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Screening for seedling salt tolerance in chickpea (*Cicer arietinum* L.)

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Abstract
The present study was undertaken for screening of seedling in 50 chickpea genotypes on root length and shoot length traits of chickpea. Seven lines have been putatively identified to be tolerant to seedling salt stress. They are ICCV 92311, ICCV 10315, ICCV 00102, IG5856 (4 % reduction in seedling parameters studies under salt stress) are the most tolerant along with the established checks ICCV10, CSG 8962 and JG62. Selection for genotypes with tolerance to salt stress will ensure greater establishment of seedlings in saline soils and better yield under salt stress. Current study help to Identification of new donors for salinity salt tolerance.

Keywords: Salt tolerance, root length, shoot length, chickpea

Introduction

Chickpea (*Cicer arietinum* L.; Family: Fabaceae) a self-pollinated, diploid (2n=16), cool season pulse crop with a genome size of ~738-Mb with an estimated 28,269 genes (Varshney *et al.,* 2013) [24] and is widely grown in more than 50 countries representing all the continents (Upadhyay *et al.*, 2011) [22]. Chickpea is grown mostly in South Asia and Sub-Saharan Africa, which accounts for more than 75% of the world chickpea area. Global chickpea production has increased from 7.68 million tonnes (1961) to 13.73 million tonnes (2014) (FAOSTAT, 2016). India ranks first in terms of cultivated area and production. However, there is a slight increase in the chickpea cultivation area in India from 9.27 million hectares (1961) to 9.92 million hectares (2014), but production increased significantly from 6.25 million tonnes (1961) to 9.88 million tonnes (2014) due to significant increase in the productivity from 0.67 t/ha (1961) to 0.99 t/ha (2014). Other major chickpea producing countries are Australia (629,400 tonnes), Myanmar (562,163 tonnes), Ethiopia (458,682 tonnes), Turkey (450,000 tonnes), and Pakistan (399,030 tonnes) (2014) (FAOSTAT, 2016). More than 95% of the area of production and consumption of chickpea is shared by the developing countries. Chickpea is grown mainly in South East Asian countries. Kabuli (white seeded) and Desi (brown seeded) are two main types of cultivated chickpea, presenting two diverse gene pools (Nawroz and Hero, 2011) [19].

Chickpea is highly self-pollinated with a crossing rate of less than 1% (Singh *et al.*, 2008) [21]. Chickpea is an important source of protein in diet and therefore an important role in food economy of the country. It is also a rich source of zinc, folate and protein and high in dietary fiber and for this reason a healthy source of carbohydrates for persons with insulin sensitivity or diabetes. It is low in fats and mainly of this is polyunsaturated. According to International Crop Research Institute for the Sami Arid Tropics (ICRISAT), it has an average of 23% protein, 64% total carbohydrates (47% starch, 6% soluble sugars), 5% fats, 6% crude fibers, 3% ash. High mineral content is alsoreportedthat is phosphorous (340mg/100g), Ca, Mg (140mg/100g), Fe (7mg/100g) and zinc (3mg/100g).

Due to high degree of self-pollination, chickpea has very narrow genetic base among cultivated chickpea accessions which is limiting the genetic improvement of chickpea through breeding efforts. To know the level of natural variation among cultivated chickpea and wild accessions at molecular level it is essential to develop pre-breeding and breeding strategies for chickpea. Diversity analysis is essential to understand *per se* the variability present in germplasm collection that can be practically put to use in plant breeding programmes for recombination breeding. Now simple sequence repeats or SSRs are the preferred marker in most areas of molecular genetics as they are highly polymorphic even between closely

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related lines, require very low amount of DNA and are transferable between populations. SNPs/SSRs are generally co-dominant markers and are brilliant for studies of population genetics and mapping (Jarne and Lagoda, 1996; Goldstein and Pollock, 1997) [12, 10]. SNPs/SSR genotypic data from a number of loci have potential to provide distinctive allelic profiles for establishing genotypes identity (Bharadwaj et al., 2010; 2011, Chaudhary et al., 2012) [3-4, 7].

Soil salinity is a major constraint that limits crop productivity and almost 80 million ha of the worlds' arable land is prone to this stress (Flowers et al., 2010) [9]. Globally, 20 per cent (45 million ha) of irrigated and 2 per cent (32 million ha) of dry land are constrained by salinity (Munns et al., 2008). This is predicted to expand to 50 per cent worldwide by the second half of the 21st century (Ladeiro et al., 2012) [14]. In conjunction with the predicted marked expansion of salinity-affected area, an additional two billion people are anticipated to inhabit the planet by 2050. Therefore, soil salinity is a major stumbling block to meeting the predicted global food demand by 2050. Abiotic stresses account for about 6.4 million tonnes in crop yield losses every year, where soil salinity is a major environmental stress (Jha et al., 2014) [13]. The enormity of the current challenge of sustaining or increasing productivity to meet yield demands in the face of increasing salinity has been well highlighted (Munns et al., 2008) [18]. This translates into an urgent requirement for improved crop production by almost 70 per cent. However, salinity limits the plant growth and severely affects the reproductive processes, resulting in lowered crop yields and chickpea is intrinsically salt sensitive unlike cereals (Flowers et al., 2010) [9]. The imperative is therefore to elucidate the genetic architecture of salinity tolerance and in particular the molecular mechanisms underpinning the tolerance responses.

Existence of large genetic variation within a gene pool would enable genotypes conferring desirable traits to be identified, including those that are able to withstand adverse saline conditions (Vadez et al., 2007) [23] Salinity tolerance is conferred by several physiological factors which have been recorded in response to salt stress to identify tolerant/sensitive genotypes (Moses et al., 2008) [16]. To enable more strategic and precise selection of tolerant genotypes, frontier genomics technologies, such as gene expression profiling, have the potential to identify robust transcripts/candidate gene(s) and their alleles that condition tolerance (Hiremath et al., 2011) [11]. Recent advances have substantially illuminated the mechanisms of salinity tolerance in chickpea, thereby paving the way towards the strategic incorporation of tolerance-imparting component traits into elite genetic backgrounds. Here, we offer a critical overview of the different genomics approaches that have been used to address salinity in chickpea. Later follows a brief discussion on targeted breeding aided by genomics tools, concluding with the future research needs for developing salinity tolerant chickpea.

Material methods
The present investigation was carried out to study the effect of salinity on seedling growth of chickpea genotypes grown under salt stress. Screening of 50 chickpea genotypes consisting released varieties, pre released advance breeding lines, germplasm collections, land races and wild derivatives lines of both. The crop was raised in large paper glasses filled uniformly with 700g of farm soil with a pH of 8.1. The recommended cultural practices for chickpea were followed to raise the crop. The saline treatment was applied as an 80mM solution of NaCl in sufficient volume to wet the soil to field capacity, this corresponds to 175ml of 80mM of NaCl solution for 700g soil. Non-saline controls were also maintained and the experiment was repeated twice with two replications each time.

Phenological Parameters
Shoot length
The length of the main shoot was measured in cms from the ground level to the tip of the plant.
A meter scale was used for this purpose.

Root Length
The length of the nodal root was measured in cms from the initiation of root to the tip of the nodal root. A meter scale was used for this purpose.

Results and Discussion
Screening of 50 genotypes state that tolerant plants showed a reduction of 35 % in shoot growth and 5 % in root growth while sensitive plant showed 73 % in shoot and 51 % in root growth compared to the unstressed conditions (Table 1 and Fig 1). Shoot growth is also reduced by salinity due to the inhibitory effect of salt on cell division and enlargement in the growing point (Kaymakanova, 2009). Among the genotypes 35 were highly susceptible for seedling salt stress, they have not germinated at all. The effect of salinity on germination of seeds can be either by creating osmotic potential which prevent water uptake or by toxic effects of ion on embryo viability of the seeds (Houle et al., 2001). Rest all except seven were susceptible to seedling salt stress. Among them four lines that have been putatively identified to be tolerant to seedling salt stress are ICCV 92311, ICCV 10315, ICCV 00102, IG5856 (4 % damage only under salt stress) along with the established checks ICCV10, CSG 8962 and JG62. Under salt stress conditions (80 mM NaCl) these lines outperformed others (Fig 2). Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield (Greenway and Munns, 1980). Genotypic variation for salt tolerance exists in chickpea germplasm. Selection for genotypes with tolerance to salt stress will ensure greater establishment of seedlings in saline soils and better yield under salt stress. Current study help to Identification of markers linked to salinity tolerance which will aid in MAS and MABC programmes.

Table 1: Top lines with less root and shoot damage in saline conditions

Entry	ROOT			SHOOT		
Name	Normal Root Length	Root Length under stress	Difference in Root Length	Normal shoot length	Shoot length	difference in shoot length
CSG 8962 (Ch)	23	10.5	12.5	16.5	15.2	1.3
ICCV10 (Ch)	29	17.3	11.7	14.5	13.9	0.6
IG 5856	24	7.1	16.9	11.5	7.2	4.3
ICCV 92311	24	6.2	17.8	18.5	9.3	9.2
ICCV 10315	23	5.9	17.1	13.6	4.9	8.7

Fig1. Seedling root profile of resistant genotype for salinity.
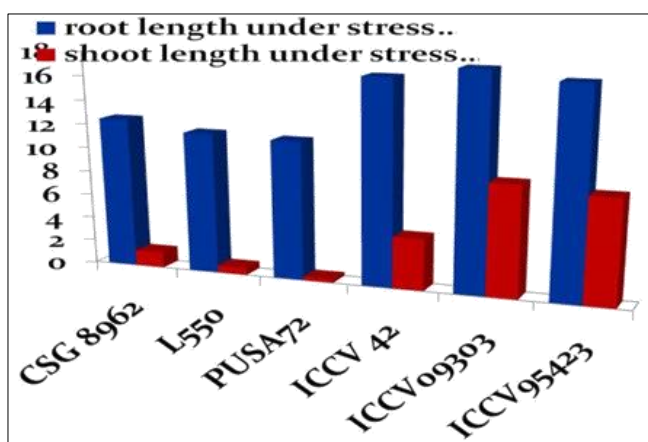
Fig 2: Comparison of root and shoot length under stress

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