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GENETIC ASPECTS OF RESISTANCE TO
PSEUDOMONAS SOLANACEARUM E.F. SMITH
IN POTATO

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PROPOSITIONS

1. Although not stated by the authors, the results by Rowe & Sequeira (1969)
   also imply that resistance to *Pseudomonas solanacearum* in *Solanum phureja*
   is very complex and epistasis is important in the inheritance of
   resistance.

> (Sequeira, L. and P.R. Rowe,1969. Selection and utilization of
> *Solanum phureja* clones with high resistance to different strains of
> *Pseudomonas solanacearum*. Am.Potato J.46:151-162.)

2. To protect potato against *Pseudomonas solanacearum*, resistance to bacterial
   wilt alone is not adequate.

3. Genetic engineering may be able to provide a general type of resistance to
   Pseudomonas solanacearum by inserting the cecropin production encoding gene
   into the potato genome. Such resistance, however, may not be durable.

4. The genes bringing about the resistance to *Pseudomonas solanacearum* in
   potato are not true genes for resistance in the context of gene-for-gene
   relationship.

5. Strain specificity in the potato-*Pseudomonas solanacearum* relationship is
   a reflection of strong host genotype x environment and/or pathogen genotype
   x environment interaction.

6. Scientists can be either generalists or specialists. A good specialist may
   be either an inventor or a policy maker. A good generalist may often be a
   policy maker, but hardly an inventor.

7. True beauty is something very natural and appealing to every one. Any
   beauty that is vulnerable to discrimination among people is not a true
   beauty.

8. The level of development of a nation or a country can be judged, among
   other things, by the amount of packaging and printing materials it wastes
   per unit of time and per unit of population.

9. Human survival depends on agriculture for food supply. Agricultural
   development, however, can not lead any nation to prosperity.

10. One's identity depends on one's contribution to one's community, but
    belongs to one's people.

11. Chocolate is consumed most where cocoa is not grown.

Pham X. Tung

1992

De publicatie van dit proefschrift is mede mogelijk gemaakt door een financiële bijdrage van het LEB fonds (Landbouw Export Bureau)

P.S. Dit inlegvel behoort bij het proefschrift "Genetic aspects of resistance to <u>Pseudomonas solanacearum</u> EF Smith in potato" door Pham Xuan Tung

ABSTRACT

The genetic control of resistance to *Pseudomonas solanacearum* in potato is complex and may involve both genes with major effects and genes with minor effects. However, no evidence of a gene-for-gene relationship between the host and the pathogen has been as yet documented. Strain specificity in the potato-*P. solanacearum* pathosystem is of the polygenic quantitative type and is probably a reflection of differential adaptation of host genotype and pathogen genotype to environments. Thus the resistance is chacterized by strong host x pathogen x environment interaction and tends to break down whenever faced with environmental conditions the host is not well adapted to. Expression of the resistance is heavily dependent on the adaptive potential of the carrier host genotype to a particular environment. Under heat stress conditions, heat tolerance strengthens expression of resistance. Interaction between genes for resistance and genes for adaptation was evident. In the inheritance of resistance, both additive and non-additive gene actions are significant. The relative magnitudes of the estimates of genetic variance components indicated that non-additive gene effects are more important. The narrow-sense heritability of resistance is low, but broad-sense heritability is relatively high. This indicates that clonal selection would be successful in developing clonal cultivars, but progress in population improvement would be slow.

CONTENT

Chapter I

GENERAL INTRODUCTION

Bacterial wilt (BW), caused by *Pseudomonas solanacearum* E.F. Smith, is one of the most destructive bacterial diseases of many crop plants belonging to over 35 families (Kelman, 1953) in the tropical, subtropical and warm temperate regions. Major economic hosts are tomato, potato, tobacco, pepper, eggplant, peanut, banana and ginger. Losses of up to 90 % in peanut (Machmud, 1985), 95 % in tomato, 80 % in eggplants and 50 % in tobacco (Agati, 1949) have been reported. High disease severity is mostly observed under warm humid growing conditions. For most of the pathogen strains the optimum temperature for disease development is between 25-35 °C.

For the potatoes, BW is considered the second important disease in the tropics after late blight (*Phytophthora infestans* (Mont.) Bary). Losses of up to 70% have been reported (Singha, 1985). The disease tends to become a major obstacle in the expansion of potato production into the lower elevation areas where high temperatures are prevalent and environmental conditions are most inducive for disease development.

The most common practical control measures are crop rotation and use of clean planting materials (Persley et al, 1985; Vander Zaag, 1985; and French 1988). Field after flooded rice (Acasio et al.,1983; and French, 1988) and a five-month bare fallow (Jackson et al.,1979) are also suitable for planting potato in the lowland tropics. Chemical control has not been proven to be economically and practically feasible and effective against BW (Enfinger et al.,1979; and Katayama et al.,1984). The use of microbial antagonists, such as fluorescent pseudomonads, *Bacillus polymixa* and spontaneous avirulent mutants of *P. solanacearum* for biological control seems promising (Kempe & Sequeira, 1983; Geels & Shippers, 1983; Aspiras et al.,1985; and Ciampi-Panno et al, 1989), but has not yet gained any practical application.

The use of resistant cultivars is probably the simplest and the most economical way of controlling the disease, especially in areas where cultivated lands are limitted and cultivation is intensive. However, this approach has been successful in some cases , but not in others. The resistance tends to break down under warm temperatures (Sequeira, 1979; Schmiediche 1985a & b; and Schmiediche & Martin, 1983). Strong host-pathogen-environment interactions in the potato-*P. solanacearum* pathosystem has been a major

challenging problem in breeding potatoes for resistance to wilt. Over the last two decades, the problem has not been solved and resistant materials have been gaining limited use mostly in restricted highland areas, where BW is usually not at high pressure (Schmiediche, 1985b).

As no reliable resistance is found in the common potato, *Solanum tuberosum* L. (Thung, 1947; and Nielsen & Haynes, 1960), breeding for resistance to BW during the 1970's had been concentrated on exploitation of the resistance derived from a few diploid *S. phureja* Juz. & Buk. clones (Sequeira and Rowe,1969; and Sequeira, 1979). The resistance, however, has been shown to be very unstable due to its major drawbacks: strain specificity and temperature sensitivity (Sequeira & Rowe, 1969; Sequeira, 1979; French & De Lindo, 1982; Schmiediche, 1985a & b; Schmiediche & Martin, 1983).

Despite the many efforts made to search for and to breed for a more stable type of resistance , little is known about the genetics of resistance to wilt. A genetic model of three independent dominant major genes (Rowe & Sequiera, 1970; and Rowe et al., 1972) does not seem to fit the complex situations of host-pathogen interaction. A series of later experiments gave contradictory results which do not support the hypothesis (Sequeira, 1979). More recent practical observations show that expression of resistance probably depends on the adaptability of the resistant host genotype to a particular environment (Kloos & Fernandez, 1986; Schmiediche, 1985a; and Tung et al., 1990b). Under hot conditions, certain heat tolerant potato clones, not known for any resistance, tend to display a higher level of resistance than those with some resistance known *a priori* (Schmiediche, 1985a; and Tung, unpublished). Genes for adaptation are seemingly involved and the resistance seems to be much more complex than it was initially thought. The contradictory results of genetic investigations by Rowe and co-workers and involvement of genes for adaptation (Tung et al.,1990b) indicate that resistance is probably polygenic in nature. Acosta & Obrero (1978) also showed that resistance to BW in diploid *S. phureja* is a polygenic trait rather than being under a simple genetic control. Preliminary evidence from the breeding program at the International Potato Center (CIP) alco indicates that BW resistance in some of the wild *Solanum* species is probably polygenic (Schmiediche, 1985b). Although practical breeding keeps going on and some encouraging results have been obtained (Schmiediche, 1985 a & b), more research is needed to fully characterize the

genetic aspects of the potato-*P.solanacearum* relationship. Detailed understanding of the genetics of the pathosystem is necessary for development of a general and durable type of resistance to BW.

The investigations described in this thesis deal with several genetic aspects of resistance to BW in the tetraploid potato. Resistance to wilt is derived from different source species and evaluated either separately or as being pooled together in a common genetic background of a clone or a population. Chapter II gives a brief review of the most recent achievements published in the liturature regarding the genetic aspects and mechanisms of pathogenicity of the pathogen and of resistance in the host. Chapter III demonstrates how important a heat tolerant genetic background of potato clones is for expression of BW resistance under heat stress conditions and its stability over environments. Chapter IV describes the effects of heat tolerance genes, resistance genes and cytoplasms on expression of resistance to BW in F_1 segregating populations. Chapter V shows that BW resistance in potato is in fact a partially dominant character with significant non-additive gene effects which are important for expression of resistance. Chapter VI presents estimates of genetic variance components and heritability obtained for a tetraploid potato population with a broadened genetic background for resistance and adaptation and their implication for resistance breeding. Finally, a general discussion is presented in Chapter VII to tie together the most important facts and understandings relevant to this thesis in attempt to attain to major conclusions and perspectives for further research and breeding for resistance.

Chapter II

THE POTATO-*PSEUDOMONAS SOLANACEARUM* RELATIONSHIP:

ASPECTS OF RESISTANCE AND PATHOGENICITY

THE PATHOGEN

Intra-specific variation

P. solanacearum is characterized by its wide host range and tremendous intra-specific variation (Buddenhagen & Kelman, 1964; French ,1979; Hayward, 1964; and Hayward, 1988). Presently, it is classified into four races and five biotypes (Hayward, 1988). The race classification is based on host range (Buddenhagen & Kelman, 1964). Races 1 and 3 attack potato under natural conditions. Race 1 is prevalent in the mid and lower elevations of the tropics and causes wilt in a large number of host plants (Kelman, 1953). Race 3 specializes on potato at high elevations and latitudes, but occasionally infects tomato. Race 2 predominantly affects bananas, plantains and Heliconias (Buddenhagen & Kelman, 1964), but may infect potato upon artificial inoculation (Thurston & French,1972; Sequeira & Rowe, 1969). Race 4 has recently been discovered in China to affect only mulbery; it is only weakly virulent on potato and eggplant (He et al, 1983).

Hayward (1964) classified 185 isolates of *P. solanacearum* into four biotypes (biovars, Bv) according to their ability to oxidize three disaccharides (lactose, maltose and cellobiose) and three hexose alcohols (manitol, sorbitol and dulcitol). Bv I is characterized by its inability to utilize either of these carbohydrate groups. Bv II is capable of oxidizing the disaccharides, but not the hexose alcohols. Bv III ,however, can use both groups of carbohydrates and Bv IV only the alcohols. Bv V coincides with Race 4 and utilizes all the three disaccharides and manitol, but not dulcitol and sorbitol (He et al, 1983).

In relation to host range, Bvs I & III have a series of host plants belonging to a large number of families. Bv II attacts only the species of *Solanaceae*, and Bv IV only members of *Solanaceae* and *Zingiberaceae*, and Bv V only mulbery (and potato and eggplant upon artificial inoculation). In almost all cases, Bv II coincides with race 3 and is often referred to as "potato race". Bv I, III, and IV do not appear to have a strict natural relationship to pathogenic capabilities. Thus race 1 is the most complex in host range and includes Bv I, III and IV in Africa, America, Asia, Australia and the Pacific

islands. Race 2 encompasses Bv II and III and has until recently been restricted to Cental and South Americas and half way around the world into the Southern Philippines (French, 1979 and 1985; and Buddenhagen, 1985).

Recent research has shown that strains of *P. solanacearum* can be divided into two major divisions based on their coefficients of similarity (Cook et al, 1989). Division I includes all members of race 1 -Bv III and IV, and race 4 -Bv V. Division II includes members of race 1 -Bv I, and race 2 and race 3. All members of Division II originate in the Americas: race 2 on Heliconias and bananas in Central America, and race 3 on potatoes in South America. Race 1 - Bv I is probably a variant of race 2 or race 3, as indicated by their coefficients of similarity. Occurrence of race 2 and race 3 outside of South America is probably a result of distribution of infected potato tubers and banana rhyzomes. Members of Division I evolved independently elsewhere around the world and on diverse hosts. Geographic isolation is suggested to be the reason why *P.solanacearum* has evolved as two separate groups of strains (Cook et al.,1989).

Mechanism of pathogenicity

Unlike in some other bacterial diseases, such as the bacterial blight of soybean (*P. syringae* pv *glycinea*) , halo blight of bean (*P. syringae* pv *phaseolicola*), where involvement of a phytotoxin is well known as a virulence factor, the mechanism of pathogenicity in BW disease has not been clearly determined. Occlusion of xylem vessels in the stem of host plants by extracellular polysaccharides (EPS) produced by the bacterium has been suggested to be the most important pathogenicity mechanism (Kelman, 1953; Buddenhagen & Kelman, 1964; and Husain & Kelman, 1958). However, research results are contradictory.

Staskawicz et al.(1983) found that mutants, that are deficient in EPS production, also lack their ability to cause wilt on potato plants. Similar results were obtained by Denny et al.(1988) on tomato. Drigues et al.(1985) demonstrated essential differences in biochemical properties between EPS produced by virulent and avirulent strains suggesting that EPS is important in pathogenicity of *P. solanacearum*. There is, however, evidence that EPS-

deficient mutants can still wilt tomato plants and are almost as virulent as the wild type parent strains (Boucher et al.,1985; and Xu et al.,1988 & 1990). More recent research (Denny et al.,1990 & 1991) provides conclusive evidence that EPS is indeed an important pathogenicity factor required by *P. solanacearum* to wilt host plants. The role of EPS as a major pathogenicity determinant has also been established in corn-*Erwinia stewartii* pathosystem (Torres-Cabassa et al.,1987; and Coplin & Majerczak, 1990).

There has been no experimental evidence of any relationship between pathogenicity and other biochemical activities such as production of plant growth substances or synthesis of phytoalexins or phytotoxins in the vascular wilt disease of potato. Some extracellular enzymes seem to play a certain partial role in pathogenesis. Reduced polygalacturonase and endoglucanase activities have been shown to be associated with reduced virulence of *P. solanacearum* on tomato, but the genes encoding these enzymes are not strictly necessary for pathogenicity (Daniels et al.,1988; Robert et al.,1988; and Allen et al.,1991).

Genetics of pathogenicity

During the last few years, molecular genetics has revealed that the genetics of pathogenicity of *P. solanacearum* is very complex. There are probably several groups of genes which control the ability to cause wilt.

Impairments in EPS production *in planta* is well correlated with the rate at which EPS-impaired and EPS-deficient mutants of *P. solanacearum* wilt tomato plants (Denny et al., 1988 & 1990). The genes encoding EPS production (eps genes) are mapped on two neighboring regions of the bacterial chromosome (Denny et al., 1991). The first region spans over a 9 kb deoxyribonucleic acid (DNA) sequence and includes at least two genes the expression of which requires another functional gene (phcA, phenotypic conversion A), that, when mutated, affects expression of multiple traits related to pathogenicity (Brumbley & Denny, 1990). The second region has a minimum size of 2.6 kb and likely contains a single gene which is also regulated by phenotypic conversion. The two regions probably do not contain all genes for EPS production in *P. solanacearum*. There are also other genes with similar

function that are induced by nutrient limitation (Denny et al., 1991). Besides the amount of EPS produced, the rate of EPS production is also an important virulent factor (Denny et al.,1991). All these findings confirm that EPS is important in pathogenicity.

Mutational analysis has also led to identification of another group of genes which specifies both the ability of the bacterium to elicit the hypersensitive response (HR) in an incompatible interaction and to cause wilt disease in a compatible interaction (hrp genes). Most of these genes are clustered and carried by a megaplasmid (>1000 kb)(Boucher et al.,1986 & 1987). They map on a region of 22 kb (Boucher et al.,1987; Arlat et al.,1989). There are at least nine transcriptional units which are all expressed on minimal medium with addition of plant extracts. On rich medium, the effects of plant extracts on expression of hrp genes is not clearly obtained. This indicates that hrp genes are responsible for plant-bacterium recognition (Arlat et al.,1989). There is a second cluster of 7 kb which shares no homology with the first cluster and contains at least two possible transcriptional units (Huang et al.,1990). Mutants defective in both gene clusters usually differ from the wild type parent strains in several aspects including loss of ability to induce the HR on tobacco leaves, loss of pathogenicity, methionine auxotrophy, and production of a brown pigment. These pleiotropic effects have been shown to be associated with a large deletion or loss of the megaplasmid (Boucher et al.,1986).

Southern hybridization and complementation analyses further reveal that hrp genes are highly conserved with respect to structural and functional homology among strains of *P. solanacearum* and *X. campestris* pathovars (Boucher et al., 1987 & 1988; Cook et al., 1989; Barlow et al., 1990; and Arlat et al., 1991). This indicates that the two pathogens employ common strategies and mechanisms to attack plants and that host plants might share some common mechanisms of HR to these two pathogens.

Though involvement of hrp genes in HR and pathogenicity is clear, little is known about their biological functions in pathogenesis. They are not involved in regulation of production or export of extracellular enzymes and EPS (Willis et al.,1991; Arlat et al., 1991). In *P. syringae* pv *glycinea* hrp mutations inhibit the transcriptional induction or function of other genes

involved in the plant-bacterium interaction (Huynh et al.,1989). In *P. syringae* pv *tabacci* , hrp mutants which do not induce the HR on tobacco are still able to induce plant genes that have been associated with disease resistance (Jakobek & Lindgren, 1990; and Lindgren et al.,1989). Apparently, hrp genes seem to play a central role in the ability of phytopathogenic bacteria to interact with plants.

The existence of two other groups of genes : disease specific (dsp genes) and avirulence genes (avr genes) proposed by Boucher et al.(1987) has not been extensively studied and experimentally established. Ma et al.(1988) showed that some of the gens located in a 12.8 kb bacterial DNA fragment carried by a cosmid clone were involved in host specificity at the species level, but no presumptive avirulence genes seem to exist.

With the present knowledge, there seem to be at least two major independent sets of genetic factors essential for pathogenicity in *P. solanacearum*. One set involves the genes necessary for the coordinated production of degradative extracellular enzymes (e.g. polygalacturonase and endoglucanase) and EPS. The other includes the hrp genes. Detailed characterization of these two sets of genetic determinants of pathogenicity and determination of component genes and their biological functions are yet to be done in order to fully understand the genetics of pathogenicity of *P. solanacearum*. This understanding is important since by interfering with or suppressing expression of these genes new methods of protection of susceptible crops from BW would be developed.

HOST RESISTANCE

Almost all studies concerning the various aspects of resistance to BW in potatoes have been carried out on the resistance derived from *S. phureja*. This is probably because this resistance has been the first source found to be reliable and the source most extensively used.

Mechanism of resistance

In spite of the many efforts to search and to breed for resistance, little is known about its mechanism. There has been no conclusive evidence of any pre-existing or induced resistance mechanisms. In resistant potato clones, *P. solanacearum* multiplies at a relatively slow rate and does not spread rapidly from the point of inoculation. In contrast, in susceptible clones , the bacterium multiplies rapidly and kills the plants within a few days (Sequeira, 1979; and Bowman & Sequeira, 1982). Inhibitors of bacterial growth produced in potato plants seem to affect *P. solanacearum* in a general way rather than in correlation with resistance (Zalewski & Sequeira, 1973 & 1975; Sequeira, 1979). Agglutination of the bacterial cells by plant lectins was first an encouraging indication of a mechanism of resistance (Sequeira & Graham, 1977). However, there is no correlation between patterns of agglutination and those of resistant or susceptible interactions. Differences in agglutination could be correlated with the amount of slime produced by each individual bacterial strain rather than with its inherent pathogenicity on a particular potato clone (Sequeira, 1979; and Dudvick & Sequeira, 1984). Vander Plank (1978) has discussed and ruled out the possibility that plant lectins are involved in plant-microbe recognition and thus disease specific reaction. Attachment to and envelopment of bacterial cells in host cell walls seem to be associated with the type of induced resistance known as HR in an incompatible interaction (Sequeira et al.,1977). This attachment and envelopment of bacterial cells by plant cell wall components, however, is also a general phenomenon and does not correlate with a particular level of resistance (Sequeira, 1979). Slusarenko & Wood (1983) indeed demonstrated that differential attachment of cells of two races of *P.syringea* pv *phaseolicola* to bean cell walls in intercellular spaces is not responsible for the specific resistance of cv. Red Mexican to the race 1 isolate they used. There is no evidence of association between other chemical compounds such as phytoalexins or other phenolic compounds and resistance to BW. The nature of resistance is not yet clearly understood. Several different resistance mechanisms are probably involved in the ability to resist wilt.

Genetics of resistance

Inheritance of resistance to BW in *S. phureja* was first investigated by Rowe

and co-workers (Rowe & Sequeira, 1970; and Rowe et al.,1972). They reported a model of three independent dominant major genes which control the resistance: one acts as a basic gene for resistance and the other two for specificity agaist two race 1 isolates of *P. solanacearum*. Zalewski (cit. Sequeira, 1979) later postulated four major genes for resistance to wilt. Results of later experiments, however, did not support the initial hypothesis (Sequeira, 1979). Logically, a simple system of three dominant major genes does not seem to fit the complex situations in host-pathogen-environment interaction and the tremendous variability in pathotypes of *P. solanacearum*. Little is known about the genetics of BW resistance in other *Solanum* spp.. Preliminary evidence from the CIP breeding program indicates that the genetic base for resistance is different in each species and is probably polygenic in some of them (Schmiediche, 1985b). In fact, Acosta & Obrero (1978) also reported that resistance to BW in diploid *S. phureja* is more of a polygenic type than being under simple genetic control. Polygenic resistance to *P. solanacearum* has also been reported in tomato (Acosta et al.,1963) and tobacco (Smith & Clayton, 1948). As more evidence becomes available (see next sections and Chapters), the genetics of resistance to BW in the potato appears to be much more complex than initially thought.

Specificity of resistance

Race-cultivar specificity does not hold in the potato-*P. solanacearum* relationship. Races of *P. solanacearum* are classified on the basis of host ranges which apparently are overlapping. Race 2, for example, specializes on bananas and Helconias, but occasionally affects potatoes (Sequeira & Rowe, 1969). Similarly, race 4 is said to specialize on mulbery, but is also pathogenic to eggplant and potato (He et al.,1983). Race 1 covers a wide host range including, in addition to potato, many plant species belonging to a number of families (Buddenhagen & Kelman, 1964). Strong host-pathogen interaction observed in the potato-*P. solanacearum* relationship is due to strain specificity (Sequeira & Rowe, 1969; Sequeira, 1979; and French & De Lindo, 1982). Strains belonging to a particular race or biotype differ greatly in their pathogenic potential as well as in other characteristics (McLaughlin & Sequeira, 1989; and Prior et al.,1990).
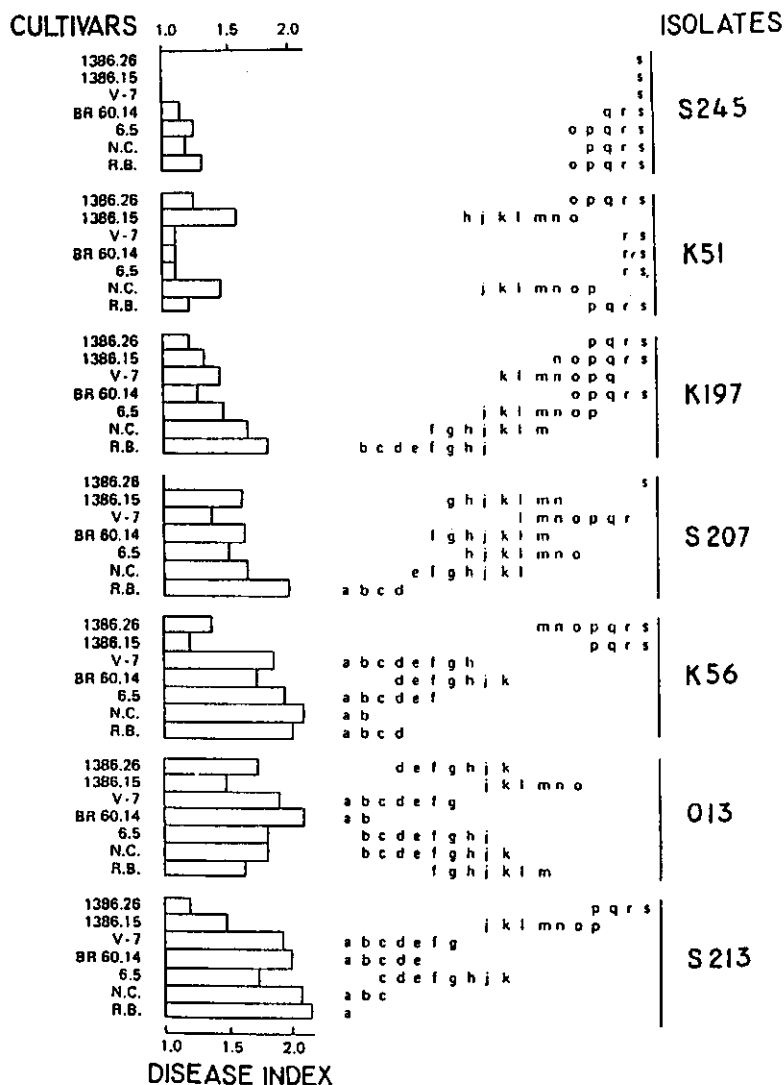
Figure 1. Disease indices for the interaction of seven potato cultivars and seven strains of *P.solanacearum*. Horisontal bars show average readings on a scale ranging from 1=no symptoms to 5=complete wilt or plant death. Bars followed by the same letters are not significantly different, P=0.01. (French and De Lindo, 1982).

Host-pathogen interaction, and hence strain specificity, in the potato-*P. solanacearum* pathosystem seems to be more of the polygenic type as described by Parlevliet & Zadoks (1977). French & De Lindo (1982) tested seven potato clones for resistance to four strains of race 3 and three strains of race 1 of *P. solanacearum* . Disease reaction was recorded 15 days after inoculation based on an index scale from 1 to 5, 1 being the most resistant (no wilt symptoms) and 5 the most susceptible (complete wilt). Significant host-pathogen interaction was found and the ranking order of potato clones in terms of reaction to disease tended to change from strain to strain (Fig. 1). A closer examination of the results, however, indicate that though interaction is significant, the effects due to host genotypes and pathogen strains are more important. Potato clones varied in level of resistance to different strains, but the variation is more or less between the scores 1 and 2, displaying various levels of incomplete resistance/susceptibility to the strains. Complete resistant or susceptible reactions typical for major gene resistance is not observed in this case. Similar results were obtained by Thurston & Lozano (1968) in testing several *S. phureja* clones for resistance to a race 2 and a race 3 isolate. Tung et al. (1990c) also showed that interaction effects , though they may be highly significant, comprise only a small fraction of the total variation in a test, and the main effects due to host genotypes and pathogen isolates are the major source of variation. Many genes seem to be involved in controlling resistance expression and the resistance is quantitative. Hypersensitive resistance or immuntity to BW is not known as yet in potatoes.

Interaction with environment

Abundant evidence indicates that expression of resistance to BW is strongly influenced by environmental conditions. The resistance tends to break down under high temperatures (Thurston & Lozano, 1968; Sequeira & Rowe, 1969; Ciampi & Sequeira, 1980a; French & De Lindo, 1982; Schmiediche, 1985a & b; and Schmiediche & Martin, 1983). This is also true with the resistance derived from other sources (Tung et al.,1990b & c; and this thesis). Low light intensity and shortened photoperiods reduce resistance to wilt in potato (Sequeira & Rowe, 1969) and in tomato (Kranz & Thurston, 1968). When tests for

resistance of a set of potato clones to different isolates of the bacterium are conducted over a range of environmental conditions, host-pathogen-environment interaction tends to become even more complex. This strong host-pathogen-environment interaction has been a major obstacle to breeding for stable resistance. So far, there has been no single resistant potato cultivar which can be grown widely under diverse growing conditions, as far as resistance to BW is concerned.

Recent observations suggest that resistance to BW in potato might be a function of adaptation (Kloos & Fernandez, 1986; Schmiediche, 1985a). Genes for adaptation seem involved in resistance expression as heat tolerant potato clones contribute greatly to the level of resistance of their F_1 progenies under hot conditions (Tung et al.,1990b). Also, potato clones/populations with a widened genetic background for both BW resistance and adaptation showed higher levels of resistance which , in addition, tends to be more stable over environments (Tung et al.,1990b; and this thesis).

Resistance to latent infection

BW resistant potato clones often yield tubers which are latently infected without any visible above-ground symptoms . Potato clones differ in their ability to resist latent infection, but few have been identified as resistant (Ciampi & Sequiera, 1980b; Ciampi et al., 1980; and Granada, 1988). In a sense, this BW "resistance" contains some elements of tolerance to P. *solanacearum*. Little is known about resistance to latent infection. It is not associated with resistance to wilt and is probably controlled by different genetic factors (Ciampi & Sequiera, 1980b). Almost nothing is known about the mechanism and genetics of resistance to latent infection.

Breeding for resistance

Early studies of Thung (1947) and Haynes (1960) indicated that resistance to BW in *Solanum tuberosum* L. is probably inadequate to control the disease. Breeding efforts during the early 1970's in the University of Wisconsin had

been concentrated on exploitation of the resistance derived from several *S. phureja* Juz. & Buk. clones (Sequeira & Rowe, 1969, Sequeira, 1979). From the breeding progam, a large number of resistant tetraploid potato clones have been bred and distributed worldwide (Sequeira, 1979; and Schmiediche, 1985b), among them the series coded BR, MS, PS, and PSW are probably the best known. The pedigrees of several BR-clones are presented in Figure 1.1. Unfortunately, only few of those clones have been successful as released cultivars.
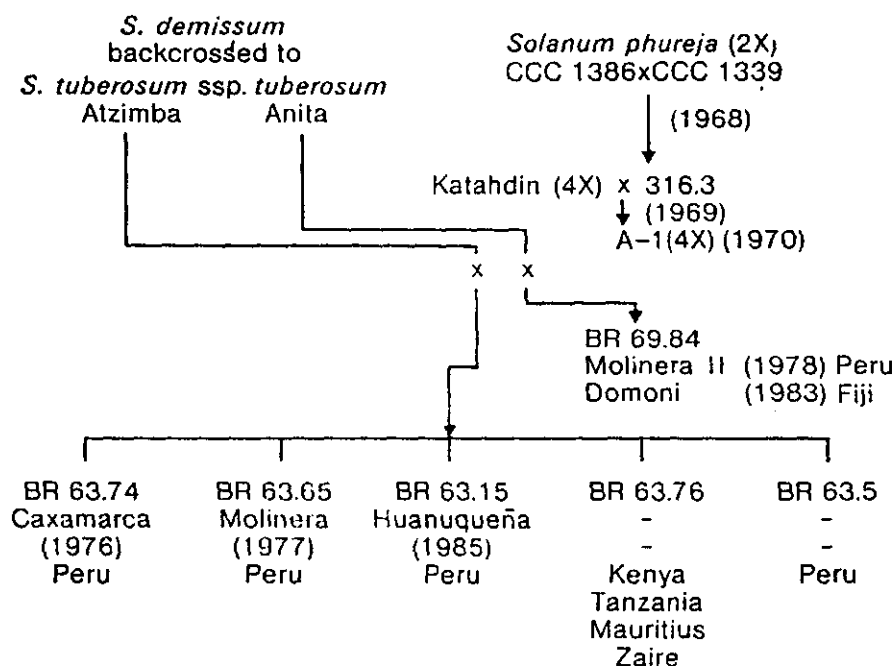
Figure 1. Pedigrees of several BR-clones with resistance to *P.solanacearum* derived from *S.phureja* (Schmiediche, 1985b).

The *S. phureja*-based resistance has proven to be strain specific and sensitive to temperature (Sequeira, 1989; Schmiediche, 1985a & b). To overcome the problem and to develop a broadly-based general type of resistance, many efforts have been made in search for other sources of resistance among the wild *Solanum spp.*. High levels of resistance have been reported in a number of accessions of *S. demissum* Lindl.; *S. chacoense* Bitt.,

S. raphanifolium Card. & Hawk, *S. acaule* Bitt., *S. commersonii* Dun., *S. microdontum* Bitt., *S. stenotomum* Juz. & Buk.. (Martin, 1979; CIP, 1986; French & Sequeira, 1988). At the CIP, incorporation of resistance genes from several specific sources into a common tetraploid pool with a wide adaptive potential has been successful and has resulted in several groups of advanced resistant materials (Schmiediche, 1985a). These materials are intended for further breeding and selection in national breeding progams in the tropics. Practical evidence, however, indicates that much work still needs be done for further improvement of such broadly-based resistant materials as far as other horticultural characteristics and resistance to other diseases and pests, such as viruses, root-knot nematodes and mites are concerned. Although these materials are better adapted to high temperatures at the lower elevations, their resistance has not been widely tested and confirmed to be reliable under large-scale cultivation.

For development of a general type of resistance to BW, genetic engineering provides a promising approach. *Agrobacterium* mediated transfer of a gene from the silkmoth, *Hyalophora cecropia* , encoding for the antibacterial protein cecropin, has been successful (Schmiediche et al.,1988). A number of transformed potato plants have shown high levels of resistance to *P. solanacearum* under quarantine greenhouse conditions (Schmiediche, personal communication). Genetic engineering has several advantages. Firstly, by this method a single gene or a small group of genes can be incorporated in the genome of an adapted cultivar without major reshufling of the genetic make-up of other economically important traits. Secondly, it seems possible to obtain resistance to several bacterial diseases, such as BW and *Erwinia* soft rots, simultaneously by a single insertion of the gene for cecropin production. This is something extremely difficult to achieve through traditional breeding using plant genes for resistance. Cecropin is a general antibacterial coumpound (Schmiediche et al., 1988). This approach of using recombinant DNA technique in potato breeding, however, may imply some risks for environment and human beings. Effects of a foreign gene insertion on the total metabolic activities of the potato plant as well as product quality need further investigations before the genetically transformed cultivars can be grown in the field. On the short and medium terms, the probability of success of this approach is unknown and classical breeding for resistance should be continued.

Chapter III

EFFECT OF HEAT TOLERANCE ON EXPRESSION OF RESISTANCE

TO *PSEUDOMONAS SOLANACEARUM* E.F. SMITH IN POTATO

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Additional key words: Adaptation, host-pathogen interaction, *Solanum* spp., stability parameters.

Summary

Twelve potato clones with different genetic background for resistance to bacterial wilt and adaptation were tested for resistance to a race 1 and a race 3 isolate of the pathogen at three locations in the Philippines representing different ranges of ambient temperature. The results showed that the genes for heat tolerance are crucial for resistance. Stability analysis indicated that clones with both resistance and heat tolerance genes displayed higher and more stable resistance to the race 1 isolate than those clones having only resistance genes. The later group tended to have higher values of both regression of disease index on environmental index and deviation from the regression in the stability analysis. Host-pathogen interaction effects were found to be statistically significant but small compared to main effects of isolates and clones. The involvement of genes with different effects on wilt resistance is discussed.

Introduction

The *Solanum phureja* Juz. & Buk. source of resistance to bacterial wilt (BW) of potato (*S. tuberosum* L.) caused by *Pseudomonas solanacearum* E.F. Smith is temperature sensitive and strain specific (Sequeira & Rowe, 1969; Rowe et al., 1972; French, 1972; Thurston & Lozano, 1968; Ciampi & Sequeira, 1980; French & De Lindo, 1982). Little is known about the behaviour of resistance from other species, such as *S.chacoense* Bitt., *S. raphanifolium* Card. & Hawk, and *S. microdontum* Bitt. which has recently been identified and utilized (Schmiediche, 1985a & b; Schmiediche & Martin, 1983). The incorporation of resistance genes from several sources was thought to reduce the effects of strain specificity and that wide adaptation would improve the stability of resistance over environments (Sequeira, 1979; Schmiediche, 1985). Tung et al.(1990b) found that hybrid potato populations involving parental genotypes with a widened genetic background for resistance coupled with heat tolerance had a high level of resistance to BW under lowland tropic conditions. There was evidence that genes for heat tolerance play an important role in resistance expression under hot conditions.

The objective of this study was to determine how the genetic background for resistance and adaptation affects the stability of resistance under diverse enviromental conditions. The reaction of a set of potato genotypes (clones) to *P. solanacearum* at three locations in the Philippines are presented.

Materials and methods

Twelve potato genotypes were tested for resistance to two isolates of *Pseudomonas solanacearum* at three locations, Los Banos [150 m above sea level (asl), August 1989], Santa Lucia (800 m asl, July 1989) and La Trinidad (1200m asl, November 1989). The clones were classified into four groups of three according to their genetic background for resistance and adaptation (Table 1). The clones AVRDC-1287.19, BR-63.74, MS-35.22R and Cruza-148 have been the major sources of resistance in the CIP breeding program (Schmiediche, 1985a), the first one having a high level of heat tolerance. Clones 378597.1 and 381064.7 are selections derived through several cycles of selection for wilt resistance and heat tolerance. Clone 381064.7 has the widest genetic background for resistance, coming from at least three known specific sources, whereas the resistance of 378597.1 derives from *S. phureja* only. The clones of groups III and IV have no known resistance to BW but are heat tolerant (Group III) or adapted to cool temperatures (Group IV).

Table 1. Potato clones used in this study; their major chracteristics and sources of resistance.

Group	Description	Clone	Source of resistance
I	Heat tolerant, BW resistant	AVRDC-1287.19	*S. chacoense,S.raphnifolium*
		378597.1	*S. phureja*
		381064.7	*S. phureja*, AVRDC-1287.19
II	Heat sensitive, BW resistant	BR-63.74	*S. phureja*
		MS-35.22R	*S. phureja*
		Cruza-148	unknown
III	Heat tolerant, BW susceptible	Cosima	-
		DTO-28	-
		LT-8	-
IV	Heat sensitive, BW susceptible	Conchita	-
		I-1039	-
		I-1035	-

The potato isolates of *P. solanacearum* used were WP-17 and WP-156 provided by the Plant Pathology Laboratory of the Institute of Plant Breeding, University of the Philippines at Los Banos. WP-17 belongs to Biovar (Bv) III and race 1 and WP-156 is a Bv II and race 3 isolate. Inoculum was produced using Kelman's (1954) standard triphenyl tetrazolium chloride medium.

Twenty plants per clone were raised by planting rooted apical cuttings in 3.5-inch clay pots containing heat-disinfected soil. Plants were irrigated with tap water at 2-3 day intervals and watered with a solution of 14% N, 6% P and 12% K every week. Mancozeb (0.3%) and cypermethrin (0.1%) were sprayed weekly to control fungi and insects. After 4 weeks, ten plants of each clone were inoculated with isolate WP-17 and ten with WP-156, representing ten replicates per treatment. The third and the fourth leaves, from the soil surface, of each plant were cut off with a scalpel dipped into a suspension of the bacterium containing 10^8 cells/ml. A separate scalpel was used for each isolate. The inoculated plants were incubated in the screenhouse where the maximum and minimum temperatures were recorded daily. Disease incidence was recorded 15 days after inoculation using the index scale used by French & De Lindo (1982): 1= no symptoms; 2=one or two leaves wilted; 3= up to half of the leaves wilted; 3= up to 3/4 of the leaves wilted and 5= plant completely wilted or dead.

Square-root transformed values of disease indices were used for analysis of variance of a split-plot design fully randomized for factor A (isolate) as the main plot and factor B (clones) split on A as subplot, at each location. Stability of resistance of the clones was assessed by Eberhart & Russell's (1966) method, where the disease indices are regressed on the environmental index taken as the difference between the mean index of all clones at a particular location and the grand mean over all locations. The environmental indices are thus deviations of each location from the overall mean. To compare performance of groups, clones were used as samples and groups as treatments. The comparision is statistically acceptable because a X^2 test for homogeneity showed that variances were homogeneous for all locations.

Results

During the test periods, the average maximum/minimum temperatures (°C) in the

screenhouses were 34.6/25.5, 30.3/20.7 and 29.5/12.3 for Los Banos, Santa Lucia and La Trinidad, respectively. Mean temperatures were correspondingly 30.1, 25.5 and 20.9.

The general tendency was as expected : disease incidence was highest in the hottest environment. Under the lower temperature conditions of La Trinidad, wilt incidence was much less severe (Table 2, Fig. 1). This tendency of disease development was more pronounced with the race 1 isolate WP-17 (Table 2). In all three locations, WP-156, a race 3 isolate, maintained its virulence and aggressiveness with very little variation with temperature. It was more virulent than WP-17 (Table 2, Fig. 1).Analysis over locations showed highly significant (P<0.01) differences between locations, isolates and clones. All types of interactions (location x isolate, location x clone, isolate x clone and location x isolate x clone) were highly significant.

Table 2. Disease indices (1-5) of twelve potato clones inoculated with isolates WP-17 (race 1) and WP-156 (race 3) of *P.solanacearum* at three locations.

| Group | Clone | Los Banos (150 m asl[a]) | | Santa Lucia (800 m asl) | | La Trinidad (1200 m asl) | |
		WP-17	WP-156	WP-17	WP-156	WP-17	WP-156
I	AVRDC-1287.19	2.1c	4.8ab	1.6b	3.9c	1.0e	4.8ab
	378597.1	3.5b	4.6b	1.7b	4.8ab	1.0e	3.9c
	381064.7	3.1b	3.9c	1.3b	4.2bc	1.2e	3.8c
II	BR-63.74	5.0a	4.9a	1.8b	3.0d	3.5bcd	4.9ab
	MS-35.22R	5.0a	5.0a	4.2a	4.8ab	1.1e	4.2bc
	Cruza-148	5.0a	5.0a	1.6b	4.8ab	1.0e	4.2bc
III	Cosima	4.6a	5.0a	4.4a	5.0a	5.0a	5.0a
	DTO-28	5.0a	5.0a	4.4a	4.6abc	2.9d	4.9ab
	LT-8	4.6a	5.0a	5.0a	4.7ab	4.2abc	4.9ab
IV	Conchita	5.0a	5.0a	4.7a	5.0a	1.8e	3.5c
	I-1039	5.0a	5.0a	5.0a	5.0a	3.4d	5.0a
	I-1035	5.0a	5.0a	4.9a	5.0a	4.3ab	4.7ab
Grand means		4.4	4.9	3.4	4.6	2.5	4.5
Location means		4.6		4.0		3.5	
CV[b](a) %		6.88		9.88		7.84	
CV (b) %		5.84		9.25		9.09	

Note: In a column, values followed by a common letter are not significantly different at P=0.01 by Least Significant Difference test.
[a], above sea level; [b] , coefficient of variation.

In Los Banos, high temperatures were seen to be detrimental to resistance expression. Although group I clones withstood wilt caused by WP-17 better than the others, it seemed that the resistance was overcome (Table 2, Fig. 1). The

resistance in group II clones was overcome by both isolates (Table 2, Fig.1). There was no significant difference in disease index among clones of groups II, III and IV. At Santa Lucia and La Trinidad, wilt incidence caused by WP-17 on groups I and II was reduced as temperature fell (Table 2, Fig.1). No differences were found between clones of group I for their resistance against WP-17 at either location (Table 2).
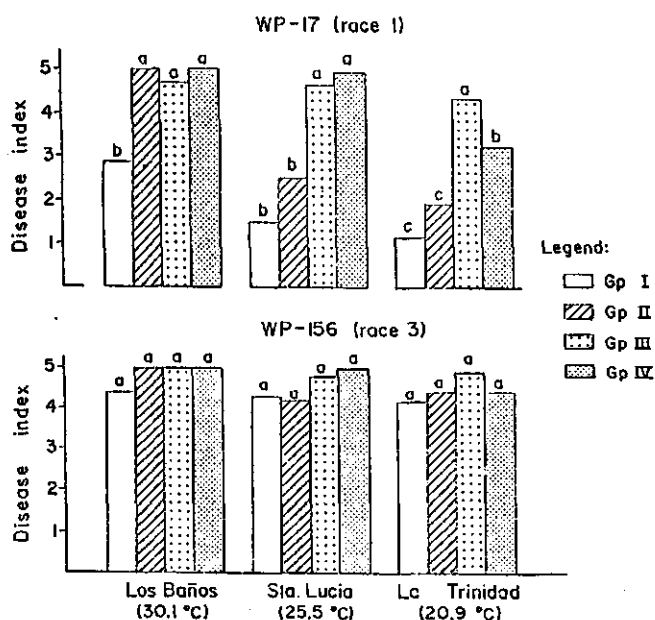
Fig.1. Reaction of four Groups of potato clones to a race 1 (WP-17) and a race 3 (WP-156) isolate of *P.solanacearum* at three locations. Bars with a common letter are not significantly different at P= 0.01 by Least Significant Difference test. For Group characteristics, see Table 1.

Against WP-156, the resistance in clone AVRDC-1287.19 tended to recover in Santa Lucia, but was unexpectedly broken again at La Trinidad. A similar behaviour was observed for BR-63-74 agaist both isolates. This situation could be explained if one assumes that these clones might possess some modifying genes as,indeed, had been suggested previously by Rowe et al. (1972). The effects of those modifying genes thus seem unpredictable under the diverse circumstances of these experiments. It was interesting that some clones of group III and IV (DTO-28 and Conchita) showed some level of resistance under

the cooler conditions of La Trinidad, although they have no known resistance. Because the expression of this resistance was well in accordance with the general tendency, the significant departure of the two clones suggests that they might possess some unknown resistance genes or rather genes with novel effects on resistance to BW under the cooler growing conditions.

When groups of clones were assigned as treatments for analysis, there were significant differences between groups in their resistance to WP-17 , but not for resistance to WP-156 (Fig. 1). On average, group I clones were most resistant over all locations (Fig. 1).

Table 3. Stability parameters for resistance to two isolates of *P. solanacearum* of twelve potato clones tested at three locations. x= mean disease index, b= regression coefficient and S^2d= deviation from regression.

Clone	WP-17			WP-156			Overall		
	x	b	S^2d	x	b	S^2d	x	b	S^2d
AVRDC-1287.19	1.6	0.582	0.000	4.5	0.805	0.462**	3.0	0.499	0.095*
378597.1	2.1	1.344	0.072	4.4	1.250	0.304**	3.3	1.427	0.000
381064.7	1.9	1.036	0.331**	4.0	-0.054	0.056	2.9	0.879	0.276**
BR-63.74	3.4	0.878	0.707**	4.3	1.695	2.164**	3.9	0.723	2.702**
MS-35.22R	3.4	2.029	1.137**	4.7	1.821	0.073	4.1	2.082	0.276**
Cruza-148	2.5	2.168	0.921**	4.7	1.711	0.037	3.6	2.153	0.188**
Cosima	4.7	-0.198	0.064	5.0	0.000	0.000	4.8	-0.174	0.007
DTO-28	4.1	1.098	0.150	4.8	0.561	0.035	4.5	0.977	0.000
LT-8	4.6	0.192	0.203**	4.9	0.472	0.001	4.7	0.219	0.001
Conchita	3.8	1.653	1.353**	4.5	3.052	0.777*	4.2	2.076	0.734**
I-1039	4.5	0.821	0.459**	5.0	0.000	0.000	4.7	0.706	0.093*
I-1035	4.7	0.323	0.002	4.9	0.612	0.002	4.8	0.442	0.009
LSD (0.05)	0.8			0.6			0.6		

* and **, significant at P<0.05 and P<0.01, respectively.

Because there were indications that clones with different genetic backgrounds for resistance and adaptation tended to respond differently to wilt between the environments, a stability analysis for resistance was attempted using the procedure of Eberhart & Russell (1966). Stability parameters for the twelve genotypes are presented in Table 3, and the regression of disease index of four clones on environmental index is illustrated in Fig.2. Regression coefficients (b) were significantly different from each other for isolate WP-17 and for both isolates taken together,but not for WP-156 alone. In general, genotypes with a good level of heat tolerance

tended to have small regression coefficients with small deviations from regression (S^2d). This is true for clones of both groups I and III , the resistant and susceptible groups of heat tolerant clones (Table 3). The clones of group II tended to have high regression coefficients and deviations from the regression. Group IV seemed to vary most , for Conchita departed strongly from the general tendency. This clone seemed to have some level of resistance which may well be expressed only under very cool temperatures (Table 2) and it is thus characterized by high values of b and S^2d (Table 3), similar to those of group II. In the model of Eberhart and Russell, a cultivar with a unit regression coefficient and deviations from regression approaching zero is said to be stable in terms of yield performance over a set test environments. In terms of resistance to disease, it can be conceived that the smaller the absolute values of the regression coefficient and deviation from regression, the more stable the the resistance. The mean performance (x) indicates the average level of resistance of a particular genotype. Thus group I clones have the highest level of resistance , which was more stable and predictable than that of group II clones in terms of resistance to the race 1 isolate, WP-17 (Table 3, Fig. 2). Low values of regression on environmental index have no meaning if resistance is absent or at too low a level. This can be seen with isolate WP-156 to which no appreciable resistance was found for any of the clones at any locations,as well as in the case of genotypes of groups III and IV (Table 1 & 3, Fig.2).

Discussion

This study showed that resistance to BW, both derived from *S. phureja* and in general, tends to break down at high temperature as was indicated by the performance of clones of the two resistant groups with different backgrounds for resistance. There was further evidence that genes for adaptation are involved in expressing resistance. Clones with genes for both resistance and heat tolerance resisted wilt better under hot conditions than those with resistance alone. This was true even with clone 378597.1 which has only resistance genes from *S. phureja* , but is heat tolerant. It has also been observed that under very hot conditions some heat tolerant clones with no known resistance could withstand wilt better than those known to have some specific genes for resistance to BW (Tung, unpublished). Cv. Conchita has no

known genes for resistance , yet showed a high level of resistance compared to other clones at La Trinidad, where it has been grown for over 30 years. It is not yet clear which among those genes for environmental adaptation confer the resistance.
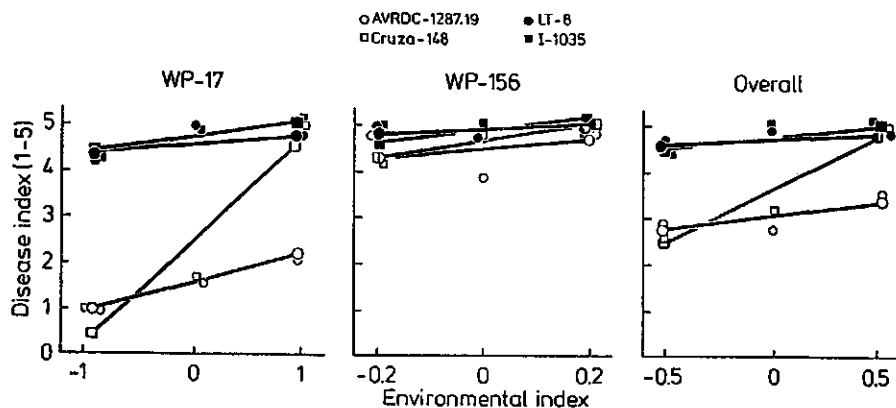
Fig. 2. Regression of disease index on environmental index (deviation from the overall mean disease index) for four potato clones inoculated with two isolates of *P. solanacearum* at three locations. Note: the higher environmental index corresponds to the higher ambient temperature.

It could not be clearly shown whether genotypes with a range of resistance genes from several species have a more stable resistance than genotypes with resistance from one species, because the number of host genotypes, pathogen strains and environments used was small. Theoretically, however, it would be expected that a wide genetic background would make the resistance more stable. Clone 381064.7 has *S. chacoense, S. raphanifolium* (AVRDC-1287.19) and *S. phureja* (BR-63.74) as its resistance ancestors, but was not consistently significantly superior to AVRDC-1287.19 and 378597.1. It is, however, not known whether clone 381064.7 has more resistance genes than the other two clones.

As found earlier (Tung et al., 1990b), host-pathogen interaction, although statistically highly significant, was small compared to main effects due to host genotypes and pathogen isolates as indicated by the much larger magnitude of the mean squares for isolates and clones (Table 4).

Table 4. Mean squares for isolates, clones and isolate x clone interaction at three locations.

Source	Degree of freedom	Mean squares		
		Los Banos	Santa Lucia	La Trinidad
Isolate (A)	1	0.745**	6.403**	17.663**
Error(a)	18	0.025	0.043	0.023
Clone (B)	11	4.860**	15.657**	15.212**
A x B	11	0.240**	0.697**	0.692**
Error(b)	198	0.020	0.037	0.032

** , significant at $P<0.01$.

Changes in pathogenicity of the pathogen and in resistance of the host were again the major source of variation. Parlevliet & Zadoks (1977) showed that this kind of distribution of the total variation would mean that genes with minor effects are involved in conditioning the resistance to wilt. However, the fact that all the host genotypes resistant to isolate WP-17 were fully susceptible to isolate WP-156 indicates that the latter isolate possesses certain different gene(s) for pathogenicity which can overcome the effects of the genes for resistance in the population of clones tested. Whether the higher pathogenicity of WP-156 is owing to a large number of genes with minor effects all combined or is due to a major gene(s) effects needs further investigation. It seems more likely, however, that the isolate might possess a strong major virulence gene(s) which has no counterpart(s) for resistance in the potato clones used because they all suffered in a similar way while the isolate maintained its high pathogenicity throughout. Evidently, a corresponding major resistance gene(s) is absent in the population of clones tested and such a gene(s) is (are) required to built up any resistance to WP-156 and similar strains of *P.solanacearum*.

The procedure of Eberhart & Russell (1966) successfully analyzed stability of resistance to wilt disease when using the mild strain WP-17. Genotypes with

both regression coefficients and deviations from the regression approaching zero are the most stable. Against extremely aggressive strain of the bacterium, e.g. WP-156, the method may not be of value until corresponding resistance genes or more sensitive evaluation procedures are available.

Acknowledgement

Chapter IV

EFFECTS OF RESISTANCE GENES, HEAT TOLERANCE GENES AND

CYTOPLASMS ON EXPRESSION OF RESISTANCE TO

RESISTANCE TO *PSEUDOMONAS SOLANACEARUM* E.F.SMITH IN POTATO

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Key words: bacterial wilt resistance, heat tolerance, interaction, potato, *Pseudomonas solanacearum*, *Solanum* spp.

Abstract

Effects of resistance genes and heat tolerance genes on expression of resistance to bacterial wilt caused by *Pseudomonas solanacearun* were investigated in 30 F_1 progenies from parents with different levels of bacterial wilt resistance and heat tolerance . A race 1 and a race 3 isolate of the bacterium were used for inoculation under screenhouse conditions at two locations. Results obtained indicated that with reduction in levels of parental resistance, resistance in the F_1 progenies also reduced. Under hot conditions , a reduction in the background heat tolerance also resulted in lower levels of resistance expression. The effect of heat tolerance tended to diminish at lower temperatures leaving the effect of resistance genes more consistent. There existed a strong interaction between resistance genes and genes for heat tolerance. The nature of resistance to bacterial wilt in potato and implications for breeding for resistance were discussed.

Effects of reciprocal crosses on expression of resistance to a race 1 isolate under hot screenhouse conditions, were studied in 5 sets of reciprocal F_1 progenies involving different resistant and susceptible parents . The reciprocal differences observed were not significant suggesting absence of cytoplasmic effects on expression of resistance.

Introduction

Breeding for resistance to ba6cterial wilt (BW), caused by *Pseudomonas solanacearum* (E.F. Smith), has been a difficult objective. Resistance is generally highly strain specific and greatly influenced by environmental conditions , especially temperature (Sequeira & Rowe, 1969; Thurston & Lozano, 1968; Ciampi & Sequeira, 1980; French & De Lindo, 1982; Tung et al.,1990a). Schmiediche (1985a) and Kloos & Fernandez (1986) suggested that resistance to BW is probably a function of environmental adaptation. There is evidence that genes for adaptation are involved in conferring the resistance. Heat tolerant parent clones gave higher proportions of resistant plants in their progenies under lowland tropic conditions (Tung et al.,1990b).

Potato clones with genes for both BW resistance and heat tolerance expressed a higher level of resistance under diverse environmental conditions (Tung et al., 1992a). There is also evidence that under hot conditions certain heat tolerant potato clones with no known resistance could withstand BW better than other well-known resistant clones (Tung, unpublished). Resistance to BW thus seems very complex with genes for adaptation probably being involved in conditioning its expression. The extent to which heat tolerance genes help strengthen resistance under heat conditions was still not clear. Also the most effective means of combining BW resistance with heat tolerance to produce superior progenies , which are much desired in breeding potato for production in the warm tropics, needs to be determined .

The objective of the experiments described in this paper was to investigate the expression of resistance in F_1 progenies from parents with different degrees of BW resistance and heat tolerance and possible cytoplasmic effects on the expression of resistance in F_1 progenies from reciprocal crosses between different parents.

Materials and Methods

In the first study, a set of potato clones (Table 1) with known genetic background for BW resistance and adaptation were used to produce sets of F_1 progenies with different average degrees of BW resistance and heat tolerance. Because most of the resistant materials currently being used in breeding programs derived their resistance from only a few original ancestors, some of the resistant parents used in this study are also more or less related. For instance, BR-63.5, BR-63.74 and BR-63.76 are full-sibs, BR-63.74 is a grandparent of 381064.3 and 381064.7, while AVRDC-1287.19 is the male parent of the latter two. In addition all clones coded BR- have virtually been developed from only two original resistant diploid *S. phureja* clones (Schmiediche, 1985b).

The hybridization was conducted in the summer of 1989 at the CIP-Region VII Germpasm and Training Center at Sta. Lucia, the Philippines (800 m above sea level,asl, 14° N latitude). Crosses were made between parents which either lack or possess one or both desirable traits to be investigated: BW

resistance and heat tolerance. This means four classes of parents (Table 1) and hence 16 types of single crosses including within- and between-class combinations. As there are crosses with similar reciprocal combinations of parental attributes, the number of types of crosses (hereafter called groups) to be tested was reduced to a meaningful 10 (Table 2), of which the Groups Va and Vb were similar in terms of degree of the traits of concern but differed from each other by the type of their resistant parents. Three crosses were made with their parent clones chosen at random from a desired class to represent a Group. There were thus a total of 30 single crosses forming 10 Groups differing in their levels of BW resistance and heat tolerance.

Table 1. Parents of the crosses studied in this article. The parents are classified according to their resistance or susceptibility to bacterial wilt (BW) and tolerance or sensitivity to heat (H).

  + symbol for the desirable traits, resistance or tolerance,

  - symbol for the undesirable traits, susceptibility or sensitivity.

Class	Parent clone	Class charateristics BW/H	Source of resistance
1	378597.1	+/+	S. phureja
	AVRDC-1287.19	+/+	S. raphanifolium S. chacoense
	381064.3	+/+	S. phureja, AVRDC-1287.19
	381064.7	+/+	S. phureja, AVRDC-1287.19
2	BR-63.5	+/-	S. phureja
	BR-63.74	+/-	S. phureja
	BR-63.76	+/-	S. phureja
	BR-112.113	+/-	S. phureja
	Cruza-148	+/-	Unknown
3	LT-8	-/+	
	DTO-28	-/+	
	DTO-33	-/+	
	7XY.1	-/+	
	Atlantic	-/+	
4	I-1039	-/-	
	I-1035	-/-	
	CFK-69.1	-/-	
	Serrana	-/-	
	P-7	-/-	
	Conchita	-/-	

For the sake of assessing the effects of differences in degree of resistance and/or heat tolerance on levels of resistance expression, Groups were tentatively joined in two ways into two clusters of three Categories: 1, according to their degree of resistance regardless of their degree of heat tolerance, Resistance categories and 2, according to their degree of heat tolerance regardless of their degree of resistance, Heat tolerance categories (Table 2). This categorization assumes that the interaction effects between resistance genes and those for adaptation in all F_1's are all of the same magnitude and positive and negative effects will cancel out each other. Thus Resistance Categories will, on the average, have the same level of heat tolerance. Similarly, Heat tolerance Categories will have the same average level of BW resistance. Otherwise, this categorization is just a rough approximation which facilitates assessing the average effects of levels of resistance and heat tolerance .

Table 2. Groups and categories of F_1's obtained from 9 basically different combinations of parental clones. BW, bacterial wilt; H, heat. + = BW resistant/ H tolerant; - = BW susceptible/ H sensitive.

Group	Parental attributes Female BW/H	Male BW/H	Group characteristics	Categories for Resistance	Tolerance
I	+/+	+/+	++/++	A	P
II	+/-	+/+	++/-+	A	Q
III	+/-	+/-	++/--	A	R
IV	+/+	-/+	+-/++	B	P
Va	+/+	-/-	+-/+-	B	Q
Vb	+/-	-/+	+-/-+	B	Q
VI	+/-	-/-	+-/--	B	R
VII	-/+	-/+	--/++	C	P
VIII	-/+	-/-	--/+-	C	Q
IX	-/-	-/-	--/--	C	R

The crosses were tested for resistance to a race 1 (WP-17) and a race 3 (WP-156) isolate under screenhouse conditions at two locations: Los Banos (150 m asl) and Sta. Lucia (800 m asl). The BW resistance tests were conducted during the periods February 28 - March 15, and March 10 - 25,1990, at Los Banos and Sta. Lucia, respectively. At each location the F_1 progenies were

tested in a split-plot randomized complete block design (RCBD) with isolates of *P.solanacearum* as main plots and the potato progenies as subplots in three replications of 30 plants.

F_1 true seeds were sown in heat sterilized volcanic soil in 0.50 m x 0.50 m boxes. Seedlings were transplanted, at the first true leaf stage, into 8 cm diameter clay-pots, containing the same type of soil. The plants were irrigated with tap water at 2-day intervals and fertilized weekly with a 1% solution of 14% N, 5% P and 12% K. Mancozeb (0.3%) and cypermethrin (0.1%) were sprayed every week to prevent infestation by fungi and insect pests.The test plants were trimmed to have one main stem one week prior to inoculation with a sterilized knife. Four weeks after transplanting , the plants were inoculated by clipping off the leaves at the third and the fourth positions from the soil surface with a sterilized scalpel dipped into a 10^8 cells/ml inoculum suspension of the bacterium. For each isolate, a separate scalpel was used. For each test, plants (5 per isolate) of the susceptible clone 7XY.1, raised from apical cuttings of a disease free stock, were also inoculated to check for effectiveness of the inocula. This clone has been observed to wilt within 5 to 7 days after inoculation (DAI). During the incubation period, the inoculated plants were maintained in the screenhouse, and the daily maximum and minimum temperatures were recorded. On the 15 DAI, when check plants were completely wilted disease incidence was recorded as percentage of plant survival in the populations and the disease index of individual plants was determined based on the following index scale: 1, no wilt symptoms; 2, one or two leaves wilted; 3, half of the leaves wilted; 4, 3/4 of the leaves wilted; and 5, plant completely wilted or dead.

The second study was conducted to investigate the possible effect of the heat tolerant cytoplasm on the level of resistance to wilt. Four pairs of reciprocal F_1 progenies were produced using the clones BR-63.5 and BR-112.113 as resistant parents which have heat sensitive BW resistance derived from *S. phureja*. The clones I-1039 and DTO-33 were used as heat sensitive and tolerant parents ,respectively, both being BW susceptible. The progenies were tested for resistance to isolate WP-17 in a screenhouse at Los Banos during the period 5-20 February, 1990, along with the reciprocal crosses of susceptible parents as checks. The experiment was conducted in an RCBD with two replications of 50 plants. All cultural practices, inoculation method, and

temperature reading followed the same procedure as described in the first study. Percentage of survival was recorded at 3-day intervals and disease index at 15 DAI.

For al experiments, Arc-sin transformation of percentage of survival was used for analysis of variance. For the disease indices, the average value of the plants of each replicate was determined. As this values are usually normally distributed, no transformation was attempted.

Results

During the first study, the average max/min temperatures were $33.4/24.5^{\circ}$ C (mean 28.9° C) at Los Banos and $29.7/19.8^{\circ}$ C (mean 24.8° C) at Sta. Lucia. Under these conditions, though not favourable for tuberization, the potato plants still used to grow well, and hardly any abnormal growth or plant death occurred before inoculation. So wilting which occurred after inoculation was considered to be due entirely to P. *solanacearum* infection.

In general, wilt incidence was much higher at Los Banos than at Sta. Lucia for both % survival (Table 3) and disease index (Table 4). As found earlier (Tung et al., 1992), isolate WP-156 was much more virulent than WP-17 (Tables 3 & 4). Statistical analysis showed highly significant differences between locations, isolates and potato progenies. All types of interaction: isolate x progeny, isolate x location, progeny x location, isolate x progeny x location, were highly significant. The main effects due to isolates and progenies , however, were the major source of total variation, similar to previous findings (Tung et al., 1990b & 1991).

Against isolate WP-17, there was a great variation among progenies at both locations (Tables 3 & 4). Though some differences between progenies belonging to different Groups were not statistically significant, Groups I, II and IV, which have a high level of resistance coupled with heat tolerance, tended to show the highest level of resistance in both % survival and wilt index (Tables 3 & 4, Fig. 1). In the absence of heat tolerance, resistance in progenies of Groups III and VI tended to diminish under hot conditions at Los Banos and recovered significantly under Sta. Lucia conditions. Heat tolerance alone did

not help in resisting wilt indicated by the low performance of progenies of Groups VII and VIII (Tables 3 & 4, Fig. 1). Under very hot conditions at Los Banos, however, Group VII seemed to be better than Groups III and VI which have resistance from one (VI) or both (III) parents, but lack heat tolerance. In general, in progenies without any resistance and/or heat tolerance (Groups VI to IX) wilting was almost complete at 15 DAI (Tables 3 & 4, Fig 1).

Against isolate 156, the resistance was almost lost at both locations. Trends in resistance expression among the F_1 progenies similar to that with WP-17 were also observed (Tables 3 & 4, Fig. 1). Although the differences between progenies were greatly reduced, especially under Los Banos conditions, it was those with higher levels of BW resistance and heat tolerance in their pedigrees which tended to show less wilting (Tables 3 & 4, Fig.1).

Results obtained from this study clearly showed the general impact of genes for resistance and heat tolerance on expression of resistance to BW in potato.

Effects of resistance

The effects of resistance genes could be examined more closely by comparing the means of Resistance Categories (A, B and C,Table 2). These have a similar average degree of heat tolerance but differed from each other by their genetic levels of resistance (Table 2). A Mann-Whitney test (Steel & Torrie, 1960) revealed that there were significant differences in % survival between progenies of Categories A and B against both isolates at both Sta. Lucia and Los Banos (Table 5). The differences between them in wilt index were found to be significant only at Sta. Lucia and against WP-156 but the general tendency was that progenies of A tended to give a better average index than did those of B (Table 5, Fig. 1). The non-significance in other cases was probably due to large significant variation among Groups within Categories. It is clear that under milder temperatures at Sta. Lucia , effects of resistance level expressed more consistently. The indifference between the two Categories against WP-17 indicate that under the hot conditions , the effect of heat tolerance genes have well expressed which greatly masked the difference in level of resistance of these Categories.

Except for wilt index for WP-156 at Los Banos, the differences between Categories A and C were significant in all cases (Table 5). An explanation for these differences is straightforward since these two Categories were the extreme situations: A had the highest level of parental resistance and C had

no resistance at all. The differences in % survival between Categories B and C were statistically significant only at Sta. Lucia and, against isolate WP-17 and diminished under very hot conditions at Los Banos and/or against WP-156, in which situations resistance seemed to be lost (Table 5 and Fig.1). The general tendency was that B tended to show higher levels of resistance than C (Fig.1, Table 5).
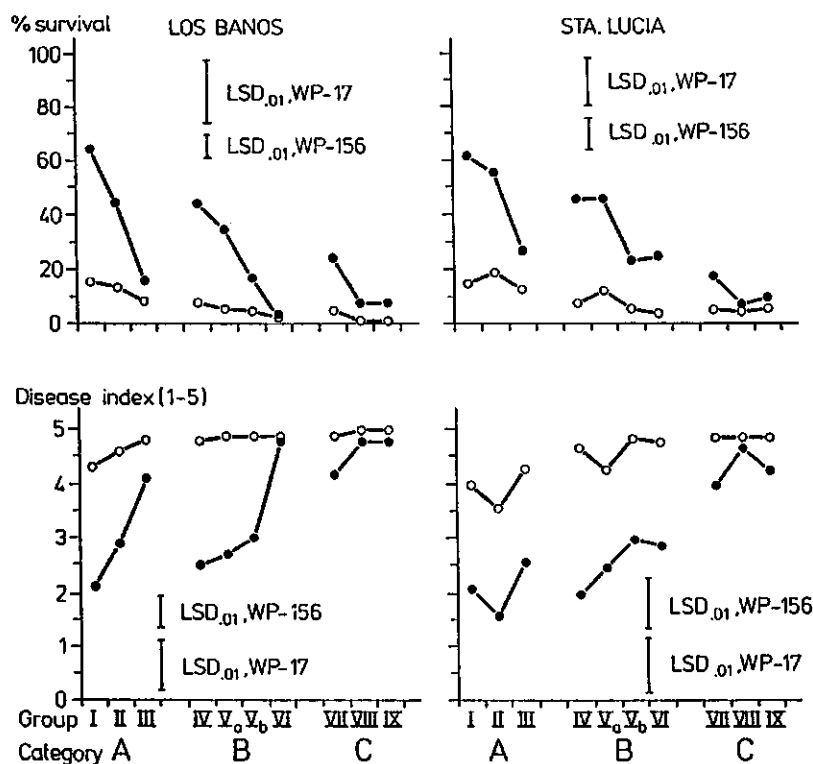
Figure 1. Group averages for survival (1-100%) and wilt index (1-5) of 10 groups of F_1 progenies (of Tables 3 & 4), differing in level of BW resistance and heat tolerance, inoculated with isolates WP-17 (.) and WP-156 (o) of P.solanacearum at Los Banos (left) and Sta. Lucia (right).

　　　　Categories: A= resistance comes from both parents; B=resistance from one parent and C= resistance from neither parent.
　　　　Groups: I,IV & VII, heat tolerance from both parents; II,Va, Vb & VIII, heat tolerance from one parent, and III,VI & IX , heat tolerance from neither parent.

Table 3. Survival (%) of 30 F_1 progenies 15 days after inoculation with two isolates of *P. solanacearum* at two locations under screenhouse conditions. For Group characteristics, see Table 2.

Group	Cross	Los Banos WP-17	WP-156	Sta. Lucia WP-17	WP-156
I	381064.3 x 378597.1	44.9 b-e	17.8 ab	55.3 a-c	23.8a
(++/++)	381064.7 x AVRDC-1287.19	71.3 a	17.9 ab	65.4 a	12.0 a-c
	AVRDC-1287.19 x 378597.1	69.2 ab	10.4 a-c	61.4 ab	7.8 a-c
II	BR-63.5 x 381064.7	27.3 d-h	10.6 a-c	48.7 a-c	18.4 a-c
(++/-+)	BR-112.113 x AVRDC-1287.19	56.4 a-c	11.0 a-c	58.2 ab	19.4 ab
	BR-63.74 x 381064.3	51.8 a-d	20.3 a	60.7 ab	18.3 a-c
III	BR-63.74 x BR- 63.76	16.2 g-i	8.1 a-d	28.7 b-g	7.9 a-c
(++/--)	BR-63.74 x BR-112.113	27.0 d-h	12.2 a-c	30.0 b-f	17.7 a-c
	Cruza-148 x BR-63.5	8.2 ij	8.0 a-d	22.1 d-h	12.7 a-c
IV	381064.7 x DTO-33	42.3 c-f	6.0 a-d	46.1 a-c	9.3 a-c
(+-/++)	381064.3 x Atlantic	39.7 c-g	11.3 a-c	39.9 b-f	13.5 a-c
	AVRDC-1287.19 x LT-8	51.3 a-d	6.3 a-d	51.4 a-c	2.3 b-c
Va	378597.1 x I-1035	22.8 e-h	0.0 d	30.1 b-f	10.8 a-c
(+-/+-)	381064.7 x I-1039	41.2 c-f	9.0 a-c	55.4 a-c	13.6 a-c
	381064.3 x I-1035	40.0 c-f	7.7 a-d	53.1 a-c	9.8 a-c
Vb	BR-63.5 x DTO-33	19.0 f-i	11.7 a-c	31.4 b-f	10.9 a-c
(+-/-+)	BR-63.5 x 7XY.1	10.1 h-j	3.2 cd	17.2 f-h	7.3 a-c
	BR-63.74 x Atlantic	23.2 e-h	0.0 d	24.1 c-g	0.0 c
VI	BR-63.74 x I-1039	4.7 jk	1.5 cd	23.8 c-g	0.0 c
(+-/--)	BR-63.5 x I-1035	8.2 ij	2.3 cd	31.4 b-f	7.1 a-c
	Cruza-148 x I-1039	0.0 k	o.o d	18.8 e-h	4.8 bc
VII	DTO-28 x Atlantic	24.0 e-h	9.0 a-c	10.3 g-i	0.0 c
(--/++)	DTO-33 x Atlantic	13.4 g-i	6.7 a-d	18.8 e-h	6.6 a-c
	LT-8 x DTO-33	29.9 d-g	0.0 d	23.4 c-g	5.0 a-c
VIII	I-1035 x DTO-33	9.3 ij	o.o d	17.7 f-h	10.0 a-c
(--/-+)	DTO-28 x I-1039	7.7 ij	2.9 cd	0.0 i	0.0 c
	LT-8 x I-1039	5.6 jk	0.0 d	6.3 hi	1.7 bc
IX	P-7 x I-1039	16.7 g-i	0.0 d	8.5 hi	4.7 bc
(--/--)	Serrana x I-1035	0.0 k	0.0 d	0.0 i	0.0 c
	Conchita x CFK-69.1	7.9 ij	1.8 cd	22.4 d-h	7.2 a-c
CV (i)%		18.76		19.09	
CV (p)%		19.94		15.66	

Note: In a column, values followed by a common letter are not significantly different at P=0.01 by Duncan's multiple range test.
CV(i) = coefficient of variation for isolates,
CV(p) = coefficient of variation for progenies.

Table 4. Disease index of 30 F_1 progenies 15 days after inoculation with two isolates of *P. solanacearum* at two locations under screenhouse conditions. For Group characteristics, see Table 2.

Group	Cross	Los Banos		Sta. Lucia	
		WP-17	WP-156	WP-17	WP-156
I	381064.3 x 378597.1	2.5 d-f	4.1 b	2.4 e-i	3.3 gh
(++/++)	381064.7 x AVRDC-1287.19	2.0 ef	4.1 b	1.8 h-j	4.3 c-f
	AVRDC-1287.19 x 378597.1	1.9 f	4.7 ab	2.0 g-j	4.5 a-d
II	BR-63.5 x 381064.7	3.6 a-d	4.8 ab	1.9 g-j	3.8 fg
(++/-+)	BR-112.113 x AVRDC-1287.19	2.4 d-f	4.7 ab	1.4 j	3.8 fg
	BR-63.74 x 381064.3	2.7 d-f	4.3 ab	1.5 ij	3.2 h
III	BR-63.74 x BR-63.76	4.4 a-c	4.7 ab	2.4 e-i	4.8 a-c
(++/--)	BR-63.74 x BR-112.113	3.3 b-e	4.7 ab	2.4 e-i	3.9 ef
	Cruza-148 x BR-63.5	4.6 a-c	4.9 a	3.0 d-f	4.3 c-f
IV	381064.7 x DTO-33	2.6 d-f	4.8 ab	1.9 g-j	4.7 a-c
(+-/++)	381064.3 x Atlantic	2.6 d-f	4.7 ab	2.1 f-j	4.4 b-e
	AVRDC-1287.19 x LT-8	2.3 ef	4.9 a	1.9 g-j	5.0 a
Va	378597.1 x I-1035	3.1 c-f	5.0 a	2.6 d-h	4.4 b-e
(+-/+-)	381064.7 x I-1039	2.6 d-f	4.9 a	2.5 e-h	4.0 d-f
	381064.3 x I-1035	2.5 d-f	4.9 a	2.4 e-i	4.4 b-i
Vb	BR-63.5 x DTO-33	3.2 c-f	4.8 ab	2.4 e-i	4.8 a-c
(+-/-+)	BR-63.5 x 7XY.1	2.9 d-f	4.9 a	3.9 a-c	4.9 a-b
	BR-63.74 x Atlantic	2.9 d-f	5.0 a	2.8 d-g	5.0 a
VI	BR-63.74 x I-1039	4.8 ab	4.9 a	3.2 de	5.0 a
(+-/--)	BR-63-5 x I-1035	4.5 a-c	4.9 a	2.5 e-h	4.7 a-c
	Cruza-148 x I-1039	5.0 a	5.0 a	3.0 d-f	4.8 a-c
VII	DTO-28 x Atlantic	4.4 a-c	4.8 ab	4.4 a-c	5.0 a
(--/++)	DTO-33 x Atlantic	4.6 a-c	4.8 ab	4.2 a-c	4.8 a-c
	LT-8 x DTO-33	3.5 a-d	5.0 a	3.5 cd	4.9 ab
VIII	I-1035 x DTO 33	4.7 ab	5.0 a	4.2 a-c	4.8 a-c
(--/-+)	DTO-28 x I-1039	4.8 ab	4.9 a	5.0 a	5.0 a
	LT-8 x I-1039	4.8 ab	5.0 a	4.9 ab	4.9 ab
IX	P-7 x I-1039	4.6 a-c	5.0 a	4.7 ab	4.9 ab
(--/--)	Serrana x I-1035	5.0 a	5.0 a	5.0 a	5.0 a
	Conchita x CFK-69.1	4.9 a	4.9 a	3.1 de	4.8 a-c
CV (i) %		3.41		7.73	
CV (p) %		2.17		6.25	

Note: In a column, values followed by a common letter are not significantly different at P=0.01 by Duncan's multiple range test.

CV (i) % = coefficient of variation for isolates,

CV (p) % = coefficient of variation for progenies.

Table 5. Effects of resistance (R)/susceptibility (S) of parents (P_1 and P_2) on % survival (upper part) and disease index (lower part) in F_1 progenies. Roman figures refer to the groups in tables 3 and 4.

F_1 groups pooled (Category)	Parental characteristics		Los Banos		Sta. Lucia		Overall category mean
	P_1	P_2	WP-17	WP-156	WP-17	WP-156	
% survival [*]							
I+II+III (A)	R	R	41.4a	12.9a	47.8a	15.2a	29.3a
IV+V+VI (B)	R	S	25.2b	4.9b	35.4b	7.2b	18.2ab
VII+VIII+IX (C)	S	S	12.7c	2.2b	11.9c	3.8b	7.6b
	Mean		26.4	6.6	31.7	8.7	18.3
Disease index (1-5) [*]							
I+II+III (A)	R	R	3.0a	4.6a	2.1a	4.0a	3.4a
IV+V+VI (B)	R	S	3.4a	4.9a	2.6a	4.7b	3.9a
VII+VIII+IX (C)	S	S	4.6b	4.9a	4.3b	4.9b	4.7b
	Mean		3.7	4.8	3.0	4.5	4.0

[*] In a column, values followed by a common letter are not significantly different at P= 0.05 by Mann-Whitney test.

As it was suggested earlier (Tung et al., 1992) that there is no effective gene(s) against isolate WP-156 in the population of potato clones used, the resistance to BW in this study also seemed to be broken down by this isolate. It is likely that the presence of some level of resistance against WP-156 in the progenies (Tables 3, 4 & 5) was due to a certain residual effects the resistance which is effective against WP-17. The differences between Categories A and B in Los Banos might thus indicate that the residual resistance was to some extent correlated with the genetic level of resistance of the progenies as A always tended to resist wilt better (Table 5, Fig. 1).

Effect of heat tolerance

As mentioned in the foregoing section, there was a large variation among the Groups within Resistance Categories (Fig.1). Groups within these Categories differed in their genetic levels of heat tolerance (Table 2). Although some of the between Group differences were not statistically significant, there was a general tendency that within a Category, Groups with higher levels of heat tolerance tended to show higher levels of resistance to wilt caused by both isolates at both locations, for both % survival and indices (Fig.1). The

effect of heat tolerance genes was especially dramatic under Los Banos conditions and seemed to reduce at Sta. Lucia, where progenies of Group II and Va , having heat tolerance from only one parent, tended to be slightly better, against WP-156, than Group I which had twice the degree of heat tolerance. The differences were, however, not statistically significant.

When Groups with similar degree of heat tolerance were pooled in another way into Heat tolerance Categories (P,Q and R, Table 6), the effects of heat tolerance became even more clear. These three Categories differed significantly from each other in resisting wilt caused by WP-17 at Los Banos. At Sta. Lucia, these differences were leveled off and significance was found only for % survival and between P and R, and between Q and R (Table 6). With WP-156, no significant difference was found in any case as resistance was overcome by the isolate's extreme virulence.

Interaction

Large variation among Groups within Categories (Fig. 1) resulted in certain significant differences between them, on the one hand, and insignificant differences between Groups belonging to different Categories, on the other hand. While significant differences between Groups within Resistance Categories were attributable to the effects of heat tolerance genes, the indifference between Groups of different Categories indicated that there was a large amount of interaction between genes for resistance and genes for heat tolerance. This interaction effect also seemed to decrease considerably under milder temperatures at Sta. Lucia , where effects of heat tolerance became less pronounced and resistance was thus more clearly expressed. The significant differences between progenies within Groups (Tables 3 & 4) might be associated with the combining ability of the particular parent clones, as reported elsewhere (Tung et al., 1990a).

Whereas significant differences in overall means were detected between Resistance Categories (Table 5, last column), none was found between Heat tolerance Categories (Table 6,last column). This strongly suggests that though heat tolerance can greatly improve expression of resistance under heat stress conditions , tolerance alone would not make up any resistance to wilt and that resistance genes are indispensable despite the fact that their expression may be strongly affected by changes in environmental conditions.

Table 6. Effects of heat tolerance (T)/sensitivity (S) of parents (P_1 and P_2) on % survival (upper part) and disease index (lower part) in F_1 progenies. Roman figures refer to the groups in tables 3 and 4.

F_1 groups pooled (Category)	Parental characteristics		Los Banos		Sta. Lucia		Overall category mean
	P_1	P_2	WP-17	WP-156	WP-17	WP-156	
% survival (*)							
I+IV+VII (P)	T	T	42.9a	9.5a	41.2a	8.9a	25.6a
II+V+VIII (Q)	T	S	26.3b	6.4a	33.4a	10.2a	18.7a
III+VI+IX (R)	S	S	9.9c	3.8a	20.5b	6.9a	10.3a
		Mean	26.4	6.6	31.7	8.7	18.3
Disease index (1-5) (*)							
I+IV+VII (P)	T	T	2.9a	4.7a	2.7a	4.5a	3.7a
II+V+VIII (Q)	T	S	3.4b	4.8a	3.0a	4.4a	3.9a
III+VI+IX (R)	S	S	4.6c	4.9a	3.3a	4.7a	4.4a
		Mean	3.6	4.8	3.0	4.5	4.0

(*) In a column, values followed a common letter are not significantly different at P=0.05 by Mann-Whitney test.

Effect of reciprocal crosses

During the period of the test for effect of reciprocal crosses at Los Banos, the max/min temperatures in the screenhouse averaged 32.5/23.4° C with a mean of 27.9° C.

Under the conditions of this experiment, no significant effect of reciprocal crosses of any pair was detected in terms of both % survival and disease index (Table 7). It was the effect of a particular parent clone which gave the general impact of resistance expression (Table 7, Fig.2). The clones BR-112.113 and BR-63.5 have been recognized for their resistance to BW under cool conditions. However, they usually succumb to the disease under hot conditions. In this experiment, BR-112.113 showed to be the better resistant parent compared to BR-63.5 at the 15 DAI (Table 7, Fig.2),especially in the absence of heat tolerance. A similar comparision could be made between DTO-33 and I-1039, as the first was superior in giving more resistant offsprings (Table 7). The results obtained confirm with our previous finding (Tung et al., 1990a) that combining ability is an important feature of BW resistance in potato and heat tolerance plays a crucial role in expression of resistance

under hot conditions. The survival curves of the progenies showed that though the effect of parent clones was apparent during the first 15 DAI, the differences between them tended to level off at later dates. This was true for both resistant and susceptible parents, heat tolerant or sensitive. The resistance to BW thus seems to be the ability to slow down the bacterial multiplication and to delay wilt.

Table 7. Average % survival and disease index of five reciprocal sets of F_1 progenies 15 days after inoculation with a race 1 isolate of *P.solanacearum*. For symbols BW, H, + and -, see caption of Table 1.

Reciprocal set	Cross	Characteristics BW/H of Female	F_1	% survival	Disease index (1-5)
1	BR-63.5 x DTO-33	+/-	+-/-+	41.1 abc	3.4 abc
	DTO-33 x BR-63.5	-/+	-+/+-	34.8 abc	3.7 abc
2	BR-112.113 x DTO-33	+/-	+-/-+	47.7 ab	2.8 bc
	DTO-33 x BR-112.113	-/+	-+/+-	52.3 a	2.6 c
3	BR-63.5 x I-1039	+/-	+-/--	10.5 cd	4.1 ab
	I-1039 x BR-63.5	-/-	-+/--	13.6 cd	3.9 abc
4	BR-112.113 x I-1039	+/-	+-/--	36.5 abc	3.9 abc
	I-1039 x BR-112.113	-/-	-+/--	48.5 ab	3.2 bc
5	DTO-33 x I 1039	-/+	--/+-	6.8 d	4.8 a
	I-1039 x DTO-33	-/-	--/-+	4.1 d	4.9 a
CV (%)				15.02	10.04

Note: In a column, values followed by a common letter are not significantly different at P=0.05 by Duncan's multiple range test.

Discussion

Rowe and co-workers (Rowe & Sequeira, 1970 and Rowe et al.,1972) reported on a system of three independent and dominant major genes that control specific resistance to two race 1 strains of *P.solanacearum* in the diploid *S.phureja*. Results from their later experiments, however, did not support their initial hypothesis (Sequeira, 1979). Since then, no further efforts have been dedicated to genetic analysis of BW resistance in potatoes, probably due to the very complex nature of the resistance: strain specificity and sensitivity to fluctuations in environmental conditions.
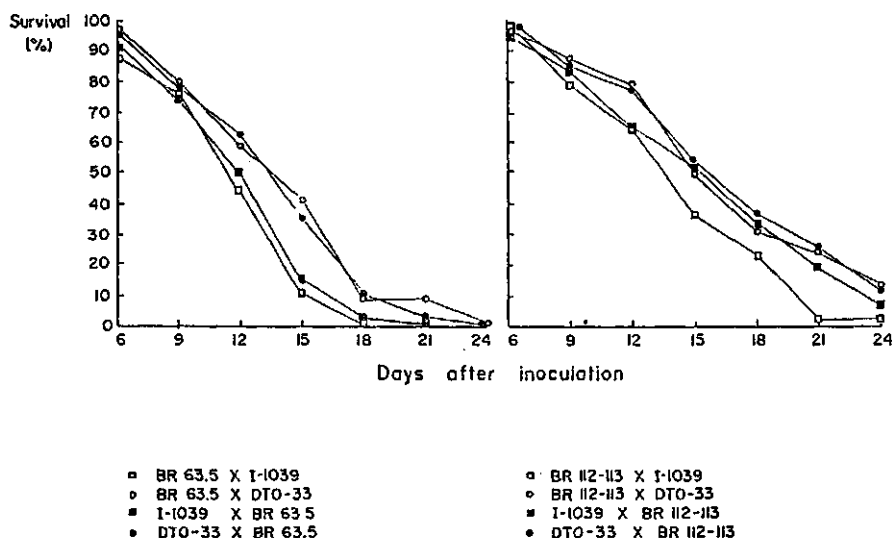
Figure 2. Survival patterns of four pairs of reciprocal crosses after
inoculation with isolate WP-17 of *P.solanacearum*.

In all cases, one parent (coded BR-) is BW resistant;

■ & □ , both parents are heat sensitive;

● & ○ , one parent is heat tolerant;

Squares vs circles exhibits effects of heat tolerance;

Black vs white compares reciprocal crosses.

Results from this study confirm our previous finding that genes for adaptation
are involved in conferring BW resistance (Tung et al.,1990a, b & 1992). This
is indicated by the considerable effect of heat tolerance genes in
conditioning resistance under heat stress conditions and their interaction
with genes for resistance. Full susceptibility to isolate WP-156 further
indicates absence of a gene(s) with a major effect in conditioning resistance
to wilt caused by this isolate in the potato populations tested. There is
obviously a strong gene(s) for resistance to WP-17 present in these
populations. It is thus evident that the genetics of resistance to *P.
solanacearum* in potatoes is very complex with both genes with major and minor
effects involved. Strong interaction between genes for heat tolerance and
those for resistance implies the presence of a large amount of favourable non-
additive (epistatic) gene effects in a high resistance expression. In this

study, there was further evidence that a widened genetic background for resistance and adaptation would provide a higher level of resistance which is likely more stable under diverse circumstances. This was examplified by the performance of progenies of the clones 381064.3,381064.7,and AVRDC-1287.19, which have undergone several cycles of selection for resistance and adaptation to warm conditions and combine resistance from at least two (AVRDC-1287.19) to three (381064.3, 381064.7) specific sources (Table 1).

All the aforementioned suggests that breeding at the population level by incorporating multiple sources of resistance and heat tolerance should be effective in producing superior genotypes suitable for potato production in the lowland tropics where high levels of BW resistance and heat tolerance are much needed. Recurrent selection in a population with a wide genetic background for resistance and adaptation should be a promising approach. An example is the great improvement in resistance to *Corynebacterium insidiosum* McCull Jens. (bacterial wilt) in alfalfa after just three cycles of recurrent selection (Barnes et al.,1971). If residual resistance to *P.solanacearum* is of any significance, incorporation of multiple sources of resistance in a genotype should also be an advantage in circumstances where mild compatible pathotypes/strains are prevalent, because residual effects seem to correlate with level of resistance. This advantage would be desirable especially under the conditions of subsistence agriculture in the developing world.

The foregoing discussion did not consider latent infection of tubers with *P. solanacearum* as it was shown by Ciampi and Sequeira (1980) that wilt symptoms are generally not correlated with latent infection. They suggested that latent infection of tubers is controlled by different genetic factors. Setting latent infection aside and taking into account the tremendous variation in pathotypes of *P. solanacearum* and involvement of genes for adaptation , one has to think of the BW resistance as quantitative and probably polygenic in nature.

The effect of reciprocal crosses, as tested in this study, on expression of resistance to BW did not show to be significant. The effect of a particular parental genotype appeared to be more important. Effects of reciprocal crosses need, however, further investigations, preferably in a controlled environment, since cytoplasmic effects are usually a sensitive objective demanding more sensitive test procedures.

Chapter V

INHERITANCE OF RESISTANCE

TO *PSEUDOMONAS SOLANACEARUM* E.F.SMITH IN POTATO

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Abstract

Inheritance of bacterial wilt resistance in tetraploid potato was investigated in segregating progenies of parent clones with resistance derived from different specific sources and different types of adaptation. A race 1 and a race 3 isolate of *Pseudomonas solanacearum* were used to test the resistance under warm temperature. Results obtained indicated partial dominance of resistance. Significant general and specific combining abilities showed that both additive and non-additive gene actions are important in conditioning the resistance expression. There was evidence that epistasis is an important component of the non-additive gene action in the inheritance of the resistance. Other aspects of the resistance and implications for breeding are discussed.

Introduction

The first attempts to search for resistance to bacterial wilt (BW), caused by *Pseudomonas solanacearum* E. F. Smith, in potato were probably made by Thung (1947) and Nielsen & Haynes (1960). It was found that the resistance in *Solanum tuberosum* L., the common potato, is probably not adequate to control the disease. The first source of high resistance was found by Thurston & Lozano (1968) in several Colombian *S. phureja* Juz. & Buk. clones. The resistance has , however, been found to be strain specific and temperature sensitive (Thurston & Lozano, 1968; Sequeira & Rowe, 1969; Ciampi & Sequeira, 1980; French & De Lindo, 1982; and Tung et al., 1992 a & b).

Rowe and co-workers (Rowe & Sequeira, 1970; and Rowe et al., 1972) reported on a system of three independent and dominant major genes which control resistance to BW in *S. phureja*. There was also evidence of effects of modifying genes. Results from their later experiments, however, did not support their initial hypothesis (Sequeira, 1979). Since then, no further efforts have been made to understand the genetics of resistance, probably because of the very complex nature of the resistance itself. The great complexities of tetrasomic inheritance might have further discouraged investigations on the genetics of resistance in tetraploid potatoes. Despite the fact that additional sources of resistance have been found and utilized in breeding programs during the past two decades (Schmiediche, 1985a;

Schmiediche & Martin, 1983; French & Sequeira, 1988), the genetics of BW resistance in potatoes has remained a question still to be answered.

More recent evidence shows that resistance to BW in potatoes is very complex in nature. It is probably a function of environmental adaptation (Schmiediche, 1985a; and Kloos & Fernandez, 1986) with genes for adaptation being involved (Tung et al., 1990b and 1992a & b). Research results have indicated that the genic system controlling the resistance may involve genes with major and and genes with minor effects. In addition, there is a large amount of interaction between genes for resistance and those for adaptation (Tung et al., 1992a & b) and combining ability seems to be a considerable feature of the resistance (Tung et al., 1990b). Also, potato clones with a widened genetic background for both BW resistance and adaptation tend to display a higher level of resistance which is more stable over environments. The resistance thus seems to be of a polygenic and quantitative type.

The objective of this study was to investigate the type of gene action and combining abilities in the inheritance of the resistance to BW derived from several specific sources and introduced into the tetraploid potato.

Materials and Methods

A simple and convenient way of testing for presence of additivity and/or non-additivity of gene action in inheritance of any trait is to intercross two parent clones, to self them, and to compare the mean of the hybrid progeny with the overall mean of the two self progenies. As the hybrid progeny must be at least as heterozygous as the self progenies, or even more so, any significant departure from the mean of the two self progenies will reflect the interaction between the genes by which the parent clones differ. The interaction can be dominance or dominance-based epistasis or both. This procedure reflects the fundamental concept underlying several biometrical methods of quantitative genetics such as the diallel cross (Hayman, 1954), North Carolina Design I and II (Comstock & Robinson, 1952), and generation mean analysis (Mather & Jinks, 1971). In fact, it was successfully employed by Killick & Malcolmson (1973) in investigating the genetic control of resistance to late blight, *Phytophthora infestans* (Mont.) De Bary in potatoes. The experiments 1 and 2 in this study made use of this procedure with the parent clones also included in the test for resistance with the expectation that the data to be obtained would provide additional information about the effect of selfing and intercrossing on the level of resistance.

Experiment 1

The potato clones AVRDC-1287.19, BR-112.113, BR-63.74, I-1039 and LT-7 were used. The first three clones have been the initial resistant stocks in CIP and several national breeding programs and have a relatively simple genetic background for resistance to BW. The resistance of clone AVRDC-1287.19 has been derived from *S.chacoense* Bitt. and *S. raphanifolium* Card. & Hawk and is well combined with a high level of heat tolerance. BR-112.113 and BR-63.74 have the typical *S. phureja*-based resistance which usually breaks down under high temperatures. LT-7 has been known primarily for its high level of heat tolerance, but recently was found to be highly resistant to a race 1 isolate, WP-17, of *P. solanacearum* (Tung et al., 1990a). I-1039 is a susceptible cultivar agronomically well adapted to tropical highlands, but largely heat sensitive.

Six F_1 progenies were produced by intercrossing both AVRDC-1287.19 and BR-112.113 with each of the other three clones. All parents were also selfed. There were thus six genetic sets, each set including the parent clones P_1 and P_2 (P's), the self progenies P_1-self and P_2-self (P-selfs) and the F_1 (see Table 1). All sets were tested for resistance to isolate WP-17 under screen-house conditions at Los Banos, the Philippines (150 m above sea level, asl), during the period 25 January - 8 February, 1990. The experiment was conducted in a completely randomized design with two replications of 50 plants for each self and F_1 progeny and 10 plants per parent clones. As individual plants of a clone are genetically identical, planting medium and cultural practices were uniformly applied and replicate means were to be used for analysis, the extent to which the difference in plot size between parents and progenies would affect the residual variance was assumed to be at a minimum and negligible.

Seedlings of F_1 and self progenies, raised in heat sterilized volcanic subsoil, were transplanted individually at the first true leaf stage in 8 cm-diameter clay pots containing the same type of soil. At the same time disease-free tuberlets of the parent clones were planted directly in the pots. Ten tuberlets of the susceptible clone 7XY.1 were also planted to serve as a check for effectiveness of the inoculum. The plants were irrigated with tap water at two-day intervals and fertilized once a week with a 1% solution of 14 % N, 5 % P, and 12 % K. Mancozeb (0.3 %) and cypermethrin (0.1 %) were sprayed weekly to prevent infestation by fungi and insects. All plants were trimmed to single-stemmed one week prior to inoculation. Four weeks after planting, the plants were inoculated by cutting off the leaves at the third and the fourth positions from the crown with a scalpel dipped into an inoculum

suspension of the bacterium containing 10^8 cells/ml. The inoculated plants were maintained in the screen-house where maximum/minimum temperatures were recorded daily. Fifteen days after inoculation, when check plants were all completely wilted or dead, wilt symptoms of individual plants were scored following the index scale used by French and De Lindo (1982): 1, no symptoms; 2, one or two leaves wilted; 3, up to half of the leaves wilted; 4, up to 3/4 of the leaves wilted and 5, plant completely wilted or dead.

Experiment 2

This experiment was the first attempt to investigate the inheritance of resistance to BW in an advanced population of potato clones derived through several cycles of selection for resistance , combined from several specific sources, and for adaptation to warm conditions. The underlying hypothesis was that the inheritance pattern might have been altered in some way following the changes in the genetic make-up of the resistance.

The potato clones used were numbered 384008.4, 384012.2, 384015.30 and 381064.12. They have at least three known specific resistance sources, namely *S. phureja*, *S. chacoense* and *S. raphanifolium* in their ancestry and were selected from the population of clones mentioned above. The last cycle of selection for resistance to *P. solanacearum* under field conditions and for adaptation to warm temperatures was done in Mindanao (800 m asl), the Philippines, where race 3 is prevalent. All of these clones have BR-63.74 as initial resistant ancestor three generations backwards, and AVRDC-1287.19 as the most recent resistant parent or grand-parent. Clones 384015.30 and 301064.12, though selected in different years, have full sibs as female parent and AVRDC-1287.19 as male parent. The clones were selfed and intercrossed to produce three genetic sets (see Table 2) comparable to those in experiment 1. Parent clones, F_1 and self progenies were tested for resistance to a race 3 isolate (DT-10) of *P. solanacearum* in Ductrong (1000 m asl), Vietnam, during the period 10-25 April, 1991.

A test nursery was constructed with 3.5 x 5.0 m houses roofed with transparent polyethylene sheets at the test site. In each house, 0.70 x 2.0 x 0.15 m soil beds, raised 0.7 m above the ground, were made and filled up with a 15:1 mixture of heat sterilized volcanic subsoil and decomposed chicken manure. Seedlings of F_1 and self progenies were transplanted at the first true leaf stage into the raised beds in three completely randomized replicates of 20 plants per progeny. Rooted cuttings of parent clones were also transplanted

on the same day with 10 plants in each of three replicates per clone. All cultural practices,inoculation methods, temperature reading, and assessment of reaction to disease were as described in experiment 1.

Experiment 3

Combining ability for resistance to *P. solanacearum* was investigated for a set of nine clones. Five resistant clones were crossed as females with each of four susceptible male clones following the Mating Design II (Comstock & Robinson, 1952).The resistant parents were AVRDC-1287.19, BR-112.113, BR-63.74, BR-63.76 and Cruza-148. The first three clones were described previously. BR-63.76, a full sib of BR-63.74, and Cruza-148, with an unknown source of resistance , have also been utilized as initial resistant stocks at CIP and several national breeding programs. The susceptible parents were CFK-69.1 and I-1039, both heat sensitive, and DTO-33 and LT-8, both heat tolerant. The 20 F_1 progenies were tested for resistance to isolate DT-10 under the nursery conditions at the same location and during the same period of time as described in experiment 2. The experiment was conducted in a completely randomized design with three replications of 20 plants per progeny. Ten plants per parent clones were also tested for their own level of resistance. All other experimental operations were as described in experiments 1 and 2.

For all experiments, the plot mean disease indices were used for analysis of variance . For experiment 3, the % survival was also taken for combining ability analysis after an arcsin-transformation. Non-orthogonal sets of single degree-of-freedom contrasts were employed to compare F_1 vs P-selfs, F_1 vs P's and P-selfs vs P's in experiments 1 and 2. The tests criterion was adjusted to $F' = F/(k-1)$, where F was the value obtained directly from the comparison, and k= the number of entries involved in each comparison. F' thus had k-1 and N-1 dgrees of freedom for the test of significance of the differences, where N=the total number of observations in each comparison(Meddis, 1975).

Results

In Los Banos, high temperatures were prevalent. The average maximum/minimum temperatures (°C) were 32.7/22.8, the mean being 27.8. In Ductrong , temperatures were moderately warm, the average max/min being 30.2/18.7 and the mean 24.5. Under these conditions, disease incidence was high at both locations, but high and intermediate levels of resistance were found in Los

Banos and Ductrong against the race 1 and race 3 isolates used, respectively (Tables 1, 2 & 3). At both locations, there were significant differences between entries tested in all experiments.

Table 1. Mean wilt index (1-5) of five potato clones (P), their self progenies (P-self), and six F_1 s 15 days after inoculation with a race 1 isolate of *P. solanacearum* and pertinent comparisons based on analysis of variance.

Entry		Set 1	Set 2	Set 3	Set4	Set 5	Set 6
	P_1	AVRDC-1287.19	AVRDC-1287.19	AVRDC-1287.19	BR-112.113	BR-112.113	BR-112.113
	P_2	I-1039	BR-63.74	LT-7	I-1039	BR-63.74	LT-7
P_1		1.35 d[1]	1.35 c	1.35 c	3.10 c	3.10 c	3.10 a
P_2		4.45 a	4.70 a	1.00 c	4.45 a	4.70 a	1.00 c
Mean of P's		2.90	3.03	1.18	3.78	3.90	2.05
P_1self		2.83 c	2.83 b	2.83 a	3.57 bc	3.57 bc	3.57 a
P_2self		4.72 a	4.47 a	1.92 b	4.72 a	4.47 a	1.92 b
Mean of P-selfs		3.78	3.65	2.38	4.15	4.02	2.65
F_1		3.28 b	3.08 b	2.22 b	4.13 ab	3.93 b	2.27 b

Source of variation	df	Mean squares					
		Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
F_1 vs P-self	1	0.3267*	0.4332*	0.0320	0.1680	0.0108	0.3008*
F_1 vs P's	1	0.1925	0.0043	1.4560**	0.0003	0.0588	0.0645
P's vs P-self	1	1.5313**	0.7813**	2.8800**	0.1850	0.0288	0.9661**
Error	5	0.0161	0.0152	0.0122	0.0196	0.0216	0.0151

[1] For P's, P-selfs and F_1 of each set, values followed by a common letter are not significantly different at P=0.05 by Duncan's multiple range test.
* and **, significant at P<0.05 and P<0.01, respectively.

Inheritance of resistance

In experiment 1, F_1 progenies of all six genetic sets displayed an intermediate level of resistance between the respective P-selfs, but tended to come closer to the more resistant one (Table 1, upper part). In fact, in five sets disease indices of F_1's were not statistically different from that of the more resistant P-self. A similar situation was also observed in experiment 2. In two of the three sets, F_1's showed intermediate levels of resistance which were not significantly different from that of the more resistant P-self (Table 2, upper part). An exception was observed in set 1 where the F_1 progeny tended to be more resistant than both respective P-selfs, though not significantly different from the more resistant one. This higher resistance expression might be associated with a certain interaction between

the parents and will be discussed later. In general, the results obtained
indicated that resistance to BW is a partially dominant character.

Table 2. Mean wilt index (1-5) of four potato clones (P), their self progenies
(P-self) and three F_1s 15 days after inoculation with a race 3 isolate of
P.solanacearum and pertinent comparisons based on analysis of
variance.

Entry		Set 1	Set 2	Set 3
	P_1	384012.2	384008.4	384015.30
	P_2	384015.30	381064.12	381064.12
P_1		3.20 ab[1]	2.67 b	2.83 ab
P_2		2.83 b	1.83 c	1.83 c
Mean of P's		3.02	2.25	2.33
P_1-self		3.35 a	3.28 a	3.10 a
P_2-self		3.10 ab	2.47 b	2.47 b
Mean of P-sel		3.23	2.88	2.98
F_1		2.77 b	2.92 ab	2.98 ab

Source of variation	df	Mean squares		
		Set 1	Set 2	Set 3
F_1 vs P-self	1	0.4201*	0.0035	0.0800
F_1 vs P's	1	0.1205	0.8892*	0.8450*
P-self vs P's	1	0.1302	1.1719*	0.6075*
Error	10	0.0253	0.0423	0.0302

[1] For P's, P-selfs and F_1 of each set, values followed by a common letter are
     not significantly different at P=0.01, by Duncan's multiple range test.
* and ** , significant at P<0.05 and P<0.01, respectively.

The linear contrasts of F_1 vs P-selfs showed significant departure of F_1's
from the mean of the respective P-selfs in three of the six sets of experiment
1 (Table 1, lower part) and in one of the three sets of experiment 2 (Table
3, lower part). In experiment 1, F_1's of AVRDC-1287.19 significantly differed
from the mean of respective P-selfs in sets 1 and 2, but not in set 3.
Similarly, F_1's of BR-112.113 departed significantly from P-selfs' mean in set
6, but not in the other two (Table 1, lower part). In a statistical sense, the
variation in level of resistance in sets 3,4 and 5 could be ascribed to
statistical additive effects and that in sets 1,2 and 6 to non-additive
effects. As statistical additivity or non-additivity reflects a corresponding
genetical additivity or non-additivity it was apparent that gene effects were
additive in some crosses and non-additive in the others. In experiment 2,
clone 384015.30 was involved in two crosses, but yet combined additively in

one (set 1) and non-additively in the other (set 3). In contrast, 381064.12 combined additively in both crosses in which it was involved (Table 2, lower part). More detailed interpretation of the data seemed almost imposible and it may be concluded that in the inheritance of BW resistance in potato, both additive and non-additive gene actions are important. The additivity and non-additivity of gene action would consequently result in general and specific combining ability as it has been proposed (Tung et al. ,1990b). Though the resistance behaved as a partially dominant trait, F_1 progenies of some parental clones may unexpectedly show higher or lower levels of resistance than P-selfs or P's as a consequence of specific combining ability.

Effects of selfing and intercrossing

Except for BR-63.74, P-selfs tended to display mean levels of resistance lower than those of their respective P's in both experiments 1 and 2 (Table 1 & 2, upper part). However, significant differences between P-selfs and their respective P's were found only for AVRDC-1287.19, LT-7 (Table 1, upper part) and 381064.12 (Table 2, upper part) which were both highly resistant and heat tolerant. With susceptible clones or clones with low levels of resistance there were no significant differences between P-selfs and P's (Table 1 & 2, upper part). The contrasts P-selfs vs P's indicated significantly lower average levels of resistance in P-selfs as compared to the mean levels of the respective two P's in sets 1, 2, 3 and 6 in experiment 1 (Table 1, lower part) and in sets 2 and 3 in experiment 2 (Table 2, lower part). These results suggest that selfing or inbreeding had caused a certain reduction in level of resistance in the parent clones. However, the degree to which resistance expression was depressed seemed to be significant only with highly resistant clones. Under a particular set of environmental conditions, this situation would not be observed if the resistance in the parent clones is lost as typified by the case with Cruza-148 and BR-clones (Table 1 and 2).

The contrasts F_1 vs P's showed that in both experiments most of the F_1's tested had a mean disease index not significantly different from that of the two respective P's (Tables 1 & 2, lower part). Considerably lower levels of resistance of F_1 were found in crosses AVRDC-1287.19 x LT-7, 384008.4 x 381064.12, and 384015.30 x 381064.12, where the parent clones were either highly or moderately resistant. It appeared that while inbreeding, as a result of selfing, may seem to reduce the level of resistance passed on from parent clones to self progenies, the loss of heterozygosity does not seem to be the major cause of such reduction. On the contrary, if it is, one would expect

that F_1's means should always be equal to the means of their two parent clones as the F_1's are at least as heterozygous as the parents, or even more so. This was not the case with the resistant clones used in this study, however. Because parent clones are highly selected and vegetative propagation preserves the whole genotypes, the loss of resistance upon selfing or intercrossing seems more a consequence of loss of certain favorable epistatic associations upon break-down of the intact parental genotypes rather than being a result of reduced heterozygosity.

Because AVRDC-1287.19 and LT-7 were developed by two different breeding programs using different breeding materials, it is probable that they possess different alleles for resistance. If intralocus interactions were very large one would expect that their cross would display non-additivity. Since this was not the case, it seemed that non-additive gene action in the inheritance of BW resistance is largely of the epistatic type.

Effects of adaptation

It was of interest to note that, as found earlier (Tung et al.,1992a), resistance to wilt was largely reduced in clone BR-112.113 and almost lost in BR-63.74 (Table 1, upper part) as these two clones are heat sensitive. AVRDC-1287.19 and LT-7 are both resistant and heat tolerant and indeed showed a high level of resistance to the race 1 isolate used in experiment 1. The contrasts F_1 vs P-selfs demonstrated that BR-112.113 combined additively in crosses with I-1039 and BR-63.74 which are heat sensitive, but non-additively in the cross with LT-7 which is heat tolerant. In contrast, AVRDC-1287.19 combined additively with LT-7, but not so with I-1039 and BR-63.74 (Table 1, lower part). These data suggest that additive gene effects might be of importance when the parents are of a similar type of (or have similar genes for) adaptation. Conversely, non-additive gene effects might be large when parents differ greatly in this aspect. It is thus conceivable that genes for adaptation are involved in conditioning the resistance expression. There indeed has been evidence which is well in accordance with such an argument (Tung et al., 1990b; Kloos & Fernandez, 1986; and Schmiediche, 1985).

Combining ability

Analysis of variance applied to results of experiment 3 demonstrated highly significant general combining ability (GCA) of both resistant and susceptible parent clones as well as specific combining ability (SCA) in their F_1's

(Table 4). For wilt index, GCA effects were significant for four of the five resistant parents, and three of the four susceptible parents (Table 5). For % survival, only three resistant and 2 susceptible parents showed significant effects of GCA (Table 5). Under the conditions of this experiment AVRDC-1287.19 and BR-63.76 seemed to be the better general combiners among the resistant parents. Similarly, among the susceptible parents, CFK-69.1 and LT-8 appeared to be the better ones.

Table 3. Disease index (1-5, upper value) and % survival (lower value) of 20 F_1 progenies from crosses between five resistant and four susceptible clones, 15 days after inoculation with a race 3 isolate of *P. solanacearum*.

Resistant parent	Susceptible parent				Mean	Index of parent clone
	CFK-69.1	I-1039	DTO-33	LT-8		
Cruza-148	4.22	4.27	3.80	3.40	3.92	3.90
	5.00	5.00	13.30	12.00	8.80	
BR-63.74	2.97	4.00	3.82	2.97	3.44	3.70
	25.00	13.30	8.30	26.70	18.30	
BR-112.113	2.82	4.12	3.30	3.15	3.27	2.60
	31.70	13.30	23.30	26.70	15.80	
BR-63.76	3.10	3.60	3.03	2.82	3.19	3.00
	25.00	20.00	23.30	31.70	25.00	
AVRDC-1287.19	2.88	3.18	2.28	2.67	2.74	2.30
	25.00	16.70	46.70	35.00	30.90	
Mean	3.20	3.83	3.25	3.00		
	22.30	13.70	23.00	26.42		
Index of parent clone	3.90	4.60	4.20	3.40		

$LSD_{0.05}$ = 0,35 and 3.1 for disease index and % survival, respectively.

Effects of SCA were significant in nine of the 20 crosses for wilt index and in three for % survival (Table 5). The cross AVRDC-1287.19 displayed the highest SCA effect in increasing the level of resistance. Assuming parents are fixed and summing up the squared effects separately for each parent and for all crosses, it appeared that resistant parents varied in GCA more than did the susceptible ones and SCA was important in conditioning resistance expression.

Table 4. Mean squares for disease index and % survival for five resistant and four susceptible parents and their interactions in 20 crosses made in Mating Design II by analysis of combining ability for resistance to a race 3 isolate of *P.solanacearum*.

Source	df	Mean squares	
		Index	% survival
Resistant parents	4	2.0611**	460.0356**
Susceptible parents	3	1.1242**	374.6074**
Interaction	12	0.2673**	81.0852**
Remainder	40	0.0621	20.8406

**, significant at P<0.01.

Table 5. General combining ability (GCA) effects (in margins) of nine potato clones and their specific combining ability (SCA) effects in 20 crosses (in middle) for resistance to a race 3 isolate of *P. solanacearum*, 15 days after inoculation. Disease index: upper value; % survival [1]: lower value.

	SCA effects				GCA
	CFK-69.1	I-1039	DTO-33	LT-8	effects
Cruza-148	0.41**	-0.16	-0.05	-0.20*	0.61**
	-7.42**	-0.41	3.03	4.80	9.30**
BR-63.74	-0.35**	0.06	0.44**	-0.15	0.13**
	5.19	1.78	-8.57**	1.60	-2.60
BR-112.113	-0.41**	0.26**	0.02	0.12	0.03
	4.80	-1.08	-0.70	-3.01	2.10
BR-63.76	0.08	-0.05	-0.03	0.00	-0.18**
	-0.52	3.00	-1.66	-0.82	3.10*
AVRDC-1287.19	0.26**	-0.11	-0.39**	0.24**	-0.57**
	-2.06	- 3.28	7.91**	-2.57	6.80**
GCA effects	-0.12**	0.51**	-0.07	-0.32**	
	0.60	-6.40**	0.06	5.20**	

[1] Arcsin- transformed values.

* and **, significantly different from zero at P<0.05 and P<0.01, respectively.

Distribution of phenotypes

Distributions of plants of F_1s and self progenies tesed in experiments 1 and 2, and some of the progenies tested in experiment 3 are presented in Figs. 1, 2 and 3, respectively.
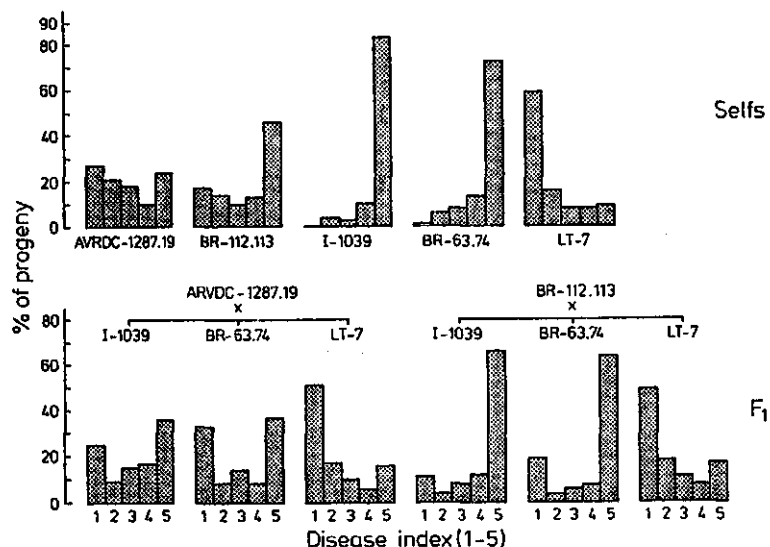

Figure 1. Distribution of plants (%) among disease indices (1-5) in self and F_1 progenies of five potato clones, 15 days after inoculation with a race 1 isolate of *P. solanacearum* (WP-17) at Los Banos. For disease index of parent clones see Table 1.

A common feature observed in all three experiments was that the progenies tended to segregate to the extreme values of indices, though not completely so and in no case a clearcut bimodal distribution was found. The reason for this tendency was not at all clear, but either the presence of a gene with a major effect or the break-down of the intact parental genotypes upon cross-breeding or both might be the possible cause of such distribution patterns.

There has been some evidence that in the population of the parental clones used certain resistance gene(s) has a major effect on expression of resistance

to isolate WP-17 (Tung et al.,1992 a & b). The effect of this gene(s) seemed to be strong enough to maitain similar distribution patterns in the progenies tested in these experiments (Fig. 1, 2 & 3). Strain specificity and effects of different environmental conditions in this case did not seem to be strong enough to cause much difference in distributions of progenies tested in experiments 1 & 3. Also, no clearcut distinction was found between distributions of progenies of clones with a simpler genetic background for resistance (in experiment 1 & 3) and those of clones with a more complex one (experiment 2).
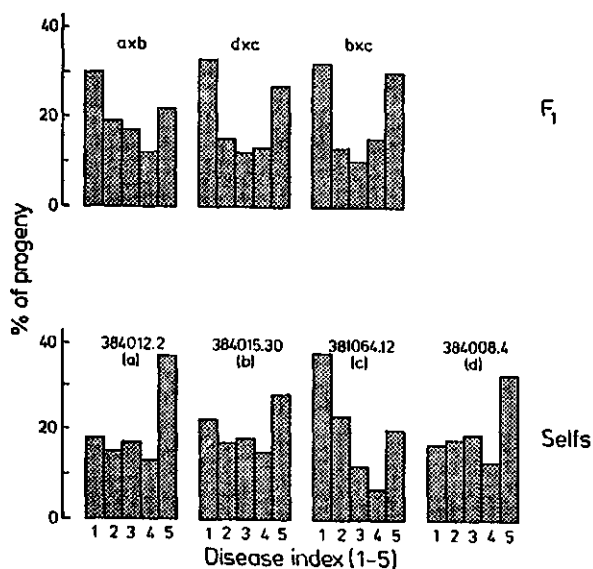

Figure 2. Distribution of plants (%) among five disease indices (1-5) in self and F$_1$ progenies of four resistant potato clones, 15 days after inoculation with a race 3 isolate of *P. solanacearum* (DT-10) at Ductrong. For disease index of parent clones see Table 2.
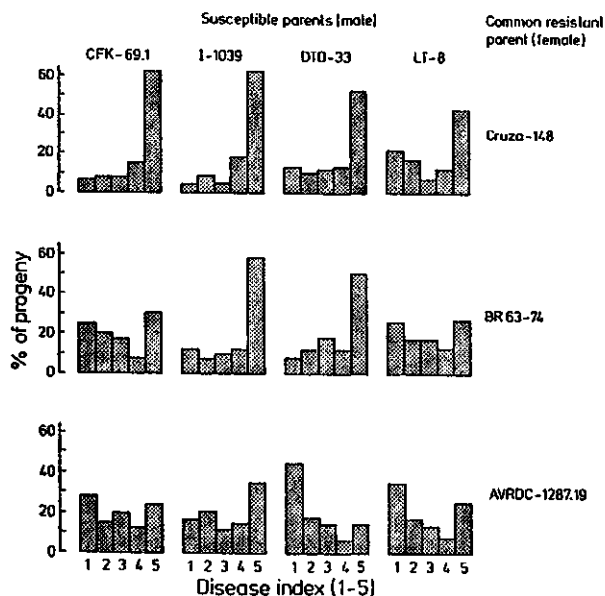
Figure 3. Distribution of plants (%) among disease indices (1-5) in 12 crosses between three resistant and four susceptible potato clones, 15 days after inoculation with a race 3 isolate of *P. solanacearum* (DT-10) at Ductrong. For disease index of parent clones see Table 3.

Discussion

Results from this study did not permit a detailed inference about the genetic architecture of the resistance to BW in the tetraploid potato. It did, however, provide some basic information about the genetic properties of the resistance which would be useful to potato breeders. It was in accordance with Rowe and co-workers' finding that the resistance is a dominant character, whatever the source used in this study it has come from and involves genes with both major and minor effects (the latter being called modifiers by Rowe et al., 1972). The dominance, however, is more of a partial type and a system of three major genes does not fit the complex nature of the resistance. Both additive and non-additive gene actions are important in inheritance of resistance, a large proportion of the latter being of the epistatic type. This is indicated by the fact that some susceptible parent, or rather parents not known for any resistance, tended to increase or to decrease the level of

resistance in their progenies beyond expectation based on their GCA thus displaying highly significant SCA effects. Because the resistance used to disappear whenever the carrier genotype confronts new ecological niches, presence of significant interaction between resistant and susceptible parents further implies that genes for adaptation are involved in conditioning resistance expression.

The reduction of resistance to wilt resulting from selfing and intercrossing of highly resistant parent clones is most likely due to breakdown of the intact parental genotypes and loss of favourable epistatic associations. This seems well in line with Wright's (1956) theory that in species with prevalent uniparental reproduction and occasional cross-breeding, clonal selection, natural or artificial, will result in advantageous genotypes with certain " adaptive peaks ", and breakdown of such peaks upon cross-breeding. The outcome is thus usually the lower performance of segregating offsprings. The tendency of the progenies, selfed or F_1, to segregate to the extreme performances in this study might in part be a consequence of such a phenomenon.

It is noteworthy that recent research at CIP shows that resistant accessions of various wild *Solanum* species have been collected from areas where *P. solanacearum* has never been found (CIP, 1986). It is thus a matter of reasoning, that true resistance genes have never been moulded by natural selection. This raises the question, why are there genes which exert major effects on resistance expression and what is their nature ? Plausibly, certain genes for adaptation might have turned out to have the novel (pleiotropic) effects in conferring the resistance once the potato plant has come into contact with the pathogen under a certain set of environmental conditions. Those genes are then eventually termed genes for resistance and the resistance itself tends to disappear whenever demanded to meet new ecological conditions as those in the lower elevations of the tropics. As BW resistance is associated with fitness, breeding for resistance, which has taken place only during the recent two decades, would seem a difficult and challenging task. Attempts to transfer resistance from wild *Solanum* species into the common potato would result in excessive recombinations which are most vulnerable to breakdown upon intercrossing. Furthermore, as pointed out by Mather (1960), over the short term, the unit of transmission and adjustment is not so much the genes as the chromosome segments carrying genes affecting more than one character. From an evolutionary point of view, adjustments and adaptations imposed on the polygenic systems in the wild species by natural selection in the past will obviously channel and limit response to selection, whether

environments of the lower elevations in the tropics will indeed demand readjustment and reorganization of the genic systems conditioning both resistance and adaptation. As a consequence, progress during the first few cycles of selection would rather be slow and inconsistent.

The foregoing discussion indicates that more effort needs further be devoted to breeding for BW resistance as potato production is steadily expanding in the warm tropics. For attaining a high level of stable resistance, recurrent selection employing both additive and non-additive gene actions in a population with the widest genetic background possible for both resistance and adaptation seems to be the most promising approach. Clonal selection is still the most effective method in developing resistant cultivars for restricted local areas but testing for resistance and adaptation should be done right at the location where the cultivars are destined to grow. The best resistant true potato seed progenies can be developed only by progeny testing under local conditions.

Chapter VI

GENETIC VARIATION FOR BACTERIAL WILT RESISTANCE

IN A POPULATION OF TETRAPLOID POTATO

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Key words: bacterial wilt, genetic variance, heritability, *Pseudomonas solanacearum*, resistance, *Solanum* spp..

Abstract

Genetic variance components and heritability were estimated for resistance to bacterial wilt in a population of tetraploid potato with resistance derived from several specific sources.   Both additive and non-additive variance components were significant. Their relative magnitudes indicated the importance of non-additive gene action in the genetic control of the resistance. Narrow-sense heritability was relatively low for both disease index and % survival indicating that progress in population development would be slow. Broad-sense heritability was, however, relatively high which promises success of clonal selection in developing clonal cultivars.

Introduction

Since no reliable resistance to bacterial wilt (BW), caused by *Pseudomonas solanacearum* E.F. Smith, is found in the common potato (*Solanum tuberosum* L.), during the last twenty years, breeding for resistance has been based chiefly on the resistance derived from *S. phureja* Juz. & Buk. The resistance, however, has been shown to be very unstable due to its strong host-pathogen-environment interaction (Sequeira & Rowe, 1969; Ciampi & Sequeira, 1980; French & De Lindo, 1982; and Tung et al.,1990c and 1992 a & b). Extensive more recent evidence shows that in general the resistance is very complex in nature. It is apparently of a polygenic and quantitative type involving genes with major effects as well as genes with minor effects. There is also evidence that in the inheritance of resistance to wilt non-additive gene action,including epistasis, is important (Tung et al.,1992a, b & c). Moreover, the genetic background for adaptation is of crucial importance for expression of resistance (Kloos & Fernandez, 1986; Schmiediche, 1985a & b; and Tung et al.,1990a and 1992 a & b).

Incorporation of several sources of resistance into a genotype/population with wide adaptation has been thought to stabilize the resistance expression under diverse pathogenic conditions (Sequeira, 1979; Schmiediche & Martin, 1983).During the last decade, additional sources of resistance to BW have been identified and utilized in the breeding programs at the International Potato Center (CIP) and the University of Wisconsin (Schmiediche & Martin, 1983; Schmiediche, 1985a & b; French & Sequeira, 1988).

Preliminary evidence from the CIP breeding program indicates that the genetic base for resistance to BW is different in each resistant wild species and that there is probably polygenic resistance in some of them (Schmiediche, 1985b). The concept of wide spectrum resistance thus has become realistic. Breeding efforts during the last few years have resulted in a new tetraploid population with resistance derived from several specific sources. The population is intended for further breeding and selection in national breeding programs in the tropics. The objective of this study was to evaluate the genetic variation for resistance to BW in this population in terms of types and magnitudes of genetic variances.

Materials and Methods

The reference population comprises tetraploid clones derived through three or four cycles of selection for BW resistance and adaptation to warm growing conditions. Resistance is known to be incorporated from three specific sources, namely *S. phureja* Juz. & Buk., *S. raphanifolium* Card. & Hawk, and *S. chacoense* Bitt. The last cycle of evaluation and selection for resistance to *P. solanacearum* was done under field conditions in a mid-elevation area of Mindanao (800 m above sea level, asl), the Philippines, where race 3 is prevalent. Because selection was relatively mild, it was assumed that wide genetic variation is still present in this population.

Six clones were crossed as males to each of other six female clones (Table 2) to produce 36 F_1' following the Design II mating scheme (Comstock & Robinson, 1952). The clones were chosen at random avoiding all common parentage. The progenies along with their parent clones were evaluated for resistance to a race 3 isolate (DT-10) during the summer of 1991, at Dalat (1500 m asl) and Ductrong (1000 m asl), Vietnam.

Test for resistance

At each location, a test nursery was constructed with 3 x 5 m houses roofed with transparent polyethylene sheets. In each house, 0.70 x 2.00 x 0.15 m soil beds, raised 0.70 m above the ground, were made and filled up with a 15:1 mixture of heat sterilized volcanic soil and decomposed chicken manure. F_1 seedlings and rooted cuttings of parental clones, raised from apical cuttings, were transplanted into the soil beds in a randomized complete block design with three replications of 20 plants per entry. Twenty rooted cuttings of the susceptible clone 7XY.1 were also planted to serve as check for the

effectiveness of the inoculum. The plants were irrigated with tap water at 2-day intervals and fertilized weekly with a 1 % solution of 14 % N, 5 % P and 12 % K. Mancozeb (0.3 %) and cypermethrin (0.1%) were sprayed every week to prevent infestation by fungi and insects. All the test plants were trimmed to single-stemmed one week prior to inoculation. After four weeks, the plants were inoculated by cutting off the leaves at the third and the fourth positions from the crown with a scalpel dipped into an inoculum suspension of the bacterium containing 10^8 cells/ml. Fifteen days after inoculation, when check plants were all completely wilted or dead, wilt symptoms of individual plants were recorded based on the index scale used by French & De Lindo (1982): 1= no symptoms; 2 = one or two leaves wilted; 3 = up to half of the leaves wilted; 4 = up to 3/4 of the leaves wilted; and 5 = plant completely wilted or dead. For F_1s , % of plant survival of each plot was also recorded. The test was performed during the periods 15-30 April and 2-17 May, 1991 at Dalat and Ductrong, respectively. During the test periods, maximum/minimum temperatures in the test nurseries were recorded daily.

Estimation of genetic parameters

For statistical analysis, disease index of individual plants was transformed to $\sqrt{(x + 0.5)}$ and % plant survival of each plot to arcsin $\sqrt{(\%)}$. Plot means were used for all analyses of variance (ANOVA) and covariance.

The pooled ANOVA for parents and progenies over locations is shown in Table 1. The ANOVA for F_1s and derivation of variance components followed the method described by Comstock & Robinson (1952). The parent-offspring covariance for disease index was computed using parental means and means of the corresponding six F_1's. The analysis of covariance was analogous to the ANOVA for parents. The genetic model assumed: 1) non-inbred parents, 2) no maternal effects, 3) linkage equilibrium, 4) tetrasomic inheritance with random chromosome assortment (i.e. absence of double reduction, $\alpha= 0$) and no preferential pairing, and 5) random choice of parents. Whether these assumptions are valid or not will be discussed later. Under these assumptions and, just for the sake of simplicity, if the higher order epistasis is ignorable:

$$\sigma^2_m = \sigma^2_f = \text{covHS} = 1/4\ \sigma^2_A + 1/36\sigma^2_D + 1/16\ \sigma^2_{AA} + 1/144\ \sigma^2_{AD} + 1/1296\ \sigma^2_{DD}$$

$$\sigma^2_{mf} = \text{covFS} - 2\text{covHS} = 1/6\ \sigma^2_D + 1/12\ \sigma^2_T + 1/36\ \sigma^2_F + 1/8\ \sigma^2_{AA} + 1/72\ \sigma^2_{AD} + 31/648\ \sigma^2_{DD}$$

$$\sigma_{po} = 1/2\ \sigma^2_A + 1/6\ \sigma^2_D + 1/4\ \sigma^2_{AA} + 1/12\ \sigma^2_{AD} + 1/36\ \sigma^2_{DD}$$

(Kempthorne,1955)

and,

$$\sigma^2_c = \sigma^2_A + \sigma^2_D + \sigma^2_T + \sigma^2_F + \sigma^2_{AA} + \sigma^2_{AD} + \sigma^2_{DD}$$

where covHS, covFS, σ_{po} are covariance of half sibs, full sibs and parent and offspring, respectively; σ^2_A, σ^2_D, σ^2_T, σ^2_F are variance components due to additive, diallelic, triallelic, tetrallelic intralocus gene effects, respectively; σ^2_{AA}, σ^2_{AD}, σ^2_{DD} are variance components due to additive x additive, additive x dominance and dominance x dominance interlocus interaction effects, respectively. Higher order epistasis may, in reality, be important, and all further interpretation of epistatic gene effects will certainly not imply its absence.

Table 1. Over-location analysis of variance for Mating Design II with parent clones included.

Source	df	Expectation of mean squares [a]
Location	1	-
Rep/Location	4	-
Clone	11	$\sigma^2 + r\sigma^2_{ce} + re\sigma^2_c$
Clone x Location	11	$\sigma^2 + r\sigma^2_{ce}$
Progeny	35	-
Male	5	$\sigma^2 + r\sigma^2_{mfe} + rf\sigma^2_{me} + re\sigma^2_{mf} + ref\sigma^2_m$
Female	5	$\sigma^2 + r\sigma^2_{mfe} + rm\sigma^2_{fe} + re\sigma^2_{mf} + rem\sigma^2_f$
Male x Female	25	$\sigma^2 + r\sigma^2_{mfe} + re\sigma^2_{mf}$
Clone vs. Progeny	1	-
Male x Location	5	$\sigma^2 + r\sigma^2_{mfe} + rf\sigma^2_{me}$
Female x Location	5	$\sigma^2 + r\sigma^2_{mfe} + rm\sigma^2_{fe}$
Male x Female x Location	25	$\sigma^2 + r\sigma^2_{mfe}$
Pooled error	188	σ^2

[a] σ^2_{mfe}, σ^2_{mf}, σ^2_{me}, σ^2_{fe}, σ^2_m, σ^2_f, σ^2_c, σ^2_{ce} are variance components associated with effects due to male x female x location, male x female, male x location, female x location, males, females, clone and clone x location, respectively; coefficients r, e, m, and f denote the number of replications (r=3), locations (e=2), males (m=6) and females (f=6), respectively.

Because the number of males equalled the number of females, σ^2_A was estimated as $2(\sigma^2_m + \sigma^2_f)$ which equals 4 covHS and is probably the least biased estimate when epistasis is important. In this case the estimate is overestimated by $1/9\ \sigma^2_D + 1/4\ \sigma^2_{AA} + 1/36\sigma^2_{AD} + 1/324\ \sigma^2_{DD}$. The most reasonable estimate of the total non-additive variance for disease index is then $\sigma^2_{non-add.} = \sigma^2_c - \sigma^2_A$, which is underestimated by the amount of variance by which σ^2_A is inflated. For % survival, $\sigma^2_{non-add.} = 6\ \sigma^2_{mf}$ which is underestimated by $1/2\ \sigma^2_T + 5/6\sigma^2_F + 1/4\sigma^2_{AA} + 11/12\ \sigma^2_{AD} + 77/108\ \sigma^2_{DD}$.

Narrow-sense heritability (h^2) for disease index was estimated as $\sigma^2_A / \sigma^2_{P(c)}$, where $\sigma^2_{P(c)} = \sigma^2_c + \sigma^2_{ce}/e + \sigma^2 / er$ which is the phenotypic variance of

parent clones. Narrow-sense heritability for % survival was computed as $h^2 = 1/2 \ \sigma^2_A / \ \sigma^2_{P(fs)}$ where the denominator is the phenotypic variance between full-sib families and equals $\sigma^2_m + \sigma^2_f + \sigma^2_{mf} + (\sigma^2_{me} + \sigma^2_{fe} + \sigma^2_{mfe}) \ / \ e + \sigma^2 \ / \ er$. Narrow-sense heritability on an individual plant basis (h^2_i) was estimated as $\sigma^2_A \ / \ [\sigma^2_{P(fs)} + \sigma^2_w]$, where σ^2_w is the within full-sib family variance obtained by a one-way within and between plot ANOVA. Since data were obtained as early as 15 days after inoculation when the most susceptible plants had not started to decay, inoculum built-up in the soil, due to inoculum release from susceptible decayed plants, was not likely to occur. So there certainly no considerable differences in inoculum levels beween any two plants within and/or between any two experimental plots. Therefore, the resistance or susceptibility expressions of individual plants within plot was certainly due mostly to the genetic differences beween them and ,as usual, to some undetectable within plot environmental variation. The sum $[\sigma^2_{P(fs)} + \sigma^2_v]$ thus expresses the total phenotypic variance of the full-sib families evaluated. Broad-sense heritability was obtained as $H^2 = \sigma^2_c \ / \ \sigma^2_{P(c)}$. Standard errors (SE) of variance components were computed as described by Becker (1984) and SE of covariance between parent and offspring by the method of Mode & Robinson (1959).

Results and discussion

The average max/min temperatures (°C) during the tests for resistance were 24.9/15.8 and 29.3/18.2 in the test nurseries at Dalat and Ductrong, respectively. The mean disease index and % survival of each F_1 are presented in Table 2 and index of parent clones in Table 3. A wide variation in level of resistance to wilt existed among the parent clones and the F_1's which indicates that selection has been relatively mild and a considerable genetic variation exists in this population. Error variances were homogeneous for both locations, so variance components were derived from the pooled ANOVA and are presented in Table 4.

For disease index, of the five variance components derived from the ANOVA which involve additive gene effects, i. e. σ^2_m, σ^2_f, σ^2_{me}, σ^2_{fe}, and σ_{po}, only the last one was significant (Table 4). The components σ^2_{mf} was significant and of a much larger magnitude suggesting importance of non-additive gene action which largely specified the magnitude of genotype x environment interaction as indicated by the large σ^2_{mfe} and σ^2_{ce}. Significance of σ_{po} (Table 4), however, indicated that additive gene action is also important. The insignificance of σ^2_m and σ^2_f can probably be ascribed to the much smaller proportion of additive variance (coefficient= 1/4), as well as other non-additive elements, in each

of them compared to that of σ_{po} (coefficient of additive component = 1/2), and/or to the inaccuracy of its estimation . The estimate of additive variance, σ^2_A , was indeed significant and was thrice its pooled SE. The equation σ^2_c -[6 σ^2_{mf} + (6 covHS - σ_{po})] = 1/2 σ^2_T + 5/6 σ^2_F + 1/8 σ^2_{AA} + 33/72 σ^2_{AD} + 477/648 σ^2_{DD} = 0.00717 which is 1,5 times as large as σ^2_A, indicates that either tri- , tetrallelic or epistatic variance or all of the three are important in conditioning resistance to wilt. Results previously reported (Tung et al.,1992b & c) indicated that BW resistance in potato is a partially dominant character and in its inheritance interlocus gene interactions are important. If one further assumes that one resistance allele is enough to confer the full effect of a locus on resistance expression and tri- and tetrallelic variance components are then negligible, the above equation indicates that epistatic gene effects are large. Separation of individual non-additive components of variance was not attempted because restrictions on the genetic model which exlude anyone or another component did not seem reasonable. Therefore, all non-additive variance components were included in one term, $\sigma^2_{non-add.}$,which is 4.5 times larger than σ^2_A (Table 4). The relative magnitudes of σ^2_A and $\sigma^2_{non-add.}$ suggests the importance of non-additive gene effects in the inheritance of BW resistance in this population. This seems well in accordance with evidence reported previously (Tung et al., 1992b & c).

For % survival, a similar situation was observed (Table 4) but the relative magnitudes of σ^2_A and $\sigma^2_{non-add.}$ were different with the latter only twice larger than the first one. This change can be ascribed to the fact that $\sigma^2_{non-add.}$ = 6 σ^2_{mf} , was obviously underestimated while the estimate of σ^2_A inflated.

Narrow-sense heritability (h^2) was low for disease index and relatively higher for % survival (Table 4), but the latter estimate was certainly inflated by the overestimated numerator (1/2 σ^2_A) and the underestimated denominator ($\sigma^2_{P(fs)}$) . Another estimate of h^2 for disease index obtained from doubling the regression of offspring on parent was of a similar magnitude (0.11 ± 0.04). On an individual plant basis, narrow-sense heritability (h^2_i) for disease index was extremely low (Table 4). Broad-sense heritability (H^2), however, was relatively high: its value was five times and 16 times larger than h^2 and h^2_i, respectively. All of this suggests that clonal selection, which can capture all genetic variances, would be successful in developing clonal cultivars, but progress in population improvement would be very slow.

Table 2. Mean disease index (1-5) and % plant survival of 36 F_1 populations 15 days after inoculation with a race 3 (DT-10) isolate of *P. solanacearum* at two locations. Ductrong: warm conditions; Dalat: cool conditions.

Crosses		Disease index		% survival	
Female	Male	Ductrong	Dalat	Ductrong	Dalat
384558.10	384007.3	2.73 j-p	2.40 n	16.7 c-h	40.0 b-f
	386481.3	2.92 i-p	2.57 l-n	26.7 b-c	40.0 b-f
	381064.12	2.63 n-o	2.08 o	28.3 bc	50.0 a
	384010.1	3.35 a-c	3.20 a-d	13.3 e-h	21.7 i-l
	384559.6	3.30 a-e	2.90 c-f	11.7 f-h	26.7 g-k
	384015.30	3.00 h-m	2.60 k-n	20.0 b-f	33.3 c-g
384000.3	384007.3	3.65 ab	3.45 a-c	5.0 i	18.3 k-l
	386481.3	3.35 c-g	2.82 e-i	16.7 c-h	26.7 g-k
	381064.12	3.08 e-i	2.72 h-m	22.7 b-f	33.3 c-g
	384010.1	3.23 b-f	3.50 ab	15.0 d-h	11.7 l-m
	384559.6	2.93 i-o	2.98 g-l	20.0 b-f	21.7 i-l
	384015.30	2.73 j-p	3.02 d-j	25.0 b-d	25.0 g-k
384008.5	380007.3	3.65 ab	3.63 a	13.3 e-h	11.7 lm
	386481.3	3.38 a-e	3.33 a-d	8.3 hi	18.3 kl
	381064.12	3.28 b-f	3.07 c-f	13.3 e-h	28.3 e-j
	384010.1	2.85 k-p	2.83 e-i	20.0 b-f	23.3 f-g
	384559.6	2.98 g-l	2.78 e-g	15.0 d-h	20.0 i-l
	384015.30	3.15 f-k	2.80 e-i	18.3 c-g	36.7 b-e
384002.2	384007.3	3.78 a	3.47 a-e	5.0 i	20.0 i-l
	386481.3	3.67 ab	2.93 c-f	10.0 gh	31.7 d-g
	381064.12	2.80 k-p	2.63 l-n	25.0 b-d	31.7 d-g
	384010.1	2.60 op	3.00 k-n	21.7 b-f	23.3 fg
	384559.6	2.58 p	3.08 c-f	30.0 ab	21.7 i-l
	384015.30	3.02 h-m	2.80 e-i	25.0 b-d	26.7 g-h
384004.2	384007.3	3.07 e-i	3.02 d-j	8.3 hi	8.3 m
	386481.3	3.28 b-f	2.92 c-f	18.3 c-g	28.3 e-j
	381064.12	2.93 i-o	2.72 h-m	23.3 b-e	35.0 c-i
	384010.1	3.05 f-l	3.21 a-d	16.7 c-h	19.3 k-l
	384559.6	2.77 l-p	2.78 e-g	26.7 b-c	26.7 g-k
	384015.30	2.12 q	2.93 c-f	40.0 a	31.7 d-g
384011.3	384007.3	2.85 d-i	3.25 a-d	21.7 b-f	20.0 i-l
	386481.3	3.12 f-j	3.03 d-j	15.0 d-h	28.3 e-j
	381064.12	2.75 l-p	2.45 mn	26.7 b-d	33.3 c-g
	384010.1	3.52 a-c	3.02 d-j	13.3 e-h	28.3 e-j
	384559.6	3.00 i-m	2.88 c-i	16.7 c-h	26.7 g-k
	384015.30	3.10 f-l	2.93 c-f	21.7 b-f	28.3 e-g
Mean		3.08	2.96	18.7	26.1

Note: In a column, values followed by a common letter are not significantly different at P=0.05 by Duncan's multiple range test.

Table 3. Disease index (1-5) of twelve parent clones 15
days after inoculation with a race 3 isolate of *P.*
solanacearum (DT-10) at two locations. Ductrong: warm conditions;
Dalat: cooled conditions.

Clone	Index [a]	
	Ductrong	Dalat
384558.10	2.57 e	1.98 f
384000.3	3.18 cd	2.90 c
384008.5	3.90 b	3.20 b
384002.2	3.43 c	2.73 c
384004.2	4.48 a	3.25 b
384011.3	3.48 c	3.15 b
384007.3	4.08 b	3.15 b
386481.3	3.30 cd	2.15 de
381064.12	1.72 f	1.58 g
384010.1	3.97 b	3.72 a
384559.6	2.62 e	2.02 ef
384015.30	2.99 d	2.30 d

[a] In a column, values followed by a common letter are not
significantly different at P=0.05 by Duncan's multiple range
test.

Table 4. Estimates of variance components and heritability for resistance to
a race 3 isolate of *P. solanacearum* in a population of tetraploid.

Variance component[a]	For disease index	For % survival
σ^2_m	0.00120 ± 0.00123	8.50 ± 7.05
σ^2_f	0.00096 ± 0.00100	3.13 ± 4.60
σ^2_{mf}	0.00202 ± 0.00100	7.99 ± 5.34
σ^2_{me}	0.00053 ± 0.00064	2.41 ± 3.43
σ^2_{fe}	0.00044 ± 0.00060	1.59 ± 3.02
σ^2_{mfe}	0.00100 ± 0.00020	13.10 ± 5.11
σ^2_c	0.02397 ± 0.01410	-
σ^2_{ce}	0.02427 ± 0.01827	-
σ_{pe}	0.00252 ± 0.00083	-
σ^2_A	0.00432 ± 0.00121	23.28 ± 5.96
$\sigma^2_{non-add.}$	0.01977 ± 0.00436	47.96 ± 13.10
h^2	0.12 ± 0.03[b]	0.39 ± 0.10[c]
h^2_i	0.04 ± 0.01[d]	-
H^2	0.65 ± 0.38[e]	-

[a] For designation of variance components see text and footnote of Table 1.

[b] $h^2 = \sigma^2_A / \sigma^2_{P(c)}$

[c] $h^2 = 1/2\, \sigma^2_A / \sigma^2_{P(fm)}$

[d] $h^2_i = \sigma^2_A / [\sigma^2_{P(fm)} + \sigma^2_w]$

[e] $H^2 = \sigma^2_c / \sigma^2_{P(c)}$

Assuming experimental conditions similar to that of this study, at 10% selection intensity, gain from selection of individual resistant plants would be: 1.755 x 0.04 x 0.32977 (standardized selection differential x heritability x phenotypic standard deviation,SD) = 0.0231 SD units. If selection is based on performance of full-sib families (average disease index) and 10% of full-sib families are selected and their parent clones are retained for recombination, the gain would be: 1,755 x 0.12 x 0.00776 = 0.0016 SD units. Selection of individual plants thus would be more effective than that based on performance of full-sib families. These figures assume truncation selection practiced among the survivors and further assume that screening procedures are efficient enough to allow no escapes. For % plant survival, if the 10 best of 100 full-sib families were selected and parent clones recombined in a recurrent selection program using bulked pollen, the gain would be: 1.755 x 0.37 x 5.563 = 3.612 SD units. The phenotypic SD is in the Arcsin-transformed state and is roughly equivalent to 1 % survival. So the gain expected after each cycle of selection would roughly be 3.6 % . This type of selection may be useful especially in development of a population destined for selection of parent clones for production of resistant true potato seed progenies.

Tung et al. (1992c) showed that break-down of parental genotypes upon cross-breeding might result in F_1's with a tendency to segregate to the extremes of the distribution of phenotypes due to loss of a considerable amount of favorable epistasis. In these experiments, diverse types of distribution were observed including those approaching a bell shape distribution. These results coincided with some observations by Rowe & Sequeira (1970) on the diploid *S. phureja* and further confirm the hypothesis. Some of the distributions are presented in Fig.1. Roughly, 7 % of the F_1's tested showed bell shape distribution. In line with Wright's (1956) theory of natural selection, vegetative propagation seem to have preserved favorable gene complexes and temporary linkage blocks which tend to break down upon cross-breeding resulting in the various types of phenotypic distribution observed.
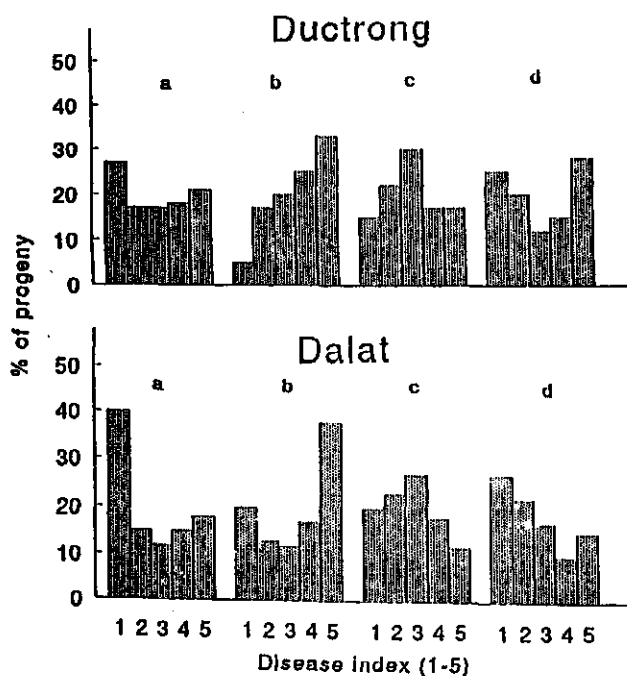
a= 384558.10 x 386481.3   b= 384000.3 x 384007.3
c= 384008.5 x 384559.6   d= 384002.2 x 384015.30

Figure 1. Distribution of plants (%) among disease indices (1-5) in four
F_1 progenies 15 days after inoculated with a race 3 isolate of
P.solanacearum at two locations. Parent clones were randomly selected
from an advanced population with a widened genetic background for
resistance and adaptation to warm temperarures.

A question arises as to the reliability and applicability of the genetic
interpretation and prediction made in this study. Several of the assumptions
made in the genetic interpretation of the results were not completely
satisfied. Because the parent clones were not from a random mating population,
linkage equilibrium no doubt was not present. The parents were further
selected to some extent, though fixation was obviously not reached since the
population is only newly formed and selection has been mild. The parents were
more or less related because resistance has been derived from only a few
common sources. They were certainly , however, not inbred because no selfing
or sibbing has been applied through the course of the breeding program. These
all might have affected the experimental results and tend to reduce their

reliability, but to what extent is not clear. Griffing (1963) pointed out that if selection is mild to moderate and heritability is low the bias generated by use of selected materials is not excessively great. Nei (1963) showed that under selection the genetic variance components associated with additive gene effects are most affected and gradually reduced in magnitude.

For practical breeding purposes,it may be concluded that there is a considerable amount of genetic variation in the reference population studied. Any application of and prediction based on the results reported herein are to be restricted to this population. Breeding schemes designed to make use of both additive and non-additive gene actions seem most suitable for further improvement of this population. Open options need be available for introduction of new sources of resistance which are desirable to generate wide genetic variability and development of a broad-based, stable and, perhaps, durable resistance.

Acknowledgement

Chapter VII

GENERAL   DISCUSSION

Resistance and expression of resistance

Resistance to BW in potato was shown to be a partially resistant character (Chapter V). The number of genes involved is uncertain but there is evidence that both genes with major effects and genes with minor effects are involved in expression of resistance to wilt (Chapter III & IV). To this extent, results from the investigations presented in this thesis are in a general agreement with the hypothesis of Rowe and co-workers (Rowe & Sequeira,1970, Rowe et al.,1972). The inheritance of resistance and its expression are, however, complex. Both additive and non-additive gene actions are of significance in inheritance of resistance to wilt (Chapter V & VI). Their relative magnitudes (Chapter VI), however, indicate that the latter component is more important. As a result, significant GCA and SCA for resistance were found (Chapter V). This conforms with results of a previous investigation (Tung et al.,1990b) which showed that combining ability for resistance to BW is an apparent feature of certain potato clones.

The evidence of large amounts of interaction between genes for resistance and genes for adaptation (Chapter IV & V) further confirms the hypothesis that genes for adaptation are involved in conditioning the resistance expression (Tung et al., 1990b). It also indicates that epistatic gene effects are probably large in inheritance of resistance to BW. These epistatic gene effects are certainly conserved by artificial clonal selection for resistance and vegetative propagation of the selected resistant clones in breeding programs. Breakdown of the intact parental genotypes upon cross-breeding will result in partial loss of favourable epistatic associations for resistance. The consequence is a large reduction in level of resistance in self and F_1 progenies of resistant parent clones (Chapter V). The large difference between narrow-sense and broad-sense heritabilities (Chapter VI) is certainly due to the much larger magnitude of non-additive variance which probably includes substantial epistatic variance.

The importance of the adaptive potential of a resistant genotype on expression of resistance was clearly demonstrated (Chapter III, IV & V). Resistance expression depends heavily on the adaptability of the carrier genotype to a particular environment. Heat tolerance strengthens resistance expression under hot conditions and its effects tend to diminish as temperature goes down (Chapter III & IV). Resistance to wilt is thus more stable over environments when coupled with heat tolerance, or rather wide adaptation, in a genotype.

Resistance genes

Unlike in several other plant-bacterium pathogenic systems such as bean-*P. syringae* pv *phaseolicola*, soybean-*P. syringae* pv *glycinea*, cotton-*X. campestris* pv *malvacearum* and rice-*X. campestris* pv *oryzea*, there is no evidence of a gene-for-gene relationship between potato and *P. solanacearum*. Avirulence genes, as being functional *sensu* Parlevliet (1981), have been proposed by Boucher et al.(1987), but have never been demonstrated for *P.solanacearun*, at least until recently (Ma et al.,1988). Mutant strains of *P. solanacearum* which lose the ability to cause wilt on a susceptible host genotype also lose their virulence on other host genotypes and are always impaired for a number of morphological and physiological characteristics (Boucher et al., 1987; and Driguez et al., 1985). Mutations from avirulence to virulence have never been caught in sight (Buddenhagen, 1985).

The facts that almost all resistant accessions of wild *Solanum* spp. have originated in areas where *P. solanacearum* has never been found, strongly suggests that until recently there has been no coevolution between the host and the pathogen. A gene-for-gene relationship *sensu* Flor (1971) does not seem to be applicable to the potato-*P. solanacearum* system as judged from an evolutionary point of view. Functional genes for avirulence are thus unlikely to exist in *P. solanacearum*. Resistance genes, true in the sense that they solely confer resistance to BW, and are moulded by natural selection throughout the course of coevolution of the host and the pathogen, thus also may not exist. *P. solanacearum* seems to attack potato as it attacks other solanaceous plants, i.e. through the mechanisms of general compatibility. Probably certain genes, other than those "for resistance alone", can act pleiotropically to confer the resistance to wilt in potato. They are eventually termed "genes for resistance" once a certain level of resistance is detected. It is not difficult to conceive that some of those genes are most likely genes for adaptation since resistance tends to break down whenever the carrier host genotype faces new ecological conditions. The major or minor status of these genes may depend on the particular genotype of the pathogen, the genetic background of the carrier host genotype as well as the particular environmental conditions which influence their expression. This is probably the reason why Zalewski (cit. Sequeira,1979) found four instead of three major genes (Rowe & Sequeira,1970; and and Rowe et al.,1972) govern the resistance to BW in *S.phureja* and why contradictory results were obtained from their later genetic studies (Sequeira, 1979).

Specificity of resistance

Strain specificity, and not race-cultivar specificity, is a common feature observed in many tests of potato cultivars for resistance to BW which stands against the fact that the gene-for-gene concept is not applicable to the potato-*P.solanacearum* system as discussed in the previous section. There seem to be two reasons which explain why is there strain specificity in such a pathosystem where no true resistance genes in the host and functional avirulence genes in the pathogen seem to exist. The first reason derives from the understanding that a large number of genes are involved in the pathogenicity of the pathogen. Strains of *P.solanacearum* are known for their tremendous infra-specific variability in pathogenic potential. Similarly, several mechanisms, involving many genes, seem to take part in expression of resistance to BW in the host. Variations in pathogenic capability in the pathogen and in various components of resistance in the host will inevitably lead to interactions in the context of minor gene (polygenes) quantitative resistance described by Parlevliet & Zadoks (1977). There is no reason that such interactions should not occur.

The second reason is also a highly probable one. The host genotype x pathogen genotype interaction in the potato-*P.solanacearum* system often seem to be artifactual. The host and the pathogen are both sensitive to environmental changes. Therefore, host-genotype x pathogen genotype interaction may also be a result of host genotype x environment and/or pathogen genotype x environment interactions as discussed by Kulkarni & Chopra (1982). Tung et al.(1990c) observed that the ranking order of host genotypes in resistance to a particular pathogen genotype tended to change when tests were conducted under different environmental conditions. Further, host genotypes and pathogen genotypes responded greatly to environmental changes (main effects in a factorial test) and the effects of host x pathogen interaction remained small and were of the magnitudes of experimental errors. Apparently, strain specificity in potato-*P.solanacearum* seems to be a consequence of differential adaptation of particular host and pathogen genotypes to environments as discussed by Crute (1985). Similar situations have been reported for maize-*Bipolaris maydis* and maize-*Collethotrichum graminicola* by Jenns et al.(1982) and for lettuce-*Bremia lactucae* by Norwood et al. (1984).

The above two explainations of specificity of resistance to BW may at first seem unrelated, but intuitively they are just one. Any variation in the genetic components of resistance in the host and of pathogenicity in the

pathogen will be reflected as the variation in their adaptive potentials (resistance and pathogenicity under a particular set of environmental conditions). The consequence is the very strong host-pathogen-environment interaction and the vulnerabiliity of the resistance of potatoes to BW, being addressed everywhere.

Breeding for resistance

Strong host x pathogen x environment interaction in the potato-*P.solanacearum* relationship offers inherent difficulties for breeding for resistance to BW. Practical evidence during the last two decades indicates that breeding for resistance, though successful to some extent, has made a relatively slow progress. Some fundamental problems are already discussed in Chapter V and VI. The variability in pathotypes of *P.solanacearum* requires a broadly-based type of resistance to cope with, but more emphasis should be put on adaptation aspects of the host genotype. Wide adaptation is necessary for stable expression of resistance.

Selection

Breeding procedures will largely depend on the concrete situations a breeding program is faced with: sources of resistance available, prevalence of a particular strain of the bacterium, ecological conditions, agronomic requirements, etc. For any case, the relative magnitudes of genetic variance components and heritabilities suggest that clonal selection will be successful in developing clonal cultivars for specific growing conditions. However, such cultivars would not offer stable resistance if selection is not based on a broad genetic background for resistance and adaptation. For population improvement, breeding procedures should be designed to make use of both additive and non-additive gene actions. Open options should be kept for continuous introduction of resistance from new sources. In a broadly-based population, reccurent selection of individual resistant plants would offer a better progress. Full-sib family selection with recombination of selected parent clones would be successful in development of a population from which the best parent clones may be selected for production of resistant true potato seed progenies.

Screening

The success of any method of breeding for resistance is a function of

efficiency of the screening method applied for selection of supperior genotypes (Mendoza, 1988). Various inoculation techniques were reviewed by French & Nyderger (1988). In the context of this thesis, the use of an appropriate isolate/strain of *P.solanacearum* is probably the most important factor affecting the effectiveness of selection for high stable resistance, assuming an effective inoculation technique. Variability in pathogenicity according to environmental changes, especially temperature, has been shown to be great in most strains of *P.solanacearum*. A highly virulent strain which maintains virulence and aggressiveness over a wide range of enrironmental conditions (such as isolate WP-156) would be the most suitable for tesing and selecting for stable resistance. The test may seem very rigorous, but may assure a high level of stable resistance. This proposition is derived from the concept that host x pathogen interaction in the potato-*P.seudomonas solanacearum* system is a reflection of differential adaptation of host genotype and pathogen genotype to environments, discussed in the previous section. A strain with a great variability according to environmental changes is not suitable for testing and selecting for stable resistance. Resistance to such strain would likely be suitable for use under restricted local conditions as ever since observed.

Threshold and adult plant resistance

Resistance to BW is probably the ability to restrict bacterial proliferation in side the plant system and to delay wilt (Bowman & Sequeira, 1982). Denny & Baek (1991) presented some evidence that there is a threshold level of EPS production that is necessary for complete wilt. Resistance evaluation experiments may have to take into account some aspects of disease development related to resistance to help identify useful cultivars in case no high level of complete resistance is available. The experiment of Nielsen & Haynes (1960) illustrates this point (Fig. 1). The cv Prisca was more resistant to wilt than cv Cobbler with respect to the ability to delay appearance of wilt symptoms, although the final disease incidence scored for both cvs was roughly equal. So the threshold level of EPS production might be higher in Prisca than in Cobbler. There is evidence that resistance to BW tends to increase in mature plants of some potato clones (Tung et al.,1990a). A good level of resistance thus may be obtained with the combination of the ability to delay wilt and adult plant resistance. An early maturing cultivar which can delay appearance of wilt symptoms until it becomes mature and resistant may be as useful as a highly resistant cultivar under the conditions of subsistence agriculture.
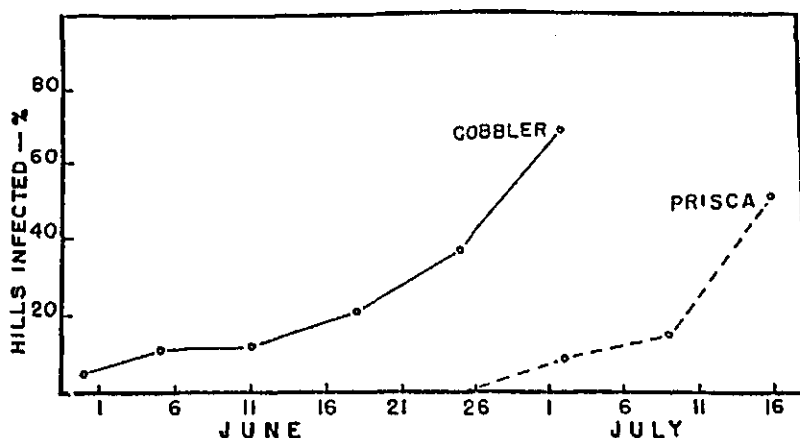
Figure 1. Comparative disease development in susceptible Irish Cobbler and resistant Prisca. The curves are based upon the cumulative proportion of disease hills of the two clones growing under the same conditions in soil naturally infectied with *P.solanacearum*. (Nielsen & Haynes, 1960).

Latent infection

Almost nothing is known about the mechanisms and genetics of resistance to latent infection. Further, latent infection seems to be a neglected objective in potato breeding which is touched upon only recently (Granada, 1988). The reason is perhaps because latent infection does not seem to be associated with above ground symptoms (Ciampi & Sequeira, 1980b). As more and more potatoes are grown in the tropics, latent infection appears to be a serious problem because of the danger of spreading the disease by latently infected tubers. Perhaps some efforts need be dedicated to research on genetics and breeding for resistance to latent infection in the near future.

SUMMARY

Pseudomonas solanacearum, the causal agent of bacterial wilt(BW) of potato, possesses a complicated genetic control of pathogenicity. A large number of genes contribute to its virulence and aggressiveness toward the host. The exact biochemical nature of resistance to BW is not known, but several mechanisms are probably involved. The complicated aspects of host x pathogen x environment interaction in the potato-*P.solanacearum* system are probably the result of differential adaptation of host genotype and pathogen genotype to environments. Because there has been no coevolution of the host and the pathogen, true resistance genes in the host and functional avirulence genes in the pathogen are not likely to exist. No evidence of a gene-for-gene relationship in the pathosystem has yet been documented. Probably, certain genes, other than those only for resistance, act pleiotropically to confer the phenotypic resistance to wilt. Those genes are most likely genes for adaptation and thus resistance tends to break down whenever the host carrier genetype confronts new ecological conditions to which it is not well adapted.

It was clearly shown that expression of resistance to BW is indeed dependent on the adaptive potential of a potato genotype to a particular environment. The resistance was well expressed in a heat tolerant genotype under heat stress conditions, and not so in a heat sensitive one. Thus in a wide genetic background for adaptation, resistance tended to be more stable over environments. The interaction between genes for resistance and genes for adaptation was evident, but no significant effect of cytoplasms was found.

In the inheritance of BW resistance, both additive and non-additive gene actions are significant. The relative magnitudes of estimates of genetic variance components, however, indicated that non-additive gene effects are more important. There was evidence that epistatic gene action is important in expression of resistance. Combining abilities of potato clones for resistance thus appeared to be significant. The low narrow-sense heritability found implies a slow progress in resistance improvement at the population level. This population improvement is important in broadening the genetic base for resistance and adaptation in the cultivated potato. In a broadly-based population, selection of individual resistant plants would be more effective than selection based on family performance. Clones selected on the basis of their full-sib family performance would be useful in development of a population from which good parents may be selected for production of resistant true potato seed progenies. Clonal selection is expected to be successful because of the high broad-sense heritability found. However, clonal cultivars

with high levels of stable resistance to BW would not likely be obtained if selection is not based on a broadly-based genetic background for resistance and adaptation.

The choice of an appropriate strain/isolate of *P.solanacearum* appears to be of crucial significance in the development of a general and stable type of resistance to wilt. A strain/isolate which maintains virulence and aggressiveness over a wide range of environmental conditions would be the most suitable for testing and selecting for stable resistance.

SAMENVATTING

De pathogeniteit van Pseudomonas solanacearum, de bacterie die verwelkingsziekte veroorzaakt bij de aardappel, heeft een gecompliceerd overervingspatroon. Een groot aantal genen draagt bij tot de virulentie en agressiviteit van de bacterie ten opzichte van de waardplant. De biochemische aard van de resistentie is niet exakt bekend, maar waarschijnlijk zijn verscheidene mechanismen in het spel. De gecompliceerdheid van de interactie tussen waardplant-pathogeen-milieu in het aardappel-Pseudomonas systeem is waarschijnlijk het resultaat van differentiële adaptatie van waardplantgenotype en pathogeengenotype aan diverse milieu-omstandigheden.

Omdat er geen coëvolutie heeft plaats gehad van waardplant en pathogeen, menen wij te mogen stellen, dat echte resistentiegenen in de waardplant en functionele avirulentiegenen in het pathogeen waarschijnlijk niet bestaan. Totnutoe is er nog geen bewijs gevonden van het bestaan van een gen-om-gen relatie in het pathosysteem.

Het is waarschijnlijk, dat andere genen dan aparte resistentiegenen via pleiotropische werking de fenotypische resistentie tegen de verwelkingsziekte bewerken.

Die genen zijn zeer waarschijnlijk genen voor adaptatie. Daaraan is het vermoedelijk toe te schrijven, dat de resistentie de neiging heeft te bezwijken, wanneer de waardplant met deze genen wordt geconfronteerd met nieuwe ecologische condities, waaraan het onvoldoende is aangepast.

Duidelijk werd aangetoond, dat expressie van resistentie tegen verwelkingsziekte inderdaad afhankelijk is van het adaptieve potentieel van een aardappelgenotype ten opzichte van een bepaald milieu. De resistentie kwam goed tot expressie in een hittetolerant genotype onder condities van hittestress en duidelijk minder in een hittegevoelig genotype. Zodoende was er de tendens, dat met een brede genetische achtergrond voor adaptatie de resistentie een betere stabiliteit vertoonde onder verschillende milieu-omstandigheden. De interactie tussen genen voor resistentie en genen voor adaptatie was evident. Cytoplasmatische effecten bleken niet significant.

Bij de overerving van resistentie tegen verwelkingsziekte bleken zowel additieve als non-additieve genwerkingen significant. De relatieve grootten van schattingen van genetische variantiecomponenten toonden echter aan, dat niet-additieve geneffecten belangrijker zijn.

Er werden aanwijzingen gevonden voor het belang van epistatische genenwerking bij de expressie van resistentie. Zodoende werden er significante combinatiegeschiktheden van aardappelklonen voor resistentie gevonden. De lage "narrow-sense heritability" die werd gevonden, houdt in, dat er bij resistentieverbetering op populatieniveau slechts langzame voortgang kan worden gemaakt. Populatieverbetering is belangrijk voor het verbreden van de genetische basis voor resistentie en adaptatie in de cultuuraardapel. In een populatie-op-brede-basis zou selectie van individuele resistente planten effectiever zijn dan selectie op basis van de prestaties van families.

Klonen, die geselecteerd zijn op basis van de prestatie van hun full sib-families, zouden nuttig zijn voor het ontwikkelen van een populatie, waaruit goede ouders kunnen worden geselecteerd voor de productie van resistente zaadnakomelingschappen. Klonale selectie zal naar verwachting succes opleveren vanwege de hoge "broad-sense heritability" die werd gevonden. Echter het is onwaarschijnlijk, dat klonale cultivars met hoge niveau's van stabiele resistentie tegen verwelkingsziekte worden verkregen, als de selectie niet is gebaseerd op een genetisch-brede achtergrond voor resistentie en adaptatie. Bij de ontwikkeling van een algemeen en stabiel type resistentie tegen verwelkingsziekte is de keuze van een geëigende stam of isolaat van Pseudomonas solanacearum van cruciale betekenis. Een stam/isolaat, die (dat) virulentie en agressiviteit weet te handhaven over een breed scala van milieu-omstandigheden zou het meest geschikt zijn voor het toetsen en selecteren op stabiele resistentie.

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