

SHORT COMMUNICATION

Intra and interspecific variability of *in vitro* culture response in *Lycopersicon* (tomatoes)

Guillermo Pratta, Roxana Zorzoli and Liliana Amelia Picardi

ABSTRACT

Intra and interspecific variability was measured in the genus *Lycopersicon* for the traits: *productivity rate* (PR, total number of regenerated shoots/total number of cultures), *regeneration percentage* (%R, number of cultures regenerating shoots or primordia/total number of cultures) and *callus percentage* (%C, number of cultures only producing callus/total number of cultures). Leaf explants from various genotypes of *L. esculentum*, *L. esculentum* var. *cerasiforme*, *L. pimpinellifolium* and *L. peruvianum* were placed on Murashige and Skoog (*Physiol. Plant.* 15: 473-493, 1962) medium + 0.175 mg/l IAA + 2.25 mg/l BA. Significant differences among species and among genotypes within the same species were found, while genotypes from different species showed similar responses.

INTRODUCTION

The genus *Lycopersicon* comprises the cultivated tomato (*L. esculentum* Mill.) and a few related wild species (Warnock, 1988). In the process of adaptation to different environments, the latter acquired characteristics that the cultivated species may have lost during the human search for commercial traits, such as productivity, adequate fruit shape and size, and homogeneity. Examples of these agronomically desirable characteristics are insect and disease resistance, soil salinity and adverse weather condition tolerance, higher soluble solids and vitamin C content, etc. (Rick, 1979; Bretó *et al.*, 1993).

The unilateral incompatibility among self-compatible (subgenus *Eulycopersicon*) and self-

incompatible (subgenus *Eriopersicon*) species of the genus, as well as the small number and low fertility of the interspecific hybrids that can be obtained (Hogeboom, 1972; Rick, 1983), usually prevent combinations of genotypes with desired traits. In this regard, *in vitro* plant tissue culture techniques are useful to break down these barriers. Plant breeders can resort to immature hybrid embryo rescue or *in vitro* fertilization to overcome incompatibility (Hogeboom, 1972), while regeneration from somatic explants may be used to increase the number of selected genotypes. When using this technique, direct regeneration is preferred in order to minimize somaclonal variation (Evans and Sharp, 1983; Lee and Phillips, 1988).

Micropropagation in *Lycopersicon* could be a useful option when a large number of rare genotypes (such as interspecific hybrids) is required. As in other crops (Baroncelli *et al.*, 1973; Bayliss and Dunn, 1979; Pence *et al.*, 1979; Jarret *et al.*, 1980), different responses (callus production, regeneration of whole plants, roots and pseudo-fruit differentiation) have been reported in

tomato, depending on the genotypes, explants, culture media and incubation conditions (Kartha et al., 1976; Tal et al., 1977; Kut and Evans, 1982; Kurtz and Lieneberger, 1983; Locky, 1983; Zorzoli et al., 1993a, b).

In vitro performance of wild *Lycopersicon* species should be compared to that of the cultivated tomato before including them as parental strains in a non-traditional breeding program. The objective of this research was to evaluate *in vitro* culture response in some genotypes of the genus *Lycopersicon*, so as to estimate intra and interspecific variability for the traits regeneration capacity and callus production.

MATERIAL AND METHODS

Several genotypes of the self-compatible species *L. esculentum* Mill., its wild relatives *L. esculentum* var. *cerasiforme* (Dun.) Gray and *L. pimpinellifolium* (Jusl.) Mill., and from the self-incompatible *L. peruvianum* (L.) Mill. were planted (Table I). Sowing was made in March, in a greenhouse at Campo Experimental José F. Villarino, Zavalla, Santa Fe, Argentina (33° South latitude and 61° West longitude). Following Zorzoli et al.'s (1993a) technique, explants were taken from the third leaf below the apical meristem, 40 days after emergence. Culture medium consisted of vitamins and inorganic salts, 30 g/l sucrose, 0.175 mg/l IAA, 2.25 mg/l BA and 9 g/l agar (Murashige and Skoog, 1962). Explants were disinfected by washing in 96° ethyl alcohol, soaking 8 min in a 4% active chlorine commercial sodium hypochlorite solution and rinsing three times in sterile distilled water. The cultures were incubated in an acclimatized room at 25 ± 2°C with a photoperiod of 16 h (50 microEinstein/m².sec).

The design was completely randomized, with five plants per genotype and a mean of six replications per plant (N = 540). Data were taken 45 days after the initiation of the cultures. The regeneration capacity was estimated through both the productivity rate (PR, total number of regenerated shoots/total number of cultures) and the regeneration percentage (%R, number of cultures regenerating shoots or primordia/total number of cultures). These variables were transformed by $\ln(\text{PR} + 0.001)$ and $\arcsin\sqrt{\%R}$, respectively. The transformed means of species and genotypes within species were compared through an ANOVA (Snedecor, 1964). The

linear correlation between PR and %R was calculated, and the genotypes were classified according to the range of variability of both variables (Zorzoli et al., 1993a). Callus production was analyzed through the variable callus percentage (%C, number of cultures only producing callus/total number of cultures). Genotype - %C independence was measured through the χ^2 test (Snedecor, 1964).

RESULTS AND DISCUSSION

Table II shows the values of PR, %R and %C for the different genotypes. In general, all of them (except Pi2) produced calli early; some of these calli began developing primordia, and afterwards, shoots. The results confirmed, as already described for other genotypes (Locky, 1983), that organogenesis is indirect for the tomato species utilized.

Highly significant differences (F = 7.44 for PR and F = 26.20 for %R; P < 0.01) were found for *in vitro* regeneration capacity among *Lycopersicon* species. *L. peruvianum* showed the highest PR and %R within the group (also being precocious, since it was the first species to redifferentiate), *L. pimpinellifolium* had the lowest values, while *L. esculentum* and its wild form *L. esculentum* var. *cerasiforme* demonstrated an intermediate performance. Similar results were reported by Kut and Evans (1982), who defined *L. pimpinellifolium* as a recalcitrant species. With respect to *L. peruvianum*, Tal et al. (1977) suggested that the high morphogenic potential might be due to its high degree of heterozygosity. Consequently, the lower regeneration values of the autogamous species could be caused by a lack of heterozygosity.

Significant differences among genotypes within species were found in *L. esculentum* (F = 10.53 for PR and F = 8.19 for %R; P < 0.01), *L. esculentum* var. *cerasiforme* (F = 7.13 for PR; P < 0.01 and F = 4.00 for %R; P < 0.05) and *L. peruvianum* (F = 13.45 for PR; P < 0.01 and F = 3.87 for %R; P < 0.05). Genotypes from *L.*

Table I - *Lycopersicon* species and genotypes within species employed in the analysis. L.A.-identified wild materials were kindly provided by Dr. Charles Rick (Davis, University of California).

<i>L. esculentum</i> (E)	<i>L. esc. var. ceras</i> (C)	<i>L. pimpinellifolium</i> (Pi)	<i>L. peruvianum</i> (Pe)
cv. Rin (E1)	cv. L.A. 1385 (C1)	cv. L.A. 1246 (Pi1)	cv. L.A. 111 (Pe1)
cv. Nor (E2)	cv. L.A. 2664 (C2)	cv. L.A. 2181 (Pi2)	cv. L.A. 1333 (Pe2)
cv. Kitec (E3)	cv. L.A. 1673 (C3)	cv. L.A. 722 (Pi3)	cv. L.A. 1292 (Pe3)
cv. Platense Italiano (E4)	cv. Z. 1994 (C4)	cv. Z. 1995 (Pi4)	cv. L.A. 2151 (Pe4)
cv. Caimanta (E5)			

Table II - PR, %R and %C mean values for *Lycopersicon* species and genotypes.

Genotypes	PR	%R	%C
E1	0.50	58	32
E2	0.04	6	68
E3	0.45	33	66
E4	0.19	65	35
E5	0.08	21	69
E	0.25	39	54
C1	0.65	64	36
C2	0.18	41	46
C3	0.00	0	100
C4	0.00	48	50
C	0.21	40	58
Pi1	0.00	11	88
Pi2	0.00	0	0
Pi3	0.13	40	59
Pi4	0.08	33	61
Pi	0.05	27	52
Pe1	0.78	97	3
Pe2	0.51	100	0
Pe3	4.41	100	0
Pe4	0.37	77	22
Pe	1.56	94	6

For genotype abbreviations see Table I. PR: Productivity rate; %R: regeneration percentage; %C: callus percentage.

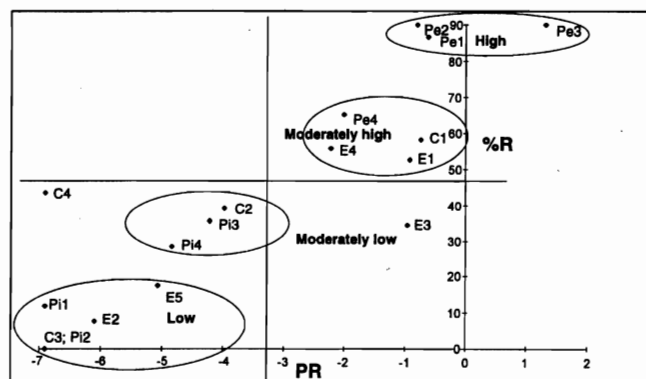


Figure 1 - Distribution of the transformed values of the genotypes along the X (PR) and Y (%R) axis. The mean transformed values of PR and %R (-3.23 and 47.52, respectively) permitted a new division into four quadrants along the X and Y axes. The genotypes were classified according to their position at these quadrants.

pimpinellifolium did not show statistically significant differences ($F = 1.00$ for PR and $F = 1.19$ for %R; non-significant). Though Pe3 was an extremely productive strain (Table II), genotypes from *L. peruvianum* could be considered more homogeneous than those from self-compatible species, since they all had high regeneration capacity. The greater F value of this alogamous species might indicate greater within (rather than among) line differences (Bretó *et al.*, 1993). The greater variability

observed within the self-compatible species *L. esculentum* and *L. esculentum* var. *cerasiforme* (in the sense that these genotypes present a wider range of performances) could be explained by the fact that, by selfing, genetic drift would have originated segregating lines within each species. However, it must be taken into consideration that in this experiment, the sample of self-compatible genotypes was more numerous than that of self-incompatible ones.

The highly significant linear correlation ($r = 0.64$; $P < 0.01$) between PR and %R suggests that the ability to regenerate is tightly linked to *in vitro* productivity.

The classification of the genotypes, taking into account both variables simultaneously, would indicate the existence of different responses to *in vitro* culture. Non-conventional clusters (Figure 1) were obtained using this criterion, i.e., the genotypes of different species behaved similarly. Four groups could be clearly defined, since highly significant differences for PR ($F = 17.40$; $P < 0.01$) and %R ($F = 73.16$; $P < 0.01$) were found between them: I) genotypes with high regeneration capacity ($PR > 0.50$; $\%R > 80$): Pe3, Pe2 and Pe1; II) genotypes with moderately high regeneration capacity ($0.15 < PR < 0.65$; $55 < \%R < 80$): Pe4, E1, C4 and E4; III) genotypes with moderately low regeneration capacity ($0.05 < PR < 0.20$; $30 < \%R < 55$): C2, Pi3 and Pi4; and IV) genotypes with low regeneration capacity ($PR < 0.10$; $\%R < 30$): E5, E2, Pi1, Pi2 and C3. The variable that gave the clearest differences for clustering was %R. Though C4 and E3 responses were opposite to the other ones (because they showed contrasting PR and %R), they could be included in group III, since they appeared to show a moderately low performance when the variables were considered together. Therefore, clusters in Figure 1 would exemplify the previous considerations about intraspecific variability.

Highly significant genotypic differences were also found for %C ($\chi^2 = 20.14$; $P < 0.01$). Table II shows, as expected, that low regeneration capacity generally corresponds to high %C (the exceptions are those genotypes showing poor or even null morphogenic response, such as E2 or Pi2). These results would indicate that dedifferentiation is a common fact occurring as a response to *in vitro* culture in the genus *Lycopersicon*, while redifferentiation is restricted to certain genotypes (Tal *et al.*, 1977; Locky, 1983).

In vitro performance would imply that distinct physiological processes, causing a corresponding associated response, take place in certain genotypes (Kut and Evans, 1982; Kurtz and Lieneberger, 1983; Locky, 1983). The presence of different genic systems regulating the trait, and then the existence of intra and

interspecific variability for *in vitro* culture response would be indicated by these data. There are species and genotypes within species with high regeneration capacity, and others with high efficiency in callus production. These factors must be considered when planning intra and interspecific crosses, so as to obtain a greater benefit from the great genetic potential that these wild species represent for a breeding program.

RESUMO

A variabilidade intra e interespecífica foi avaliada no gênero *Lycopersicon* para as variáveis: taxa de produtividade (PR, número total de brotos/número total de culturas), percentagem de regeneração (%R, número de culturas que regeneraram brotos ou primórdios/número total de culturas) e percentagem de calo (%C, número de culturas que só desenvolveram calo/número total de culturas). Os explantes foliares de vários genótipos de *L. esculentum*, *L. esculentum* var. *ceraciforme*, *L. pimpinellifolium* e *L. peruvianum* foram colocados em meio de cultura Murashige and Skoog (*Physiol. Plant.* 15: 473-493, 1962) + 0.175 mg/l AIA + 2.25 mg/l BA. Detectaram-se diferenças significativas entre espécies e entre genótipos de uma mesma espécie, apresentando genótipos de diferentes espécies a mesma resposta.

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