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ORIGINAL PAPER

Genetic analysis of physiological components of salt tolerance conferred by *Solanum* rootstocks. What is the rootstock doing for the scion?

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Abstract Grafting desirable crop varieties on stress-tolerant rootstocks provides an opportunity to increase crop salt tolerance. Here, a commercial hybrid tomato variety was grafted on two populations of recombinant inbred lines developed from a salt-sensitive genotype of *Solanum lycopersicum* var. *cerasiforme*, as female parent, and two salt-tolerant lines, as male parents, from *S. pimpinellifolium*, the P population, and *S. cheesmaniae,* the C population, to identify an easy screening method for identifying rootstocks conferring salt tolerance in terms of fruit yield. Potential physiological components of salt tolerance were assessed in the scion: leaf biomass, [Na^+], nutrition, water relations and xylem ABA concentration. A significant correlation between scion fruit yield and scion leaf fresh weight, water potential or the ABA concentration was found in the C population under salinity, but the only detected QTL did not support this relationship. The rootstocks of the P population clearly affected seven traits related to the sodium, phosphorous and copper concentrations and water content of the scion leaf, showing heritability estimates around 0.4 or higher. According to heritability estimates in the P population, up to five QTLs were detected per trait. QTLs contributing over 15% to the total variance were found for P and Cu concentrations and water content of the scion leaf, and the proportion of fresh root weight. Correlation and QTL analysis suggests that rootstock-mediated improvement of fruit yield in the P population under salinity is mainly explained by the rootstock's ability to minimise perturbations in scion water status.

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Introduction

Over 6% of the world's total land area is affected either by salinity or the associated condition of sodicity. Of the 1,500 million ha of land farmed by dryland agriculture, 32 million are affected by secondary salinity and of the 230 million ha of irrigated land, 45 million ha are also salt affected (FAO 2005). To sustain increases in food production in many regions of the world affected by salinity, increased salt tolerance of crops and horticultural species is needed (Munns 2005).

Cultivated tomato is widely adapted to different climates, but its growth and development is rather sensitive to salinity (Cuartero et al. 2006). Although several tomato wild species have been utilized for genetic and physiological characterization of salinity tolerance and for breeding purposes, improvement of tomato salt tolerance via conventional breeding has been minimal, thus new strategies are necessary (Cuartero et al. 2006; Foolad 2007). An alternative approach to improving selection efficiency is to identify genetic markers that are associated with genes or QTLs that control the traits of interest. In a previous study, salt tolerance in terms of fruit yield has been studied by QTL analysis using two *Solanum* populations of F_7 lines

(recombinant inbred lines or RILs) developed from a salt-sensitive genotype of *Solanum lycopersicum* var. *cerasiforme*, as female parent, and two salt-tolerant lines from *S. pimpinellifolium,* and *S. cheesmaniae* (Villalta et al. 2007) as male parents. Contrary to expectations, it was found that the wild allele (i.e. from the wild salt-tolerant genotype) was advantageous only at one total fruit yield QTL (*tw10.1*, on chromosome 10 near the salt-specific *fn10.1*). In fact, advantageous alleles at all fruit weight QTLs came from the cultivated, salt-sensitive, species.

Grafting is a biotechnological tool used since ancient times to improve the amount and uniformity of crop yield, and currently most fruit crops and many horticultural species are grown as scion–rootstock combinations. Although this strategy triples the work required by breeders (selection for rootstock, scion and the combination), it may allow desired features such as salt tolerance to be conferred by a suitable rootstock, while retaining excellent fruit yield and quality traits of the scion (Estañ et al. 2005; Martinez-Rodriguez et al. 2008). With this aim, Estañ et al. (2009) genetically analyzed the rootstock effect on the fruit yield of a grafted tomato variety under salinity using the same two populations of recombinant inbred lines (at F_9 generation) as rootstocks. The rootstock effect was heritable (H^2 near 0.3) and governed by at least 8 QTLs. Only two fruit yield QTLs on chromosomes P9 and C11 might correspond to fruit yield QTLs of the non-grafted lines, indicating their root system dependence. Since the advantageous allele generally came from the wild, salt-tolerant species, it was concluded that a more efficient utilization of wild germplasm would be via the improvement of rootstocks that confer salt tolerance, instead of introgression of beneficial QTL alleles into the genome of the cultivated tomato. Introgression would be a more long-term, expensive strategy if fruit tree species (where rootstock utilization is the rule) were the target crop.

With the aim of developing easy screening methods to identify suitable rootstocks conferring salt tolerance, fruit yield (Estañ et al. 2009) and different physiological traits were analyzed in the shoot of grafted plants that utilized two populations of RILs as rootstocks. In this study, the genetic relationship of potential physiological components of salt tolerance conferred by the rootstock were investigated by correlation analyses and searching for co-location of physiological and fruit yield QTLs.

Materials and methods

Two populations of F_9 lines (Villalta et al. 2007), developed from a salt-sensitive genotype *S. lycopersicum* var. *cerasiforme* (formerly *L. esculentum*) as female parent and two salt-tolerant lines from *S. pimpinellifolium* L. (formerly *L. pimpinellifolium*), and *S. cheesmaniae* (L. Riley) Fosberg (formerly *L. cheesmanii),* as male parents (the P population and C populations, respectively) were used to study vegetative and physiological components of salt tolerance.

Two vegetative traits, the proportions of the fresh root weight (FRWp) and dried root weight (DRWp), in relation to the weight of the aerial part of the plant, were evaluated using the two populations of RILs (non-grafted plants) under the same saline (15 dS m^{-1}) and greenhouse conditions as used previously (Villalta et al. 2007). Three plants per RIL from 139 F8 P lines, and 91 F8 C lines were evaluated 1 month after the saline treatment, 4 months after germination. This will be referred to as a non-grafted experiment.

The physiological traits were evaluated in leaves of a grafted commercial tomato hybrid using 123 F_9 P lines, and 100 F_9 C lines, as rootstock. Since the physiological basis of salt tolerance is different depending on the population (Villalta et al. 2008), each one was accordingly evaluated for a different set of traits in a different experiment (the P and C graft experiments).

The commercial tomato hybrid *S.* cv. Boludo (Bol) was used as scion in both grafting experiments. Boludo was also grafted onto roots derived from a different plant of the same genotype (Bol/Bol). Bol and Bol/Bol, non-grafted and self-grafted, plants were included as controls and to evaluate any physiological changes induced by the grafting process per se.

Grafting experiments were carried out as follows: seeds were germinated in a growth chamber under controlled conditions (28°C and 90% relative humidity in darkness). Grafting was performed when seedlings had developed 3–4 true leaves, seedlings were excised at the cotyledons, using the shoot as scion and the remaining cut stump as rootstock. Grafts were made immediately after cutting the plants and grafting clips were used to adhere the graft union. After grafting, seedlings were grown in the same growth chamber, where the environmental conditions were optimised for the growth of grafted plants: 25/18°C, 70 and 90% relative humidity in light (16 h) and dark conditions, respectively. During the light period, the irradiance tried to simulate the natural diurnal changes, with maximum and minimum photosynthetic photon flux density (400/700 nm) at plant level of 385 and 135 μmol m^{-2} s^{-1}, respectively except for the first day in which the grafted plants remained in the dark.

Six plants per line were transferred to a polyethylene greenhouse, in Murcia, Spain, after the grafts had established (2 weeks after grafting). The experimental design was in a randomized complete block replicated three times (two plants per line and per block). Plants were grown in inert substrate, using a drip irrigation system, with 4 l h^{-1} drippers, and normal fertilization for tomato culture (Cadahia 1995). One stem per plant was allowed to develop by

eliminating all axillary buds. The growing period was February–July. The temperatures varied between 16 ± 2 and 36 ± 2°C (during the culture) and the relative humidity between 40% and 75%.

Irrigation water of both experiments came from the "Transvase" Tajo-Segura rivers, CE = 0.85 dS m^{-1}. The salt treatment applied to the C population was 75 mM NaCl (CE = 8.6 dS m^{-1}) and to the P population, 125 mM NaCl (13.7 dS m^{-1}). The salt treatments were applied from 15 days after transplanting to the end of the experiment (120 days).

Twenty physiological traits were measured in Boludo grafted on the P population, six plants per RIL. These traits corresponded to nine leaf features: Na, K, Ca, Mg, P, S, Fe and Cu concentration (mmol kg^{-1} of dried weight) in the second (g2LNa, g2LK, g2LCa, g2LMg, g2LP, g2LS), and the fifth (g5LNa, g5LK, g5LCa, g5LMg, 5LP, g5LS, g2LFe, g5LFe, g2LCu, g5LCu) leaves and the water content (WC in mg g^{-1} of dried weight) of the two parts of these leaves, the rachis and the leaflets (g2RaWC, g5RaWC, g2LWC and g5LWC, respectively). Tissue samples of leaves were washed with deionized water, fresh weight determined, oven dried for 48 h at 80°C, weighed (dry weight) and prepared for mineral analysis by digestion in a HNO_3:$HClO_4$ (2:1, v/v) solution. Inorganic solutes were determined by inductively coupled plasma spectrometry (ICP) (Ionomic Service; CEBAS–CSIC, Murcia, Spain).

Three other traits were measured in Boludo leaves grafted on the C population where a previous study (Villalta et al. 2008) suggested involvement of ABA in gene effects on chromosome C1. About 50 days after the start of the salt treatment, the second leaf over the fourth truss (with actively growing fruits) of three plants per RIL was used for the determination of the leaf weight (gLFW in grams), as a measure of the invigorating rootstock effect on the grafted variety, and two physiological (leaf water potential and ABA concentration) traits in the graft. Leaf water potential (gHP in bar) was measured using a Scholander-type pressure chamber. Leaf xylem sap was obtained by applying a N_2-based pneumatic pressure slightly greater than the leaf water potential (Pérez-Alfocea et al. 2000). The sap was collected using a pipette, immediately frozen with liquid nitrogen and stored at −80°C until analysis. Sap ABA concentration (gABA in pmol mL^{-1}) was measured with a radioimmunoassay (Quarrie et al. 1988), using the monoclonal antibody AFRC MAC 252 (kindly provided by Dr. G Butcher, Babraham Bioscience Technologies, Cambridge, UK).

Broad sense heritability (H^2) was calculated for traits measured in both populations assuming individuals from the ninth self-pollinated generation were nearly homozygous for all loci. Heritability was calculated as reported previously by Villalta et al. (2007), using the formula: $H^2 = V_g/(V_g + V_e)$ where V_g and V_e are the estimates of genotype and environmental variance, respectively, by restricted maximum likelihood (REML).

Pearson's correlation coefficients were calculated between each trait and the scion fruit yield traits reported previously (Estañ et al. 2009): the weight (gFW) and number (gFN) of fruits and the total fruit weight (gTW). RIL means for fruit yield and physiological traits previously evaluated in the non-grafted P and C populations under saline conditions (Villalta et al. 2007, 2008, respectively) were also considered in correlation and QTL analysis.

Marker analyses and linkage maps used for the QTL analysis have been previously reported by Villalta et al. (2005) and updated in Villalta et al. (2007, 2008) and Estañ et al. (2009). A total of 156 markers genotyped for the P population and 134 markers for the C one were considered for QTL analysis. QTL analyses were carried out using the interval mapping procedure in MapQTL (Van Ooijen and Maliepaard 1996). Permutation tests were used to know the LOD scores that corresponded to overall 5 and 1% significance levels. Only QTLs detected above the 5% experimental-wise significance level are reported. When more than one QTL was detected within the same linkage group, composite interval mapping methodology was tried by using QTL-Cartographer (Basten et al. 2002), and stepwise selection of cofactors, to remove the variation that was associated with the other linked QTL. Epistatic interactions between linked QTLs were tested by two-way ANOVA using the QTL-linked markers.

Results

The rootstock clearly affected some scion traits. Thus, seven traits related to the Na, P and Cu concentrations and water content of the scion leaf showed heritability estimates around 0.4 or higher (Table 1). Analyses of the same trait (e.g. ion concentration) in two separate leaves (leaves 2 and 5) accounted for a similar amount of heritability.

Significant relationships (as determined by correlation analysis) between physiological and yield traits of the grafted RILs (gFW, gFN and gTW, reported by Estañ et al. 2009) are shown in Table 2. As suggested by the value of the correlation coefficients, gFN is mostly related to 2RaWC and 2LCa in the P population and gHP in the C population. In the case of gFW, the closest traits are g5LWC, g2LWC and g2RaWC in the P population and gLFW in the C population. Thus, gTW is mainly related to 2RaWC and gLFW, and, inversely to 2LCa and gHP.

The correlation between these traits and others that were significantly associated with the scion fruit yield here, and the others reported from previous salt tolerance experiments (Villalta et al. 2007, 2008) were also investigated.

Table 1 Variance components and heritability estimates (H^2) of traits

Population	Trait	V_g	V_e	H^2
C	gLHP	1.16	3.85	0.23
C	gLFW	63.23	106.31	0.37
C	gABA	302.70	1,144.50	0.21
C	FRWp	13.69	43.24	0.24
C	DRWp	9.60	31.64	0.23
P	g5LNa	237,072.00	354,496.50	**0.40**
P	g2LNa	273,567.00	388,590.00	**0.41**
P	g5LK	1,671.80	12,543.00	0.12
P	g2LK	655.41	12,415.80	0.05
P	g5LCa	226.73	15,745.90	0.01
P	g2LCa	1,403.80	44,734.50	0.03
P	g5LMg	153.86	1,289.30	0.11
P	g2LMg	768.43	4,261.30	0.15
P	g5LP	270.94	371.96	**0.42**
P	g2LP	468.40	515.79	**0.48**
P	g5LS	249.94	705.05	0.26
P	g2LS	654.06	2,098.70	0.24
P	g5LFe	0.05	0.51	0.09
P	g2LFe	0.00	1.98	0.00
P	g5LCu	0.00	0.01	0.37
P	g2LCu	0.01	0.01	**0.43**
P	g2RaWC	0.36	0.56	0.39
P	g5RaWC	0.18	0.38	0.32
P	g2LWC	0.50	0.45	**0.53**
P	g5LWC	0.55	0.54	**0.51**
P	FRWp	8.63	23.91	0.27
P	DRWp	6.96	44.54	0.14

Heritabilities equal or larger than 0.4 are indicated in bold

V_g genetic variance, V_e non-genetic variance

The correlation coefficients of the significant ($P \leq 0.05$) trait comparisons are presented in Table 3. In general, the highest coefficients were observed between Leaf 2 and Leaf 5 measurements of the same trait, and the two leaf components (leaflets and rachis). High coefficients were also found between the water content of the leaf and its Na concentration (particularly for leaf 5), between Ca and Mg concentrations for both leaves, between Cu and P concentration of Leaf 2, and, inversely, between Ca and Na concentrations of Leaf 5. In the C population, the strongest correlation involved gHP and gLFW. Notably, Na or K leaf concentrations of the grafted and the non-grafted RILs (from Villalta et al. 2008) were not significantly related and, contrary to expectations, the proportion of the root weight was not related to the scion fruit yield under salinity. In spite of the close relationship between gLFW and gTW in the grafted C RILs, and the relationship between leaf area (LA) and total fruit yield in the non-grafted Rils (Villalta et al. 2007), gLFW and LA were not correlated.

Table 2 Correlation coefficients of traits significantly correlated with the scion fruit yield

Population	Trait	gFW	gFN	gTW
P	g2LNa	**0.30**		0.18
P	g5LNa	**0.38**		0.25
P	g2LK		0.19	0.19
P	g5LK	0.19	0.19	0.22
P	g2LCa	**−0.26**	**−0.37**	**−0.41**
P	g5LCa	**−0.28**	**−0.26**	**−0.32**
P	g2LMg			−0.19
P	g5LMg	−0.25	−0.15	−0.21
P	g5LP	**−0.29**		
P	g5LFe	**−0.27**	−0.18	−0.25
P	g2LCu		**0.26**	0.22
P	g5LCu		**0.28**	0.23
P	g2RaWC	**0.39**	**0.35**	**0.44**
P	g2LWC	**0.39**	0.20	**0.31**
P	g5RaWC	**0.29**		0.20
P	g5LWC	**0.41**	0.24	**0.34**
C	gHP		**−0.36**	**−0.43**
C	gLFW	**0.38**	**0.33**	**0.56**
C	gABA		0.21	

Highly significant correlations ($P < 0.001$) are indicated in bold

A total of 59 QTLs controlling all evaluated traits were detected in both populations (Table 4), mainly in the P population. Thirteen of them are likely involved in the genetic control of the two levels of the same evaluated feature, or the proportions of both the fresh and dried root weights. In general, individual contributions of the reported QTLs were medium size (individual contributions from 7 up to 23.1% at most) and only 38 QTLs remained significant when considering the 1% threshold. Notably, some QTLs clusters in certain genomic regions, most of them containing fruit yield QTLs (linkage groups C1, P5a, P3 and P9 in Fig. 1).

Since two linked QTLs were detected by interval mapping for g5LP, g2LCu and FRWp on linkage groups p5a and p1b, composite interval mapping was used for locating multiple QTL there. QTLs *g5Lp5.1* and *g5Lp5.2* did not increase their LOD scores when considering QTL-linked markers SSRW115_240 and TG69_600 as cofactors but they remained significant (LOD scores were 2.21 and 2.49, respectively). Notably, these QTLs (QTL-linked markers SSR27-310 and SSRW43_460) were found to be epistatic ($P \leq 0.03$; Fig. 2). QTLs *g2LCu1.2* and *g2LCu1.1* increased their LOD scores (2.15 and 2.35, respectively) when considering QTL-Linked markers SSR41_200 and SSR9_180 as cofactors. And regarding linked QTLs for FRWp on p1b, *frwp1.1* and *frwp1.2,* both remained significant (LOD scores were 2.22 and 4.03, respectively) when using QTL-linked marker CT167_530 as cofactor.

Table 3 Correlation coefficients of significantly associated traits

g2LNa	g5LNa	g2LK	g5LK	g2LCa	g5LCa	g2LMg	g5LMg	g5LP	g5LFe	g2LCu	g5LCu	g2RaWC	g2LWC	g5RaWC	g5LWC	**TRAITS**	gLFW	gABA
																gHP	**−0.65**	−0.28
																SKC		0.23
																NLS	0.26	
				0.20								−0.18				LKC		0.24
	−0.22											−0.19				LKN		
					−0.18			0.19		0.20	0.21					SNC		
												0.18				DSW		
0.21	0.25											0.23	0.23	0.23	0.23	LA		
0.22	0.26	−0.18								0.23	0.26	0.21	0.26	0.21	0.23	TN		
0.22	0.19				−0.18				−0.21							SD		
−0.19									0.20	0.20	0.20					SH		
										0.18	0.20					FH		
		0.22								0.29	**0.30**					FRW		
0.29	0.24												0.20	0.18	0.20	L		
−0.23	−0.21							0.22					−0.19	−0.18	−0.22	a		
0.33	0.29							−0.22				0.21	0.25	0.24	0.26	b		
0.21	0.18			−0.18		−0.22		−0.26				0.23	0.25		0.26	Cl		0.28
										−0.32	−0.29	−0.19				FW		
	0.94		**0.34**		**−0.48**		**−0.35**	**−0.51**	**−0.46**	−0.19		**0.57**	**0.86**	**0.65**	**0.74**	g2LNa		
			0.36	−0.23	**−0.59**	−0.24	**−0.43**	**−0.53**	**−0.50**			**0.64**	**0.87**	**0.72**	**0.84**	g5LNa		
			0.67	**−0.30**						**0.33**	**0.31**	0.18				g2LK		
				−0.19	**−0.38**	−0.18	**−0.33**		**−0.31**			0.24	0.23	0.28	0.19	g5LK		
					0.58	**0.71**	**0.37**	0.21	**0.22**		−0.23	**−0.41**		−0.26	**−0.34**	g2LCa		
						0.39	**0.70**	**0.37**	**0.56**			**−0.38**	**−0.44**	**−0.43**	**−0.50**	g5LCa		
							0.65	**0.31**	0.19	0.18		−0.26		−0.21	−0.25	g2LMg		
								0.34	**0.42**			−0.23	−0.26	−0.25	−0.29	g5LMg		
								0.89		**0.58**	**0.53**		**−0.33**		**−0.31**	g2LP		
									0.31	**0.39**	**0.37**	**−0.35**	**−0.53**	**−0.41**	**−0.54**	g5LP		
										0.23	0.23	0.23	**0.38**	**0.36**	0.25	g2LS		
									0.30		0.18		0.23	0.27		g5LS		
									0.62	**0.46**	**0.38**		**−0.31**		**−0.36**	g2LFe		
											0.25	−0.29	**−0.38**	-0.27	**−0.45**	g5LFe		
											0.93	0.25				g2LCu		
												0.29		0.21		g5LCu		
													0.71	**0.68**	**0.75**	g2RaWC		
														0.74	**0.89**	g2LWC		
															0.80	g5RaWC		

Highly significant correlations ($P < 0.001$) are indicated in bold. Traits without the prefix g were evaluated in the non-grafted populations (Villalta et al. 2007, 2008)
SKC stem K^+ concentration, *NLS* Na^+ leaf sensitivity, *LKC* leaf K^+ concentration, *LKN* K^+/Na^+ ratio in leaves, *SNC* stem Na^+ concentration, *DSW* dried stem weight, *LA* leaf area, *TN* transported Na^+, *SD* stem diameter, *SH* number of days from sowing date till harvesting date, *FH* number of days from flowering date till harvesting date, *FRW* fresh root weight; *L*, *a*, *b* leaf colour parameters defined by Hunter coordinates; *Cl* Cl^- leaf concentration, *FW* fruit weight

The *S. pimpinellifolium* allele is most frequently associated with increasing additive values (negative *a* values in Table 4) in traits related to the P and Cu leaf concentrations, and leaf water content, while the lycopersicum allele is most frequently associated with increasing values for vegetative traits, gABA and S, Ca, Fe and Mg leaf concentrations.

Although the trait heritabilities were similar and not very low in the C population ($H^2 > 0.200$), only two minor putative QTLs were detected. However, in the P population where a broad spectrum of heritability values were available, the heritability estimates and the number of detected QTLs were significantly correlated ($P < 0.0001$, $r^2 = 0.67$).

Discussion

A plant's response to environmental stress is modulated by many physiological and agronomical characteristics, which may be controlled by the actions of several to many genes

Table 4 List of QTLs detected by using interval mapping procedure (5% overall significance level) for proportion of fresh and dried root weight (FRWp and DRWp, respectively) in the non-grafted RILs, and in the Boludo-grafted RILs for the other traits (with the g-prefix)

Population	Trait	Chr	Sig.5	Sig.1	Map	LOD	Marker(s)	QTL	PEV	*a*
C	gABA	c1	1.6	2.1	32.6	1.73	TG48_510	*gABA1.1*	8.3	7.80
C	FRWp	c11b	1.7	2.2	18.4	1.83	TG30_320	*frwp11.1*	8.4	1.77
P	g2LP	p1b	1.5	2.2	0	2	SSR30_300	*g2LP1.1*	7.3	5.23
P	g2LP	p2	1.7	2.3	30.1	2.36	SSRW104_900-CT156_870	*g2LP2.1*	10.2	−6.49
P	g2LP	p7	1.8	2.5	26	5.38	SSR22_344	*g2LP7.1*	19.5	−8.59
P	g2LP	p7	1.8	2.5	46.9	3.46	SSR34_750-SSRW565_396	*g2LP7.2*	17.4	−8.15
P	g5LP	p1b	1.5	2.1	0	3.33	SSR30_300	***g5LP1.1***	11.9	8.52
P	g5LP	p2	1.6	2.3	30.1	2.49	SSRW104_900-CT156_870	***g5LP2.1***	11.4	−8.79
P	g5LP	p5a	1.5	2.2	22.6	2.46	SSR27_310-SSR35_298	*g5LP5.1*	12.9	−8.92
P	g5LP	p5a	1.5	2.2	34.5	2.7	SSR21_900-SSRW43_460-TG69_600	*g5LP5.2*	9.7	−7.77
P	g5LP	p7	1.8	2.4	20.2	3.5	SSRW285_290-SSR22_344	***g5LP7.1***	15.3	−9.71
P	g2LS	p2	1.7	2.2	47.4	2.22	SSR9_220	*g2LS2.1*	8.7	−6.28
P	g2LS	p5a	1.4	2	35.9	3.5	SSRW344_430	*g2LS5.1*	12.8	7.60
P	g2LS	p8	1.6	2.3	30.2	2.26	SSR37_530-SSRW344_750	*g2LS8.1*	12	7.30
P	g5LS	p1b	1.5	2	2.6	1.93	SSRW44_700	*g5LS1.1*	7.2	9.74
P	g5LS	p2	1.8	2.4	47.4	1.8	SSR9_220	***g5LS2.1***	7.1	−9.65
P	g5LS	p5a	1.5	2.3	35.9	1.95	SSRW344_430	***g5LS5.1***	7.4	9.84
P	g5LFe	p1b	1.6	2.5	0	2.06	SSR30_300	*g5LFe1.1*	7.5	0.19
P	g5LFe	p6	1.7	2.3	32.7	1.86	SSRW11_490-CT283_700	*g5LFe6.1*	8.9	0.20
P	g5LFe	p8	1.8	2.3	52.6	2.58	SSRW38_250	*g5LFe8.1*	9.6	−0.21
P	g2LCu	p1b	1.6	2.2	32.2	1.88	SSRW75_175-SSR41_200	*g2LCu1.2*	9	−0.02
P	g2LCu	p1b	1.6	2.2	42.6	1.81	SSR41_200-NHX4	*g2LCu1.1*	7.9	−0.02
P	g2LCu	p4b	1.1	1.8	0.7	1.92	SSR31_130	*g2LCu4.1*	7.3	−0.02
P	g2LCu	p7	1.8	2.5	46.9	1.85	SSR34_750-SSRW565_396	*g2LCu7.1*	10.2	−0.03
P	g2LCu	p12	1.3	2.1	0	2.59	SSRW223_370	*g2LCu12.1*	9.5	−0.03
P	g5LCu	p3	1.7	2.3	49.9	2.06	TG134_118	*g5LCu3.1*	7.6	−0.03
P	g5LCu	p4b	1.1	1.8	0.7	1.87	SSR31_130	***g5LCu4.1***	7.2	−0.03
P	g5LCu	p7	1.7	2.4	51.9	2.22	SSR34_750-SSRW565_396	*g5LCu7.1*	15.5	−0.04
P	g5LCu	p12	1.3	2.1	2.6	2.88	SSRW94_450	***g5LCu12.1***	10.4	−0.03
P	g2LCa	p11	1.2	2.1	0	2.28	SSRW76_230	*g2LCa11.1*	8.6	19.72
P	g5LCa	p1b	1.5	2.1	7.2	1.98	CT167_530	*g5LCa1.1*	7.6	33.35
P	g2RaWC	p1b	1.6	2.2	0	2.25	SSR30_300	*g2RaWC1.1*	8.2	−0.21
P	g2RaWC	p3	1.7	2.3	49.9	4.01	TG134_118	*g2RaWC3.1*	14.2	−0.27
P	g2RaWC	p5a	1.4	2.3	33.1	2.78	SSR17_320	*g2RaWC5.1*	10.1	0.23
P	g2RaWC	p9	1.6	2.5	22.1	2.98	SSRW69_130-SSRW19_220	*g2RaWC9.1*	12.7	0.25
P	g5RaWC	p3	1.6	2.3	49.9	4.03	TG134_118	***g5RaWC3.1***	14.3	−0.20
P	g5RaWC	p5a	1.5	2.3	33.1	2.39	SSR17_320	***g5RaWC5.1***	8.8	0.16
P	g5RaWC	p7	1.8	2.7	83.5	1.86	SSR24_750	*g5RaWC7.1*	8.5	−0.16
P	g5RaWC	p8	1.7	2.6	25.2	2.66	SSR37_530-SSRW344_750	*g5RaWC8.1*	13.9	0.20
P	g5RaWC	p9	1.5	2.3	22.1	1.93	SSRW69_130-SSRW19_220	***g5RaWC9.1***	8.3	0.15
P	G2LWC	p1b	1.5	2.3	0	2.46	SSR30_300	*g2LWC1.1*	8.9	−0.24
P	G2LWC	p3	1.6	2.4	49.9	2.69	TG134_118	*g2LWC3.1*	9.8	−0.25
P	g2LWC	p5a	1.6	2.1	35.1	5.42	TG69_600	*g2LWC5.1*	18.6	0.35
P	g2LWC	p7	1.7	2.5	65.4	2.13	SSRW565_396	*g2LWC7.1*	7.9	−0.22
P	g5LWC	p1b	1.5	2.3	0	2.53	SSR30_300	***g5LWC1.1***	9.1	−0.25
P	g5LWC	p3	1.5	2.2	49.9	3.99	TG134_118	***g5LWC3.1***	14.2	−0.31
P	g5LWC	p5a	1.5	2.3	35.9	4.38	SSRW344_430	***g5LWC5.1***	15.7	0.34

Table 4 continued

Population	Trait	Chr	Sig.5	Sig.1	Map	LOD	Marker(s)	QTL	PEV	*a*
P	g5LWC	p7	1.8	2.5	65.4	2.16	SSRW565_396	***g5LWC7.1***	8.1	−0.24
P	g5LWC	p9	1.5	2.1	24.4	1.75	SSRW19_220	*g5LWC9.1*	6.4	0.21
P	g2LNa	p1b	1.5	2.2	0	2.47	SSR30_300	*g2LNa1.1*	8.9	−171.04
P	g2LNa	p5a	1.4	2	35.9	3.89	SSRW344_430	*g2LNa5.1*	14	217.98
P	g5LNa	p1b	1.5	2.2	0	3.04	SSR30_300	***g5LNa1.1***	10.9	−204.63
P	g5LNa	p5a	1.4	2	38.1	3.68	S_440	***g5LNa5.1***	13.2	237.65
P	g2LK	p4b	1	1.5	6.3	1.83	TG43_750-L_900	*g2LK4.1*	7	−18.75
P	g5LK	p6	1.7	2.4	3.3	1.93	SSRW47_220	*g5LK6.1*	7	17.11
P	g5LMg	p1b	1.5	2	7.2	2.15	CT167_530	*g5LMg1.1*	8.3	13.14
P	FRWp	p1b	1.7	2.5	2.6	2.22	SSRW44_700	*frwp1.1*	7.7	1.25
P	FRWp	p1b	1.7	2.5	17.2	4.01	CT167_530-SSRW75_175	*frwp1.2*	23.1	2.08
P	FRWp	p5a	1.5	2.1	34.5	7.42	SSRW43_460	*frwp5.1*	22.7	−2.08
P	DRWp	p1b	1.6	2.3	17.2	2.09	CT167_530-SSRW75_175	***drwp1.2***	12.2	1.71
P	DRWp	p2	1.7	2.4	60.4	2.22	SSR12_140	*drwp2.1*	8.5	1.46
P	DRWp	p5a	1.4	2	34.5	4.44	SSRW43_460	***drwp5.1***	14.1	−1.86

RILs were grown under salinity and belong to two connected populations, the P and the C populations (Pop). The Map position (map) of QTL peaks in the *Solanum* P or C chromosomes (Chr) (Villalta et al. 2008) is indicated in cM. The 5% (Sig.5) and 1% (Sig.1) significance LOD scores for each trait-linkage group combination estimated from 1,000 permutation tests each are also included. Co-location of QTLs governing related traits (i.e. dried vs. fresh or leaf 5 vs. 2) are indicated by the QTL name in bold

a the estimated additive value, *PEV* the percentage of explained variance

whose expressions are influenced by various environmental factors. In addition, stress tolerance is a developmentally regulated, stage-specific phenomenon; since tolerance at one stage of plant development is often not correlated with tolerance at other developmental stages (Cuartero et al. 2006). Efforts have been mainly made to identify QTLs for salt tolerance during seed germination and vegetative growth (Foolad 2007). However, in the case of crop plants, it is ultimately the yield under specific field conditions that will determine whether or not a gene or combination of genes (or QTLs) is of agronomic importance. Estañ et al. (2009) studied the genetic factors of the rootstock that conferred salt tolerance in terms of fruit yield of the scion (a hybrid tomato variety). Since fruit yield integrates a number of plant processes and can be both temporally and financially expensive to assess, we have studied other parameters which could be used to select rootstocks able to induce salt tolerance of the shoot genotype. The results reported here are centred on the rootstock and its effects on the vegetative and physiological traits that might explain such salt tolerance.

Salinity imposes on plants an osmotically induced water stress, by decreasing the availability of water in the external solution. Furthermore, ion-specific effects emerge from the gradual accumulation of salts in plant tissues, inducing nutritional alterations. Of the 16 traits that were significantly correlated with total fruit yield (gTW in Table 2), g2LCa, g2RaWC, gHP and gLFW showed relatively high coefficients. In spite of the relatively high correlation between gLFW and gTW, and the medium size heritability estimate of gLFW in the C population, no QTL has been detected for this trait (nor for gHP). This should prevent gLFW being used as a sole criterion to indirectly select rootstocks conferring salt tolerance. Taking into account both trait heritabilities and the number of QTLs involved, two criteria that are closely related in the P but not in the C population, it seems clear that the best predictors of gTW are related to the leaf water content, at least in the P population.

The ability of the rootstock to minimise any salinity-induced decrease in scion leaf water status appears to contribute to a higher fruit yield, consistent with interpretation of previous results in tomato. Increased average number of lignified cells in the xylem of salinised roots (compared to control plants) was interpreted by Sánchez-Aguayo et al. (2004) as an anatomical adaptation to salinity aiming to improve water flow through the plant. Another possible (non-exclusive) alternative might consist of invigorating rootstocks, i.e. rootstocks conferring an increased vascular cylinder area relative to that of the non-grafted variety, as an insurance against disruption of water column integrity under salinity. Alleles from wild germplasm might be valuable to enhance both of these rootstock features: vascular cylinder area and xylem cell lignification under salinity.

Although the main effect of long-term salinity is generally associated with ion-specific effects, salt tolerance is

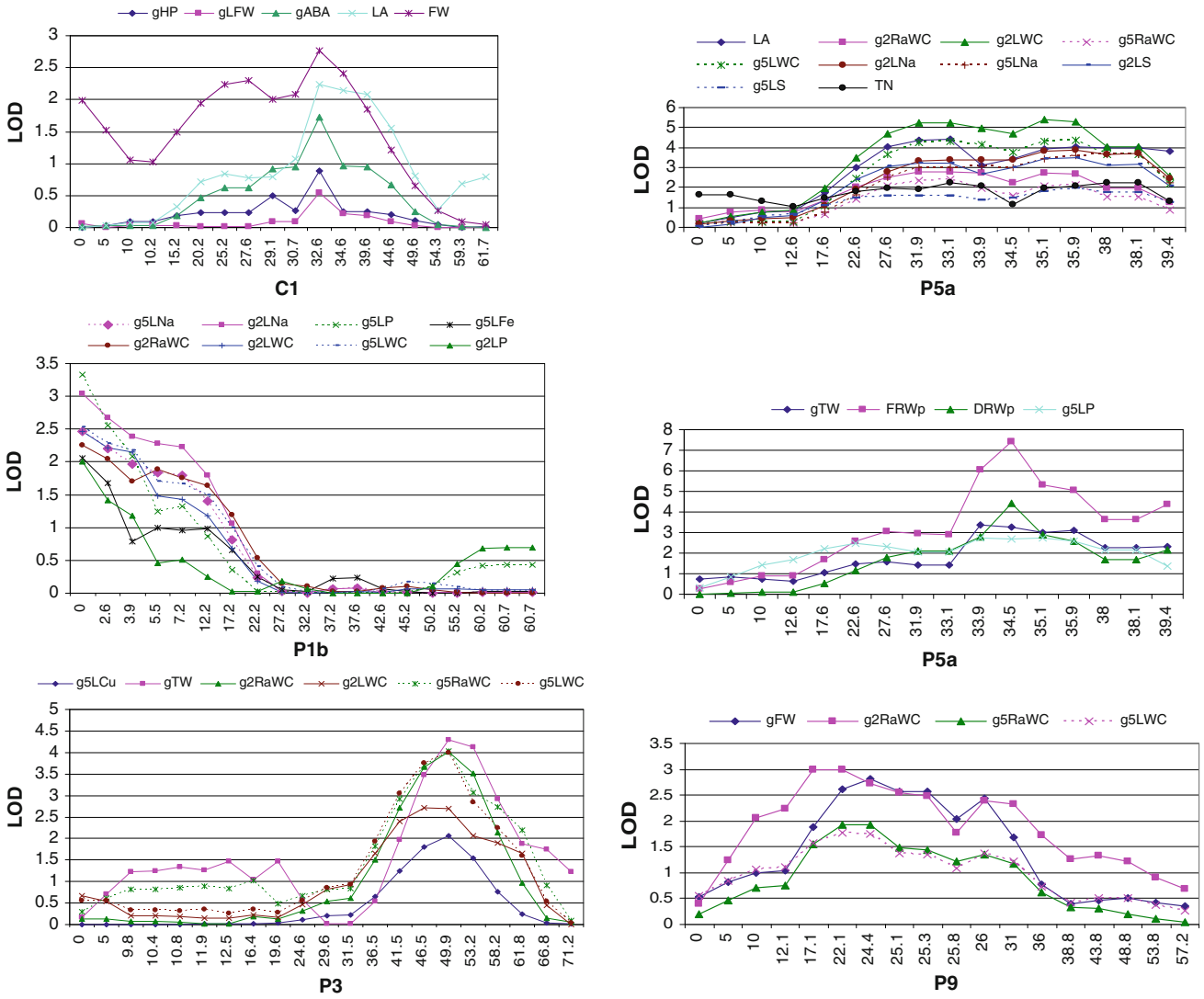

Fig. 1 LOD function along the linkage groups (C1, P5a, P1b, P3 and P9) containing clustering of QTLs here reported by using interval mapping procedure and MapQTL (Van Ooijen and Maliepaard 1996). In the case of C1, non-significant LOD function for traits gHP and gLFW, potentially related to gABA, were also included. Map positions in cM are indicated at the *X* axis where linkage group is indicated. FW and gFW traits correspond to the fruit weight of the non-grafted and the grafted RIL plant, respectively. The total fruit yield of the grafted RIL plant corresponds to gTW

not always associated with lower accumulation of sodium (Collins et al. 2008) but rather to the capacity to maintain ionic regulation (e.g. Estañ et al. 2005; Albacete et al. 2009). Indeed, the salt tolerance of wild tomato species has generally been related to the halophytic feature of Na^+ transport in the xylem (Pérez-Alfocea et al. 2000) and accumulation in the shoot (Bolarin et al. 1991; Tester and Davenport 2003) such that its osmotic contribution to maintaining leaf water status (Pérez-Alfocea et al. 1993, 2000) outweighs its detrimental salt-specific effects on biochemical processes contributing to growth and yield (Husain et al. 2003; Muñoz-Mayor et al. 2008). In the P population, it is interesting that leaf $[Na^+]$ and water content are significantly correlated, and gLNa QTLs co-locates with certain gLWC QTLs (Table 4) providing a genetic rationale to the association of both scion traits under salinity.

The positive relationship between fruit yield and water content (and Na accumulation) was accompanied by negative correlations with the leaf concentration of some nutrients, as P, Mg^{2+}, Fe^{3+}, and particularly, Ca^{2+} (Table 2). Correlation analysis of traits in Table 3 shows that $[Ca^{2+}]$ is positively correlated with $[Mg^{2+}]$ and $[Fe^{3+}]$ in the scion leaf suggesting their similar chemical properties influenced common uptake and transport patterns. Leaf $[Na^+]$ and $[Ca^{2+}]$ are inversely related explaining, at least in part, the negative correlation found between gTW and $[Ca^{2+}]$ in the absence of common QTLs for both traits. Since heritability

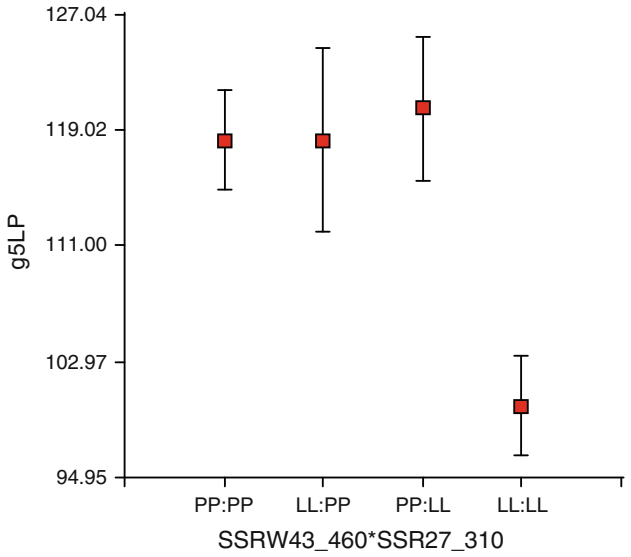

Fig. 2 Epistatic interaction affecting g5LP (*Y* axis, in mmol kg^{-1} of dried weight) between markers SSRW43_460 (linked to *g5LP5.2*) and SSR27_310 (linked to *g5LP5.1*). P corresponds to the *S. pimpinellifolium* allele and L to the *S. lycopersicum* allele

estimates for leaf [Ca^{2+}] are very low, the genetic base of the rootstock effect on this trait must be very narrow suggesting it is mostly a scion property. It is well known that high concentrations of Na^+ can lead to Ca^{2+} deficiency by replacing cell wall (and membrane) bound Ca^{2+} and by reducing root pressure-driven Ca^{2+} translocation to the shoot. In fact, elevated levels of ambient Ca^{2+} have long been known to relieve salinity stress (Marschner 1995).

Why is the leaf concentration of an important macronutrient such as P negatively related to fruit yield (gFW)? Similar to Ca^{2+}, the leaf [P] is inversely related to both leaf water content and [Na^+], suggesting it is also being diluted or translocated to somewhere else (e.g. growing tissues). But contrary to Ca^{2+}, the estimated broad sense heritability for leaf P concentration is high (such as the number of detected QTLs is), suggesting that the efficiency of leaf P accumulation under salinity will respond to selection.

Clustering of QTLs: co-location of physiological and fruit yield QTLs as an explanatory tool

Although the size of the QTL confidence interval limits genetic inferences from QTL analysis because of the large number of candidate genes included in a minimum of 10 cM, the position of the maximum significance for the QTL detection might be a good indication of the position of the responsible gene (Price 2006). The position of the maximum LOD score for some fruit yield QTLs was the same as that for some physiological QTLs (Fig. 1) suggesting a genetic rationale for some relationships. The fruit yield QTLs (gTW) were located on chromosomes 3, 5 and 9 (*gtw3.1*, *gtw5.1* and *gtw9.1*, respectively) (Estañ et al. 2009). Four QTLs (*g2RaWC3.1*, *g5RaWC3.1*, *g5LWC3.1* and *g5LCu3.1*) show their maximum LOD score where *gtw3.1* does. Moreover, the *S. pimpinellifolium* allele increases the trait value at all of them (Table 4). These results suggest the presence of closely linked genes or pleiotropic gene effects over these traits. Similarly, *gFW9.1* shows its maximum LOD score at the same position as *g5LW9.1*. Besides, the LOD curves of *g5LWC9.1*, *g5RaWC9.1* and *g2RaWC9.1* are very similar to that of *gfw9.1*, and the same allele (the *S. lycopersicum* one) increases the phenotypic values at these four QTLs. Again, this suggests that the main cause of a higher scion fruit yield is related to a greater capacity of the rootstock to minimise perturbation in scion water supply. In the case of *gtw5.1*, the results are not so easy to interpret because although many QTLs cluster on P5a, none shows the same position of maximum LOD score as *gtw5.1*. The closest QTLs are *g5LP5.2*, *frwp5.1* and *drwp5.1*; nevertheless, the *S. pimpinellifolium* allele is increasing the phenotypic values at them while decreasing it at *gtw5.1*. The *S. lycopersicum* allele increases the phenotypic value at *gtw5.1* and at the other QTLs clustered on P5a which are controlling the following correlated traits: leaf area (LA) and transported Na^+ (TN) of the non-grafted RILs, and g2RaWC, g2LWC, g5RaWC, g5LWC, g2LNa, g5LNa, g2LS and g5LS of the grafted RILs. Therefore, an explanation of the negative relationship between the P leaf concentration and fruit yield might be the close linkage (0.6 cM) between *gtw5.1* and *g5LP5.2* which differ in the increasing allele. The Mendelian dominant epistasis found affecting P leaf (5) concentration (Fig. 2) in which one locus, SSRW43_460 (linked to *g5LP5.2)* suppresses the allelic effects at SSR27_310 (linked to *g5LP5.1)* would facilitate to improve simultaneously scion fruit yield and leaf P concentration because it would require just to obtain the recombinant haplotype containing the *S. pimpinellifolium* allele at *g5LP5.1* and the *S. lycopersicum* allele at both *gtw5.1* and *g5LP5.2*. Notably, Estañ et al. (2009) also found dominant epistasis controlling rootstock effects in tomato and these interactions were unveiled using composite interval mapping methodology too.

During vegetative growth, ABA-mediated adaptive responses are critical to plant survival during drought, salt and cold stress. ABA accumulates as the soil dries or when salt stress occurs, and is apparently critical to stomatal control although its involvement in growth regulation is more ambiguous (reviewed in Dodd 2005). However, a major QTL originally reported for maize leaf ABA concentration (Tuberosa et al. 1998) was later shown to affect root size and architecture (Giuliani et al. 2005) and grain yield (Landi et al. 2007). For these reasons, QTL analysis of xylem ABA concentration (gABA) and other putatively related traits (water potential and leaf biomass) were also

considered as potentially relevant to tomato fruit yield under salinity, particularly when using the C population (Villalta et al. 2008). Although high ABA concentrations can decrease shoot growth but maintain root growth of maize grown at low water potential (Saab et al. 1990), no significant correlation between gABA and any measure of root weight ratio (FRWp or DRWp) was detected in the non-grafted C population. Only one putative QTL, *gABA1.1* was detected on chromosome C1 (Fig. 1). The position of its maximum LOD score (32.6 cM, where TG48-510 is located) is the same as those of *fw1.1* (Villalta et al. 2007), and *la1.1* and *dlw1.1* (Villalta et al. 2008); nevertheless, no fruit yield QTL for the grafted RIL was detected in this position (Estañ et al. 2009), suggesting *fw1.1* does not exclusively depend on the root system. Quarrie et al. (1997) also reported a QTL for ABA accumulation coincident with a QTL for leaf size in rice such that the association of high ABA with smaller leaves was interpreted as a consequence of genetic linkage. In our case, the *S. lycopersicum* allele at both, *gABA1.1* and *la1.1,* is associated with higher ABA concentration and larger leaf area (LA in non-grafted experiment), respectively, although no significant correlation was found between gABA and LA (Table 3). Moreover, no significant QTL for gFLW has been detected, in agreement with the lack of association between leaf biomass and ABA concentration in the grafted C population. Therefore, our results agree with those showing that xylem ABA concentration had no clear effect on maize leaf elongation response to soil water deficit (Voisin et al. 2006), and that xylem ABA concentration alone (independent of its ratio with other xylem hormone concentrations) was not significantly correlated with tomato leaf biomass under salinity (Albacete et al. 2009) .

An association between gABA and fruit yield (gFN in Table 2) has been found that would be better interpreted through the association of gABA with gHP rather than with gLFW. However, QTL analysis of these traits in the C population does not support the positive association between fruit yield and gLFW or gHP in the grafting experiment using the C RILs as rootstocks, suggesting these traits would be mostly controlled by the non-additive component of the genetic variance.

Estañ et al. (2009) reported that only two fruit yield QTLs of 8, on chromosomes P9 and C11, might correspond to fruit yield QTLs of the non-grafted lines indicating their exclusive dependence from the root system. Present results have shown that the rootstock-dependent fruit yield QTL on chromosome P9 co-locates with a QTL for leaf water content (*g5LW9.1*), and that another on chromosome C11b locates near a QTL controlling the proportion of fresh root weight (*frwp11.1*). Therefore, those genomic regions seem agronomically important for the adaptive development of the root system in tomato and deserve further attention.

In conclusion, the improvement in fruit yield under salinity attained by grafting is explained, at least partially, by the rootstock's ability to minimise perturbations in scion water status and leaf fresh weight. This conclusion is supported by the co-location of scion water content and yield QTLs in the P population, providing a valuable criterion for indirect selection of rootstocks conferring salt tolerance. To our knowledge, this is the first QTL study of rootstock effects on physiological scion traits trying to identify a genetic base for correlations which is not possible when using variation from collected germplasm.

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