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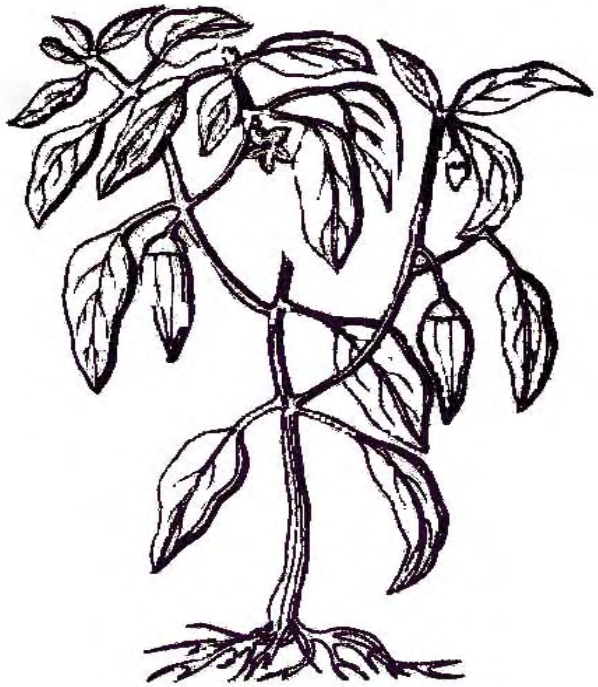

University of Turin
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Plant Breeding And Seed Production

No. 12 1993

CAPSICUM & EGGPLANT NEWSLETTER

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DI.VA.P.R.A.

Plant Breeding and Seed Production

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CONTENTS

FOREWORD

As you have probably noticed, according to the decision taken during the EUCARPIA Meeting of Rome (September 1992), our Newsletter has changed both the title and its look. In fact, we believed that the old cover seemed a little old-fashioned (after ten years of honourable service ...) and decided to add eggplant to the title of the journal, since for a long time we have been including also articles on this Solanaceous plant.

Another important change is the enlargement of the space available for each contribution: since many Authors complained that two pages were too little, now you have at your disposal four pages per article (tables, graphs and literature included).

This issue includes three invited papers. Two of them deal with the preservation of pepper and eggplant germplasm and the breeding for quality in Capsicum. They have been written respectively by Liwayway M. Engle and Paul W. Bosland: we thank them very much for their kind co-operation. The third invited paper inaugurates a new survey: the situation of pepper and eggplant breeding in different countries. The first report has been writ-Len by our Referee Ramiro Gil Ortega and concerns Spain. We will appreciate any ideas on countries to be considered in the next issues and people to contact for writing the articles.

Please, remember that a subscription fee to the Newsletter will be very appreciated. The fees have not been changed and are still U.S.$ 20 for normal subscribers and U.S.$ 100 for supporters. Remember also that now it is possible to book your own copy of the journal: just fill in the order form you will find on page 103 and send it to us. In the meantime your subscription fee should be paid directly to EUCARPIA Secretariat (please note that the address has been again modified: the new one is P.O.Box 315, 6700 AH Wageningen, The Netherlands). We call the attention of our subscribers to the possibility to pay the fee by

American Express, Diners Club, Eurocard and Visa. In this case the fee must be paid in Dutch currency (Dfl. 35 for normal and Dfl. 175 for supporter subscription). People who decide to pay with their credit card have to send their card number, the expiring date, the issuing bank and their signature to the EUCARPIA Secretariat (you can use the voucher on page 105). We prefer this way of paying because of the high bank charges for each payment coming from abroad. Please, definitely do not send cheques to us in Turin, as we are not allowed by Italian law to run any financial activities.

As usual, we have not modified any of the accepted and published papers: therefore, the Authors themselves are responsible for both the scientific content and the form of their own reports.

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

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

Lastly, we are happy to announce that, starting from the present issue, co-operation between our Newsletter and the Food and Agriculture Organization (FAO) has started. We hope that in this way the journal's circulation will become more wide-spread.

We remind you that the deadline for the submission of articles to be included in the next issue of the Newsletter (No. 13, 1994) is February 28, 1994.

Piero Belletti and Luciana Quagliotti

Turin, 31st May 1993

LIST OF THE AUTHORS

LIST OF THE CONTRIBUTIONS

Capsicum and Eggplant Newsletter, 12 (1993). 13-24. Invited paper.

THE PRESERVATION OF PEPPER AND EGGPLANT GERMPLASM

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Introduction

Pepper and eggplant are two of the principal crops of the Asian Vegetable Research and Development Center (AVRDC). The inclusion of these two crops among the principal crops of the Center was partly based on the crops' popularity in the tropics, nutritional value, adaptability to the hot wet tropics, potential to increase farmer's income, and adaptability into the existing cropping systems. To support research activities on these two crops, AVRDC maintains a germplasm collection both for distribution and conservation. As of the January 1993, there were 5,959 accessions of Capsicum consisting of nine species and 194 accessions of the Center's new principal crop Solanum melongena and related species. The Center has been designated by the International Board for Plant Genetic Resources (IBPGR) to store the duplicate global base collection of peppers. The other global base collection is at CATIE, Costa Rica. An active collection, for distribution purposes, complements about a third of the base collection at AVRDC. These materials are available in limited quantities for germplasm exchange. Requests should be accompanied by the proper import permit so that seed treatment can be applied, if necessary, and a phytosanitary certificate issued.

The preservation of assembled germplasm is the core of genetic conservation (Chang, 1985). For species with orthodox seeds, like pepper and eggplant, seed preservation is the most efficient means of maintaining large numbers of accessions and making them available for distribution.

The goal in seed preservation is to maintain genetic integrity of the population and to maximize the longevity of the stored seeds at minimal cost. Longevity is affected by two factors: the status of the seeds before entry into storage and the conditions surrounding the seeds during storage. This paper will discuss these two factors along with other considerations in the conservation of germplasm. Many of the factors discussed are general to plant genetic conservation. However, where special procedures apply to pepper and eggplant, these are presented.

Quantity of seeds to produce

New introductions frequently arrive with insufficient quantity of seeds to be directly stored for preservation. This is especially true of peppers collected from home gardens and wild populations where only a few mature fruits can be collected (Pickersgill, 1986). At least one cycle of seed multiplication is needed to produce sufficient and viable seeds for preservation and distribution.

It is best to produce large quantities of seed sufficient for preservation and distribution in as few cycles as possible. Frequent regeneration of seeds is not only costly but may also result to questionable genetic fidelity (Breese, 1989) possibly due to mechanical errors, genetic drift when the sample size is small as well as genetic shifts caused by loss of unadapted genotypes.

For genetically uniform material, IBPGR recommends storage of 4,000 seeds, with a minimum of 3,000 (Hanson, 1985). The recommended amount for heterogeneous material is 12,000 with 4,000 as minimum. At AVRDC, the practice is to produce at least 20,000 seeds per accession of pepper and eggplant. This quantity is divided into two groups: 12,000 for the base collection under long-term storage condition and the remainder for the active collection under medium-term preservation. To produce the target amount of seeds, 30 plants of each accession need to be established if the accession is uniform.

Preserving genetic structure

The seed increase operation should consider the need to preserve the genetic structure of the original population.

All the domesticated peppers are self-compatible. Predominant self-pollination ensures that cultivars and landraces usually appear quite uniform (Pickersgill, 1986). However, varying degrees of natural cross-pollination has been known to occur. Quagliotti (1979) in a survey of literature reported 1 to 68% natural cross-pollination. Tanksley (1984) reported a high of 91%. The degree of cross-pollination is influenced by distance of plants, wind direction and insect activity with the last contributing the most to crosspollination (Murthy and Murthy, 1962; Francheschetti, 1972). Eggplant is also a predominantly self-pollinated crop but cross-pollination had been reported from 0.2 to 47% (Quagliotti, 1979) with insects playing a major role. Selfing can be greatly increased by hand pollination. To preserve the genetic integrity of each population, cross pollination between different accessions should be prevented. At AVRDC, peppers and eggplants are multiplied under insect-proof nylon net cages to prevent the entry of insects that may cause cross pollination. A row between plots is left vacant to separate accessions. Furthermore, plants are staked and pruned if necessary to prevent intermingling of branches. These procedures also minimize the possibility of mixing fruits harvested from adjacent plots.

The use of net cages however has posed several problems. Aside from being expensive and laborious to construct, the shading that results affects the growth of the plants and may distort characterization data. The environment inside the net cage is also so favorable to mites that severe infestation is frequent and often difficult to control even with repeated spraying of miticides. On the other hand, growing in net cages results in the exclusion of insects that can cause damage to

the fruits, e.g. fruit borer that would cause reduced seed yields as well as reduced seed vigor (Krishnasamy, 1990).

Several workers reported that peppers normally show a great amount of phenotypic variation and although some accessions may look uniform based on morpho-agronomic traits, they may carry hidden heterogeneity, evidenced by heteromorphy for chromosomal markers (Quagliotti et al., 1972; Pickersgill, 1986). Our data show that out of 2118 accessions characterized 60% can be considered as homogeneous and only 6% as highly heterogeneous.

For a heterogeneous population the number of plants used in seed increase should be sufficient to preserve its genetic composition. In cases where a sample showing distinct morphologic variants, e.g., red versus yellow mature fruits, is separated into two accessions a second planting may be necessary to meet the required amount of seeds. An inherently heterogeneous population is harvested as a bulk.

Systematic characterization using morpho-agronomic characteristics may begin with the first seed increase. Such characterization data not only provides information on the potential utility of the accession but serves as identifying information that can be used to check on the correct identity of the accession during regeneration.

Processing and handling seeds for preservation

The aim of preservation is maintenance of high seed viability. High viability not only helps maximize the possibility of an adequate number of healthy, vigorous seedlings being established, but also minimizes the risk of genetic damage that have been observed in several instances (Ellis, 1991). Many national genebanks consider loss of seed viability during storage one of their most serious problems, even though some of them have good storage facilities. It appears that improper pre storage processing may be the cause of such rapid loss of viability (Engle and Chang, 1991).

Precautions during processing and handling are not only meant to maintain high viability but also to avoid mechanical mixtures.

Harvesting. Harvested fruits should be transported and dried in net bags to minimize accidental mixing of different accessions. Each accession should be processed separately from the others.

One of the factors that affect longevity of stored seeds is the quality at harvest. Seeds should therefore be harvested at stage of highest quality. In both pepper and eggplant, better quality seed is obtained from a slightly advanced harvest than from a delayed harvest. For 'Tabasco' type pepper (Capsicum frutescens), germination was greater and faster in red fruits harvested early than in

15

immature orange fruits or those harvested post red mature in the growing season (Edwards and Sundstrom, 1986). Some seed producers leave the fruit on the plant until the abscission layer just behind the calyx is fully developed. However, over-ripe fruits should be avoided. In domesticated peppers which lack dormancy, the seeds may germinate within the moist interior of the fruit while it is still on the mother plant. Such pregerminated seeds will be inviable when dried for storage (Pickersgill, 1986).

Recently, experiments by Demir and Ellis (1992) contradict the widely accepted hypothesis of Harrington (1972) that a seed attains its highest quality (i.e., viability and vigor and therefore storability) at physiological maturity and that deterioration as a result of aging starts thereafter. Their experiments showed that in C. annuum maximum potential longevity was achieved 63 to 65 days after anthesis. This is 10 to 12 days after mass maturity that they defined as the end of the seed-filling phase. Similar results in tomato led them to conclude that delayed harvesting results in little or no reduction in seed quality in those seed crops in which seed m.c. is maintained naturally within fruits at values approaching those of fully imbibed seeds.

Seeds for preservation should be free from injury, surface infestation by microbes, or insect infestation. In addition, they should be harvested from plants that have not suffered stress due to water deficit, water excess, or nutritional deficiency in the field.

Seed-borne mycoflora has been described in several publications. Among seedborne mycoflora, Fusarium moniliforme caused 6 to 8% loss in germination of pepper seeds (Hashmi, 1989). Pepper seed infected with Colletotrichum spp. often has poor germination or viability (Grover and Bansal, 1970).

Leaving the seeds in the fruit for a month after harvesting before removing them greatly increases the germination percentage in pepper although 1000 seed weight remains practically unchanged. The minimum post-harvest ripening time that can be considered effective is 20 days. On the other hand, it is worth prolonging the post-harvest ripening period to 30 days (Quagliotti, 1977). In 'Tabasco' pepper optimum post-ripening occurred after 21 days at 250C (Edwards and Sundstrom, 1986).

In eggplant, the germinability of seed occurs 40 days post anthesis on the plant and increased germinability can be observed after 20 days post harvest ripening at room temperature applied to the fruit harvested 40 days after anthesis. When 100 seeds fresh and dry weight approach maximum values, germination rises abruptly from below 10% to above 80% within a very short period (Osman, 1989).

Seed extraction. For large-fruited and low yielding accessions of pepper the fruits are usually split open with a knife and the seeds extracted manually.

Hot peppers cause a big problem during extraction. Capsaicinoids can irritate the hands of those who do the extraction manually and indirectly the eyes, nose, and other sensitive tissues of the body. Dried fruits when processed produce highly irritating fine particles. Even fresh fruits in large quantities release fumes that irritate the lining of the nose and cause watery eyes. Many workers find it uncomfortable to wear gloves and masks when extracting seeds. They prefer to work outside the building or to use fans that can minimize the discomfort caused by the strong fumes. For sensitive hands, we have tried dipping hands in starch, milk and soapy water after work. Starch seems to alleviate the discomfort. A mild solution of Chlorox has also been suggested (Poulos, personal communication) but is also harsh on sensitive skin. Doing the extraction inside table top cabinets with fume absorbers may help.

For small-fruited and high yielding accessions of pepper, a meat grinder can be used to cut the fruits into small pieces and the seeds collected by water extraction (Tay, 1989). Alternatively, the fruits inside the net bags can be threshed in water by trampling. The seeds are then washed clean of the accompanying pulp. During washing, underdeveloped seeds usually float and can be removed.

Eggplant fruits can be softened by beating or rolling for easier extraction. Seeds can then be manually separated from the pulp and washed dean with water.

At every step, care should be exercised to minimize deterioration and mechanical damage to the seeds or mixtures.

Drying seeds. Seed moisture content (m.c.) is one of the principal factors in determining seed longevity. Lowering the moisture content of seeds result in improved seed longevity. For example, for many crops when m.c. is decreased from twelve to five percent, the seed storage life is increased 127 times (Zhang and Tao, 1989).

The relationship between longevity and m.c. is logarithmic i.e., the benefit to longevity of one per cent reduction in m.c. increases as m.c. is lowered., However, once the lower critical m.c. is reached, a change in m.c. has very little effect on longevity. There is therefore a practical limit to desiccation for seed storage. For a wide range of species the critical values of seed moisture are close to those in equilibrium with 10% RH (Ellis et al., 1989). Equilibrating seeds against RH of 25% or lower greatly extends seed longevity, even at ambient temperature (Ellis et al., 1989, 1990; Vertucci and Roos, 1990). Equilibrium m.c. for eggplant at 250C is 6.3% at 30%RH and 11.9% at 75%RH (Hanson, 1985).

As a rule of thumb, for every one percent increase in seed m.c., the life of the seed is halved. This rule applies to a range between five and 14% (Harrington, 1972).

For long-term preservation seeds must be dried down to 5% ± 1 m.c., wet basis (Cromarty et al., 1985). More recently drying to less than three percent is recommended for some oily seeds (Ellis et al., 1990). It is not known if such ultra dried condition would provide added benefit to the preservation of pepper or eggplant seeds.

Drying can be achieved by use of heat, dehumidifier or desiccant.

The use of heat at 35 to 450C, however, could result in seed deterioration, particularly for certain vegetable seeds (Tao, 1988). IBPGR recommends that seeds are best dried in a drying room maintained at 150C and 10 to 15 %RH with good circulation. At AVRDC, this condition is attained with the use of a sorption type air dehumidifier with refrigeration to lower the temperature and remove heat generated by the air dehumidifier. The pepper and eggplant seeds in net bags are placed in open trays inside the drying room for two weeks to dry down to at least 8% m.c. Pepper and eggplant are among the species whose seed m.c. should be determined by modified low constant temperature oven method (Hanson, 1985).

Another method is to use silica gel for drying. Seeds are placed with silica gel in a closed container. The seeds are kept in porous bags to separate them from the silica gel. Depending on species, seed m.c. can be lowered to three to seven percent within a month. A 1:1 seed to silica gel ratio (by weight) is generally used (Zhang and Tao, 1989).

Freeze-drying has been reported as another method to lower seed moisture to the two to five percent range by avoiding potential damage due to heat and over desiccation. Seed storability at warm temperatures may be improved by this technique (Woodstock et al., 1983).

Emphasis on lowering seed m.c. to below 10% and the availability of free solar energy has prompted many to sun-dry or to use mechanical drying temperatures that exceed 400C (Engle and Chang, 1991). This could lower longevity of seeds. Experience at AVRDC reveals that temperature of cement floors exposed to the sun can attain temperatures of 420C even on a cool-dry day (min 14.60C, 48%RH, max 250C, 98%RH ambient conditions). Such temperature can have deleterious effect on the viability of the seeds during storage.

Packaging. Packaging is done to keep each accession separate, to prevent contamination of the seeds from insects and diseases and to minimize absorption of water by the dried seeds. Most seeds are hygroscopic, and under refrigerated conditions, RH may fluctuate.

In practice, three types of packaging are used for long-term preservation: glass, metal and aluminum foil laminates. Theoretically, any material that is

impermeable to water vapor (hermetic type) is suitable for packaging. They prevent absorption by the seeds of water vapor from the atmosphere after drying.

We find the use of aluminum polyethylene envelopes (AL-PE) convenient for both the base and active collection. Seeds of pepper and eggplants for distribution are packed in AL-PE envelopes in 50-seed quantities. This allows the retrieval of a specified amount of seeds of each accession without opening and closing containers. It also provides protection from absorption of moisture by the seeds due to inadequate dehumidification of the cold store and during transport.

Storage conditions

Seed preservation is achieved in several ways. It is safest and cheapest if life processes are reduced to the minimum level i.e., seeds are put in a quiescent state. To prolong seed viability for long periods, the environmental conditions surrounding the seeds should be controlled.

Temperature. Temperature is another major factor that determines seed longevity. As a rule of thumb, for each 5^0C increase in seed temperature, the life of the seed is halved. This rule applies between one and 50^0C. The adverse effect of high temperature extends from physiological maturity to harvest, during transport, drying, and from open-shelf storage to cold storage (Harrington, 1972). The Preferred Standard for long-term seed storage is -18^0C (Cromarty et al., 1985).

Temperature-moisture interaction. The two rules based on temperature and moisture apply independently. For instance, seeds with 10% moisture and stored at 20^0C will survive as long as those with 8% moisture stored at 30^0C (Harrington, 1972). Ellis and Roberts (1980) have furnished predictions on seed longevity. It also appears plausible that various crop species react differently to changes in temperature and moisture. The rule of thumb to indicate safe storage condition is "the sum of percent RH and temperature in Fahrenheit (%RH + OF) should not exceed 100" (Thompson, 1979).

For medium-term storage the temperature ranges from 0 to 10^oC, RH from 15 to 50%, and seeds are stored in fairly airtight containers. The projected seed longevity ranges from 10 to 30 years or longer.

Other factors. High oxygen content tends to hasten loss in viability, especially in seeds with high m.c.. On the other hand, high C02 or N2 content or a vacuum may retard deterioration. Seed exposed to ultraviolet light will deteriorate faster and radiation can damage seed in storage. Recently, Benson (1990) assessed the role of oxidative stress and free radical-mediated damage in the safe

conservation of plant genetic resources as well as the possible effects of antioxidants and recovery/repair mechanisms.

Corollary activities

To ensure that seed storage conditions are maintained at desired levels, regular monitoring is required. Seed m.c. and viability need to be tested before sealing and over time during storage. Temperature and RH in the storage rooms need to be monitored as well. The amount of seed in store should be monitored especially for active collections that are used for distribution purposes. The amount of seed in store and seed viability are two factors that are considered in deciding when regeneration of seed lots should be done.

Roberts (1975) has suggested that when seed viability drops to 85%, regeneration is warranted. For some crops, high initial seed viability is difficult to attain. For example, initial viability testing in pepper has shown that only about 75% of the accessions tested have initial percent germination higher than 85 (AVRDC, 1992). After one year in short-term storage, 19% of those which showed <85% germination showed improved germination of >85%. Dormancy may therefore be a factor. However, germination testing in both cases followed the recommended procedure of pretreatment with one percent sodium hypochlorite for 10 min followed by washing three times with distilled water and wetting with
0.2% potassium nitrate before incubation at alternating 30^0C (8 hr) fight, 20^0C (16 hr) dark (Ellis et al., - 1985)

For viability monitoring in pepper, the International Seed Testing Association (Anon., 1985) recommends first and final counting at 7 and 14 days, respectively. However, Bellettiand Quagliotti (1989) found that some seed lots showed a high frequency of fresh ungerminated seeds at 14 days.

Regeneration and viability monitoring are two of the most laborious, time consuming and expensive activities in germplasm conservation. Regeneration becomes formidable when the collection is a large one. In the case of viability, monitoring methods used routinely are destructive so seeds that have been painstakingly multiplied have to be sacrificed during this activity. More practical approaches such as the adoption of the core collection concept (Frankel and Brown, 1984) during regeneration and the use of check varieties or clusters (AVRDC, 1992) in monitoring viability may have to be considered.

Conclusion

From the above it can be seen that the preservation of pepper and eggplant germplasm encompasses a web of complex interrelated activities. Many of the procedures and conditions followed are based on general precepts on preservation of germplasm. Only a few are based on actual research and data obtained for the two crops.

The stringent requirements for preservation of germplasm necessitate the availability of facilities and funds designed for long-term operation as well as trained and dedicated personnel.

Genetic erosion due to loss of viability can be serious in the maintenance and storage of germplasm. Such loss is demoralizing after all the effort, risk, and cost of launching a well-planned collecting expedition, and documentation of the collections. Appropriate handling, storage procedures and management are necessary to preserve the genetic diversity gained through collection.

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21

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BREEDING FOR QUALITY IN CAPSICUM

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Capsicum fruits are popular and used in cuisines all over the world. There are many different varieties, forms; and uses of Capsicum (Smith et al., 1987, Bosland, 1992a, and Cheng, 1989). This variation is also reflected in the goals and objectives needed to breed for quality. The strategy of the Capsicum breeder is to assemble into a cultivar the superior genetic potential for yield, protection against production hazards, and improved quality. Quality means different things to different people. To some extent the definition of quality, like that of beauty, lies in the eyes of the beholder. That is, factors that are important quality considerations for one particular use may not be important in other uses. Different aspects of quality standards vary according to uses by growers, shippers, sellers, and consumers. A grower judges quality in yield, fruit color, and lack of disease and pests. As the Capsicum moves through market channels, other quality parameters are recognized. These include appearance, storage quality, processing quality, and nutritional quality. The major dichotomy in the perspective of Capsicum quality is in consumption as fresh (vegetable) versus processed product (spice). Each purpose requires different qualities, so it is impossible to give a single definition of a quality Capsicum. A Capsicum suitable for the fresh market, for example, may be unsatisfactory for the manufacture of dried chile powder. A Capsicum suitable for dried chile powder may be unsatisfactory for paprika use. Because all quality attributes can not be readily addressed, this paper will be devoted primarily to breeding of Capsicum for the quality components of dried red chile and paprika; red fruit color, vitamins, and pungency.

Breeding Quality of Red Chile and Paprika

The quality of red chile and paprika products is based on visual and extractable red color, pungency level, and to a lesser degree, nutrition. One of the obvious ways to improve the quality of processed products is to improve the quality of the raw material from which these products are derived. This usually means improved cultivars. The acreage of cultivars used for red chile and paprika production continues to increase in the world. Production growth has mainly come from the processed food industry, where large quantities of red chile and paprika are used in prepared meals, seasoning blends, and in the canning industry.

Red chile is the mature red fruit of pungent capsicums, whereas international spice traders use the term "paprika" for non-pungent (sweet), red Capsicum powder. Even though paprika is considered a spice product in international trade, it is consumed as an important dried vegetable in European and North African diets. Red chile and paprika are dehydrated, and sold as whole

fruits or ground into powder. There is a wide range of shapes and appearance in the dried pods from which red chile powder is made. In the United States, red chile is produced from the New Mexican chile type, sometimes called Anaheim type. However, red chile powder may be produced from cayenne, mirasol, Asian, etc. types. There is also a wide diversity in chi16 types from which paprika powder is made. Again, in the United States, paprika is made from the New Mexican-type chile, whereas in Europe, paprika is made from two principal fruit types: 1) a round fruit about the size of a peach, and called Spanish or Moroccan paprika, and 2) a longer, more conical and pointed type grown in the Balkan countries, called Hungarian paprika (Bosland, 1992a). The Hungarian word for plants in the genus Capsicum is "paprika." Thus, Hungarian paprika may be pungent or non-pungent, depending on the cultivar (Somos, 1984).

Color is one of the most important attributes of red chile and paprika. However, little attention has been given to Capsicum color control. Four different genes ($_{y, C1}$, $_{C2, C1}$) with epistatic interactions have been reported to control color in mature fruits (Hurtado-Hernandez, I.H. and P.G. Smith, 1985, Shifriss, C. and M. Pilovsky, 1992). Approximately 20 carotenoids contribute to the color of Capsicum powder. Unfortunately, the inheritance of the different carotenoids and the genetics of color intensity have not been elucidated. There is little, if any, research reported on the genetics or breeding for carotenoid content in chile (Bosland, 1992b). A. Levy in Israel is currently investigating this area, therefore, information on the breeding for carotenoid content may be forthcoming.

Carotenoid compounds are yellow to red pigments composed of isoprene units, and are normally fat-soluble colors (Bunnell and Bauernfeind, 1962). The keto-carotenoids, capsanthin, capsorubin and cryptocapsin are unique Capsicum carotenoids. The major red color in Capsicum comes from capsanthin and capsorubin, while the yellow-orange color is from beta-carotene and violaxanthin. Capsanthin, the major carotenoid in ripe fruits, contributes up to 60% of the total carotenoids. Capsanthin and capsorubin increase proportionally with advanced stages of ripeness, with capsanthin being the more stable of the two (Kanner et al., 1977, and Harkay-Vinkler, 1974). The amount of carotenoids in fruit tissue at harvest depends on factors such as cultivar, maturity stage, and growing conditions (Reeves, 1987).

Extractable color is measured by a spectrophotometric process, designated ASTA units (American Spice Trade Association, 1985). Generally, the higher the ASTA color value, the greater the effect on the brightness or richness of the final product. A Capsicum powder with 120 ASTA color units would give a brighter red to a finished product than an equivalent amount of an 80 ASTA color. Another term to describe red color in oleoresin, a red oily mixture of carotenoids and other non-volatile compounds, is the standard international color unit (SICU), where 100,000 SICU is equal to 2,500 ASTA units.

Surface color measurement of Capsicum powder or fruits is based on the Hunter tristimulus color system (L.a.b.) method (Conrad et al., 1987). L is the degree of whiteness-darkness on a scale of 100 to 0; a measures red when positive and green when negative; and b measures yellow when positive and blue when negative. Cultivar, stage of development at harvest, granulation and processing

are all contributing factors to the final color quality of the product. ASTA color affects the brightness of a product, while the surface color has an impact on the hue of the product. Hue sets the kind of color, e.g. brownish-red, orange-red, or red-red. An orange-red cultivar can have a high level of red and yellow pigments, giving a high ASTA reading but a low L.a.b. reading. Green capsicums do not have capsanthin. Brown capsicums, such as "chocolate-colored" Mexican pasilla and mulato, simultaneously contain chlorophyll and capsanthin, a mixture of red and green that produces a brown color.

Having the appropriate cultivar, the right maturity stage, and the best growing conditions, does not insure good quality red chile and paprika powder. The capsicums must be processed and stored correctly to maintain high quality. For storage, drying to safe moisture levels (10%) is necessary. Traditionally, Capsicum was dehydrated by sun-drying. This method was replaced by controlled artificial drying, now practiced by virtually all commercial processors. Red color retention mainly depends on prevention of oxidative attack of the powder that reduces original color (Lease and Lease, 1956).

When Capsicum is used as a primary ingredient, it contributes flavor, texture and color to the dish, as well as nutrition. Capsicums are good sources of many essential nutrients. Capsicums produce high amounts of provitamin A, vitamins C, E, P, B1 (thiamine), B2 (riboflavin), and B3 (niacin). Vitamin A is not found in capsicums. Capsicum contains the provitamins, alpha-, beta-, gammacarotene, and cryptoxanthin, which are all transformed in the human liver into vitamin A. In the mature fruit, the beta-carotene content of most cultivars is approximately 10% of the total carotenoid. However, there is a wide variation among cultivars in the total carotenoid content as well as the ratio of carotenoid components (Davies, et al., 1970).

After total energy deficiency, vitamin A and protein deficiencies are estimated to be the most common dietary problems in the world (Pitt, 1979). However, if the United States daily vitamin A requirement is utilized, the vitamin A requirement is met by consumption of only 3 to 4 g (1/2 Tbsp) ground red Capsicum (Lantz, 1943). In addition, evidence from epidemiological studies indicates that higher intake of carotene or vitamin A may reduce the risk of cancer (National Academy of Science, 1982; Ziegler et al, 1986). Beta-carotene is the most plentiful form of provitamin A and can be cleaved to form two molecules of retinol, the physiologically active form of vitamin A. Hartwell (1971) lists 14 references where Capsicum has been cited as a therapeutic agent for cancer. Capsicum does have a strong anti-oxidative property and the binding of free radicals may be the course of action (Colditz, 1987).

Capsicums are also among the richest known plant sources of vitamin C. Vitamin C was first purified from Capsicum in 1928 by Hungarian biochemist Albert Szent-Gy6rgyi, who later won a Nobel Prize for his work with vitamin C in Capsicum. Green Capsicum has the highest amount of vitamin C, which decreases with maturity. Fresh fruits may contain up to 340mg/100g of vitamin C (Sviribeley and Szent-Gyorgyi, 1933; Jachemoiviez, Th., 1941). A 156 g serving, equal to one medium-sized fruit provides 130 percent of the recommended daily amount of vitamin C in the United States. In addition, the serving contains 5

varieties. As the market for hot Capsicum powder becomes more sophisticated, the capsaicinoid profile may have to be manipulated genetically to fit specific parameters. In our laboratory, research is underway to determine the genetic mechanism(s) for manipulating the capsaicinoid profile.

Dried red Capsicum powder is classified into five groups based on pungency level: non-pungent or paprika (0 to 700 scoville heat units), mildly pungent (700 to 3,000), moderately pungent (3,000 to 25,000), highly pungent (25,000 to 70,000) and very highly pungent (> 80,000 scoville heat units). The very highly pungent powder is mainly the type of Capsicum grown and exported from Asia.

The pungency level of Capsicum has genetic and environmental components. The capsaicinoid content is affected by the genetic make-up of the Capsicum, weather conditions, growing conditions, and fruit age. Plant breeders can selectively develop cultivars with varying degrees of pungency. Also, growers can somewhat control pungency by the amount of stress to which they subject their plants. Capsicum is hottest after it has survived a more stressful growing environment (Quagliotti, 1971).

Whether the capsicums are intended for fresh market or for processing, a chain of quality should be maintained from field production to consumption. High quality Capsicum begins with the selection of the proper cultivar and the purchase of quality seed. Good cultural practices such as fertilization, irrigation and disease management in the field are critical to producing a high quality crop. The level of stress that the crop endures in the field will influence yield, fruit color, nutritional quality, pungency, and diseases.

A knowledge of quality will be important in developing an applicable Capsicum breeding program in the future. Breeding for quality is a moving target. As market demands change so will the breeding program. Continued growth of Capsicum acreage in the world will entail the development of new Capsicum cultivars adapted to each area's specific ecological conditions. New red chile and paprika cultivars will incorporate high color, a color that is stable through the drying process, higher nutritional quality and a uniform pungency.

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PEPPER GROWING IN SPAIN

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CROP IMPORTANCE

After the F-A-0. yearbook (1991), Spain with a pepper production of 795,000 t(peppers for powder paprika not included) was in 1989 the fourth world producer after China, Turkey and Nigeria but, it was in Spain where the highest average yield (29 t/ha) was obtained due to the intensive cultivation.

The importance of peppers among other vegetable crops in Spain is well represented by the following data (MINISTERIO DE AGRICULTURA,1989). Pepper in 1989 was one of the most important crops under protected cultivation (9,100 ha; 462,200 t). Total surface devoted to pepper growing was 33,800 ha, including 5,800 ha for canning and freezing, 6,600 ha for powder paprika and 1,000 ha for hot peppers. Total production reached the amount of 891,400 t. The processed production (canned, freezed and powder paprika) about 25% of total production is, very probably, the highest rate in the world. Exports in 1989 raised to 226,100 t in fresh, 17,000 t canned and freezed and 12,400 t as paprika powder.

In the last 20 years the surface was increased in 13%, while the production was doubled. This was due mainly to the large increase of protected cultivation in the Mediterranean coast (Almeria, Murcia and Valencia) and in the Canary Islands. For open air growing, Murcia and Extremadura were the main regions for paprika production, while the Ebro Valley area (Arag6n, Navarra y La Rioja) together with Toledo were the main places for canning pepper production.

MAIN CULTIVATION PROBLEMS

In general terms, virus and fungus diseases are the main production constraints. Poor fruit setting follows in importance in protected and open air cultivations as well. The poorly airconditioned plastic greenhouses give few possibilities to manage temperatures within the optimum interval for right fruit setting on peppers. Also, it is relatively frequent to obtain poor fruit setting in the open air cultivation during summer time, when anthesis and temperatures over 35^0C coincide.

In the last years labour which is getting scarce and very expensive, arose as a new and important problem. Processing factories ask for cheap raw materials whose costs are higher as the fruit size gets lower. For paprika cultivation small fruited cultivars are being used. That is why paprika powder factories, more heavily than canning industries, are suffering a very strong competition from countries where labour is much cheaper (North Africa, South America and East and Central Europe). To solve this problem, mechanical harvesting, firstly, and direct sowing, secondly, are being developed in the main open air growing areas.
The future of the Spanish pepper processing factories, especially those devoted to powder paprika, depends on the results of those - trials together with the breeding and introduction of pepper varieties adapted to mechanization. In connection with this point, Bulgarian paprika cultivar 'Buketen', some U.S.A varieties and Spanish materials are currently being tested in our trials.

DISEASES

As it was previously pointed out, the large amount of pathogens which severely attack pepper plants is the main problem on pepper growing.

The most important diseases are those difficult to control when they have already attacked the pepper fields. In this sense, in Spain we should point out the following: Pepper Mild Mottle Virus (PMMV) and Potato Virus Y (PVY) in plastic greenhouse cultivation and PVY and Verticillium wilt (Verticillium *dahliae* Kleb.) in open-air cultivation. Pathotypes PMMV-1-2(BOUKEKA,1983) and PVY-O(GEBR9 SELASSIE et al.,1985) are predominant in greenhouse cultivation. Pathotypes PMMV-1-2-3 and PVY-1, although scarcely, have also been isolated (LUIS ARTEAGA and GIL ORTEGA, 1986,1992).

To the previously cited three diseases we should add the very new problems for Spanish crops caused by the Tomato Spotted Wilt Virus (TSWV), transmitted by the thrips *Frankliniella occidentalis*. TSWV is mainly spread over the Mediterranean coast and South of Spain. screening pepper germplasm for resistance to TSWV have recently being *started* at several Spanish research centers.

Other important diseases satisfactorily controlled either by means of chemical treatments or by different cultivation methods are caused by wireworms, fruitworms, aphids, Phytophthora blight *(Phytoghthara* wet-soil wilt, powdery mildew *(Leveillula taurica), etc...*

CULTIVARS

To describe the cultivar situation in Spain the cultivar classification proposed by POCHARD in 1966 (Table 1) will be adopted.

Table 1 - Big and sweet f ruit cultivar classif ication by f ruit traits _(POCHARDj 1966).

Group	Standard variety
A. Quadrangular shape	
A_1 Smooth surface, bulging fruit base, thick wall.	Yolo Wonder
A_2 Externally evident lob divisions, non-bulging fruit base, quite thick wall.	Quadarto d'Asti
A_3 Wrinkled surface, non-bulging fruit base, base-thick wall.	Carre Doux
A_4 Thin wall, less than 100 g per fruit.	Severka
B. Rectangular shape	
B_1 Rate length/wideness (1/w) less than 2.	Museau de Boeuf (Morro de Vaca) Doux d'Espagne
B_2 Rate 1/w more than 2.	(Toledo) Ruby King
B_3 Tronkconic shape, around 100 g per fruit.	Doux Aurore
B_4 Less than 100 g per fruit,	(Jade)
C. Triangular shape	Corno de Toro
C_1 Very long and pointed.	Doux d'Alger
C^2 Very long and blunt pointed.	Najerano
C_3 Half-long, wide fruit base.	Cserdas
C_4 Short, often erect.	(Pico)
F. Tomato shaped	Topepo
N. Subspheric shape	Nora
P. Heart shaped	Perfection (Morron)

Most cultivars with Spanish origin are sweet and belong to types A, B, C and P. Type B varieties have high plants, 3-4 lobed fruits with medium thick flesh, weighing from 150 to 200 g. Type A varieties are less common than the previous ones. Plants have a more compact growing habit but fruits are thicker fleshed and weighing around 150 g. Types A and B cultivars are collected green skinned for salads and, red skinned for roasting, being the latter the main Spanish consumption. Type C, cultivars have very thin fleshed

fruits, weighing around 90 g. They are collected at green stage and usually consumed fried. Finally, most type P varieties are used for the canning industry. Paprika varieties with different fruit shapes are used.

Cultivars for the fresh market.

The main traits required in commercial varieties for fresh market (exports and Spanish consumption) are:

easy fruit setting,
acceptable early and total yields, uniformity,
and diseases and transportation resistance.

For types A and B, F, hybrid varieties have a better f ruit setting than standard ones. For this reason hybrids are preferred at least for plastic greenhouse crops where a high income allows to afford the high cost of F, hybrid seeds. Most of F, hybrid cultivars carry the gene IL,1 for Tomato Mosaic *Virus (ToMV)* resistance. Presently, new hybrids with gene 'L_3' for PMMV-1-2 resistance (BOUKEMA,1983) or gene '$vy^{1'}$='$y^{a'}$ (COOK,1963) for PVY-O resistance are being introduced.

Type B_2 cultivars are predominant in greenhouse cultivation. The main varieties are 'Atol $F_1(L_3)$, 'Clovis $F_1(L_1)$, 'Drago $F_1(L_1)$ and 'Rossita F_1'(L_3).

Type A, varieties follow in importance for greenhouse cultivation. Presently, 'Spartacus F,f, 'Latino F,I(L,) and 'Polka F,,'(L,) are the predominant varieties.

All the previously cited F, hybrid varieties give red fruits at ripeness but recently yellow ripen fruit varieties are being introduced. Among them, 'Heldor F,I(L_1), 'Asimi F,1 and 'Zarco F_1(L_1 and vy_1) belong to B, type while '*0robelle* F,1 (L_1 and vy_1), 'Aureola F,1 and 'Inia F,I(L,) correspond to A_1 type.

Seeds from all these F1 hybrids are imported by multinational companies.

In open air cultivation the main varieties are Spanish land varieties as 'Toledo', 'Grande de Plaza' and 'Morro de Vaca'. 'Toledo', also known as 'Infantes' or 'De Litrol, belongs to a intermediate type between B_2 and B_3 It shows partial resistance to V. dahiae (PALAZON, personal communication). 'Grande de Plaza', also known as 'Morr6n de Plaza', belongs to type P and, 'Morro de Vacal to type B,. Seeds of all these varieties in many cases are still multiplied by growers themselves. Pedigree selection processes have recently being started at Ebro Valley research stations on 'Toledo' and 'Grande de Plaza'.

Type C_1 varieties are 'Dulce Italianol', a Spanish land variety,

and, for greenhouse cultivation, 'Italico F_1 '(L_1) and Abdera F_1 (L_1)

Cultivars for the canning factories.

Main requirements in cultivars for canning factories are: high yield, keeping resistance (fruit cracking resistance), resistance to the canning process (thick flesh fruits), adaptability to processing and canning (appropriate fruit shape and easy peeling), good flavour quality, adaptability to mechanical growing and harvesting and diseases resistance.

Due to the strong incidence the seed price has on the final cost of the raw material, F_1 hybrids are not used f or this type of cultivation.

Canning specialties are numerous. Pepper fruits are processed as whole roasted, stripped roasted, pickled, dehydrated, deep frozen, precooked, etc,. - We shall refer only to whole roasted fruits which is the most important specialty. This speciality is made with fruits of three different groups of Spanish land varieties named 'Morron', 'Piquillo de Lodosal and 'Pico de Mendavial. 'Morron, group varieties belong to type P. They are very similar to the 'Pimiento'('Perfection') popper varieties used in U.S.A. (GREENLEAF et al.,1969) and to the 'Calahorral varieties grown in Argentina. The plants are vigorous and produce very late in the season, very thick fleshed (7mm) fruits weighing around 150 g. Our research team obtained cultivar 'Luesial by pedigree selection within 'Morron' land variety (GIL ORTEGA et al.,1986). Presently, 'Luesial is the most popular variety for the 'Morron' group canning industries. 'Luesial is partially resistant both to V. *dahliae* and fruit cracking, tolerant both to PVY (at least to pathotypes, 0 and 1) and to internal mould (Alternaria sp.) and shows very uniform ripening.

'Pico' and 'Piquillo'groups varieties belong to type C4- The plants are vigorous and give an early production with two lobed, thin fleshed fruits weighing 70 g ('Pico') and 40 g ('Piquillol). 'Piquillo' fruits are very much appreciated for its flavour. Several breeding lines very recently obtained by pedigree selection within 'Pico' and 'Piquillo' land varieties are being tested at the Ebro Valley area. Next breeding objective on these two varieties is the introduction of partial resistance to V. dahliae.

Cultivars for the paprika powder industries

Main requirements in cultivars for paprika are: high yield, high colorant content and stability, keeping resistance,

adaptability to mechanical growing and diseases resistance.

Paprika cultivars have the advantage that paprika powder industries do not require any special fruit shape or size, although thin-fleshed fruits, with low water content are preferred for making cheaper the dehydration process.

As in canning *industries F,* hybrids are *not* used as cultivars but Spanish land varieties. The most traditional cultivar, Nora' is grown in the region of Murcia. 'Mora' which is also known as 'Americano' or 'Bola', belongs to type N and shows tolerance to PVY. Presently, 'Negrall, a cultivar obtained by mutation of Nora' for higher colour content, is the most popular variety in that region.

Paprika production in Extremadura, the other important region for paprika production is based on cultivar 'Ocal', also known as 'Agridulce'. It belongs to type C_1. Several lines very recently obtained by pedigree selection within local' are being introduced at this area.

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EVALUATION AND INCREASE OF USDA CAPSICUM GERMPLASM.

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Germplasm collections and their evaluation are an important aspect of plant breeding. These collections contain the genetic material needed for breeding such traits as disease and pest resistance, drought and heat tolerance, fruit color and pungency.

At New Mexico State University, in cooperation with the USDA National Plant Germplasm System, a systematic evaluation and increase of Capsicum germplasm is underway. This germplasm and the information generated from its evaluation is available to all interested scientists free of charge. One goal of the project is to examine the possibility of a core collection of Capsigurn germplasm. This core collection would be a "selected and limited set of accessions, optimally representing the genetic diversity of cultivated and wild species of Capsicum, and providing well known genetic standards" (von Bothmer, 1990). The objectives of this core collection will be:

- to increase the efficiency of evaluation and thus of utilization of existing collections,
- to provide for a manageable and representative selection of available Capsicum germplasm for use in research and plant breeding, and
- to provide adequate material for the needs of standardization in scientific work with Capsicum.

In this way, a valuable genetic resource can be utilized and protected against loss or extinction.

Capsicum seed has been obtained from many genetic resource centers, e.g., Mexico, Colombia, and Costa Rica. When a seed increase is made, each plant accession is evaluated using a modified International Board of Plant Genetic Resources (IBPGR) descriptor list. Characteristics recorded are plant identification number, source, cultivar name, species, grow-out year, total plant number, cotyledon color, stem number, plant habit, stem color, presence of anthocyanin in nodes, stem pubescence, leaf texture, number flowers/axil, corolla color, corolla spots, anther color, filament color, stigma exertion, determinate habit, calyx constriction, peduncle insertion, fruit position, peduncle length, immature fruit color, presence of anthocyanin in immature fruit, mature fruit color, fruit corkiness, fruit shape, fruit neck constriction, calyx margin, blossom end shape, fruit persistence, fasciculated fruit set, locule number, pungency, seed color, as well as notes regarding dwarfism, male sterility, and accession uniformity.

Plants are established in field plots from transplants. When plants begin to flower, the flowers are removed. Fabric cages are placed over each accession. These cages protect the plants from cross-pollination by insects, thereby allowing maintenance of the genetic integrity of the accession. The cages are lifted periodically to record the characteristics listed above. Each characteristic is assigned a numerical equivalent, and with the aid of a hand-held portable computer, a polycorder, the data is electronically recorded while the evaluators are in the field. In this manner more accessions are evaluated faster than by handwriting the information. This also reduces the possibility of input errors while transferring the data to the computer database.

This information is then forwarded to the USDA Germplasm Resources Information Network (GRIN), a nationwide database system. The harvested seed is sent to the Plant Introduction Station for dispersal. In addition, a sample of each accession is sent to the seed storage vault at Ft. Collins, Colorado as a back-up supply of germplasm. The information and seeds are then available to researchers and plant breeders from those sources.

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VARIATION AMONG LANDRACES OF PEPPERS IN NIGERIA

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Pepper is an important ingredient in Nigerian diet. It is a vegetable of national importance ranking among the top 5% 'of the most important vegetables consumed all over the country. Generally, improved varieties are not readily available, therefore landraces of the cultivated species Capsicum annum and Capsicum frutescence are mostly produced by farmers 'in mixed cropping systems, with low crop yield of about 0.50.8 tons/ha,

The landraces exhibit considerable variation in morphological and physiological characters which can be exploited in genetic improvement programme for enhanced yield and overall pepper production in the country. In 1992 a total of 36 accessions of peppers obtained from series of surveys and collection of local vegetable germplasm carried out in the country, were evaluated on the field in a randomized complete block design to identify suitable genotypes and sources of useful genes for inclusion in a comprehensive national breeding programme on the crop.

Variation in plant characters and crop performance was recorded (Table 1). The population contained four major fruit types consisting of round, elongated cayen, bird eye and bell shaped fruits. Variants of each form were also found in the population. Multiple axillary fruit bearing character (2-3 fruits/axil) was preva-lent in the round-fruited types unlike others with solitary and occasional two or more fruits in the leaf axils. The immature fruit colour was light green for most accessions except for three ornamental like accessions ‘ED81/133’, ‘TB81/144’ and ‘OL86/11’, which had purple colour.

There was significant difference in the performance and a number of yield components among the cultivars. Outstanding genotypes in various characters are therefore potentially suitable as sources of useful genes in breeding high yielding varieties. Four accessions were outstanding for fruit yield, ‘JD91/96’ , ‘ JD91/6’; ‘JD91/7’ and IOL85/57’,while two accessions 10L85/511and ITB81/6171 were early maturing.

A maximum of thirteen harvests covering 92 days was recorded for the majority of the accessions and fruit yield per harvest was low. Therefore, most of the accessions may be best suited for small scale production and backyard farming systems where prolonged harvest period is desired. Sources of genes for the production of substantial quantity of fruits within a relatively short harvest period will be needed to

develop suitable varieties for mechanical harvesting. The most prevailent disease was the pepper mosaic virus and the cultivars showed varying degrees of resistance and susceptibility to the disease. The following accessions exhibited field resistance to the virus%'ED87/231, ITB81/66211 ITB61/6171 and IJD91/92'.

All the fruit types were hot and pungent but the bird eye fruits were the most pungent while the bell shaped fruits types were the least pungent.

TABLE 1: Mean and range for different characters of pepper accessions

	Mean	Range	Rating Scale
Fruit yield (g) per plant	119.48+5.81	30.8-290.5	
Fruit weight g/1000	1314.6+15.6	648-2275.9	
No. of fruits per plant	82.19+5.6	16.7-273.4	
Plant height at maturity (cm)	55.6+2.8	35-95	
Plant canopy spread	58.8+2.1	49-81	
Days to 50% flowering	94.5+2.9	66-117	
No. of fruits/axil	.1.0	1-3	
Fruit length (cm)	5.4+1.2	2.5-14	
Fruit girth (cm)	5.7+1.2	2.0-10.5	
Virus infection	3	1-4	1 = Resistant 4 = Highly Suceptible

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STUDIES ON STIGMA POSITION OF SEVENTEEN PEPPER ACCESSIONS

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Summary

Pepper (Capsicum spp.) belongs to the autogamous, species; however, a considerable amount of cross pollination occurs mainly through insects and to a lesser extent by wind. Providing isolation distance is often diff icult where peppers are cultivated on adjacent farms as well as on seed production farms where there is limited land. Flower traits that would facilitate self pollination would be invaluable for pepper seed production and breeding programs. Among pepper accessions there is noticeable heterostyly where flowers differ in the position of the stigma in relation to anthers. This preliminary study investigated the extent of varietal differences in stigma position under different environmental conditions. Of major concern was to observe whether high temperature affected stigma position.

Seventeen pepper accessions were planted in observational plots under three different environments. Ten flowers from each plant were measured for their pistil and stamen length and values for stigma position were calculated. Daily maximum temperature at each location was recorded during the period of the experiment. Cultivars significantly varied in their stigma position. The variety'P.I. 241650'and'Long Fruit A' had the inserted stigma position, whereas, the others showed exserted stigmas. Simple regression analysis indicated that stigma position was not a function of the weekly mean maximum temperature prior to anthesis. Some plants developed both homomorphic and heteromorphic flowers.

Introduction

The amount of natural cross pollination in pepper may vary between 1-91% depending on genotype, location and insect activity (Franceschetti 1972; Murthy and Murthy 1962; Odland and Porter 1941; Tanksley, 1984). Outcrossing may effect undesirable genetic variability in a cultivar, underscoring the importance of controlling cross pollination in purity maintenance of pepper seed stocks. Flower traits that facilitate self pollination may be invaluable for pepper seed production and breeding programs. Two such traits are homostyly and cleistogamy. Among pepper cultivars there is noticeable heterostyly where flowers differ in the position of the stigma in relation to anthers. Three kinds of flowers were identified; short styled, medium styled and long styled. The long styled flowers in which the stigma extends beyond the stamens favors cross pollination whereas a high degree of self pollination is expected from short-styled flowers (Cochran, 1938; Erwin, 1931). This preliminary study investigated the extent of varietal differences in stigma position under different environmental conditions.

Materials and Methods

Seventeen pepper accessions were planted in observational plots under three different environmental conditions, namely, the field and, two different screenhouses at the Asian Vegetable Research and Development Center, Taiwan. The number of plants per variety tested in these locations were 10, 6 and 4, respectively. Ten flowers from each plant were measured for their pistil and stamen length. Lengths were determined at full anthesis by measuring up to the tip of the stigma and anthers from the base of the ovary. Stigma position was calculated as the difference between pistil and stamen length. Negative values represented the inserted stigma position while positive values indicated an exserted stigma position. Because high temperature was thought to play a role in style elongation, daily maximum temperature at each location was recorded during the period of the experiment, and the mean maximum temperature for one week duration prior to anthesis was the statistic used for analysis. Mean maximum temperature varied between 29-330C in the field and 35-420C in the two screenhouses. Locations were treated as replications and an analysis of covariance was used to test the hypotheses of no differences among accessions for stigma position with temperature as the covariate. Simple regression equations were estimated for stigma position of each accession as a function of temperature, and simple correlations were compared among flower traits.

Results and Discussion

Cultivars significantly varied in their stigma position. The variety P.I. 241650' and 'Long Fruit A' had the inserted stigma position whereas the others showed exerted stigmas. Among these two major groups, eight significantly different discreet classes in stigma position were found based on a Fishers Protected LSD means comparison test (Table 1). Two classes had overlapping values. The range in phenotypic expression suggested a quantitative expression for the character.

Overall, the covariate, mean maximum temperature for one-week duration prior to anthesis, did not show any significant effect on stigma position. The regression coefficients were significant in the individual functional equations for half the accessions but there was no clear trend in slopes to attribute to temperature effects (Figure 1). The relationship between temperature and pistil or stamen length was significant but of very low correlation ($r < 0.20$). Pistil and stamen length; however, showed a significant and moderately high correlation ($r = 0.62$), indicating that environmental factors affecting stamen length similarly affected pistil length. Variety x temperature interactions for stigma position were significant for seven accessions only. These accessions were'CH-6 Num 216','Gwangju','P.I. 102883','Jawahar 218', 'Ludhiana Long Selection', 'P.I. 125807' and 'CA 8'. It was observed that all the accessions which had shown variety x temperature interaction had some plants with either exserted and inserted stigmas or with all three flower types. In the case of 'CA 8' and 'Punjab Lai', all the plants produced homomorphic and heteromorphic flowers, even at the same temperature. Other factors, either genetic or environmental, probably influenced stigma position of these accessions. The production of flowers of all three morphological types may be characteristic of these two accessions.

Although most of the plants of 'Long Fruit A' had the inserted stigma position a few plants produced either flowers with exerted stigmas or flowers with all three morphological types. Hence, variability for stigma position of this variety may be attributed to heterogeneity of the plants. The variety 'P.I. 241650', a .C. frutescens accession, was very stable for the inserted stigma position.

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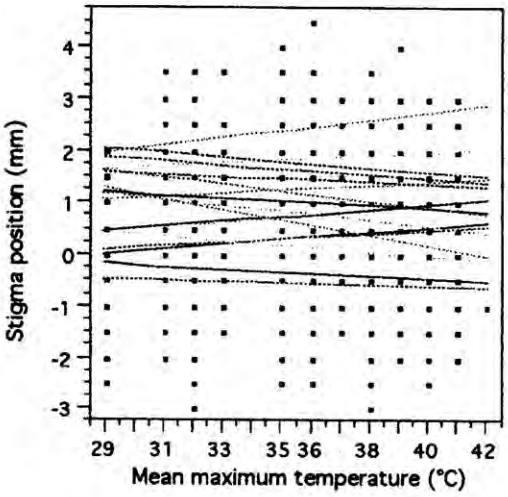
Table 1. Mean stigma position of seventeen pepper accessions

Accession	Stigma position †	
PI 241650	- 0.55	a
Long Fruit A	- 0.32	b
Punjab Lal	+ 0.16	c
CH-6 Num 216	+ 0.26	c
PI 102883	+ 0.31	c
CA 8	+ 0.71	d
PI 125807	+ 0.73	d
Jawahar 218	+ 0.80	d
PI 163201	+ 0.88	d e
MI 2	+ 1.01	e
PI 105444	+ 1.24	f
Gwangju	+ 1.24	f
KA 2	+ 1.45	g
KKU Cluster	+ 1.64	h
PI 138557	+ 1.74	h i
Japan # 2	+ 1.82	i
Ludhiana Long Selection	+ 2.41	j

†means followed by the same letter are not signficantly different according to Fisher's Protected LSD test (P=0.05)
- value = inserted stigma
+ value = exserted stigma



Accession	Regression equation	(slope) Prob > |t|
CA 8	$\hat{Y}$ = -0.1002x + 4.1809	0.0000
Punjab Lal	$\hat{Y}$ = -0.0426x + 1.6661	0.0016
Ludhiana Long Sel.	$\hat{Y}$ = 0.0719x - 0.1471	0.0025
KKU Cluster	$\hat{Y}$ = -0.0376x + 2.9537	0.0104
PI 105444	$\hat{Y}$ = -0.0652x + 3.5080	0.0121
CH-6 NUM 216	$\hat{Y}$ = 0.0482x - 1.4023	0.0152
PI 163201	$\hat{Y}$ = -0.0597 + 2.9142	0.0326
Japan #2	$\hat{Y}$ = 0.0291x + 0.7914	0.0339
MI 2	$\hat{Y}$ = -0.0290x + 2.0381	0.0342
PI 125807	$\hat{Y}$ = 0.0454x - 0.8655	0.0582
Jawahar 218	$\hat{Y}$ = 0.0458x - 0.8882	0.0595
PI 138557	$\hat{Y}$ = -0.0423x + 3.2591	0.0598
PI 102883	$\hat{Y}$ = 0.0330x - 0.8560	0.0897
Gwangju	$\hat{Y}$ = 0.0296x + 0.1962	0.1002
KA 2	$\hat{Y}$ = -0.0219x + 2.2173	0.2111
Long Fruit A	$\hat{Y}$ = -0.0264x + 0.5877	0.2568
PI 241650	$\hat{Y}$ = -0.0116x - 0.1383	0.4524
Combined model	$\hat{Y}$ = -0.0009x + 0.9225	0.8775

Figure 1. Fitted regression lines for stigma position as a function of maximum temperature during one week prior to anthesis for seventeen pepper accessions.

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STUDIES ON PERCENT OUTCROSSING IN 'CLOSED FLOWER' LINE 'UFBG 8209-1' K. D. A PereraO, J. M. Poulos+

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Summary - The closed flower *(cf)* or cleistogamous pepper line 'UFBG 8209-1' has been reported as a potential - source for increasing the extent of self-pollination in pepper cultivars. This study was conducted to investigate the percentage of outcrossing in the cleistogamous line UFBG 8209-1 under field conditions at AVRDC. Four sib-lines of UFBG 8209-1 were field transplanted in alternate rows with the high anthocyanin - variety 'Lorai' which produces purple cotyledons, foliage, flowers and fruit morphological markers. The variety 'Lorai' also has pendant fruit, whereas, 'UFBG 8209-1' has upright fruits. Ten plants were randomly selected from each of the cleistogamous lines and three individual fruits from each plant, differing in harvest interval (early, mid, late season), were tagged for seed collection. Seeds obtained from these fruits were put to progeny tests and outcrosses in each seed lot were scored at the cotyledon and adult plant stage based on the various dominant morphological markers.

Results showed that the use of purple cotyledon as a genetic marker was fairly accurate for identifying outcross progenies despite low expressivity of anthocyanin at the seedling stage. Only nine of 1,541 seedlings (0.58%) had been misclassified at the seedling stage. Outcrosses based on true foliage and flower color, fruit pedicel position and plant growth habit were easier to score but required a longer growing season. Fifty percent of the progenies of two sib-lines of UFBG 8209-1 showed outcrosses while one sib- line had progenies completely absent of outcrosses. Among the progenies of 120 fruits sampled the total percent outcrossing was 2.21 %. Outcrosses per progeny line varied between 0-56%, indicating that some of the flowers in the purported cleistogamous genotypes were open during pollination. Further selection and testing is underway to confirm the stability of the cleistogamous trait in the sib-family that had no outcross progenies.

Introduction - The amount of natural cross-pollination in pepper may vary depending on genotype, location and insect activity (Franceschetti, 1972; Murt~y and Murthy, 1962; Tanksley, S.D., 1984). High rates of outcrossing pose problems in assuring inbreeding and maintaining genetic purity of varieties in seed production and breeding programs. Traits that facilitate self-pollination are very useful in pepper improvement programs. One such trait is cleistogamy or the closed flower trait (gene symbol *cf),* the inheritance of which was reported as a single recessive gene in pepper line, 'UFBG 8209-1' (Subramanya, 1984). In preliminary field and screenhouse observation at AVRDC where 'UFBG 8209-1' is being used in the pepper-breeding program, it was observed that some of the flowers of this line opened partially or completely, but it was not clear whether flowers opened before or after pollination. Therefore, this study investigated the percentage of outcrossing in the cleistogamous line 'UFBG 8209-1'.

Materials and Methods - Four sib-lines of 'UFBG 8209-1'1 and the high-anthocyanin variety 'Lorai' which produces purple cotyledons, foliage, flowers and immature fruit morphological markers were sown at AVRDC on 6 September 1991. In addition to these dominant anthocyanin markers the variety 'Lorai' has growth - characteristics distinct from those of 'UFBG 8209-1' such as pendant fruits and tall plant stature with long internodes. The line 'UFBG 8209-1' produces green leaves, white colored closed petals, light . green/yellow immature fruits and upright fruits. Plants are of short stature with short internodes. Of the four sib-lines of 'UFBG 8209-1', three were derived from single plants and the other was a bulk population, -" all derived from the original accession.

The four sib-lines of 'UFBG 8209-1' were transplanted in alternate rows with 'Lorai'. There were 30 plants on each single row bed. Ten plan;ts were randomly selected from each of the cleistogamous lines and three individual fruits from each plant, differing in time intervals to anthesis, were tagged for seed collection. Seeds obtained from these fruits were put to progeny tests and outcrossing in each seed lot was scored at the cotyledon stage B,nd confirmed at the adult plant stage based on the various domina

morphological markers. These seeds were sown on 25 June 1992 in plastic flats with seeds of 'Lorai' and the four sib-lines of 'UFBG 8209-1'for comparison,

Results and Discussion

Results showed that with very careful observation purple cotyledon was a fairly accurate marker for identifying the outcross progenies despite low expressivity of anthocyanin at the seedling stage. Outcrosses based on true foliage and flower color, fruit pedicel position and plant growth habit were easier to score but required a longer growing season. The space requirement was the same because plants were forced to maturity in the seedling nursery rather than transplanted to larger pots. Most of the outcrossed plants developed strong anthocyanin pigmentation along the stem and leaves, grew taller and produced purple tinted open flower petals and purple colored pendant immature fruits. Only nine of 1,541 seedlings (0.58%) had been misclassified at the seedling stage. Seven of these grew taller, produced open flowers with white petals, had pendant but light green fruits similar to the shape of 'Lorai'. These were obvious outcrosses that may have resulted from outcrossing with 'Lorai' plants that were heterozygous for the anthocyanin genes or due to the possibility of low penetrance of anthocyanin genes. The other two plants showed the anthocyanin characters described above.

Fifty percent of the progenies of two sib-lines derived from 'UFBG 8209-1-1(op)-3' and 'UFBG 8209-11(op)-7' showed outcrosses while the progenies of the entire sib4amily of 'UFBG 8209-1-1(op)-,C were completely absent of outcrosses (Table 1). Thirteen of the 120 fruits had progenies that were outcrosses (10.8%). Among the seedlings of , 120 fruits sampled the total percent outcrossing was 2.2%. Percent outcrossing per progeny line varied between 0-56%, indicating that some of the flowers in the purported cleistogamous genotypes were open during pollination (Table 2). The sib-family 'UFBG 8209-1-1 (op)-4' which did not show any outcross progenies either was strictly self -pollinating or at least its flowers were not contaminated by pollen from the variety'Lorai'. This cleistogamous line may be used as breeding material to increase the degree of self -pollination in pepper cultivars. The stability of cleistogamy in this selection will be confirmed by further testing with whole-plant seed lots, rather than with seed from few individual fruits. Different spatial arrangements of the cleistogamous plants surrounded by the high anthocyanin marker plants could also be considered.

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Table 1. Number of outcrosses derived from random sampling of cleistogamous sib-families

Pedigree of sib-family	Number of outcrosses (%I derived among Plants [a]		derived among fruits		progenies per sib family [c]
UFBG 8209-1 -1 (op)-3	5	(50)	5	(16-6)	17(4.5)
U FBG 8209-1 -1 (op) -4	0	(0)	0	(0)	0 (0)
UFBG 8209-1 -1 (op)-7	5	(50)	6	(20)	9(2.2)
UFBG 8209-1-BK1(op)	2	(20)	2	(6.7)	8(2.1)

[a] random sample of 10 plants per sib-family
[b]random sample of three fruits per plant (= 30 fruits per sib-family)
[c]random sample of approximately 20 seedlings per fruit (= approximately 600 seedlings per family)

Table 2. Percent outcrosses among progenies derived from single fruits

Pedigree of progeny linest	Harvest intervaltt	Outcrosses (%)
UFBG 8209-1 -1 (op)-3-8 (op)	1	5.3
UFBG 8209-1-1(op)-3-9(op)	2	56.3
UFBG8209-1-1(op)-3-21 (op)	2	7.7
UFBG 8209-1 -1 (op)-3-24(op)	1	29.4
UFBG 8209-1 -1 (op)-3-26(op)	3	9.1
UFBG 8209-1 -1 (op)-7-1 (op)	2	5.0
UFBG 8209-1 -1 (op)-7-2(op)	2	23.1
UFBG 8209-1 -1 (op)-7-2(op)	3	10.5
U FBG 8209-1 -1 (op)-7-9(op)	1	6.3
UFBG 8209-1 -1 (op)-7-14(op)	1	14.3
UFBG 8209-1-1(op)-7-22(op)	3	5.6
UFBG 8209-1 -BK1 (op)-I 1 (op)	1	50.0
UFIBG 8209-1 -BK1 (op)-27(op)	2	5.9

these 13 of 120 fruits had outcross progenies (10.8%); 34 of 1541 seedlings were outcrosses (2.2%)
l = early season, 2 = mid-season, 3 = late season

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SELECTION OF SPICE PAPRIKA BREEDING LINES

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Paprika (Capsicum annuum L.) is an important spice in the international Trade. There is great demand of paprika powder and oleoresin in Europe and USA. Ecological conditions favour the growing of spice paprika in India. The cultivation of red chilli in India is being done on commercial scale and a large part of its production is also exported. Paprika is mostly used as ground product and is also an imported basic material for producing capsicum oleoresin and has a tremendous demand for export. As yet in India there is no spice paprika -variety grown commercially. Thus the breeding of both spice and vegetable paprika *varieties was taken up at this* station since April 1988. The breeding objectives were aimed at consumers, growers and processing requirements for developing suitable varieties, Since It *is not possible* to introduce all the r4esired characters into one variety) therefore a large number of varieties well adopted to different agroclimatic conditions will have to be developed.

In the breeding programme a large number of paprika germplasm from home and abroad was collected, evaluated, and-selfed. For paprika variety development pungent and non-pungent types were- selected. Crossing was attempted successfully in the diallel sets *of both pungent* and non-pungent inbred. Isolation of male sterile lines is also in progress, which in future will be utilized to develop hybrids among the best combiners. At-present observations are given for the most desirable horticultural traits required in the paprika varieties, developed by selection using single seed descent method, The colour value of' the grinded paprika was calculated as per Woodbury (1977),

Fruit characteristics and yield of the lines selected given in the tables revealed that genotype 'Kt-P1-19 is an Iceotype which borne all the desired traits important in the sweet spice paprika. Its fruit has excellent firmness, pendent habit, two locules and 68.7% availability of high coloured skin *for* processing. Field trials for its adaptability will be conducted with other chilli cultivars in different agroclimatic zones within the country; these lines are being used to develop high

coloured pungent and non-pungent varieties, Some of the selections of their crosses-are in advance stage. Its being the first achievement in order to attend paprika cultivation within the country, has tremendous potentialities of export.

Characteristics of the selected genotypes

Genotypes	Fruit size		Mature Fruit	Pungency	Color Unit	
	Length cm	Diamenter cm	Yield g/ha		ASTA*	EOA**
Kt-Pl-8	15.2	2.6	488.0	Sweet	178.35	66337.5
Kt-Pl-18	18.0	2.7	509.7	Sweet	174.25	64812.5
Kt-Pl-19	16.8	3.1	864.8	Sweet	233.70	86925.0
Kt-02	11.0	2.2	530.6	Mild	141.40	52612.5
Kt-03	13.0	1.3	340.4	High	95.30	35456.2
Kt-04	15.4	1.4	354.4	High	138.30	51468.7
NPKT-2	13.2	1.4	361.8	Mild	94.30	35075.0
Agni(F1)	10.6	1.3	222.7	Moderate	136.10	50630.0
					C.D. at 5% 21.156	211.56

*ASTA = American Spice Trade Association
** EOA = Essential Oils Association

'Kt-Pl-19' has also been identified by the Spice Board as as Standard genotype for taking up its production commercially

Average fruit characteristics

Genotypes	Fruit Weight			Percentage Recovery from dry fruit		
	Fresh (g)	Dry (g)	Drying %	Skin	Seed	Pedicle
Kt-Pl-8	32.5	3.8	11.6	66.0	27.0	7.0
Kt-Pl-18	48.7	8.6	17.6	66.6	27.0	6.4
Kt-Pl-19	48.5	9.5	19.5	68.7	25.0	6.3
Kt-01	28.5	2.8	9.8	68.4	22.1	9.5
Kt-02	25.4	3.8	14.9	65.7	29.3	5.0
Kt-03	18.3	1.7	9.2	57.2	30.8	12.0
Kt-04	22.4	3.5	15.6	68.0	26.5	5.5

Morphological characteristics of the promising line following Joshi et. al. (1988) are as follows -

Kt Pl-18 Indeterminate, pendent bearing non-pungent fruits between ancho and to Anaheim group, tapering to a pointed tip, green turning red at maturity, productive.

Kt-Pl-_19 Indeterminate, pendent bearingo non-pungent fruits belong to anahein group, tapering to slightly curved tip, highly productive.

Study was also carried out for number and weight of seeds, which is an urgent requirement for producing seed to ensure seed availability for commercial production. It was found that one fruit of Kt-Pl-19 gives an average 212.8 seeds weighing 3.140 9 wet and.1.495g dry, Genotype 'Kt-P1-8' bears an average 165.8 seeds weighing 2.608g wet and 1.399g) dry seeds per fruit. The above study reveals that with an average mature fruit yield of 250-300q,/ha . gives 48q Cry fruits per hactare. This with a 66% skin recovery yields 31.6g. dry basic product for processing Into ground Paprika or oleoresin. This in. terms of economics is quite remunerative enterprise for the farmers, For a dual benefit he can also sale 7-8 q of seeds if the crop is produced in isolation for taking up next commercial crop.

Further, research on 'the development of disease resistant highly productive paprika varieties, selection of genotypes with fruits which do not loose its elasticity and remain intact while packing as most of the cultivars crack or split when picked dry, Standardization' of seed production modernization of cultural practices and drying tech. suitable is in progress, Seeds of these breeding lines can be had an request for research use*.

ACKNOWLEDGEMENTS

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INHERITANCE OF EARLINESS IN *RED PEPPER* (Capsicum annuum L.)

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Earliness which is determined by the date of first fruit set or flower opening (Bahoo, 1961) is an important character in an *intensive and multiple cropping system and is of* particular importance in areas like Kashmir where growing seasons being short makes it necessary to develop varieties which should not only matures early but should fit well in short growing seasons and different cropping system. The present study is in this regard,

Inheritance of earliness was studied from six generations P1 , P2 , F1 , P2 BC1 and BC2) of three intervarietal crosses viz. Shalimar Long x SPE-1 (SL x SPE-1),Shalimar Long x Selection-1 (SL x, Sel-1) SC1X SPE1.. The experiment was conducted during kharief 1989 at Vegetable Farm, S.K,, University of Agricultural Sciences and Technology Shalimar,
Srinagar, India. Earliness was determined by recording number of days taken from seed sowing to date of first fruit set. The generating means were analyzed following Mather and Jinks - (1971).

The F1 mean performance in all the three crosses *indicated over - dominance of* earliness over lateness. The *significant* chi-square value of simple additive-dominance model suggested the involvement of *epistatic components.* All the components in this model were significant with negative dominance *component, which* further supported dominance of early parent. *in six* parameter model, the additive component in all the crosses and dominance component in *the cross SL x Sel-1* were significant. Among interaction components, the dominance x dominance in all the crosses and additive x additive in crosses SL x SPE-1 and SL x Sel-1 were significant. The magnitude - of non-additive gene effects were however *large* in compassion to additive effects. Further in cross SL x SP E-1 the dominance and *dominance x* dominance components were reinforcing each other leading to *the* complementary *gene action* which infact has been reflected in *early* fruiting of F1 hybrids. Although genes with non-additive effects have been found more *important,* however significance of additive gene effects cannot be ignored. Hence under such situation recurrent *selection would be more* suitable for obtaining desirable gains. Involvement of both additive and non additive genes in the inheritance of *earliness has also* been reported by Ahmad et al.(1964) and Khadi (1986).

Table 1 : Mean and their standard Eror for different generations of three crosses. Character-Earliness

Generation	Crosses		
	SL x SPE-1	SL x Sel-1	SC-1 x spe-1
F1	86.20 ± 0.75	86.20 ± 0.75	76.90 ± 0.43
F2	103.53 ± 0.46	103.37 ± 0.71	103.25 ± 0.51
F1	84.06 ± 0.80	83.05 ± 0.95	84.40 ± 0.49
F2	99.72 ± 0.57	98.36 ± 0.54	87.83 ± 0.52
BC1	91.08 ± 0.46	84.72 ± 0.51	82.73 ± 0.50
BC2	101.41 ± 0.60	95.87 ± 0.63	94.54 ± 0.54

Table 2 : Three-parameter model m, [d] and [h] of three crosses.

Parameter	Crosses		
	SL x SPE-1	SL x Sel-1	SC-1 x SPE-1
m	97.06** ± 0.39	95.74** ± 0.47	88.49** ± 0.30
[d]	23.06** ± 0.15	18.92** ± 0.76	12.92** ± 0.30
[h]	-4.87** ± 0.81	-5.85** ± 0.95	-5.11** ± 0.57
χ^2	2043.71**	676.94**	84.33**

Table 3 : Six-parameter model m [d] [h] [i] [j] & [l] of three crosses

Parameter	Crosses		
	SL x SPE-1	SL x Sel-1	SC-1 x SPE-1
m	108.76** ± 2.78	127.05** ± 2.74	86.85** ± 2.57
[d]	8.66** ± 0.49	8.58** ± 0.52	13.17** ± 0.33
[h]	-11.47** ± 6.64	-70.76** ± 6.72	6.35 ± 6.16
[I]	-13.90** ± 2.74	-32.26** ± 2.69	3.22 ± 2.93
[j]	1.66 ± 0.88	2.57* ± 1.03	-2.73 ± 1.60
[l]	-13.23** ± 4.21	26.76** ± 4.46	-8.81* ± 3.78

** , * significant at 1% and 5% respectively

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CORREIATION STUDIES IN SWEET PEPPER (Capsicum annuum L)

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Correlation helps in determining the association between two characters. This is helpful to form the basis of selection. It is generally due to pleotropy or gene linkage. Genetic linkage can be manipulated by hybridization and selection to obtain desired recombination's.

Correlation studies were conducted on the basis of four generations Pl. P2,Fl and F2 of eight varieties and their 28 hybrids of sweet pepper, Genotypic, phenotypic and environmental correlations were computed on single plant basis, However, the data was recorded from 10, plants for parents and F1 while 40 plants for F2 populations.

These studies indicated that generally genotypic correlation coefficients were higher than phenotypic ones (Table.I) Environmental correlation coefficient was significant only between number of fruits per plant and total yield per plant, Venkata Rao and Chhonkar (1981) reported that genotypic correlations were greater in magnitude than the phenotypic correlations in Chilli, Plant height was having positive correlations with number of branches-per plant, number of fruits per plant and yield per plant. While number of branches per plant showed similar correlations with number of fruits per plant as well as yield. Number of fruits per plant were significantly correlated with yield, Depsetre et. al, (1985) also found positive correlation between number of fruits per plant and total yield in pepper. A significant correlation was found between days to first harvesting and early yield. Plant height, number of branches per plant, numbers of fruits per plant being closely associated with yield per plant are *the* major components contributing towards total yield,

TABLE I.Genotypic, phenotypic and environmental correlation coefficients between different characters in -*weet,pepper

Character		No9of Total branches/ plant	No*Of fruits/ plant	Days to first harvesting plant	Early Yield/ plant	
Plant height	G	0*757	0*771	-00016	o,165	-0*792
	1P	Oe715	0*716	-00004	o.167	0,735
	E		0*279	-0*084	00186	0,314
No,tof branches /plant	G		Oe7O9	09069	0,164	Oo745
	F		0*659	600*007	0.162	
	0*693					
	E		0,236	00011	-0,142	
	01198					
Nosof fruits /plant	G			-09265	0,0369	
	0088i"					
	P			-0,,243	00363	
	0,869 **					
	E			-o,,o67	0.304	
	0.746 *4					
Days to first harve-, sting	G				-0085e*	
	0.214					
	P				0, 77:i**	
	0.207					
	E				0,052	
	00144					

* t *0- signif icant at 5% and 1% level

F, E FER ENC E S.

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BETA-ORANGE MUTANT IN PEPPER (Capsicum annuum L.)

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In 1965 dry seeds of the variety Pasardzhishka kapia were irradiated with 120 Gy X-rays.In 1966 620 M2 progenies containing 25 plants were grown and mutations were selected. In 1967 in M3 progeny No 357 previously screened for chlorophyll mutation 23 plants out of 85 were found to be with orange mature fruits. All local varieties possess red mature fruits. The mutant character was confirmed in M4,M5,M6,etc.The immature fruits of the mutant are green as in the initial variety. The habitus of the mutant and the vegetation period do not differ from the initial variety.

The mutant is slightly overyielding the present check variety Kurtovska kapia (table 1).In 1991 the mutant was released as variety under the name Orangeva kapia (Orangekapia).

Biochemical investigations revealed that the orange fruits contain 2.0-2.5 time more beta- carotene (provitamin A) than the red one with practically the same amount of vitamin C,dry matter and shugar (table 2).

Applying thin layer chromatography (TLC) of SiO2and Al2O3 the pigment spectrum of mature fruits was studied (fig.1).The data indicate that the pigment spectrum of the orange and the red fruits is of the same kind - presence of betacarotene, yellow hydroxy carotenoids and red cyclopentyl ketones.The lack of most of the hydroxy carotenoids in the spectrum of the orange fruits (components 2,7,9) evidently is a result of complete transformation in other xanthophylls of the carotenoid biasynthetic pathway. Concerning the quantitative content of the different carotinoids the mutant differ significantly from the red control. The main pigment of the variety Orangeva kapia is the beta-carotene.Our data indicate recessive monogenic inheritance of the orange fruit colour. Most probably a gene has mutated that determines the hydroxylation of the beta- carotene to beta-criptoxanthin.As a result of the decreased activity of the mutated gene the fruits accumulate beta- carotene.The other stages of the carotenoid biosynthetic pathway are not changed.The lack of many of the hydroxy carotenoides in the orange fruits is indication of participation of various oxydases in the hydroxylation of the beta- carotene and the obtained xanthophylls.In this connection it is very interesting that the orange fruits are characterized with increased relative content of capsorubin (component 6 in relation to those of capsanthin (component 5) in comparison to the red fruits. One may assume that this is a result of quick oxydation of the obtained small amounts of antheraxanthin (precursor of capsanthin)in violaxanthin (precursor of capsorubin).

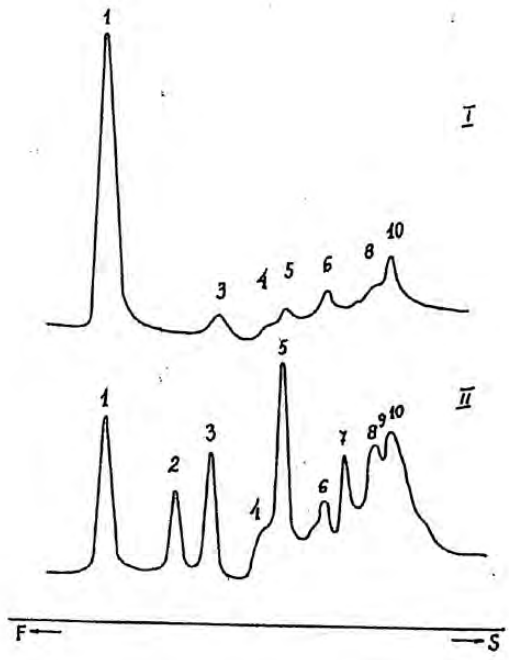

Fig.l. TLC-carotenoid spectra of orange(I) and red(II) fruits The spectra are registered with an ERY-65(Zeiss,Jena)densitometer at 430 nm. Carotenoids:l-beta-carotene;4,5,6,8,10-cyclopenthyl ketones (5-capsanthin,6-capsorubin,etc.)2,3,7,9-yellow and orange hydroxy carotenoids without epoxide groups

Table 1 Performance of the mutant variety

Variants	Year		Total yield %
		kg/ha	
Kurtovska kapia	1983	31950	100.00
Orangeva kapia		33500	104.85
Kurtovska kapia.		19e4	27450100.00
Orangeva kapia			29500107.47

Table 2 Biochemical data of red an orange fruits

Variants	Dry matter	Vitamin C	Shugar	Beta-carotene	
	%	mg %		%	mg %
Kurtovska kapia	12.04	168.20	6.18		4.680
Orangeva kapia	11.95	153.12	5.08		9.100

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ANTHER CULTURE OF SEVERAL SWEET AND HOT PEPPER GENOTYPES

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Abstract

Four F 1 hybrids and 13 cultivars or breeding lines of sweet and hot pepper were grown in the greenhouse and used as anther donor genotypes. Anther culture was performed following the method described by Dumas de Vaulx et al. (1981) with modifications regarding the growth regulators (0-5 mg/l 2,4D and 0.5 mg/I KIN or 0.5 mg/1 2.4D and 0.5 mg/l 6-BAP). Results showed that in some case embryos were obtained only in medium containing 6-BAP. A total of 268 embryos were derived from 2937 ' in vitro cultured anthers of 15 genotypes and 181 mature plants were obtained from 12 genotypes. Among the regenerated androgenetic plants about an equal number of haploid and diploid plants were found.

Introduction

In vitro anther culture of pepper (Capsicum annuum L.) is emphasized as a tool for obtaining doubled-haploid plants for practical breeding purpose, but several evidences indicates the importance of genotype for the production of haploid plants from cultured pepper anthers. Dumas de Vaulx et al. (1981) reported that the better response was obtained from large-fruited cultivars or from F 1 hybrids between large and small fruited cultivars.

Here we report about the effect of different growth regulator combination on the androgenetic response of several hybrid F 1 inbred cultivar and breeding lines of sweet and hot pepper.

Materials and Methods

Four F 1 hybrids, ('Yolo Wonder' x 'PM687' , 'OSIR' , 'ZARCO' , 'BO04' tree varieties of hot pepper (P28, P32, P44) and 10 varieties or breeding lines of sweet pepper were used.

Anthers were excised from flower buds of greenhouse grown-plant when the corolla was lightly longer than the clayx, at this stage the majority of microspores were at the late mononucleate or early binucleate phase.

The basal medium C used for anther culture was that described by Dumas de Vaulx et al. (1981) with modifications regarding growth regulators (0.5 mg/ 1 2 4D and 0.5 mg/l KIN or 0.5 mg/l 2,4D and 0.6 mg/l 6-BAP). The petri dishes were incubated at 2 35*C -for 8 days in the dark, then anthers were placed at 250C, 30)µE m min for 4 days and finally the anthers were transferred to medium R. Two R media containing 0.1

mg/I BAP or 0.1 mg1I KIN were employed . Anther coming from C medium containing BAP or KIN were transferred, respectively, to R medium supplemented with the same cytokinin.

Embryos appeared after 2-4 weeks of culture on medium R. Well developed embryos were transferred to V 3 hormone-free medium for further development. Plantlets with normal root development were transplanted in soil under high humidity. The ploidy of the androgenetic plants was determined by counting the number of the chromosomes in root tips and the number chloroplast in guard cells.

Results

The anther donor genotypes had a strong effect on the androgenetic response. Two cultivars (P29 e P41) did not produce any embryos; two sweet and one hot pepper lines produced embryos which failed to develop into plantlets (tab. 1). A total of 181 plants were obtained from the remaining genotypes with a frequency ranging from 20 to 1.3 plant per 100 cultured anthers. Although KIN confirmed the best cytokins, it is to point out that also BAP was capable to promote the androgenetic process. In addition, two genotypes (P32 and P37) produces embryos only when cultured in BAP containing medium (tab. 1).

These results showed that genotypes and growth condition of the donor plant are important factors to take into account. Besides, the cytokinin employed may improve the responsiveness of "recalcitrant" genotypes especially when anther culture is applied for practical pepper breeding.

The number of chloroplasts in the guard cell resulted correlated with the ploidy level. Among 153 androgenetic plant analysed almost an equal proportion of haploids and diploids was found regardless of the two media used (tab. 2).

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Tab. 1 – Number of cultured anthers, number and frequency of responding anthers and regenerated ambryos and number of mature plants obtained on medium containing kinetin (KIN) or 6-benzylaminopurine (BAP) from 17 genotypes (H = hot pepper)

Genotypes	No. of cultured Anther			responding anther				embryos					No. of plants		
				number		frequency%		number			frequency%				
	KIN	BAP	Tot	KIN	BAP	KIN	BAP	KIN	BAP	Tot.	KIN	BAP	KIN	BAP	Tot.
P17 F1	506	20	526	68	4	13.4	20.0	183	5	188	35.2	25	123	4	127
OSIR F1	49	55	104	5	1	10.2	1.8	13	1	14	26.5	1.8	8	1	9
Zarco F1	55	56	111	1	0	1.8	0	1	0	1	1.8	0	1	0	1
B004 F1	189	-	189	3	0	1.6	-	4	0	4	2.1	0	2	0	2
P16	14	15	29	1	0	7.0	0	2	0	2	14.3	0	0	0	0
P28 (H.)	85	91	176	1	0	1.2	0	1	0	1	1.2	0	0	0	0
P29	55	59	114	0	0	0	0	0	0	0	0	0	0	0	0
P32 (H.)	70	58	128	0	6	0	10.3	0	6	6	0	10.4	0	5	5
P34	59	5	64	3	0	5.1	0	5	0	5	8.5	0	3	0	3
P35	49	-	49	1	0	2.0	-	1	0	1	2.0	0	1	0	1
P36	137	36	173	2	0	5.6	0	4	0	4	2.9	0	2	0	2
P37	74	69	143	0	1	0	1.4	0	1	1	0	1.5	0	0	0
P38	125	123	248	2	2	1.6	1.6	5	3	8	3.7	2.4	4	2	6
P39	109	127	236	2	0	1.8	1.6	2	0	2	1.8	0	1	0	1
P40	175	131	306	4	0	2.9	0	5	0	5	2.9	0	4	0	4
P41	49	-	49	0	0	0	0	0	0	0	0	0	0	0	0
P44 (H.)	175	117	292	13	8	7.4	608	17	9	26	9.7	7.7	13	7	20
TOTAL	1975	962	2937	100	100	6.0	3.0	253	15	268	12.56	2.5	162	19	181

Tab. 2 – Ploidy level of anther-derived plants for each genotype and culture medium employed (2n = diploid plant; n = haploid plant)

Genotypes	Total No of plants	Medium containing kinetin					Medium containing 6-Benzylaminopourine				
		No of plants	2n plant		n plant		No of plants	2n Plant		N plant	
			No	%	No	%		No	%	No	%
P17 F1	102	98	55	56.1	43	43.9	4	3	75	1	25
P44 (H.)	19	13	7	53.8	6	46.2	6	3	50	3	50
OSIR F1	9	8	3	37.5	5	62.5	1	1	100	0	-
P38	5	3	0	-	3	100	2	2	100	0	-
P32	5	0	0	-	0	-	5	-	20	4	40
P40	4	4	0	-	4	100	0	-	-	0	-
P34	3	3	1	33.3	2	66.6	0	-	-	0	-
P36	2	2	0	-	2	100	0	-	-		--
B004 F1	2	2	1	50	1	50	0	-	-	0	-
P35	1	1	0	-	1	100	0	-	-	0	-
ZARCO F1	1	1	1	100	0	-	0	-	-	0	-
TOTAL	153	135	68	50.4	67	49.6	18	10	55.6	8	44.4

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USE OF DETACHED LEAVES AND SHOOTS FOR SCREENING PEPPER GERMPLASM FOR RESISTANCE TO MAJOR DISEASES

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Introduction

When evaluating breeding materials for resistance to a certain disease, usually breeders are also interested in the performance as to other traits such as yield and reaction to other major diseases. It is however difficult to evaluate the plants against other traits when conventional screening techniques are employed because susceptible plants are killed or given serious damage by the parasite inoculated. Here we report a method to use detached shoots or leaves in screening for resistance to Phytophthora rot, bacterial leaf spot, and a strain of Tobacco Mosaic Virus, which would make it possible to screen the same individual plants against multiple diseases and evaluate for other horticultural traits.

Materials and methods

System for extending freshness of leaves or shoots. In order to maintain freshness of the detached leaves and shoots for a period of time long enough for the symptoms to develop fully, lower end of leaf pedicels and shoots were placed on silicon plugs and the plugs were placed on holes of a plastic pipe, through which tap water is flowing continuously, lined in acryl boxes.

Plant materials. Resistant and susceptible genotypes were chosen on the basis of the results previously obtained in our lab. or elsewhere. 'Early CalWonder(ECW)', '3-25-27' and 'Redland Sweet Sue' were chosen as the susceptible, hypersensitive, partially resistant varieties, respectively, for bacterial leaf spot disease(Hibberd 1989). In studies on the reaction to Phytophthora capsici, 'P1201234'(Barksdale 1984), 'CM331'(Verkmortel 1989), and 'Kimjang' were used as the resistant checks, while three other varieties were included as the susceptible checks. 'Taean' and 'Saegochu'(Kang and Choi 1976) were used as the susceptible and resistant variety to Tobacco Mosaic Virus. 'Long Fruit' and 'P1260549' were added to each category, respectively.

Inoculurn preparation and inoculation methods. Mixture of races 1 and 3 of Xanthomonas campestris pv. vesicatoria were prepared at the densities of 103, 105, 108 cfu/ml and these inocula were infiltrated to intact leaves as well as to those detached and maintained in the system described above.

For Phytophthora blight, 10 cm-long top shoots were used instead of leaves. The shoots were artificially wounded in advance by making two 1-cm longitudinal slit on the stem 2 cm from the bottom end with a razor blade and the wound was covered with cotton, which were previously wet with about 1.0 ml of inoculum containing 105 zoospores. The isolate (E95) of the pathogen was provided by the Agricultural Sciences Institute, Suwon, Korea. Disease

development was compared with that in the conventional screening method of drenching the inoculum at the seedling stage(Kimble and Grogan 1960).

An isolate of tomato streak strain(Kang and Choi 1976) of TMV received from the Agricultural Science Institute was multiplied on Nicotiana tabaccurn L. var. 'Samsun'. Virus inoculum was prepared by homogenizing 1 part of infected tobacco leaves in 5 parts of 0.1 M phosphate buffer(pH 7.0) Plants at 6-leaf stage were inoculated by gently rubbing the inoculum to the two to three middle leaves that were previously dusted with carborundum(600 mesh). From a half of the plants of both cultivar entered in the virus study, middle leaves were removed and the leaves were inoculated in the same manner as above and maintained in the flowing water as in the screening against bacterial leaf spot.

Results and discussions

Disease was rated based on a 0-5 scale, where 0 = no symptom; 1 = brownish lesion at the inoculation site; 2 = stem lesion extending 1-3 cm from the inoculation site; 3 = stem lesion progressed up to half of the branch or plant with wilting sign; 4 = stem lesion progressed up to the apex of the branch or plant mostly with severe wilting sign; and 5 branch or plant dead. The scores read in time course after inoculation are shown in Fig. 1.

In the conventional method, two entries, 'Dahong' and 'Putgochu', showed susceptible reaction, while the other four revealed resistant reaction. Two additional genotypes, 'S7-7' and 'Kimjang', were classified as susceptible when inoculation was made onto detached shoots. Time period from inoculation to full development of disease was shortened from 15 days of conventional soil-drenching to 7 to 11 days when detached top shoots are used for screening. Such results implies that the new method is replaceable to the conventional drenching at the seedling stage, and moreover without killing the susceptible plants. A higher selection pressure in the new method may be attributed to the possible exclusion of the host resistance associated with delayed or interrupted penetration. Age-related expression of the resistance mechanism(Hwang and Kim 1990) may, on the other hand, have affected somewhat toward narrowing the differences between the two methods.

In the genotype with vertical resistance to bacterial spot, hypersensitive reaction(Cook and Guevara 1982), was expressed, as expected, on the inoculated leaves in two days from inoculation, when the bacteria were infiltrated at the density of 108 cfu/ml to leaves detached or those on intact plant. The other two genotypes remained symptomless until two days after inoculation, but later symptom appeared and spread in similar rates. It was therefore not possible to discern these two genotypes at this density. However, at the densities of 105 and 103 cfu/mI, two resistance types showed reactions visually different from each other, and from the susceptibility in 15 and 30 days from inoculation, respectively: no symptom in the vertical resistance; mild development of spots in the partial resistance; and severer spots development in susceptible genotype(Table 1). Symptoms of partially resistant and susceptible genotypes were severer at the density of 10^5 than at 10^3 cfu/mI.

Local lesion was observed 5 days after inoculation of TMV on both leaves detached and those kept intact on the plants in resistant varieties, while no symptom appeared in the

susceptible varieties by the time(Table 2). Based on this result it may be concluded that resistant varieties can be distinguished from susceptible ones by inoculation on single leaves detached and maintained in the system described above, especially, if the resistance is simply inherited and expressed in a hypersensitive fashion as in the case shown here(Kang and Choi 1976). If leaves on intact plants were inoculated, it was possible to confirm the host susceptibility by the mosaic symptom appearing in two to three weeks after inoculation. But such confirmation was not possible, when the detached leaves were used for screening.

From the results hitherto explained, it became possible to use detached leaves or shoots for screening for two (bacterial spot and Phytophthora. rot) and some strains of another(virus complex) of the four major diseases in Korea(Kang 1989), if their freshness are maintained in the system above described. The remained one major disease, anthracnose, can also be screened with the detached fruits. It would be feasible to learn the reaction of single individual plants to multiple diseases and to evaluate them for other horticultural traits, if the detaching stage are properly chosen in order not to give a serious effect on the growth and biomass production of the plants.

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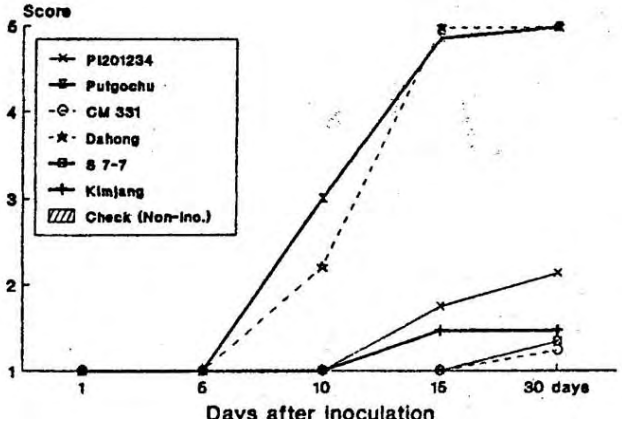

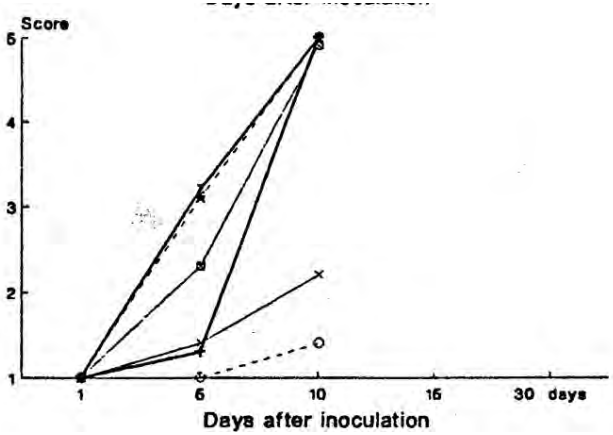

Fig.1. Mean scores of pepper genotypes in time course span from inoculation of *Phytophthora capsaici* by drenching to the seedlings (top) and by cotton-immersion on the artificial wound of the branches detached and placed on the flowing tab water (bottom).

Table 1. Symptom* development of different genotypes as reacted to X. campestris pv vesicatoria infiltrated on leaves detached and those on intact plants.

Leaves	Genotype (variety)	Inoculum density(cfu) 10^8/ml	10^5/ml	10^3/ml
Detached	Vertical Res. (3-25-27)	+++++	-	-
	Partial Res. (RSS)	-	+++	+
	Sus.(ECW-30R)	-	+++++	+++++
On intact plant	Vertical Res. (3-25-27)	+++++	-	-
	Partial Res. (RSS)	-	+++	-
	Sus.(ECW-30R)	-	+++++	++
Optimum stage for symptom reading(DAI**)		2	15	30

* Symptom : non(-) to severe(+++++)
** DAI : Days after Inoculation.

Table 2. Symptom development of different genotypes as reacted to tomato streak strain of TMV inoculated on leaves detached and those on intact plants.

Variety	Genotype known	Symptom developed on leaves: Detached	on intact plants
Saegochu	Resistant	Local lesion	Local lesion
PI260549	Resistant	Local lesion	Local lesion
Taean	Susceptible	None	None(→ Mosaic*)
Long Fruit	Susceptible	None	None(→ Mosaic*)

* Mosaic symptom was expressed in susceptible varieties 1 to 2 week later than the local lesion in the resistant varieties.

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PRESENCE OF PVY-1-2 PATHOTYPE IN PEPPER CROPS IN SPAIN

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Potato virus Y (PVY) is one of the most frequent viruses found in field and protected pepper crops in Spain.

Based on the differential reaction on a set of known pepper cvs., PVY strains affecting peppers in France were classified into three pathotypes: PVY-0, PVY- I and PVY-1-2 (Gebre Selassie et al., 1985). The varieties used to distinguish these pathotypes are: 'Yolo Wonder' -- susceptible to the three pathotypes, 'Yolo Y' - resistant to PVY-0, Florida VR2' resistant to PVY-l and PVY-1-2 and 'Serrano Veracruz' - resistant to the three pathotypes.

In Spain PVY-0 was found as the predominant pathotype while PVY-1 had a reduced expansion (Luis Arteaga and Gil Ortega, 1986). The study and characterization of another PVY isolate found in a pepper sample from MAlaga in 1988, let us confirm also the presence of PVY-1-2 in pepper crops in Spain.

Isolates showing higher virulence than PVY-1, were also detected in USA (Cook, 1963; Zitter, 1972; Smith, 1974), Argentina and Brasil (Von Der Pahlen and Nagai, 1973) and Australia (Thomas gW., 1989), but comparative characterization of them is still to be done.

Unlike the French isolates classified as PVY-1-2, which originated either from weed species or from successive inoculations of a PVY-l isolate on a PVY-1 resistant pepper cultivar (Gebre Selassie et al., 1985), the Spanish isolate originates from a pepper field crop sample.

It is probable that the introduction of PVY-0 and PVY-1 resistant cultivars can bring up a change in the presence rate of PVY-0 pathotype in favor of PVY-1 and PVY-1-2.

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IDENTIFICATION AND CHARACTERIZATION OF A PEPPER VEINAL MOTTLE VIRUS STRAIN IN CAMEROON

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Pepper veinal mottle potyvirus (PVMV) is the most damaging pepper *(Capsicum spp.)* disease in West African crops (Lana and Adegbola, 1977). This virus is transmitted non persistently by at least four aphid species: *Mysus persicae, Aphis gossypii, A. craccivora* and A. spiraecola (Atiri and Dele, 1985). Since the first report identifying this virus as PVMV in Ghana (Brunt and Kenten, 1971), several strains have been described in different African countries (Fig. 1), based particularly on their biological properties (Brunt *et al,* 1978; Fauquet and Thouvenel, 1980). During a survey of virus diseases of Solanaceous and Legurninoseous crops in the Western province of Cameroon in 1990-1991, a virus disease characterized by veinbanding followed by severe mosaic, was observed on pepper plants in different farms at Foumbot, 350 Km far from the capital Yaounde. The plants were infected one-hundred percent. Therefore a study was initiated to characterize this severe virus disease. Based on the following observations, PVMV was identified as the causal agent of the pepper disease observed in the Western province of Cameroon.

Symptoms on indicator plants

Ile indicator plants tested were Capsicum *annuum cv.* Yolo Wonder-, *Chenopodium arnaranticolor, C. quinoa; Datura stramonium; Lycopersicon esculentum cv.* Monalbo; *Nicotiana benthamiana; N. clevelandii; N. glutinosa; N. tabacum cv.* Xanthi nc.; *Petunia hybrida; Physalis floridana* an.d *Solanum melongena cv.* Violette de Barbentane. These plants were mechanically inoculated with different PVMV isolates from Cameroon and the PVMV type strain kindly supplied by Pr. Brunt (Glasshouse Crops Research Institute, Littlehampton-Great Britain). The plants were grown in an insect-proof glasshouse at 22-21^{o}C. The infectivity of the isolates from Cameroon on the selected host plants was similar. Then, one isolate coined PVMV-Fbot was used in further tests. The symptoms induced by this isolate and by the PVMV type strain on the above indicator hosts are summarized in Table 1.

Virus purification

PVMV-Fbot was maintained in N. *benthamiana* by mechanical inoculation. Twelve to fourteen days postinoculation, apical leaves showing disease symptoms were harvested and the virus was purified as for potato virus Y (PVY) using the method of Gebre-Selassie et *al. (1985).* The yield of purified virus was about 20 mg/kg fresh material.

Electron microscopy

Electron microscopy of leaf cell extracts or purified virus were negatively stained with 1% ammonium molybdate, pH 7 and were examined with a Philips CM10 electron microscope. Crude sap from infected N. *benthamiana* contained many flexuous filamentous particles as well as fragments of pinwheels plates. Purified preparations also contained flexuous particles of about 760 nm long and 12 nm wide.

Serology

Purified preparations were mixed with an equal volume of Freund's incomplete adjuvant and injected intramusculary into a rabbit. Eight injections of 500 ~tg/ml were performed weekly. A serum of a titre of 1/4096 in double immunodiffusion test with purified preparation (1 mg/ml) to both PVMV-Fbot and type strain was obtained and stored at -20 C after mixing with an equal volume of glycerol. In DAS ELISA, PVMV-Fbot and the type strain reacted in a similar manner. PVMV-Fbot does not react with a PVY serum neither in double immunodiffusion nor in DAS ELISA tests.

The different results so far obtained (symptoms on a host range, electron microscopy, serology...) indicated that the pepper virus isolated in Cameroon is a strain of PVMV. Electron microscopy showed that the virus particles were similar in shape and size to those described by Brunt and Kenten (197 1). PVMV-Fbot and PVMV type strain caused very similar symptoms although some differences were noted on certain hosts. PVMV-Fbot is serologically related to PVMV type strain. In Nigeria, Ladipo and Roberts (1979) reported a strain of PVMV from tobacco, which showed some differences with the type strain of PVMV. These authors, as Brunt et al (1978) have indicated before, suggest that there may be a wide range of PVMV strains in West Africa and that these may fall into groups on the basis of their host ranges and serological properties. Owing to the host range

and serological reactions, PVMV-Fbot seems to be more closely related to the type strain (from Ghana) than to the Nigeria strain of PVMV previously described.

Because the damages induced by PVMV in pepper and other crops are very important, Brunt *et al.* (1978), have suggested the use of immune or tolerant cultivars may be the best way to control this virus. Indeed, a few years ago, certain lines of pepper were reported in our research center by Gebre-Selassie *et al* (1986) to be resistant to PVMV. In order to combat this severe disease, it will be very interesting to test these pepper lines in the different Africa countries in which PVMV occurs.

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Table 1: Reactions of pepper veinal mottle (PVN14V) strains on selected indicator plant species.

PV?vlV strains

Indicator plant	PV,%IV-Fbot	PVMV type strain
Capsicwn annuum cv. Yolo Wonder		
Chenopo&wn amaranticolor		
Chenopodium quinoa		
Dauira straswniwn		
Lycopersicon esculenrum cv. Nlonalbo		
Nicodana bentharniana		
Nicoriana clevelan&i		
Nicodana gludnos		
Nicotiana rabacwn cv. Xanthi nc		
Pewia hybrida		
Physaftsfloridana		
Solanwn melongena		

a In each instance, the reaction of the inoculated leaves is shown on the left and the systemic reaction (noninoculated leaves) on the right. - = no symptoms observed, VB-Mo = veinbanding followed by mottle, 11 = local lesions, *IM = mosaic,* M-N = mosaic followed by necrosis, Chl chlorosis, VC-M = veinclearing followed by mosaic,RS = ringspot b no systemic infection detected by infectivity tests on a susceptible indicator host.

Figure 1: Incidence of pepper veinal mottle virus in Africa:
vcountries in which the virus is reported.

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IDENTIFICATION OF VIRUS ISOLATES AND OF TOBACCO ETCH VIRUS (TEV) PATHOTYPES INFECTING GREEN PEPPER IN CAUJERI VALL6 (GUANTANAMO, CUBA)

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Tobacco etch virus (TEV) was reported in Cuba as one of the most important viral diseases in green pepper *Capsicum annuum* (Fernandez 1976, 1977). Main symtoms of the disease are dwarfing when young plants are infected, mosaic with leaf deformation, mid rib shortening with leaf blade and edge wrinkling and small leaves lacking in bilateral symetry. Fruits are scarce, small and misshapen with dark green stripes. In infected fields, both yield and commercial quality are hardly affected. This disease is naturally spread by Aphids, but can be artificially inoculated by mechanical means. In fields, TEV remains in weeds as *Datura stramonium, Portulaca oleracea, Cassia* tora among others.

Literature reports several TEV pathotypes : the common strain (TEV-C) infects Yolo Wonder but not Florida VR2, other virulent strains can infect Florida VF12 but not Avelar, and more virulent pathotypes can infect all these cultivars, (Greenleaf 1986). The TEV pathotypes from Cuba remain unknown up to date.

At Cauieri valley, Guahtanamo, pepper is traditionnally planted out of season, taking advantage of the local altitude. During the 1991 summer, fields planted with "Espanol" pepper became infected by a very aggressive viral disease, which symtoms looked like those of TEV. In order to determine the causal agent, four isolates (CALI I to CAI-14) were obtained from diseased plant samples and differential hosts including pepper (Yolo Wonder), *Lycopersicum esculentum, Datura stramonium, fficoliana tabaccum* (Xanthi), *Chenopodium arnaranticolor, Vigna unguiculata* and Cucumber were inoculated. Mosaic symptoms, characteritic for TEV were observed in *D. stramonium* for all the tour isolates. One isolate (CALI 3) also produced local lesions in V. *unguiculata* and mosaic in *cucumber,* indicating the presence of CMV. Presence of TEV in the four isolates and of CMV + TEV in CAU 3 was then confirmed with serological DAS-ELISA tests.

In order to determine the TEV pathotypes, the isolates were reextracted from the Datura and inoculated to three pepper varieties (Yolo Wonder, Forida VR2 and Avelar). Results are shown in table 1. Symptoms appeared 10 to 13 days after inoculation in Yolo Wonder and Florida VR2. 3 to 4 weeks after inoculation in Avelar.

From these results. it is concluded that highly virulent pathotypes of TEV (infecting Florida and Avelar) are present in the Caujeri valley. CAU 4 isolate produced the most severe symptoms in all the pepper varieties and it was chosen for- screening purposes. Additionnal work is in progress in order to obtain resistant varieties to that pathotype.

Table I : Determination of TEV pathotypes isolated in Caujeri valley, Cuba. (Number of plants with mosaic symptoms / number of inoculated plants)

PEPPER VARIETY	TEVISOLATE			
	CAU1	CAU2	CAL13	CAU4
Yolo Wonder	17/17	19/19	19/19	16/16
Florida VR2	16/16	16/16	17/17	17/17
Avelar	15/17	17/17	15/18	19/19

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NATURAL OCCURRENCE OF TWO TOSPOVIRUS SPECIES INFECTING CAPSICUM SPP. IN BRAZIL.

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Tomato spotted wilt virus (TSWV) has been reported as the causal agent of one of the most economically destructive viral diseases of Capsicum spp. in Brazil. Crop losses ranging between 49-69% have been reported in sweet-pepper due to TSWV infection (Cupertino et aL, 1984). A recent taxonomic review (Cle Avila et al., 1993) has indicated that TSWV is not a sole member of the genus Tospovirus as initially proposed. Three additional viral species denoted as tomato chlorotic spot virus (TCSV), 'groundnut ring spot virus (GRSV) and Impatiens necrotic spot virus (INSV) were proposed based on their divergence of the nucleocapsid protein encoded by the small (S)-RNA. Because this taxonomic arrangement has only recently blossomed, no information's are available about natural occurrence of those tospoviruses on Capsicum spp. In the present study, we report a serological identification of two Tospovirus species as causal agents of the 'spotted wilt disease' of Capsicum spp. in Brazil.

Capsicum spp. plants showing characteristic 'spotted wilt'symptoms (chlorosis and necrosis of the new growth; necrotic, often concentric lesions on leaves, stems and fruits; and an overall plant stunting) were collected from two different geographical regions of Brazil (Federal District and Santa Catarina State). The isolates were analyzed in a double antibody sandwich ELISA (DAS-ELISA) essentially as described by Clark & Adams (1977) against four Tospovirus species (TSWV, TCSV, GRSV and INSV) antisera.

Our results strongly indicated that the 'spotted wilt' of Capsicum spp. is a complex disease under Brazilian conditions. Apart of the previously reported TSWV (Kitajima,1986), TCSV was also identified as natural pathogen of Capsicum spp. TCSV was mainly detected in Santa Catarina State and TSWV in the Federal District. GRSV and INSV were not detected infecting Capsicum spp. The tospovirus distribution in different geographical regions may suggest that different thrips species are involved in the dissemination of these viruses. A representative nation-wide survey of tospoviruses occurring in Brazil is now underway. The main concern of several Capsicum spp. breeding programmes is to incorporate a stable and durable type of resistance to the 'spotted wilt disease'. The genotypic variability present in the genus Tospovirus must be considered by those breeding programmes to avoid selection of virusspecific resistant lines.

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CAPSICUM CHINENSE PI 159236: A SOURCE OF RESISTANCE TO *PHYTOPHTHORA CAPSICI* AND TOMATO SPOTTED WILT VIRUS (TSWV).

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Phytophthora capsici and tomato spotted wilt virus (TSWV) are among the most important pathogens of Capsicum spp. in Brazil. Sources combining resistance for TSWV and P. capsici are still not available. CNPH/EMBRAPA and UnB have conducted a breeding programme searching for sources of multiple disease resistance in Capsicum spp..

Fifty accessions of Capsicum spp. were evaluated for resistance to one isolate of *Phytopthora* capsici as described by Reifschneider *et aL* (1992). The same collection was mechanically inoculated with a TSWV isolate obtained from naturally infected *Capsicum annuum* plants in the Brasilia (DF) area. The isolate was maintained in *Nicotiana rustica* plants. Inoculation was made by griding leaves of N. *rustica* in 0.1M potassium phosphate buffer (pH 7.0) containing 0.01M sodium sulfite and by rubbing the extracts on leaves of 25 days-old Capsicum spp. plants (Cupertino *et af.,* 1988). Plants were reinoculated 1 week later to ensure infection. Plants were scored visually for TSWV symptoms up to 8 weeks after inoculation.

Only C. *chinense PI* 159236 line was found to be resistant to both pathogens. Inheritance studies are being conducted to determine the genetic basis of the resistance to P. capsici and TSWV presented by this line.

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SCREENING SWEET PEPPER ACCESSIONS FOR RESISTANCE TO BACTERIAL WILT

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Bacterial wilt of pepper is an Important disease in Japan. However, there are a few reports concerned with this disease. This report identifies resistant varieties to bacterial wilt in sweet pepper.

Seventy-four accessions of sweet pepper and 7 accessions of chile pepper Including 'Pant C-l' and 'White Khandarl' were used In this study.
' Pant C-l' and ' White Khandarl' were reported as resistant to bacterial wilt by Peter et al. (1984).

'Nine seedlings per accessions were transplanted to an infested field of Pseudomonas solanacearum at NIVOT. In order to ensure the incidence of the disease, an inoculum suspension of P. solanacearum (isolated from a diseased plant in this field) was poured Into the soil at the base of each plant with root wounding.

For the evaluation of resistance, each plant was scored for bacterial wilt symptoms at 4 weeks and 8 weeks after Inoculation. The scale of resistance ranged from 0 to 4 where: 0= no symptoms; 1= wilting of few leaves; 2= wilting of almost all leaves; 3= wilting of all leaves; and 4= death.

All the plants of 33 accessions including 'Pimiento' and 'California Wonder' wilted 4 weeks after inoculation. These accessions were regarded as susceptible. At 8 weeks after inoculation the disease indices of 14 accessions including commercial varieties were 0, and the accessions were regarded as resistant. The disease indices of 12 accessions were lower than 2, these were regarded as moderately resistant (Table 1). Twenty-three accessions among these 26 accessions were Japanese varieties. Fruit shape of 15 varieties was bell and of the others was elongate. 'Mie Midori' is assumed to be the origin of bacterial wilt resistance In bell type varieties since almost all of the resistant varieties of bell type were derived from this variety.

Although ' Pant C-1 ' was reported as resistant to bacterial wilt, It was susceptible in this study.

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Table 1. Resistance to bacterial wilt in pepper.

variety or strain	4 weeks after inoculation wilted plant' plant M	inde0 index	8 weeks after inoculation wilted plant W	index	variety or strain	4 weeks after --inoculation wilted plant M	index	8 inoculation wilted disease W	
(Bell type Japanese sweet pepper varieties)					(Elongate type Japanese sweet pepper varieties)				
Kyo Nami	0. 0	0. 00	0. 0	0. 00	Shishito	0. 0	0. 00		
	0. 0	0. 00							
Nishi Midori	0.0	0.00	0.0	0.00	Pikkoro Shishito	0.0	0.00		
	0.0	0.00							
Shimousa 2	0.0	0.00	0.0	0 , 00	Nakagawa Shishito	0.0	0.00		
	0.0	0~00							
Shinsakigake 2	0.0	0.00	0.0	0.00	Tokyo Sennari 2	0.0	0.00		
	0.0	0.00							
Mie Midori	0.0	0.00	0.0	0.00	Fushimi Amanaga	0.0	0.00		
	0.0	0.00							
Ise	0.0	0.00	0.0	0.00	Sapporo Onaga Nanban	0.0	0.00	0.0	0.00
Akashi	0.0	0.00	0.0	0.00	Suiko Shishito	11.1	0.22	11.1	0.44
Ishii Midori	0.0	0. 00	11. 1	0. 11	Genki-Amanaga -------- 0.0 ------ 0.00 ----- 100.0 -----				
1. 44									
Shinsakigake	0.0	0.00	11.1	0.33	(Sweet pepper except Chinese and Japanese varieties)				
Harusen	0.0	0.00	33.3	0.44	Rio Grande Giant	11.1	0.22	77.8	2.22
Akino	0.0	0.00	33.3	0.78	Antibois	55.6	1.44	100.0	3.78
Tosa Green A	0.0	0.00	44.4	1.44	Ruby King	66.7	1.33	88.9	3.78
Shosuke	33.3	0.89	44.4	1.56	Chinese Giant	100.0	2.56	100.0	3.89
Kyo Midori	0.0	0.00	66.7	1.67	Agronomico 10	77.8	1.89	100.0	4.00
Tosa Hime	22.2	0.56	55.6	1.78	LS3760	77.8	2.11	100.0	4.00
Green 100	22.2	O~22	88.9	2.33	LSlU0	88.9	2.11	100.0	4.00
Nishiki	33.3	0.44	88.9	3.00	Yolo Wonder	88,9	3.11	100.0	4.00
Original	33.3	0.67	100.0	3.22	No.10	100.0	2.44	100.0	4.00
Hijir;	33.3	0.67	88.9	3.22	Gigante Vermelho	100.0	2.78	100.0	4.00
Chihaya	66.7	1.56	100.0	3.67	Galben Superior	100.0	3.22	100.0	4.00
Suigyoku 2	77.8	1.78	100.0	3.78	Pinomorko Chile	100.0	3.89	100.0	4.00
Green 300	33.3	0.44	100.0	3.89	California Wonder	100.0	3.89	100.0	4.00
New Face	44. 4	1 . 11	100. 0	4. 00	LS2187	100. 0	3.89	100. 0	4. 00
Kagura	77.8	2,78	100.0	4.00	Pimiento	100.0	4.00	100.0	4.00
Ryokuou	77.8	2.78	100.0	4.00	Earliest-Red-Sweet-100.0 ------ 4.00 ----- 100.0 -----				
4.00									
Saitama	88.9	3.00	100.0	4.00	(Chinese sweet pepper varieties)				
Daiou	88.9	3.33	100.0	4.00	Chen Q;ao Qie Men	0.0	0.00	44.4	1.00
Mansaku	100.0	2.67	100.0	4.00	Fong bang Qie Men	11.1	0.11	55.6	1.44
Chigusa	100.0	2.89	100.0	4.00	Zhong Jiao 2	66.7	1.33	88.9	3.00
Wase Uwamuki	100.0	2.89	100.0	4.00	Jiao Pei 3	55.6	0.88	100.0	3.33
Heian Elko	100.0	3.00	100.0	4.00	Ying Kolu Qie Men	100.0	3.11	100.0	4.00
Bell Homare	100.0	3.67	100.0	4.00	Qie Men	100.0	3.33	100.0	4.00
Gokuwase Ojishi	100.0	3.78	100.0	4.00	Tong Fong 17	100.0	3.78	100.0	4.00
Ace	i0o. 0	3. 89	100. 0	4. 00	Qing-Jiao ---------- 100.0 ------ 3. 89 ----- 100. 0 ----- 4.				
00									
Myojo	100.0	3.89	100.0	4.00	(Chile pepper varieties)				
Ryokuko Wase	100.0	3.89	100.0	4~00	NuMex Sunset	100.0	3.89		
	100.0	4.00							
New Ace	100.0	4.00	100.0	4.00	NuMex Joe E.Parker	100.0	4.00		
	100.0	4.00							
Satsuki	100.0	4.00	100.0	4.00	1 NuMex Sunrise	100.0	4.00		
	100.0	4.00							
Sakigake	100.0	4.00	100.0	4.00	NuMex 6-4	100.0	4.00		
	100.0	4.00							
Ishii	100.0	4.00	100.0	4.00	Pasilla	100.0	4.00		
	100.0	4.00							
Takakura Gokuwase	100.0	4.00	100.0	4.00	Pant C-1	44.4	1.00	77.8	3.11
Gokuwase Shuryoku	100.0	4.00	100.0	4.00	White Khandari	0.0	0.00	0.0	0.00

z: percentage of wilted plant

Y: rated on a 0(no symptoms) to 4(death) scale

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REACTION OF Capsicum spp. FRUITS TO *Colletotrichum g1deosporidides.*

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Anthracnose is one of the most common and destructive diseases of sweet pepper in Brazil. The pathogen attacks fruits in the field and during the postharvest period, which varies from 2 to 7 days. Almost all Brazilian cultivars have in their genetic background a high contribution of the cv. Cascadura, which is highly susceptible to C. *gloeosporioides.* Despite the importance of anthracnose, none of the national breeding programmes have focusect primarily on this disease. The aim of this experiment was to identify sources of resistance to anthracnose.

Fifty-two lines of Capsicum spp. were grown under field conditions in Brasilia-DF and fruits harvested at the ripe stage. Twenty fruits per genotype were washed in tap water and superficially disinfested with a 70% alcohol solution . Fruits were inoculated by puncturing their surface once with a ten-pinned inoculator and a 15 kI droplet of inoculum (2.5 x 104 conidia/ml) placed in the injured area. The inoculated fruits were kept at 25 C and 100% RH inside covered plastic boxes, in a completely randomized design. Evaluation was done 4 days after, based on the average diameter of the lesions. Cluster analysis was used to separate the lines into 3 groups (resistant, intermediate and susceptible). Th irteen out the 52 lines evaluated were considered as resistant; 18 as intermediate and 31 as susceptible. Brazilian cultivars Agronomico 10G and Cascadura Ikeda, very popular among growers, were considered as susceptible to anthracnose (Table 1). Brazilian cvs. Agronomico 10G and Cascadura Ikeda, very popular among growers, were rated as susceptible. Later, five selected genotypes (AVRDC 13, AVRDC 16, CNPH 2689, CNPH 2727 and CNPH 2682) were evaluated at two different fruit ripening stages (green- and red-ripe). At the green ripe stage, the fruits of all the five genotypes were highly resistant, while at the red-ripe stage the genotype CNPH 2689 was susceptible and the other 4 were considered as partly resistant (lesion diameter ranging from 0.56 to 0.96 cm).

Table 1 - Fruit reaction of 52 Capsicum spp, genotypes to Colletotrichum gloeosporidides.

Genotype	Lesion diameter (mm)	Reaction*	Genotype	Lesion diameter (mm)	Reaction*
CNPH 2686	0.11	R	CNPH 2810	1.27	S
AVRDC 17	0.11	R	AVRDC 19	1.29	S
AVRDC 15	0.12	R	CNPH 2809	1.30	S
AVRDC 24	0.14	R	AVRIDC 26	1.31	S
CNPH 2727	0.16	R	AVRIDC 18	1.34	S
AVRDC 16	0.21	R	PI 201234	1.35	S
CNPH 2682	0.22	R	AVRDC 3	1.37	S
AVRDC 2	0.28	R	ESPUERA G.	1.51	S
CNPH 2730	0.29	R	CNPH-3	1.51	S
CNPH 2679	0.30	R	1-16	1.53	S
AVRIDC 13	0.33	R	CNPH 2806	1.59	S
AVRIDC 9	0.33	R	AGRONOMICO	1.63	S
AVRDC 15	0.34	R	1-7	1.63	S
AVRDC 27	0.47	1	1-17	1.67	S
AVRDC 28	0.55	1	CNPH 2805	1.67	S
AVRDC 1	0.58	1	CASCADURAI.	1.68	S
SERRANO	0.65	1	CNPH 2808	1.69	S
AVRDC 29	0.67	1	AVRIDC 25	1.70	S
CNPH 2689	0.70	1	PI 201232	1.70	S
AVRDC10	0.80	1	CNPH 2807	1.76	S
AVRDC 20	0.93	1	CNPH196	1.68	S
1-15	1.11	S	LAMUYO F1	1.81	S
1-18	1.21	S	CNPH 2690	1.82	S
AVRDC 22	1.24	S	1-4	1.87	S
MALLORCA	1.25	S	GIG. AMAZ.	1.89	S
AVRIDC 14	1.26	S	CNPH 2811	1.93	S

Reaction: R=resistant; kintermediate; S=susceptible.

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EVALUATION OF PEPPER GENOTYPES TO Leveillula taurica Lev.(Arn.) RESISTANCE IN TUNISIA

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Tunisia climate is very favourable to the powdery mildew fungus development on pepper foliage. Leveillula taurica is the agent causing the disease which find there the conditions necessary to growth during all the pepper culture seasons. The infected leaves that shed prematurely, have a defavourable effect on the fruit production.

Thirteen pepper genotypes were tested to Leveillula taurica resistance under natural conditions of infection.

The seedlings were planted in greenhouse in January 31, 1991 at the C.R.G.R. station near Sousse. Phytosanitary treatment was applied only for aphids control; fongicide treatment was not used to permit a maximum multiplication of the fungus spores. The pepper fruits had remained on their plant as far as they become reds for increase the genotype sensibility.

The powdery-mildew symptoms on the sensible plants and on the borderlines were evident by end of May. This inoculum had persisted strongly on the pepper plants till the end of the trial.

Only one notation of the plant infection level was made at the end of the trial in July 17, 1991 according to the notation scale established by Molot and Lecoq (1986). All the foliage of each plant was scored visually in giving a note ranging from 0 (without symptoms) to 5 (80 to 100% of the leaves area infested).

The experiment was conducted on a randomised complete block design with 6 blocks of 4 plants. The data was treated by analysis of variance.

The genotype sensibility classification is represented in Table.l. The tunisian varieties were the most susceptible to L.taurica. The haplodiplold HV106 was as sensible as'Beldi',-semmeneland'Nabeul 2. However, P5 and HV2 were more resistant than the tunisian varieties and HV106 genotype. PM 681 and PM 687 were partially resistant. The PM 803, HV12 and HV13 genotypes were yet very resistant under our climatic conditions.

The L.taurica mycelium growth is rapidly stoped into the PM 803 cells making up some small necrosis that engender a premature leave drops.

Nevertheless, on the leave area of HV12 and HV13 only some chlorotic small spots take place without parasite mycelium growth. These leaves persist on the plant to the old age.

This study shows that the haplodiplolds HV12 and HV13 represent a valuable source of resistance to Leveillula taurica fungus.

Table.l. Comparison of 13 pepper genotype resistance naturally infested in greenhouse with Leveillula taurica.

genotypes	mean notations (1)	origin/seed source
Baker'	3.76A (2)	variety/ INRAT (Tunisia)
Meski'	3.31 AB	local variety/ INRAT (Tunisia)
'Nabeu12	2.98 BC	11
Beldi'	2.87 BC	
Semmone'	2.71 SC	
MV106	2.62 C	havlodiploid/ INRA (Montfavet, France)
P5	1.37 D	a line/ INRAT (Tunisia)
HV2	1.33 D	haplodiplold/ INRA (Montfavet, France)
PH 687	0.92 DE	from India/ INRA (Montfavet, France)
PH 681	0.46 EF	11 .1 .1 11 1.
PH 803	0.00 F	from Haute Volta/ INRA (Montfavet, France)
HV12	0.00 F	haplodiplold/ INRA (Montfavot, France)
KV13	0.00 F	1. tv 11 ti

(1) Rach value is the mean of 24 plant notes. The notation scale range from 0 to 5 which: O= healthy plant and 5= 80 to 100% plant foliage infested (Molot and Lecoq, 1986).
(2) Means separations according'to Duncan test, level 5%

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A SEEDLING SCREENING TECHNIQUE FOR FOLIAR BLIGHT (PHYTOPHTHORA CAPSICI) OF CAPSICUM

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Foliar blight of chile (gajLsirum spp.), caused by Phytoghthora capsicl, is an economically important disease in hot and humid climates of the world. Studies on foliar blight of chile are limited. - No chile cultivars resistant to foliar blight are available. This may be due to the unavailability of a reliable disease screening procedure. We have developed a rapid and inexpensive, but efficient, seedling screening technique for foliar blight of chile using zoospores as inoculum.

Test plants. Seeds of 'Criollo de Morelos 334', 'NuMex R Naky', 'Jalapefio M', 'Keystone Resistant Giant #3', and 'NuMex Sweet' were sown in 9 cm x 9 cm plant containers filled with a peat moss-vermiculite mix and placed on a plant propagation pad in a greenhouse to facilitate seed germination. The temperature inside the greenhouse had a diurnal range of 28 ± 1 C to 15 ± 1 C. The trays were watered daily, and a slow-release complete fertilizer was placed on the surface of each cell after germination.

Inoculating and incubation. The preparation of P. capsici inoculum followed the procedures of Bosland and Lindsey (1991) except that the inoculum concentration was adjusted to 5,000 zoospores per ml. Six- to eight-week-old seedlings were inoculated by placing 100-200 microliters of inoculum on the leaves. The trays containing the seedlings were placed inside a polyethylene tunnel. A humidifier was placed inside the tunnel to provide continuous wetness of foliage during the incubation period. The temperature inside the chamber fluctuated diurnally from 15 to 43 C.

Disease assessment and interaction phenotype scale. The plants were observed for symptom development 4 days after inoculation. Disease scoring was stopped when the infected leaves dropped off. Plants were scored using an interaction phenotype scale, where; 0 = no symptom, 1 = small necrotic lesions, 2 = 1-5% of total leaf area with scalded or had light tan lesions, 3 = > 5 to < 10% leaf area with lesions, 4 = > 10 to < 25% with lesions, 5 = > 25 to < 50% with lesions, 6 = > 50% with lesions, 7 = brown water soaked lesions around the petiole, 8 = dark lesions girdling around the stem, 9 = defoliated. A completely randomized experiment with three replicates of 6 seedlings per replicate was used.

The commercial cultivars tested were similarly susceptible to foliar blight. Scalding and light tan lesions were observed on all fully expanded leaves. Blight symptoms were observed on young leaves. The disease progressed to the petioles and main stem causing the leaves to drop off. Ten days after inoculation, all aerial parts of the plant were blighted. "Criollo de Morelos 334' was highly resistant to foliar blight. No symptoms were observed on leaves or stems. This accession has also proven to be resistant to root rot when inoculated with the same isolate of -P. cagsici (Bosland and Lindsey, 1991). This screening technique enabled the differentiation between susceptible and resistant plants. Continuous leaf wetness

during the incubation period contributed to the rapid development of the disease. The development of an epidemic by flagellated spores, like Phytophthora , depends on the presence of free water (Schlub, 1983). Hence, leaf wetness is a prerequisite for infection. The air was saturated inside the tunnel throughout the incubation and dew formed on the leaves. This condition is necessary to provide high humidity around the leaf surface as free water evaporates creating a microclimate favorable for sporangial formation (Harrison, 1992). f!. gagsici is a high temperature organism which requires an optimum temperature of 26-32 C for growth (Waterhouse et al., 1983). Though the temperature fluctuated inside the tunnel, a temperature as high as 43 C did not deter infection and disease development. By using a micropipette to place the inoculum on the leaves, the chance of zoospores entering the soil and the lower stem is delayed. Infection of the main stem and roots had been a problem in previous inoculations. With P. cagsici, the use of zoospores suspension has proven to be a more effective inoculum than mycelia preparation (Reifschneider et al., 1986). The use of sporanglal suspension may delay disease onset and development because they may germinate indirectly by releasing zoospores (Ribeiro, 1983). It is also important to recognize that the relative cheapness of the incubation chamber used in this study did not impair the differentiation between susceptible and resistant plants. This technique allows the plant breeder to screen at the seedling stage, thereby providing continuity of work because one can use the same resistant plants in breeding when the plants reach flowering. Resistance to foliar blight is controlled by a different genetic system than root rot resistance (Barksdale et al., 1984). We are now screening advanced lines with root rot resistance and populations of 'Criollo de Morelos 334' (Le, F,, F2, BCj) to better understand the genetics of resistance to foliar blight and root rot.

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ENHANCED Capsicum GERMPLASM RESISTANT TO VERTICILLIUM VALT

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Verticillium dahliae Kleb., is a soil-borne fungal pathogen that threatens Caosicum world-wide. To date, Verticillium wilt of Capsicum is without an effective and economical contr6l. Therefore, genetic resistance is a mechanism that has been suggested to solve this problem. Several attempts, however without success, have been done to find genetic resistance to Verticillium wilt in Capsicum germplasm (Woolliams et al., 1962; Lippert & Hall, 1963; Pest! et al., 1985; Lindsey & Iglesias, 1986). At New Mexico State University a source of genetic resistance to Verticillium wilt was found in USDA P.I. 215699, a -Q. annuuaccession (Gonzdlez-Saldn & Bosland, 1992). A soil infestation method with 2000 microsclerotia of V. dahliae per gram of soil and strict control over the soil temperature (25 ± VC), differentiates between susceptible and resistant plants.

A Verticillium wilt resistant population of Capsicum was developed from P.1 215699. A resistant plant that was selfed increased the percentage of resistant plants from 38% to 68%. Four cycles of selfing with disease screening and selection at each cycle were done. The experiments were performed using the soil infestation method with soil temperature tanks (Gonzalez-Saidn & Bosland, 1992). The variables statistically evaluated 70 days after planting were: interaction phenotype (IP) with a scale ranging from 1 to 9, where: 1 = no aerial symptoms and 9 = death, and plant height in centimeters. The percentage of resistant plants was calculated as the number of plants with IP = 1 divided by the total number of plants in that family and then multiplied by 100.

The results indicated that after four generations of screening and selection, P.I. 215699 still continues to segregate for Verticillium wilt resistance. However, the percentage of resistant plants increased to approximately 70% (Graph 1.). On the other hand, the height versus interaction phenotype correlation coefficient
0. 17) indicates that no correlation is present between plant height and resistance.

The segregation ratios for Verticillium wilt resistance in P.I. 215699 suggests that Verticillium wilt resistance is a quantitative trait. Additive and dominance genetic variance effects were determined for a better understanding of the genetic inheritance of Verticillium wilt resistance in this accession. A preliminary joint 3-factor scaling test (Mather and Jinks, 1982) to estimates the parameters [m] (mid-parent value), [d] (additive effects) and [h] (dominance effects) revealed that the data did not fit a simple additive-dominance model. Epistasis was suspected to be present and a joint 6- factor model was therefore tested. The joint 6-factor model estimates [m], [d], [h] and three epistatic interactions parameters additive x additive 01, additive x dominance al, and

dominance [11 (Mather and Jinks, 1982). All epistatic interactions parameters were significant. These preliminary results indicated that both additive and epistasis effects (Non-additive effects) were involved in the genetic control of Verticillium wilt resistance in P.I. 215699 and agree with the complexity and difficulty when working with this chile disease. Heritabilities were h2b.=0.81 and h 2 0. 53. Nevertheless, resistance is being introgressed from P.I. 215699 (68% of resistant plants) into jalapeno, bell, and New Mexican type Capsicums. For bell and jalapenpo types the F213C, have been screened, and the highly resistant plants were saved to generate the F213C2. In the New Mexican type, the F2 was screened, and the highly resistant plants were saved and backcrossed to produce the F2BC,.

INCREASE IN VERTICILLIUM WILT RESISTANCE
P.I. 215699

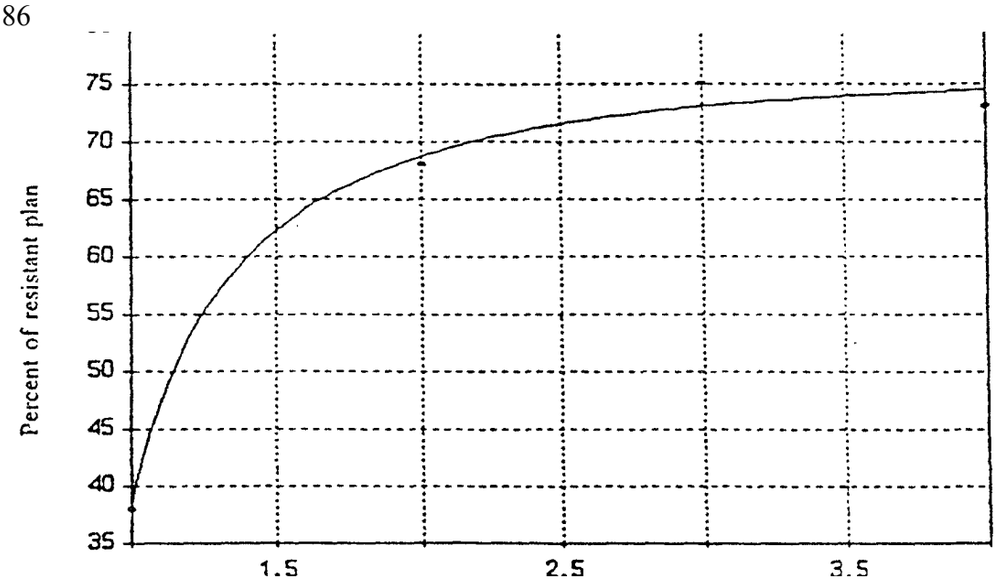
y = sqrt(a + bIX2), r2 = 0.992625768
a 5810.8599
b-4370.182

12

Generations

GRAPH 1. In each generation screening and selection was conducted.

86



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Production of hybrid pepper seeds with

Bombus terrestris as pollenizer.

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Long time pepper breeders, particularly those working with male sterility have been interested in increasing hybrid seed yield via insects pollination, particularly honey bees (Daskalov 1976, Csillery 1986 and others). Improving hybridization by insects pollination can save hand labour routinely used for producing commercial hybrid seeds. Using male sterile plants in different pollen parent combinations under open field conditions, yielded from 0 to about 100 seeds per fruit. In Israel, with honey bees, we did not receive yet higher yield than 55 seeds per fruit (Shifriss 1986).

Materials and methods

Parent plants: two male sterile lines, one "determinant" short and early (1788), and the second "indeterminant", tall which matures late (1647), 50 plants per line were planted in 4 rows. Between two male sterile plants, a normal fertile plant (line 3552) was planted and the spacing within and between the rows was 0.3 and 1 meter respectively. The experiment was performed under insect proof conditions.

Bombus terrestrss: a hive of the Bombus was supplied by Kibbuts Sede Eliahu Plant Protection Laboratories during the middle of July when the three parents were at full flowering. Following three weeks of pollination the hive was removed from the house. At fruit ripening a single harvest was made and the seeds per fruit were counted.

Results and Discussion

Comparable with 55 hybrid seeds per fruit, which were obtained in Israel under open field conditions, the Bombus has low pollination efficiency (Table 1). Specific low seed/fruit yield was obtained in 1788 though both females (1788 and 1647) had same number of open flowers and received the same number of visits from the Bombus.

In addition, when male sterile 1788 plants are hand pollinated they set

normal number of seeds. The low seed yield in 1788 is probably due to specific incompability between its flower and the structure of the Bombus.

Though the Bombus is relatively inefficient as pollenizer, still additional factors worth testing like the use of prolific pollen parents (female sterile mutants?) and more spacing between females and the fertile plants

Table 1. Hybrid seed yield of peppers obtained by Bombus terrestris as pollenizer.

Male sterile parents	No. of seeded fruits	No. of seeds Average/fruit	No. of seeds Average/plant	No. of seedless fruits Average/plant
1788	6.4 + 0.8	11.4 + 1.1	71.2 + 9.9	5.6
1647	5.4 + 0.6	21.5 + 1.9	116.5 + 13.2	4.7

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PRELIMINARY RESULTS ON REGENERATION AND GENETIC TRANSFORMATION OF SOLANUM SODOMEUM.

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Eggplant is susceptible to pests and diseases which reduce its production. Several agronomic traits (resistance to Verticillium dahlie, low temperatures, bacteria, nematodes) have been identified in the wild species S. torvum, S. integrifolium. S. sodomeum and S. sisymbrifoliurn (1,2). A method to transfer desirable traits from the wild species into the cultivated ones is somatic hybridization because protoplast fusion may offer the possibility of bypassing complex sexsual barriers (3).

This paper concerns the study on regeneration and transformation via Agrobacterium tumefaciens of S. sodomeum to introduce marker genes useful for somatic hybrid selection.

Seeds of accession no. 113 of S. sodorneurn were sterilized and placed onto sterile filter paper at 28'C (dark). After germination they were transferred to Murashige and Skoog's medium (4) containing 2% sucrose (MS20) at 250C, 16/8 light/dark cycle.

In the regeneration experiment MS20 medium supplemented with 2 mg/l glycine was used as basal medium. 6-benzylaminopurine (BAP), zeatin (ZEA) and thiadiazuron (TDZ) were tested at 0.1, 0.5, 1 or 3 mg/l. A total of 24 leaf explants (8/plate) were used for each treatment, after 4 weeks the number of shoots per explant was collected. At the concentrations tested, BAP (I mg/1) and ZEA (0.5 mg/1) promoted a proficient shoot differentation (Table 1). On the basis of these results regeneration medium supplemented with I mg/I BAP and 0.5 mg/I ZEA was utilized in genetic transformation experiments.

Two strains of A. turnefaciens were used for transformation: GV2260 pGUSINT (5) and LB4404 pRG2 (6). Bacteria were grown at 28'C, in dark, in LB medium supplemented with the appropriate antibiotics at 150 rpm. After 24 h, bacterial suspensions were centrifuged for 15 min at 3000 rev./min and the pellet resuspended in MS20 medium. Explants were immersed for 2 min in Agrobacterium suspension, blotted dry on sterile filter paper and placed upside down on MS20 plates. After a 2 days incubation at 28'C they were transferred to regeneration medium with 30 mg/l of Kanamycin and 500 mg/l of Cefotaxime. In the last experiment Kanamycin concentration was increased at 50 mg/l. Every 15 days explants were transferred to fresh medium with a lower concentration of Cefotaxime.

The effect of the following factors were evaluated: preculture and

co-cultivation media, explant sources, acetosyringone treatments and bacteria densities. The preculture and co-cultivation on medium with growth regulators allowed to recover Kanamycin resistant calli from leaf explants infected with pGUSINT strain (Table 11). The higher transformation efficiency was obtained at 0.04 O.D., no shoots was regenerated from these leaf-derived calli.

Cotyledons and hypocotyls resulted more suitable explant to obtain organogenetic callus after infection with Agrobacterium strains pGUSINT and pRG2. Acetosyringone improved the transformation efficiency of pGUSINT strain (Table Illa and Table Hlb).

Also the bacterial density affected the transformation frequency of hypocotyls and cotyledons. The O.D. (550) of 0.01 and 0.05 permitted to obtain an higher number of Kanamycin resistant calli and an higher shoot differentiation (Table IV). Bacterial density resulted significantly correlated to the transformation frequencies (Fig la-b and 2a-b). At the present the putative transgenic shoots are on selective rooting medium containing 25 mg/l Kanamycin, until now 17 shoots have rooted. Further enzymatic (GUS and NPTII) and molecular (Southern-blot) analyses are necessary to investigate the expression and integration of transgenes in S. sodomeum.

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Table 1. Response of a. soclomeu leaf section explants of to various combinations of RAP, ZEA and TDZ.

mg/l	Explants Explants; %	Explants No. of with rigen. %	No. of with shoots Xj: s	Explants shoots* %	Explants with rigen. %	Explants with shoots X± s	No. of shoots* %	Explants with rigen. %	with shootsshoots* Xt s
0.1	25	12	2.510.7	56	31	3.4±1.1	96	0	
0.5	94	81	4.8-t2.3	81	81	6.712.1	94	0	
1.0	88	88	6.6-t1.4	75	69	5.5.j1.2	80	0	
3.0	75	62	3.2±1.4	75	75	3.3-tI.I	72	0	

*** Only explants with shoots have been considered.**

Table 11. Regeneration frequency in a. sodomeum leaf explants using pGUSINT strain at different densities (O.D.550). The explants; were incubated on NE20 with (+) or without (-) growth regulators.

Growth regulat.	O.D tested	Explan ts No.	Explants with calli No.	Calli	Transformation efficiency No.	No. shoots %	X't s
0.1		46	0				
*	0.1	104	43	43		41	0
*	0.04	56	so	so		89	0

Table Illa. Effect of acetosyrinaone on cotyledon explants transformation using pGUSINT and pRG2 strains.

Strain	Treatment-	Explants tested No.	Calli shoots No.	Transformation efficiency %	CaWExplants; with regen. No.	shoots X-ts	with No.
PGUSINT	1	44	50	114	21	2.4+1.4	18
	2	79	99	125	34	3.2±1.1	34
	3	40	34	85	12	2.0+0.7	8
pRG2	1	42	38	90	21	2.1+1.1	21
	2	41	24	58	16	1.9±0.7	12
	3	42	17	40	9	2.2±1.1	9

Table Illb. Effect of acetosyringone on hypocotyl explants transformation using pGUSINT and pRG2 strains.

Strain	Treatment-	tested No.	Calli No.	Explants efficiency %	with regen. No.	shoots Vts	with shoots No.
Transformation	-- - -------	Calli					
pGUSINT	1	63	48	76	17	1.9+0.9	17
	2	64	64	100	26	2.9±3.2	26
	3	73	57	78	22	1.1±0.3	22
pRG2	1	70	53	76	10	2.2±1.0	8
	2	68	64	94	24	2.5±1.3	19
	3	75	97	129	14	1.9±0.9	14

-Treatment 1 - without acetosyringone, 2 = acetosyringone (200 ILM) in infection medium, 3 = explants incubated in NIS20 supplemented with acetosyringone (50 gW for 2 hours.

BAP 7FA TDZ

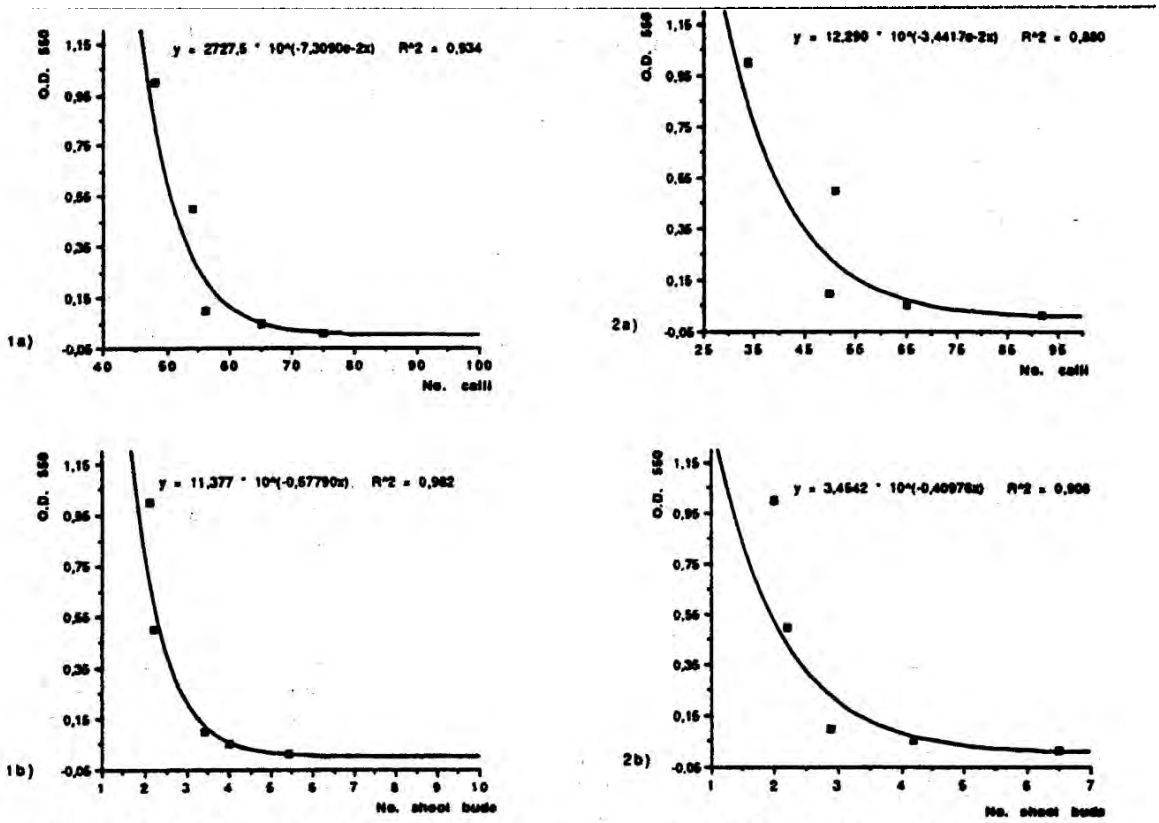

Fig. 1a,b - Effect of bacterial density on cotyledon explants transformation.

Fig. 2a,b - Effect of bacterial density on hypocotyl transformation.

Table IV. Effect of bacterial density (O.D. 550) on hypocotyl and cotyledon explants transformation using pGUSINT strain.

	COTYLEDONS					HYPOCOTYLS				
O.D.	No. explants tested	Explants with calli No.	Calli No.	Transformation efficiency %	No.shoots X±s	No. explant tested	Explants with calli No.	Calli No.	Transformation efficiency %	No. shoots X±s
0.01	36	33	75	200	4.2±2.9	50	50	92	184	1.7±1.2
0.05	37	37	65	176	4.4±3.2	50	49	65	130	2.4±1.9
0.1	36	34	56	155	1.0±0.0	50	25	50	100	1.2±0.4
0.5	36	31	54	150	3.7±2.5	52	28	51	98	1.0±0.0
1.0	36	32	48	133	1.6±0.6	50	26	34	68	1.1±0.4

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NEW SOURCES OF RESISTANCE TO BACTERIAL WILT IN EGGPLANT

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Introduction

Eggplant (Solanum melongena L.) is one of the important vegetable crops cultivated in India. Several eggplant varieties and land races are being cultivated in different parts of the country depending on the regional specificity for its colour, shape and size.

Recently the threat posed by bacterial wilt (Pseudomonas solanacearum) has limited its successful cultivation in important eggplant cultivated areas. Losses between 75 and 81 per cent have been recorded in India (Sequeira and Kelman, 1976).

Development of resistant varieties seems to be the best way to combat this disease, since no other methods can practically control this disease. A systematic breeding programme is being carried out at this Institute for over two decades with a view to develop wilt resistant varieties, catering to different consumer preferences. As a result, three varieties with purple long fruits viz., 'Arka Nidhi', 'Arka Keshav' and 'Arka Neelakanthl highly resistant to bacterial wilt have been released for commercial cultivation.

Screening for additional sources of resistance to bacterial wilt was carried out in order to isolate bacterial, wilt resistance in all types catering to various consumer preferences like green long and green round.

Materials and Methods

Sixty four lines were field screened for wilt resistance during the year 1991. Among the lines tested, four lines viz., IHR 85, 1HR 180, IHR 181 and IHR 182 had high levels of resistance. A confirmatory study was undertaken during 1992 to assess the stability of resistance in these lines, along with 3 susceptible checks, viz., 'Arka Kusumakar', 'Manjarigota I and 'Black Beauty'. Forty days old eggplant seedlings were planted out at s acing of 90 x 40 cm in wilt sick soil (bacterial conc. 10^7 cfu/g) , and fertilized with 120 kg N: 80 kg P: 50 Kg K per hectare.

Three replications of 10 seedlings each were maintained for each entry. Inoculations were made with the bacterial suspension (at 0.7 OD) after 20 days of transplanting by employing puncturing method as suggested by Winstead and Kelman (1952).Bacterial suspension was prepared by cutting

small pieces out of the collar region of infected susceptible plants, washing them in clean water and dipping cut pieces in distilled water just sufficient to cover the pieces for a period of 6 hours. The bacterial suspension thus obtained was filtered through Whatm.an filter paper No. 40. The optical density was adju6ted to 0.7 OD by adding required quantity of distilled water and the reading was noted at 600 nm using Spectronic-20 spectrophotometer.

The data on plant survival was recorded up to 125 days after planting at 30 days interval. First observation was recorded on the day the wilting symptoms were observed in susceptible checks. Susceptibility to bacterial wilt was further confirmed by ooze tests.

Results and Discussion

The results indicated that wilting in all the check varieties viz. 'Arka Kusumakarl, 'Manjarigotal and 'Black Beauty' was 100 per cent by 65th day after transplanting (Table 1). The first wilting symptoms were observed in all the susceptible checks by 35th day after transplanting.

Survival in IHR 180 and IHR 181 was 100 % even after 125 days of planting, whereas in IHR 182 and IHR 85 it. ranged from 70 to 80 % respectively. Complete wilting in the check varieties indicated that the screening technique adopted was perfect and no susceptible plant escaped infection.

Cent per cent survival in the lines IHR 180 and IHR 181 even after 125 days of planting indicated that the resistance in both these lines was stable and complete. Higher per cent of survival in IHR 182 and IHR 85 indicated high levels of resistance in these lines. As all the four lines exhibit high levels of resistance to wilt they can be utilized as new sources of resistance in breeding varieties with green round, green long and pink long types.

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Table 1: Percent survival in eggplant lines

Lines/varieties	Days after transplanting				Ranarks
	35	65	95	125	
'Arka Kusumakar	90	0	0	0	
'Manjarigotal	97	0	0	0	
IHR 85	100	97	90	80	Collectionf rcrn U.S.A. (BC No. 164453), pink long fruits
IRR 180	100	100	100	100	Local collection fran Bellary Dist. Karnataka. Fruits green, round large (500g)
IHR 181	100	100	100	100	Local collection fran costal Karnataka. Fruits green, round, large (400 g) calyx thorny, creamy patches at stylar ena..
IHR 182	100	70	70	70	Local collection fran costal Karnataka. Fruits green,long, calyx thorny.
'Black Beauty'	93	0	0	0	

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