Meeke AKAK Salima

AGRO.

258

Chetelat RT, Rick CM, DeVerna JW (1989) Isoenzyme analysis, chromosome pairing and fertility of *Lycopersicon esculentum* × *Solanum lycopersicoides* diploid backcross hybrids. Genome 32:783–790

Derks FHM, Wijbrandi J, Koornneef M, Colijn-Hooymans CM (1991) Organelle analysis of symmetric and asymmetric hybrids between *Lycopersicon peruvianum* and *Lycopersicon esculentum*. Theor Appl Genet 81:199–204

DeVerna JW, Chetelat RT, Rick CM (1987) Cytogenetic, electrophoretic and morphological analysis of progeny of sesquidiploid *Lycopersicon esculentum-Solanum lycopersicoides* hybrids × *L. pennellii*. Biol Zent 106:417–428

Frandsen NO (1986) Die Plastidenzahl als Merkmal bei der Kartoffel. Theor Appl Genet 38:153–167

Gadish I, Zamir D (1987) Differential zygotic abortion in an interspecific *Lycopersicon* cross. Genome 29:156–159

Hosticka LP, Hanson MR (1984) Induction of plastid mutation in tomatoes by nitromethylurea. J Hered 75:455–450

Jacobsen E, Reinhout P, Bergervoet JEM, Looff J de, Abidin PE, Huigen DJ, Ramanna MS (1992) Isolation and characterization of potato-tomato somatic hybrids using an amylose-free potato mutant as parental genotype. Theor Appl Genet 85:159–164

Jong JH de, Wolters AMA, Kok JM, Verhaar H, Eden J van (1993) Chromosome paring and potential for intergeneric recombination in some hypotetraploid somatic hybrids of *Lycopersicon esculentum* (+) *Solanum tuberosum*. Genome (in press)

Karp A, Risiott R, Jones MGK, Bright SWJ (1984) Chromosome doubling in monohaploid and dihaploid potatoes by regeneration from cultured leaf explants. Plant Cell Tissue Organ Cult 3:363–373

Kofer W, Glimelius K, Bonnett HT (1991) Modification of mitochondrial DNA cause changes in floral development in homeotic-like mutants of tobacco. Plant Cell 3:759–769

Koornneef M, Hanhart CJ, Martinelli L (1987) A genetic analysis of cell culture traits in tomato. Theor Appl Genet 74:633–641

Koornneef M, van Diepen JAM, Hanhart CJ, Kieboom-de Waart AC, Martinelli L, Schoenmakers HCH, Wijbrandi J (1989) Chromosomal instability in cell- and tissue culture of tomato haploids and diploids. Euphytica 43:179–186

Koornneef M, Bade J, Hanhart C, Horsman K, Schel J, Soppe W, Verkerk R, Zabel P (1993) Characterization and mapping of a gene controlling shoot regeneration in tomato. Plant J 3:131–141

Laat AMM de, Verhoeven HA, Sree Ramulu K, Dijkhuis P (1989) Chromosome transplantation and applications of flow cytometry in plants. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry vol 9. Springer Verlag, Berlin, pp 343–359

Lee CH, Power JB (1988) Intra- and interspecific gametosomatic hybridisation within the genus Petunia. Plant Cell Tissue Organ Cult 12:197–200

Melchers G, Sacristan MD, Holder AA (1978) Somatic hybrid plants of potato and tomato regenerated from fused protoplasts. Carlsberg Res Commun 43:203–218

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Neal CA, Topolenski LD (1983) Effects of the basal medium on growth of immature tomato embryo's in vitro. J Am Soc Hortic Sci 108:434–438

Nettancourt D de, Devreux M, Ianeri U, Cresti M, Pacini E, Sarfatti G (1974) Genetic and ultrastructural aspects of self and cross incompatibility in interspecific hybrids between self-compatible *Lycopersicon esculentum and* self-incompatible *L. peruvianum*. Theor Appl Genet 44:278–288

Nootebos MG, Tan MMC, Kool AJ (1989) Differences in nuclear DNA content in various organs of *Lycopersicon esculentum*. In: Science for plant breeding. Book of poster abstracts, XII Eucarpia Congr. Göttingen, p 30

Pental D, Mukhopadhyay A, Grover A, Pradhan AK (1988) A selection method for the synthesis of triploid hybrids by fusion of microspore protoplasts (n) with somatic cell protoplats (2n). The Appl Genet 76:237–243

Pirrie A, Power JB (1986) The production of fertile, triploid somatic hybrid plants (*Nicotiana glutinosa* (n) + *N. tabacum* (2n)) via gametic:somatic protoplast fusion. Theor Appl Genet 72:48–52

Pijnacker LP, Ferweda MA (1984) Giemsa C-banding of potato chromosomes. Can J Genet Cytol 26:415–419

Ramanna MS, Mutsaerts MCA (1971) Unusual behaviour of growing pollen tubes in the styles and ovules of *Spinacia oleracea* L. Euphytica 20:145–151

Rick CM (1963) Differential zygotic lethality in a tomato species hybrid. Genetics 48:1497–1507

Rick CM, DeVerna JW, Chetelat RT, Stevens MA (1986) Meiosis in sesquidiploid hybrids of *Lycopersicon esculentum* and *Solanum lycopersicoides*. Proc Natl Acad Sci USA 83: 3580–3585

Rick CM, Chetelat RT, DeVerna JW (1988) Recombination in sesquidiploid hybrids of *Lycopersicon esculentum* × *Solanum lycoperisicodies* and derivates. Theor Appl Genet 76:647–655

Schoenmakers HCH, Koornneef M, Alefs SJHM, Gerrits, WFM, Kop D vd, Cherel I, Caboche M (1991) Isolation and characterization of nitrate reductase deficient mutants in tomato (*Lycopersicon esculentum* Mill.). Mol Gen Genet 227: 458–464

Schoenmakers HCH, Nobel EM, Koornneef M (1992) Use of leaky nitrate reductase deficient mutants of tomato (*Lycopersicon esculentum* Mill.) for selection of somatic hybrid cell lines with wild type potato (*Solanum tuberosum* L.) Plant Cell Tissue Organ Cult 31:151–154

Schoenmakers HCH, Wolters AMA, Haan A de, Saiedi AK, Koornneef M (1993) Asymmetric somatic hybridization between tomato (*Lycopersicon esculentum* Mill.) and gamma-irradiated potato (*Solanum tuberosum* L.): a quantitative analysis. Theor Appl Genet (in press)

Shepard JF, Bidney D, Barsby T, Kemble R (1983) Genetic transfer in plants through interspecific protoplast fusion. Science 219:683–688

Smith JA, Desborough SL (1986) An interpretation of genomic balance in seed formation in interspecific hybrids of *Solanum*. Theor Appl Genet 72:346–352

Soost RK (1958) Progenies from sesquidiploid F_1 hybrids of *Lycopersicon esculentum* and *L. peruvianum*. J Hered 49: 208–213

Thomas Br, Pratt D (1981) Efficient hybridization between *Lycopersicon esculentum* and *L. peruvianum* via embryo callus. Theor Appl Genet 59:215–219

Uijtewaal BA (1987) Ploidy variability in greenhouse cultured and in vitro propagated potato monohaploids (2n = x = 12) as determined by flow cytometry. Plant Cell Rep 6:252–255

Verhoeven HA, Sree Ramulu K, Gilissen LJW, Famelaer I, Dijkhuis P, Blaas J (1991) Partial genome transfer through micronuclei in plants. Acta Bot Neerl 40:97–113

Vries SE de, Ferweda MA, Loonen AEHM, Pijnacker LP, Feenstra WJ (1987) Chromosomes in somatic hybrids between *Nicotiana plumbaginifolia* and monoploid potato. Theor Appl Genet 75:170–176

Wolters AMA, Schoenmakers HCH, van der Meulen-Muisers JJM, van der Knaap E, Derks FHM, Koornneef M, Zelcer A (1991) Limited DNA elimination from the irradiated potato parent in fusion products of albino *Lycopersicon esculentum* and *Solanum tuberosum*. Theor Appl Genet 83:225–232

Theor Appl Genet (1993) 87:337–342

THEORETICAL AND APPLIED GENETICS

Study of the three-genome hybrid *Lycopersicon esculentum* Mill. – *L. chilense* Dun. – *L. peruvianum* var '*humifusum*' Mill. and its use as a source for resistance

Z. V. Vulkova, V. G. Sotirova

Academy Doncho Kostoff Institute of Genetics, BAS, Sofia III 3 Bulgaria

Received: 22 April 1992 / Accepted: 4 January 1993

Abstract. *L. peruvianum* var 'humifusum' is reproductively the most isolated of the species of the genus *Lycopersicon*. It can be crossed with the cultivated tomato using *L. chilense* as an intermediary. After a series of backcrosses of the three-genome hybrid F_1 (*L. esculentum* × *L. chilense*) × *L. peruvianum* var 'humifusum' with *L. esculentum*, accompanied by selection for resistance to some economically important diseases, several lines were established. One of these lines, Cm 180, which showed resistance to *Clavibacter michiganensis* subsp. *michiganensis*, was subjected to genetic analysis. This resistance was found to be controlled by a single dominant gene (*Cm*) that was not allelic to the gene originating from *L. hirsutum* f. *glabratum*. This *Cm* gene was genetically mapped on chromosome 4. The germ plasm of *L. peruvianum* var 'humifusum' in combination with *L. chilense* was transferred into *L. esculentum*. Different breeding lines possessing resistance to various diseases and pests could be developed from this material.

Key words: Remote hybridization – *Lycopersicon* – Resistance genes – Mapping – *Clavibacter michiganensis* subsp. *michiganensis*

Introduction

Strict reproductive barriers exist between *L. peruvianum* var 'humifusum' and most of the other species of the genus *Lycopersicon*. This species can be crossed only with *L. chilense* and three races of *L. peruvianum* (Rick and Lamm 1955; Rick 1963, 1979). As the representatives of *Eriopersicon* – *L. peruvianum*, *L. peruvianum* var 'humifusum', *L. chilense*, *L. hirsutum* f. *typicum* and *L. hirsutum* f. *glabratum* – are sources of valuable genes for resistance to tomato diseases and pests, they are generally included in breeding programmes aimed at developing resistant cultivars (Hassan et al. 1968; Thyr 1969; Lindhout 1987; Sotirova and Beleva 1977; Sotirova and Bogatsevska 1988, etc). There are different estimates of number of the genes controlling resistance to *Clavibacter michiganensis* subsp. *michiganensis*. Some authors report that the resistance is controlled by a single dominant gene and modifier genes (Laterrot 1974; Laterrot and Rat 1987), while others state that the inheritance of resistance to *Clavibacter michiganensis* subsp. *michiganensis* is polygenic (Heinz 1972; Thyr 1976; De Jong and Honma 1976).

The object of the research presented here was to study the characteristics of the three-genome hybrid *L. esculentum-L. chilense-L. peruvianum* var 'humifusum'. We also assessed the genetics of the resistance of line Cm 180, which was developed from the three-genome hybrid, to *Clavibacter michiganensis* subsp. *michiganensis*. Finally, we screened and studied the resistance of 23 lines developed from the same hybrid to economically important diseases.

Materials and methods

A series of *L. esculentum* cultivars and lines, among which were isogenic line gf (green fruit in cv 'Ailsa Craig'), *L. chilense* LA 460 and *L. peruvianum* var 'humifusum' PI 127829, were included in remote hybridization. The crosses were made in a greenhouse. About 300–600 emasculated flower buds from *L. esculentum*

Communicated by M. Koornneef

were pollinated with pollen from *L. chilense* and *L. peruvianum* var 'humifusum'.

The line Cm 180 was developed from the three-genome hybrid after six consecutive backcrosses with *L. esculentum* (cv 'Drujba'); its pedigree is shown in Fig. 1. The line 'Okitso Sozai 1–20' originates from *L. hirsutum* f. *glabratum* and *L. esculentum* (Kuryama and Kuniyssu 1974). Both lines are resistant to *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). The susceptible cv 'Roma' and Rick's susceptible lines with morphological markers for all of the tomato chromosomes except chromosomes 2 and 5 were used in hybridization experiments with Cm 1180. The marker lines with the respective markers indicated are:

LA 1491: chromosome 1 – scurfy (*scf*), diageotropica (*dgt*)
chromosome 8 – sparsa (*spa*), anthocyanin loser (*al*)
LA 1182: chromosome 3 – sunny (*sy*), solanifolia (*sf*)
chromosome 12 – albescent (*alb*), multifurcata (*mua*)
LA 780: chromosome 6 – yellow virescent (*yv*), potato leaf (*c*)
chromosome 10 – hairs absent (*h*), anthocyanin gainer (*ag*)
LA 1164: chromosome 7 – variabilis (*var*), notabilis (*not*)
chromosome 9 – marmorata (*marm*), Hoffman's anthocyanin-less (*ah*)
LA 784: chromosome 4 – fulgens (*ful*), entire (*e*)
chromosome 11 – hairless (*hl*), anthocyaninless (*a*)
LA 886: chromosome 4 – fulgens (*ful*), entire (*e*)

The F_1, F_2 and BC_1 progenies of the hybrid Cm 180 × cv 'Roma' and F_1 and F_2 progenies of the hybrid Cm 180 × 'Okitso Sozai' were studied for the genetic relationship between *Cmm* resistance in Cm 180 and 'Okitso Sozai'. The map position of the gene conferring resistance to *Cmm* was derived from F_2 and BC_1 progenies of the hybrids of Cm 180 with the different marker lines.

Pollen viability was assayed by staining pollen with 1% acetocarmine.

The testing for resistance to *Cmm* was performed under greenhouse conditions. The inoculation was carried out at the third to fourth true leaf phase with a suspension prepared from 36- to 48-h-old cultures of *Cmm* at a concentration of 10^8 cfu/ml after the method of De Jong and Honma (1976). The inoculated plants were grown under conditions favouring the development of the bacterium: a temperature of 24 °C and a relative humidity of 80%. Symptom expression was recorded using the classification of Laterrot (1974): 1 – no symptoms of the disease; 3 – one wilted leaf or several wilted leaflets; 5 – several wilted leaves; 7 – 50–80% of the leaves have wilted; 9 – 90% of the leaves have wilted and the plant is dying. Plants classified as 1 and 3 are taken as being resistant; those classified as 5, 7 and 9, as being susceptible.

Screening and selection of lines for resistance to Tomato mosaic virus (ToMV), *Pseudompnas syringae* pv. tomato race 0 and race 1 (Pst), *Fusarium oxysporum* f. sp. *lycopersici* race 1 and race 2 (F), *Verticillium dahliae* (Ve), *Oidium lycopersici*, *Meloidogyne incognita* and White fly (*Trialeurodes vaporariorum*) was started in the F_2 of BC_3. The inoculation and disease scoring was carried out using specific methods for the given disease (Cirulli 1974; Vito and Lamberti 1978; Sotirova and Georgiev 1981; Stamova 1987; Bogatsevska 1988).

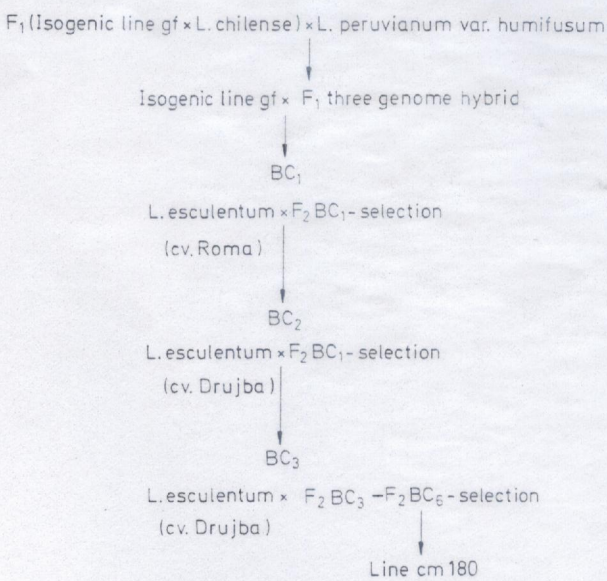

Fig. 1. The pedigree of line Cm 180

Results

After all our efforts for obtaining a direct crossing between *L. esculentum* and *L. peruvianum* var 'humifusum' failed we used *L. chilense* as a bridge species. The hybrid F_1 (isogenic line gf × *L. chilense*) was crossed with *L. peruvianum* var 'humifusum; and 4 plants were produced that differed from one another in phenotypic characteristics. However, all had yellow fruits and the typical aroma and ripe fruit abscission of *L. peruvianum* var 'humifusum'. The percentage of acetocarmine-stained pollen ranged from 44 to 84. The backcrosses to line gf was successful only when the gf line was used as a female parent, in which case 5 BC_1 plants were produced. The percentage of acetocarmine-stained pollen varied from 55 to 83. All 5 plants were self compatible. It was possible to cross BC_1 plants only as male parents with *L. esculentum*, and only as female parents with both *L. chilense* and *L. peruvianum* var 'humifusum'. The data are presented in Table 1.

Seeds with normal germination were produced after selfing of the 5 BC_1 plants, and 35 F_2 BC_1 plants were grown. The percentage of acetocarmine-stained pollen in individual plants varied from 27 to 70. Under conditions of spontaneous selfing in a greenhouse all plants with high pollen viability had fruits with normal seed set.

Line Cm 180 was obtained after a series of backcrosses with *L. esculentum* cv 'Drujba' combined with selection in each back-cross generation.

Genetic analysis of the resistance to Clavibacter michiganensis subsp. michiganensis found in line Cm-180

The hybrid F_1 (Cm 180 × 'Roma') was resistant to *Cmm*. In the F_2 generation the ratio of resistant:suscep-

Table 1. Reproductive relationship of three-genome hybrid [(F_1 isogenic gf line × *L. chilense*) × *L. peruvianum* var 'humifusum']

Hybrid combinations and controls	Number of pollinated flowers	Number of fruits having seeds	Total number of seeds
F_1 (gf × *L. chilense*) × *L. peruvianum* var humifusum	283	4	4
Line gf Ailsa Craig × F_1 [(gf × *L. chilense*) × *L. peruvianum* var humifusum]	50	5	9
BC_1 – selfing	130	71	1682
Line gf-Alisa Craig × BC_1	60	16	89
BC_1 × *L. chilense*	24	4	4
BC_1 × *L. peruvianum* var humifusum	110	7	102
Line gf-Ailsa Craig	10	10	145
L. chilense	10	10	94
L. peruvianum var humifusum	10	10	132

Table 2. Segregation in F_2 and BC_1 – resistant:susceptible

Combinations and controls	Segregation[a] R^+:S	Expected ratio	χ^2	$P >$
F_2 (Roma × Cm 180)	81: 27	3:1	0.0	0.05
BC_1 (Roma × Cm 180) × Roma	79:101	1:1	2.69	0.05
BC_1 (LA 1182 × Cm 180) × LA 1182	174:156	1:1	0.98	0.05
BC_1 (LA 1491 × Cm 180) × LA 1491	166:148	1:1	1.03	0.05
Cm 180	148 –	1:0	–	–
Roma	– 85	0:1	–	–
LA 1182	– 70	0:1	–	–
LA 1491	– 120	0:1	–	–

[a] $^+$R, Resistant; S, susceptible

tible plants was close to 3:1; in the BC_1, 1:1 (Table 2). These results show that resistance was determined by dominant alleles at one locus, which we designated by the symbol "*Cm*". However, modifier genes must have an influence on the action of *Cm* since the group of resistant plants were not uniform in their resistance and included plants classified as 1 and 3. We cannot answer the question of whether *Cm* originated from the genome of *L. chilense* or from the genome of *L. peruvianum* var 'humifusum' since both wild species are resistant to *Cmm*. The action of the *Cm* gene was stable at temperatures ranging from 25 °–28 °C; temperatures above 30 °C reduced the number of resistant plants.

All plants of the hybrid of Cm 180 × 'Okitso Sozai 1-20' were resistant to *Cmm*. In the resulting F_2 generation 27 % ($n = 240$) of the plants observed were susceptible, indicating that resistance in both lines is controlled by different genes.

Of the 12 tomato chromosomes 10 were investigated for linkage of the *Cm* gene. After F_1 plants had been selfed, individuals of each F_2 population were scored for marker segregation and susceptibility to the pathogen. The ratio between the phenotypical groups was close to 9:3:3:1, and chi-square analysis showed that there was no significant linkage between *Cm* and the marker genes (Table 3). However, segregating populations derived from crosses of Cm 180 with LA 784 and LA 886, the *Cm* locus demonstrated significant linkage with the chromosome 4 markers *ful* and *e* (Table 4). The estimates of recombination percentages shown in Table 4 indicate that the *Cm* gene is located between *ful* and *e* approximately 12 cM from *ful*.

Lines resistant to economically important diseases and pests developed on the basis of the three-genome hybrid L. esculentum-L. chilense-L. peruvianum var 'humifususm'

Introgressive hybridization with *L. esculentum* accompanied by constant selection for resistance to disease resulted in the development of 23 F_4 lines. All of these lines were tested for resistance to the pathogens mentioned in the Material and methods, and most were found to be resistant (R) or moderately resistant (MR). Single lines were susceptible (S). The greatest number of lines were resistant to *Cmm* and to *M. incognita*: of the 23 lines tested 14 were resistant and only 1 was susceptible (Table 5). Most of the lines were moderately resistant to the remaining pathogens. Resistance to *Oidium lycopersici* and White fly was shown by the least

Table 3. F_2 segregation of Cm and marker genes located on 9 different tomato chromosomes

Marker lines	Chromosomes	Genes symbols	Segregation[a]				χ^2 association	$P >$
			$^+$RM	rM	Rm	rm$^+$		
LA 1491	1	*dgt*	54	18	18	6	0	0.05
		scf	48	18	24	6	0.58	0.05
LA 1182	3	*sy*	170	59	48	23	1.20	0.05
		sf	167	52	51	18	0.15	0.05
LA 780	6	*yv*	111	30	42	18	0.19	0.05
		c	126	35	27	13	2.04	0.05
LA 1164	7	*var*	110	47	36	13	0.23	0.05
		not	116	42	41	13	0.13	0.05
LA 1491	8	*spa*	51	18	23	5	0.71	0.05
		al	63	16	10	7	3.29	0.05
LA 1164	9	*ah*	134	30	39	13	1.11	0.05
		marm	109	41	52	14	0.90	0.05
LA 780	10	*h*	123	33	29	16	3.88	0.025
		ag	116	28	36	21	4.94	0.025
LA 784	11	*hl*	171	46	42	21	3.91	0.025
		a	178	49	35	18	3.57	0.05
LA 1182	12	*alb*	136	49	82	26	0.21	0.05
		mua	171	51	47	24	3.28	0.05

[a] $^+$RM, Resistant without the marker phenotype; rM, susceptible without the marker phenotype; Rm, resistant with the marker phenotype; rm, susceptible with the marker phenotype

Table 4. Phenotype segregation and recombination percentage calculated between Cm and genes fulgens (*ful*) and entire (*e*) on chromosome 4

Combination	Genes	Number of plants[a]					Recombination percentage ± SD
		$^+$RM	rM	Rm	rm$^+$		
BC (Cm 180 × LA 784) × LA 784	*Cm-ful*	71	10	8	39	128	14.1 ± 3.1
F_2 (Cm 180 × LA 784)		158	8	16	38	220	11.4 ± 2.3
BC (Cm 180 × LA 886) × LA 886		85	13	6	62	166	11.4 ± 2.5
BC (Cm 180 × LA 784) × LA 784	*Cm-e*	51	9	28	40	128	28.9 ± 4.0
F_2 (Cm 180 × LA 784)		131	33	23	33	220	27.9 ± 3.7[c]
BC (Cm 180 × LA 886) × LA 886		67	13	34	52	166	28.3 ± 3.5
		Ful E	ful E	Ful e	ful e[b]		
BC (Cm 180 × LA 784) × LA 784	*ful-e*	47	13	33	35	128	35.9 ± 4.2
F_2 (Cm 180 × LA 784)		136	27	45	12	220	45.9 ± 4.8[c]
BC (Cm 180 × LA 886) × LA 886		63	17	34	52	166	30.7 ± 3.6

[a] $^+$RM, Resistant without the marker phenotype; rM, susceptible without the marker phenotype; Rm, resistant with the marker phenotype; rm susceptible with the marker phenotype

[b] *Ful E*, Dominant genes; *ful e*, marker genes

[c] Estimated with the Product Ratio method (Stevens 1939)

number of lines. Lines LCH 50, LCH 62, LCH 108 and, LCH 174 are distinguished by abundant pollen production and a high percentage of fruit set under unfavourable climatic conditions. Their fruits are small and two-loculed. Four lines, LCH 90, LCH 91, LCH 93 and LCH 94, are distinguished by larger fruits with excellent flavour quality.

Discussion

The three-genome hybrid F_1 (L. esculentum-L. chilense-L. peruvianum var 'humifusum')

L. chilense could serve as a bridge between *L. esculentum* and *L. peruvianum* var 'humifusum' due to its closer

Table 5. Resistance of F_4 lines derived from BC_3 genotypes of the three-genome hybrid

Lines	Resistance/susceptibility to pathogens									
	TMoV	Cmm	Pst Race		F Race		*Ve*	*O. lycopersici*	*M. incognita*	White fly
			0	1	1	2				
LCH 36/1	R	R	R	MR	R	R	R	R	R	R
LCH 36/3	R	R	R	MR	R	R	R	R	R	R
LCH 36/5	R	R	R	MR	R	R	R	R	R	R
LCH 44/11	MR	R	MR	MR	R	R	R	MR	R	MR
LCH 44/17	MR	R	MR	MR	R	MR	MR	MR	R	MR
LCH 47	MR	R	MR	MR	MR	S	MR	MR	R	MR
LCH 48	S	S	S	S	S	S	S	S	S	S
LCH 50	MR	R	R	R	MR	S	MR	S	R	MR
LCH 54	MR	R	R	R	MR	S	MR	S	R	MR
LCH 58	S	MR	S	S	MR	S	MR	S	MR	S
LCH 60	MR	S	S	S	MR	MR	MR	S	MR	MR
LCH 62	MR	R	MR	S	MR	S	MR	S	R	S
LCH 90	S	MR	S	S	S	S	S	S	MR	R
LCH 91	S	MR	S	S	MR	S	MR	S	MR	S
LCH 93	S	MR	S	S	MR	S	MR	S	MR	S
LCH 94	S	MR	MR	MR	MR	S	MR	S	MR	S
LCH 101	MR	MR	MR	MR	MR	MR	MR	S	MR	S
LCH 106	R	R	R	R	R	MR	MR	S	R	S
LCH 108/3	MR	R	MR	MR	MR	S	MR	MR	R	MR
LCH 108/4	MR	R	MR	MR	MR	S	MR	MR	R	MR
LCH 174	R	R	MR	R	R	R	R	S	R	R
LCH 286	R	R	R	MR	R	R	R	R	R	R
LCH 287	R	R	R	MR	R	R	R	R	R	R
Drujba	S	S	S	S	S	S	S	S	S	S

R, Resistant; S, susceptible; MR, moderately resistant

relationship to the two species. The three-genome hybrid F_1 (line gf × *L. chilense*) × *L. peruvianum* var 'humifusum' was crossed both with the isogenic line gf (green fruit) and with *L. peruvianum* var 'humifusum'. With respect to the reproductive relationships of BC_1 one interesting characteristic must be pointed out: the 5 BC_1F_1 plants were all self-compatible. The fact that only self-compatible plants were obtained could be due to the preference for male generative cells that the genes for self-compatibility (Hogenboom 1979).

Genetic study of the resistance to Clavibacter michiganensis subsp. michiganensis in line Cm-180

Resistance to *Cmm* has been studied earlier in 'Bulgaria 12' and in the species *L. pimpinellifolium* that took part in the development of the Cm 180 line. According to Laterrot (1974) the resistance is monofactorial in both cases. According to Thyr (1976) both in *L. pimpinellifolium* and in 'Bulgaria 12' resistance is controlled by one to four partially dominant alleles, while according to De Jong and Honma (1976), by one recessive and three dominant genes. The resistance in line 'Okitso Sozai 1-20' is believed to be controlled by one major gene plus modifier genes (Laterrot and Rat 1987). The investigation of Sotirova and Beleva (1978) showed that the mode of inheritance of the resistance to *Cmm* depends on the sources used. In hybrids with *L. pimpinellifolium* the resistance is inherited with partial dominance in the direction of the susceptible parent, whereas in hybrids with *L. hirsutum*, partial dominance is in the direction of the resistant parent. Stamova (1987) studied lines carrying germ plasm from *L. chilense* and found that the resistance to *Cmm* is dominantly inherited. According to Lindhout (1989) resistance in *L. peruvianum* var 'humifusum LA 2151' is controlled by two or three recessive genes.

Our results show that the resistance in our material is controlled by a dominant allele at one locus. We believe that the effect of modifier genes on the major gene *Cm* influences the degree of the resistance.

The test for allelism between Cm 180 and 'Okitso Sozai 1-20' showed that the genes for resistance of these two lines are different. The gene *Cm* conferring resistance to *Cmm* in line Cm 180 is located on chromosome 4 between the markers *ful* and *e*.

For the first time the germ plasm of *L. peruvianum* var 'humifusum' in combination with *L. chilense* has been transferred into *L. esculentum*. The participation of two wild species as donors of genes for resistance enabled us to commence on a large breeding programme in which 23 different lines were studied for

resistance to some pathogens. The testing of selected lines from the three-genome hybrid showed that some of them are distinguishable by different combinations to disease resistance. Lines LCH 174, LCH 286 and LCH 287 are of particular interest because they possess complex resistance. Lines LCH 44, LCH 47, LCH 50, LCH 54, LCH 62 and LCH 106 deserve attention because they are moderately resistant and promise satisfactory durability. Botev (1986) reported resistance of progenies from a three-genome hybrid to *F. oxysporum* f. sp. *lycopersici* race 1 and 2. The lines which present the greatest promise will be included in breeding programmes for the development of F_1 hybrids with complex resistance to diseases and pests.

References

Bogatzevska N (1988) *Pseudomonas syringae* pv. tomato the pathogen causing bacterial speck of tomatoes. Plant Sci 7:25 91–96

Botev A (1986) Evaluation of genus *Lycopersicon* for breeding of resistance to *Fusarium oxysporum* (Schlecht) f. sp. *lycopersici* (Sacc) Synd & Hans. PhD thesis, University of Plovdiv

Cirulli M (1974) Resistenza del pomodoro verso le malattie. Genet Agric 28:317–356

De Jong, Honma S (1976) Evaluation of screening techniques and determination of criteria for assessing resistance to *Corynebacterium michiganense* in tomato. Euphytica 25: 405–414

De Jong, Honma S (1976) Inheritance of resistance to *Corynebacterium michiganense* in tomato. J Hered 67:79–84

Hassan AA, Strider DL, Konsler TL (1968) Application of cotyledonory symptoms in screening for resistance of tomato to bacterial canker and host range studied. Phytopathology 58:233–239

Heinz J (1972) Notice of release of bacterial canker resistant tomato breeding line H-2990. US Dep Agric Commun No.

Hogenboom NG (1979) Incompatibility and incongruity in *Lycopersicon*. In: Hawkes JG, Lester RN, Skelding AD (eds) The biology and taxonomy of the *Solanaceae*. Academic Press, London, pp 435–444

Kuriyama T, Kuniyssu K (1974) Studies on the breeding of disease resistant tomato by interspecific hybridization. III. On the breeding of a new tomato line resistant to bacterial canker by *Corynebacterium michiganense*. Bull Ornamental Groups Res Sta Jpn Ser A 2:105–107

Laterrot H (1974) Value of the resistance of the tomato Bulgaria 12 to *Corynebacterium michiganense* (Smith) Jensen. In: Eucarpia tomato working group. Bari

Laterrot H, Rat B (1987) Stability of the resistance to *Corynebacterium michiganense* of the tomato line 'Okitso Sozai 1-20'. In: Modern trends in tomato genetics and breeding (Eucarpia tomato working group). Salerno

Lindhout P (1987) Resistance against *Corynebacterium michiganense* found in *Lycopersicon peruvianum*. In: Modern trends in tomato genetics and breeding (Eucarpia tomato working group). Salerno

Lindhout P (1989) Investigation of tomato breeding for resistance to *Corynebacterium michiganense*. In: XII Eucarpia Congr. Göttingen

Rick CM (1963) Barriers to interbreeding in *Lycopersicon peruvianum*. Evolution 17:216–232

Rick CM (1979) Biosystematic studies in *Lycopersicon* and closely related species of *Solanum*. In: Hawkes JG, Lester RN, Skelding AD (eds) The biology and taxonomy of the Solanaceae. Academic Press, London. pp 667–677

Rick CM, Lamm R (1955) Biosystematic studies on the status of *Lycopersicon chilense*. Am J Bot 44:663–675

Sotirova V, Beleva L (1977) Resistance of wild species tomatoes to *Corynebacterium michiganense* (Smith) Jensen. Hortic Vitic Sci 14:63–71

Sotirova V, Beleva L (1978) Resistance of interspecific tomato hybrids to *Corynebacterium michiganense* (Smith) Jensen. In: Eucarpia of tomato working group. Leningrad

Sotirova V, Bogatzevska N (1988) New sources of resistance to *Pseudomonas syringae* pv. tomato (Okabe) Young et al. CR Acad Bulg sci T 41:1541–1543

Sotirova V, Georgiev Chr (1981) Resistance of wild species of varying origins to White greenhouse fly (*Trialeurodes vaporariorum* West). Genet Plant Breed 14:372–378

Stamova L (1987) Breeding of resistance in tomato (*Lycopersicon esculentum* Mill.). PhD thesis, University of Plovdiv

Stevens WL (1939) Tables of the recombination fraction estimated from the product ratio. J Genet 39:171–180

Thyr BD (1969) Additional sources of resistance to Bacterial canker of tomato. Plant Dis Rep 53:234–237

Thyr BD (1976) Inheritance of resistance to *Corynebacterium michiganense* in tomato. Phytopathology 66:1116–1119

Vito M, Lamberti F (1978) Resistance of tomato varieties and hybrids to italian population of *Meloidogyne incognita*. In: Eucarpia of tomato working group. Leningrad

Theor Appl Genet (1993) 87:343–346

THEORETICAL AND APPLIED GENETICS

Screening of pollen grains vis-à-vis whole plants of oilseed brassicas for tolerance to salt

R. K. Tyagi *, N. S. Rangaswamy

Department of Botany, University of Delhi, Delhi 110 007, India

Received: 2 September 1992 / Accepted: 20 April 1993

Abstract. Pollen grains and whole plants of 11 cultivars of oilseed brassicas (*B. juncea*, *B. campestris*, *B. carinata*) were screened for salt tolerance. Whereas pollen germination percentage in sitting drop cultures served as a reliable index of pollen tolerance to NaCl, pollen-tube growth did not. Seed yield in plants of the same 11 cultivars raised in artificially salinized soils also proved to be a good index of whole plant tolerance to soil salinity. A close correspondence between pollen (gametophyte) and whole plant (sporophyte) responses to salinity was discovered. Our studies show that tolerance to salt is yet another trait expressed in both the sporophyte and gametophyte.

Key words: Oilseed brassicas – Tolerance to salt – Pollen germination as index – Comparison with seed yield

Introduction

The use of pollen grains in place of whole plants as a screening system is based on reports that more than 60% of the genes expressed in an individual of a species are also expressed in its pollen grains (Tanksley et al. 1981; Willing and Mascarenhas 1984). The screening of whole plants of a species for desirable traits would demand a greater infrastructural input (space, time and labor) than the screening of pollen grains. Also, as individual plants produce a large quantity of pollen grains, a variety of factors can be tested on pollen produced by a given genotype. Pollen grains of certain taxa have been used in studies on tolerance to ozone, patho- and phyto-toxins, herbicides, heavy metals, salinity, and low and high temperatures (Ottaviano and Mulcahy 1989; Searcy and Mulcahy 1990; Hormaza and Herrero 1992; Shivanna and Sawhney 1993). Some of the tolerance traits are expressed in both the gametophyte and sporophyte.

We present results from the screening of pollen grains of 11 cultivars of oilseed brassicas for tolerance to NaCl and report the comparative response of whole plants grown in artificially salinized soils.

Materials and methods

Three cultivars of *Brassica juncea* Coss. and Czern. ('Krishna', 'Pusa Barani', and 'Pusa Bold'), 2 each of *B. campestris* L. var 'brown sarson' ('DBS-1' and 'Pusa Kalyani') and var 'yellow sarson' ('DYS-3' and 'YST-151'), and 4 of *B. carinata* A. Braun ('BICRIDA-169', 'BICRIDA-172', 'Carinata-337', and Carinata-435') were studied. Plants of all 11 cultivars were raised in the field from seeds procured from the Division of Genetics, Indian Agricultural Research Institute, New Delhi.

Tolerance of pollen to NaCl

Pollen grains from several anthers (that had been allowed to dehisce in the laboratory under a 40-W incandescent lamp) were cultured in vitro in basal medium of Roberts et al. (1983). Sitting drop cultures (Shivanna and Rangaswamy 1992) were raised; three concentrations of NaCl (4, 6, 8 dS/m[1]) were tested. For each treatment either four or six cultures of each cultivar were raised on different occasions. The cultures were maintained at 20° ± 2 °C under continuous laboratory light for 4 h, at the end of which the cultures were terminated by adding a drop of 1%

* On study leave from the National Bureau of Plant Genetic Resources, New Delhi

Communicated by G. S. Khush

Correspondence to: N. S. Rangaswamy

[1] dS/m = m mhos/cm; 1 dS/m NaCl ≃ 0.05% NaCl