Breeding Science 49 : 63~68 (1999)

Resistance to Bacterial Wilt (*Pseudomonas solanacearum*) of Potato Evaluated by Survival and Yield Performance at High Temperatures

Junko A. Watanabe [1], Matilde Orrillo [2] and Kazuo N. Watanabe *[1]

[1] *Faculty of Bioscience and Technology, Kinki University,* 930 Nishimitani, Uchita-cho, Naga-gun, Wakayama 649-6493, Japan
[2] *The International Potato Center (CIP),* Apartado 1558, Lima, Peru

Summary
Survival and yield performance of the diploid potato genotypes selected during field trials in the coastal desert area of Lima, Peru, were evaluated under subtropical conditions in San Ramon, Peru, in a field with severe bacterial wilt (BW) infestation. Some selected diploid genotypes, which had been found to be resistant also to other biotic stresses in field trials in Lima, showed comparable yields to those of the tetraploid standard local cultivars under the subtropical conditions. Based on analysis of variance, the effects of the resistance to BW on the survival and yield were not consistent. These facts were considered to result from interactions between the BW resistance, adaptation (e. g. heat tolerance) and environmental factors. Observations of the latent infection provided an insight into how the expression of BW resistance is modified by environmental and pathogenic factors under field conditions.

Key Words : potato, bacterial wilt, yield performance, latent infection, adaptation.

Introduction

Bacterial wilt (BW) caused by *Pseudomonas solanacearum* is responsible for severe potato crop loss under tropical and subtropical conditions (French 1985). The damage can be enhanced especially by interaction with other pathogens and pests, such as bacterial soft rot (*Erwinia* spp.) and root-knot nematodes (*Meloidogyne* spp.) (Schmiediche 1988).

Wild diploid potato species including *Solanum raphanifolium*, *S. microdontum*, *S. sparsipilum*, *S. chacoense*, as well as the cultivated diploid species *S. phureja* have been used in breeding programs for resistance to BW (Schmiediche 1983, CIP 1987). Resistance to BW is a quantitatively inherited trait (Rowe and Sequeira 1970, Schmiediche 1988, Tung *et al.* 1990a), which prevents rapid improvement of resistance at the tetraploid level (Schmiediche 1988). Diploid breeding material lines with a significantly higher level of quantitative resistance have been generated (Watanabe *et al.* 1992b). They transmitted the resistance to BW to tetraploid progeny via first division restitution (FDR) 2n pollen (Watanabe *et al.* 1992 b). Based on the frequency of the resistant progenies in the 4x × 2x crosses and the genetic mode of FDR 2n pollen, at least five to six loci were assumed to be associated with the resistance to BW (Watanabe *et al.* 1992a).

Since the resistance to BW is temperature sensitive (Harison 1961, French 1985) and strain specific (Sequeira and Rowe 1969, French and De Lindo 1982), the level of resistance varies depending on the interaction among host, pathogen and environment (Tung *et al.* 1990b). Changes in the pathogenicity of the isolates with the temperature can be the major source of variation in resistance (Tung *et al.* 1990b). On the other hand, genes for adaptation to the environment as well as genes for heat tolerance were also involved in the transmission and expression of resistance to BW (Tung *et al.* 1990a, b).

The final objective of our germplasm enhancement program is to generate genotypes showing stable resistance to multiple biotic stresses and with a rusticly but reasonably yielding performance under harsh environmental conditions. In general, the diploid landraces give a lower yield than tetraploid cultivars (Anonymous 1989, Yamamoto 1980). However, the yield of selected diploid landraces is comparable to that of the tetraploid standard varieties when they are grown in the irrigated desert area of coastal Peru (Watanabe *et al.* 1996a, b). Diploid germplasm with some resistance to biotic stresses has been produced by our group in Peru (Watanabe *et al.* 1994, 1996b). Considering the components of the quantitative resistance to BW, the genotypes showing resistance to multiple pests (other than to BW) and characterized by short-day adaptation and crossability to tetraploids were selected under greenhouse tests for BW resistance, followed by field trials (Watanabe *et al.* 1996a).

The purpose of this study is to evaluate the yielding capacity under subtropical conditions, of the diploid genotypes pre-selected in field trials in the coastal desert area of Peru. Expression of the resistance to BW and latent BW infection were examined.

Materials and Methods

Plant materials

The potato genotypes used are listed in Tables 1 and 2.

Communicated by K. Yonezawa
Received July 21, 1998. Revision received December 28, 1998.
Accepted January 27, 1999.
*Corresponding author

Their pedigree and other characteristics have been reported by Iwanaga *et al.* (1989) and Watanabe *et al.* (1994, 1995, 1996a, 1996b). The tetraploid cultivars, 'LT-7' and 'AVRDC 1287.19' were considered to be heat-tolerant (Tung *et al.* 1990a, b).

Methods

Greenhouse evaluation of BW resistance: CIP isolate 204 belonging to race 3 of *Pseudomonas solanacearum* was inoculated to at least 20 plants per genotype produced from tubers derived from the same seedlings in the greenhouse (Watanabe *et al.* 1992a).

Field evaluation of BW resistance: The same set of genotypes as that used for greenhouse inoculations was employed for artificially inoculated field tests under subtropical conditions at San Ramon, Peru. The field was infested with a mixture of races 1 and 3, which are common in the region, including CIP isolate 204 to enhance the infestation. Five tubers per treatment with six replications were planted with a completely randomized block design for each diploid genotype, with susceptible controls planted in the same manner in adjacent rows to monitor the uniformity of infection with *P. solanacearum*. Clones with more than 50% survival were considered to be resistant.

Latent infection test: Possible latent infection was tested in the putative resistant genotypes using the method of Elphinestone and Aley (1993) at San Ramon in September, 1991, in September, 1993 and in April, 1995. September and April correspond to the end of the dry and wet (rainy) seasons at San Ramon, respectively, and the yield and infection level during the two seasons could be different due to the level of humidity which is the principal determinant of potato growth and bacterial wilt infection.

Evaluation of resistance to root-knot nematodes (RKN: *Meloidogyne incognita*, race 3): The inoculation method and resistance scoring were the same as those described by Iwanaga *et al.* (1989).

Yield trials: Using selected genotypes, a field trial was conducted in the BW-infested field in San Ramon, Peru, described above. Harvest was performed in September 1991 and 1993, 90 days after planting. Yield was obtained only from the plants that survived at harvest.

Table 1. Yield of selected genotypes with or without resistance to latent infection of bacterial wilt, greenhouse resistance to bacterial wilt and root knot nenatodes, their ploidy, and results of latent infection tests at San Ramon in September, 1991.

Genotype	Ploidy	Mean yield (kg/hill)	Mean yield at Lima[1)] (kg/hill)	Latent infection	Resistance to BW in greenhouse (cutting evaluation[2)])	Resistance to RKN[3,4)]
LT-7	4x	0.396		+	S	S
AVRDC1287.19	4x	0.375		+	MR	S
DG82.23	2x	0.319	1.60	+	S	S
86.97.48	2x	0.299	1.06	+	S	S
88.123.8	2x	0.278		+	S	S
88.142.32	2x	0.268	0.39	+	S	S
86.105.12	2x	0.258		+	R	S
86.29.43	2x	0.235		+	S	S
DG81.68	2x	0.214		+	S	S
Yungay	4x	0.213		+	S	S
88.140.29	2x	0.209	1.51	+	S	MR
86.61.43	2x	0.195	0.73	+	S	S
AA-3	4x	0.191		+	S	S
DW84.1457	2x	0.164		+	S	S
86.54.18	4x	0.155		+	S	R
AA-2	4x	0.137		+	S	S
85.37.38	2x	0.089		−	R	R
5.119	4x	0.088		−	R	R
84.194.30	2x	0.079	0.48	−	R	S
86.57.7	2x	0.062		+	S	S
6.115	4x	0.052		−	R	R
CCC1386.26	2x	0.005		+	R	S
CCC1386.26CD	4x	0		+	R	S
I-12.1	4x	0		−	R	R
LSD(0.05)		0.168				
LSD(0.01)		0.222				

1) For mean yield at Lima, refer to Watanabe *et al.* (1996 b).
2) and 3) abbreviations: R, resistant; MR, moderately resistant; and S, susceptible, respectively.
4) For the levels of resistance to RKN, refer to Watanabe *et al.* (1994) and Iwanaga *et al.* (1989).

Table 2. Yield of selected potato genotypes with or without greenhouse resistance to bacterial wilt, latent infection of bacterial wilt and resistance to root knot nematodes

Genotypes	Ploidy	Mean yield (kg/ hill) in Sep., 1993	Mean yield at Lima	Latent infection in Sep., 1993.	Mean yield (kg/ plant) in Apl., 1995	Latent infection in Apl., 1995.	Resistance to BW in greenhouse (cutting evaluation[1)])	Resistance to RKN[2),3)]
90.28.41	2x	0.547	0.332	–	0.450	–	R	S
90.28.39	2x	0.410		–	1.233	–	R	S
90.36.47	4x	0.345	0.375	–	0.000	–	R	S
90.34.31	2x	0.310		+	0.650	+	R	S
90.33.35	2x	0.241		+	0.230	+	S	S
90.30.32	2x	0.234		+	0.500	+	R	S
90.30.57	2x	0.147					R	S
90.27.33	2x	0.132		+		+	S	S
90.10.57	2x	0.073					S	S
90. 8.42	2x	0.033		–	0.000	–	R	MR
90.30.53	2x	0.033		+			R	S
Atzimba	4x	0.024	0.336	+	0.000	+	S	S
90.35.47	4x	0.007		+	0.000	+	R	S
90. 3.39	2x	0.003		+			R	S
90.12.52	2x	0.000	0.471	+	0.000	+	R	MR
90.14.38	2x	0.000		–			R	S
90.30.45	2x	0.000		+			S	S
LSD(0.05)		0.142			NS			
LSD(0.01)		0.190						

1),2) and 3) Refer to notes in Table 1.

The plants that wilted before harvest did not produce tubers or produced only decayed ones.

Results

Yield trials in 1991

There was a significant difference in yield among the genotypes in the BW-infested field (Table 3). The tetraploid cultivar 'Yungay' was used as the control. The statistical difference in yield among the genotypes was not due to the difference in the ploidy levels. The heat-tolerant tetraploid controls, 'LT-7' and 'AVRDC 1287.19' gave a high yield, whereas, the tetraploids 'CCC 1386.26 CD' and 'I-12.1' did not produce any yield (Table 1). Some diploid genotypes, such as 'DG 82.23', '86.97.48' and '88.140.29', which were evaluated as BW-susceptible in greenhouse tests and were also tested during the field trials in Lima, gave a yield comparable to that of 'Yungay' in San Ramon.

The resistance to BW based on greenhouse tests showed a negative correlation with the yielding capacity (Tables 1 and 3). Most of the susceptible genotypes gave higher yields than the resistant genotypes. Moderately resistant 'AVRDC 1287.19' and greenhouse-resistant '86.105.12' showed a comparable yield to that of 'Yungay', while most of the resistant genotypes gave a low yield or did not produce any yield.

Resistance to root-knot nematodes (RKN) also showed a negative correlation with yield. Genotypes susceptible to RKN gave significantly higher yields the than the RKN-resistant genotypes. The moderately field-resistant 2x '88.140.29' showed a yield comparable to that of 'Yungay'. However, most of the resistant genotypes gave a low yield or did not produce any yield.

Table 3. Comparison of yield of selected diploid and tetraploid potato genotypes with or without resistance to bacterial wilt and root knot nematodes. The field test was performed at San Ramon, Peru in June-September 1991.

Factor	D.F.	S.S.	M.S.	Fvalue
Genotype	23	1.8215	0.0792	3.8245***
Ploidy	1	0.0327	0.0327	1.0857
Resistance to BW	1	0.4657	0.4657	17.220***
Resistance to RKN	1	0.3038	0.3038	10.779**
Block	5	0.3560	0.0701	2.4440*
Error	112	1.1510	0.0103	
Total	143	4.3063		

*, ** and *** significant at the 5%, 1% and 0.1% levels, respectively.

Latent infection in 1991

All the genotypes susceptible based on the greenhouse evaluation for BW resistance showed a latent BW infection (Table 1). Five of the nine BW resistant or moderately resistant genotypes identified in the greenhouse tests did not show any latent infection, and four of them were also resistant to RKN. In contrast, all the genotypes that showed a latent BW infection were not

Table 4. Comparison of yield of selected diploid and tetraploid potato genotypes with or without resistance to bacterial wilt and root knot nematodes. The field test was performed at San Ramon in June - September 1993.

Factor	D.F.	S.S.	M.S.	Fvalue
Genotype	16	1.5620	0.0972	12.953***
Ploidy	1	0.1235	0.1235	4.3226*
Resistance to BW	1	0.1193	0.1193	4.1635*
Resistance to RKN	1	0.0737	0.0737	2.5125
Block	2	0.0399	0.0200	0.657
Error	43	0.0053		
Total	64	1.9237		

* and *** significant at the 5% and 0.1% levels, respectively.

resistant to RKN .

Yield trials in 1993

Diploid genotypes gave a significantly higher yield than tetraploid genotypes (Table 4). Most of the diploid genotypes showed a yield comparable to that of 'Atzimba', the tetraploid standard cultivar (Table 2). The diploid clone '90.28.41' and the tetraploid clone '90.36.47', which were selected in the greenhouse test for BW resistance and yield trial in Lima, also showed a yield comparable to that of 'Atzimba'. High yielding genotypes in September, 1993 tended to give a high yield in April, 1995 (Table 2), but the R^2 value of the linear regression was not significantly high (R^2=0.432).

Unlike in 1991, the BW resistant genotypes gave significantly higher yields than the susceptible genotypes in the growing season of 1993 in the BW-infested field (Table 3). However, RKN-resistant genotypes did not give significantly higher yields than RKN-susceptible genotypes in 1993.

Latent infection in 1993

All the genotypes susceptible to BW except for '90.10.57' for which data were not available showed a latent BW infection (Table 2). Five of the twelve resistant genotypes did not show any latent infection. The same tendency was observed in the 1995 growing season. Among all the genotypes tested, one genotype, '90.8.42' was resistant to both BW (greenhouse test) and RKN. The genotype '90.8.42' was free of latent infection in both the 1993 and 1995 growing seasons.

Discussion

2x genotypes can outyield 4x genotypes under subtropical conditions.

Some diploid genotypes selected for BW resistance followed by field trials at Lima (Watanabe *et al.* 1996a, b) showed a comparable yield to that of the local standard cultivars under subtropical conditions. Ortiz *et al.* (1991) reported that the yield in the coastal desert area was correlated with the yield in the highlands. A diploid genotype '90.12.52' which gave a higher yield than the standard cultivar in Lima, however, did not produce any yield at San Ramon (Table 2), presumably due to the difference in the environmental conditions. In general, high Genotype (G) x Environmental (E) interaction has been observed in yield components in potatoes (Ross 1986). Watanabe *et al.* (1996a, b) reported a large genotype x year variation in yield tests on the same genotypes as in Lima over several years. San Ramon is located in a subtropical area with a much higher average temperature and relative humidity, which are the main constraints on potato development (Moorby 1978). These environmental factors in San Ramon may have been responsible for such a large yield reduction in '90.12.52'.

Our results indicated that there was no consistent relationship between ploidy levels and yield (Tables 3 and 4) under subtropical conditions. Although there was no significant difference between the diploids and tetraploids in 1991 (Table 3), the diploids gave a significantly higher yield than the tetraploids in 1993 (Table 4). Therefore, it is assumed that selected diploid genotypes may display a high yielding capacity under subtropical conditions as well as in the coastal desert area (Watanabe *et al.* 1996a, b).

Factors associated with BW field resistance

Interestingly, it seems that the resistance to RKN can be associated with the resistance to BW. Wilting caused by RKN makes potato cultivars more susceptible to BW, especially in hot climates (Ortiz *et al.* 1994). Therefore combined resistance to BW and RKN should be a desirable trait for potato growers in tropical and subtropical regions. In addition, Tung *et al.* (1990a) suggested that widening of the genetic base for both resistance and adaptation is important in breeding for resistance to BW.

Heat tolerance seems to play an important role in both yield and resistance to BW. Heat tolerant 'LT-7' and 'AVRDC 1287.19' showed the highest and the second highest yields in 1991, respectively. Since they were not scored as resistant in the greenhouse evaluation of BW resistance in our study, it seems that their heat tolerance promoted the overall tolerance to BW. When the plants were grown in the hot tropics, the resistance to BW derived from *S. phureja* was markedly reduced in the absence of heat tolerance (Schmiediche 1983). In contrast, a heat tolerant parent derived from *S. chacoense*, *S raphanifolium* and *S. tuberosum* produced a higher frequency of BW resistant progenies (Tung *et al.* 1990b). Therefore, simply classifying genotypes into R and S under different environmental conditions would not be appropriate.

Resistance to latent BW infection and field resistance to BW

Almost all the genotypes susceptible to BW in the greenhouse showed a latent infection in the field in

1992, 1993 and 1995 (Tables 1 and 2). The genotypes selected as resistant in the greenhouse were not completely free from latent infection in the field. In 1991. Four of the nine resistant lines showed a latent infection, and in 1993 and 1995 even higher latent infection rates. Tubers with latent infection should be discarded to avoid the infection of new crops because farmers use some of the harvested tubers for the subsequent potato crop. Therefore, susceptible as well as resistant genotypes with latent infection should not be selected even when they can produce considerably high yields.

In breeding for field resistance to *P. solanacearum*, several procedures have been described and compared for mass screening of seedlings or cuttings (Gonzales *et al.* 1973, Gitaitis *et al.* 1983, French 1986) in order to select the BW resistant potato materials. Nevertheless, efficient screening methods still remain to be developed (Mendoza 1988). Further optimum screening test for resistance to BW under greenhouse conditions is necessary in order to predict the resistance in the field.

Conclusion

It seems that adaptation, especially heat tolerance, could interact considerably with field resistance to BW under the subtropical conditions. Further studies on the interaction between the resistance to BW and heat tolerance would be required to make the best use of the germplasm. To produce genotypes resistant to BW or other biotic stresses with a sufficiently high yielding capacity under specific field conditions, a breeding program should include studies on 1) evaluation methods to select stably resistant genotypes based on preliminary tests in the greenhouse, and 2) the degree and patterns of genotype x environment interaction of the resistance.

Acknowledgments

The authors would like to thank Profs. Jari P. T. Valkonen, Genetic Centre, Swedish University of Agricultural Sciences; K. Hosaka, Kobe University; S. A. Slack, Cornell Univ. for their constructive suggestions and editorial assistance. The main part of the work has been conducted under the umbrella of the International Potato Center. KNW acknowledges the support from a grant from Japan Science Promotion Association, JSPS-RFTF96L00603 and a grant-in-aid from Ministry of Education, Science and Culture 09760007.

Literature Cited

Anonymous (1989) Potatoes. In: Lost crops of the Incas. 93-104. National Academy Press, Washington D. C.

CIP (1987) Annual Report, 210. International Potato Center, Lima, Peru.

Elphinestone, J. G. and P. Aley (1993) Integrated control of bacterial wilt of potato in the warm tropics of Peru. *In*: G. Hartman (ed.), "Proceeding of International Conference on Bacterial Wilt", 276-283. ACIAR, Canberra, Australia.

French, E. R. (1985) Interaction between strains of *Pseudomonas solanacearum*, its hosts and environments. *In*: G. J. Perseley (ed.), "Bacterial Wilt Disease in Asia and the South Pacific", 145. Australian Center for International Agriculture Research (ACIAR), Australia.

—— (1986) Efficacy of five methods of inoculating potato plants with *Pseudomonas solanacearum*. Phytopathology 76: 1078 (Abst.).

—— and L. De Lindo (1982) Resistance to *Pseudomonas solanacearum* in potato: Strain specificity and temperature sensitivity. Phytopathology 72: 1408-1412.

Gitaitis, R. D., S. R. Ghate, C. A. Jaworsky and S. Phatak (1983) Evaluation of a mass inoculation method of potatoes with *Pseudomonas solanacearum*. Am. Potato J. 60: 625-630.

Gonzales, L. C., L. Sequeira and P. R. Rowe (1973) A root inoculation technique to screen potato seedlings for resistance to *Pseudomonas solanacearum*. Am. Potato J. 50: 96-104.

Harison, D. E. (1961) Bacterial wilt of potatoes: Field symptoms of the disease and studies on the causal organisms: *Pseudomonas solanacearum* var. Asiaticum. Aust. J. Agric. Res. 12: 845-871.

Iwanaga, M., P. Jatala, R. Ortiz and E. Guevara (1989) Use of FDR 2n pollen to transfer resistance to root-knot nematodes into cultivated 4x potatoes. J. Amer. Soc. Hort. Sci. 114: 1008-1013.

Moorby, J. (1978) The physiology of growth and tuber yield. *In*: P. M. Harris (ed.), The Potato Crop, 154-194. Chapman & Hall, London, UK.

Mendoza, H. A. (1988) Progress in resistance breeding in potatoes as a function of efficiency of screening procedures. *In*: Bacterial Disease of the Potato. Report of Planning Conference, 39-64. CIP, Lima, Peru.

Ortiz, R., M. Iwanaga and S. J. Peloquin (1994) Breeding potatoes for developing countries using wild tuber bearing *Solanum* spp. and ploidy manipulations. J. Genet & Breed. 48: 89-98.

——, S. J. Peloquin R. Freyre and M. Iwanaga (1991) Efficiency of potato breeding using FDR 2n gametes for multitrait selection and progeny testing. Theor. Appl. Genet. 82: 602-608.

Ross, H. (1986) Potato Breeding - Problems and Perspectives, 95-96. Verlag Paul Parey, Berlin, Germany.

Rowe, P. R. and L. Sequeira (1970) Inheritance of resistance to *Pseudomonas solanacearum* in *Solanum phureja*. Phytopathology 60: 1499-1501.

Schmiediche, P. (1983) Breeding bacterial wilt (*Pseudomonas solanacearum*) resistance germplasm. *In*: "Present and Future Strategies for Potato Breeding and Improvement, Report of the Planning Conference", 44-55. International Potato Center, Lima, Peru.

—— (1988) Breeding for resistance to *Pseudonomas solanacearum*. *In*: "Report of the Planning Conference on Bacterial Disease of the Potato", 19-28. International Potato Center, Lima, Peru.

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: 451-462.

Tung, P. X., E. T. Rasco, P. Van der Zaag and P. Schmiediche (1990a) Resistance to *Pseudomonas solanacearum* in the potato: I. Effects of sources of resistance and adaptation. Euphytica 45: 203-210.

——, ——, —— and —— (1990b) Resistance to *Pseudomonas solanacearum* in the potato: II. Aspects of host-pathogen-environment interaction. Euphytica 45: 211-215.

Watanabe, K., H. M. El-Nashaar and M. Iwanaga (1992a) Transmission of bacterial wilt resistance by first division restitution (FDR) 2n pollen via 4x × 2x crosses in potatoes. Euphytica 60: 21-26.

——, M, Iwanaga, H. El-Nashaar and P. Jatala (1992b) Transmission of resistance on root-knot nematodes and bacterial wilt by 2n pollen in potatoes. In: G. Kimber (ed.), "Proceeding of the Second International Symposium on Chromosomes Engineering on Plants". 128-132. College of Agriculture, University Extension of Missouri-Columbia, Missouri, USA.

——, M. Orrillo, S. Perez, J. Crusado and J. A. Watanabe (1996b) Testing yield of diploid potato breeding lines for cultivars development. Breed. Sci. 46: 245-249.

——, ——, M. Iwanaga, R. Ortiz, R. Freyre and S. Perez (1994) Diploid potato germplasm from wild and land race genetic resources. Am. Potato J. 71: 599-604.

——, ——, S. Vega, A. M. Golmirzaie, S. Perez, J. Crusado and J. A. Watanabe (1996a) Generation of heat resistant, diploid potato germplasm with short-day adaptation from diverse genetic stocks. Breed. Sci. 46:329-336.

——, ——, ——, M. Iwanaga, R. Ortiz, R. Freyre, G. Yerk, S. J. Peloquin and K. Ishiki (1995) Selection of diploid potato clones from diploid (haploid × wild species) F_1 hybrid families for short day conditions Breed. Sci. 45: 341-347.

Yamamoto, N. (1980) Environmental exploitation on the eastern slope of the southern central Andes: A case study of the agro-pastoral society of Marcapata, Cuzco, Peru. Sci. Rept. Natl. Museum Anthropology, Japan 5: 121-189.