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Inheritance of *in vitro* plant regeneration ability in the tomato

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ABSTRACT

Lycopersicon pimpinellifolium, with high plant regeneration ability, used as a pollen donor, was crossed with five recalcitrant *L. esculentum* genotypes. Statistical analysis was based upon frequency data of calluses that regenerated plants (R) in F_1, F_2, BC_1 and on reciprocal crosses, with genotypes that did not regenerate plants (NR). F_1 hybrids showed a dominance effect; no differences were observed in reciprocal crosses. The F_2 rate was close to 9:7 (R:NR) and BC_1 expressed a 1:3 (R:NR) rate. The results suggest an interaction of two dominant genes, indicating that the transfer of high plant regeneration ability from wild to commercial varieties would be relatively easy, since a qualitatively inherited dominant trait is involved.

INTRODUCTION

Studies on the genetics of regeneration in tomatoes have demonstrated that this trait is highly heritable (Frankenberger *et al.*, 1981) with a dominance effect (Ohki *et al.*, 1978; Adams and Quiros, 1985; Wijbrandi *et al.*, 1988). Models of qualitative inheritance of plant regeneration have been described for alfalfa (Wan *et al.*, 1988; Seitzkris and Bingham, 1988), corn (Tomes and Smith, 1985; Hodges *et al.*, 1986) and wheat (Galiba *et al.*, 1986; Higgins and Mathias, 1987; Kaleikau *et al.*, 1989; Ou *et al.*, 1989). In studies with rye, it was concluded that plant regeneration ability is determined by a polygenic system and has a recessive character (Rakoczy-Trojanowska and Malepszy, 1993). Despite the various studies carried out on plant regeneration, only a few papers have proposed in detail the mode of inheritance of regeneration ability using F_1, F_2, back crosses (BC) and reciprocal crosses (Reish and Bingham, 1980; Nadolska-Orczyk and Malepszy, 1989).

The objective of the present study was to establish a model for the inheritance of the plant regeneration trait on the basis of the F_1, F_2 and BC_1 generations obtained from crosses between the wild species *Lycopersicon pimpinellifolium* and five cultivars of *L. esculentum*. The choice of *L. pimpinellifolium* was due to the high regeneration capacity presented by one of its accessions and to the fact that this species crosses easily with cultivated tomato plants, producing fertile and viable seeds.

MATERIAL AND METHODS

Plant material

To initiate genetic studies of regeneration, genotypes with high and low regeneration potential were identified and cross-pollinated in the greenhouse after emasculation. The high regeneration genotype *L. pimpinellifolium*, WV-700 (P1), was selected previously by Faria and Illg (1991), as were the five low *L. esculentum* regeneration parents known as: VFN-8 (P2), Petomech (P3), Santa Rita (P4), CNPH-080 (P5) and Red Cherry (P6). The F_1, F_2 and BC_1 generations were analyzed for plant regeneration capacity.

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In vitro procedures

Hypocotyl segments (3 mm) from seedlings germinated *in vitro* were utilized as explant sources. Culture medium (mg) was based on Murashige and Skoog (1962), with the salt concentration reduced by half in all experiments. Calluses were induced for two weeks on MS medium supplemented with 1 mg/l IAA and 2.25 mg/l BA. Plant regeneration from callus was achieved by transfers for two months to the same basic medium, but containing 0.5 mg/l IAA and 5 mg/l BA, as described by Garcia-Reina and Luque (1988).

Data analysis

Genetic analysis was done on the basis of the frequency of calluses that regenerated (R) and did not regenerate (NR) plants in segregating generations F_2 and BC_1. For segregation analysis, two fruits of each progeny were selected at random and all of their seeds were germinated *in vitro* to obtain one callus per individual hypocotyl explant. A randomized complete-block design was used in all experiments. Chi-square analyses, with the level of significance set at 0.05, were used for testing the goodness of fit for the appropriate genetic model for plant regeneration capacity.

RESULTS AND DISCUSSION

The interspecific crosses of *L. pimpinellifolium* with *L. esculentum* genotypes were successful as these species do not show any type of incompatibility (Hogenboom, 1972; Rick, 1982). The results of the crosses, showing the number of calluses that effectively regenerated plants (R) or not (NR) in the F_1, F_2, and BC generations, are presented in Table I. The F_1 hybrids demonstrated a high plant regeneration ability (73-90%) in the five family sets analyzed, with dominance effects. Dominance for plant regeneration from calluses culture has been previously reported in the tomato literature (Kut and Evans, 1982; Tan *et al.*, 1987; Wijbrandi *et al.*, 1988) and for other crops such as corn (Hodges *et al.*, 1986; Prioli, 1987; Novak *et al.*, 1988), alfalfa (Reish and Bingham, 1980; Wan *et al.*, 1988; Seitzkris and Bingham, 1988) and wheat (Kaleikau *et al.*, 1989; Ou *et al.*, 1989). Wijbrandi *et al.* (1988) observed that fusion of protoplasts from *L. peruvianum*, which has a high plant regeneration ability, with genotypes of *L. esculentum*, in which this trait is not present, favored the formation of calluses with plant regeneration ability. These investigators suggested that plant regeneration can be used as a dominant marker for the selection of somatic hybrids. Recently, a gene controlling shoot regeneration in the tomato has been mapped on chromosome 3 of *L. peruvianum* (Koornneef *et al.*, 1993). The frequency of plant regeneration for F_1 hybrids did not exceed that of the parental genotype WV-700 (*L. pimpinellifolium*), indicating the absence of a heterosis effect on the crosses performed (Table I). In contrast, Ohki *et al.* (1978) reported that hybrids between two tomato cultivars showed heterosis for plant regeneration. The observed proportion of calluses that regenerated plants (R) or not (NR) was close to 9:7 (R:NR) in the F_2 generation and to 1:3 (R:NR) in BC_1. Neither value was significant at the 5% level of probability by the chi-square test for the five family sets analyzed (Table I). These results suggest the involvement of two independent genes interacting in the expression of plant regeneration.

The segregation results obtained in the present study indicate that plant regeneration ability is a qualitative trait, agreeing with results reported by other studies on the genetic control of regeneration in the tomato (Frankenberger *et al.*, 1981; Koornneef *et al.*, 1987).

In RFLP analyses, Miller and Tanksley (1990) concluded that *L. pimpinellifolium* is one of the closest species to *L. esculentum*, thus suggesting that the genetic variation detected in this species can be easily utilized for tomato improvement.

On the basis of the present results, indicating that plant regeneration is controlled by two independent interacting genes, we suggest that it should be relatively easy to transfer plant regeneration ability from *L. pimpinellifolium* to commercial *L. esculentum* varieties, in order to maximize the plant regeneration response *in vitro*.

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RESUMO

O genótipo WV-700 (*Lycopersicon pimpinellifolium*) com uma alta capacidade para regeneração de plantas foi cruzado com cinco genótipos de *L. esculentum* recalcitrantes para esta característica. As análises estatísticas foram

Table I - Genetic analysis for plant regeneration ability in the five tomato cross families.

Genotype	Generation	Explant number	Observed		Expected		χ^2 value[4]
			R[2]	NR[3]	R	NR	
P1		60	55	5	1	0	
P2		60	5	55	0	1	
P1 x P2	F_1	60	54	6	1	0	
	F_2 (A)[1]	192	97	95	9	7	2.56
	(B)	201	120	81	9	7	0.97
	BC (A)	129	29	100	1	3	0.44
	(B)	144	34	110	1	3	0.15
P3		60	3	57	1	0	
P1 x P3	F_1	60	47	13	1	0	
P3 x P1	F_1	60	44	16	1	0	
	F_2 (A)	189	102	87	9	7	0.40
	(B)	171	92	79	9	7	0.42
	BC (A)	114	26	88	1	3	0.29
	(B)	138	27	111	1	3	2.17
P4		60	3	57	1	0	
P1 x P4	F_1	60	45	15	1	0	
	F_2 (A)	195	115	80	9	7	0.59
	(B)	210	131	79	9	7	3.19
	BC (A)	138	26	112	1	3	2.79
	(B)	126	25	101	1	3	1.78
P5		60	0	60	1	0	
P1 x P5	F_1	60	52	8	1	0	
P5 x P1	F_1	60	54	6	1	0	
	F_2 (A)	174	100	74	9	7	0.11
	(B)	186	108	78	9	7	0.25
	BC (A)	111	20	91	1	3	2.89
	(B)	156	34	122	1	3	0.86
P6		60	4	56	0	1	
P1 x P6	F_1	60	49	11	1	0	
	F_2 (A)	162	98	64	9	7	1.19
	(B)	168	93	75	9	7	0.05
	BC (A)	123	27	96	1	3	0.61
	(B)	138	30	108	1	3	0.78

[1]A and B: Are progenies in different fruits from the same generation.
[2]R: Regenerated plants.
[3]NR: No regenerated plants.
[4] Differences not significant at the level of 0.05.
P1 = *Lycopersicon pimpinellifolium*, WV-700; P2-6 = *L. esculentum*, P2 = VFN-8, P3 = Petomech, P4 = Santa Rita, P5 = CNPH-080, P6 = Red Cherry.

baseadas na freqüência de calos que regeneraram plantas (R) nas gerações F_1, F_2 e retrocruzamentos (RC) com os genótipos que não regeneram plantas (NR). Os híbridos F_1 apresentaram uma alta capacidade de regeneração de plantas, mostrando um efeito de dominância. A freqüência de regeneração na geração F_2 foi próxima a 9:7 (R:NR) e nos RC próximo de 1:3 (R:NR). Esses resultados sugerem a interação de dois genes dominantes, indicando que a transferência da capacidade de regeneração de plantas do tomateiro selvagem para variedades comerciais deve ser relativamente fácil, já que essa característica é dominante e controlada qualitativamente.

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