DOI:10.34101/ACTAAGRAR/1/12274

Evaluation of chickpea (*Cicer arietinum* L.) in response to salinity stress

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SUMMARY

Soil salinity is a severe and expanding soil degradation problem that affects 80 million ha of arable lands globally. Chickpea (Cicer arietinum L.) is very sensitive to saline conditions; the most susceptible genotypes may die in just 25 mM NaCl in hydroponics. Approximately 8–10% yield loss in chickpea production is estimated due to salinity stress. However, it is still not established why chickpea is so susceptible to salt affection. Salinity (NaCl) impedes germination of seeds, though chickpea varieties considerably differ from one another in this respect. Some chickpea genotypes are more tolerant in the stage of germination, tolerating even 320 mM NaCl. The reasons of this variation are unrevealed; there is a shortage of knowledge about the germination abilities of chickpea genotypes in saline conditions. Nevertheless, the effect of salt stress on vegetative growth can be analysed in hydroponics, in pot or field conditions, regardless the experimental environment, the ranking of genotypes regarding salt resistance is coherent. Chickpea genotypes can be different in their ability to retain water, maybe under salt affection; the more salt tolerant lines can maintain higher water content in the shoots, while the more sensitive ones cannot. The identification of salt tolerant chickpea landraces based on developing genetic variability is a suitable strategy to combat against salinity problems arising in arid and semi-arid areas.

Keywords: *abiotic stress; legumes; salt affection; chickpea genotypes*

INTRODUCTION

The reduction of freshwater use is required from the agricultural sector, and at the same time, higher rate of the use of low-quality waters is necessary to fulfil the increasing demand for domestic and industrial waters. Therefore, in the future, the more intensive use of saline waters cannot be avoided in agricultural production. As a consequence of the decreasing quality of water resources and increasing salinity in agriculture lands, soil fertility is being reduced. High concentrations of salts such as sodium chloride (the most dominant), calcium sulphate and sodium carbonate are characteristic to saline waters and saline soils (Shahid et al., 2018). In terms of plant growth, salinity is among the most serious abiotic stresses (Ceritoğlu et al., 2020).

Salinity stress has deleterious impacts on several physiological, biochemical functions, which might hinder plant growth and development and cause eventually death or a dramatic reduction in the crop production depending on stress severity. High concentration of soluble salts in the root zoon reduces the ability of plants to uptake sufficient quantity of water and nutrients due to the osmotic effect, which will imbalance plant water relations causing wilting, as a consequence of decline in the cell water potential affecting the gas exchange process.

Continuous exposure of plants to salinity stress results in a remarkable accumulation of the determinant toxic ions (Na^+ and Cl^-) leading to chlorosis and bleaching of leaves, which will reduce their photosynthetic capacity and eventually dry matter accumulation.

Chickpea *(Cicer arietinum* L.) is among the first grain crops that people started to cultivate. Recently, chickpea production is in the 3rd mass lace among food legumes in the world after beans (*Phaseolus* spp.) and field pea (*Pisum sativum* L.). Chickpea is produced over 50 countries, but the chickpea growing area exceeds 20,000 ha only in 22 countries, while 10,000 to 20,000 ha are covered with chickpea in 19 countries. 8.4 million tons is the total annual world production. The main chickpea producing countries are as follows: India (65% of annual production), Pakistan (10%), Turkey (7%), Iran (3%), Myanmar (2%), Mexico (1.5%), and Australia (1.5%) (FAO, 2018). Ethiopia, Iraq, Jordan, Morocco Syria; Canada, Tanzania and Malawi are also considerable chickpea producer countries.

Regarding grain legumes, chickpea is the second in importance, it is a good resource of essential amino acids and protein (20–25%). Soil fertility is increased by chickpea due to its unique ability to biologically fix atmospheric nitrogen. In terms of global production of pulse crops in semi-arid regions, chickpea is the most important food grain (Roy et al., 2010). The high chickpea production potential (over 14.79 million tons) cannot be fully realized due to, among others, salinity and drought stress (Kashiwagi et al., 2015).

LITERATURE REVIEW

Economic importance of Chickpea

Chickpea can be used in human nutrition as well as for animals feed due to its richness in protein (20–25%) and CHO (49–55%), plus vitamins and minerals (Grasso et al., 2021). After frying, the sprouted seeds of chickpea become edible for humans. The plant parts and pod husk can be utilized for milking animals. The crop also enhances soil fertility by fixing about 70–90 kg N ha^{-1} (Rupela, 1987). Vinegar with high medicinal value can be prepared from an acidic liquid (oxalic

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DOI: 10.34101/ACTAAGRAR/1/12274

and malic acids) which can be taken from the granular hairs of chickpea plant at flowering stage.

Chickpea seed, as a supplement rich in protein, is an important ingredient in cereal-based diets, especially for the people of the developing countries as animal protein is too expensive for them or because they are vegetarian by choice.

Botanical classification of Chickpea

Chickpea is ranked into family of *Fabaceae* (*Leguminoseae*) and into the genus of *Cicer*. 39 species, 38 wild species and one cultivated species (*Cicer arietinum* L.) of chickpeas are known. It possesses 2n=16 chromosomes. According to Vavilove (1926), the origin of cultivated chickpea is the region of the Mediterranean Sea and Southwest Asia.

Desi and Kabuli are the two main types of chickpeas. The Desi type has generally small seeds (< 200 mg per seed), the seed coats are coloured and its shape is angular. The Kabuli type is characterised by larger seeds (> 350 mg per seed), its seed coats have cream colour and a shape of a 'rams-head'. Kabuli and Desi types can be hybridised, but Desi is strongly preferred by the consumers to Kabuli.

Van der Maesen (1987) divided genus Cicer into 4 sections on the basis of geographical distribution, morphology, and lifespan:

- Horizontal or firm erect stems branching from base or middle are characteristic for Monocicer.
- Thin stem creepers and small flowers are characteristic for Chamaecicer.
- The leaf rachis ends in a tendril or a leaflet in section Polycicer.
- Branched stems with large flowers and woody base persistent spiny leaf rachis are the characteristics of Acanthocicer.

The genetic diversity is large within this species, Varshney et al. (2021) published huge polymorphisms in chickpea by investigating 3366 germplasm, including cultivated and wild types as well.

Environmental requirements of Chickpea

Chickpea is an annual crop, in South and Southeast Asia, it is traditionally produced after the rainy season; while in West Asia Middle East, and South Mediterranean Region, in the winter rainfall season, while in North America and North Mediterranean in springtime (Khan et al., 2015). Chickpea is considered a cool season crop that demands temperatures of 21–27 °C daytime and 18–21 °C at night to perform optimally (McVay et al., 2013). In terms of texture, it prefers well-drained clay loam to sandy loam soils with pH>7. Sufficient soil moisture content is needed for good germination and seed development, later, its water and nutrient uptake can be limited when the season is dry. The chickpea growing area under irrigation is increasing as this crop is responsive to proper water management. Nevertheless, chickpea fields are generally not irrigated. Chickpea is susceptible for floods and water logging even in short periods; therefore, it is necessary to secure appropriate drainage to avoid the reduction of growth and the chance of root and stem rots (Farooq et al., 2017). Gaur et al. (2010) reported that the highest yield and quality of chickpea seeds have been produced in areas where the precipitation pattern is well distributed without heavy rainfalls. In terms of plant nutrition, the usual recommendation is 20–30 kg nitrogen, 40–60 kg phosphorus, and 17–30 kg potassium substances per ha (Kurdali, 1996).

Stress sensitivity

According to Nene and Reddy (1987) and Reed et al. (1987), diseases such as ascochyta blight (*Ascochyta rabiei*), fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), botrytis grey mould (*Botrytis cinerea*); and pests such as leaf miner (*Liriomyza cicerina* and Helicoverpa pod borer (*Helicoverpa armigera* and *H. punctigera*) are the main biotic constraints to the production of chickpea. Soil salinity (salt affection), drought, low temperatures are the main abiotic stress factors endangering the productivity of chickpea. Flowers et al. (2010) estimated approximately 8–10% yield loss in chickpea production globally due to salinity stress.

Chickpea is very susceptible to salt stress at both the vegetative and reproductive stages, especially during the early growing and maturing stages. In terms of environmental salt stress, the most critical stage is the seedling growth (Pujol et al., 2000). Even salt tolerant chickpea varieties are destroyed within 75 days after they were exposed to 40 mM of NaCl (Samineni et al., 2011). According to Flowers et al. (2009), there is a significant variation among the different chickpea genotypes, the most sensitive ones failed to grow even in 25 mM NaCl. However, the more tolerant genotypes survived the salt stress induced by 100 mM NaCl in hydroponics.

Depending on the extent and severity of salinity stress, changes in several physiological and metabolic processes can occur in plants resulting in limited crop production. In the first phase, plant growth is repressed by soil salinity through osmotic stress, later ion toxicity occurs (Rahnama et al., 2010; James et al., 2011).

Some chickpea genotypes are more tolerant in the stage of germination, tolerating even 320 mM NaCl. Cl^- had higher concentrations in shoots than Na^+ in a salty medium, as the anion was secreted from glandular hairs on leaves, stems and pods. Salinity induces osmotic adjustment as it is larger in the nodules than in the roots or leaves. Due to this mechanism, it also reduces the amount of water available for the plants. If the plants are exposed to NaCl levels considered moderate for other crops, chickpea can react with growth reductions. Serraj et al. (2004) grew treated 234 chickpea plants with 80 mm NaCl solution in a Vertisol and found a 60% biomass reduction 40 days after sowing. Abido and Zsombik (2017) found genotype-dependent salt tolerance of the 3 studied Hungarian wheat landraces (Gamási, Fáti and Kartali) under 0,5,10 and 15 dSm^{-1} NaCl-induced salinity stress.

DOI:10.34101/ACTAAGRAR/1/12274

Higher salinity affected more the germination parameters, seedling growth traits and biochemical indicators.

In chickpea, increased senescence can be induced by salinity stress (Katerji et al., 2001) as well as increased ethylene and its precursor 1-aminocycloprane-1-carboxylic acid (ACC) production in nodules and roots (Nandwal et al., 2007). In chickpea grown in NaCl (100 mM), the concentration of photosynthetic pigments declined resulted in 60% lower photosynthetic activity (Murumkar and Chavan, 1993). Epitalawage et al. (2003) found differences in the impacts of salinity on chlorophyll fluorescence in the cases of different chickpea genotypes. Maliro et al. (2008) figured out the symptoms of leaf necrosis when Na^+ and/or Cl^- levels were higher in tissues than the plants can tolerate. This was probably in conjunction with ion toxicity that resulted in elimination of chlorophyll in leaf cells. They also established that the visually detectable scores of necrosis could be used as an index of salt tolerance or resistance. Kukreja et al. (2005) found that 10 dS m^{-1} salinity induced 180% increase in hydrogen peroxide of in chickpea (CSG-8962) roots and 170% increase of lipid peroxidation. However, in another study by Eyidogan and Oz (2007), after four days treatment of chickpea with 100 mM NaCl in hydroponics, the H_2O_2-content increased (by 170%) in leaves, but it decreased in roots (by 20%). For normal growth, reactive oxygen species must be scavenged (Sairam et al., 2006). Under salt stress, antioxidant enzymes in chickpea increased in activity and expression (Eyidogan and Oz, 2007). In the roots of 60-day-old chickpeas exposed to 10 dS m^{-1} for three days, the antioxidant enzyme, superoxide dismutase, increased by 150%, catalase by 360%, ascorbate peroxidase by 240%, peroxidase by 220%, glutathione transferase by 140%, and glutathione reductase by 126% (Kukreja et al., 2005). Nevertheless, these changes in enzyme activities have not prevented membrane damage in roots (Kukreja et al., 2005) or in leaves (Eyidogan and Oz, 2007), as it could be figured out by determining an increased malondialdehyde (MDA) content due to a 2–4 days exposure to salinity. However, there are no studies evaluating the correlation between antioxidant production and plant growth, hence yield of different chickpea genotypes.

Leaf soluble carbohydrates and proline contents were found to be more consistent with salt tolerance responses of the genotypes by Arefian et al. (2014). They established that the first 2-week-long period was the most critical after salt stress was initiated. In their experiment, Zawude and Shanko (2017) determined the most tolerant genotype among 5 chickpea landraces under study to salinity stress induced by 4 NaCl salinity levels. They figured out considerable differences in some traits like shoot fresh weight, shoot dry weight, shoot length, root fresh weight, root dry weight, root length, seedling root and shoot reduction under salt stress condition. From this study, it can be concluded that the identification of salt tolerant landraces based on developing genetic variability is a suitable strategy to combat against salinity problems arising in arid and semi-arid areas.

The global and regional importance of abiotic stress conditions (e.g., chemical toxicity, salinity, oxidative stress, extreme temperatures and drought) is very high especially in terms of the genetically-based response to them. Osmotic stress in conjunction with salinihation and drought are among the threats endangering agriculture the most involves. The alterations induced by drought stress can affect plant physiology, morphology, and biochemistry in various extent depending on duration and harshness of drought, furthermore, on the species and developmental stage of the plant (Basu et al., 2016). Various strategies are adopted by plants to prevent water loss, to preserve water even in the case of low value of water potential. These strategies help plants to survive the periods with unfavourable water supply resulting in low water content in tissues (Verslues et al., 2006).

Various ions creating soil-salt-alkalisation complex can be found in alkaline soils with high salt content (Läuchli and Lüttge, 2002). Under saline conditions, owing to low osmotic potential, water availability from the soil by plants is limited, hence plants cannot satisfy their demands for metabolic processes or maintain turgidity. Nevertheless, various physiological and biochemical mechanisms are developed in plants in order to survive in saline soils: uptaking and compartmentalisation biosynthesis of osmoprotectants and compatible solutes, ion transport, activation and synthesis of antioxidant enzymes, polyamines and hormonal modulation are the primary mechanisms, among others (Reddy et al., 1992; Roy et al., 2014). Stress responses in the early stages are dominantly involve the closure of stomata, triggered by abscisic acid. Nevertheless, the main carbon sink manifested in the instant expansion of roots and young leaves is affected earlier and more intensely than the carbon source of photosynthesis (Muller et al., 2011) that can result in sufficient water uptake due to the increased root growth, even under drought conditions. This mechanism provides the chance to maintain plant productivity in case of short periods of water insufficiency. Nevertheless, severe decreases in the CO_2 uptake and photosynthesis rate, redirection of assimilate transport for root growth improvement can be expected (Muller et al., 2011; Li et al., 2018). The response of three pepper cultivars (Fokusz, Bobita F1 and Carma) to salinity stress was evaluated. Results revealed no significant differences in the total seedlings' dry weight and the rate of seedling growth among the investigated varieties. The rate water loss was significantly higher in the cultivar Bobita F1 (89.61%), while it was lower in the other studied varieties. The early seedling vigor in terms of initial growth rate was significantly higher in the Carma compared with the others (Massimi and Radocz, 2022).

In case of long duration of stress, the strategy of adaptation may not be sufficient to maintain plant growth and ensure productivity, hence other mechanisms like cell wall hardening, detoxification by reactive oxygen species (ROS) detoxification enzymes, accumulation of compatible solutes and protective proteins, and metabolic changes are taking place in plants to establish drought tolerance. Recently there are considerable achievements in studying the different responses to water shortage involving the expression of stress-specific genes, stomata closure, accumulation of osmolytes, and regulation of antioxidant systems (Tatrai et al., 2016). In terms of stress tolerance, the mechanisms essential for plant survival are in conjunction with significant changes in the patterns of metabolites and proteins, hence the analysis of these changes induced by salt stress provides a good possibility for plant breeders in order to increase stress tolerance and/or resistance of plants (Frolov et al., 2017), as well as to develop plant protectants against stress (Lamaoui et al., 2018). Among the main interacting factors of stress tolerance, beyond genetics and breeding, plant and cell physiology, molecular biology must be mentioned, although the recently available knowledge is often incomplete and inconsistent in this respect.

CONCLUSIONS

A series of morphological, physiological, biochemical and molecular changes in plants are induced by abiotic stress negatively affecting their growth and productivity. Due to the challenges caused by global population growth and climate change, a priority task is to explore the cultivation of plants with a wide stress tolerance. The need for irrigation due to drought raises the potential for salt stress, which affects plant production. Due to the excellent content properties of chickpeas, it is the basis of a healthy diet, and the examination of its sensitivity to stress promotes the possibilities of its cultivation. During vegetative growth, chickpea is highly susceptible to salinity manifested in huge decline in dry matter production, but we still have no enough knowledge about different growth stages' sensitivity.

The available research results justify that the effect of salt stress on vegetative growth can be analysed in hydroponics, in pot or field conditions, regardless the experimental environment, the rank order of salt resistance of genotypes is consistent. However, it is still not established why chickpea is so susceptible to salt affection. Chickpea genotypes can be different in their ability to retain water, maybe under salt affection, the more salt tolerant lines can maintain higher water content in the shoots, while the more susceptible ones cannot.

ACKNOWLEDGEMENTS

Project no. TKP2021-NKTA-32 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-NKTA funding scheme.

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