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Salt sensitivity in chickpea: Growth, photosynthesis, seed yield components and tissue ion regulation in contrasting genotypes

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ABSTRACT

Chickpea is a relatively salt sensitive species but shows genotypic variation for salt tolerance, measured as grain yield per plant in mild-to-moderately saline soil. This experiment was designed to evaluate some physiological responses to salinity in three contrasting genotypes. One tolerant (Genesis836), one moderately tolerant (JG11) and one sensitive (Rupali) genotype were grown for 108 d in non-saline nutrient solution (controls) and two levels of salinity treatment (30 and 60 mM NaCl). No plants survived to maturity in the 60 mM NaCl treatment; however, Genesis836 survived longer (87 d) than JG11 (67 d) while Rupali died after 27 d; only Genesis836 flowered, but no pods were filled. At 30 mM NaCl, Genesis836 produced a few filled pods, whereas JG11 and Rupali did not. Genotypic differences in plant dry mass at the vegetative stage were evident only at 60 mM NaCl, while at maturity differences were evident at 30 mM NaCl. Photosynthesis was maintained to different degrees by the three genotypes (e.g. at 30 mM NaCl, 35–81% of controls; highest in Genesis836); photosynthesis was restricted predominately due to non-stomatal limitations as the intercellular CO_2 concentration was only modestly affected (94–99% of controls). Photosystem II damage was evident in the less tolerant genotypes (e.g. at 30 mM NaCl, actual quantum efficiency of photosystem II values were 63–96% of controls). Across treatments, shoot dry mass was negatively correlated with both Na^+ and Cl^- shoot concentrations. However, the sensitive genotype (Rupali) had equal or lower concentrations of these ions in green leaves, stems or roots compared to tolerant genotypes (JG11 and Genesis836); ion 'exclusion' does not explain variation for salt tolerance among these three chickpea genotypes. The large difference between Rupali (sensitive) and Genesis836 (tolerant) in the salt-induced reduction in net photosynthesis via non-stomatal limitations and the assessed damage to photosystem II, but with similar leaf ion concentrations, provides evidence that variation in 'tissue tolerance' of Na^+ and/or Cl^- in leaves contributes to the differential salt tolerance of these chickpea genotypes.

Introduction

Chickpea (*Cicer arietinum* L.) is an important cool-season pulse crop particularly in the Indian subcontinent and some other countries. Salinity is a major environmental stress limiting crop productivity in arid and semi-arid regions worldwide which is where chickpea is widely grown (Ryan, 1997). Chickpea is a salt sensitive species with an estimated worldwide yield loss of 8–10% due to salinity and complete crop failure can occur in the worst-affected soils (Flowers et al., 2010). Salinity impairs shoot growth of chickpea while reproductive processes are considered even more sensitive (Lauter and Munns, 1986; Vadez et al., 2012). Nonetheless, the causes of salt sensitivity in chickpea at different growth stages are not clear (Flowers et al., 2010).

Considerable variation in salt sensitivity/tolerance in chickpea was observed in salinized soils in pot experiments with plants grown to maturity (Turner et al., 2013; Vadez et al., 2007); however, there is need to understand salt sensitivity of chickpea as well as the tolerance mechanisms. Salt stress results in high accumulation of ions (mainly Na^+ and Cl^-) in different tissues which impairs cellular functioning and restricts plant growth and ultimately yield is reduced (Munns, 2002). Growth reductions in chickpea in saline conditions have been associated with accumulation of high Na^+ and/or Cl^- in shoots (Flowers et al., 2010); however,

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the responses of chickpea leaf photosynthesis to high Na^+ and/or Cl^- concentrations requires additional understanding. There is also need to explore, under controlled conditions, if contrasting chickpea genotypes vary for ion regulation in different tissues. Salinity tolerance in plants is conferred by numerous physiological traits and identifying causes of salt sensitivity and tolerance mechanisms in chickpea through exploiting genotypic differences would assist development of improved varieties for salt tolerance via a breeding approach to 'pyramid' these traits (Flowers and Yeo, 1986; Flowers et al., 2010).

Like in other plants (Munns, 2002), salinity impairs photosynthesis in chickpea (Murumkar and Chavan, 1993; Soussi et al., 1998). Photosynthesis is reduced in salt stressed plants through stomatal (i.e. closure) and/or non-stomatal (e.g. mesophyll conductance to CO_2 and damage to photosynthetic machinery) factors, resulting in reduced growth and eventually declining yield (Chaves et al., 2009). Gas-exchange measurements are used to estimate stomatal limitations under different environmental conditions (Chaves et al., 2009) whereas chlorophyll fluorescence measurements are a tool for determining damage to the photosynthetic apparatus which can be caused by different stresses (Murchie and Lawson, 2013). The present work combined studies of leaf gas-exchange and chlorophyll fluorescence parameters to explore the causes of photosynthetic limitations due to salt stress in three contrasting chickpea genotypes.

There are conflicting reports on the toxicity of Na^+ or Cl^- in chickpea; for instance Lauter and Munns (1986, 1987) suggested that growth reduction is closely related with high shoot Na^+ concentration; however, Vadez et al. (2007) found no correlation between shoot Na^+ (% dry mass) at the vegetative stage and seed yield in salinized soil. Using several of the same genotypes as Vadez et al. (2007), Na^+ concentration in the youngest fully-expanded leaves was reported to correlate negatively ($r^2 = 0.32$) with seed yield under salt stress (Turner et al., 2013). Additionally, some reports indicate that Cl^-, as well as Na^+, may be the cause of ion toxicity in chickpea (Dua, 1997; Mamo et al., 1996; Samineni et al., 2011). Grewal (2010), using only one genotype, found a negative correlation between chickpea shoot growth and leaf concentrations of both Na^+ and Cl^-. Evaluation of Na^+ and Cl^- accumulation in different tissues is important when considering the potential toxicity of Na^+ and/or Cl^-. Plant tolerance to salinity involves 'excluding' (i.e. restricting the rate of entry) these ions from shoots and tolerating excess ions that arrive in leaves by sequestering these into vacuoles (Munns and Tester, 2008). Chickpea typically contains lower concentrations of Na^+ in shoots than in roots, whereas the opposite is true for Cl^-, when expressed on a dry mass basis (Dua, 1997; Samineni et al., 2011; Sharma and Kumar, 1992; Sleimi et al., 2001); however, it is not known if genetic variation exists for ion regulation in different tissues. The present study evaluated tissue ions in three genotypes with contrasting salt tolerances.

The use of nutrient solution culture in experiments on salinity tolerance can provide advantages for several types of experiments, so the present study compared three contrasting chickpea genotypes from a previous report that used soil pot culture (Turner et al., 2013). Nutrient solution culture enables uniform root-zone treatments, avoiding the variations observed in soil and aiding future mechanistic work on physiological traits. Furthermore, root development in chickpea is considered more sensitive to salinity than shoot development (Ashraf and Waheed, 1993; Tejera et al., 2006) and nutrient solution culture enables easier access for root studies. In the present study, one tolerant, one moderately tolerant and one sensitive chickpea genotype were exposed to NaCl salinity to evaluate variation for: (1) growth and yield at the vegetative stage and at maturity in nutrient solution culture, (2) photosynthetic limitations in leaves, and (3) ion (Na^+, K^+ and Cl^-) concentrations in different tissues. We hypothesized that salt sensitivity in chickpea is due to high accumulation of ions (Na^+ and/or Cl^-) which impair photosynthesis and reduce growth of chickpea. In addition, genotype differences for NaCl tolerance might be related to differences in tissue ion concentrations (i.e., ion 'exclusion' resulting in low Na^+ and/or Cl^- concentrations in leaves) or the capacity to maintain function when tissues contain high Na^+ and/or Cl^- concentrations (i.e., 'tissue tolerance' of ions).

Materials and methods

Plant materials and growth conditions

Three chickpea genotypes, one tolerant (Genesis836), one moderately tolerant (JG11) and one sensitive (Rupali), were selected for this study based on previous seed-yield data from pot experiments with salinized soil (Turner et al., 2013). The present experiment was conducted in a phytotron (temperature-controlled glasshouse; $20/15 \pm 2\,^{\circ}C$ day/night) during winter and spring 2012 (July to November) at The University of Western Australia, Perth WA, Australia (31°57′S, 115°47′E). Plants received natural light (transmitted through glass panels); no supplementary light was needed and the timing of the experiment coincided with the local winter-spring growing season for annual broadacre crops. Plastic pots (4.5 L) covered with aluminium foil were used to grow plants in continuously-aerated nutrient solution. The composition of the full-strength nutrient solution, in deionized water, was (mM): 5.0 Ca^{2+}, 5.0 K^+, 0.625 NH_4^+, 0.4 Mg^{2+}, 0.2 Na^+, 5.4 SO_4^{2-}, 4.4 NO_3^-, 0.2 $H_2PO_4^-$, 0.1 SiO_3^{2-}, 0.1 Fe-sequestrene, 0.05 Cl^-, 0.025 BO_3^{3-}, 0.002 Mn^{2+}, 0.002 Zn^{2+}, 0.0005 Cu^{2+}, 0.0005 MoO_4^{2-} and 0.001 Ni^{2+} (Samineni et al., 2011). The solution was buffered with 1.0 mM MES (2-[*N*-morpholino]ethanesulfonic acid) and adjusted to pH 6.5 using KOH.

Seeds were washed with commercial bleach added to deionized water (final concentration of active ingredient: 0.042% (w/v) sodium hypochlorite) for 5 min, rinsed twice in tap water, the seed coat was pricked, and imbibed overnight in aerated solution of 0.5 mM $CaSO_4$ in darkness. Imbibed seeds were germinated on mesh on 10% concentration aerated nutrient solution in the dark for 2 d and seedlings were then transferred to 25% concentration aerated nutrient solution and exposed to light. After 7 d in 25% concentrated solution, three individual healthy seedlings were transferred to each pot containing full concentration aerated nutrient solution to grow for another 4 d before the start of the NaCl treatments.

Treatment application

NaCl treatments (30 and 60 mM) were started 13 d after imbibition and control pots without NaCl treatment (but containing 0.2 mM Na^+ from Na_2SiO_3 and 0.05 mM Cl^- in the micronutrient stock) were also continued. NaCl was added in 15 mM increments daily until the desired concentration. Solution in all pots was renewed on a weekly basis and topped up with deionized water as required (initially every second day, then daily in the final four weeks). Pots were arranged in a completely randomized design with three replicates and the pots were moved randomly every week to minimize any possible effects of environmental variation within the phytotron. Solution pH was measured every 2nd day and maintained at about 6.5 by additions of KOH as required. Initially, no KOH additions were required as plants were small and the solution was buffered with MES, with small amounts subsequently needed and particularly in control pots with larger plants towards the end of the experiment (up to 2 mL of 1 M KOH per week in the final four weeks).