Sources of high tolerance to salinity in pea (*Pisum sativum* L.).

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Key words: Field pea, NaCl, phenotypic screening, germplasm

Summary

This study was aimed at identification of parental germplasm that could be used for improvement of tolerance to sodium chloride (NaCl) in field pea. An initial screening experiment of 780 globally-distributed *Pisum* L. accessions identified significant variation in response to applied NaCl, based on plant symptoms. Lines with relatively higher tolerance as compared to commercial varieties grown in Australia were most frequently identified within landraces originating from the central, eastern and southern provinces of China. The most tolerant identified accession was an unadapted landrace 'ATC1836' originating from Greece. Variation for salinity tolerance was validated using a sub-set of 70 accession lines. Salinity-induced toxicity symptoms were closely associated with reductions of plant growth rate, height, shoot and root dry matter and with increased concentration of Na^+ at the plant growing tip. The level of salinity tolerance based on these factors varied substantially and provides an important basis for genetic improvement of field pea for Australia.

Introduction

Field pea (*Pisum sativum* L.) is broadly adapted and widely grown as a dryland grain crop in rotation with cereals. The annual field pea acreage over the last 20 years has fluctuated between 400 and 500,000ha in Australia (ABARE, 2012). Over 70% of this production is within the lower rainfall cropping regions of southern Australia, in which rainfall during the growing season is typically in the 160-230 mm range. A high proportion of the soils in these regions have dense clay sub-soil layers that are both highly sodic (i.e. exchangeable sodium percentage (ESP) >15%) and alkaline (i.e. pH > 8.5) (Rengasamy, 2002 & 2006). Within such regions, soil sodicity-, salinity- and alkalinity-induced nutrient deficiencies (for micronutrients such as Fe, Mn, Cu, Zn and P) and toxicities (i.e. due to boron, carbonate and aluminate) can act together to limit crop growth and grain yield of field pea. A range of dissolved salts, can contribute to salinity stress, however NaCl is the most prevalent and important salt for improving salinity tolerance in Australia (Rengasamy, 2002; Munns & Tester, 2008). Individual sub-soil constraints occur transiently varying in spatial and temporal degrees over the soil profile. In field pea, relatively high tolerance to boron toxicity has been identified (Bagheri et al., 1994 & 1996; Leonforte et al., 2009) and appears to be highly heritable (Bagheri et al., 1996; Leonforte et al., 2009). Tolerance is associated with exclusion of boron from the roots (Bargheri et al., 1996). However, no investigation of variation for tolerance to high soil NaCl has been undertaken for *Pisum sativum* L. Research in other major grain crops such as wheat (Munns et al., 2006) has highlighted the difficulty of using yield-based response measurements in field studies as a measure of tolerance. This is due to the complexity of interactions with abiotic and biotic stress factors, variability of NaCl in the soil profile, and differential responses according to both growth stage and genotype. Studies of biomass reduction and grain yield changes in response to salinity under controlled environment conditions indicate that pulse species are generally much more sensitive than other major dryland crops grown in Australia, such as barley (Maas, 1986, Saxena et al., 1994), wheat (Francois and Maas, 1994) and canola (Steppuhn, et al., 2001). Indeed, the threshold salinity level for pea has been estimated to be very low at 1.5dSm^{-1} (Dua et al., 1989). Soils subject to transient salinity in cropping regions of Australia generally exhibit maximal NaCl concentration in B and C soil horizons between 4 to 16dSm^{-1} (Rengasamy, 2002). It is therefore highly likely that salinity levels significantly limit grain yield of pulse crops such as field pea in low rainfall environments.

From separate studies, faba bean (Cordovilla et al., 1995) and pea (Hernandez et al., 2000; El-Hamdoui et al., 2003; Gomez et al., 2004), appear to be less sensitive to NaCl than chickpea (Sadiki & Rabhi 2001) and lentil (Maher et al., 2003). Comparison of salinity tolerance studies within pulse crops indicates that lentil (Jana, 1979) and faba bean (Hamid & Talibuddin, 1976; Al-Tahir & Al-Abdussalam, 1997) may be more sensitive at germination and as seedlings than at subsequent growth stages. However, the converse is likely to be true for chickpea (Kumar, 1985), and there is no information for pea regarding variation in germination response. For cool-season grain legumes, pot-based phenotypic screening has successfully identified sources of relatively higher seedling tolerance to NaCl in both lentil (Maher et al., 2003) and chickpea (Serraj et al., 2004; Sadiki & Rabih, 2001). The present study was hence aimed at identification of sources of relatively high tolerance to NaCl for genetic improvement of Australia's field pea crop.

Materials and Methods

Experiment 1: Identifying sources of high salinity tolerance within **Pisum** ***L.***

Three sets of *Pisum* germplasm were sourced from the Australian Temperate Field Crops Collection (ATFCC) at Horsham, VICDPI and included accessions that represented the global collection based on geographic origin (418 lines), Chinese landrace accessions (355 lines) based on origin according to province and major Australian field varieties (7 lines). The experiment was conducted over spring (September-November in the southern hemisphere) in a semi-controlled environment (i.e. large plastic igloo) at Horsham. Six plants of each accession were sown with equidistant spacing in 13 cm diameter pots into a sand and gravel medium to a depth of 2 cm. The gravel medium was composed from a 1:1 ratio of coarse river sand and 5 mm bluestone chips. Each pot was treated daily with rainwater from sowing until emergence. From four days post-emergence, seedlings were watered with a complete nutrient solution (i.e. nitrosol, NPK ratio 12.2: 2.9: 8.5), in addition to supplementation with a calcium source (i.e. calcium nitrate). From 10 days post-emergence, NaCl dissolved in water as a concentrated stock solution was added to the watering solution. The required NaCl concentration was tested using an EC meter and was applied at an initial rate of 4dsm^{-1}. The concentration of applied NaCl was increased by 4dsm^{-1} at each watering time, up to 16dsm^{-1}, and maintained at this concentration until assessment. All watering with the nutrient and salt solution was undertaken over 3 day-intervals at a rate of 200ml per pot applied directly to the growing medium surface. Individual plants were assessed for percentage necrosis at 9 weeks, and for a general salinity tolerance scores at 12 weeks post-sowing. The salinity tolerance score and screening method were based on a visual growth response scale (1-10), developed for lentil (Maher et al., 2003) and adapted for pea as described in Table 1.

The experiment was designed as a pot experiment with two replicates, in each of which pots were randomized in a grid of 80 ranges by 7 rows. A null-salt application treatment was included in order to identify any confounding effects caused by the growing climate, disease or nutritional deficiencies. The null-salt treatment consisted of pots sown in six ranges randomly located within each experimental block. A residual estimation maximum likelihood (REML) analysis of the mean salt score for each pot was performed to obtain predicted means adjusted for range and row in the experiment. Statistical analysis was conducted on the mean symptom score for each accession. Mean symptom scores were based on at least three plants per pot. A total of 33 accessions were excluded from the analysis, as fewer than three plants per pot had germinated. To determine significant differences in mean salinity scores for accessions based on country of origin, an analysis of variance (ANOVA) was undertaken by contrasting between the national sources that had ten or more accessions represented in the screening experiment. ANOVA was also performed on the predicted mean salinity tolerance scores for 335 accessions for which site of origin was specified to 20 individual Chinese provinces.

Experiment 2: Identification and validation of lines with relatively high salinity tolerance for breeding application

For this experiment, 4 germplasm sets totalling 70 lines were used, with the following characteristics:

Set 1). Forty two accessions identified from experiment 1, based on the criteria of: low salinity symptom score (< 6), geographic diversity in origin and diversity of plant features (e.g. internode length, leaf type, and branching habit).

Set 2). Eighteen breeding lines identified from the field pea program of Pulse Breeding Australia (PBA), with relatively higher NaCl tolerance following routine screening (Leonforte et al., 2009).

Set 3) Six Australian cultivars (Kaspa, Helena, Parafield, Excell, Yarrum and Sturt), that showed varying sensitivity in experiment 1.

Set 4). Three accessions identified from experiment 1 that showed significantly higher sensitivity on the basis of salinity tolerance score.

The sowing process and NaCl application was conducted using the same methodology as described in experiment 1. Experiment 2 was designed as a split plot consisting of 4 replicates with the the main plot treatment being application of salt as NaCl at a level of 18dSm^{-1} or no application of salt and the sub-plot treatment being 70 germplasm accessions described above.

Once the final EC reading of the solution reached 18dSm^{-1} for the plus-salt treatment, a salinity symptom score (i.e. as described above) and plant height (i.e. length of the primary stem from the base of the stem to the last node) were recorded every 7th day for four weeks (i.e. 7th, 14th, 21st, 28th day) for each plant in each pot. The total plant and root matter were harvested at final assessment, dried in an oven at 70°C for 3 days, and dry matter content was recorded. Total Na^{+} concentration was measured on leaflets from the final growth node of plants at the final growth stage using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Zarcinas et al., 1987) from samples digested in nitric acid/hydrogen peroxide solution. The growth rate for the main stem (i.e. cm/day) was calculated from the increase in length of the primary or main stem, and was divided by the number of growing days. Following REML analysis, the percent reduction in dry matter and growth rate or plant height was calculated from the predicted means. A principal component analysis was undertaken using Genstat11, and a bi-plot was developed to graphically represent the relationships between lines and measured variates.

Results

Experiment 1: Identification of source of high salinity tolerance within **Pisum** *L.*

The salinity symptom scores for the null-salt treated pots were consistently measured as 1, indicating that biotic or abiotic factors were not confounding salinity symptoms as assessed in this experiment. There were major differences for symptom scores between the 747 accessions that could be compared in the plus salt treatment (Appendix: Table 1). Over 80% of these accessions were very sensitive (i.e. symptom score was equal to, or above 7) (Table 2) in this screening experiment. Of the 36 accessions that had a salinity score of 4 or less, thirty originated from China. Accessions from Greece and China had the lowest symptom scores (Table 3, Figure 2) and these were significantly lower than accessions from Afghanistan, Ethiopia, Finland, the former Soviet Union, Sweden and the United States (Table 4). There was no significant difference for salinity score on the basis of Chinese province of origin (Table 5). The regions in which higher tolerance was identified (i.e. salinity symptom score < 4) were located in neighbouring provinces in the central-eastern region (i.e. Shaanxi, Henan and Anhui) and the south central region (i.e. Yunnan, Guizhou and Guangxi) and in Qinghai province (Figure 1). Variation for salinity tolerance within specific geographic locations allocated to country or to Chinese province was typically large (Figures 2 and 3). Forty two accessions were selected for further investigation in Experiment 2, on the basis of a low salinity symptom score (i.e. less than 6), (Figure 4), diverse geographic origin and diverse plant morphology (e.g. internode length, leaf type, etc).

Experiment 2. Identification and validation of lines with relatively high salinity tolerance for breeding.

A significant reduction in growth rate and plant height was associated with the application of NaCl after 7, 14, 21 and 28 days (Table 5). Lines grown in the null-salt treatment did not display any salinity symptoms, and associated tolerance score values were consistently equal to 1 (Table 6). The plant height of the main stem increased significantly at 7, 14, 21 and 28 days for the null-salt treatment. However in the plus salt treatment there was no significant increase in mean plant height of lines after day 14 (Table 6). The mean plant growth rate did not significantly change over the evaluation period in the null-salt treatment but significantly decreased from day 7 to 14 to 21 with application of NaCl. There was a significant reduction in both total shoot dry matter and root dry matter at day 28 associated with the application of NaCl (Table 6). An Anova analysis indicated a significant interaction (e.g. F pr <0.001) between salt treatment (i.e. plus, minus) and accession line treatment for plant height, plant symptom score and plant growth rate for each consecutive recording (i.e. days 7, 14, 21, 28) and between shoot dry matter and total root dry matter at day 28,

Linear regression analysis (Table 7) indicates that accession growth rates for the salt treatment were closely correlated with the null-salt treatment at day 7, weakly correlated at day 14, and not correlated by day 21 and 28. The accession plant heights from the salt treatment were closely correlated with the plant heights in the null-salt treatment for each day of assessment. There was also a significant correlation between total shoot DM at day 28 in the presence or absence of applied salt.

A principal component bi-plot graph of accessions by variates measured on each day of assessment in the presence of salt treatment indicates that tolerance scores were highly consistent between assessment times. High salinity symptom scores (i.e. sensitivity) were closely associated with parameters such as higher Na^+ and lower K^+ concentration in the growing tip tissue, lower growth rate and root and shoot dry matter at day 28. Variation in growth rates at days 7 and 21 and plant height in general did not appear to be as closely correlated with the accession salinity symptom scores (Figure 5)

Based on salinity symptom scores, both unadapted and adapted breeding lines with relatively higher tolerance compared to commercial varieties were identified (Figure 6). The rate at which toxicity symptoms developed over time varied significantly between lines (Figure 6). In general, tested varieties showed relatively moderate to severe toxicity symptoms from day 14 when compared to the more tolerant lines identified in experiment 1 (Figure 6). The most sensitive Australian variety on the basis of symptom development was Kaspa (Figure 6). Accession ATC1836 showed the slowest rate of symptom development (Figure 6). Some of the adapted material with lesser symptoms (i.e. low salinity symptom score) also maintained higher growth rates when compared to the unadapted lines with low symptom scores (Figure 7). However all accessions showed an initial rapid reduction in growth rate from day 7, which became more gradual from day 14 to 21 (Figure 7).

There was a significant association between for accession salinity symptom score between experiments (R^2=0.43; F pr. < 1%). Notable accessions with a low salinity symptom score in experiment 1 (symptoms score < 4) and slower rate of salinity symptom development in experiment 2 included ATC01836 andATC1093 from Greece, ATC1091 from Albania and ATC04226, ATC06592, ATC06642 and ATC07157 from China.

Discussion

Following screening of a diverse pea germplasm set from the ATFCC, several sources of high tolerance to NaCl have been identified within the species. On the basis of country-of-origin, China provided the largest proportion of qualified accessions. Within China the central west (Henan, Anhui, Shaanxi, Guangxi), southern (Guizhou, Yunnan) and western (Qinghai) provinces showed higher proportions of tolerant accessions. These provinces are all located along three major river basins of the Yellow, Yangtze and Pearl rivers where crop irrigation has been common practice for over 2000 years and is likely associated with soil salinisation. Recent studies have highlighted the genetic distinctiveness and diversity of Chinese pea germplasm compared to the global pea germplasm collection (Zong et al., 2009), implying that pea germplasm in China has undergone strong directional selection, potentially in isolation from the Fertile Crescent region where peas are thought to have originated (Ambrose 1995). The higher frequency of salinity tolerance discovered in China in this study may be linked to divergence in natural selection between China and other global regions. However separate introgression from wild species or independent domestication also cannot be discounted. The high variability for salinity tolerance associated with province of origin in China or country is not surprising, as peas have been historically grown in diverse environments that vary significantly in terms of altitude, climate, soil type and farming systems (irrigated vs. dryland).

Pea germplasm with relatively higher salinity tolerance was morphologically diverse, and not specifically associated with non-domesticated or adapted types within the species. The most tolerant accession (ATC1836) was obtained from Greece. Unfortunately, ATC1836 displayed several negative traits that will reduce its value as a parental line. These include a high number of basal branching, relatively low early vegetative growth, very long plant internodes, thin and wiry stems, a very late flowering habit, low seed number per pod, small seed size and a dark and patterned seed coat. However the moderate salt tolerance identified in adapted plant backgrounds (i.e. OZP0812) provides a basis for selecting recurrent parents to use in targeted breeding (Figure 6).

Exposure to salt treatment significantly reduced plant height and growth rate over time, and resulted in rapid onset of plant toxicity symptoms and early plant death. Importantly, however, this study identified in the pea germplasm a wide diversity of responses in terms of the rate of symptom development and growth responses that can be exploited in breeding higher tolerance. Relative salt tolerance based on tissue-specific symptoms was validated and highly consistent across experiments. The growth response to salinity in pea appears to be similar to the biphasic response observed with other crops, which is characterised by an initial sudden reduction in growth, mostly likely due to the exposure to a solution of low osmotic potential and then followed by a gradual response to increasing salt toxicity (Rawson et al., 1988; Cramer et al., 1994; Munns et al., 1995; Fortmeier and Schubert, 1995; Yeo et al., 1991; Rivelli et al., 2002).

Plant height as controlled by internode length, does not appear to be directly associated with NaCl sensitivity in pea. This is an important finding, as international breeding efforts are focused on the development of semi-dwarf types with a high harvest index. Selection for higher plant biomass may, however, be useful in order to dilute and delay Na^+ toxicity effects (Almodares et al., 2011). For peas, scope exists to increase plant biomass as a salt stress avoidance mechanism, even within semi-dwarf plant backgrounds via the selection for, increased internode length and leaflet number, larger plant structures (i.e. tendrils, stipules) and greater basal and aerial branches (e.g. ATC1836). In Australia early flowering time is generally a breeding priority for improving reliability of yield of field pea, as production is mostly within short season environments (Sadras et al., 2012). Despite this the rate and timing of maximum biomass accumulation can still be significantly increased during reproductive development (Mahli et al., 2007) in early flowering germplasm.

The growth symptoms used as a basis for symptom assessment were not confounded by the growing conditions, and developed rapidly in response to application of salt. As expected germplasm with higher salinity symptoms also displayed the greatest reduction in root and shoot dry matter. The Na^+ and K^+ concentrations at the growing tips were closely correlated with symptom scores, indicating that plant symptoms are highly predictive of Na^+- induced plant tissue toxicity. As growing tips of more tolerant pea lines had lower Na^+ and inversely higher K^+ concentration, Na^+ exclusion (Demidchik et al., 2002) may be involved as a mechanism. Knowledge of how Na^+ transport may be regulated in pea and at what point (e.g. roots (Lauchli et al., 2005), xylem (Davenport et al., 2005; Pitman 1984; Munns, 2005) or phloem tissue (Munns & Rawson, 1999; Wolf et al., 1991)) requires further investigation to facilitate selection.

Consecutive salinity score assessments did not vary significantly and little advantage was gained in assessing symptoms after 14 days of salt exposure. However, the optimum timing for assessment is likely to vary with the concentration of exogenous salt and the climate for growth (e.g. temperature and applied water), as these factors have an interactive effect on osmotic regulation and transpiration rate (Blum 2005). For pea the low cost and rapid semi-hydroponic screening methodology described in this study appears very effective to identify new sources of NaCl tolerance in the species. Interestingly the major field pea variety grown (Kaspa) in Australia appears to be quite sensitive to salinity even when compared to other commercial varieties. This may partially explain the unreliability of this variety in some short season climates (Sadras et al., 2012) and the sometimes unexpected poor growth and early senescence observed in field testing in Western Australia (I. Pritchard, personal communication) where salinity is likely to be more severe. On this basis any incremental gain in salinity tolerance could have a major impact on crop reliability in Australia.

The positive variation for salinity tolerance in pea appears substantial and available across diverse plant backgrounds and origins. Significant genetic improvement based on direct phenotypic selection alone is therefore highly likely to be possible. Consequently targeted backcross and recurrent selection breeding is being undertaken using variation identified in this study. Further research to validate how this tolerance varies across ontogeny, understand genetic control and identify major genes or DNA molecular markers in field pea are now planned.

Acknowledgments

We thank Dr D. Enneking (Department of Education, South Australia) for his contribution to identifying the initial diverse set of pea accessions for screening from the ATFCC and Ms D. Noy and Mr G. Ambrose for their technical assistance in conducting the research experiments.

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Tables

Table 1. Description of salinity symptom score.

Salinity symptom score (1-10)	**Description**
1	Plant healthy green, no obvious salinity symptoms
2	Beginning to yellow, not very many symptoms
3	Some chlorosis bottom half of plant, no necrosis, overall yellowing
4	Necrosis beginning on bottom half of plant.
5	Chlorosis and necrosis bottom half of plant, yellowing overall (50% affected).
6	Chlorosis becoming more severe on upper part of plant, not necrotic on upper plant
7	Chlorosis and necrosis more than half of plant
8	More necrosis than 7, but still some green leaves.
9	Stem and very youngest leaves green, rest dead (all leaves may be dead)
9.5	Only top of stem (or small part stem) and very youngest leaves still green, rest dead (all leaves may be dead)
10	Plant dead

Table 2. Distribution of salinity symptom scores: 1 (no symptoms) to 10 (dead) for 747 accessions from the Australian Temperate Field Crops Collection screened at seedling stage for salinity tolerance.

Range of symptom scores	**Number of accessions**

1-2	1
2-3	7
3-4	28
4-5	23
5-6	70
6-7	89
7-8	185
8-9	143
9-10	201

Table 3. Mean and range of salinity symptom scores of *Pisum* L. accessions grouped on the basis of global region and individual country of recorded origin in the Australian temperate field crops collection centre.

Origin	**Number of accessions**	**Mean salinity tolerance score (1-10)**	**Range of salinity tolerance score (1-10)**
AFRICA	**60**	**8.3**	**5.7-10**
Burundi	1	10	10
Ethiopia	53	8.3ab	5.7 – 10
Kenya	1	7.5	7.5
Madagascar	1	10	10
Rwanda	1	7.6	7.6
Tanzania	1	8.9	8.9
Uganda	1	9.8	9.8
Yemen	1	7.5	7.5
Zaire	1	7.2	7.2
ASIA	**428**	**7.5**	**2.2-10**
Afghanistan	38	8.1ab	4.9 – 10
China	355	7.3a	2.2 – 10
India	16	8.1	5.8 – 10
Mongolia	2	8.1	6.3 – 10
Nepal	8	8.3	7.4 – 10
Pakistan	7	8.5	4.0 - 10.1
Taiwan	1	8.8	8.8
EASTERN EUROPE	**58**	**8.8**	**4.5-10**
Armenia	1	7.4	7.4
Belarus	1	8.7	8.7
Bulgaria	4	8.0	5.6 – 9.3
Estonia	1	5.7	5.7
Former Soviet Union	10	9.6ab	8.4 – 10
Georgia	8	8.6	7.6 – 10
Kazakhstan	7	8.7	6.7 – 10
Kyrgyzstan	3	7.8	5.3 – 10
Romania	2	8.8	7.6 – 10
Russian Federation	5	9.3	8.3 – 10
Tajikistan	7	8.5	6.3 – 10
Ukraine	3	9.8	9.5 – 10
Uzbekistan	5	8.6	4.5 – 10

MEDITERANEAN	**77**	**7.3**	**1.1-10**
Albania	3	7.4	2.4 – 10
Algeria	2	7.9	7.9 – 7.9
Egypt	2	7.6	5.7 – 9.4
Greece	34	7.0a	1.1 – 10
Italy	3	7.8	7.1 – 8.7
Libya	1	7.9	7.9
Morocco	5	7.4	4.7 – 8.7
Portugal	2	7.9	6.6 – 9.2
Spain	3	7.5	6.2 – 9.4
Tunisia	1	7.6	7.6
Turkey	21	7.4ab	4.0 – 10
MIDDLE EAST	**11**	**8.3**	**6.2-10**
Iran	3	9.4	8.9 – 10
Israel	2	7.3	6.4 – 8.2
Palestine	2	9.4	8.7 – 10
Syria	4	7.5	6.2 – 8.2
NORTH AMERICA	**15**	**8.4**	**6.0-10**
Canada	4	8.2	6.5 – 9.5
Mexico	1	8.1	8.1
United States	11	8.5ab	6.0 – 10
SOUTH AMERICA	**5**	**7.7**	**5.7-10**
Bolivia	1	5.7	5.7
Chile	1	8.5	8.5
Colombia	1	10.0	10
Malaysia	1	6.0	6.0
Peru	1	5.7	5.7
WESTERN EUROPE	**113**	**8.3**	**4.6-10**
Austria	1	10	10.0
Finland	10	9.0ab	7.0 – 10
France	10	8.0	5.2 – 10
Germany	4	7.7	6.6 – 8.9
Iceland	1	7.7	7.7
Netherlands	9	7.6	4.9 – 10
Poland	4	7.3	4.6 – 10
Sweden	66	8.4ab	5.3 - 10.4
United Kingdom	5	6.9	4.9 – 9.5
Yugoslavia	3	8.5	6.9 - 10
Australian cultivars	**8**	**8.6**	**7.0-10.6**

* Means with the different letters are significantly different (F prob. < 5%) and only presented for countries represented by 10 or more accessions.

Table 4. P value (F pr.), for contrasts between China and other individual countries of recorded origin in the Australian temperate field crops collection centre.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Country	10	193.0	19.3	6.8	<.001
China versus Afghanistan	1	18.6	18.6	6.6	0.01
China versus Ethiopia	1	43.6	43.6	15.4	<.001
China versus Finland	1	26.0	26.0	9.2	0.003

China versus Former Soviet Union	1	50.4	50.4	17.8	<.001
China versus France	1	4.2	4.2	1.5	0.225
China versus Greece	1	4.1	4.1	1.5	0.229
China versus India	1	8.4	8.4	3.0	0.084
China versus Sweden	1	70.3	70.3	24.9	<.001
China versus Turkey	1	0.1	0.1	0.0	0.85
China versus United States	1	15.2	15.2	5.4	0.021
Residual	613	1732.7	2.8		
Total	623	1925.7			

Table 5. Mean and range of salinity symptom scores of *Pisum* L. accessions grouped on the basis of Chinese province of recorded origin in the Australian temperate field crops collection.

Origin (Province)	**Number of accessions**	**Mean salinity tolerance score (1-10)**	**Range for salinity tolerance score (1-10)**	**Number of accessions with salinity tolerance score < 4**
Henan	9	5.0	2.2-7.5	3
Shaanxi	51	5.9	2.3-9.8	15
Anhui	11	6.3	3.5-8.5	1
Guangxi	35	7.2	3.6-9.6	1
Guizhou	14	7.3	2.8-9.9	2
Yunnan	85	7.9	2.9-10.0	3
Qinghai	39	8.3	3.1-10.0	1
Jiangsu	1	4.7	-	0
Sichuan	16	7.0	4.4-9.7	0
Hubei	10	7.2	5.2-8.5	0
Guangdong	2	7.4	6.2-8.6	0
Nei Mongol	38	7.5	7.7-9.6	0
Beijing	2	7.7	5.6-9.8	0
Heilongjiang	1	7.8	-	0
Shanghai	2	7.8	6.0-9.6	0
Gansu	4	8.1	6.7-9.0	0
Xinjiang	4	8.2	6.1-9.5	0
Xizang	9	8.5	6.9-10.0	0
Ningxia	2	8.5	7.8-9.2	0

Table 6. Mean plant height, salinity symptom score, growth rate at day 7, 14, 21 and 28 and final shoot and root dry matter at day 28 for plus versus minus salinity treatments.

Trait	**Salt treatment**	**Day 7**	**Day 14**	**Day 21**	**Day 28**

Plant height (main stem: cm)	Salt (Nil)	26.2 a	39.7 ad	51.7 ae	60 af
	Salt (Plus)	16.4 b	20.1 bc	20.4 bc	21.7 bc
Salinity tolerance score (1-10)	Salt (Nil)	1.0 a	1.0 a	1.0 a	1.0 a
	Salt (Plus)	1.4 b	2.6 bc	4.9 bd	7.6 be
Plant Growth rate (cm/day: main stem)	Salt (Nil)	1.87 a	1.85 a	1.82 a	1.64 a
	Salt (Plus)	1.17 b	0.53 bc	0.14 bd	0.18 bd
Total shoot DM (g)	Salt (Nil)				6.63 a
	Salt (Plus)				2.21 b
Total root DM (g)	Salt (Nil)				5.43 a
	Salt (Plus)				2.51 b

*Means with the same letters are not significantly different (F prob. < 5%).

Table 7. The R^2 values based on linear regressions of accession growth rate, plant height, root DM and shoot DM in plus versus minus salt treatments.

Day	**Plant growth rate (cm/day)**	**Plant height (main stem: cm)**	**Total root DM (g)**	**Total shoot DM (g)**
7	0.84*	0.84*		
14	0.15*	0.77*		
21	NS	0.72*		
28	NS	0.70*	0.19*	0.46*

* F prob. < 1%

Figures

Figure 1. Map of China highlighting provinces in which tolerant land races (T) (i.e. mean salinity symptom score of less than 4) were identified and provinces in which only sensitive land races (S) were identified (i.e. mean salinity symptom score of 4 and above).

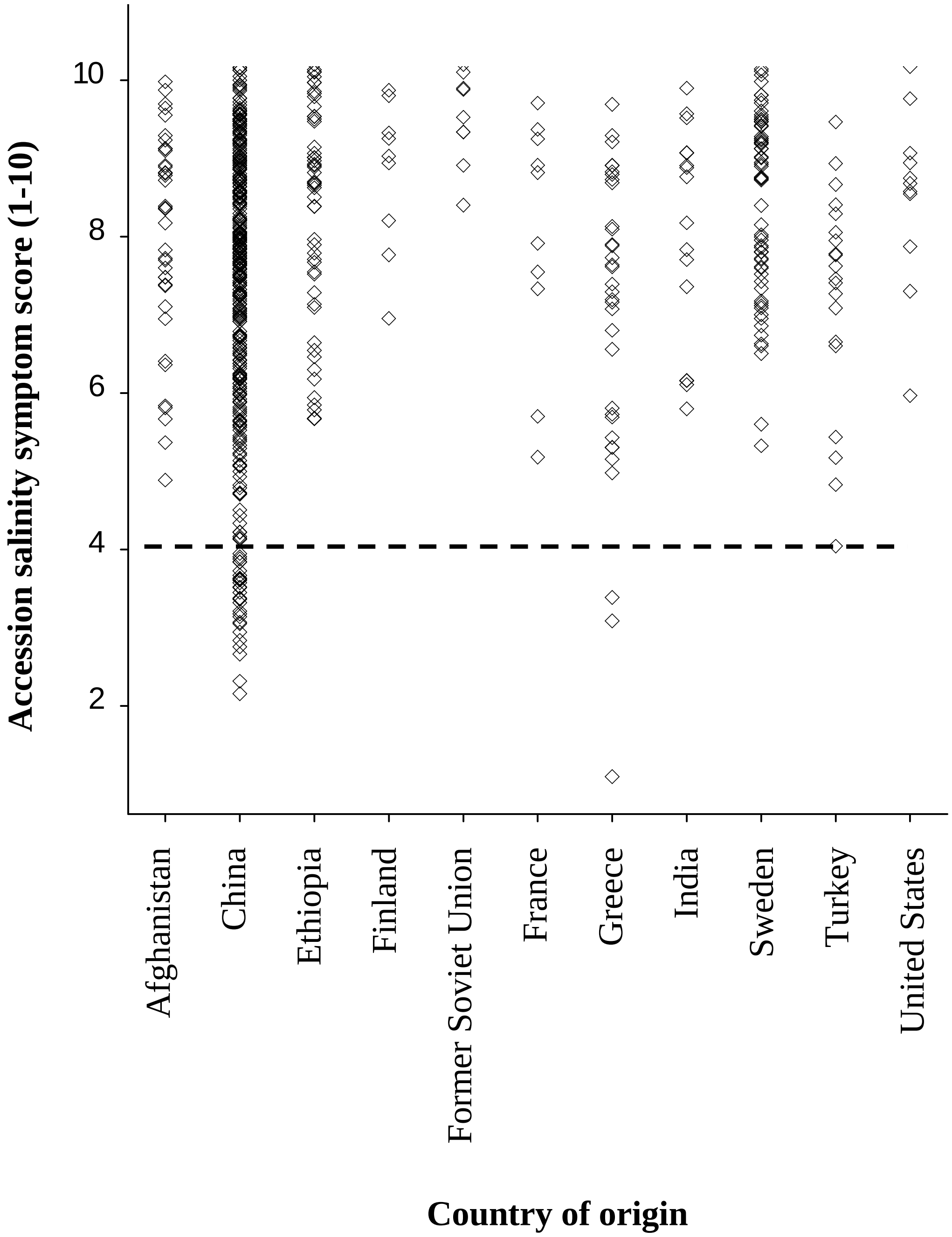

Figure 2. Variation in salinity symptom scores within countries of origin that could be statistically compared (country represented by more than 20 accessions).

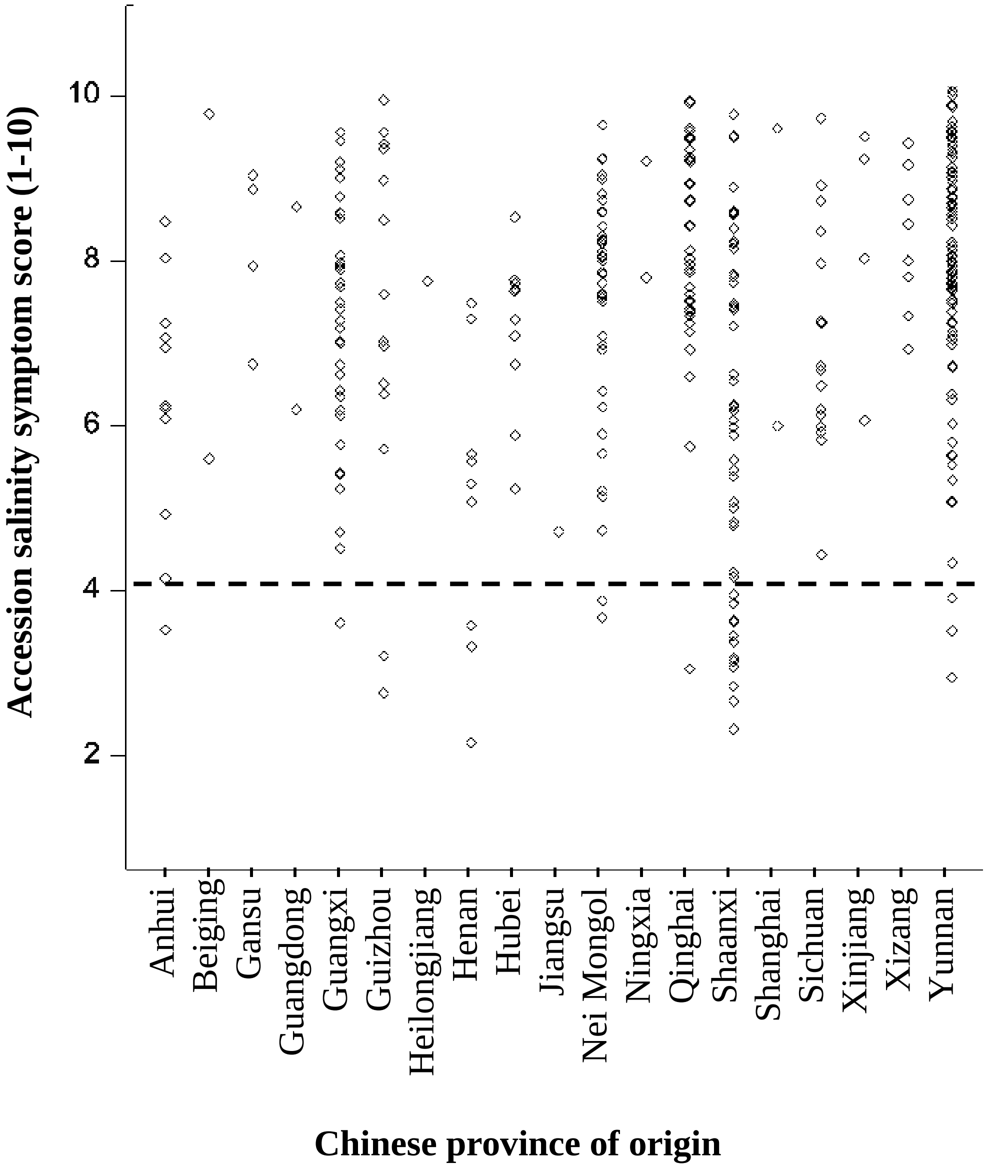

Figure 3. Variation in salinity symptom scores within Chinese province of origin that could be statistically compared (province represented by more than 20 accessions).

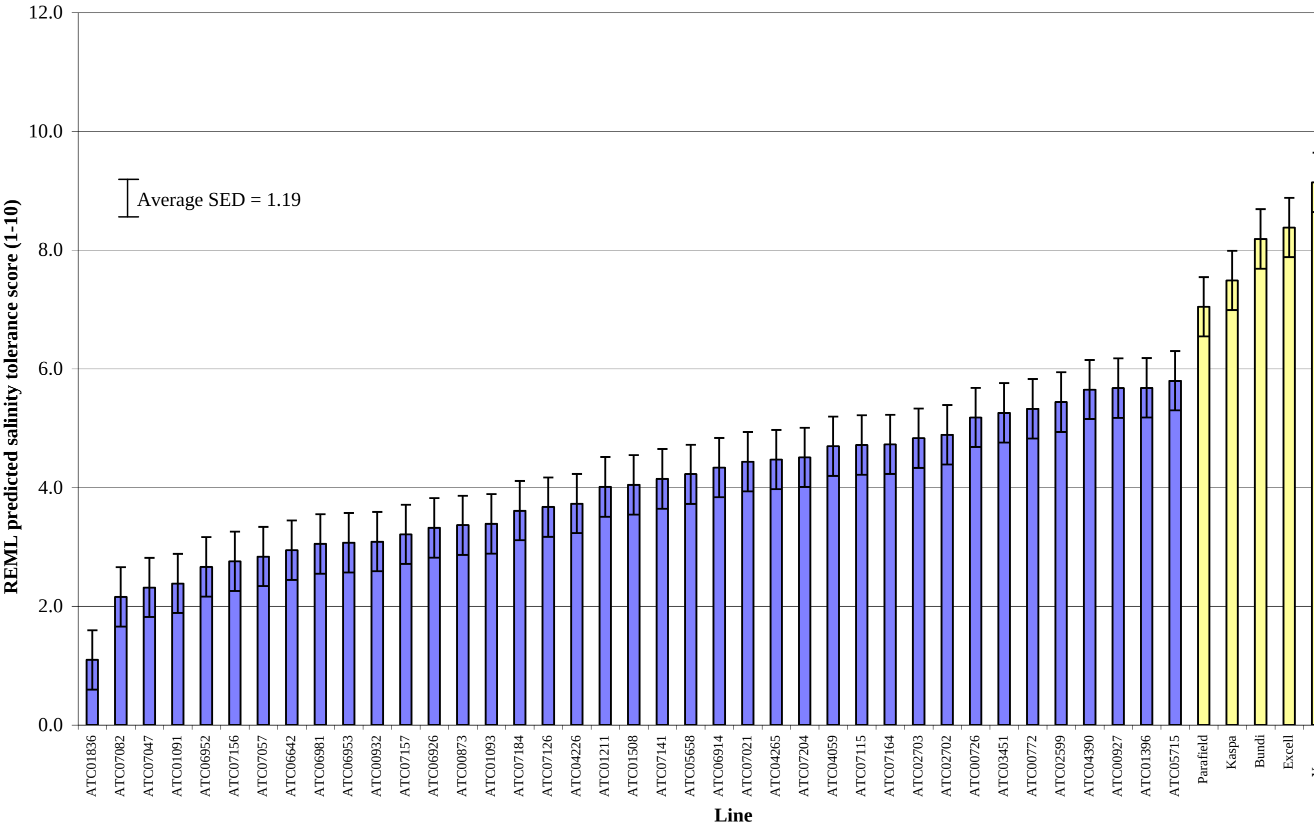

Figure 4. Salinity symptom scores for accessions that showed higher tolerance in experiment 1 and selected for validation and further investigation of whole plant growth responses in experiment 2. Australian commercial varieties are highlighted in the lighter coloured shading.

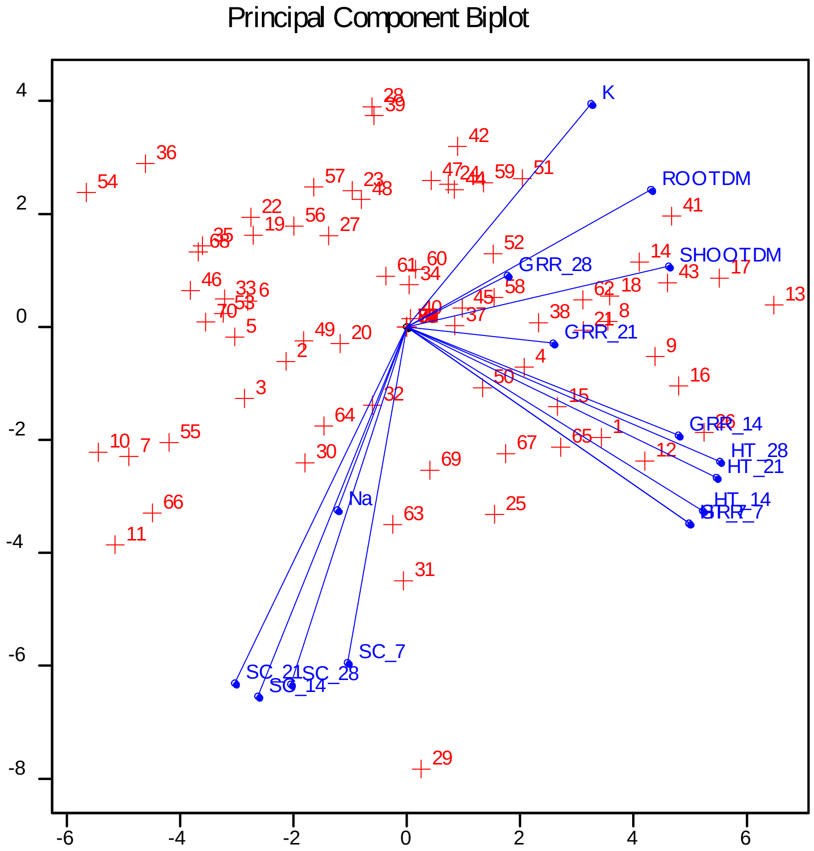

Figure 5. Principal component biplot between lines (represented by number) and the variates measured in plus salt treatment: growth rate (GRR), final root dry matter (ROOTDM), final shoot dry matter (SHOOTDM), final plant growing tip Na^+ concentration (NA) and K^+ concentration (K), plant height (HT), and salinity symptom score (SC) for days day 7,14,21 and 28.

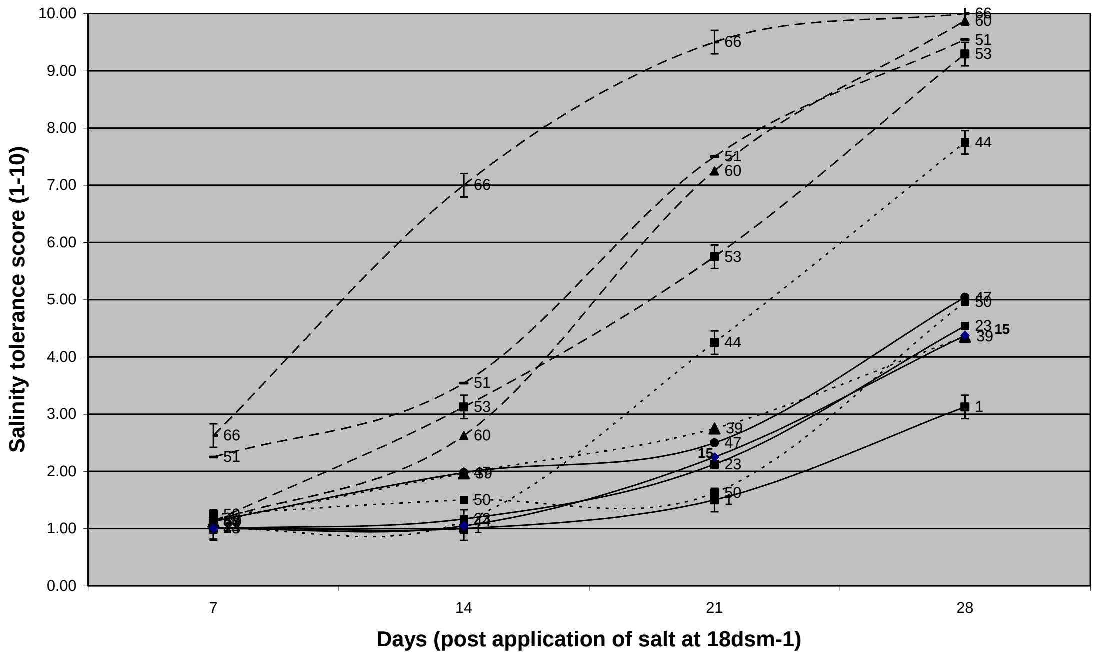

Figure 6. Salinity symptom scores from day 7 to 28 in plus salt treatment for unadapted accessions as indicated by the solid lines () for 1 (ATC01836), 15 (ATC01093), 23(ATC07021), 39 (ATC04226), adapted breeding lines as indicated by

the dotted lines (···) for 47 (OZP0812), 50 (99-410-2-14-2) and Australian commercial varieties as indicated by the broken lines (—) for 51 (Parafield), 53 (Helena), 60 (Yarrum), 66 (Kaspa).

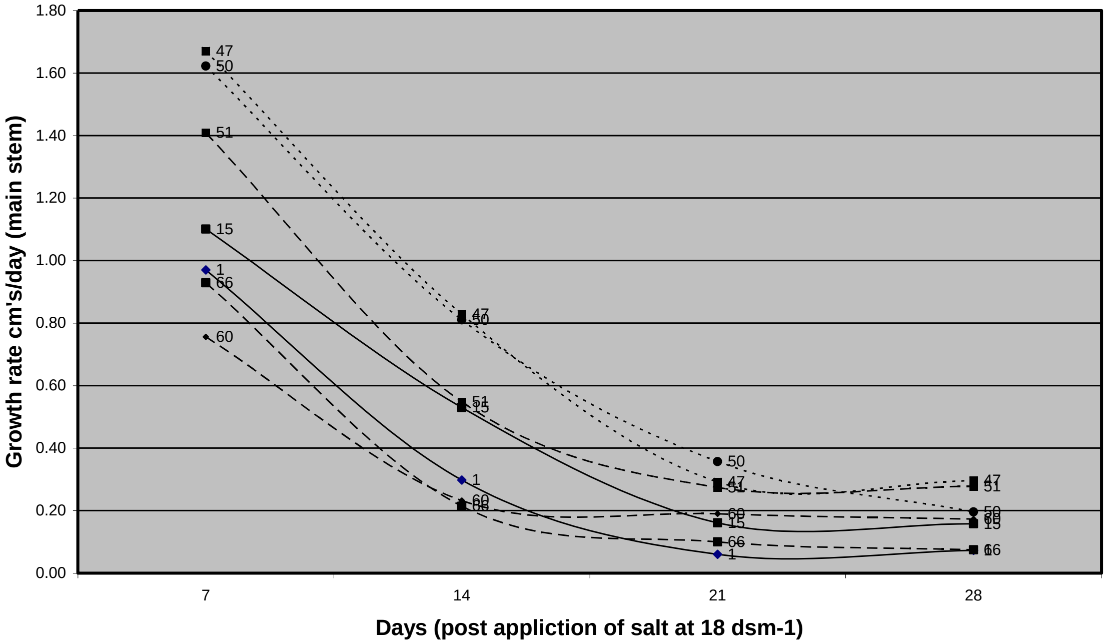

Figure 7. Main stem growth rate (cm/day) from day 7 to 28 in plus salt treatment for unadapted accessions as indicated by the solid lines () for 1 (ATC01836), 15 (ATC01093), adapted breeding lines as indicated by the dotted (···) for 47 (OZP0812), 50 (99-410-2-14-2) and Australian commercial varieties as indicated by the broken lines (—): 51 (Parafield), 60 (Yarrum), 66 (Kaspa).

Appendix

Table 1. Mean salinity symptom scores based on a 1 (no symptoms) to 10 (dead) scale for 780 accessions screened during the vegetative period following application of NaCl. Australian commercial varieties are highlighted in bold.

Mean salinity tolerance score	**Accession: Australian Temperate Field Crops Collection ID number or variety name.**
≤ 2	ATC01836
≤ 3	ATC07082, ATC07047, ATC01091, ATC06952, ATC07156, ATC07057, ATC06642
≤ 4	ATC06981, ATC06953, ATC00932, ATC07073, ATC07072, ATC07157, ATC06926, ATC00873, ATC07062, ATC07048, ATC01093, ATC07045, ATC06912, ATC07142, ATC07083, ATC07184, ATC06946, ATC07068, ATC07035, ATC07126, ATC04226, ATC07046, ATC07069, ATC07129, ATC06898, ATC07051, ATC01211, ATC01508
≤ 5	ATC00872, ATC07141, ATC07074, ATC07053, ATC05658, ATC06914, ATC07021, ATC04265, ATC07204, ATC03362, ATC04059, ATC07200, ATC07115, ATC07164, ATC07060, ATC07043, ATC02703, ATC02702, ATC02707, ATC07140, ATC02549, ATC01479, ATC07067
≤ 6	ATC06913, ATC07065, ATC07086, ATC07034, ATC07100, ATC00754, ATC01501, ATC00726, ATC07170, ATC07191, ATC00721, ATC03451, ATC06931, ATC00756, ATC00947, ATC00772, ATC06632, ATC04198, ATC01191, ATC07041, ATC07017, ATC07186, ATC00933, ATC02599, ATC07049, ATC07033, ATC00107, ATC07085, ATC07044, ATC06956, ATC00941, ATC06647, ATC04509, ATC06648, ATC04390, ATC07087, ATC07090, ATC04037, ATC00912, ATC01299, ATC00927, ATC01396, ATC01770, ATC00722, ATC00377, ATC07121, ATC03803, ATC05657, ATC06976, ATC07210, ATC01333, ATC06932, ATC05715, ATC02413, ATC00740, ATC07030, ATC00977, ATC01734, ATC07145, ATC07050, ATC07099, ATC07118, ATC03430, ATC02922, ATC06943, ATC04209, ATC07025, ATC07000, ATC00018, ATC06639
≤ 7	ATC06960, ATC07071, ATC07135, ATC05714, ATC07181, ATC07028, ATC05746, ATC05743, ATC00946, ATC07122, ATC01395, ATC07208, ATC06959, ATC07024, ATC07139, ATC03847, ATC07108, ATC07052, ATC07138, ATC07054, ATC04108, ATC01615, ATC06653, ATC02706, ATC07183, ATC02565, ATC06650, ATC07149, ATC00964, ATC07165, ATC07205, ATC01648, ATC01306, ATC00550, ATC07117, ATC04238, ATC02358, ATC07150, ATC06949, ATC00982, ATC02577, ATC01215, ATC04239, ATC03387, ATC06985, ATC00104, ATC00920, ATC01037, ATC01528, ATC07206, ATC07066, ATC00939, ATC00957, ATC01463, ATC01503, ATC07022, ATC04394, ATC06903, ATC07029, ATC06628, ATC07203, ATC00887, ATC07213, ATC07080, ATC06614, ATC01084, ATC02936, ATC03465, ATC02561, ATC07094, ATC07293, ATC07167, ATC03976, ATC07130, ATC03095, ATC00954, ATC00991, ATC00534, ATC07147, ATC07311, ATC06643, ATC07161, ATC07176, ATC00768, ATC07173, ATC07155, ATC06634 **Parafield**, ATC00762
≤ 8	ATC07133, ATC05762, ATC07103, ATC07218, ATC05990, ATC03464, ATC02356, ATC06635, ATC01475, ATC00966,

	ATC00968, ATC02352, ATC04056, ATC07284, ATC01321, ATC06629, ATC02638, ATC01459, ATC07179, ATC00778, ATC00973, ATC07056, ATC01728, ATC07134, ATC07119, ATC06972, ATC07312, ATC06652, ATC04208, ATC07027, ATC00613, ATC07193, ATC07212, ATC00976, ATC06930, ATC00758, ATC02523, ATC07001, ATC07075, ATC00724, ATC00897, ATC01272, ATC07268, ATC00975, ATC07319, ATC07287, ATC00965, ATC01659, ATC01476, ATC06944, ATC01517, ATC07283, ATC07189, ATC00896, ATC04519, ATC07037, ATC00547, ATC01510, ATC01736, ATC07042, ATC06627, ATC06924, ATC06915, ATC01468, ATC00909, ATC07197, **Kaspa**, ATC07277, ATC07163, ATC07305, ATC01436, ATC07272, ATC01079, ATC00989, ATC06654, ATC07111, ATC02708, ATC00718, ATC01040, ATC04612, ATC07102, ATC07276, ATC07120, ATC04234, ATC03988, ATC07127, ATC00667, ATC00962, ATC05141, ATC00888, ATC00955, ATC01023, ATC01263, ATC07214, ATC01730, ATC05136, ATC06918, ATC00969, ATC07143, ATC00972, ATC07318, ATC06904, ATC01392, ATC06965, ATC07195, ATC06638, ATC02408, ATC02376, ATC01044, ATC01394, ATC01555, ATC07216, ATC06895, ATC01076, ATC01687, ATC05137, ATC01083, ATC00738, ATC07101, ATC07304, ATC07182, ATC06656, ATC06945, ATC02394, ATC01791, ATC00895, ATC07144, ATC00540, ATC03429, ATC06637, ATC01393, ATC07077, ATC07297, ATC02351, ATC07064, ATC02555, ATC07010, ATC02366, ATC02573, ATC06937, ATC07038, ATC05781, ATC06633, ATC06900, ATC07013, ATC07098, ATC04387, ATC02536, ATC02520, ATC07006, ATC04062, ATC02391, ATC06988, ATC02434, ATC02437, ATC07178, ATC04197, ATC01089, ATC00934, ATC00719, ATC04035, ATC06996, ATC07201, ATC06917, ATC04064, ATC00935, ATC06975, ATC00707, ATC07019, ATC01438, ATC07192, ATC06916, ATC03846, ATC06644, ATC07114, ATC02649, ATC06992, ATC03414, ATC02343, ATC06971, ATC07095, ATC02374, ATC07136, ATC07172, ATC06907
≤ 9	ATC07168, ATC01502, ATC06893, ATC07202, ATC07185, ATC01036, ATC00931, ATC07160, ATC07273, ATC06636, ATC01088, ATC07040, ATC02444, ATC03489, ATC07032, ATC00742, ATC01042, **Bund**i, ATC03755, ATC03844, ATC06951, ATC00541, ATC07113, ATC06646, ATC06611, ATC05752, ATC05744, ATC07089, ATC01630, ATC07112, ATC03355, ATC07128, ATC03416, ATC00995, ATC03472, ATC07023, ATC02308, ATC00952, ATC02564, ATC03462, **Excell**, ATC01391, ATC07039, ATC01455, ATC01564, ATC02354, ATC02653, ATC04998, ATC02369, ATC01491, ATC07107, ATC06989, ATC04207, ATC07292, ATC07298, ATC07011, ATC05089, ATC07137, ATC07153, ATC03801, ATC03445, ATC07309, ATC00925, ATC07187, ATC07215, ATC04322, ATC02504, ATC07299, ATC07124, ATC07207, ATC07055, ATC07209, ATC02933, ATC07166, ATC07125, ATC06624, ATC07070, ATC07162, ATC04359, ATC01614, ATC06905, ATC06958, ATC02894, ATC01749, ATC01034, ATC07317, ATC02427, ATC01058, ATC01294, ATC02422, ATC07300, ATC06641, ATC00524, ATC00548, ATC04038, ATC04120, ATC06979, ATC02567, ATC04465, ATC07026, ATC00919, ATC07081, ATC02393, ATC04383, ATC06982, ATC07092, ATC02372, ATC05721, ATC02383, ATC02926, ATC00385, ATC06655, ATC02438, ATC01521, ATC07314, ATC06899, ATC07174, ATC04937, ATC03816, ATC01452, ATC06936, ATC01481, ATC01748, ATC00730, ATC00953, ATC00948, ATC05139, ATC06901, ATC07076, ATC05399, ATC05133, ATC06902, ATC05780, ATC07058, ATC02412, ATC01576, ATC05153, ATC02388, ATC00898, ATC07020, ATC01498, ATC01772, ATC0757, ATC01467, ATC02710, ATC03101, ATC00731, ATC01825, ATC07285, ATC01656, ATC02381, ATC01742, ATC05138, ATC01541, ATC07289, ATC00536, ATC07274, ATC03447, ATC01087, ATC06645, ATC07159, ATC01471, ATC05720, ATC07169, ATC07106, ATC07016, ATC00944, ATC01305, ATC01287, ATC06911, ATC02905, ATC02436, ATC04259, ATC06995, ATC00539, ATC07104
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Title:
Sources of high tolerance to salinity in pea (Pisum sativum L.)

Date:
2013-01-01

Citation:
Leonforte, A., Forster, J. W., Redden, R. J., Nicolas, M. E. & Salisbury, P. A. (2013). Sources of high tolerance to salinity in pea (Pisum sativum L.). EUPHYTICA, 189 (2), pp.203-216. https://doi.org/10.1007/s10681-012-0771-4.

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