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Salt tolerance in *Lycopersicon* species. VI. Genotype-by-salinity interaction in quantitative trait loci detection: constitutive and response QTLs

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Abstract A study of genotype-by-salinity interaction was carried out to compare the behavior of quantitative trait loci (QTLs) in two F_2 populations derived from crosses between the cherry tomato, *Lycopersicon esculentum* Mill. var. cerasiforme, and two wild relatives *Lycopersicon pimpinellifolium* (Jusl.) Mill. and *Lycopersicon chesmannii* f. *minor* (Hook. f.) Mull., grown at two environmental conditions (optimum and high salinity). QTLs for earliness and fruit yield could be classified into four groups: "response-sensitive", those detected only under control conditions or whose contribution significantly decreased in salinity; "response-tolerant", detected only in salinity or in which the direction of their additive effects changed; "constitutive", detected in both growing conditions; and "altered" QTLs, those where the degree of dominance changed according to the presence or absence of salt. Epistatic interactions were also influenced by the salt treatment. This differential allele effect at some (non-constitutive) QTLs induced by salt stress will make selection under an "optimum environment" unfruitful for the "response-tolerant" QTLs. Similarly, selection under salinity will ignore "response-sensitive" QTLs. Given that salinity is highly variable in the field, marker-assisted selection should take into account not only the "response-tolerant" but also the "response-sensitive" QTLs although there might be cases where selection in some QTLs for both conditions is not feasible. Comparing both populations, very few QTLs showed the same behavior.

Key words Yield components · Earliness · G × E · Gene effects · Wide adaptation · Genetic markers · MAS · Epistasis

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Introduction

Soil salinization, the concentration of salts in the surface, or near-surface, zones of soils, is a major process of land degradation, leading to falling crop yields and the loss of land from production in a range of environments. Human-induced salinization also contributes to desertification processes in the world's drylands and more temperate environments because it is particularly associated with irrigation schemes (Thomas and Middleton 1993).

Breeding for salt tolerance has been recognized as a suitable approach to improve crop productivity in salt-affected areas (Epstein et al. 1980). However, progress so far has been modest. Two reasons for this are specially relevant: (1) breeding for salt tolerance has been mostly carried out by introgressing polygenes into the domesticated species from salt-tolerant wild species which are poorly productive and with many undesirable characteristics, and (2) the spatial and temporal variability in salinity affected fields. The first problem could be reduced using marker-assisted selection (MAS), as suggested by Monforte et al. (1996). The second problem has brought about the controversy among breeders on the choice of a suitable environment for evaluation and selection in salt-tolerance breeding programs. Thus, Richards (1983) concluded that the best strategy was to select for yield under low- or non-saline conditions, which has been supported by the results from some experiments (Rawson et al. 1988; Kelman and Qualset 1991). Other authors suggest that selection must be carried out under conditions of salinity (Johnson et al. 1992; Saranga et al. 1992). A fact frequently ignored for convenience is that the genotype-by salt treatment interaction is usually large and significant in most experiments (Azhar and McNeilly 1988; Asíns et al. 1993 b; Igartua 1995). Data at the molecular level also support the existence of this interaction, such as differential mRNA expression (Gibson

et al. 1984; Gulick and Dvorák 1990), synthesis *de novo* or the accumulation of proteins, commonly named "osmotin" (reviewed by Hurkman 1992), and the disappearance of certain isozymes and peptides (Asíns et al. 1993 a, b). In attempting to study the merits of potential strategies for salinity tolerance breeding, Igartua (1995) points out that this objective can be compared to what has been known as breeding for "wide adaptation.", making it necessary to test a subset of environments, including stress and non-stress locations. Hence, the controversy about the choice of an optimum selection environment must be focused on whether, in spite of differential gene expression, selection under non-saline conditions can ensure a minimum response under salt conditions or, conversely, genes acting on non-salinity conditions are different from those acting under salinity, then causing the selection under non-salinity to be inefficient. One way to asses this, is to select under conditions of salt and non-salt treatment and then compare both responses. Several experiments of this kind have been carried out, giving conflicting conclusions (see Igartua 1995 for a more detailed discussion). Another approach is to use QTL analysis based on genetic markers to study the salt-induced differential genotypic expression or genotype × environment (G × E) interaction. If different QTLs are detected depending upon the presence/absence of salinity, it could be used in the design of a marker-assisted selection (MAS) scheme which, taking into account all of QTLs of the trait, would help to broaden "adaptation" from non-saline to saline conditions.

The objectives of the present work were to carry out a comparative study, by means of QTL analysis, of the genetic control of tomato fruit yield under both saline and non-saline irrigation conditions, and to compare the results of this G × E interaction among families differing in their salt-tolerant parental species.

Materials and methods

Families were derived from three interspecific crosses between *Lycopersicon* spp: cross A, *L. esculentum* cv "Madrigal" and *L. pimpinellifolium* line L1 (Bretó et al. 1994); cross B, *L. esculentum* var cerasiforme line E9 (a cherry tomato cultivar) and *L. pimpinellifolium* line L5 (Asíns et al. 1993 a); and cross C, line E9 and *L. chesmannii* line L2 (Asíns et al. 1993 a).

Parentals, F_1 and F_2 populations, except for the F_2 from the A cross, were grown under both control and salt-treatment conditions. For control conditions plants were cultured in individual pots filled with peat plus sand and irrigated with tap water (approximately 2 dS/m). Plants under salt treatment were grown on sand in a greenhouse with both photoperiod (12-h light) and temperature (25 + 10°C) control and irrigated with one-half Hoagland solution plus 171.1 mM NaCl (conductivity 15 dS/m). The sunlight, measured as the photosynthetic photon flux density (PPFD) inside the greenhouse on June 3rd., was very low (200 $\mu mol\, m^{-2}\, s^{-1}$) because the plaques had lost transparency. Earliness (EA) and the fruit-yield components, total fruit weight (TW), average fruit weight (FW) and fruit number (FN) were measured at 9 week after plants started producing fruit, as described in Monforte et al. (1997).

Means, variances, contrasts for non-additive (NA), and epistatic (EP) effects, the proportion of transgressive individuals, marker linkage analysis, QTL detection and analysis, and epistatic interactions between markers were calculated as described in Monforte et al. (1997).

Genotype-by-environment interaction was studied by two-way ANOVA using genotypes (including parentals and F_1s) and treatments as classification levels.

Results

Several statistics for the measured traits and genotype-by-environment (G × E) interactions for EA, TW, FN and FW in the three families are presented in Table 1. G × E was highly significant ($P < 0.0001$) in all traits and families except for EA and FN in the B family. No transgressive segregants were observed for any trait under control conditions for the B family. The contrast for epistatic interactions (EP) in B under control conditions was highly significant ($P < 0.01$) for EA and TW, and significant ($P < 0.05$) for FN (data not shown). The F_1 from family C flowered and yielded tomatoes very late under salinity conditions. Moreover, 77 out of 200 F_2 plants produced tomatoes under control conditions while only 50 out of 178 F_2 plants did so under salt treatment, which is significantly different. Non-yielding plants in C F_2 were kept in the greenhouse and a proportion of them yielded fruit from 13 to 23 weeks after the conventional yielding plants finished their 9-weeks of production (data not shown). Therefore, QTLs were analyzed only in non-late-yielding plants of this F_2 to follow the same criteria under control conditions as Monforte et al. (1997) did for C plants under salinity.

The QTLs detected are summarized in Table 2; they are named by an abbreviation of the trait and a significantly associated nearby marker(s). The genotypic value of the homozygote for the "wild" allele (a), the dominance deviation of the heterozygote from the mean of the homozygotes (d), and the percentage of explained phenotypic variance at a single locus (R^2) are also shown. The QTLs that could not be detected in one cross, because of the lack of marker polymorphism, are enclosed between parentheses. The most likely positions of the QTLs detected under control conditions according to MAPMAKER/QTL are similar to those found under conditions of salinity. The only differences were in the exact position of three fruit-weight QTLs that were placed in one extreme of the interval under salinity and in the middle under control conditions. In addition, QTLs for total weight which were most likely located near fruit-number QTLs in salinity conditions, moved closer to the fruit-weight QTLs under control conditions, due to an increase in the contribution of the FW component for that environmental condition.

The direction of additive effects (a) of yield-related QTLs in early yielding plants of the C cross was towards "esculentum" alleles, as happended under salt treatment (Monforte et al. 1997). The direction of the

Table 1 Means and standard deviations for the characters. NA indicates non-additivity (+) positive (−) negative effects, and genotype-by-environment interactions (G × E) at significance levels: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$. (†) data not available. The A family is Madrigal × L1; B is E9 × L2. (#) only one plant of L2 yielded fruits under conditions of salinity

Generation	Family	Earliness		Total weight		Fruit number		Fruit weight	
		Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control
Madrigal		0.00 ± 1.09	0.50 ± 0.55	26.63 ± 11.6	272.66 ± 80.6	1.33 ± 0.51	5.15 ± 2.24	21.78 ± 11.2	56.63 ± 13.9
E9		1.50 ± 1.18	1.10 ± 0.90	6.42 ± 9.86	57.91 ± 22.9	0.70 ± 1.00	2.62 ± 1.09	10.32 ± 4.62	23.09 ± 5.67
L1		133.00 ± 1.03	− 1.00 ± 0.00	12.02 ± 2.51	70.03 ± 30.7	22.00 ± 4.10	89.67 ± 38.3	0.75 ± 0.10	1.15 ± 0.25
L2#		20.00	20.50 ± 0.71	1.10	20.09 ± 5.58	3.00	107.00 ± 36.73	0.37	0.27 ± 0.05
L5		− 2.53 ± 0.96	− 3.47 ± 0.88	15.30 ± 8.86	19.16 ± 12.19	29.07 ± 14.19	27.73 ± 17.46	0.51 ± 0.07	0.69 ± 0.07
	A	− 5.33 ± 0.52	3.33 ± 1.33	49.35 ± 22.7	137.03 ± 35.50	16.33 ± 7.06	31.83 ± 8.93	3.78 ± 0.72	7.20 ± 6.50
F1	B	0.14 ± 3.77	− 3.07 ± 0.96	4.52 ± 3.79	32.04 ± 16.56	2.33 ± 1.53	10.67 ± 5.42	1.72 ± 0.68	3.06 ± 0.55
	C	21.71 ± 1.44	9.14 ± 12.2	27.95 ± 22.36	18.14 ± 16.48	18.87 ± 12.69	8.13 ± 8.13	1.48 ± 0.27	2.09 ± 0.42
	A	0.88 ± 4.00	†	30.09 ± 25.1	†	13.40 ± 10.30	†	3.17 ± 1.60	†
F2	B	− 2.35 ± 2.15	− 3.77 ± 1.41	11.18 ± 10.41	34.08 ± 21.58	6.01 ± 5.62	11.22 ± 7.18	2.03 ± 0.98	3.27 ± 2.10
	C	8.23 ± 12.7	9.14 ± 12.2	8.82 ± 11.7	14.21 ± 11.07	6.35 ± 8.33	8.18 ± 6.82	1.64 ± 0.95	2.33 ± 1.62
	A	** (−)	** (−)	** (−)			** (−)	** (−)	** (−)
NA	B		** (−)	** (−)		** (−)			*** (−)
	A	***		***		***		***	
G × E	B			***				***	
	C	***		***		***		***	

additive effects of fruit-weight and fruit-number QTLs were according to parental performance in the B F_2 under control conditions, except for *fnTG63*. Total-weight QTLs showed the same directions as their respective yield-component QTLs.

The proportion of phenotypic variance (R^2) explained by the markers or QTLs was usually below 10%. Some QTLs had a large contribution to the trait, especially *fwTG48-TG180* (58%), *fnTG23* (21.4%) and *fwTG134* (12.56%).

QTLs for fruit weight were quite constant between crosses under control treatment; the three QTLs in the C F_2 were also detected in the B cross. There was no common QTL for either FN or EA (Table 2). Salinity greatly affected the QTL analysis of earliness and fruit-yield components. Very few QTLs showed no change, and none of them coincided between the B and C families.

Significant epistatic interactions, according to the presence/absence of salinity, are summarized in Table 3 for the B family. As in the case of salinity (Monforte et al. 1997), the effect of these interactions was to increase the range of the means of the two-locus genotypes relative to the one-locus genotypes (data dot shown). Epistatic interactions changed with the environment; although some genomic regions are involved in espistatic interactions affecting the same trait under both environments, only one interaction, that for fruit weight (TG30-TG43), was common. Noteworthily, the marker TG43, associated with FN and TW constitutively, but not with FW, interacted with almost all FW QTLs in both treatments. Most interactions detected under control conditions, and some under salinity, involved a genomic region and more than one unlinked marker, which implies that multilocus associations are involved in the phenotypic expression of the traits.

Discussion

The success of the plant breeder should be judged not on the total number of genotypes grown in any one year, but rather on how efficient and reliable the selection procedures are in identifying superior individual genotypes and progenies. There is little to be gained, and much effort wasted, in growing numerous individuals at the start of any program if 90% of the available variation is discarded in the early generations as a consequence of poor or unproven selection procedures which are often no better than random selection (Brown et al. 1987). Nowadays, marker-assisted selection (MAS) can be used to improve efficiency in this process by allowing an increase of the frequency of favourable putative genotypes in the segregant populations before they have to be phenotypically evaluated or assayed in field trials. However, several

Table 2 QTLs are named by an abbreviation of the trait and the significantly associated nearby marker(s). QTLs enclosed between parenthesis were detected in only one family because the marker was not polymorphic in the other one. The value of the *t* statistic of the genotype-mean comparison and the LOD score when intervals were analyzed, and the genotypic value of the homozygote for the "wild" allele (a) and dominance deviations (d) are also shown . The R^2 at a single locus is expressed in %

Trait	Family	Control							Salinity					
		QTL	*t*	LOD	a	d	[d/a]	R^2	*t*	LOD	a	d	[d/a]	R^2
Earliness	B	*eaTG24-TG51*	2.85	1.83	− 0.49	0.00	0.00	10.00	2.22	1.19	− 0.52	0.44	0.85	7.16
		eaTG30	2.78		− 0.40	− 0.40	1.00	8.21	2.26		− 0.87	0.01	0.01	7.97
		(*eaTG43*)							3.10		− 1.15	− 0.14	0.12	14.30
		eaTG48-TG180							2.17	1.60	− 0.37	1.23	3.27	13.00
	C	*eaTG23*							3.06		1.91	− 0.27	0.14	14.04
Total weight	B	(*twEst4*)							2.18		− 2.68	− 0.84	0.31	3.55
		twTG24-TG51							2.47	1.10	3.99	− 0.82	0.20	6.92
		(*twTG43*)	2.18		7.59	0.31	0.04	5.09	2.89		4.40	2.80	0.63	8.20
		twTG48-TG180	2.46	1.31	− 8.73	− 3.08	0.35	7.20	2.31	1.10	2.75	1.95	0.71	4.00
		twTG123-TG182							2.88	2.88	− 1.92	5.61	2.91	7.85
	C	(*twPgm2-TG182*)							1.54	1.55	− 2.20	− 2.31	1.05	13.50
		(*twTG20*)							2.07		− 1.57	1.94	1.23	7.59
		twTG23	3.41		5.66	− 1.75	0.30	22.41						
		twTG24-TG51							2.15	1.40	− 1.87	2.80	1.78	12.00
		twTG48-TG180	2.95	1.87	− 2.95	− 3.40	1.15	11.00	2.33	1.29	− 2.55	0.16	0.06	11.00
		twTG68	1.88		− 1.24	4.12	3.32	5.92						
		twTG134	1.98		− 3.47	− 0.01	0.00	7.63	2.13		− 0.31	3.87	12.48	12.17
Fruit number	B	*fn-Aco1-TG68*							1.96	1.03	− 1.73	− 0.18	0.10	4.80
		(*fnEst4*)							2.56		− 1.47	0.86	0.58	4.72
		(*fnTG18*)	2.09		2.27	0.62	0.27	3.98						
		fnTG24-TG51							2.42	1.37	1.45	1.83	1.26	6.30
		fnTG30	2.44		2.38	1.09	0.45	5.43	2.78		1.30	3.30	2.52	8.37
		(*fnTG43*)	1.96		2.05	0.95	0.46	4.06	2.56		2.16	0.85	0.39	6.29
		fnTG48-TG180							2.52	1.40	1.73	0.86	0.49	5.00
		fnTG63	2.10		− 1.38	− 2.36	1.71	4.21						
		fnTG123-TG183							1.94		− 0.94	2.01	2.13	3.78
		fnTG134	2.03		1.98	− 1.65	0.83	5.77	2.53		2.23	− 0.81	0.36	8.34
	C	(*fnTG20*)							1.95		− 0.60	1.08	1.80	7.33
		fnTG23	3.3		− 2.21	− 0.64	0.29	21.43						
		fnTG24-TG51							1.41	0.98	− 0.01	1.52	151.00	8.70
		(*fnTG28*)	1.75		− 0.00	1.73	3.45	5.84						
		fnTG48-TG180	1.96	1.62	− 0.28	2.31	8.25	9.80	2.36		− 0.77	1.03	1.60	9.40
		fnTG134							2.61		− 0.11	2.14	18.65	17.15
Fruit weight	B	(*fwEst4*)							2.72		0.12	− 0.50	4.16	7.50
		fwAco1-TG68	2.49	1.72	− 0.37	− 0.77	2.08	9.00	2.49	1.68	0.33	− 0.18	0.55	6.40
		fwTG24-TG51	3.01	3.94	− 0.69	− 0.62	0.91	15.50	3.65	6.30	− 1.03	− 0.85	0.85	29.80
		fwTG30	2.64		− 0.61	0.15	0.23	6.84						
		fwTG48-TG180	3.50	10.28	− 1.92	− 1.10	0.57	58.00	2.90	1.99	− 0.43	− 0.49	1.13	14.00
		fwTG134	4.62		− 0.83	− 0.29	0.35	15.97	3.21		− 0.61	− 0.21	0.34	19.30
	C	(*fwPgm2-TG182*)							3.62	3.22	− 0.62	0.12	0.20	25.80
		fwTG24-TG51	1.86	1.99	− 1.07	− 0.87	0.85	11.80	2.20	1.08	− 0.24	0.35	1.41	9.40
		fwTG48-TG180	3.30	3.14	− 1.17	− 0.65	0.55	17.79	2.02	0.92	− 0.32	− 0.21	0.66	8.30
		fwTG134	2.47		− 0.99	− 0.47	0.47	12.56						

Table 3 Epistatic interactions at the two-locus level for the B family. Bold markers are significantly associated to the trait in at least one family and treatment

Trait	Treatment	
	Salinity	Control
Earliness	**TG180**-TG63	*Aco1*-**TG30**
		Aco1-**TG180**
		Est4-TG123
		TG18-TG134
		TG30-TG180
		TG30-TG68
		TG43-TG63
		TG63-TG134
Total weight	***Est4-Aco1***	***Aco1*-TG180**
	***Est4*-TG180**	**TG24**-TG18
	TG24-TG23	**TG134**-TG18
	TG43-TG123	**TG43**-TG18
	TG180-TG63	**TG48**-TG18
	TG134-*Aco1*	**TG48-TG68**
		TG48-TG123
		TG180-TG134
Fruit number	***Est4*-TG180**	***Aco1*-TG180**
	TG43-TG123	
	TG48-TG18	
Fruit weight	***Aco1-Est4***	***Aco1*-TG180**
	***Aco1*-TG43**	TG18-TG43
	***Est4*-TG24**	TG23-TG43
	***Est4*-TG68**	**TG24**-TG43
	TG24-TG30	**TG30**-TG43
	TG24-TG48	**TG68**-TG43
	TG30-TG43	**TG180**-TG43
	TG48-TG43	**TG180**-TG63
	TG48-TG68	**TG134**-TG123
	TG68-TG134	**TG134**-TG23

factors may reduce this efficiency, for instance the genetic variability at a QTL (Monforte et al. 1997). Additionally, many problems, particularly in breeding for resistance, are associated with the fact that breeders are manipulating genetic variability to overcome an abiotic or biotic problem that also changes with time and/or space. Salinity is an excellent example of this problem. Hence, we have used this factor to study the effect of genotype-by-environment (G × E) interaction on the genetic control of agronomically important traits such as earliness and fruit yield by means of QTL analysis and to examine how this interaction affects the MAS scheme for the improvement of salt tolerance. The use of F_2 populations, instead of backcross populations or recombinant inbred lines, has enabled us to study gene action at individual QTLs. Taking advantage of this approach, earliness and fruit-yield QTLs could be classified into four groups (Table 4):

"*Constitutive* QTLs", those that showed no important change in their gene action and were detected in both the control and under conditions of salinity. They are the least numerous group and none were common to B and C families.

Table 4 Classification of the detected QTLs according to their response to salt treatment. QTLs enclosed between parentheses were detected in only one cross due to lack of marker polymorphism in the other

Constitutive		Altered gene action		Response sensitive			Response tolerant	
B	C	B	C	B	C	B	B	C
		eaTG24-TG51					(*eaTG43*)	*eaTG23*
		eaTG30					*eaTG48-TG180*	
(*twTG43*)		*twTG134*	*twTG134*		*twTG23*		(*twEst4*)	(*twTG20*)
			twTG48-TG180		*twAco1-TG68*		*twTG48-TG180*	(*twPgm2-TG182*)
							twTG24-TG51	*twTG24-TG51*
							twTG123-TG182	
(*fnTG43*)	*fnTG48-TG180*	*fnTG30*		(*fnTG18*)	*fnTG23*		(*fnEst4*)	(*fnTG20*)
fnTG134				*fnTG63*	(*fnTG28*)		*fnAco1-TG68*	*fnTG24-TG51*
							fnTG24-TG51	*fnTG134*
							fnTG48-TG180	
							fnTG123-TG182	
fwTG24-TG51			*fwTG24-TG51*	*fwTG30*	*fwTG134*		(*fwEst4*)	(*fwPgm2-TG182*)
fwTG134				*fwTG48-TG180*	*fwTG48-TG180*		*fwAco1-TG68*	

"*Altered* QTLs", those that were detected under both conditions but where the degree of dominance changes depending on the presence/absence of salinity.
"*Response-sensitive* QTLs", those only detected under control condition or those whose contribution to the phenotypic value significantly decrease under conditions of salinity, such as *fwTG48-TG180*.
"*Response-tolerant* QTLs", those only detected under salinity or those where there is a change in the direction of gene effects depending upon the presence/absence of salinity, such as *fwTG68-Aco1*.

No constitutive QTLs for earliness were detected. Gene effects at all EA QTLs are affected by salinity; therefore, salinity seems to play a very important role in the regulation of this trait.

Three factors may be involved in the lack of detection of QTLs in one of the treatments:

(1) Sampling errors. This would also include differences in the recombination fraction. If in one of the treatments there is a random increase of the recombination fraction between a marker and a QTL of low contribution, this may prevent its detection.
(2) A change in the contribution of QTL to the genetic variance of the trait. This change could be as drastic as the lack of expression of the genes at the QTL under a certain growing condition. Another possibility would be that the differences in performance between alleles at the QTL under one environment disappear under the other, simply because none of them is better than the other in that environment.
(3) Epistatic interactions. Gene interactions affect the trait value. (Bretó et al. 1994; Lark et al. 1995; Monforte et al. 1997) and these interactions change depending upon the treatment. Might they mask allelic variation at QTL?. We think that this is possible because Lark et al. (1995) have shown that trait variation at a locus may be conditional upon a specific allele at another. If expression of this epistatic locus is blocked by the environment (a stress condition for instance) variation at the QTL will disappear.

Sampling errors could be important in QTLs with a low contribution, whereas the differential detection or contribution of the QTLs with large effects ($> 10\%$) should be more readily explicable by a change in the contribution of the QTL to the genetic variance. Present data does not allow us to study the relative importance of cause (3) to explain the lack of detection of QTLs in one of the treatments, mainly because powerful statistical methods for epistasis are not available in QTL analysis.

Sometimes, one of the two yield-components (FW or FN) was associated to a marker (s) locus, but the TW QTL was not detected in any treatment; hence, this lack of detection must be attributed to sampling errors and/or small changes in the contribution of the total weight components (FW and FN). These causes can also be applied to other QTLs with a low contribution ($< 5\%$), such as *fnTG18*, *fnTG63* in B, and *fnTG28* and *twTG68* in C. However, there are many other "response" QTLs that show an important contribution to the trait. A clear example, although less radical, of a sensitive QTL is *fwTG48-TG180*, which has a large contribution to fruit weight under control conditions but it is greatly reduced under salinity. This QTL is a very important one; Alpert et al. (1995) showed that it can be considered as a major QTL for fruit weight, contributing up to 47% of the phenotypic variance. We also reported a QTL with an important contribution to this trait under salinity at the same chromosomal location (Monforte et al. 1996, 1997), and in the present experiment we confirmed its importance, contributing up to 58% in family B (Table 2). The genetic background of family C must be the cause of the much lower effect of the very same "esculentum" allele in this family under both growing conditions. The important reduction of fruit weight under salinity can be explained, at least in part, by a reduction of the effect of the "esculentum" allele at this QTL. Genetic variability at this QTL within the cultivated species could be used to search for other allelic variants less sensitive to salinity.

Most detected QTLs are response-tolerant QTLs. These will determine an important proportion of the breeding value of the genotype under salt treatment. Some of them make a relatively important contribution to the trait (*eaTG23*, *eaTG43, eaTG180-TG48, twTG24-TG51, twTG123-TG182, fnTG24-TG51, fnTG134, fwPgm2-TG182, fwEst4*); so, their detection only under conditions of salinity must be due to an important change in the effects of the "esculentum" and wild alleles, or to differential gene expression, in the sense that they were not expressed under control conditions. The study of tolerant QTLs demonstrated that both cultivated and wild parentals present genes that could be masked by the rest of the genotype but which are involved in the tolerant response to salt stress.

Within tolerant QTLs, *fwTG68-Aco1* deserves a special mention. Its additive effect was to "esculentum" alleles in the control but changed to "pimpinellifolium" under conditions of salinity, showing a drastic change in gene action. It was detected by Paterson et al. (1991) in non-stress conditions where the direction was towards the "esculentum" allele. Eshed and Zamir (1995) also found it under non-saline conditions, but here the direction was towards the wild allele. *FwTG68-Aco1* must be an orthologous QTL present in both cultivated and wild tomato species, but no "esculentum" nor "pimpinellifolium" allele from those we have sampled is good for both the presence and absence of salinity. QTLs like this one limit adaptation to both environments, although genetic variability may exist at this QTL to overcome this problem. This refinforces the importance of treatment or environment in QTL detection and in the consequent design of the MAS scheme.

From the breeder's point of view, if only one environment is used for evaluations, salinity will make more QTLs available for response to selection, while response-sensitive QTLs will stay mute. The fact that fruit yield under control conditions showed a correlated response to selection by MAS based on QTL analysis under salinity (Monforte et al. 1996) is explained by the existence of constitutive QTLs; however, the large number of response-tolerant QTLs compared with the response-sensitive QTLs, suggests that fruit yield under salinity will not respond as efficiently to selection by a MAS scheme based on a QTL analysis under an optimum environment. Response-sensitive and -tolerant QTLs are specially important if breeding for wide-salinity adaptation is intended. If breeding for wide-salinity adaption is pursued, MAS should be directed not only to constitutive and response-tolerant QTLs but also to response-sensitive and altered QTLs, although in some cases selection for both saline and non-saline environments at some QTLs (*fwTG-68-Aco1*, for instance) may not be compatible.

Salinity changes the mode of gene action of that group of QTLs which we have named altered QTLs. This means that G × E interaction can change intralocus gene interactions. There are four genomic regions, TG24-TG51(58), TG48-TG180, TG134 and TG30, that are involved in fruit number and fruit weight, and two of them are also involved in earliness. We cannot distinguish between several closely linked QTLs or one QTL with pleiotropic effects, although under a given growing condition and family, their most likely position was different. The most likely position of these QTLs by interval mapping has not been ascertain because they may change from the middle of the interval to towards one flanking marker, and vice versa, depending on the family and the treatment. Nevertheless, we think this is not relevant because it can be explained first by the usually large size of the confidence intervals for the QTL positions (Hyne et al. 1995) and second by differences in genetic recombination. However, if in fact there is only one QTL then the pleiotropic effects must depend on the environment. The most dramatic change affects *fwTG24-TG51* in family C, from recessiveness under control conditions to overdominance under conditions of salinity. From control to salinity the changes are, from recessiveness to additivity, from additivity to dominance, overdominance or underdominance, and three mute QTLs for fruit number under control conditions showed overdominance under salinity. These results suggest that non-additive effects are very important under stress conditions. We think these changes affecting the mode of gene action should be taken into account to establish the best breeding strategy. Thus, a kind of combined selection, based on selection among plants depending on their performance under non-stress environment and the performance of their hybrid progeny under the stress condition, might be an advisable breeding strategy to improve resistance to salinity, or to any stress factor that changes the relative importance of the additive vs the non-additive components of the genotypic variance.

It has been shown that gene interactions changed depending on the family or genetic background (Monforte et al. 1997), and it is now shown they also change as a consequence of the presence/absence of salinity. Some markers (like TG43 for FW in cross B) have shown epistatic interactions with many QTLs involved in a trait (mainly under control condition); however, the marker itself is not significantly associated with the trait. This constitutes clear example of the epistatic expression of a QTL, as Lark et al. (1995) have found in soybean. Another important observation is that many marker loci (individually associated, or not associated, to a trait) are involved in more than one interaction, especially in fruit weight (Table 3). This suggests that multilocus associations are involved in the final expression of agronomically important traits, although we have only be able to study the two-locus level.

Our QTL-analysis approach to salt tolerance pursued two main objectives: to apply MAS in the improvement of salt tolerance of the tomato using wild related species (Bretó et al. 1993; Monforte et al. 1996), and to dissect the character "fruit yield". This dissection has allowed us to study important phenomena in quantitative genetics, such as heterosis and transgressive segregation, and to investigate the possible causes that make MAS inefficient: i.e. allelic variation at a QTL and epistasis (Monforte et al. 1997), and genotype-by-environment interaction (the present paper). Our conclusion completely agrees with Allard's description of biological complexity (Allard 1960): "virtually all phenotypic effects are not related to the gene in any simple way. Rather they result from a chain of physico-chemical reactions and interactions initiated by genes but leading through complex chains of events, controlled or modified by other genes and the external environment, to the final phenotype". Can we ignore this complexity when applying MAS? Is it possible to include the complexity (allelic variation, epistasis and G × E) in a MAS index? Will the selection response make the cost of applying this MAS index worthwhile? The dissection of quantitative traits using molecular markers has created a powerful method of analysis for these important characters; however, there still remains significant loose ends, especially regarding the relative importance of epistasis, and the extent to which stress environments increase the relative contribution of the non-additive components of genetic variance.

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References

Alpert KB, Grandillo S, Tanksley SD (1995) fw 2.2: a major QTL controlling fruit weight is common to both red- and green-fruited tomato species. Theor Appl Genet 91 : 994–1000

Allard, RW (1960) Principles of plant breeding. John Wiley. Chichester

Asíns MJ, Bretó MP, Carbonell EA (1993 a) Salt tolerance in *Lycopersicon species*. I. Character definition and changes in gene expression. Theor Appl Genet 86 : 737–743

Asíns MJ, Bretó MP, Cambra M, Carbonell EA (1993 b) Salt tolerance in *Lycopersicon* species. II. Genetics effects and a search for associated traits. Theor Appl Genet 86 : 769–774

Azhar FM, McNeilly T (1988) The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L.) moench seedlings. Plant Breed 101 : 114–121

Bretó MP, Asíns MJ, Carbonell EA (1993) Genetic variability in *Lycopersicon* species and their genetic relationships. Theor Appl Genet 86 : 113–120

Bretó MP, Asíns MJ, Carbonell EA (1994) Salt tolerance in *Lycopersicon* species. III. Detection of QTLs by means of molecular markers. Theor Appl Genet 88 : 395–401

Brown J, Caligari PD, Mackay GR, Swan GEL (1987) The efficiency of visual selection in the early generations of a potato-breeding program. Ann Appl Biol 110 : 257–263

Epstein E, Norlyn JD, Rush DW, Kingsbury RW, Kelley DB, Cunningham GA, Wrona AF (1980) Saline culture of crops: a genetic approach. Science 210 : 399–404

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennelli* in the cultivated tomato enables the identification and fine mapping of yield-associated QTLs. Genetics 141 : 1147–1162

Gibson TS, Spiers J, Brady CJ (1984) Salt tolerance in plants. II. In vitro translation of mRNAs from salt-tolerant and salt-sensitive plants on wheat-germ ribosomes. Responses to ions and compatible organic solutes. Plant Cell Environ 7 : 579–587

Gulick PJ, Dvorák J (1990) Selective enrichment of cDNAs from salt-stressed-induced genes in the wheat grass, *Lophopoyrum elongatum*, by the formamide-phenol emulsion reassociation technique. Gene 95 : 173–177

Hurkman WJ (1992) Effect of salt stress on plant gene expression: a review. Plant and Soil 146 : 145–151

Hyne V, Kearsey MJ, Pike DJ, Snape JW (1995) QTL analysis unreliability and bias in estimation procedures. Mol Breed 1 : 273–282

Igartua E (1995) Choice of selection environments for improving crops yields in saline areas. Theor Appl Genet 91 : 1061–1021

Johnson DW, Smith SE, Dobreuz AK (1992) Selection for increased forage yield in alfalfa at different NaCl levels. Euphytica 60 : 27–35

Kelman WM, Qualset CO (1991)Breeding for salinity stressed environments: recombinant inbrd wheat lines under saline irrigation. Crop Sci 31 : 1436–1442

Lark KG, Chase K, Adler F, Mansur LM, Orf JH (1995) Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proc Natl Acad Sci USA 92 : 4656–4660

Monforte AJ, Asíns MJ, Carbonell EA (1996) Salt tolerance in *Lycopersicon* species. IV. High efficiency of marker-assisted selection to obtain salt tolerant breeding lines Theor Appl Genet 93 : 765–772

Monforte AJ, Asíns MJ, Carbonell EA (1997) Salt tolerance in *Lycopersicon* species. V. May genetic variability at quantitative trait loci affect their analysis? Theor Appl Genet (in press)

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparisons across species, generations and environments. Genetics 127 : 181–197

Rawson HM, Richards RA, Mumms R (1988) An examination of selection criteria for salt tolerance in wheat, barley and Triticale genotypes. Aust J Agric Res 39 : 759–772

Richards RA (1983) Should selection for yield in saline regions be made on saline or non-saline soils? Euphytica 32 : 431–438

Saranga Y, Cabanes A, Zamir D, Marani A, Rudich J (1992) Breeding tomatoes for salt tolerance: inheritance of salt tolerance and related traits in interspecific populations. Theor Appl Genet 84 : 390–396

Thomas DSG, Middleton NJ (1993) Salinization: new perspectives on a major desertification issue. J Arid Environ 24 : 95–105