.
The discover of PVY -PRW pathotype suggests the use of 'PRW as an additional differential cultivar when we try to distinguish between PVY-PRW and PVY-1 (Table 1). The detection of PVY -PRW pathotype could also explain some discordant results between workers who obtained different cultivar response with different PVY -1 isolates *(Paskoetal.,* 1996).
On the last 15-year period we have worked with different PVY isolates infecting peppers in Spain. Some times we have observed pathotype evolution, generally to more virulent pathotypes

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Capsicum and Eggplant Newsletter. 16 (1997): 87-90.

UTILIZATION OF *CAPSICUM* SP. RESISTANCE TO TSWV IN PEPPER BREEDING.

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Resistance to TSWV in C. *chinense* PI-152225 and PI-159236 accessions has been reported by several authors in artificial inoculation trials (Black *et al.,* 1991; Diez *et al.* , 1993; Palloix *et aI.,* 1993; Boiteux, 1995). Other materials have been found to be resistant by authors in different growing conditions or in previous inoculation trials. C. *baccatum* C- 57 and C. *chacoense* C-153 and C-280 exhibited good reaction to disease in field conditions, being the two last accessions resistant when mechanical inoculation with L- 93940 isolate was performed (Rosello *et aI.,* 1996). Accession 7204 reacted as resistant to mechanical inoculation in previous experiments (Diez *et aI.,* 1993). Nevertheless, the utilization of these resistance sources in breeding programs is conditioned by their stability under different environmental conditions, since desestabilization of resistance phenomenon has been observed in C. *chinense* PI-159236 after inoculation with a strain of TMV (Boukema, 1982). A trial was conducted with the above mentioned accessions to study their resistance using a new virulent isolate and at two temperatures.

Trials were conducted in a climatic chamber under two temperatures, *28/18*° C and *25/18*° C (day/night). The other environmental conditions were similar in both assays: 70/95 % HR (day/night), 65/80 ~mol m-2 gl of irradiance and photoperiod of 14/10 hours (light/dark). Spanish isolate T -941117 of TSWV was selected by its high virulence (Abad, 1996). Plants were inoculated at 4-6 leaves stage. Inoculation was repeated a week later. Plants were scored visually for TSWV symptoms each 10 days up to two months. ELISA test was used to confirm latent infection on plants without obvious symptoms.

All plants of the susceptible control became infected in both trials (Table 1). First systemic symptoms appeared 7 days after inoculation. Accessions C-153 and C-280 reacted as susceptible in the trial conducted at 25°C, exhibiting medium to severe symptoms of the disease. Reaction of these accessions to *28*° C was apical necrosis followed by regrowing and normal growth of the plant. Only one plant shows ELISA positive results. This behaviour is different from the shown by these accessions in previous experiments carried out by the authors using a different isolate, in which plants reacted as resistant without any apical necrosis (Rosello *et al.,* 1996). This result suggests a dependence of the isolate on the early phase of the infection. Response of C. *baccatum* C-57 was similar that the one described for C. *chacoense* accessions. C. *chinense* PI-159236 and PI-152225 were resistant after inoculations conducted at *25*° C, showing only local lesions of hipersensibility 3-4 days after inoculation. Nevertheless, necrotic lesions on the stern, on uninoculated leaves and, accasionally, on the apex developed 20 days after inoculation at *28*° C. This suggests the lack of efectiveness in the initial hipersensitivity reaction to prevent the expansion of the virus. A partial migration of the virus probably occurs, producing necrotic lesions capable to kill some plants. These necrotic lesions are likely of hipersensitive type,

as no virus was detected by ELlS Arm close tissues taken higher up the necrosis... Accession 7204 exhibited a resistant reaction at 25° C, Desestabilization of this resistance was observed at 28° C, although 7204 did not show apical necrosis nor wilt symptoms as PI- 159236 and PI-152225 (Table I),
Results of this experiment show that resistance found in *Capsicum* species represents Drily a partial solution~ as it can be desestabilized by temperature. Further search of new resistance sources no dependent of this factor need to be carried out.

Table 1: Temperature-conditioned response of some accessions of *Capsicum* to TSWV mechanical inoculation.

Accession	Temperature			
	25° C		28° C	
	Infection ratio	Symptoms	Infection ratio	Symptoms
C. annuum				
Negral (Control)	12/12 [1]	{Yr(2)[2], Lp(2), Nul(1), Sn(2), An, D(2)} → W(3)	12/12	{Yr(2), Lp(2), Nul(1), Sn(3), An, D(2)} → W(3)
C. chacoense				
C-153	10/12	Lp(1), Yr(1), Y(1) [An→R] [3]	1/12	An→R→Health [Nul(1)]
C-280	8/12	Nul(2), Yr(2), An [Y(2),Lp(2)]	1/12	An→R→Health
C. baccatum				
C-57	12/12	Nul(2), Lp(2) [Y(2), M(2)]	0/12	An→R→Health [Nul(1), Sn(1)]
C. chinense				
PI-152225	0/12	Ll; Nss.	0/12	Ll.; Nul(2), Sn(2)→ R [W(3)]
PI-159236	0/12	Ll; Nss.	0/12	Ll.; Nul(2), Sn(2)→ R [An, W(2)]
C. frutescens*?				
7204	0/12	Ll; Nss.	0/12	Ll.; Nul(2), Sn(1)→ R

1 Number of plants infected ELISA + *I* Total number of plants.
2 Typical symptoms. Number between brackets indicate severity of the symptoms: (I) slight; (2) medium; (3) severe.
3 Occasional symptoms are indicated between [ ].

Ll : Local lesion. D: Dwarfmg. Nul:Necrosis on uninoculated leaves.
Nss: No sistemics symptoms. M: Mosaic. Sn: Stem necrosis.
Y: Yellowing. W: Wilt. R: Regrew.
Lp: Leaf puckered. An: Apex necrosis.
Evolution of symptoms.
Yr:Yellow rings.
.

* Accession 7204 was provided by the Centro de Recursos Fitogeneticos (Madrid) as *Capsicum frutescens.* It was classified in the *chinense* species after Pickersgill (Moury *et al.,* 1995). Characteristics of this accession are: two pedicels per axil, green-white corolla and erect pedicels at anthesis. With these characteristics, the only trait that permits distinguish between *frutescens* and *chinense* species is the annular constriction at junction of calix and peduncle on ripe fruits. In our growing conditions, a slight constriction on some fruits has been only ocasionally observed.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the cession of the accessions tested in this study by Dr R. Gil Ortega, Centro de Recursos Fitogeneticos (Madrid) and USDA ARS Plant Genetic Resources Unit. Likewise we are extremely grateful for the financial support provided by Instituto Nacional de Investigaciones Agrarias by means of the Project N° SC93-183-C3-1.

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SOURCES OF RESISTANCE TO BACTERIAL WILT IN CAPSICUM ANNUUM

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Introduction

Bacterial wilt caused by *Ra/stonia so/anacearum* is an important disease affecting pepper *(Capsicum* spp.) production in the tropics. The combination 'of high - temperature and poor drainage favor development of this soilborne disease. Symptoms
in susceptible varieties begin with wilting of ' the lower leaves followed by a sudden, permanent wilt of the entire plant with only slight or no leaf yellowing. Good drainage, liming, and crop rotation programs of three to five years are helpful in minimizing this disease, but the most efficient method of control is the use of resistant varieties. Reports of resistance to bacterial wilt in *Capsicum* spp. have been made (Matsunaga et aI, 1993; Matsunaga and Monma, 1994; Matsunaga and Monma, 1995; Peter et aI" 1984), but several of the reported resistance sources were found to be partially or completely susceptible to Taiwan strains of the bacterial wilt pathogen. This study was conducted to confirm stability of resistance to bacterial wilt in 17 accessions rated as resistant in a previous seedling-screening test at A VRDC (A VRDC, 1994).

Materials and Methods

Seventeen pepper accessions were tested for the stability of their resistance to bacterial wilt using three methods: seedling screening in a growth room at 28 °c (GR), field screening at the Asian Vegetable Research and Development Center by transplanting inoculated seedlings (A VRDC), and field screening in a naturally infested field in Taichung, Taiwan (TSS). Code number, variety name, variety origin, and fruit type of the 17 resistant pepper entries, the resistant check, and the susceptible check are given in Table 1.

For the GR trial, plants were inoculated using a soil drenching and root severing - method. Forty plants per accession were grown in 7.5-cm polyethylene pots containing a steam-sterilized potting soil mixture (3: 1: 1: 1 soil: rice hulls: sand: compost). Seedlings were inoculated with bacterial strain Pss71 (race 1, biovar 3) at 28 days after emergence. Roots were injured with a knife by cutting through the soil 1 to 2 cm from the collar and 30 ml of bacterial suspension (108 cfu/ml) was poured into each pot. After inoculation, plants were kept in a growth room at 28 °c for observation. For the A VRDC field trial, 60 plants per accession were raised and inoculated in the same way as those for the GR trial. Inoculated seedlings were transplanted to an A VRDC field 3 days after inoculation.

For the TSS trial, 60 plants per accession were grown in a disease nursery naturally infested with *R. so/anacearum* that has been used for evaluating bacterial wilt reaction

in tomato and pepper for several years. The previous crop was *Sesbania,* spp. Percentage of wilted plants were recorded every one or two weeks after inoculation or transplanting.

Results and Discussions

Symptoms other than wilting, such as leaf necrosis, leaf yellowing, and defoliation, were observed in most of the entries tested. However, wilting was the most consistent symptom among the trials and was used as the criteria for resistance evaluation. The trial mean wilt incidences were 21.0,15.2, and 11.1%, while those of the susceptible check, OriasKossarvu, were 100, 100, and 85.4% for the GR, A VRDC, and TSS trials, respectively. Thus, the disease pressure was lower in the TSS trial. The combined " ANOV A indicated that entry and trial effects were significant ($p<0.05$), but the entry x trial interaction was not significant, indicating that entries were ranked similarly in. each trial. Among the entries tested, PBC 385 (cayenne fruit type, from Malaysia) was the only line showing no symptoms in all trials.

Isolation of the pathogen from the collar region or lower stem area was attempted for nine ()fthe 17 entries (PBC 066, PBC 204, PBC 375, PBC 384, PBC 385, PBC 473, PBC 535, PBC 631, and pgC 1341) at the end of each trial. *R. solanacearum* was isolated frO1'l1 all nine lines in each trial except forPBC 066, PBC 204, and PBC 1347 in the TSS trial. The range of colonization frequency over entries was 61-100%, 60- 100%, and 0-39% for the GR, A VRDC, and TSS trials, respectively. Thus, the apparent resistance shown by these lines is actually tolerance, since the pathogen was present in the plants but does not cause visual wilting symptoms. The phenomenon of latent infection has been observed in other solanaceous host of *R. solanacearum,* such as potato (Ciampi & Sequeira 1980), tomato (Grimault etal 1993), and eggplant (J. Wang, Unpublished data).
A VRDC has initiated international evaluation to determine the stability of the above selected resistance sources. Those who are interested in joining this evaluation, please contact the author.

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Table 1. Mean incidence of wilted plants among pepper entries evaluated for their bacterial wilt reactions in two field trials and one growth room trial.

Code	Variety name	Origin	Type	%Wilt	DMRT
C00835	Orias Kossarvu (susc. ck.)	Hungary	Wax	96.0 x	A x
PBC650	Sinagtala	Philippines	Sweet	39.4	B
PBC1350	KingGumGoChu	Korea	Chilli	36.9	BC
PBC404	PL-38475	Nigeria	Chilli	36.4	BC
PBC717	Sheetal-51	India	Chilli	30.5	BCD
PBC495	Perennial	India	Chilli	26.7	CD
PBC518	PSP-11	India	Chilli	21.4	D
PBC067	MC 5	Malaysia	Chilli	6.9	EF
PBC1347	R1-26(17)	Malaysia	Chilli	6.1	E
	MC 4 (res. ck.)	Malaysia	Chilli	3.6	EFG
PBC384		Malaysia	Chilli	3.4	EFG
PBC743	Chinda 2	Thailand	Chilli	2.7	EFG
PBC473		Indonesia	Chilli	1.6	EFG
PBC204	Cili Langkap	Malaysia	Chilli	1.6	EFG
PBC066	MC 4	Malaysia	Chilli	1.6	EFG
PBC375	Paris Minyak	Indonesia	Chilli	1.1	FG
PBC535	IR	Indonesia	Chilli	1.1	FG
PBC631	CA 8	Sri Lanka	Sweet	0.9	EFG
PBC385		Malaysia	Chilli	0.0	G

x Wilt% means are actual values. Analysis of variance and mean separations were done using transformed data by arcsin of the square root.

Capsicum and Eggplant New letter 94-97

EVALUATION OF FIELD RESISTANCE OF PEPPER LINES AND VARIETIES POSSESSING DOMINANT (Bsl, Bs2, Bs3) RESISTANCE GENES TO *XANTHOMONAS CAMPESTRIS PJI: VESICATORIA*

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Bacterial leaf spot caused by *Xanthomonas campestris pv. vesicatoria* is one of the wide sprea4,and severe ?' diseases of pepper allover the world. Resistance br~ng *toX* c. *pv. vesicatoria* has been in pro'gress Since the early 80's in Hungary (10). Two types of resistance to this pathogens have been described according to the inoculation method:

I. High concentration (5x108 cfu/ml) of bacterial suspension of race I, race 2 or race 1,2,3 infiltrated into pepper leaves possessing resistance genes Bs3, Bsl and Bs2 elicited hypersensitive response *in* the host tissue (4,2).
2. Low concentration (103 cfu/ml) of bacterial suspensions infiltrated into pepper leaves caused quantitatively controlled 'slow hypersensitive response' (5,8).

The aim of our experiment w~ to test and evaluate the field resistance of various pepper lines possessing BsI, Bs2 and Bs3 dominant resistance genes to the home population *of X. c. pv. vesicatoria.*

Material and method

Isogen 'Early Calwonder' pepper lines possessing BsI, Bs2 and Bs3 resistance genes and their susceptible forms (7), 'Florida XVR 3-25' Resistant variety (I) and two Hungarian tomato-shaped varieties were inoculate with high concentration bacterial suspension by spraying (Table I). Inoculations were done by two bio types of bacteria (Table 2), ~ characters of a home strain were previously checked in control plants (3). The infection (fi) and defolia(ion (di) of four to six true-leaves plants were evaluated at the same time, one and two weeks after the inoculation (6). Data were analyzed by their variances.

Results

Pepper lines possessing Bs2 resistance genes proved to be the most tolerant of all three races of the pathogen. Among them 'ECW-205R(538)', heterozygous to Bs2, controlled the infection with the home strain only on medium level, but its tolerance remained on high level in the case of race 2.
Lines carryingrBs3 genes showed moderate susceptibility to all races of bacteria, but line 'ECW I 30(8081/2)' was infected in the highest degree according to its infection index.

Lines carrying Bsl genes proved rather different levels 9f tolerance or susceptibility to all races. 'ECW' cultivars without any resistance genes were infected seriously by strain 'XVT48', but only mildly by strain 'XVPI4', because of decreased defoliation. Hungarian cultivars were infected seriously by both races, but the infected area of the leaves was only on average level in case of race 2.
Bs2 resistance genes prevent high-level infection by bacterial leaf spot of pepper plants in Hungary. Plants having Bs2 genes in homozygous form; even infected, suffer less: the infected area is smaller, they don't drop leaves, don't loose yield. But Bs2 genes in heterozygous form seem to insure only average field

tolerance to home population of *X. c. pv. vesicatoria.* Bs3 resistance genes do not prevent bacterial leaf spot in pepper plants in Hungary.

Table 1. **Pepper lines and varieties inoculated by strains of *X c. pv. vesicatoria***

Code	Previous Code	Origin	Resistance gene
1268	ECW	1	-
1276	ECW	2	-
1269	ECW-10	1	Bs1
1278	ECW-10R	2	Bs1
1277	ECW-10R(537)	3	Bs1
1270	ECW-20(8059/1)	1	Bs2
1271	ECW-20(8060/2)	1	Bs2
1272	ECW-20(8064/1)	1	Bs2
1280	ECW-20R	2	Bs2
1279	ECW-20R(538)	3	Bs2
1283	FL-XVR3-25(17)	3	Bs2
1273	ECW-30(8080/2)	1	Bs3
1274	ECW-30(8081/2)	1	Bs3
1275	ECW-30(8083/1)	1	Bs3
1285	ECW-30R	2	Bs3
1281	ECW-30R(539)	3	Bs3
1284	Greygo(18)	3	-
1285	Paradicsom alakú zöld szentesi	3	-

Meaning of origin code:

1 - lines selected by G. Csillery, Budakert Ud., Hungary
2 -lines from J. M. Poulos, A VRDC, Taiwan
3-lines selected in Plant Breeding Center of vetO mag Trading house Co. Ud., Hungary

Table 2.

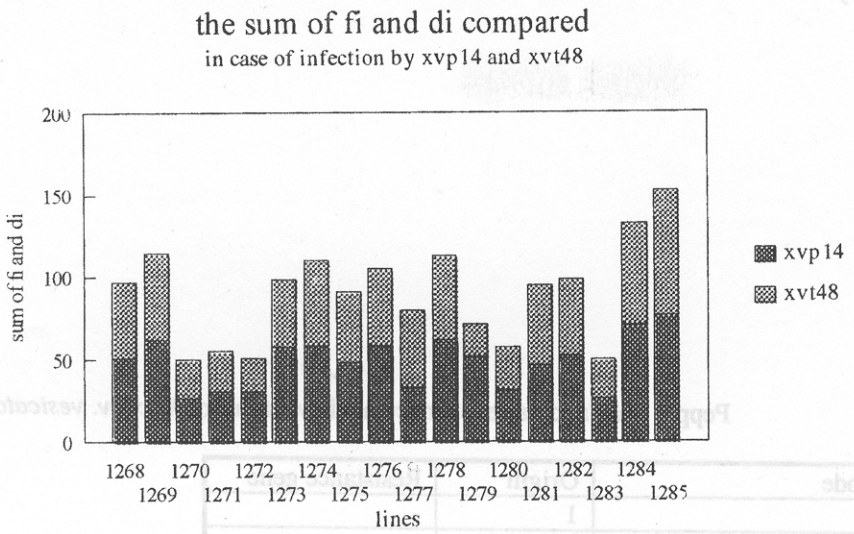
Strains *of X. c. pv. vesicatoria* used for sprain inoculation of pepper lines and varieties

Strain	**Race**	**Date of isolation**	**Origin**
XVP14	**1**	**1991**	**1**
XVT48	**2**	**?**	**2**

Meaning of origin code:

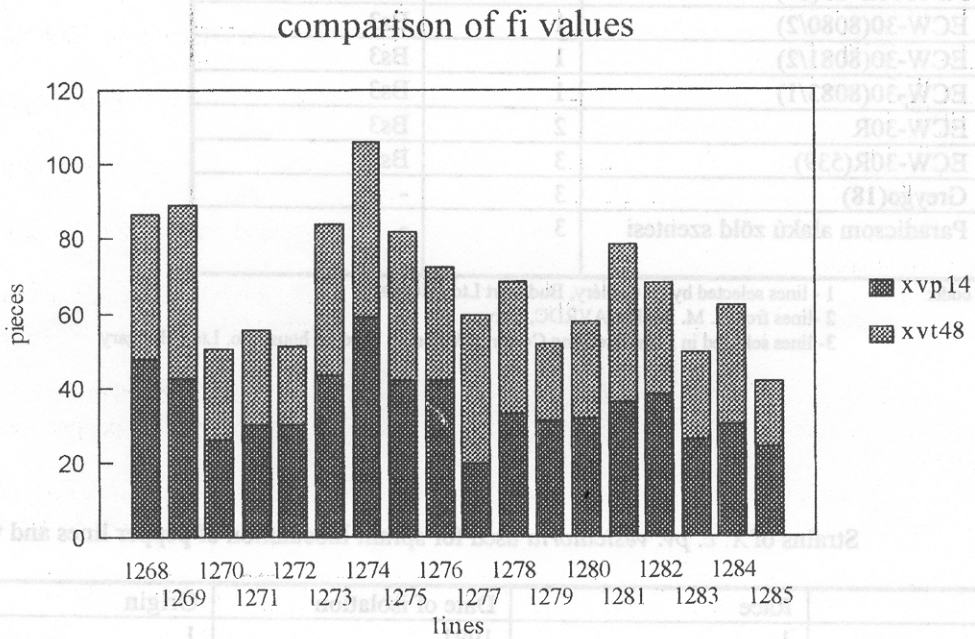
1 - M. Hevesi, University of Horticulture and Food, Hungary
2 - J. F. Wang, AVRDC Taiwan

Figure 1.



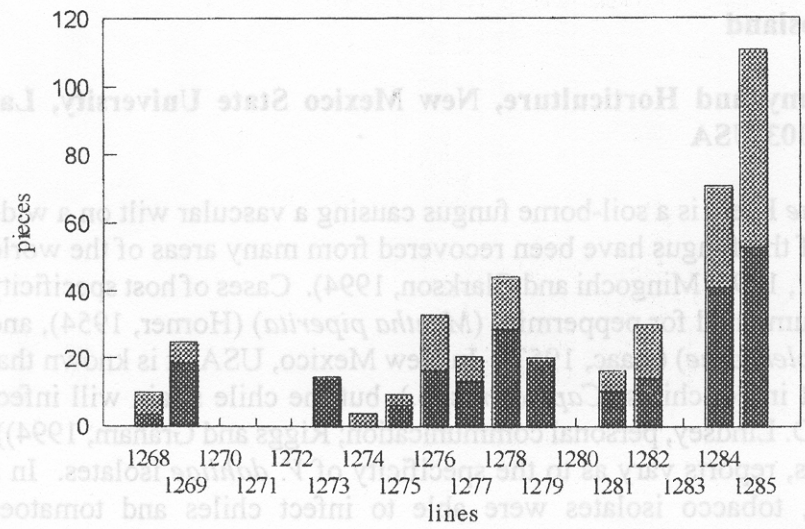
spring of '95

Figure 2.



spring of '95

Figure 3.



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HOST SPECIFICITY OF UNITED STATES TOMATO AND CHILE ISOLATES OF *Verticillium dahliae*

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Verticillium dahliae Kleb. is a soil-borne fungus causing a vascular wilt o~a wide - range of hosts. Isolates of the fungus have been recovered from many areas of the world (Taylor, 1969; Douira et al., 1995; Mingochi and Clarkson, 1994). Cases of host specificity of isolates have been documented for peppermint *(Mentha piperita)* (Homer, 1954), and brussel sprouts *(Brassica oleraceae)* (Isaac, 1957). In New Mexico, USA, it is known that the cotton strain does not infect chiles *(Capsicum* spp.1 but the chile strain will infect cotton *(Gossypium* spp.) (D. Lindsey, personal communication; Riggs and Graham 1994). Among solanaceous crops, reports vary as to the specificity of *V dahliae* isolates. In a study by Taylor (1969), tobacco isolates were able to infect chiles and tomatoes *(Lycopersicon esculentum),* but exhibited only slight symptom expressions. Among 80 isolates from Japan, Horiuchi et al. (1990) found a tomato strain that infected eggplant *(Solanum melongena),* a chile strain that infected eggplant, but no strain that infected tomato and chile. Mingochi and Clarkson (1994) reported that from six isolates of *V. dahliae* from tomato (four race1 and two race2), all were pathogenic on chiles, with some isolates causing severe symptoms. Douira et al. (1995) also reported susceptibility of chiles to some but not all tomato isolates tested. Seed companies in the United States 'have questioned whether the isolate obtained from chiles is actually a tomato strain. The following study was conducted to help resolve these contrasting reports with respect to the host specificity of tomato and chile isolates of *V. dahlia.*

Materials and Methods

The tomato strain (F 190) used in this experiment was determined to be race 1 and was isolated from tomato in California. The chile strain (PWB-18) was isolated from chile in New Mexico. The isolates were cultured on Potato Dextrose Agar (for the tomato strain) and Potato Carrot Dextrose Agar (for the chile strain) in 1bOmm x 15mm petri plates for =
14-16 days. For each isolate, the contents of two plates were blended with 200ml sterile distilled water. The inoculum concentration was determined (via dilution plating) to be 106 z colony-forming-units per milliliter of inoculum. The tomato host plants were 'Better Boy VFN' (resistant to *V. dahliae* of tomato, race 1) and 'Fantastic' (susceptible to *V dahliae of* tomato, race 1). Chile host plants were lines developed,f;rom P.I.215699 (app!ox. 75% resistant to the chile isolate of *1t.. dahliae)* and B.G.1668 (susceptible to the chile isolate). Seeds of the four hosts were sowp in vermiculite. ,All plantlets were at the cotyledon to first-true-leaf stage when inoculated (17- 24 days from sowing date).

Seedlings were removed from the planting trays and the roots washed free of vermiculite. For each of the four host genotypes, 20 seedlings (with their roots trimmed

to 1 cm) were dipped into one of the two inoculum suspensions for three minutes. This resulted in eight genotype/inoculum combinations. Seedlings were then transplanted to plastic planting trays filled with a commercial planting medium, Metro Mix 360. Each tray contained four rows of five plants each. Of the 20 seedlings in each tray, all were of the same species. Within each tray the four rows consisted of two rows of the resistant genotype and two rows of the susceptible genotype. Seedlings within a single tray were inoculated with the same strain. The four rows were randomized within each tray. There were a total of eight. trays - four with tomato plants and four with chile plants. Within each group of four trays, the plants in two trays were inoculated with the tomato isolate and the ;;; plants in the other two trays were inoculated with the chile isolate. The trays were
completely randomized within a soil temperature tank where the soil was maintained at 24C. Seedlings were watered as needed and the photoperiod adjusted to 18 hours with a light intensity of250 ~mol/m2/sec. After a three week incubation period, seedlings were scored for disease severity using an interaction phenotype scale from 1 =healthy, no symptoms to 9=death.

Results and Discussion

The tomatoes resistant to v: *dahliae* race1 ('Better Boy') had no disease symptoms when challenged with the race 1 tomato strain (Table 1). All tomatoes susceptible to *V. dahliae* race 1 ('Fantastic') were dead after infection with the race1 tomato strain. Neither of the two lines of chiles expressed any symptoms of disease from the race1 tomato strain.

When challenged with the chile isolate of v: *dahliae,* no symptoms were visible on either 'Better Boy' or 'Fantastic' plants. Plants from the resistant chile line were not affected by the chile isolate, while all of the susceptible chile seedlings were dead.

Table 1. Mean disease severity! of four genotypes screened for susceptibility to *Verticillium dahliae* isolates from chile and tomato.

Host Isolate	BB2	Fan	PepR	PepS
Tomato	1.0	9.0	1.0	1.0
Chile	1.0	1.0	1.0	9.0

1 Disease severity based on an interaction phenotype scale where 1 = healthy, no symptoms, to 9=dead.

2 BB='Better Boy' - resistant to *V dahliae* race 1 of tomato; Fan='Fantastic' - susceptible to race 1 Of tomato;

PepR= chile accession resistant to *V. dahliae* of chile;

PepS= chile accession susceptible to *V. dahliae* of chile.

This study demonstrates conclusively the inability of this tomato isolate to infect

chiles, and the inability of the chile isolate to infect tomatoes. Because the susceptible tomatoes were infected with the tomato isolate, and the susceptible chiles were infected with the chile isolate, both strains were determined to be virulent on their respective hosts. If the chile isolate had indeed been a tomato strain, then the susceptible tomato host should have expressed symptoms ofVerticillium wilt. This was not observed. Therefore, host specificity is present in both isolates tested.

There are some factors that may explain the discrepancies in results from different host specificity studies. In the report by Douira et al. (1995) *V dah/iae* isolates not pathogenic to chile were from fields where tomatoes were continuously :cropped without rotation. The isolates they found to cause disease on chile were from fields where tomatoes ~ and peppers were rotated. Several authors have suggested the importance of cropping history on the pathogenicity of isolates (Douira et al., 1995; Mingochi and Clarkson, 1994; ':: Tjamos, 1981).' Aside from cropping history, environmental factors as well as the host cultivars grown may influence the pathogenicity of *V dah/iae.* Studies involving isolates from several geographic areas and different cropping histories combined with numerous host cultivars may aid in further studies of host specificity of *V. dah/iae.*

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IDENTIFICATION OF SOURCES OF JUVENILE RESISTANCE IN *CAPSICUM SPP. TO PHYTOPHIHORA CAPSICI*

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Phytophthora root rot is an important disease affecting pepper *(Capsicum annuum* L.) in ~ Brazil and the use of genetic resistance, as part of an integrated disease management program, is considered the most effective individual method of controlling *Phytophthora - capsici* (Bosland & Lindsey, 1991). Incidence of Phytophthora root rot in young plants in seedbeds is frequently observed; infected seedlings are a major source of primary inoculum in production fields. Therefore, we attempted to identify stable sources of juvenile resistance to *Phytophthora capsici* since our previous work had already identified some durable, stable and effective sources of adult-plant resistance, which is commonly expressed in 40 day-old or older plants.

Eighty-one *Capsicum* genotypes of Embrapa-Hortalivas' germplasm collection were evaluated for Phytophthora capsici resistance by inoculating 7 plantlets, in 3 replications, 15 days after emergence. Inoculation was achieved by placing 3 mI. ofa 5xl04 *P. capsici* zoospore suspension per plant isolate (CNPH 02). Genotypes CNPH 148, 173 and 192 were used as resistant, partly resistant and susceptible checks, respectively. Evaluations were made 4 and 8 days after inoculation (dai) by determining the percentage of dead plants.

Several genotypes presented 40% or less dead plants 8 dai (Table 1) and are considered to have juvenile resistance to *P. capsici;* genotypes CNPH 1393, 2171, 2174 and 2176 were as resistant as the resistant check CNPH 148.

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Table 1. Reaction of Capsicum genotypes inoculated with *Phytophthora capsici* 15 days - after emergence as measured by the number of dead plants 8 days after inoculation..

% of dead plants *t*	Genotype (CNPH #)
0 – 10	148, 1393,2174,2171,2172,1397,2176, 1386,2175,2661
11-20	173,134,287,1387
21 – 40	961,989,992,2653, 1424, 974, 2652, 2658, 1379, 1374, 1376

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SHOOT REGENERATION FROM HYPOCOTYL SEGMENTS OF HOT PEPPER MEDIATED BY NON-DISARMED ISOLATES OF *AGROBACTERIUM*

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INTRODUCTION

The potential of utilization of genetic engineering *for* improving hot pepper cultivar has not been realized due to inability to regenerate shoots in vitro from various explants of this ~ crop. Shoot regeneration from hot pepper has been attempted "by various researchers around the world (1,2,4,5) since this step is required for regenerating transgenic hot pepper.
Although many have" reported some successes (1,2,4,5), their developed procedures were usually cultivar specific.

In this short communication we reported our attempt to regenerate shoot from local hot pepper cultivars originated from Indonesia. The procedures were developed by inoculating in vitro grown hypocotyl segments of hot pepper either with non-disarmed isolate of *Agrobacterium tumefacien* or A. *rhizogenes,* respectively.

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MATERIALS AND METHODS

Seeds of hot pepper cv. Hot Spiral, Jatilaba, Laris, and Tit Super were surface sterilized using solution of commercial bleach (20%) for 20 minutes. Subsequently, the seeds were rinsed three times with sterile water. Sterile seeds were soak overnight on sterile water to stimulate germination and transplanted on MS medium (Murashige and Skoog, 1962) without plant growth regulator (MSO). Two weeks old seedlings were harvested and their hypocotyls were isolated. The hypocotyls were cut approximately 1 cm below embryo axis, separated from their roots, and planted on MSO. In each; experiment, at least 100 independent hypocotyls were inoculated with the *Agrobacterium.*
A. *tumefacien* and A. *rhizogenes* were prepared by inoculating 1 m1 of LB medium with the respective isolate and by incubating the culture on a shaker (50 rpm). The cultures were incubated .at 28 C overnight. Single colony plates on solid LB medium were made from these overnight cultures and once growing, they were used to inoculate hot pepper hypocotyls. Inoculation of hypocotyl with the bacteria was done on the cut (apical) site. The hypocotyl cultures were incubated on culture *room* with illumination of 1500 lux *for* 24 hrs and temperature of 26 C day and night, respectively. Growth and development of inoculated hypocotyls were observed *for* a period of at least 3 months. ;

RESULTS AND DISCUSSION

The results showed hypocotyls cultured on MSO without *Agrobacterium* inoculation did not develop any further during the first three months in culture. On the other hand, inoculation with *Agrobacterium* on hypocotyls of hot pepper cultured on MSO resulted in - formation of callus from the infection site and from other part of the hypocotyl that was in contact with the *Agrobacterium.* From at least 100 inoculated hypocotyls, up to 32 to 74% of them formed callus (Table 1) while the rest either developed nothing or dead due to overwhelming growth of the *Agrobacterium.* Callus formation occurred between 5 to 14 days after inoculation (d.a.i.). Inoculation with A. *rhizogenes* as expected also resulted in the formation of roots from the inoculated site of the hypocotyls that occurred between 30 to 36 d.a.i.. The percentage of root formation was presented in Table 1. On the other hand, inoculation of the hypocotyls with A. *tumefacien* never resulted in formation of roots.

Shoot regeneration from hypocotyls of hot pepper was also obtained. Both A. *rhizogenes* and A. *tlimefacien* were able to induce direct shoot meristem regeneration from inoculated site of the hypocotyls. Initiation of shoot meristem was achieved from at least 0 to 8% (Table 1) of the total inoculated hypocotyls. Initiated shoot meristems were usually developed into single or double leaves that did not develop any further. To induce shoot elongation, the hypocotyls with the shoot initials have to be transferred onto MS medium containing 2 mg/l GA3. In this medium, the initial shoot meristems will elongate and develop into normal shoots (Figure 1).

In this experiment, we have shown the potential of using non-disarmed *Agrobacterium* to induce shoot regeneration from hypocotyl of hot pepper. Although shoot regeneration was stin low (less than 8%), the procedures was reliable enough for regenerating shoots from our local hot pepper varieties, especially since our attempt to utilize published procedures for regenerating shoot of hot pepper in vitro was unsuccessful. Subsequently we will use this strategy to develop genetic transformation procedure for hot pepper. We have introduced gene construct carrying PVY coat protein (PVY CP, see 4) into these non-disarmed A. *tumefaciens* and A. *rhizogenes,* respectively. Attempts to introduce PVY CP gene construct into hot pepper genome and regenerate transgenic hot pepper with resistance to PVY are still being conducted. Results from these attempts will be used as model to demonstrate the use of transgenic hot pepper with resistance to viruses. The results from these attempts win be presented elsewhere.

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Table 1. Development ofhypocotyl of hot pepper *(Capsicum annuum* L.) inoculated with *Agrobacterium tumefaciens* or *A. rhizogenes.* Hypocotyls were isolated from two-week-old seedlings. For each cultivar, 100 hypocotyls were inoculated with each isolate of *Agrobacterium,* respectively.

HotPepper cultivars	Strain of Agrobacterum	Percentages Callus	(%) Of explants Root	Forming Shoot
Hot Spiral	A Tumefaciens A.rhizogenes	34 37	- -	- -
Jatilaba	A Tumefaciens A.rhizogenes	32 37	- 1	- 1
Laris	A Tumefaciens A.rhizogenes	65 74	- 1	2 8
Tit Super	A Tumefaciens A.rhizogenes	40 55	- 2	1 -

Note: (-) indicated explants showed no response.

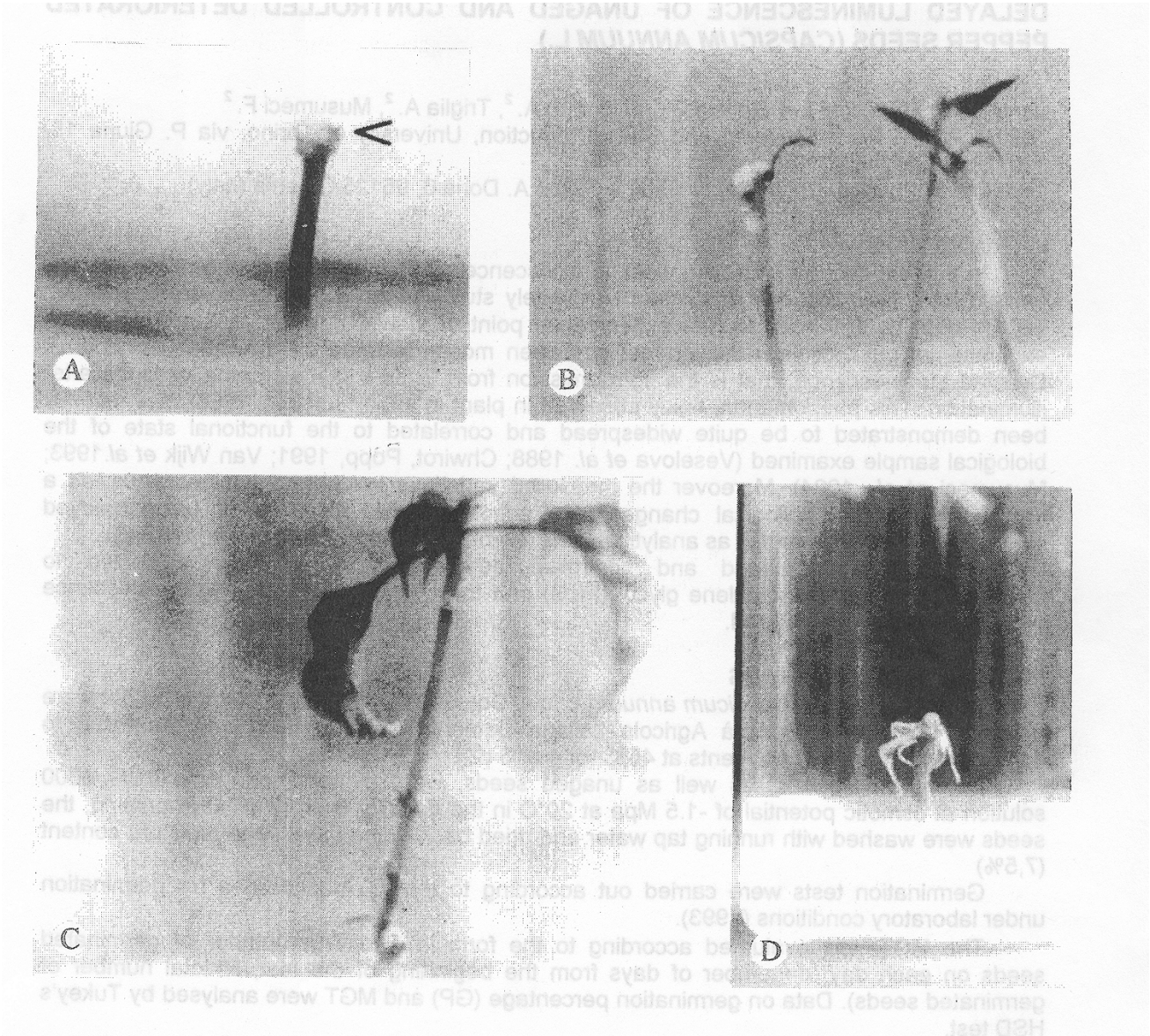

Fig 1. Development of hypocotyl of hot pepper cultures on MS medium without plant growth regulator (MOS) and inoculatd with non-disarmed isolate of Agrobacterium.(A) Hypocotyls of hot pepper cv. Laris forming shoot meristem (arrow), induced by A. rhizogenes, (B) Development of shoot meristem into leaves and (C) Elongation of shoot meristem in to normal shoot on medium containing GA#. (D) Hypocotyls of hot pepper cv. Jatilaba forming roots induced by A. rhizogenes**.**

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DELAYED LUMINESCENCE OF UNAGED AND CONTROLLED DETERIORATED PEPPER SEEDS *(CAPSICUM ANNUUM* L.)

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INTRODUCTION

In the last decades the ultra-weak luminescence, both spontaneous and photoinduced, emitted from biological systems, has been widely studied from the theoretical (Popp *et a/.,* 1988; Popp, Li, 1993) as well as experimental point of view (Triglia et ai, 1997). From an experimental point of view the interest has been mostly focused on the measurements of Delayed Luminescence, that is the light emission from living materials some seconds after illumination. This phenomenon, firstly observed in plant in 1951 (Jursinic, 1986), has recently been demonstrated to be quite widespread and correlated to the functional state of the biological sample examined (Veselova *et a/.* 1988; Chwirot, Popp, 1991; Van Wijk *et a/.1993;* Musumeci *et a/.,* 1994). Moreover the measured variations in delayed luminescence, as a function of specific biological changes, has suggested the possibility of using delayed luminescence measurements as analytical tests (Forbus *et a/.1985;* Scordino *et a/.* 1996).
In this study unaged and controlled deteriorated seeds were subjected to osmoconditioning in polyethylene glycol (PEG) and their variations in delayed luminescence emission have been analysed.

MATERIALS AND METHODS

Seeds of pepper *(Capsicum annuum* L. cv. 'Corno di Toro') from a commercial lot were obtained from SAIS (Societa Agricola Italiana Sementi, Cesena, Italy) and subjected to controlled deteriorated treatments at 45°C for 4 or 6 d.
Deteriorated seeds, as well as unaged seeds, were osmoprimed using PEG 6000 solution at osmotic potential of -1.5 Mpa at 20°C in the dark for 6 or 12 d. After priming, the seeds were washed with running tap water and dried back to the initial seed moisture content (7,5%)
Germination tests were carried out according to the 1ST A guidelines for germination under laboratory conditions (1993). The MGT was evaluated according to the formula 2 and/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.
The experimental set up, designed to measure the ultra-weak flow of photons emitted from biological systems, consisted of an aluminium dark chamber where the samples to be analysed were maintained at a constant temperature. The radiation emitted from the sample was detected by a low-noise photomultiplier (Thorn EMI type 9558 QA) working in single photon counting mode and having a spectral sensitivity ranging from 200 nm to 850 nm. In order to decrease the dark current the photomultiplier was cooled down to -20°C. In the standard experimental set up the solid angle of measurements was of the order of 0.1 sr.
Measurement consisted in illuminating the seed sample and counting the number of photons re-emitted from the sample after the light source had been switched off. Since the parameters describing the time decay could not be only connected to the state of the biological system, but also to the illumination duration, the illumination of the samples was performed using a flash lamp (METZ 45CL 1) in order to have short duration (about 3 ms). During the illumination a light shutter, above which the photomultiplier was fastened, was closed, in order

to prevent the dimpling of the photomultiplier. After the light source was switched off the shutter was opened by a pneumatic actuator. Due to this time lag of the experimental set-up, the photon counting started some tens of ms after the source was switched-off.

The counting of photons emitted by the sample, after each illumination, was stored by a channel scaler using a dwelling time chosen in order to measure the decay dynamics in the best way. In our operational mode each channel of scaler recorded the number of pulses counted in a 30 ms time interval. Every sample was made by about 3 g of seeds covering the bottom of a plastic Petri dish (diameter 6 cm). For each sample a series of five runs were performed, for which the average value was considered. A delay time ten times longer than the decay time was allowed, between two next illuminations, in order the emission from the biological sample came back to the unperturbed value. The background emission, due to the emission from the empty cuvette containing the sample, was measured in the same experimental conditions for each set of measurements and subtracted from the measurements taken from each sample.

In order to reduce the environmental influence and to avoid residual luminescence from . the materials, the biological samples were maintained in dark condition at least one hour before the measurements started. Due to the low level of the signal the spectral analysis of the emitted photons was performed by using broadband filters (40 nm FWHM, ANDOVER 110FA40) ranging from 400 nm to 750 nm.

Experimental raw data of DL emission were processed with a smoothing procedure in order to reduce random noise (Scordino *et a/.,* 1996) and data were fitted to the hyperbolic trend: I(t) = 1o *I* (t+to)m

Such a hyperbolic form has already been used in literature to describe the decay of delayed fluorescence (Jursinic, 1986) and the temporal decay of coherent systems (Popp *et a/.,* 1988). The parameters 10, to and m which characterize equation were calculated using a non-linear least-squares procedure (Musumeci *et a/.,* 1994).

Starting from the above-mentioned equation the parameter A_{tot} was evaluated as follows:

$$A_{tot} = (t).\ dt$$

with tin=0.120 sec, which corresponds to the first temporal channel of the acquisition systems. So defined the parameter Atot represents the total number of photon emitted starting from the initial acquisition time (at the end of the measurement the emission reached the background value).

RESULTS AND DISCUSSION

Data on the effect of controlled deteriorated and priming treatments on seed germination performance, together with the Atot values for different ageing and priming conditions are shown in Table 1.

Priming in PEG solutions sensibly decreased MGT of fresh seeds depending on its duration. In less deteriorated seeds (4d), osmoconditioning completely reversed the effect of ageing and even caused enhancement of MGT up to the level of unaged osmoprimed seeds. An analogous effect was observed in more deteriorated seeds (6 d ageing) but only after priming for the longest duration.

The A tot value of 6d aged seeds was higher than the one of untreated seeds, while the ageing treatment performed for 4d did not influenced it significantly. The recovery in MGT due to priming was correlated to decreased values of the Atot parameter.

Plot of total DL counts (i.e. Atot) against MGT is reported in Figure 4. In each seed sample a positive correlation between decrease in total DL count and decrease in MGT was observed.

In aged seeds priming performed for the longest duration affected seed viability by reducing the GP of about 10%. A negative effect of priming on seed viability of deteriorated seeds have been previously observed (Lanteri *et a/.,* 1996).

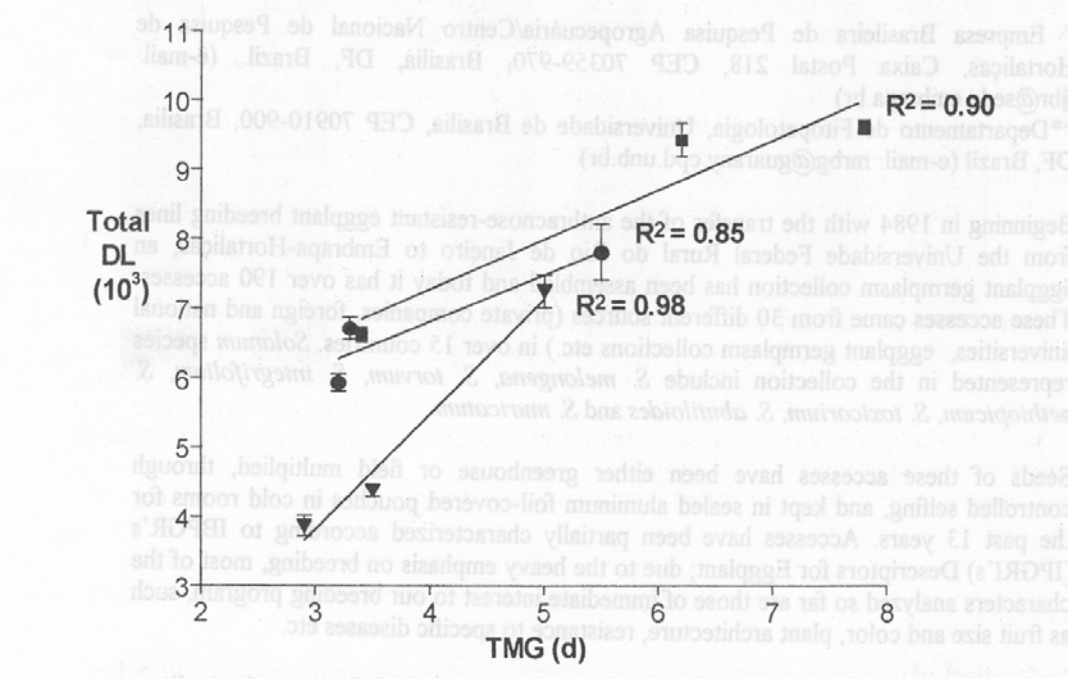
The results of DL measurements show that a correlation exists between the photoinduced delayed luminescence emitted from seeds and their germination performance,

as measured by the reduction in the mean time to germination. These results are in accordance with previous ones obtained on soya seeds (Musumeci *eta/.,* 1994).
A remarkable increase both in total DL and MGT was observed in 6d aged seeds, while the ageing treatment performed for the shortest duration (4d) did not significantly affect both parameters. In unaged as well as in 4 or 6d aged seeds a direct correlation was observed between seed reinvigoration due t9 priming and decrease in total DL emitted. Only in pepper seeds aged for the longest duration and then primed for 6d no decrease in total DL was observed, however in this seed sample only a limited improvement of the MGT was detected.
Interestingly, although the priming treatments performed for 12d exerted almost the same effect on MGT of unaged and aged pepper seeds, in the latter higher values of total DL were observed, S.nce in aged seeds the positive effect of 12d priming on MGT was accompanied by a decrease of about 10% in germination performance, the total DL emission seems to be a very sensitive parameter which correlates to the general physiological state of a seed lot.
The possibility of relating the total DL to characteristics of the germinative performance can thus provide a non-invasive and non-destructive technique in order to test seed vigour and viability. This can have a remarkable importance under an applicative point of view:
In addition, the definition of the correlation between the seed physiological state and delayed luminescence can provide both useful information about the mechanisms governing the DL phenomenon, correctly attributing the changes in the DL to specific biophysical and physiological events, and the possibility to take a deep look in the physical characteristics of the phenomenon and its role in living organisms.
In order to obtain this goal a wider methodical experimental investigation will be planned.

Table 1: Effect of controlled deterioration at 45°C for 4 and 6 days and osmopriming in PEG solution for 6 and 12d on germination percentage (GP), mean germination time (MGT), and total delayed luminescence (AtoV of pepper seeds. Within a column, means with the same letter are not significantly different (P<0.01: Tukey's HSD test)

Treatment	GP%	MGT(d)	A_{tot} (count)
Control	96.6+2.0a	5.0+0.3c	7254+236e
Ageing 4d	96.0+2.1	5.5+0.2c	7811+404e
Ageing 6d	92.0+2.5a	7.8+0.4e	9622+400f
Control +priming 6d	97.0+1.8a	3.5+0.2b	4385+90b
Control +rimng 12d	92.8+2.2a	2.9+0.1a	3866+141a
Ageing 4d+riming 6d	93.5+2.5a	3.3+0.2ab	6715+157d
Ageing 4d +priming 12d	83.0+2.8	3.2+0.2	5938+122c
Ageing 6d+ primng 6d	95.6+2.5	6.2+0.4d	9438+243e
Ageing 6d+priming 12d	85.5+2.4d	3.4+0.1b	6627+102d

Figure 4 : Relation between D.L. total emission and mean germination time of seeds unaged (") and aged for 4 (* or 6 (* days after priming treatments performed for 6 or 12 days. The data are means of five runs on the same sample, vertical bars on the symbols represent the standad error on the mean value



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Capsicum and Eggplant Newsletter. 16 (1997): 110-111.

EVALUATION, CHARACTERIZATION AND AVAILABILITY OF EGGPLANT GERMPLASM AT EMBRAPA,- HORTALICAS, BRAZIL

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Beginning in 1984 with the transfer of the anthracnose-resistant eggplant breeding lines . from the Universidade Federal Rural do Rio de Janeiro to Embrapa-Hortalilcas, an eggplant germplasm collection has been assembled and today it has over 190 accesses.These accesses came from 30 different sources (private companies, foreign and national universities, eggplant germplasm collections etc.) in over 15 countries. *Solanum* species represented in the collection include S. *melongena.* S. *torvum.* S. *integrifolium,* S. *aethiopicum,* S. *toxicarium,* S. *abutiloides* and S. *muricatum.*

Seeds of these accesses have been either greenhouse or field multiplied, through controlled selfing, and kept in sealed aluminum foil-covered pouches in cold rooms for the past 13 years. Accesses have been partially characterized according to IBPGR's (IPGRI' s) Descriptors for Eggplant; due to the heavy emphasis on breeding, most of the characters analyzed so far are those of immediate interest to our breeding program, such as fruit size and color, plant architecture, resistance to specific diseases etc.

Embrapa-Hortalilcas major interest lies on the development of disease-resistant lines, varieties and hybrids which could be released either as breeding material to public and private institutions or as finished commercial O.P. varieties and hybrids. The collection has been found to contain several accesses, which present resistance to one or more biotypes of some major pathogens. The complete collection has been screened for the identification of sources of resistance to anthracnose, caused by *Colletotrichum gloeosporioides* and bacterial wilt, caused by *Ralstonia solanacearum.* Sources of resistance (partial or complete) have been registered for the following pathogens: *Colletotrichum gloeosporioides, Fusarium* spp., *Phomopsis* sp., *Ralstoniq solanacearum (Pseudomonas solanacearum) and Verticillium dahliae.*

Embrapa-Hortalicas is in the process of re-multiplying and further characterizing its ~ accesses using circa 20 descriptors. Weare highly interested in further enhancing our collection through germplasm exchange and welcome interested individuals and institutions to contact us on this matter. Due to the importance of verticillium wilt to commercial eggplant production in Brazil, we are initiating a program to identify and incorporate resistance to this pathogen! albeit limited chances of success due to low levels of resistance identified so far by different research groups in Brazil and abroad.

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BACTERIAL WILT RESISTANCE SOURCES IN EGGPLANT, SOLANUM MELONGENA

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Introduction

Bacterial wilt, caused by *Ralstonia solanacearum,is* the major limiting factor of eggplant production in the tropics and subtropics. The pathogen is difficult to control because it is soilborne and has a wide host range. Planting resistant varieties is the most efficient method for disease control. Resistance sources to bacterial wilt have been identified and resistant breeding lines in eggplant have been developed mainly in India (Sadashiva et al., 1993). At A VRDC, germplasm of cultivated eggplant, *Solanum melongena,* has been collected worldwide. This report summarizes the results of the Center's screening program for bacterial wilt resistance in eggplant from 1992 to 1995.

Material and methods

Genetic materials included for screening came from three major sources: 1) commercial varieties from seed companies (47 varieties from 21 seed companies, mostly F I hybrids); 2) accessions from the A VRDC Genetic Resource and Seed Unit (GRSU) (more than 200 lines); and 3) accessions collected from India (95 lines). Varieties and accessions were initially evaluated for resistance t~ bacterial wilt at seedling stage in greenhouse trials. Greenhouse trials were conducted following a RCBD with three replications and 15 plants per entry in each replication. Plants were grown in 7.5 cm pots containing a steam-sterilized potting mixture (3:1:1:1 ratio of soil:rice hull: sand: compost). Seedlings were inoculated with bacterial strain Pss 97 (race 1, biovar 3) at 1 month after sowing. Roots were injured with. a knife by. Cutting through the soil 1 to 2 cm away from the collar, then 30 ml of bactenal suspension (10 cfu/ml) were poured Into each pot. After Inoculation, plants were kept In a greenhouse where temperatures ranged from 25 to 35 °c. Symptoms were rated once a week following the Winstead and Kelman scales and disease indices were calculated (Winstead and Kelman, 1952). Resistant entries were subsequently evaluated in additional greenhouse trials using the same methods to confirm their resistance

Accessions found to be tolerant or resistant to bacterial wilt during the greenhouse screening trials were further evaluated' in the field at A VRDC from 25 August to 26 October 1995. The design was a RCBD with three replications and 12 plants per entry in each replication. One-month-old seedlings were inoculated as described above and transplanted into the field three days after inoculation. Severity rating was performed as above.

Results and Discussions

Most commercial varieties tested were susceptible to bacterial wilt. Only two varieties, Slim Jim and M 701 F1, were resistant in greenhouse trials with disease indices of 3% and 7%, respectively. However, these varieties were found to be moderately resistant and moderately susceptible, respectively, in the field evaluation.

Twenty-six GRSU accessions showed high levels of bacterial wilt resistance in greenhouse trials. However, many accessions gave inconsistent disease reactions in repeat trials, and only six accessions showed consistent resistance with disease indices c; less than 10%. These were TS 3, TS 43, and TS 47A from Malaysia; and TS 69, TS 87, and TS 90 from Indonesia. Particularly TS 3, TS 43, TS 47 A, and TS 90, exhibited . excellent stable bacterial wilt resistance in all screening trials in 1994 and 1995.

India has a long history of breeding and selection of eggplant for bacterial wilt resistance. Eight accessions among the 95 accessions from India showed high levels of resistance both in greenhouse and field screening trials. The,se were Arka Nidhi, Arka Keshav, ArkaNeelkantha, BB-1, BB-44, BB-49, EP 49, and Surya.

It has been reported that bacterial wilt resistance in tomato can be unstable over locations (Hanson et al., 1996), and that stability levels vary among resistant lines of tomato (Wang et aI, 1996). It is not known how stable the bacterial wilt resistance in eggplant selected by a single virulent strain will be in other locations. Therefore, we have selected 17 bacterial wilt resistant accessions (Table 1) for international distribution and evaluation. Anyone interested in collaborating to evaluate these entries in multi location trials can contact the authors for seeds.

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Table 1. Reaction of 18 eggplant accession inoculated with Ralstonia solanacearum strain Pss 97

Acc.	Variety	Source	Description		Disease Index%b		Disease Reaction c	
Code			Color a	Chapea	GHd	Field	GHd	Field
TS3		Malaysia	G	O	5	3	R	R
TS7	MTE 2	Malaysia	P	CM	8	11	R	MR
TS47A		Malaysia	G	CL	2	5	R	R
TS 56B	Terong Hijan	Indonesia	GW	O	16	8	MR	R
TS 64	Jackpot	Netherlands	P	CM	12	10	MR	MR
TS 69	Gelatik	Indonesia	G	O	1	7	R	R
TS75		Thailand	G	CL	10	18	Mr	MR
TS 87	Glatik	Indonesia	GW	O	4	9	R	R
TS 90		Indonesia	G	O	2	2	R	R
EG014	Slim Jim	Italy	P	CS	7	11	R	MR
EG190	SM6-6	India	W	CS	2	7	R	R
EG191	Arka Keshav	India	RB	CL	3	5	R	R
EG 192	Arka Shirish	India	P	CS	35	4	MS	R
EG 193	Arka Nidhi	Indai	RB	CM	5	2	R	R
EG 195	BB49	India	GW	TD	4	9	R	R
EG203	Surya	India	DP	O	0	7	R	R
EG219	BB44	India	GW	CS	4	9	R	R
EG 120	Bonne	Taiwan	P	CL	92	100	S	S

Color: DP as dark purple, G as green, GW as green with white stripes, P as purple, RB as redish brown and W as white ; Shape : Cl as cylindrical long, CM as cylindricall medium long, CS as cylindrical short, O as oval and TD as teardrop

Dissease index= [(□Ni xI)/(Nx5}x 100%, where Ni=no. of plants with I scale, I=0-5, N= total plants tested; Symtons of each scale are :0=no symptom, 1=one leaf wilted, 2=two or thress leaves wilted, 3=all except the top two or three leaves wilted, 4= all leaves wilted, 3=all except the top two or three leaves wilted, 4=all leaves wiled and 5= plant dead.

Disease reaction R:R= DI% less than 10% MR= 10-20%; mS= 21-40%; S=more thatn 40%

TS and EG series were screened at seedling stage in different greenhouse trials.

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SCREENING FOR BACTERJAL WILT RESISTANCE IN BRINJAL

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Introduction:

Eggplant (Solanum melongena L.) is an important fruit vegetable cultivated throughout the tropics and as a summer annual in warm subtropics. One of the important factors limiting brinjal cultivation in the tropics is the incidence of bacterial wilt caused by Pseudomonas solanacearum. The use of resistant varieties is the cheapest and most effective both method of combating bacterial wilt and hence attempts have been made to evolve resistant varieties. For this task for screening for the BW reistance has to be carried out. 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 alsb, Mondal et al (1991) Sadashiva et al (1993) Sadashiva et al 1994 and Furgo et al (1994).

MATERIALS AND METHODS

The screening for resistance in 95 accessions collected from India was done in three batches. The 3 sowing dates were 26 April, 14 June, and 12 August 1994; and the 3 inoculating dates were 24 May, 13 July, and 13 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 screened in pathology growth room along with checks. The sowing oates were 9/27/94, 10/28/94 and inoculating dates were 10/27/94 and 11/28/94.

Seeds were sown in 3-inch plastic,in the greenhouse. The experiment design was RCBD with three replications. Each replication contained 15 plants (pots).

Bacterial wilt inoculum PSS 97 was obtained from the plant pathology unit. B W screening was done by soil drenching method to supplement root cutting. When seedlings were 30 days old, each pot drenched with 30 ml inoculum m adjusted to a concentration of 108 bacteria cells/ml right after the root wounding. After inoculation, soil moisture \4BS maintained at a high level and temperature was maintained at 25-32°C in the greenhouse. In growth room studies temperature was maintained at 28°C.

The inoculated plants were observed daily, and records were kept of the date of appearance of wilt symptom and of plant death. Disease reading, based on the scale of 0-5 (0= no Symptom 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-day intervals and converted to disease indices. The experiment terminated 30 days after inoculation (DAI) and Data were analyzed statistically.

At the end of 28 DAI in accessions with 80-100% plant survival, 15 plants were cut at the base of the stern and any discoloration of the vascular tissues was noted. Plants that were discolored was scored as positive.

The remaining plant stems from the above tests were used for selection media test. A stem piece weighing about 1 g was cut from the base of each remaining stem, was washed with tap water, and dried. Each piece was then surface sterilized with 70 % alcohol and put separately into glass tubes containing 5 ml steril water. Samples was incubated at 18°C for 16 h and the suspensions will be streaked out on triphenyl tetrazolium chloride (TTC) medium plates. After incubating at 30°C for 2 days, samples with plates showing typical P. solanacearum colonies was score, as positive.

Table 1: The disease indices and percentage of non-symptom plants of eggplants (Solanum melongena) after inoculated with - Pseudomonas solanacearum PSS97 for 3

ACC. NO.	VARIETY	DISEASE INDEX %	% OF NON-SYMPTOMED PLANTS	BACTERIAL WITH REACTION
1.	Arka Nidhi	0	100	R
2.	Arka Keshav	0	100	R
4.	Arka Neelkantha	0	100	R
6.	BB-1	0	100	R
7.	BB-7	18	82	MR
8.	BB-49	0	100	R
9.	EP47-Annamalai	0	100	R
16.	Local - 1	1	99	R
21.	SM6 - 6	2	98	R
41.	EP143	18	92	MR
68.	EP98	16	84	MR
141.	Surya	0	100	R
11.	BB44	0	100	R
10.	BB13-1	10	90	R
7.	BB2	9	91	R
SI20	Bonne (Sus. ck)	97.3	3	S
TS56B	TS56B (res. ck)	4	96.3	R

RESUL TS AND DISCUSSION ,

Uhder Among the 95 accessions collected in three batches
from India"the greenhouse screening, the results revealed that 12 accessions
exhibited i high level of resistance. Eight accessions showed no wilt symptoms. They were Arka Nidhi (0), Arka Keshav (OJ, Arka Neel Kantha
(0), BB-l(O), BB44(0) , BB49(0), ~EP143(18) and Surya(O). The remaining four were BB 13-1(10), BB-2(7), K.Local 1(1) and SM 6-6(2).

There are some moderate resistant lines viz BB-7(18), EP58(29) EP143(18) , EP98(16). These accessions were also tested for Ooze test, selective media, vascular discoloration, and their Disease index Area under disease progress curve and their BWR was confirmed.
Table 2 Table Detection of Latent disease indices, AVDPC and BWR of Selected

ENTRIES	OOZE TEST	SELECTIVE MEDIA	VASCULAR DISCOLOR ATION	DI	AUDPC	BWR
Arka Nidhi	-	-	+(1/15)	4.8	45.1	R
Arka Kesha	-	+(1/15)	-	2.89	16.88	R
Arka Neelkantha	-	+(1/15)	-	4.36	81.94	R
BB-1	-	-	+(2/15)	10.19	126.76	MR
BB49	-	-	-	4.33	95.63	R
Annamalai	-	-	-	9.2	166.45	R
Kerala Local 1	-	+(4/15)	-	2.66	97.3	R
SM6-6	-	+(1/15)	-	1.33	1.33	R
BB-7	+(2/15)	+(6/15)	+(3/15)	19.40	79.6	MR
EP143	-	+(8/15)	-	20.0	89.25	MR
EP98	+(4/15)	+(10/15)	+(2/15)	20.78	79.0	MS
Surya	-	-(0/15)	-	0.0	0.0	R
BB44	-	+(1/15)	-	2.10	119.55	R
BB13-1	-	+(6/15)	-	10.75	89.25	MR
BB-2	+(1/15)	+(4/15)	-	12.6	194.9	MR
TS56(B)	-	+(1.15)	-	10.62	112.3	MR
Bonne	NT	NT	+(6/9)	97.6	14527	S

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BREEDING FOR BACTERIAL-WILT RESISTANCE OF EGGPLANT IN BRAZIL

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Bacterial wilt (BW), caused by *Ralstonia solanacearnm,* is a major solanaceous disease in Brazil. For eggplants, BW is particularly destructive under high humidity and high temperature, like in lowland regions in the southeastern states as well as in the - Northeast Region. According to a regional survey, this crop has been infected by biovars I and III, and eventually by biovar 11- T (Lopes, 1992)

Because eggplant is grown mainly by small farmers with little land to allow proper crop rotation, control of BW will be more effective if resistant cultivars are available to be associated with disease-reducing crop management measures, such as soil amendment and proper irrigation. Nowadays, all cultivars found in the Brazilian seed market are very susceptible to BW. A screening methodology developed by Morgado *et al.* (1994) allowed the identification of four resistant genotypes in the eggplant germplasm collection, of the National Research Centre for Vegetable Crops (EMBRAPA-Hortali~as): 'P12', 'PIS', 'Nantou Nasu' and 'Dingaras Multiple Purple', CNPH 171, CNPH 175, CNPH 407 and CNPH 40S, respectively (Morgado *et al.,* 1992).

These genotypes were re-evaluated in greenhouse in order to establish if their resistance is strain or biovar-dependent, which would partly explain different plant reaction to the disease in different regions. The four resistant genotypes and two susceptible controls (line CNPH 006 and cv Florida Market) were challenged with 16 strains of biovars I, 11- T and III of *R. solmlacearnm* isolated from solanaceous plants in different locations. The experimenta) design was a completely randomized two-way factorial (genotypes and bacterial strains) with three replications of eight plants in two pots. Seedlings were inoculated at the two/three-true leaf stage by dipping the roots, which had been washed in tap water and severed at one-third from the lower extremity, for 1 min into the bacterial suspension of c.a, 108ufc/ml. Inoculated seedllings were then transplanted to sterile soil in 0.5-L plastic pots and disease individually scored 21 days after inoculation according to a scale from 1 (no symptoms) to 5 (dead plant).

Analysis of variance for disease severity revealed significant differences ($P<O.05$) among genotypes, bacterial strains and the interaction between genotypes and strains. No specific resistance to any of the biovars among eggplant genotypes were observed, as indicated by cluster analysis. Considering the performance of all genotypes for each - strain, 'CNPH 171' showed significant lowers disease severity than the susceptible controls for all strains, being then considered the best source of resistance in our germplasm collection. The same results have been observed when plantlets at the 2-leaf stage were wound inoculated by puncturing the stem with an entomological needle through a 10 III drop of bacterial suspension of c a. 108ufc/ml. This last inoculation method has been preferred since: (a) it is a quick method allowing screening of a large

number of plants in shorter time (b) it is more reliable resulting in fewer escapes, and (c) disease can be recorded sooner, 7-10 days after inoculation, comparing to 15-20 days necessary for root inoculation. Regarding to virulence variability among bacterial strains, RS 54 was chosen for future selection tests, since it showed such a virulence level to allow a clear differentiation between susceptible and resistant genotypes.

The Inbred Line System is currently being used at EMBRAP A-Horta1icas for introgression the BW resistance into commercial varieties. New sources of resistance are being sought in the germplasm collection.

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ANNOUNCEMENT

PRE-ANNOUNCEMENT OF THE Xth EUCARPIA MEETING ON GENETICS AND BREEDING OF CAPSICUM AND EGGPLANT.

- The Xth Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant will be held in France from the 7th to the 11th September 1998.

- All communications and posters dealing with Genetics, Breeding, Genetic Resources, Disease Resistance, Cellular and Molecular Biology, Quality and Biochemistry of Pepper and Eggplant will be welcome.

- Organisation will be performed by INRA-Avignon, with: A. Palloix, A.M. Daubeze, M.C. Daunay, V. Lefebvre, c.. Trousse, , Vegetable Genetics and Breeding Station, INRA BP94, 84143 Montfavet Cedex, FR.
- An official announcement will be sent to the earlier meeting participants. But new participants as well as regular ones can sent their addresses and all requests through E-mail to : capsicum@avignon.inra.fr

NEW BOOKS

Dave DeWitt & Paul W. Bosland, 1996 - Pepper of the world: an identification guide - Ten Speed Press, Berkeley, California, U.S.A. -ISBN 0-89815-840-0

Did you know that the total number of world varieties of pepper is between two and three thousand? Even this estimate is highly speculative, considering the propensity of the *Capsicum* genus to cross-pollinate.

315 of these varieties have been collected, grown, photographed and finally, described by Dave DeWitt and Paul Bosland through four years of work: the result is a guide in which every varieties is showed together with the following data: USDA number, Z botanical name, common name, location, seed source, pod length, pod width, immature color, mature color, comments.

The varieties have been joined in chapters according to the species: undomesticated *Capsicum* (The wild species), C. *frutescens* (Tabascos ana: more), C. *pubescens* (Rocotos and Canarios), C. *baccatum* (Los Ajis of South America), C. *chinense* (The hottest of them all) and C. *annuum* (A plethora of peppers). A further chapter deals with identification and breeding of peppers.

The authors of the book can be reached at P.D.Box 4980, Albuquerque, NM 87196, U.S.A., while the address of the Ten Speed Press is P.D.B. 7123, Berkeley, CA 94707, U.S.A.

RECIPES

Terry Berke (Asian Vegetable Research and Development Center, Tainan, Taiwan, Republic of China) sent us some recipes, in which pepper or its derivates are used. We are pleased to share these recipes with our readers, hoping that you find them interesting. Thus, we do not have to just breed pepper, but can also enjoy the results of our work! -

Olive Salsa (from October 1996 Chile Pepper Magazine)

- 1 cup pitted black olives;
- 1 cup grated mozzarela 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.*

Xnipec (pronounced schnee-peck, it means dog's nose) from "The Habanero Cookbook" by Dave DeWitt and Nancy Gerlach.

- . 2 habaneros, seeds and stems removed, chopped; .
- 2 tomatoes, chopped;
- . 1 purple onion, chopped;
- . 1/3 cup lime juice;
- . 3 Tb. cilantro *(Coriandrum cilantrum)* , chopped.

Combine ingredients in a *bowl and mix well, allow to sit for* 2 *hours to blend the flavors.* So *hot it will make your nose run and you will howl like* a *dog (this is how it got its name).*

- Chilli Fruit Sundae (from The Habanero Cookbook by Dave DeWitt and Nancy Gerlach)
- . 3 Tb. vinegar; ,
- . 2 Tb. sugar;
- . 1/2 Tsp. dried crushed chilli (red) pepper; .
- 1 cup cubed watermelon; .
- 1 cup cubed pineapple;
- . 1 cup sliced fresh strawberries.

In a *saucepan, combine the vinegar and sugar and heat until the sugar dissolves. Stir in the chilli pepper and let cool. Place the fruits in* a *bowl and pour the cooled vinegar mixture over them. Cover and chill until serving. (it will warm you and cool you at the same time).*

Ole Hawg's Breath Rub (from the July/August issue of Chile Pepper magazine) :

- . 3 Tb. sugar
- . 3 Tp.black pepper
- . 3Tb.paprika
- . 3 T!b. dry barbecue seasoning;
- . 1 Tsp, garlic powder;
- . 1 Tsp, ground chilli pepper
- . 1 Tsp, ground cinnamon
- 1/2 Tsp, ground nutmeg.

Rub liberally on your favorite cut of meat before roasting or grilling for that real Hawg's Breath flavor.

- Dr. Pepper's Perky Piquant Pungent Pickled Peppers
- . 1 pound jalapeno chopped
- . 6 garlic cloves, minced 1/2 pound carrots, chopped
- . 1/2 pound onions, chopped '
- 1 Tb. black pepper
- .2Tb. oregano
- . 1 Tsp. thyme
- . 5 bay leaves
- . 2 Tb. salt
- . 2 quarts vinegar
- . 1/2 cup vegetable oil

Bring vinegar to a boil. Add spices and oil, Pour over chopped vegetables. Let. cool and refrigerate.

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