J. Appl. Genet. 42(1), 2001, pp. 33-42

Towards the introducing of resistance to powdery mildew from *Lycopersicon hirsutum* into *L. esculentum*

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Abstract. Genes of resistance to *Oidium lycopersicum* from *Lycopersicon hirsutum* LA 1775 were introduced to *L. esculentum*. Breeding procedures were based on a one-way programme up to the F_2 generation and then four different methods were adopted to obtain F_4 and BC_4 populations. Screening tests among those hybrid populations were performed in a greenhouse and showed segregation for resistance to powdery mildew due to different genetic backgrounds of the families derived from four breeding methods that changed the status of the gene/genes responsible for resistance to powdery mildew. F_4 and BC_4 populations varied in relation to morphological traits (fruit size and weight, seed and fruit productivity, number of locules). There was a significant progress in breeding in comparison to *L. hirsutum* regarding fruit size and weight, and the number of locules. Values of two other traits: seed and fruit productivity, that are correlated with self- and cross-compatibility, were low and similar to *L. hirsutum*. Therefore, another one or two backcrosses will probably improve seed and fruit productivity.

Key words: *Lycopersicon hirsutum*, *Lycopersicon esculentum*, *Oidium lycopersicum*, resistance.

Introduction

Interspecific hybridisation within the genus *Lycopersicon* is an efficient breeding method, whereby genes of resistance to plant diseases, pests, and adverse environmental conditions can be transferred to the cultivated tomato. Among wild tomato species, *L. hirsutum* is an excellent breeding source, since it carries many useful traits, including resistance to powdery mildew caused by *Oidium lycopersicum*

Received: July 31, 2000. Accepted: December 5, 2000.
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Cooke & Massee, emend. Nordeloos & Loerakker (LINDHOUT et al. 1994, LATERROT et al. 1995, KOZIK 1999).

The resistance of *L. hirsutum* (accession G1.1560) to *O. lycopersicum* is controlled by an incompletely dominant gene *Ol-1* (VAN DER BEEK et al. 1994). The inheritance of resistance in another accession, such as *L. hirsutum* LA 1775, suggested two incompletely dominant genes with one major gene having a stronger expression than the second minor gene (KOZIK 1999). These genes were transferred with backcross pedigree methods which was expected to provide a way of improving the variety A 241 that excels in a large number of attributes but is deficient in resistance to powdery mildew.

In this study, a strain of *O. lycopersicum* was used to study the genetic control of resistance/susceptibility in individual F_4 and BC_4 hybrid populations of tomato from a cross between *L. esculentum* breeding line A 241 and wild species *L. hirsutum* LA 1775. The results of evaluation of those hybrid populations with regard to several morphological characteristics are also discussed.

Material and methods

Individual F_4 and BC_4 families from a cross between *L. esculentum* line A 241 and the accession *L. hirsutum* LA 1775 were produced at the Research Institute of Vegetable Crops at Skierniewice, Poland. *L. hirsutum* LA 1775, carrying resistance to powdery mildew, was used as the male and donor parent. A fresh market tomato, *L. esculentum* breeding line A 241 with good morphological traits and good combining ability, was used as the recurrent parent. Procedures of backcross pedigree breeding were based on a one-way program up to the F_2 generation and then four different methods were adopted to obtain F_4 and BC_4 at the end (Table 1).

Seeds of the F_4 (BC_3 × self, F_3 × self) and BC_4 ($BC_3 \times P_1$, $F_3 \times P_1$) populations, from the initial crosses between the resistant accession and susceptible parent, were produced by controlled pollinations in the greenhouse.

Table 1. Pedigrees of four groups of studied hybrid populations from a cross between *L. esculentum* line A 241 and *L. hirsutum*

Method 1	Method 2	Method 3	Method 4
	A 241 × *L. hirsutum*		
	BC_1 ($F_1 \times P_1$)		
	BC_2 ($BC_1 \times P_1$)		
	F_2 (BC_2 × self)		
F_3 (F_2 × self)	BC_3 ($F_2 \times P_1$)	F_3 (F_2 × self)	BC_3 ($F_2 \times P_1$)
BC_3 ($F_3 \times P_1$)	F_3 (BC_3 × self)	BC_3 (F_3 × self)	F_3 (BC_3 × self)
F_4 (BC_3 × self)	F_4 (F_3 × self)	BC_4 ($BC_3 \times P_1$)	BC_4 ($F_3 \times P_1$)

Screening for powdery mildew resistance

All F_4 and BC_4 plants were grown in pots and maintained under greenhouse conditions. The plants were inoculated with conidia brushed off from heavily infected tomato leaves. The plants were inoculated twice: at the second true leaf stage, and 3-4 days later. To increase humidity (up to 100%) the plants were kept for four days after the second inoculation under a low plastic tunnel with perforated film. After removal of the tunnel, humidity was kept at approximately 75%. Temperatures were set at 24/20°C day/night.

Plants were rated for disease symptoms three weeks after the second inoculation. Individual plants were visually evaluated for disease severity. The following classes were distinguished: 0 = no visible symptoms, 1 = few and small powdery dots, 2 = up to 25% of infected young leaves and lack of expansion, 3 = fungal growth luxuriant, up to 50% of infected older leaves, 4 = fungal growth luxuriant, more than 50% of infected older leaves, new colonies spreading up to the top of plants.

Plants from class 0 were considered resistant (R), from 1 and 2 were intermediate (I) because of the very low sporulation and lack of expansion, and from classes 3 and 4 were susceptible (S). Data from these three categories of F_4 and BC_4 populations were processed with STATISTICA 5.0 software for goodness-of-fit to theoretical ratios using the χ^2 test.

Morphological evaluation

After the selection for resistance to powdery mildew, plants were transplanted into 10-liter plastic rings under greenhouse conditions. The experiment was set up according to a complete randomised block design with 9 to 10 blocks containing one plant per population. Plants were headed above the fifth or sixth truss.

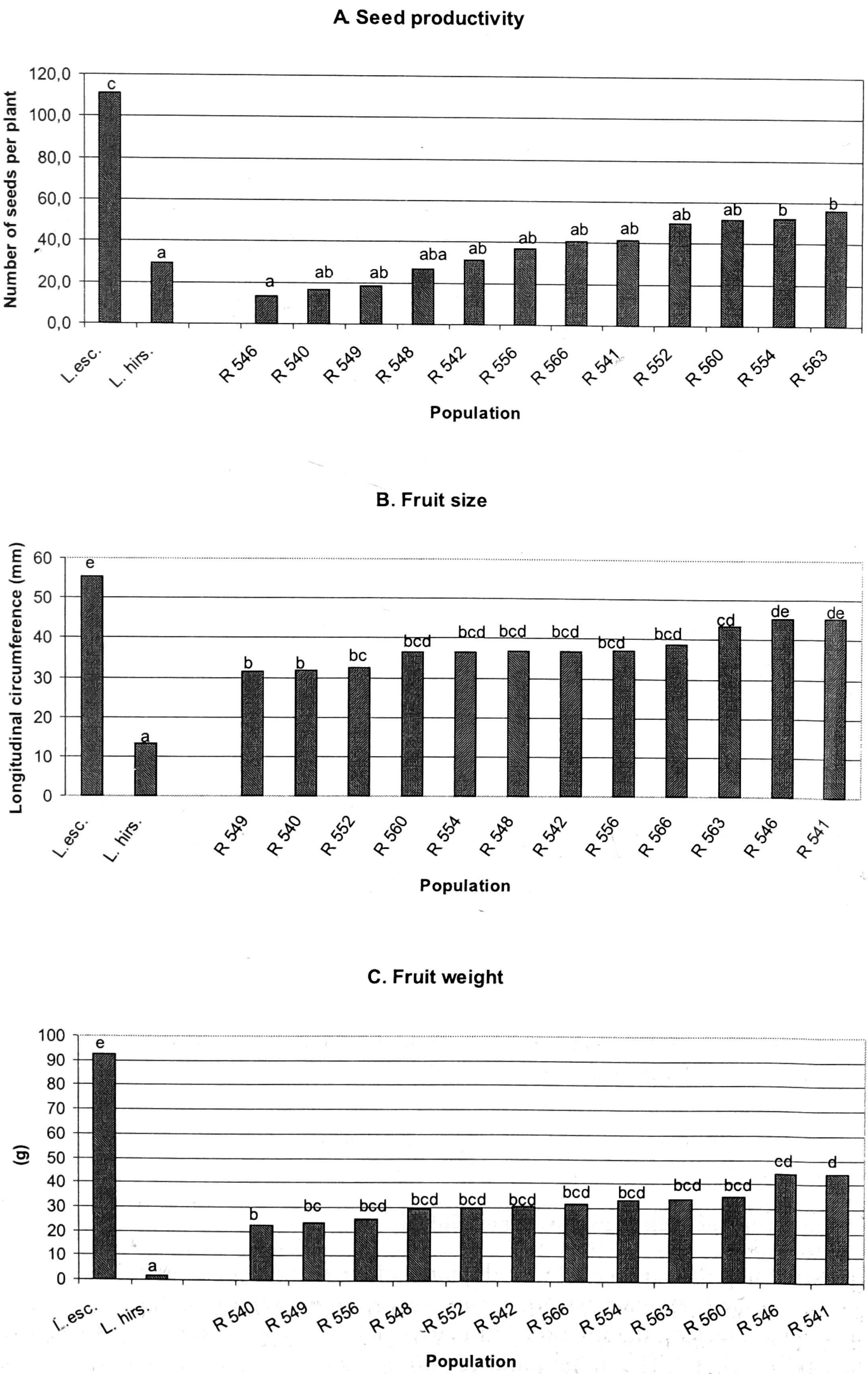
Different morphological traits were evaluated in the interspecific hybrids: the number of seeds per fruit, fruit weight, number of locules, number of fruits per plant, and fruit size (longitudinal circumference).

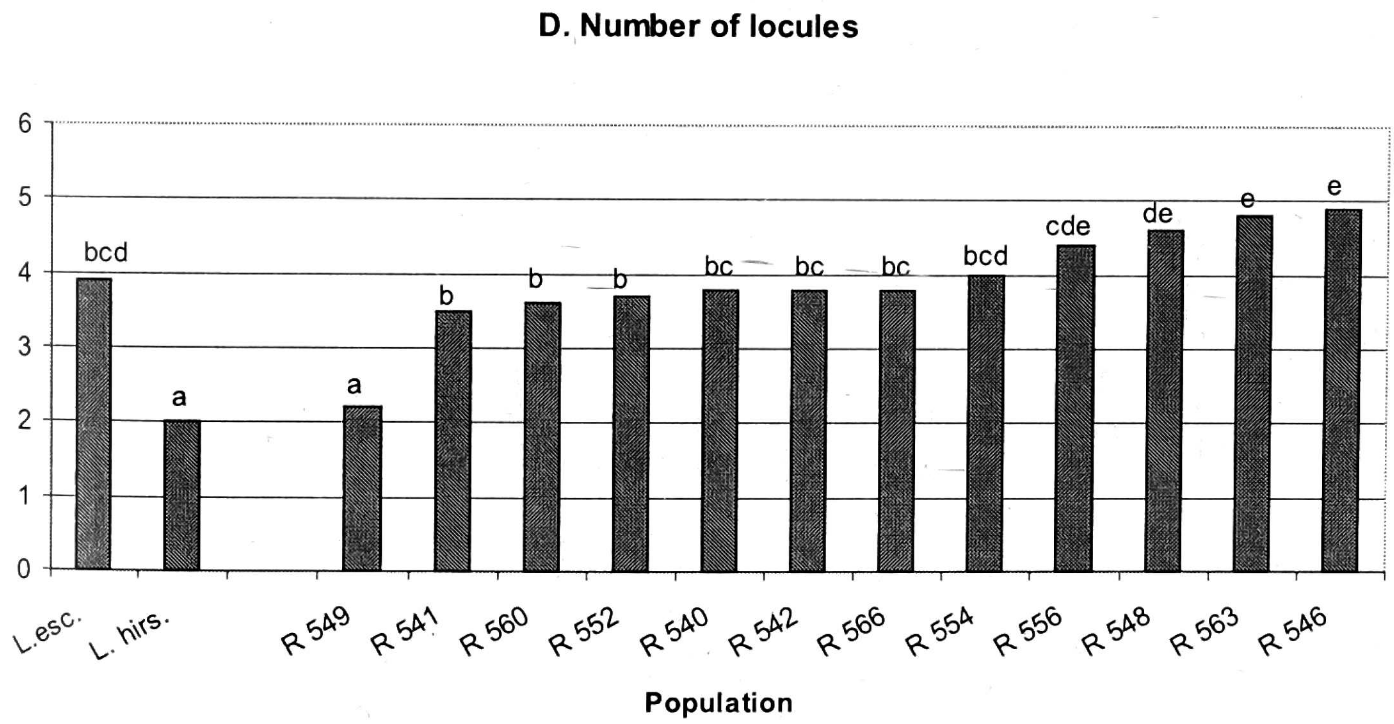
The results were elaborated statistically by an analysis of variance (ANOVA) and the significance of differences between populations was evaluated by Student's *t* test at P = 0.05. The number of fruits per plant was analysed on data transformed to square root functions.

Results

Variation in resistance to powdery mildew

The screening tests indicated that all plants of the recurrent parent A 241 were severely diseased and were assigned to the susceptible category (Table 2). This indicates that the inoculation procedure was able to infect 100% of the known susceptible plants. The donor parent *L. hirsutum* LA 1775 was resistant, with no symptoms on both leaves and stems.


A. Seed productivity
Number of seeds per plant
120,0
100,0
80,0
60,0
40,0
20,0
0,0
c
a
a
ab
ab
aba
ab
ab
ab
ab
ab
ab
b
b
L.esc.
L. hirs.
R 546
R 540
R 549
R 548
R 542
R 556
R 566
R 541
R 552
R 560
R 554
R 563
Population
B. Fruit size
Longitudinal circumference (mm)
60
50
40
30
20
10
0
e
a
b
b
bc
bcd
bcd
bcd
bcd
bcd
bcd
cd
de
de
L.esc.
L. hirs.
R 549
R 540
R 552
R 560
R 554
R 548
R 542
R 556
R 566
R 563
R 546
R 541
Population
C. Fruit weight
(g)
100
90
80
70
60
50
40
30
20
10
0
e
a
b
bc
bcd
bcd
bcd
bcd
bcd
bcd
bcd
bcd
cd
d
L.esc.
L. hirs.
R 540
R 549
R 556
R 548
R 552
R 542
R 566
R 554
R 563
R 560
R 546
R 541
Population



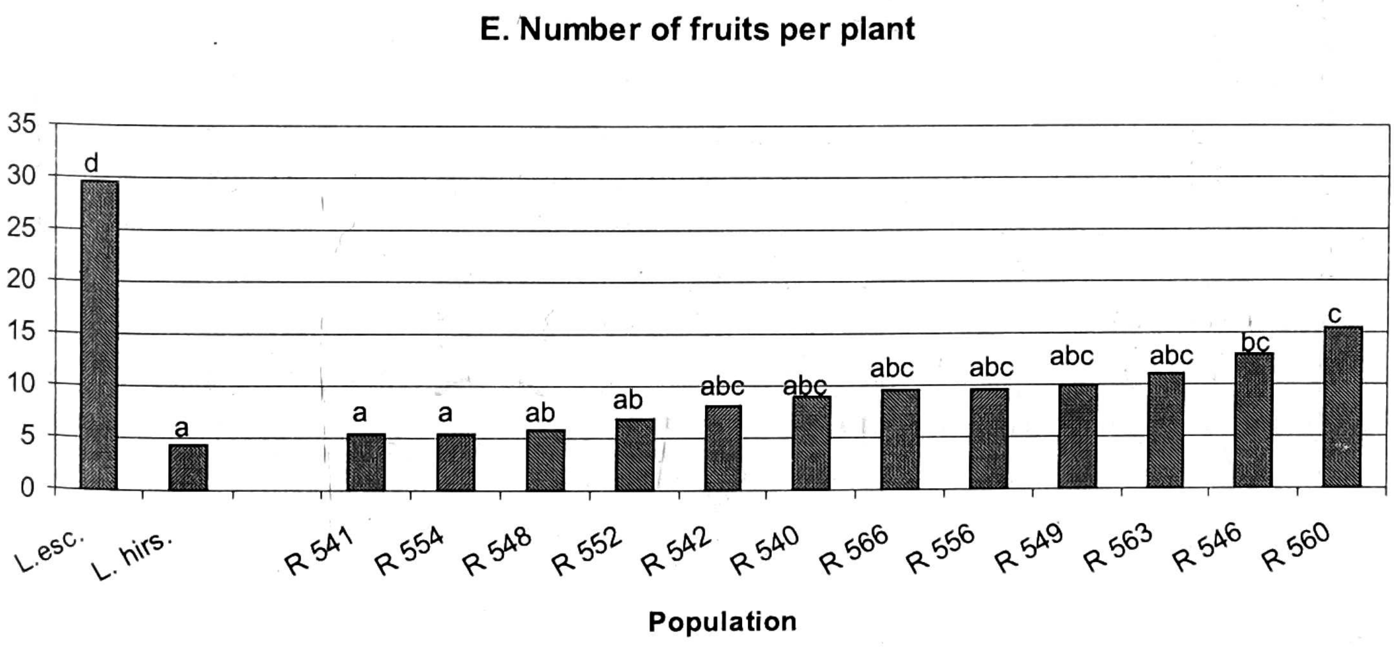


Figure 1. Morphological traits of hybrids derived from *L. esculentum* × *L. hirsutum*
Bars marked with the same letters do not differ significantly (P = 0.05) from each other

The pooled F_4 population within method 1 fell into three categories (Table 2). There were 38 healthy plants, 53 partially infected plants and 49 heavily infected plants. These data indicate a ratio of 4 resistant : 7 intermediate : 5 susceptible with a probability of 0.368 (pooled $\chi^2 = 1.998$). This ratio suggests that the previous generation BC_3, before selfing, was probably heterozygous for both resistant genes. Almost all plants of the F_4 population derived from method 2 were resistant. There were only five plants with intermediate level of resistance (class 1 and 2). All the families derived from method 3 (R 554, R 555, R 556) segregated

Table 2. Resistance reactions to *Oidium lycopersicum* of hybrid populations derived from *L. esculentum* × *L. hirsutum*

Generation/line	Number of plants				χ^2	df	P
	R	I	S	Total			
1	2	3	4	5	6	7	8
			Method 1				
$F_4/BC_3/F_3/F_2/BC_2$							
R 540	17	14	14	45	4.60	2	0.1003
R 541	12	19	15	46	0.11	2	0.9465
R 542	9	20	20	49	2.29	2	0.3182
Expected ratio	4	7	5				
Total					7.00	6	
Pooled	38	53	49	140	1.998	2	0.368
Heterogeneity					5.002	4	0.287
			Method 2				
$F_4/F_3/BC_3/F_2/BC_2$							
R 546	28	3	0	31			
R 548	51	0	0	51			
R 549	50	0	0	50			
R 552	64	2	0	66			
Expected ratio	all resistant						
Total	193	5	0	198			
			Method 3				
$BC_4/BC_3/F_3/F_2/BC_2$							
R 554	0	10	37	47	0.35	1	0.5541
R 555	0	16	35	51	1.11	1	0.2921
R 556	0	17	59	76	0.28	1	0.5967
Expected ratio		1	3				
Total					1.74	3	
Pooled	0	43	131	174	0.0076	1	0.931
Heterogeneity					1.7324	2	0.421
			Method 4				
$BC_4/F_3/BC_3/F_2/BC_2$							
R 557	0	21	33	54	2.66	1	0.1029
R 561	0	23	24	47	0.04	1	0.8415
R 563	0	28	18	46	2.18	1	0.1398
Expected ratio		1	1				
Total					4.88	3	
Pooled	0	72	75	147	0.061	1	0.805
Heterogeneity					4.819	2	0.0899

1	2	3	4	5	6	7	8
R 560	2	55	0	57			
R 566	1	35	0	36			
Expected ratio	all intermediate						
Total	3	90	0	93			
R 559	0	3	59	62			
R 562	0	3	22	25			
Expected ratio	all susceptible						
Total	0	6	81	87			
L. hirsutum	20			20			
L. esculentum	0	0	25	25			

[x] Resistant (R): class 0 (no visible symptoms). Intermediate (I): classes 1 (few and small powdery dots) and 2 (up to 25% of infected young leaves and lack of expansion). Susceptible (S): classes 3 (up to 50% of older leaves infected) and 4 (more than 50% of older leaves infected, new colonies spreading up to the top of plants, fungal growth luxuriant).

for resistance. The pooled population was 43 intermediate : 131 susceptible. These data indicate a ratio of 1 intermediate : 3 susceptible with a probability of 0.931 (pooled $\chi^2 = 0.0076$). This ratio suggests that BC_3 might have been heterozygous for both resistant genes before backcrossing with the susceptible recurrent parent A 241, which is consistent with the assumption mentioned in the case of method 1. The reaction of 7 BC_4 families within method 4 differentiated for resistance. Three families (R 557, R 561, R 563) segregated in the ratio 1 intermediate :1 susceptible. The pooled χ^2 value was equal to 0.061 ($P = 0.805$). Two other families (R 560, R 566) were classified as intermediate in their reaction to *O. lycopersicum*. The last two families (R 559, R 562) had nearly all of the plants seriously infected.

Variation in morphological traits

Student's *t* test revealed no significant differences between the four different methods used (Table 3). A considerable variation in morphological traits was observed among twelve populations used in this study. The most variable trait was the number of fruits per plant (Figure 1).

The highest number of seeds was obtained in two lines: R 554 and R 563 (52.5 and 55.3, respectively). Although their seed productivity was significantly higher than that of *L. hirsutum* (29.3), it did not reach *L. esculentum* level (111.2 seeds/fruit). All other populations did not differ significantly from *L. hirsutum* (Figure 1A).

Fruit size varied from 12.3 to 55.2 mm in circumference for *L. hirsutum* and *L. esculentum*, respectively (Figure 1B). All populations had significantly larger fruit than *L. hirsutum*, and two of them (R 541 and R 546) had a fruit size comparable with *L. esculentum*. The size of fruit for R 540, R 549 and R 552 was far from being similar to *L. esculentum*.

Table 3. Effect of breeding methods on morphological traits of F_4 and BC_4 populations derived from *Lycopersicon esculentum* × *L. hirsutum*

Method	Generation	Number of seeds per fruit	Longitudinal circumference of fruit (mm)	Fruit weight (g)	Number of locules per fruit	Number of fruits per plant
1	F_4/BC_3	29.7 a	37.8 b	32.4 b	3.7 b	7.5 ab
2	F_4/F_3	26.9 a	36.5 b	31.8 b	3.9 b	9.0 b
3	BC_4/BC_3	44.8 a	37.9 b	29.2 b	4.2 b	7.6 ab
4	BC_4/F_3	49.5 a	39.6 b	30.4 b	4.1 b	12.0 b
L.esculentum	P_1 A 241	111.2 b	55.2 c	92.5 c	3.9 b	29.5 c
L.hirsutum	P_2 LA 1775	29.3 a	12.3 a	1.6 a	2.0 a	4.3 a

The significance of differences was evaluated by Student's *t* test at P = 0.05.
Within a column, means followed by the same letter do not differ significantly.

The heaviest fruit was recorded for R 541 and R 546 (44.5 and 44.2 g, respectively), although those values were still significantly lower than for *L. esculentum* (Figure IC). All populations were significantly different from *L. hirsutum*.

Population R 549 had the smallest number of locules (2.2) per fruit, typical for *L. hirsutum* (Figure 1D). Two lines, R 546 and R 563, expressed a statistically higher number of locules (4.9 and 4.8, respectively) than the recurrent parent. Remaining populations constituted a group characterised by fruit with the number of locules typical for A 241.

The majority of tested populations showed low fruit setting not being statistically different from *L. hirsutum* (Figure 1E). The highest number of fruits was recorded for two populations: R 560 and R 546 (15.5 and 12.9). Their productivity differed significantly from the recurrent parent and *L. hirsutum* as well. The lowest number of fruits was observed for R 541 and R 554 (5.5 and 5.8 fruits/plant, respectively).

Data on all morphological traits revealed that the best population was R 546 derived from method 2. It is characterised by a longitudinal circumference typical for A 241, high fruit weight and fruit productivity and the highest number of locules.

The results generated by all four methods of breeding were very similar for all morphological traits except fruit productivity (Table 3). The mean values of all methods for seed productivity did not differ significantly from the donor parent *L. hirsutum*. It means that there was no significant progress in breeding process. A considerable progress was noticed in two other traits: longitudinal circumference and fruit weight, and there was no difference between all methods, but significant differences were noticed for both parents *L. esculentum* and *L. hirsutum*. The values of those traits were higher than A 241 but lower than *L. hirsutum*. The greatest impact on fruit productivity was recorded for method 1 and method 2, where the number of fruits per plant was significantly higher than in *L. hirsutum*. It might be associated with the use of BC_3 after F_2 in both cases.

Discussion

Previous studies have shown that resistance to powdery mildew in *L. hirsutum* LA 1775 is controlled by two partially dominant genes: one major gene with a stronger expression (particularly when homozygous), and a minor gene (KOZIK 1999). The major dominant gene, when homozygous, will give complete resistance (without any symptoms – class 0); both genes in heterozygous form and/or the minor gene when homozygous will provide an intermediate level of resistance (classes 1 and 2); double recessive homozygotes and/or genotypes with one gene in heterozygous form when the second gene is homozygous recessive will manifest susceptibility (classes 3 and 4). Data in this study confirm that plants that carry resistance to *O. lycopersicum* can be found in most of the F_4 and BC_4 families. Probably only in two families (R 559, R 562) the resistance genes were missing and almost all plants expressed susceptibility. Differences in segregation in resistance responses to powdery mildew indicate that genetic backgrounds of the families were different and depended on the homo/heterozygous form of both genes responsible for resistance to powdery mildew.

Accession LA 1775 of *L. hirsutum,* also used in this study, is moderately self-compatible and the self-compatibility is even lower than cross-compatibility (KOZIK, DYKI 2000). Data on low fruit and seed yield for all populations, as compared with the recurrent parent A 241, suggest some level of the strength of residual selfing and crossing barriers inherited from the donor parent *L. hirsutum*. Also all other investigated features differed morphologically from the recurrent parent A 241.

Generally, if backcross breeding is to be successful, the genotype of the recurrent parent must be recovered in its essential features. This is primarily a function of the number of backcrosses, although selection for the type of the recurrent parent in the early backcross generations is effective in shifting the population toward the characteristics of that parent. It is believed that six backrosses coupled with rigid selection in early generations produce plants that resemble the recurrent parent effectively (ALLARD 1960). In this study the four backcross pedigree programs with no selection concerning the type of the recurrent parent made their discrimination impossible and revealed that variation among methods was insignificant. There was a noticeable progress in breeding regarding fruit size and weight, and the number of locules. Values of two other traits: seed and fruit productivity, which are correlated with self- and cross-compatibility, were low and similar to those of *L. hirsutum*. It is possible that this accuracy may be further improved by additional one or two backcrosses with a particularly high intensity of the resistance to powdery mildew being transferred.

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