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Large number of flowers and tertiary branches, and higher reproductive success increase yields under salt stress in chickpea

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ABSTRACT

Salinity is a major problem worldwide and improving salt tolerance of chickpea (*Cicer arietinum* L.) will allow expansion of production to more marginal areas. Plant reproduction suffers under salt stress in chickpea, but it remains unclear which process is most affected and what traits discriminate tolerant from sensitive lines. Three pot experiments were carried out to compare the effects of salt application (17 g NaCl kg^{-1} Alfisol) at sowing (SS) and at the start of flowering (SF) on growth, canopy transpiration, plant architecture, and flower, pod and seed development (timing, numbers, mass, abortion). Six pairs of tolerant/sensitive lines with similar flowering times within each pair, but different among the pairs, were used. Shoot biomass was similar in tolerant and sensitive lines in the SS and SF treatments, whereas the seed yield decreased more under SS and SF treatments in the sensitive lines. The flower, pod and seed numbers within all pairs was higher in the tolerant than in the sensitive lines in the non-saline controls, but the differences in numbers of seeds and pods further increased in both the SS and SF treatments. By contrast, neither the duration of flowering or podding, nor the percentage of flower or pod abortion, discriminated tolerant from sensitive lines. In non-saline controls the numbers of primary branches was 100% higher across the sensitive lines, whereas the number of tertiary branches was 8-fold higher across tolerant lines. The relative transpiration of the tolerant lines in the salt treatments was above that for the sensitive lines in three pairs of tolerant/sensitive lines, but did not differ within two pairs. Our results demonstrate that constitutive traits, i.e. numbers of flowers and tertiary branches, and adaptive traits, i.e. high number of seeds under salt stress, are both critical aspects of salinity tolerance in chickpea.

1. Introduction

Salinity is a major and increasing problem worldwide that needs to be addressed in order to maintain agricultural production. Genetic approaches to improve crop tolerance of salinity (i.e. breeding) will be important, and especially since management options require a large investment that poor farmers are unlikely to implement. Chickpea is grown in various regions challenged by increasing soil salinity (Flowers et al., 2010). There exists genetic variation for salinity tolerance which can be used to breed superior varieties (Vadez et al., 2007; Krishnamurthy et al., 2011). However, breeding would be made more efficient by focusing on those traits that are critical, but still relatively unknown, for the salinity tolerance of chickpea (Flowers et al., 2010).

Although salinity affects shoot growth, its effect on reproductive processes is relatively more severe in chickpea. Genotypic tolerances, based on seed yield obtained under saline conditions, were related more to maintaining a large number of seeds and less to maintaining a high biomass production relative to a non-saline control (Dhingra and Varghese, 1993; Vadez et al., 2007; Krishnamurthy et al., 2011). From the early development of flower meristems until the development of seeds in the pods, abiotic stresses can affect a number of processes. Abiotic stresses are known to affect meiosis during gamete production and male sterility appears to be more common than female gamete sterility (Saini, 1997). Flower production was decreased under drought in chickpea (Nayyar et al., 2005; Fang et al., 2010), or under heat stress in groundnut (Vara Prasad et al., 2000). Flower abortion was another cause for yield decrease under drought in a study that showed that cultivated chickpea aborted a larger number of flowers than wild germplasm (Nayyar et al., 2005), or in chickpea exposed to cold where plants produced flowers but failed to set pods (Clarke and

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Siddique, 2004; Srinivasan et al., 1998). Pod abortion also was the key limitation to seed yield in chickpea exposed to drought stress (Behboudian et al., 2001; Leport et al., 1999, 2006; Fang et al., 2010). Finally, the duration and rate of seed filling can also explain yield variations under drought stress in chickpea (Davies et al., 1999). So, a number of processes during the reproductive phase can be affected by abiotic stresses. There is, unfortunately, limited knowledge on which of these processes are most affected in chickpea exposed to salinity and whether tolerant and sensitive lines differ in sensitivity of one or several of these processes to determine seed yield in saline soils.

Carbohydrate supply could be a limitation. Reproductive structures are quite demanding for carbohydrates and the supply of sucrose to the developing embryos was shown to be critical to rescue embryos of water stressed plants where photosynthesis was inhibited (Zinselmeier et al., 1999). So, reproductive failure under salt stress could be related to decreased transpiration relative to unstressed plants, where transpiration is a surrogate for photosynthesis/carbon supply to the developing embryos. Recent data (Vadez et al., 2011) also suggest that in early chickpea lines, where flowering is simultaneous with sustained shoot growth, the high yielding lines were those having both reproductive success and sustained shoot growth under saline stress. The sustained growth under saline stress could lead to increased branching and to an increased number of reproductive nodes and flowers. So, the question remains whether shoot growth and branching could lead to more reproductive structures, especially in early duration lines.

The overall objective of this work was to pinpoint traits that distinguish tolerant and sensitive lines, with a particular focus on plant architecture and reproductive biology. The work was performed with six pairs of tolerant/sensitive lines of chickpea in which flowering time was similar within each pair. A first objective was to compare effects of salt stress application at sowing and flowering on biomass and yield, with the hypothesis that salt effects would be the same in these two types of treatments if reproduction was the most sensitive plant process to salinity in chickpea. A second objective was to assess the effect of salt on phenological development (flowering/podding duration) and growth patterns (rooting, shoot branching). A third objective was to investigate the direct effects of salt treatment on reproductive structures (flower number and abortion, pod number and abortion, seed number and size). The last objective was to investigate how salinity affects plant transpiration during reproduction.

2. Material and methods

2.1. Growth conditions and treatments

Chickpea (*Cicer arietinum* L.) was grown under saline and non-saline conditions in 20 cm diameter pots filled with 4 kg of Vertisol soil (Vertic Inceptisol) collected from the ICRISAT farm, mixed with farm manure at a rate of 50:1 (soil:manure, w/w), autoclaved, sieved and sun dried. The soil [pH 8.1, CEC/clay ratio = 0.8 and an electrical conductivity = 0.10 dS m^{-1} in saturated paste extract (ECe) (El Swaify et al., 1985)] was fertilized with di-ammonium phosphate and muriate of potash, at a rate of 0.3 g and 0.2 g per kg soil, well mixed with the soil before filling the pots. Soil was inoculated with standard chickpea rhizobium inoculum at the time of sowing.

Three experiments were carried out between November and March at ICRISAT headquarters (Patancheru, AP, India, Latitude: 17°31′53 N, Longitude: 78° 15′ 54 E), two outdoors (Experiments 1 and 2) and one in a greenhouse (Experiment 3). The average maximum temperatures ranged between 25.3 and 36.8 °C and minimum temperatures between 8.4 and 22.0 °C outdoors. The average maximum temperatures ranged between 29.7 and 32.6 °C and minimum temperatures between 15.4 and 16.1 °C in the greenhouse. Four seeds were planted in each pot. These were thinned to two plants per pot at 3 weeks after sowing.

Three treatments were used: a non-saline control (C), a salt treatment applied at the time of sowing (SS), and a salt treatment applied at the beginning of flowering (SF), therefore applied at different dates depending on genotype. The two salt treatment s were equivalent and corresponded to a salt application in the irrigation water in sufficient quantity to wet the Vertisol to field capacity (1 L per 4 kg pot) and result in the equivalent of 80 mM NaCl in the solution (1.17 g NaCl kg^{-1} soil). The salt was applied in split applications. In SS, half the dose was applied at sowing by wetting the soil with 1 L of 40 mM NaCl solution, while the second dose was applied 1 week after sowing by adding 400 mL of 100 mM NaCl. In SF, half the dose was applied when all plants of a given pair of lines had started flowering, by flushing the pots with 1 L of 40 mM NaCl, and then the following day flushing again with 1 L of 80 mM NaCl. At each time, the non-saline control pots were also flushed with 1 L of water containing no salt. Lines ICC1431 and ICC6263 mistakenly received an additional L of 40 mM NaCl solution in the SF treatment, likely explaining their higher shoot, pod and seed mass decrease than the other lines. Therefore, up to flowering time, the plants of the SF treatment and the C treatment were treated the same way. After salt application in the SS and SF treatments, pots were watered with tap water containing no significant amount of NaCl, and maintained close to field capacity (determined gravimetrically) to avoid an increase in salt concentration in the soil solution, but also to avoid leaching of the salt.

2.2. Plant materials and details of experiments

Experiment 1 (Exp.1) was carried out to compare the plant architecture, rooting, and timing of pod/seed production, the number of flowers and flower abortion, along with the seed yield/pod/seed number and shoot dry mass, and to assess the effect of salt stress on the rate of transpiration at the time of flowering (SF). Primary branches were those produced on the main stem, while secondary and tertiary branches were those produced on the primary and secondary branches, respectively. Exp.1 was conducted outdoors and used five pairs of lines that were classified as salt tolerant or salt sensitive based on seed yield in saline conditions in a previous evaluation (Krishnamurthy et al., 2011): ICC1431/ICC6263 (tolerant/sensitive in each case); JG11/ICCV2; ICC9942/ICC15802; ICC3512/ICC13283; ICC7819/ICC7571. These five pairs of lines had similar flowering time within pair, i.e. 37 DAS, 49–51 DAS, 49–51 DAS, 49 DAS, and 49 DAS, respectively. This was important because previous report showed higher tolerance to salinity in early duration lines (Vadez et al., 2007). Three treatments were used (C, SS, SF), each with eight replicate pots per genotype. Four replicate pots per line and treatment were harvested at 30 days after treatment application in the SF treatment for assessing rooting and branching, whereas the other four replicates were kept until maturity.

Experiment 2 (Exp.2) was carried out to confirm the measurements of Exp.1, and contained an extra pair of tolerant/sensitive lines, with the objective to compare the yield reduction and flower/pod/seed number and abortion in the SS and SF treatments. In this experiment, no plants were grown in non-saline soil. Exp.2 was conducted outdoors and used six pairs of tolerant/sensitive lines (flowering time in parenthesis): ICC1431/ICC6263 (46–44 DAS); JG11/ICCV2 (35 DAS); ICC9942/ICC15802 (45–44 DAS); ICC3512/ICC6877 (46 DAS); ICC7819/ICC7571 (46 DAS); ICC5845/ICC13283 (46 DAS). Four replicate pots per line were used in each of the two treatments (SS, SF).