Screening chickpea (*Cicer arietinum* L.) and wild relatives germplasm from diverse country sources for salt tolerance

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Abstract

Germplasm accessions of chickpea (*Cicer arietinum* L.) and its wild relatives, originating from 22 different countries, were screened in a greenhouse for salt tolerance. A split-plot completely randomized block design was used where the main factor was salt treatment thus (i) salt (a solution of NaCl with an equivalent electrical conductivity of 6 dSm^{-1} mixed with plant nutrient and (ii) plant growth nutrient solution only (control). The second factor was genotype with 200 accessions and 5 checklines tested. Plants were grown on sand and gravel mixture in 1:1 ratio in plastic pots. Treatments commenced 15 days from the day of sowing and continued on every other two days until the 6th week. A paired t-test and analysis of variance on necrosis scores, biomass reduction (%) and weighted scores showed a significant ($p<0.001$) effect of salt treatment and variation among genotypes. Ranking by weighted scores rated 47 (23.7%) accessions, from 7 countries of 4 geographical regions (Middle East, South Asia, USA and Ethiopia), as tolerant to NaCl. Five of these accessions rated as most tolerant included CPI 060546, ILC 01302 (from Turkey), ICC6474 from Iran, ICC 8294 from India and UC 5 from United States of America. None of the wild relative accessions rated as tolerant to NaCl.

Media summary

A search of germplasm accessions as sources of genes for breeding salt tolerance in chickpea.

Key Words

accessions, necrosis, biomass reduction, geographical sources

Introduction

Chickpea (*Cicer arietinum*) is an important pulse crop for Australian export markets and a major source of protein in diets elsewhere, particularly in South Asia, the Middle East and North Africa. Chickpea production in Australia is currently limited by a fungal pathogen *Ascochyta rabiei* but varieties with resistance to the ascochyta blight disease are being released to farmers in 2005. The poor growth of many crops, including chickpea, on the alkaline soils of the Victorian Mallee, has been attributed to a combination of hostile subsoil factors such as high pH, toxic levels of boron and salinity, in particular NaCl, and sodicity (Nuttall *et al*. 2003). Saline areas world-wide have also been increasing at an alarming rate mainly due to flood irrigated agriculture (Datta, Dayal and Goswami, 1987, Dua, 1992). While significant breeding research work has been done for bioticstresses, little has been done on tolerance to abiotic stresses such as salinity. Breeding for tolerance to salinity is the most economic and environmentally acceptable option to improve chickpea production on saline soils.

A commonly used strategy in searching for a trait of interest among a large collection of germplasm is to screen a sample of accessions from diverse eco-geographical sources. Due to high genetic diversity, landraces are preferred in the screening and, where the trait is scarce, the search is extended to its wild relatives. *C. arietinum* is known to have a narrow genetic base (Berger, Abbo, and Turner, 2003). It is against this background that a germplasm screening experiment was conducted with the objectives to (i) select chickpea accessions with improved tolerance to NaCl and (ii) relate the variation in salinity tolerance among accessions to their eco-geographical source.

Materials and Methods

Experimental methods

The experiment was conducted in a greenhouse at Horsham, Victoria, Australia. The Australian Temperate Field Crops Collection (ATFCC) provided seed of 200 accessions (181 landraces and 19 wild relatives), originating from 22 countries, randomly sampled from among 8105 accessions of its chickpea collection (Table 1). The wild relative accessions included *C. reticulatum* (8), *C. echnospermum* (3), *C. bijugum*(6), *C. judaicum* (1), and *C. pinnatifidum* (1). The first two belong to the primary gene pool while the last three species belong to the tertiary gene pool for chickpea.

The experiment had a split-plot completely randomized block design with 3 replicates, where the main factor was salt treatment with (i) salt (a solution of *NaCl with an equivalent electrical conductivity* (EC_e) *of 6* dSm^{-1} mixed with plant nutrient and (ii) plant growth nutrient solution only (control). The second factor was genotype with 200 accessions and 5 check lines. The check lines were ICCV96836 (salt tolerant line), ICC01314 and ICC07489 (moderately salt tolerant) and 'Lasseter' and 'Heera' (commercial cultivars).

Plastic pots of 14cm top diameter and 150cm depth were filled with a mixture of sand and gravel in a 1:1 ratio. Five seeds were sown in each pot and watered with tap water (with negligible ECe) until germination at 5 to 8 days. Plants were thinned to two after 12 days, and salt and control treatments commenced 15 days after sowing.

The salt treatment solution (6 dSm-1) was prepared by making up a 50 litre solution comprised of tap water, 630 mL NaCl (300g NaCl/L), 50 mL $CaNO_3$ (50g $CaNO_3$/L) and 50mL 'Nitrosol?'(a commercial Liquid Plant Food). The control solution was composed of 'Nitrosol?' and $CaNO_3$ only. The EC_e for the salt solution was adjusted to 6 dSm^{-1} +/- 0.05 while that for the nutrient solution measured 1.0 +/- 0.05dS. A 200 mL marked cup was used to water the plants with the solutions every second day.

Data collection and analysis

Necrosis scores, using a scale of 1 to 10 (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, 6 = Chlorosis becoming more severe on upper half plant, not necrotic, 7 = Chlorosis and necrosis more than more than half plant, 8 = More necrosis than 7, but still some green leaves, 9 = Only stem green (only top of stem and very youngest leaves still green, rest dead/ or a bit of stem green), 10= Plant completely dead) were recorded in weeks 4, 5, and 6. After the last scoring, the above-ground parts of two plants from each pot were harvested, oven drying at 70^{o}C for 72 hours and weighed (bulked).

The following parameters were calculated:

- (i) Biomass loss (g) = Control biomass (g) – Salt treatment biomass (g)
- (ii) Biomass reduction % = (Biomass loss/ Control biomass) x 100
- (iii) Weighted score = (Necrosis $score_{salt}$ x Biomass reduction) /100

Based on the weighted score (which ranged from 0 to 10) the 200 accessions were rated as most tolerant (0 – 1), tolerant (1 < 2), moderate (2 < 4) and intolerant (4 < 10). As the necrosis score was considered a key indicator for tolerance a necrosis $score_{salt}$ of 5.0 was the cut off point for tolerant accessions even if its weighted score was below 4.0. Using GENSTAT? software a paired t-test for necrosis scores and biomass (g) was performed. A two-way analysis of variance (ANOVA) was performed for the biomass (g) while one-way ANOVA was performed for necrosis scores, biomass reduction (%) and weighted scores. Simple regression analysis and graphics were used to show the distribution of the tolerant accessions in and bewteen the countries where they were collected.

Results and Discussion

Necrosis score and Biomass reduction (%)

A paired t-test at 95% confidence interval showed that the mean necrosis scores of salt treated plants were significantly higher ($p<0.001$) than those of the control plants. None of the control plants had a mean score above 2 while among the salt treated plants, necrosis scores ranged from 1.7 to 10 with over 75% of the accessions' scores above 5.0. There were significant differences between accessions for necrosis score in the salt treatment ($p<0.001$, l.s.d = 3.0). A paired t-test at 95% confidence interval showed significant biomass reduction (%) due to salt treatment. One-way ANOVA also showed that the variation in biomass reduction (%) was significant (($p<0.001$, l.s.d = 25) among the accessions. The reduction in biomass ranged from 19% to 96%, and 14% of the accessions had less than a 50% reduction. This

A simple regression analysis showed that the higher the necrosis score the higher the biomass reduction (%) with $r^2 = 0.1433$. In other studies with chickpea plants, salt stress was found to retard growth and thus reduce biomass (Datta, Dayal and Goswami, 1987). The relationship in this present study was poor because necrosis scores were considered as the key indicator of tolerance. Any accession with a necrosis score above 5 was rated as intolerant even if its weighted score was below 4.0.

Table 1: A summary of number of accessions tolerant to salt from each country

Region	Country	Degree of salt tolerance based on weighted scores for tolerance					
		Highly tolerant	Tolerant	Moderately tolerant	Intolerant		Total accessions
	Score range	(0-1)	(1<2)	(2<4)	(4<10)		
	Species	Cultivar	Cultivar	Cultivar	Cultivar	Wild relatives	
South Asia	Afghanistan			1	4		5
South Asia	Bangladesh			1			1
North Africa	Egypt				1		1
North/East Africa	Ethiopia		1	1	14		16
Former Soviet	Former Soviet				4		4

Union	Union						
South Asia	India	1	11	9	35		56
Middle East	Iran	1	6	6	38		51
Middle East	Israel		1		1		2
Europe	Italy				1		1
Middle East	Lebanon				2		2
Central America	Mexico				1		1
North Africa	Morocco				3		3
South Asia	Myanmar				2		2
South Asia	Pakistan		1	2	3		6
Former Soviet Union	Russian Federation				5		5
South Asia	Singapore				1		1
Europe	Spain				2		2
Middle East	Syria				1	3*	4
Former Soviet Union	Tajikistan				2		2
Middle East	Turkey	2	1	1	10	16*	30
North America	United States	1			1		2
Former Soviet Union	Uzbekistan						1

Total	5	21	21	151	19*	198

*All wild relatives accessions originated from Middle East and rated as intolerant to NaCl

Weighted scores and salt tolerant accessions

One-way ANOVA results for weighted scores showed high significant variation ($p<0.001$, l.s.d = 3.0) among the accessions. Ranking of the accessions based on weighted score rated 5 accessions as most tolerant, 21 as tolerant and 21 moderately tolerant. A total of 47 (23.7%) accessions of landraces were therefore rated as tolerant to salt. The 5 most tolerant accessions which had weighted scores less than 1.0 included CPI 060546, ILC 01302 (from Turkey), ICC 6474 from Iran, ICC 8294 from India and UC 5 from United States of America. These accessions had weighted scores of 0.77, 0.89, 0.88, 0.81 and 0.98 respectively, with biomass reductions (%) of 46, 23, 29, 37 and 21 respectively. As the accessions were screened with a high concentration of NaCl (EC_e of 6 dSm^{-1}) other potentially tolerant accessions might have been rated as intolerant. None of the wild relative accessions was rated as tolerant.

Geographical sources of tolerant accessions

The accessions rated as salt tolerant were from 7 different countries of 4 geographical sources including Middle East, South Asia, North East Africa (Ethiopia) and North America (USA) (Table 1). The results therefore suggest that sampling landraces from different geographical sources is the best strategy when searching for salt tolerant genes in chickpea. However, considering the narrow genetic base of chickpea, there is a possibility that these accessions (though collected as landraces) might have originated from one common geographical region (Berger, Abbo and Turner, 2003). DNA molecular analyses are therefore necessary to check the genetic relationship among these salt tolerant accessions.

Conclusion

The 23.7% of accessions identified as salt tolerant showed that there is a high probability of obtaining germplasm for breeding salt tolerance into chickpea. Within chickpea, tolerant accessions came from countries from four different geographical areas with a high number from Middle East and South Asia. Widening the diversity of sources of accessions screened is therefore likely to increase the chances of identifying genetic material for breeding salt tolerant chickpea cultivars.

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