

Germination variation in *Arabidopsis thaliana* accessions under moderate osmotic and salt stresses

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- **Background** Water and salt stresses are two important environmental factors that limit the germination of seeds in most ecological environments. Most studies conducted so far to address the genetic basis of the above phenomenon have used stress conditions that are much more extreme than those found in natural environments. Furthermore, although an excess of ions and water restrictions have similar osmotic effects on germination, the common and divergent signalling components mediating the effects of both factors remain unknown.
- **Methods** The germination of seeds was compared under solutions of NaCl (50 mM) and polyethylene glycol (PEG, -0.6 MPa), that establish mild stress conditions, in 28 *Arabidopsis thaliana* accessions. Because Bayreuth (Bay) and Shadara (Sha) accessions showed contrasting sensitivity responses to both stresses, a quantitative trait locus (QTL) analysis was carried out using Bay \times Sha recombinant inbred lines (RILs) to identify loci involved in the control of germination under mild salt and osmotic stresses.
- **Key Results** Two loci associated with the salt sensitivity response, named *SSR1* and *SSR2* QTLs, and four loci for the osmotic sensitivity response, named *OSR1*–*OSR4* QTLs, were mapped. The effects of the *SSR1* QTL on toxic salt sensitivity, and the osmotic contribution of *OSR1*, were confirmed by heterogeneous inbred families (HIFs). Whilst the *SSR1* QTL had a significant effect under a wide range of NaCl concentrations, the *OSR1* QTL was confirmed only under moderate drought stress. Interestingly the *OSR1* QTL also showed pleiotropic effects on biomass accumulation in response to water deficit.
- **Conclusions** The regulation of germination under moderate salt and osmotic stresses involves the action of independent major loci, revealing the existence of loci specifically associated with the toxic component of salt and not just its osmotic effect. Furthermore, this work demonstrates that novel loci control germination under osmotic stress conditions simulating more realistic ecological environments as found by populations of seeds in nature.

Key words: Germination, osmotic stress, salt stress, natural genetic variation, *Arabidopsis thaliana*, quantitative trait loci (QTLs), heterogeneous inbred family (HIF).

INTRODUCTION

Seed germination and seedling establishment are the most critical stages for survival during the life cycle of an individual plant. It is well known that osmotic and matrix potentials narrow the range of germination under non-optimal conditions (Hegarty and Ross, 1978). As the soil dries, or salt levels build up, water and osmotic potentials in the soil decline, limiting the germination of seeds that become conditioned by negative water potentials. Although the underlying molecular mechanisms involved in the adaptation of seed germination to water restrictions and salt excesses are unclear as yet, the action of common and divergent components of the osmotic and salt signalling pathways has been postulated. Seed responses to abiotic stresses probably involve and integrate an interconnected signalling network to cope with drought and salinity. Plant microarray studies using roots, leaves and growing tissues demonstrate the presence of both abscisic acid (ABA)-independent and ABA-dependent regulatory systems governing different stresses (Shinozaki and Yamaguchi-Shinozaki, 2007). These pathways lead to the expression of some common downstream stress-responsive proteins involved in numerous

biological processes, and also induce the expression of many genes with regulatory functions. These genes are probably involved not only in regulating downstream stress responses but also in stress perception and signalling (Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2007).

Albeit that significant advances have been made in understanding salt tolerance mechanisms acting during vegetative growth, the molecular events that trigger germination in the presence of salts are still far from being fully understood (Zhu, 2002; Munns and Tester, 2008). Salt stress affects plant growth at various stages of development including germination and establishment, vegetative growth and finally reproduction and yield. Different tolerance mechanisms are displayed by plants to cope with salt stress (Zhu, 2002). The relative importance of these mechanisms varies between species, with the length of exposure to the salinity, the concentration of the salt and the local environmental conditions such as water supply and air humidity determining the transpiration rate and leaf water potential (Munns and Tester, 2008). One central aspect of the physiological mechanisms responsible for the salinity tolerance is the action of two distinct phases: the osmotic phase, which is the first phase immediately after

the perception of salts, and the second ion-specific phase that occurs later, starting when salt accumulation reaches toxic concentrations (Bliss *et al.*, 1986; Munns and Tester, 2008).

The quantitative property of germination allowed analysis of the natural genetic variation associated with this response by quantitative trait locus (QTL) analysis (Alonso-Blanco *et al.*, 2009). Some studies explored genetic variation in the release of seed dormancy by the action of different environmental factors through QTL mapping analysis in *Arabidopsis thaliana* (Alonso-Blanco *et al.*, 2003; Clercx *et al.*, 2004; Laserna *et al.*, 2008; Meng *et al.*, 2008; Bentsink *et al.*, 2010). Furthermore, other works documented natural variation in seed germination constrained by osmotic and salt stresses (Quesada *et al.*, 2002; Clercx *et al.*, 2004; Joosen *et al.*, 2010). Between three and five QTLs were found in seeds incubated in highly concentrated salt solutions (Quesada *et al.*, 2002; Clercx *et al.*, 2004; Joosen *et al.*, 2010), and only one was found for germination on a mannitol-osmotic solution (Clercx *et al.*, 2004). The experimental conditions used in those studies strongly impair the interpretation of the results obtained. First, the rate of germination was evaluated after 2 weeks of seed incubation in NaCl, a condition under which the osmotic effects of the salt could be underestimated due to the toxic effects displayed in long-term treatment (Munns and Tester, 2008). Secondly, the concentration of NaCl salt used was extremely high (between 100 and 250 mM) and, consequently, the conclusions regarding the biological meaning of the loci identified are restricted to extreme ecological situations. We suggest that experimental designs using moderate stresses will be more informative to understand the genetic architecture of germination under more realistic salt and drought stresses.

In spite of the extensive genetic variation present in *A. thaliana*, and the importance of the adaptation of seeds to salt and drought stresses, only a few studies have been conducted to analyse the genetic variation of these traits in this species (Alonso-Blanco *et al.*, 2009). Here the natural genetic variation in seed germination under moderate osmotic and NaCl stresses was analysed using 28 accessions. By QTL analysis using recombinant inbred lines (RILs) originating from a cross between Bay and Sha accessions, four loci related to the osmotic sensitivity response, named *OSR1–OSR4*, and two different loci associated with the salt sensitivity response, named *SSR1* and *SSR2*, were mapped. The localization and the toxic salt component of the *SSR1* QTL on the top of chromosome 1, and the osmotic component of the *OSR1* QTL in the middle of chromosome 4 were confirmed by developing heterogeneous inbred families (HIFs).

MATERIALS AND METHODS

Genetic material

Twenty-eight *Arabidopsis thaliana* accessions that have been collected from a wide range of latitudinal and environmental conditions were used to evaluate seed germination under osmotic and salt stress conditions. Seeds of different accessions and the core set of 160 RILs originating from the cross between Bay and Sha (Loudet *et al.*, 2002) were obtained from the ABRC. To confirm the presence and the allelic

effects of the *SSR1* and *OSR1* QTLs, HIFs were developed following the protocols used in Tuinstra *et al.* (1997). For the *SSR1* QTL, we used RIL98 and 183 (both polymorphic at marker F21M12); and for the *OSR1* QTL we used RIL111, RIL120 and RIL195 (polymorphic at markers MSAT4-18, CIW7 and MSAT4-15, respectively).

Germination conditions

Seeds of all genotypes used in this study were harvested from plants cultivated together in a continuous white light chamber at 22 °C. Plants were cultivated in pots (8 × 10 cm, diameter × height) filled with soil (2/3 topsoil and 1/3 vermiculite) and watered periodically with HAKAPHOS nutrient solution (Compo, Barcelona, Spain). Mature seeds were harvested and after-ripened between 12 and 18 months. The seeds were kept dry in darkness at room temperature until they were used in the experiments.

For germination experiments, seeds were sown on filter paper (Watmann, Analen SRL, Buenos Aires, Argentina) imbibed in water, NaCl or PEG6000 solutions contained in transparent plastic boxes (21 × 9 cm). Seeds were incubated at 4 °C for 5 d in darkness and then irradiated with a red pulse for 1 h. After irradiation, seeds were incubated at 25 °C in darkness for 4 d until germination was recorded. To normalize the effect of each stress on germination, the percentage sensitivity response was calculated as: osmotic sensitivity response = 100 × (germination in water – germination in PEG solution)/germination in water; and salt sensitivity response = 100 × (germination in water – germination in NaCl solution)/germination in water.

To evaluate the specific action of the *SSR1* QTL in NaCl, the salt sensitivity response of seeds exposed to different doses of LiCl and NaCl was compared. If a common mechanism of transport were acting through the cell membrane for NaCl and LiCl, similar sensitivity germination responses under both salt solutions would be expected (Mendoza *et al.*, 1994). To discriminate the osmotic from the toxic component of NaCl, different iso-osmotic concentrations of KCl salt, which is not toxic for germination, were used. All the experiments were repeated at least twice with three replicates of 25 seeds. Means between treatments were compared with the LSD test at $P < 0.05$.

QTL mapping analysis

Marker segregation data for the Bay × Sha RIL population were obtained from <http://dbsgap.versailles.inra.fr/vnat/>. We used 38 markers that cover the five chromosomes with an average genetic distance of 10.8 cM between markers (Loudet *et al.*, 2002). MAPMAKER/EXP 3.0 (Lander *et al.*, 1987) was used to construct the linkage map. Linkage groups were verified with a minimum LOD = 3 and a maximum distance = 50 cM (Kosambi function). Both the linkage map data and the phenotypic data were then imported to QTL Cartographer version 2.0 obtained from <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm> (Wang *et al.*, 2004). Between 107 and 118 RILs were used for the QTL analysis, and the percentage means were transformed (arcsine) to improve the normality of distributions when necessary. The likelihood,

location, additive effect and percentage of variance explained by each QTL were calculated using model 6 based on the composite interval mapping (CIM) method (Zeng, 1994). QTL cofactors were initially selected by using forward–backward stepwise multiple regression. Mapping was conducted with a walking speed = 0.5 cM and a window size = 3 cM. For precise determination of significant QTLs, the thresholds of LOD for each linkage group were calculated by a permutation test method (Doerge and Churchill, 1996) with 1000 permutations at the permutation significance level ($P < 0.05$). The LOD varied from 2.4 to 2.7 for the different traits. The support interval of each QTL was constructed using the 2-LOD rule, with the confidence intervals being defined by all those values falling within the 2-LOD score of the maximum value (Lynch and Walsh, 1998). To perform fine mapping of the *SSR1* and *OSR1* QTLs, a new QTL analysis was conducted for chromosome 1 and 4 using the phenotypic data and an additional set of 65 single feature polymorphism (SFP) markers mapping on the top of chromosome 1 and in the middle of chromosome 4 (West et al., 2006). Epistatic analysis, at those positions where at least one of the traits showed a significant QTL, was performed using the computer program EPISTAT (Chase et al., 1997) with log-likelihood ratio (LLR) thresholds corresponding to a significant value of $P < 0.0005$. Ten thousands trials were used in Monte Carlo simulations performed with EPISTAT to establish the statistical significance of the LLR values for the interactions detected (Chase et al., 1997).

RESULTS

Natural genetic variation in seed germination under moderate saline and osmotic stresses among accessions of *Arabidopsis thaliana*

To study natural variation on seed germination under moderate levels of salt and osmotic conditions, the germination of four *Arabidopsis* accessions was analysed in a range of different concentrations of NaCl and PEG. As expected, a wide phenotypic variation was found at intermediate levels of both stresses (Fig. 1). All accessions germinated close to 100% on water and <25% at the highest stress levels (e.g. 100 mM NaCl and -0.8 MPa using a PEG solution). The widest variation

between genotypes was found using 50 mM NaCl and -0.6 MPa, and these levels of saline and osmotic stresses were used in the subsequent experiments.

The natural genetic variation of germination was analysed under moderate saline and osmotic stresses in 28 accessions of *A. thaliana* (Fig. 2A,B; Supplementary Data, available online). Germination on water was >75% for almost all accessions with the exception of Cvi seeds that were 67% germinated in agreement with previous studies with a dormant genotype (Alonso-Blanco et al., 2003; Laserna et al., 2008). Genetic variation was observed when seeds of different accessions were incubated under mild osmotic and salt stresses (Fig. 2A,B). The stress response of seeds was normalized to their germination on water (i.e. non-dormant seeds), calculating the sensitivity response under osmotic and saline stresses for each accession (Fig. 2C). Seeds of Cvi and Bay showed the highest sensitivity responses because germination was almost completely inhibited under moderate saline and osmotic stresses (Fig. 2). In contrast, Sha and Oy-0 displayed the lowest sensitivity responses because germination was only slightly affected by the same stresses (Fig. 2). The range of variation in salt and osmotic sensitivity responses was between 0 and 92% and between 16 and 96%, respectively. A high correlation was found between osmotic and salt sensitivity responses (Fig. 2, $R^2 = 0.76$). The RLD accession was an exception because it displayed a very high osmotic sensitivity response, but a reduced salt sensitivity response (93 and 38%, respectively; Fig. 2C).

The correlation between salt and osmotic sensitivity responses of 28 accessions and their geographical distributions and climatic parameters was analysed using available information at <http://dbsgap.versailles.inra.fr/vnat/>. Low functional relationships between sensitivity responses and the geographic (latitude and longitude) and climatic (rain and temperature) parameters of their origins were found for the accessions ($R^2 < 0.2$ for all comparisons).

QTL mapping for salt and osmotic sensitivity responses in the Bay × Sha RIL population

Two independent experiments were carried out to evaluate germination under moderate osmotic and saline stresses. The RILs generated from the cross between Bay and Sha were used for the QTL mapping analysis. The germination of RIL

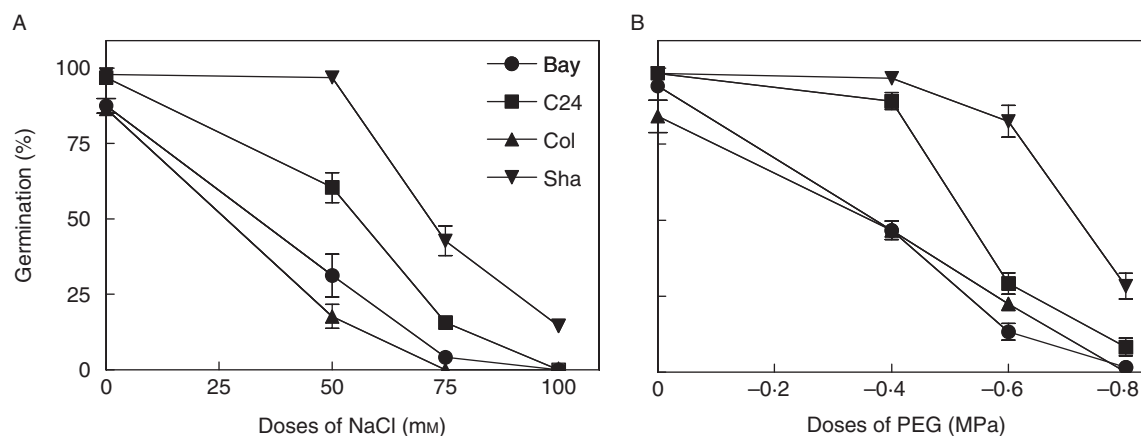


FIG. 1. Germination (%) at different doses of NaCl and PEG for Bay, C24, Col and Sha. Means \pm s.e.m. are shown.

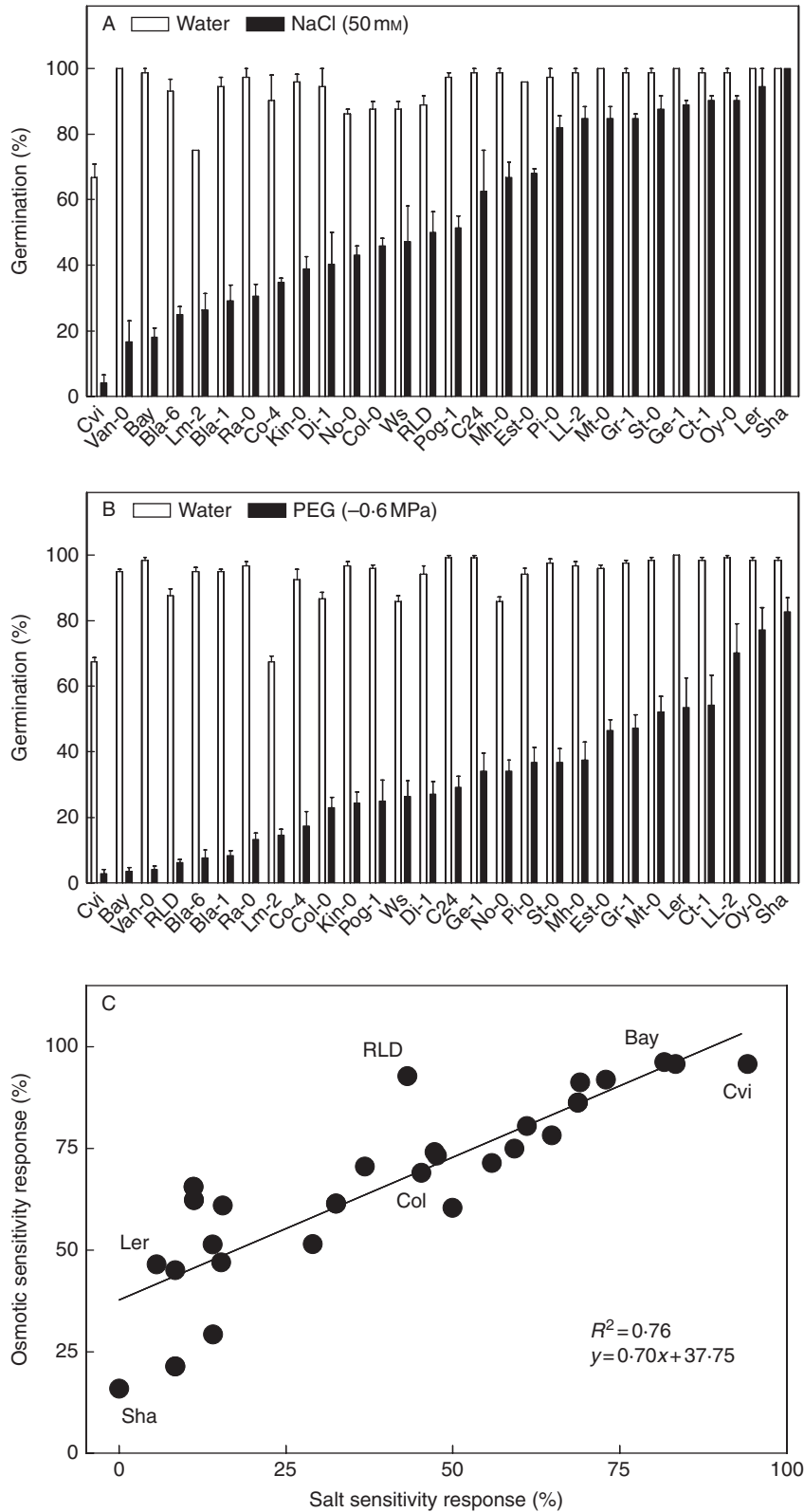


FIG. 2. Germination (%) for 28 accessions of *Arabidopsis thaliana* under water or (A) NaCl (50 mM) and (B) PEG (-0.6 MPa). Bars represent means \pm s.e.m. (C) Positive correlation between osmotic and salt sensitivity responses in 28 accessions of *A. thaliana*. Most representative accessions are indicated. Sensitivity responses (%) were calculated as $[100 \times (\text{germination in water} - \text{germination under stress}) / \text{germination in water}]$. The linear correlation equation and the R^2 are shown between the NaCl and osmotic sensitivity responses.

seeds displayed transgressive variation, suggesting that osmotic and salt sensitivity responses are under the control of several loci (Table 1; Supplementary Data). Heritabilities were >0.55 for all traits, indicating the presence of genetic variation for germination under water, osmotic and salt conditions (Table 1). The low correlation found between both stress sensitivity responses suggests that both traits are partially regulated by different loci (coefficient of correlation: 0.567). In agreement with this, specific QTLs were involved in the control of osmotic and salt sensitivity responses (Tables 2 and 3). We identified five QTLs for seeds germinating on water (i.e. GW QTLs), some of which co-localized with those mapped for osmotic and salt sensitivity responses (Tables 2 and 3).

Two QTLs termed *SSR1* and *SSR2* and four loci termed *OSR1*–*OSR4* were mapped. The arbitrary number of the QTLs corresponded to the order of relative significance of the QTL output (Tables 2 and 3). In five of them (i.e. with the exception of the *SSR2* QTL), the Bay alleles increased the sensitivity response, and these results are in agreement with the higher sensitivity responses of the Bay accession compared with the Sha accession (Fig. 2 and Table 1). Epistatic analysis, at those positions where at least one of the traits showed a significant QTL, revealed null epistatic effects for the osmotic sensitivity response and a significant

interaction between *SSR2* and MSAT5-22 marker (Table 3). The QTL analyses explained 53 and 62 % of the total phenotypic variance for osmotic and salt sensitivity responses, respectively (Tables 2 and 3).

The *SSR1* QTL was the major locus under salt stress (LOD = 6.04, and explained 20.4 % of the total phenotypic variation), and the *OSR1* QTL was the most relevant locus under osmotic stress (LOD = 6.7, and explained 26 % of the total phenotypic variation). Using a high density set of SFP markers, we fine-mapped *SSR1* and *OSR1* QTLs, reducing their confidence intervals to a region of 3.1 and 1.1 Mb, respectively (Figs 3A and 4A).

Confirmation of the *SSR1* QTL

HIFs were constructed to validate the results of the QTL analysis using the methodology proposed by Tuinstra *et al.* (1997). Two F₆ RILs were chosen as the starting material because they were heterozygous for the F21M12 marker, the closest marker to the *SSR1* QTL, and fixed as homozygous in almost all the rest of the genome. F₇ offspring from RIL98 and RIL183 were genotyped at the F21M12 marker and two independent HIFs fixed for Bay or Sha alleles were used for a posterior analysis (Fig. 3B). Comparing the salt and osmotic sensitivity responses of both independent HIFs,

TABLE 1. Quantitative genetic parameters for seed germination (%) in water, PEG (–0.6 MPa) and NaCl (50 mM), and osmotic and salt sensitivity responses for Bay and Sha parental lines and the RIL population

	Osmotic stress			Salt stress		
	Water	PEG	Osmotic sensitivity response	Water	NaCl	Salt sensitivity response
Bay	80 ± 20	22 ± 20	65 ± 35	97 ± 2	51 ± 38	46 ± 38
Sha	98 ± 3	96 ± 0	2 ± 2	100	94 ± 2	6 ± 2
RIL	70 ± 26	46 ± 30	41 ± 27	54 ± 27	14 ± 17	79 ± 21
Max–min (RIL)	100–0	99–0	100–0	100–0	84–0	100–16
H ²	0.77	0.75	0.55	0.82	0.83	0.70

Independent experiments were done for the evaluation of seed germination under osmotic and salt stresses.

TABLE 2. QTL mapping for germination in water and PEG, and osmotic sensitivity response in the Bay × Sha RIL population

QTL	Closest marker	Map position (cM)	Germination in water				Germination in PEG (–0.6 MPa)				Osmotic sensitivity response			
			LOD	Var. (%)	Add (%)	Interval (cM)	LOD	Var. (%)	Add (%)	Interval (cM)	LOD	Var. (%)	Add (%)	Interval (cM)
<i>OSR1</i>	MSAT4-15	C4/32.3	4.5	10	–15	24–43	10.4	32	–25	27–42	6.7	26	28	27–41
<i>OSR2</i>	ATHCHIB2	C3/12.8					3.5	10	–14	0–24	3.6	15	11	0–24
<i>OSR3</i>	MSAT2-36	C2/29.3	9.9	23	–21	21–36	6.3	16	–17	20–34	3.4	11	9	16–34
<i>OSR4</i>	NGA248	C1/26.1					5.4	17	–19	17–40	2.7	10	9	15–40
<i>GW1</i> *	MSAT5-12	C5/63.9	5.5	14	17	54–74								
<i>GW2</i>	MSAT4-9	C4/55.9	3.9	9	–15	48–66								
<i>GW4</i>	NGA128	C1/51.6	6.4	17	–19	31–54								
<i>GW1</i> × MSAT4-39								13						
Total phenotypic variance				73				88				62		

For each QTL, the closest marker, mapping position (indicated by the chromosome and distance in cM), maximum LOD, explained phenotypic variance (Var. %), 2 × additive effects (Add; %) and confidence interval are shown. Positive additive effects indicate a higher Bay allele contribution and negative values indicate a higher Sha allele contribution to the trait.

**GW1* co-localized with *SSR2* (see Table 3).

TABLE 3. QTL mapping for germination in water and NaCl, and salt sensitivity response in the Bay × Sha RIL population

QTL	Closest marker	Map position (cM)	Germination in water				Germination in NaCl (50 mM)				Salt sensitivity response			
			LOD	Var. (%)	Add (%)	Interval (cM)	LOD	Var. (%)	Add (%)	Interval (cM)	LOD	Var. (%)	Add (%)	Interval (cM)
SSR1	F21M12	C1/6-7	6.1	16	-17	6-21	8.7	23	-10	3-16	6.2	20	18	0-14
SSR2	MSAT5.12	C5/68.9	5.3	14	15	49-75	9.3	27	11	65-76	6.0	20	-18	64-75
SSR2 × MSAT5.22														
GW2	MSAT4.9	C4/54.9	5.4	14	-16	47-62	4.5	11	8					
GW3*	MSAT2.36	C2/18.5	6.8	18	-17	8-27								
GW4	NGA128	C1/51.6	3.1	8	-12	43-64								
Total phenotypic variance				70				61				53		

*GW3 co-localized with *OSR3* (see Table 2).

For each QTL, the closest marker, mapping position (indicated by the chromosome and distance in cM), maximum LOD, explained phenotypic variance (Var.; %), 2 × additive effects (Add; %) and confidence interval are shown. Positive additive effects indicate a higher Bay allele contribution and negative values indicate a higher Sha allele contribution to the trait.

the localization and the additive effects of the *SSR1* QTL were confirmed (Fig. 3C). The Bay alleles at the F21M12 marker were responsible for a higher salt sensitivity response than the Sha alleles. As expected for the output of the QTL analysis, no germination differences between polymorphic lines of HIF98 and HIF183 were found under osmotic stress (Fig. 3C). To investigate the specificity of the *SSR1* QTL mediating germination on NaCl solutions, we evaluated the behaviour of HIF98 and HIF183 seeds under different concentrations of LiCl, a salt used as an NaCl transport analogue (Mendoza *et al.*, 1994). Because similar germination was found between polymorphic lines at different doses of LiCl, we concluded that *SSR1* QTL is involved specifically in the signalling pathway of NaCl (Fig. 3C).

Confirmation of the *OSR1* QTL

To confirm the localization and the additive effects of the *OSR1* QTL, located in the middle region of chromosome 4, polymorphic HIFs were selected in the region of interest. Three independent HIFs, originating from RIL195, RIL120 and RIL111, known to be heterozygous at MSAT4.15, CIW7 and MSAT4.18 markers, respectively, were constructed (Fig. 4B). HIF120 confirmed the presence of the *OSR1* QTL at the CIW7 marker because the seeds carrying the Bay alleles at HIF120 showed a larger osmotic sensitivity response than those carrying Sha alleles at the same marker (Fig. 4C). HIF195 and HIF111 (polymorphic at the MSAT4.15 and MSAT4.18 flanking markers of CIW7, respectively) displayed opposite or similar additive effects for the osmotic sensitivity response (Fig. 4C).

QTL analysis detected a suggestive, but not significant, QTL for salt sensitivity response (LOD: 2.38) that co-localized with *OSR1*. In addition, it is known that NaCl has an early osmotic component and a later ion-specific component of action (Munns and Tester, 2008) that could be masking the osmotic effect of the salt. To evaluate the latter possibility, an experiment was designed that increased the chances of manifesting the osmotic component using KCl, an analogue iso-osmolar salt that lacks the toxic effects of NaCl. A germination experiment was performed using NaCl, KCl and PEG that established an identical water potential of -0.4 MPa in the imbibition medium. It was found that HIF120 seeds showed positive effects of Bay alleles on KCl and PEG sensitivity responses but not on the NaCl sensitivity response (Fig. 5). These results suggest that the *OSR1* QTL is involved in the osmotic signalling pathway when germination occurs in moderate drought or salt environments.

With the germination results in mind, we asked if the *OSR1* QTL displays pleiotropic allelic effects at other development stages. HIF120 plants were cultivated in well-watered soil (-0.03 MPa) for 45 d, and then half of them were exposed to low water potential (-0.8 MPa) conditions for 10 d. Total fresh weight and dry weight were measured to calculate the osmotic sensitivity response of biomass accumulation. It was found that the Bay alleles of HIF120 plants contributed to a higher sensitivity response than the Sha alleles (Fig. 6). These data demonstrate that the Sha alleles at the CIW7 marker also confer resistance to dehydration during vegetative

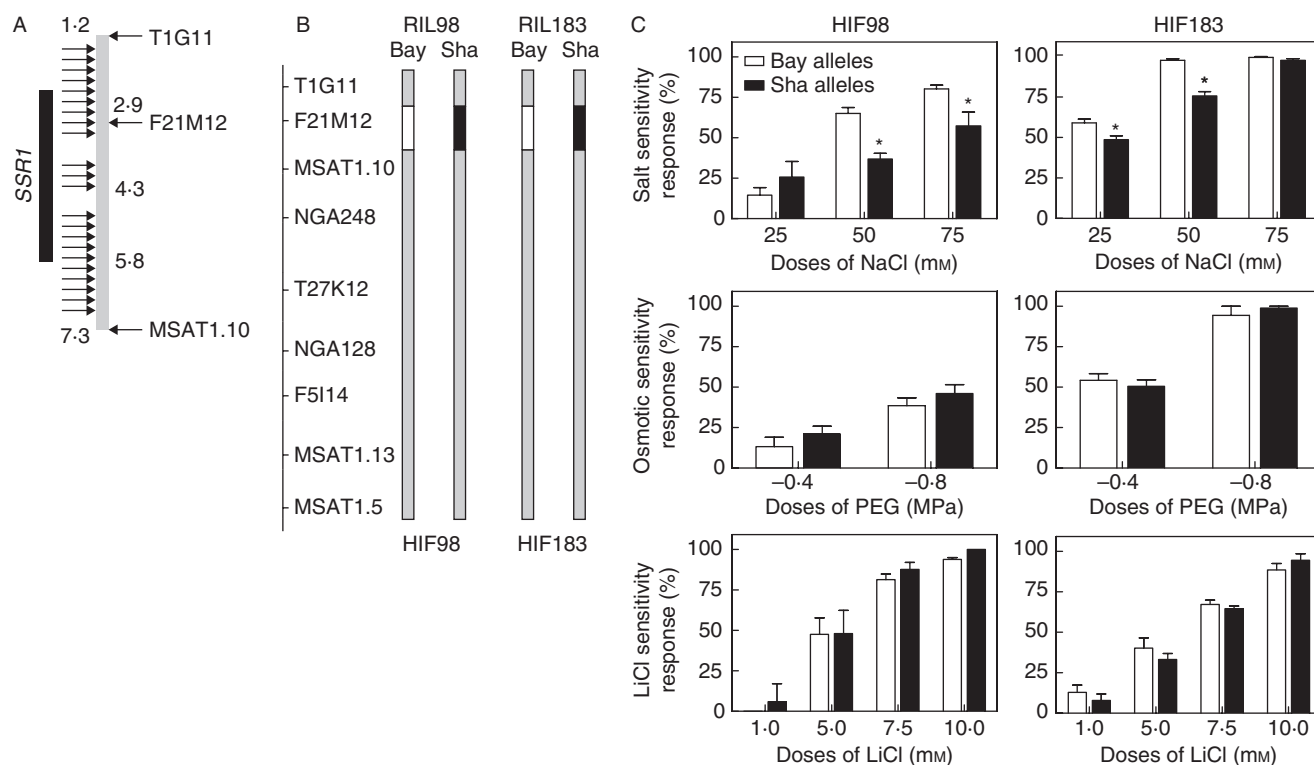


FIG. 3. Confirmation of the *SSR1* QTL in two independent HIFs at the F21M12 marker (HIF98 and HIF183). (A) Fine mapping of the *SSR1* QTL using single feature polymorphisms (SFPs) in the QTL interval and the original markers for chromosomes 1. Vertical bars represent the confidence interval of the QTL. The short arrows indicate the physical position of each SFP, and the large arrows indicate the original markers. (B) Representative scheme for HIF98 and HIF183. The segregation area for each HIF is shown as heterozygous (white and black) or homozygous (grey). (C) Dose sensitivity responses are shown for NaCl, PEG and LiCl. Bars represent means \pm s.e.m. Significant statistical differences at $P < 0.05$ between polymorphic HIFs at each dose are indicated by an asterisk.

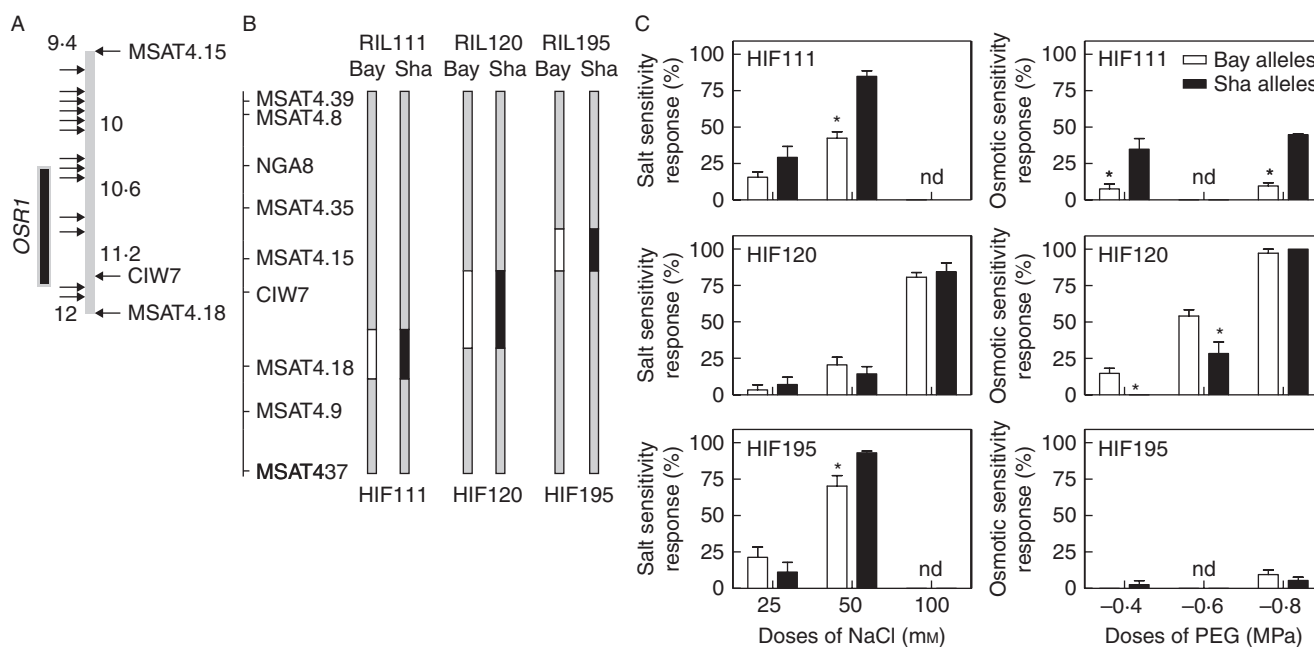


FIG. 4. Confirmation of the *OSR1* QTL by independent HIFs polymorphic at MSAT4-18 (HIF111), CIW7 (HIF120) and MSAT4-15 (HIF195) markers. (A) Fine mapping of the *OSR1* QTL using single feature polymorphisms (SFPs) in the QTL interval, and the original markers for chromosome 4. Vertical bars represent the confidence interval of the QTL. The short arrows indicate the physical position of each SFP, and the large arrows indicate the original markers. (B) Representative scheme for HIF195, HIF120 and HIF111 to confirm the *OSR1* QTL. The segregation area for each HIF is shown as heterozygous (white and black) or homozygous (grey). (C) Dose sensitivity responses are shown for PEG and NaCl. Bars represent means \pm s.e.m. nd indicates no data. Significant statistical differences at $P < 0.05$ between polymorphic HIFs at each dose are indicated by an asterisk.

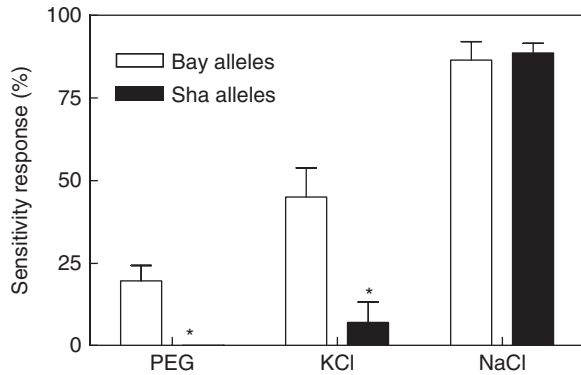


FIG. 5. Osmotic contribution of the *OSR1* QTL. Sensitivity response for HIF120 at iso-osmolar osmotic potential (-0.4 MPa) comparing the effect of PEG, KCl (100 mM) and NaCl (100 mM). Bars represent means \pm s.e.m. Significant statistical differences at $P < 0.05$ between polymorphic HIFs are indicated by an asterisk.

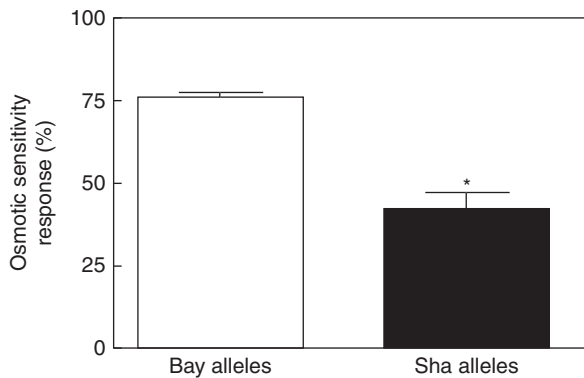


FIG. 6. The *OSR1* QTL contributes to biomass accumulation. Osmotic sensitivity response for the fresh weight/dry weight ratio in polymorphic plants corresponding to HIF120. Plants were cultivated at field capacity for 45 d and then exposed at -0.8 MPa for an additional 10 d. Then, plants were harvested and the fresh weight and dry weight were calculated. Bars represent means \pm s.e.m. Significant statistical difference at $P < 0.05$ between polymorphic HIFs is indicated by an asterisk.

growth as shown previously for the osmotic sensitivity response of seed germination.

DISCUSSION

The success of germination to produce an autotrophic plant requires the perception of environmental heterogeneity that acts as a cue for seed germination. In nature, environmental conditions are frequently sub-optimal and produce different abiotic stresses on non-dormant seeds. Seeds respond to stress conditions by inducing signal transduction pathways to adjust stress-specific adaptive responses. There are multiple stress perception and signalling pathways, some of which are specific, but others may share some signalling components (Zhu, 2002). Studying the genetic architecture of germination under mild osmotic and salt stresses, it was demonstrated here that specific loci are involved in the regulation of germination under moderate stresses.

Two groups of accessions with contrasting sensitivity responses to mild osmotic and salt stresses were found. Sha

and Oy-0 appeared to be the most resistant genotypes, while Cvi and Bay were the genotypes most sensitive to moderate stresses during seed germination (Fig. 2). Genetic variation in both stress responses was not correlated with geographical and climatic clines of the accession origins. Other factors experienced by the seeds such as the photoperiod in the maternal plant and the dispersal season could be better predictors of the geographic distributions of natural genetic variants (Donohue *et al.*, 2005a, b). The same group of contrasting accessions was identified when different traits were studied in plants previously exposed to a mild water deficit (Bouchabke *et al.*, 2008), suggesting that a common set of loci underlying dehydration tolerance could be involved in different physiological responses.

The use of moderate osmotic stress is a fruitful strategy to identify novel loci involved in the control of germination under sub-optimal conditions. Four QTLs for osmotic sensitivity response are involved in the control of germination under moderate drought conditions (Table 2). The four QTL explained 62 % of the phenotypic variance, contributing the Sha alleles to the tolerance of germination at low water potentials. *OSR1* and *OSR3* QTLs were mapped for seeds germinating on both water and PEG, whilst *OSR2* and *OSR4* QTLs were identified only when incubating the seeds on a PEG solution (Table 2). *OSR1* and *OSR3* QTLs were pleiotropic to other traits involved in the release of dormancy such as chilling and light (Meng *et al.*, 2008; Laserna *et al.*, 2008).

OSR1 was the major QTL exhibiting pleiotropic effects on seed germination and biomass accumulation at low water potentials (Figs 4 and 6). Interestingly, a QTL for salt sensitivity response co-localizing with *OSR1* (LOD: 2.38) was mapped, but its LOD score was below the cut-off threshold, casting doubts on its existence. Using iso-osmotic solutions of PEG and KCl, an NaCl salt analogue, common osmotic effects were detected between polymorphic lines at *OSR1*. Seeds of HIF120 carrying Sha alleles were more resistant than those carrying Bay alleles in PEG and KCl but not in NaCl solutions (Fig. 5). These pieces of experimental evidence suggest that the *OSR1* QTL operates as a common molecular component of the signalling network that controls germination under low water potentials generated by osmotic or saline conditions. In plants exposed to different stress environments, common regulatory nodes have been selected within the signalling network that mediates responses to multiple stresses (Shinozaki and Yamaguchi-Shinozaki, 2007; Munns and Tester, 2008). For example, coastal perennial populations of *Mimulus guttatus* are adapted to osmotic stress caused by soil salinity, and inland annual populations are adapted to osmotic stress from rapidly drying soils during the summer (Lowry *et al.*, 2009). It is proposed that common signalling components for osmotic and salt stresses, such as the *OSR1* QTL, will be relevant in the adaptation of plant populations to different ecological environments.

No other common QTLs underlying both stress sensitivity responses were identified under the present experimental conditions. *SRR1* and *SSR2* QTLs were involved in the control of germination under moderate salt concentrations (Table 3), and also mapped for seeds germinating on water with chilling and light (Table 3; Laserna *et al.*, 2008; Meng *et al.*, 2008).

Apparently, both *SSR* QTLs mediate the germination of seeds in a wide range of salt concentrations and different segregating populations of *Arabidopsis* (Quesada *et al.*, 2002; Clerkx *et al.*, 2004; Joosen *et al.*, 2010; Ren *et al.*, 2010). This suggests that *SSR1* and *SSR2* QTLs may act as conserved molecular components in the salt signalling pathway. By QTL mapping analysis for seed germination, and seedling growth under 120 mM NaCl in the *Ler* × *Sha* and *Bay* × *Sha* RIL populations, Ren *et al.* (2010) cloned the gene responsible for a QTL that co-localizes with *SSR1*. Indeed, *Response to ABA and Salt 1 (RAS1)* is a gene inducible by ABA and salt stress, and natural variation in this gene is associated with different levels of sensitivity to saline stress. In particular, a premature stop codon results in a truncated RAS1 protein in *Sha*, contributing to the increased salt tolerance in this accession during seed germination and plant growth.

The fine mapping and molecular characterization of the other *SSR* and *OSR* QTLs will provide information on their functions in the adaptation of seeds to drought and salt environments. Our work strengthens the importance of evaluating germination under moderate levels of osmotic and salt stresses in an increasing number of RIL populations recently generated using parental lines with contrasting germination sensitivity responses (O'Neill *et al.*, 2008; Balasubramanian *et al.*, 2009). These studies will help to find new loci responsible for the wide range of germination responses observed under salt and osmotic stresses.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of data for seed germination under water, PEG, NaCl and stress sensitivity responses (%) for *Arabidopsis thaliana* accessions and *Bay* × *Sha* RILs.

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