

Frequency of Potato Genotypes with Multiple Quantitative Pest Resistance Traits in 4x × 2x Crosses

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Summary

Diploid potato breeding populations with quantitatively inherited traits related to pest resistance were selected. These lines were further crossed to 4x cultivars via FDR 2n pollen to examine the level of transmission of variable traits including the resistance to bacterial wilt (BW), potato tuber moth (PTM), and root-knot nematodes (RKN). Transmission of quantitative traits including late blight (LB) resistance and the presence of glandular trichomes which confer general insect resistance in tetraploid genotypes was also examined in the 4x × 2x progeny. All the quantitative traits except for the resistance to PTM were transmitted to progeny with a high frequency of resistant progenies. Among 557 clones from 4x × 2x crosses, 114 clones showed a combination of two types of quantitative resistance traits in a single clone. Four types of combinations, BW + RKN (85 clones), LB + BW (14 clones), BW + glandular trichomes (1 clone), and RKN + glandular trichomes (14 clones), were identified. Our results indicated that: 1) the transmission of combined quantitative traits related to pest resistance was possible via FDR 2n pollen; and 2) quantitative traits related to LB resistance and the presence of glandular trichomes could be transmitted to progeny with a high frequency.

Key Words : FDR 2n pollen, 4x × 2x, transmission of resistance, quantitatively inherited traits, combined resistance.

Introduction

The potato is one of the most valuable food crops in the world. Despite utmost efforts to control biotic stresses, approximately 22% of the yield is lost per year due to diseases and pests (Ross 1986). To reduce the extensive use of pesticides and to facilitate integrated management of pests, combination of resistance to many types of biotic stresses in breeding lines is of global importance in potato breeding programs.

Most of the cultivated potatoes are tetraploid (2n = 4x = 48), while approximately seventy percent of the wild

relatives are diploid (2n = 2x = 24) (Hawkes 1994). Ploidy manipulation is essential to introgress desirable traits from the diploid wild species into the high yielding tetraploid cultivated potatoes (Ortiz *et al.* 1994, Raman *et al.* 1994, Watanabe *et al.* 1996a, b). Important traits found in the wild species include resistance and/or tolerance to major biotic and abiotic stresses such as viruses, viroids, insects, nematodes, fungi, bacteria, heat and frost (Ross 1986).

Use of first division restitution (FDR) 2n gametes has been a powerful tool for the transmission of specific desirable traits from diploid germplasm directly into the 4x cultivated potatoes, and 2n pollen has clear advantages over 2n eggs in potato improvement due to the ease in selection (Iwanaga *et al.* 1989). Previous studies demonstrated the transmission of resistance to biotic stresses to 4x cultivars via FDR 2n pollen; resistance to root-knot nematodes (RKN; *Meloidogyne incognita*, race 3) (Iwanaga *et al.* 1989, Watanabe *et al.* 1990); bacterial wilt, (BW, *Pseudomonas solanacearum*) (Watanabe *et al.* 1990, 1992); early blight, [*Alternaria solani* (Ell. & Martin) Sor.] (Herriott and Haynes 1990); potato cyst nematode (*Globodera pallida*) (Ortiz *et al.* 1997); and common scab [*Streptomyces scabies* (Thaxt.) Lambert and Loria] (Murphy *et al.* 1995). Since in these studies, the resistance was transmitted with varying frequency or heritability, estimation/prediction of the frequency of the progeny with the resistance would be important in effective use of the resistance in 4x × 2x crosses.

Previous studies mostly focused on a single quantitative resistance trait, although multiple biotic stresses are much more likely to occur in the same period under the field conditions. In this paper we report the results of transmission of resistance to single and multiple biotic stresses. Selected diploid breeding populations showed resistance to bacterial wilt (BW), potato tuber moth [PTM, *Phthorimaea operculella* (Zeller)] and root-knot nematodes (RKN). These lines were crossed to cultivated tetraploid populations and tetraploid lines with quantitative resistance to late blight [LB, *Phytophthora infestans* (Mont.) de Bary], and the presence of type A glandular trichomes on the foliage. Type A glandular trichomes are controlled by quantitative loci and confer resistance to a broad range of insects and mites (Bonierbale *et al.* 1994). The potential of transmission of combined quantitative resistance traits is discussed.

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Materials and Methods

Plant materials

Pedigrees and other characteristics of the potato genotypes used in this study have been described in the report of Watanabe *et al.* (1994, 1996a, b) for the diploid genotypes and CIP (1996) for the tetraploid genotypes. All the diploid genotypes were selected from diploid populations producing FDR 2n pollen. Crosses were made in net houses at Lima, Peru, in 1991 and 1992.

Tuber families were generated from true seeds. The potted plants generated from true seeds were harvested in net houses at Lima, Peru, in August, 1993. These tubers were used as propagules for each resistance test. Dormancy of tubers was broken using Rindite (Nasiruddin and Blake 1997) and/or gibberellic acid treatment (200 ppm, 1 hour). The number of families for each resistance test and the number of clones per family varied depending on 1) the availability of screening plant materials; 2) priority of breeding target(s) in each family; 3) expectation of resistant progenies; or 4) selection pressure on general agronomic traits.

Methods

Late blight: Planting was performed at Cajamarca, Peru, in late December, 1993. A set of single R-gene differential potato genotypes that were derived from CIP collection was used to differentiate the physiological races of the isolates. The description of the isolates, including artificially inoculated and naturally existing ones recognized from the infested field, is given in the report of Trognitz *et al.* (1997). Inoculation was made to 5 plants / replication with 3 replications for each clone 30 days after planting, consisting of a complex of isolates of *Phytophthora infestans* with 10,000 sporangia / ml according to the method of French and Mackay (1996). Visual scoring from zero to 10 was performed every week up on defoliation up to 60 days after planting with 0 indicating the absence of damage and 10 complete destruction.

Bacterial wilt: CIP isolate 204 belonging to race 3 of *Pseudomonas solanacearum* was inoculated. A minimum of 20 cuttings per clone were used from seedling tubers in the greenhouse (Watanabe *et al.* 1992, 1996b) at Lima, Peru, in February 1994. Plants were evaluated five days after inoculation up to one month or until plants wilted completely.

Potato tuber moth: Tuber resistance using 15 tubers / clone was evaluated principally using a laboratory test for antibiosis at Lima, Peru, in February, 1994 and a storage testing method for antixenosis at San Ramon, Peru, in May, 1995. Resistance was ranked into five classes, based on the visual score of feeding by the insect (Ortiz *et al.* 1990).

Root-knot nematodes: A standardized inoculation at CIP was used to evaluate seedling tubers for resistance to RKN (Iwanaga *et al.* 1989). Two weeks after trans-

planting, each plant was inoculated with 4,000 eggs of *Meloidogyne incognita* (race 3) in the controlled greenhouse at Lima, Peru, in February, 1994. Five plants per clone were used. As for the provisionally resistant clones, they were reevaluated by the same method for confirmation. The level of resistance of each plant was evaluated by visual rating eight weeks after the inoculation.

Glandular trichomes: Leaf samples were collected from net house-grown plants at Lima, Peru. They were planted in April and sampled in early June-July, 1994. Five leaves per genotype were used. Density of type A trichomes (Ryan *et al.* 1982) was determined by counting the number of trichomes in 3.2 mm² area of each leaf surface (upper and lower) at 60 × magnification with a stereomicroscope equipped with an ocular grid (Bonierbale *et al.* 1994). Quantitative biochemical assay based on the measurement of the concentration of polyphenol oxidase (PPO) was also used to determine the type A trichome properties, according to the method of Kowalski (1989).

Results and Discussion

Transmission of resistance to bacterial wilt (BW)

Among 345 progenies from BW-resistant diploid male parents (Table 1), 142 clones (41.2%) were evaluated as resistant and 20 clones as moderately resistant (5.8%). Frequency of BW-resistant (R + MR) progenies derived from the diploid clone, '381348.7' was 53.0% (160 / 302) on an average. Although the resistance to BW in the diploid clone 'J-40' was also transmitted to progenies, the frequency was much lower (4.7%). Some differences were observed in the frequency of BW resistance among the progenies from the crosses of the diploid clone, '381348.7' with several susceptible tetraploid lines. Progenies from the cross 'Kufri Sindhuri' × '381348.7' exhibited the highest frequency of resistance (R + MR) (82.9%), while cross the 'Serrana × 381348.7' generated the lowest frequency of resistant genotypes (42.4%).

Susceptible diploid genotypes also generated BW-resistant progenies. Among 375 progenies from susceptible diploid genotypes (Table 1), 137 clones showed resistance (36.5%) and 22 clones moderate resistance (5.9%). Susceptible '86.105.12' produced BW-resistant progenies with a relatively high frequency of resistance (R + MR) (51.7%), (137 / 265). Susceptible clones '85.27.13' and 'DG81.68' also generated BW-resistant progenies with moderately high frequencies of resistance (30.2% and 10.5%, respectively). BW-resistant progenies were also found among the progenies of susceptible tetraploid male parents. Among 103 progenies from them, 17 clones showed the BW resistance (16.5%).

Transmission of the BW resistance from diploid lines to tetraploid cultivars via FDR 2n pollen has been reported using various selected diploid genotypes, ex-

Table 1. Summary of frequency of progenies with resistance to bacterial wilt, potato tuber moth, and root knot nematodes

Cross combination		Resistance in male parent ^{1,2)}			BW					PTM			RKN					
Family	Female	Male	BW ³⁾	PTM ⁴⁾	RKN ⁵⁾	Number of clones				Number of clones			Number of clones					
						Tested	R	MR	(R+MR)	R	Tested	R	R	Tested	R	MR	(R+MR)	R
1)4x × 2x																		
93.001	Kufri Sindhuri	381348.7	MR	S	MR	35	28	1	82.9	80.0				25	8	7	60.0	32.0
93.005	Atlantic	381348.7	MR	S	MR	54	23	5	51.9	42.6				34	15	10	73.5	44.1
93.007	Serrana	381348.7	MR	S	MR	59	25	0	42.4	42.4				53	2	5	13.2	3.8
93.011	Atzimba	381348.7	MR	S	MR	59	22	4	44.1	37.3				50	18	9	54.0	36.0
93.013	LT-8	381348.7	MR	S	MR	47	22	4	55.3	46.8				31	14	13	87.1	45.2
93.015	LT-9	381348.7	MR	S	MR	48	20	6	54.2	41.7				26	16	9	96.2	61.5
Total (mean) progenies from 381348.7						302	140	20	53.0	46.4				219	73	53	57.5	33.3
2)4x × 4x																		
93.024	M514.6	J-40	MR	S	R	43	2	0	4.7	4.7				25	9	7	64.0	36.0
93.002	Kufri Sindhuri	85.27.13	S	S	R									51	13	11	47.1	25.5
93.009	Achirana	85.27.13	S	S	R	53	13	3	30.2	24.5				39	25	7	82.1	64.1
Total (mean) progenies from 85.27.13														90	38	18	62.2	42.2
93.003	Kufri Sindhuri	DG81.68	S	S	S						55	0	0.0					
93.004	Ultimus	DG81.68	S	S	S						44	0	0.0					
93.006	Atlantic	DG81.68	S	S	S						46	0	0.0					
93.008	Serrana	DG81.68	S	S	S						58	0	0.0					
93.010	Achirana	DG81.68	S	S	S	57	4	2	10.5	7.0				53	1	0	1.9	1.9
93.012	Atzimba	DG81.68	S	S	S						59	0	0.0					
93.014	LT-8	DG81.68	S	S	S						44	0	0.0					
93.016	LT-9	DG81.68	S	S	S						39	0	0.0					
93.017	LT-9	84.38.12	S	MR	S						58	0	0.0					
93.018	Serrana	84.38.12	S	MR	S						59	0	0.0					
93.019	Atzimba	86.105.12	S	S	R	59	25	5	50.8	42.4				58	0	4	6.9	0.0
93.020	Atlantic	86.105.12	S	S	R	42	10	3	31.0	23.8				26	0	1	3.8	0.0
93.021	AVRDC1287.19	86.105.12	S	S	R	51	28	4	62.7	54.9				45	3	7	22.2	6.7
93.022	LT-8	86.105.12	S	S	R	53	27	5	60.4	50.9				42	0	1	2.4	0.0
93.023	Serrana	86.105.12	S	S	R	60	30	0	50.0	50.0				52	0	0	0.0	0.0
Total (mean) progenies from 86.105.12						265	120	17	51.7	45.3				223	3	13	7.2	1.3
93.025	BULKM514	85.37.38	R	S	R						37	0	0.0					
Total (mean) progenies summarized by male parents																		
Resistant diploid						345	142	20	47.0	41.2	117	0	0.0	557	123	91	38.4	22.1
Susceptible diploid						375	137	22	42.4	36.5	382	0	0.0	53	1	0	1.9	1.9
Susceptible tetraploid						103	9	8	16.5	8.7				68	0	8	11.8	0.0

¹⁾ Abbreviations: BW, bacterial wilt; PTM, potato tuber moth; RKN, root knot nematodes, respectively.

²⁾ Abbreviations: R, resistant; MR, moderately/incompletely resistant; S, susceptible, respectively.

³⁾ Refer to Watanabe *et al.* (1994) and Watanabe *et al.* (1996b).

⁴⁾ Refer to this paper.

⁵⁾ Refer to Watanabe *et al.* (1994), Watanabe *et al.* (1996b) and this paper.

cept for the diploid clones '381348.7' and 'J-40' (Watanabe *et al.* 1990, 1992, 1996a, b). Our results confirmed that the use of FDR 2n pollen was an effective method to transfer BW resistance from the selected 2x breeding populations to tetraploid potatoes. Frequency of BW-resistant progenies derived from the resistant diploid lines was higher in this study than in the

previous studies. This could be due to the fact that the population in this study was based on more advanced parental clones, such as '381348.7' (Watanabe *et al.* 1995, 1996a, b) than those used in the previous studies. Frequency of resistant progenies from the 4x female parents 'Atzimba' and '7XY.1', or the 4x male parent 'Katahdin', was variable in the two studies (Watanabe *et*

al. 1990, 1992). However, in this study and both previous studies, BW-resistant diploid male parents gave the highest frequency of resistant progenies.

The level of transmission of BW resistance varied depending on the combination of parental lines. Relatively high levels of resistance were observed in many progenies of susceptible diploid and tetraploid male genotypes in this study. Especially, susceptible '86.105.12' generated resistant genotypes with a considerably high frequency (R + MR) (51.7%). These results were consistent with many previous reports (Tung *et al.* 1990, Schiemediche 1988, Watanabe *et al.* 1990, 1992). Several factors including: 1) female by male genotype interaction (Watanabe *et al.* 1992); 2) different combining ability (Tung *et al.* 1990; Watanabe *et al.* 1992); 3) minor additive genetic resistance factors (Watanabe *et al.* 1992); and 4) width of the genetic background (Tung *et al.* 1990), were proposed to explain the phenomena in the previous reports. Application of modern techniques using molecular markers may enable to explore in more detail the genetic nature of the BW resistance in potatoes.

Transmission of resistance to potato tuber moth (PTM)

No PTM-resistant progenies were found among the 499 progenies tested in our study (Table 1). In previous studies on the transmission of the PTM resistance from diploid lines derived from the cross between *Solanum sparsipilium* (spl) and advanced diploid lines, strong evidence of reciprocal difference was reported (Chaves 1984, Ortiz *et al.* 1990, Raman *et al.* 1981). Ortiz *et al.* (1990) indicated that spl cytoplasm was not essential for the expression of PTM resistance. However, transmission of the PTM resistance derived from spl to genotypes with spl cytoplasm was much more efficient than that to lines without spl cytoplasm (Ortiz *et al.* 1990). None of the female parents had a spl pedigree in our present study (CIP 1998). Our results supported the assumption that spl cytoplasm could be desirable for the enhancement of the expression of PTM resistance (Gomez *et al.* 1983). On the other hand, other germplasm accessions, such as *Solanum multidissectum* and *Solanum gourlayi*, can be used as a source of PTM resistance (Watanabe *et al.* 1995) without the requirement of a specific cytoplasmic effect, and these diploid resistant lines will be used in the 4x × 2x scheme for transferring quantitative resistance.

Transmission of resistance to root knot nematodes (RKN)

Resistant diploid lines, '381348.7', '85.27.13', '86.105.12', 'J-40' and '85.37.38' were crossed with susceptible tetraploid female parents (Table 1). Among 557 progenies from resistant diploid male parents, 123 clones were evaluated as resistant (22.1%) and 91 clones were evaluated as moderately resistant (16.3%). Frequency of resistant progenies derived from '381348.7' varied among the families. The highest frequency was found in the

cross between 'LT-9' and '381348.7' (96.2%) (R + MR), while the lowest frequency in the crosses between 'Serrana' and '381348.7' (13.2%) (R + MR). Although a similar tendency was observed among the progenies derived from '86.105.12', the average frequency (7.2%) (R + MR) was much lower than that in the progenies derived from other resistant male parents (57.5% for '381348.7' and 64.0% for 'J-40' and 62.2% for '85.27.13'). Iwanaga *et al.* (1989) reported various transmission rates ranging from 7.6% ('84.36.29') to 30.4% ('381348.7') among the progenies derived from six different resistant male parents. Therefore, it is suggested that '381348.7' could be selected as a desirable male parent for the transmission of RKN resistance. Various transmission rates reported both in this study and that of Iwanaga *et al.* (1989) could be explained by 1) female by male genotype interaction 2) different combining ability; 3) minor additive genetic resistance factors; and 4) width of the genetic background, as indicated in the section of BW resistance.

Some resistant progenies were found among the progenies of the susceptible diploid male parent 'DG81.68' and those of the susceptible tetraploid male parent 'Tollocan'. The results imply the presence of specific (recessive or additive) alleles in susceptible parents which are non-conventional sources of the quantitative resistance. This could be interpreted by the fact that there are almost always some loci for which the inferior parent contributes a superior allele (Tanksley and McCouch 1997), as reported in rice (Xiao *et al.* 1996) and tomato (Grandillo *et al.* 1996).

Our results confirmed that the FDR 2n pollen provided a very effective method to transfer RKN resistance from wild potato species to tetraploid cultivated potatoes. Compared to the results of Iwanaga *et al.* (1989), frequencies of resistant (R + MR) progenies derived from '381348.7' and '85.27.13' in our study were 57.5% (126 / 219) and 62.2% (56 / 90), respectively, while in their study, they were 22% and 13%, respectively. In fact, the same cross, 'Atzimba' × '381348.7', was tested in the two studies. Frequency of resistance reported in the study by Iwanagas group (62.8%) did not differ appreciably from that in our study (54.0%). Therefore the improved transmission rate in this study may indicate that the 4x cultivated potatoes crossed would have been good combiners with wild potatoes expressing the RKN resistance.

Transmission of resistance to late blight (LB) from 4x to 4x × 2x progeny

Transmission of field resistance to LB was evaluated in this study (Table 2). Tetraploid female parents, 'Achi-rana', 'Atzimba', 'LT-9', and '7XY.1', were moderately resistant, and the tetraploid male parent, 'Tollocan', was also moderately resistant to LB (CIP 1996). On the other hand, the diploid genotypes, 'DG81.68', '84.38.12', '86.105.12', '85.37.38', '90.21.25', '381348.7' and '86.105.4' were susceptible

Table 2. Summary of frequency of progenies with resistance to LB and presence of Type A grandular trichomes

Family	Cross combination		Resistance in male parent ^{1,2)}				Resistance in female parent ²⁾ LB ⁷⁾	LB			Grandular trichomes		
	Female	Male	PTM ³⁾	BW ⁴⁾	RKN ⁵⁾	Trichome ⁶⁾		Number of clones tested	Number of R	% of R	Number of clones tested	Number of Tri ⁹⁾	% of Tri
1)4x × 2x													
93.012	Atzimba	DG81.68	S	S	S	<i>a</i>	MR	3	1	33.3			
93.019	Atzimba	86.105.12	S	S	R	<i>a</i>	MR	22	12	54.5			
Total (mean) progenies from Atzimba								25	13	52.0			
93.010	Achirana	DG81.68	S	S	S	<i>a</i>	MR	6	2	33.3			
93.016	LT-9	DG81.68	S	S	S	<i>a</i>	MR	1	0	0.0			
93.017	LT-9	84.38.12	MR	S	S	<i>a</i>	MR	2	0	0.0			
Total (mean) progenies from LT-9								3	0	0.0			
93.028	7XY.1	86.105.4	S	S	S	<i>a</i>	MR	6	3	50.0			
93.001	Kufri Sindhuri	381348.7	S	MR	MR	<i>a</i>	S	1	0	0.0			
93.008	Serrana	DG81.68	S	S	S	<i>a</i>	S	2	0	0.0			
93.018	Serrana	84.38.12	MR	S	S	<i>a</i>	S	2	0	0.0			
93.023	Serrana	86.105.12	S	S	R	<i>a</i>	S	13	6	46.2			
93.026	Serrana	90.21.25	S	S	S	<i>a</i>	S	1	0	0.0			
Total (mean) progenies from Serrana								18	6	33.2			
93.014	LT-8	DG81.68	S	S	S	<i>a</i>	S	4	0	0.0			
93.022	LT-8	86.105.12	S	S	R	<i>a</i>	S	3	0	0.0			
Total (mean) progenies from LT-8								7	0	0.0			
93.020	Atlantic	86.105.12	S	S	R	<i>a</i>	S	7	0	0.0			
93.021	AVRDC1287.19	86.105.12	S	S	R	<i>a</i>	S	3	1	33.3			
93.024	M514.6	J-40	S	MR	R	<i>p</i>	S				60	41	68.3
93.025	BULKM514	85.37.38	S	R	R	<i>p</i>	S	1	0	0.0	60	35	58.3
Total (mean) progenies summarized by female parents													
Resistant tetraploid								40	18	45.0			
Susceptible tetraploid								37	7	18.9			
2)4x × 4x													
93.030	Atzimba	Tollocan ⁸⁾	S	S	S	<i>a</i>	MR	3	1	33.3			

For¹⁾ and²⁾ abbreviations, refer to Table 1.

For^{3,4)} and⁵⁾ references, refer to Table 1.

⁶⁾ *p*: presence of Type A grandular trichomes with high density on the foliage and *a*: absence based on the method developed by Bonierbale *et al.* (1994).

⁷⁾ Refer to CIP (1996).

⁸⁾ Tollocan was evaluated as moderately resistant to late blight at CIP (1996).

⁹⁾ Tri: presence of Type A grandular trichomes with high density on the foliage. Density was measured based on PPO and trichome density (CIP, 1992).

(CIP 1996). Progenies resistant to LB were found in families from the crosses, 'Atzimba' × 'DG81.68', 'Atzimba' × '86.105.12', 'Achirana' × 'DG81.68', '7XY.1' × '86.105.4', 'Serrana' × '86.105.12' and 'AVRDC1287.19' × '86.105.12' (Table 2). A cross 4x 'Atzimba' × 4x 'Tollocan' also generated LB-resistant progeny. Thus, resistance components to LB derived from tetraploid female parents, 'Achirana', 'Atzimba', and '7XY.1', were transmitted to progeny. Resistant progenies were also generated from crosses between susceptible parents 'AVRDC1287.19' × '86.105.12' and 'Serrana' × '86.105.12', indicating the presence of a quantitative trait resulting from the joint action of

polygenes, providing horizontal resistance to LB (Black 1970). Progenies of diploid '86.105.12' crossed to resistant 'Atzimba' showed the highest frequency of resistant progenies (54.5%). Among 25 progenies of 'Atzimba' crossed with two diploid genotypes, 13 of them (52.0%) were evaluated as resistant. On the other hand, resistant 'LT-9' did not confer resistance to LB, presumably due to the small sample size. Our results indicated that the transmission rate of LB resistance in the resistant 4x × susceptible 2x progenies (45.0%) was higher than in the resistant 4x × resistant 4x progenies (33.3%) (Summary in Table 2). Thus, the use of 4x × 2x crosses would be appropriate to combine quantitative

resistance involving LB from 4x and others from 2x.

Transmission of type A glandular trichome traits

Tingey *et al.* (1981) and Ochoa (1980) described more than 15 species of potatoes with glandular trichomes. Type A glandular trichomes immobilize insects on the foliage, leading to cessation of feeding and death (Gregory *et al.* 1986). Unlike other types of traits examined in this study, the trait of type A glandular trichomes was derived from the tetraploid parents. This was because the diploid population selected for glandular trichomes did not produce fertile 2n pollen. Our results indicated that tetraploid lines efficiently transmitted their trichome traits to their progenies. Frequency of trichome genotypes was very high (68.3% for 'M514.6' × 'J-40' and 58.3% for 'BULK M514' × '85.37.38') (Table 2), indicating quantitative inheritance (Bonierbale *et al.* 1994).

Transmission of combined traits related to pest resistance

(1) Combined resistance to BW and RKN

The levels of transmission of combined resistance to BW and RKN for each family are shown in Table 3. Potato cultivars resistant to BW become susceptible when RKN and BW infection occurs at the same time, especially in hot climates (Ortiz *et al.* 1994). Therefore, breeding for the combined resistance to these two stresses is important, especially for developing countries in subtropical or tropical regions. Out of 557 clones generated from 4x × 2x and tested for BW and RKN resistance, 85 showed a combined resistance. Progenies from the diploid line 381348.7 resistant to both BW and RKN mostly showed a high frequency of combined resistance with one exception. In families 93.001 and 93.015, the frequency of progenies with combined resistance was higher than 50%. The other families also showed a relatively high frequency of combined resistance except for the family 93.007 (3.8%). These results suggest that the transmission of combined resistance from the 2x clones to 4x population via 2n pollen is effective. Progenies with combined resistance

Table 3. Frequency of progenies with the combined quantitative resistance to BW and RKN

Family	Cross combination		Number of clones tested	Combination of resistance			
	Female	Male		BW/S ¹ +RKN/S	BW/R+RKN/S	BW/S+RKN/R	BW/R+RKN/R
4x × 2x							
93.001	Kufri Sindhuri	381348.7	25	1(4.0%)	9(36.0%)	2(8.0%)	13(52.0%)
93.005	Atlantic	381348.7	34	1(3.0%)	8(23.5%)	14(41.2%)	11(32.4%)
93.007	Serrana	381348.7	52	24(46.2%)	21(40.4%)	5(9.6%)	2(3.8%)
93.011	Atzimba	381348.7	50	15(30.0%)	8(16.0%)	13(26.0%)	14(28.0%)
93.013	LT-8	381348.7	31	2(6.5%)	2(6.4%)	12(38.7%)	15(48.4%)
93.015	LT-9	381348.7	26	0(0.0%)	1(3.9%)	11(42.3%)	14(53.9%)
Total (mean) progenies from 381348.7			218	43(19.7%)	49(22.5%)	57(26.1%)	69(31.7%)
93.019	Atzimba	86.105.12	58	28(48.3%)	26(44.8%)	1(1.7%)	3(5.2%)
93.020	Atlantic	86.105.12	26	17(65.4%)	8(30.8%)	1(3.8%)	0(0.0%)
93.021	AVRDC1287.19	86.105.12	45	10(22.2%)	25(55.6%)	5(11.1%)	5(11.1%)
93.022	LT-8	86.105.12	42	14(33.3%)	27(64.3%)	1(2.4%)	0(0.0%)
93.023	Serrana	86.105.12	52	24(46.2%)	28(53.9%)	0(0.0%)	0(0.0%)
Total (mean) progenies from 86.105.12			223	93(41.7%)	114(51.2%)	8(3.6%)	8(3.6%)
93.009	Achirana	85.27.13	38	4(10.5%)	2(5.3%)	25(65.8%)	7(18.4%)
93.010	Achirana	DG81.68	53	47(88.7%)	5(9.4%)	1(1.9%)	0(0.0%)
93.024	M514.6	J-40	25	8(32.0%)	1(4.0%)	15(60.0%)	1(4.0%)
Total(4x × 2x)			557	195(35.0%)	171(30.7%)	106(19.0%)	85(15.3%)
4x × 4x							
93.029	Serrana	Katahdin	22	17(77.3%)	5(22.7%)	0(0.0%)	0(0.0%)
93.030	Atzimba	Tollocan	46	34(73.9%)	4(8.7%)	7(15.2%)	1(2.2%)
Total(4x × 4x)			68	51(75.0%)	9(13.2%)	7(10.3%)	1(1.5%)
Total			625	246(39.4%)	180(28.8%)	113(18.1%)	86(13.8%)

¹⁾ Abbreviations: S, susceptible; R, resistant or moderately resistant, respectively.

were also generated from genotypes resistant to either BW or RKN, or susceptible to both of these, mainly due to the inheritance of quantitative traits of resistance to BW (Watanabe *et al.* 1992) and RKN (Iwanaga *et al.* 1989). In a previous study, 22 clones with combined resistance to at least two different pests (equivalent to 2.2%) were identified among 990 seedlings tested (Watanabe *et al.* 1996b). A much higher frequency of combined resistance (15.3%) was detected in this study, probably due to more advanced selections at the diploid progenitor level.

(2) Combined resistance to LB, BW and RKN

The level of transmission of the combined resistance to LB, BW, and RKN is shown in Table 4. Among 53 progenies from $4x \times 2x$ tested for these three traits, 40 progenies were resistant to at least one of the diseases and 15 showed a combined resistance to two diseases. Progenies of the family 93.019 ('Atzimba' \times '86.105.12'), family 93.021 ('AVRDC1287.19' \times '86.105.12'), and family 93.023 ('Serrana' \times '86.105.12') showed a combined resistance to LB and BW. One resistant progeny to BW and RKN without resistance to LB was generated from the cross 'Kufri Sindhuri' \times '381348.7' (family 93.001).

Progenies with the combined resistance to LB, BW, and RKN were not observed among the tested clones, presumably due to the small sample size. Combination of resistance to LB + BW and BW + RKN was observed, but there were no progenies with resistance to LB + RKN. Most of the tested clones were progenies

from '86.105.12'. Families 93.019, 93.021, and 93.023 showed a high frequency of resistance to LB, and 93.021 showed also a high frequency of resistance to RKN. Combined resistance to LB, BW, and RKN could be possibly found in future screening trials in which a larger number of clones in the family 93.021 could be used for the evaluation of resistance to these agents.

(3) BW, RKN and glandular trichomes

Only a limited number of families was tested for the level of transmission of triple combined traits, BW, RKN and glandular trichomes (Table 4). Among the 25 progenies tested, 16 (64.0%) showed a combination of two traits, and 14 (56.0%) displayed RKN resistance and had glandular trichomes. The percentage of the resistant clones among the tested clones was considered to be very high compared to the percentage usually recorded in conventional breeding programs (Ross 1986). The presence of glandular trichomes is a desirable trait for potato production in hot climates where various insects feed on the foliage of potatoes and potentially transmit virus diseases. Hot and humid climates also accelerate the damage caused by BW (French 1985) and RKN (Jatala and Mendoza 1978). Therefore, potato genotypes with combined traits for resistance to BW, RKN and presence of the trichomes would be highly desirable for growers in tropical and subtropical regions. Those genotypes could be obtained from the male diploid line, '381348.7', which produced progenies with a very high frequency of resistance to both BW and RKN.

Table 4. Frequency of progenies with the combined quantitative resistance

Combination of traits	Family	Cross combination		Number of clones tested	Combination of resistance						
		Female	Male		S ¹⁾ +S+S	R+S+S	S+R+S	S+S+R	R+R+S	S+R+R	
LB+BW+RKN											
	$4x \times 2x$										
	93.019	Atzimba	86.105.12	22	4(18.2%)	5(22.7%)	6(27.3%)	0(0.0%)	7(31.8%)	0(0.0%)	
	93.020	Atlantic	86.105.12	6	4(66.7%)	0(0.0%)	2(33.3%)	0(0.0%)	0(0.0%)	0(0.0%)	
	93.021	AVRDC1287.19	86.105.12	3	0(0.0%)	0(0.0%)	1(33.3%)	1(33.3%)	1(33.3%)	0(0.0%)	
	93.022	LT-8	86.105.12	3	0(0.0%)	0(0.0%)	3(100 %)	0(0.0%)	0(0.0%)	0(0.0%)	
	93.023	Serrana	86.105.12	12	2(16.7%)	0(0.0%)	4(33.3%)	0(0.0%)	6(50.0%)	0(0.0%)	
	Total (mean) progenies from 86.105.12			46	10(21.7%)	5(10.9%)	16(34.8%)	1(2.2%)	14(30.4%)	0(0.0%)	
	93.001	Kufri Sindhuri	381348.7	1	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(100 %)	
	93.010	Achirana	DG81.68	6	3(50.0%)	2(33.3%)	0(0.0%)	1(16.7%)	0(0.0%)	0(0.0%)	
	Total ($4x \times 2x$)			53	13(24.5%)	7(13.2%)	16(30.2%)	2(3.8%)	14(26.4%)	1(1.9%)	
	$4x \times 4x$										
	93.030	Atzimba	Tollocan	3	1(33.3%)	1(33.3%)	0(0.0%)	1(33.3%)	0(0.0%)	0(0.0%)	
	Total			56		8(14.3%)	16(28.6%)	3(5.4%)	14(25.0%)	1(1.8%)	
BW+RKN +Trichomes					S+S+a ²⁾	S+R+a	S+S+p ²⁾	R+R+a	R+S+p	S+R+p	
	93.024	M514.6	J-40	25	1(4.0%)	1(4.0%)	7(28.0%)	1(4.0%)	1(4.0%)	14(56.0%)	

¹⁾ Abbreviations: S, susceptible; R, resistant or moderately resistant to LB, BW or RKN, respectively.

²⁾ For abbreviations, refer to note 6) in Table 2.

Efficiency of multiple quantitative pest resistance traits in 4x × 2x crosses via FDR 2n pollen

Our results indicated that: 1) the transmission of combined quantitative traits related to pest resistances was possible via FDR 2n pollen; and 2) quantitative traits related to LB resistance and the presence of glandular trichomes (type A) could be transmitted to progenies with a high frequency. Transmission rate of combined quantitative pest resistance traits is considered to be very high compared with conventional 4x × 4x breeding in which the percentage of resistant progenies is often less than 5%, and the selection in such a mating scheme may require a far larger population size (Watanabe *et al.* 1991). The desirable combination of quantitative resistance traits, such as BW + RKN in the tropics and subtropics and LB + BW + RKN in the lowland tropics, would be important (CIP 1992, Ross 1986). Together with the above combination, insect resistance is important in direct protection of potato plants against insect feeding, and indirectly for insect-transmitted viruses such as PLRV and PVY. Further trials on combining the above traits with locally important quantitative characters such as resistance to common scab (Japan) and *Erwinia* soft rot (Southeast Asia) shall be conducted to develop a high level of multiple quantitative disease / pest resistance. In addition, combined pest resistance traits from diploid lines could be further transmitted to tetraploid genotypes via FDR 2n pollen to generate 4x cultivars for sustainability and higher productivity based on the supportive information from this paper.

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