

# **Salt stress in pulses: A learning from global research on salinity in crop plants**

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#### **Abstract**

**Pulse crops are overly sensitive to salinity stress as compared to cereals and other crops. The growth, development and yield of the pulses are severely affected by high concentration of salts accumulated in the soil due to inadequate drainage or other reasons. However, some of the pulse species have developed adaptive mechanism to overcome the salinity stress and hence, thorough screening of the germplasm through hydroponics or field testing is essential to identify the potential genotypes. Several cost-effective techniques of mitigating the salt stress including the physical, chemical and biological approaches have been analyzed and discussed. The mechanism of salinity stress and plant's response towards changes in morphological, physiological, biochemical and molecular features have been delineated in detail. Effective breeding approaches and progress in development of salt tolerant pulse crops have been highlighted. However, current leads in understanding the mechanism of salt stress and the genotypes developed may not be sufficient to enhance the productivity and availability of the pulse crops. The kind of efforts needed to improve pulse crops are rare and hence, require special attention. Therefore, integration of the traditional and modern breeding approaches including 'omics' technologies and biological agents are needed to address the issue of salt stress effectively. The development of stress tolerant pulse crops through genetic engineering has also shown promise. High-quality genotypic and phenotypic data including high throughput imaging approaches might lead to proper understanding of the mechanism of salt tolerance which in turn would help designing effective breeding programme for the development of pulse crops tolerant to salinity stress.**

**Key words:** Pulse crops, salinity stress, tolerance, mechanism, genetics

#### **Introduction**

Most of the intrinsic chemical, physical and biological stress conditions are the result of climate change and soil-forming factors such as weathering over very long term in which prolonged leaching, impeded drainage and possible accumulation of organic, inorganic and soluble salts have led to infertile soil conditions. Various environmental stresses such as high winds, extreme temperatures, acidity, aluminium toxicity, soil salinity and alkalinity (sodicity), drought and flood have affected the production and cultivation of agricultural crops. Among these, soil salinity is one of the most devastating environmental stresses, which causes major reduction in cultivated land area, crop productivity and quality (Eswaran et al.2003; Yamaguchi and Blumwald 2005; Shahbaz and Ashraf 2013; Singh et al. 2011a, b; 2014). The salts that accumulates in the soil includes chlorides and sulfates of sodium, calcium and magnesium. More than half of the saline soils throughout the globe contain sodic alkaline salts (FAO; http://www.fao.org/ag/agl/agll/spush/intro.htm). Therefore, salt-induced land degradation is a global issue.

A saline soil is generally defined as one in which the electrical conductivity of the saturation extract (EC<sub>e</sub>) in the root zone exceeds 4 dS  $m^{-1}$  (approximately 40 mM NaCl) at 25°C and has an exchangeable sodium of 15%. The yield of most crop plants is reduced at this EC<sub>e</sub>, though many crops exhibit yield reduction at lower EC<sub>e</sub>s (Munns 2005; Jamil et al. 2011). It has been reported that globally 20% of the total cultivated

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and 33% of the irrigated agricultural lands are afflicted by high salinity. The salinity affected areas are increasing day by day at alarming rate due to several reasons such as low precipitation, high surface evaporation, irrigation with saline water including faulty cultured practices. It has been a threat to the agriculture for over 3000 years in some parts of the world (Flowers 2006). Salinization is a natural process of increasing salts due to lack of water for adequate leaching and poor drainage leading to degradation in the quality of soil. The surface irrigation has aggravated the condition in arid and semi-arid environments (Rao et al. 2016). The salinity stress is caused by the accumulation of soluble salts like Cl ̄, SO $_4$ , HCO $_3$  ̄, Na $^+$ , Ca $^{2+}$ , and Mg $^{2+}$ and several others. These ions chiefly come from the irrigation and poor soil drainage (Acosta-Motos et al. 2017). Similarly, alkalinity stress is caused by the presence of carbonate  $(CO_3^2)$ , bicarbonate (HCO3) and hydroxyl (OH<sup>-</sup>) anions. The excess of borates, phosphates, silicates, and other bases increase concentration of these salts leading to alkalinity which drastically reduces plant growth and development. Ironically, the salinized areas are increasing at a rate of 10% annually, which is expected to cover more than 50% of the arable land by the year 2050 (Jamil et

al. 2011). In India, about 6.73 million ha (mha) area is alkalinity affected soils (2.1% of the country) of which salinity occupies 1.71 mha, alkaline soils is 3.79 mha and coastal saline, sodic and alkaline soils is about 1.25 mha (Mandal et al. 2009; ICAR and NAAS. 2010; Mandal et al. 2018). The characterization of salinity affected soil is given in Table 1. The most affected states due to salinity includes Gujarat, Uttar Pradesh, Rajasthan, West Bengal, Telangana and Andhra Pradesh. Annually, 3000-4000 ha new areas are being affected by salinity due to surface irrigation and poor drainage (Minhas and Reddy 2017). The situation is alarming and needs immediate attention.

Pulse crops are a wonderful gift of nature to the menkind. Several pulse crops such as chickpea (Cicer arietinum), pigeonpea (Cajanus cajanifolius), lentil (Lens culinaris), urad bean (Vigna mungo), mung bean (Vigna radiata), field pea (Pisum sativum), cowpea (Vigna unguiculata) moth bean (Vigna aconitifolia), French bean (Phaseolus vulgaris), lablab bean (Lablab purpureus), faba bean (Vicia faba), horse gram (Dolichos uniflorus) and grasspea or khesari (Lathyrus sativus), etc. are being grown world over including India. They play a significant role in mitigating protein



**Table 1.** Characterization of soils affected by salinity stress

Note: 1.EC is the electrical conductivity of the saturated paste extract, and reflects the concentration of salts in the saturated soil. A conductivity of 4 dS  $m<sup>1</sup>$  is equivalent to 40 mM NaCl.

2. At high ESP, the clay particles separate. The soil drains poorly, becomes waterlogged when wet and gets very hard when dry. Source: Adopted and modified from Rana Munns, 2005 (USDA Salinity Laboratory, 2005)

malnutrition and providing nutritional security to millions of poor vegetarian people of the semi-arid tropics of the world. They also assume special significance because of efficient bio-productivity in marginal soils. Pulses constitute an important component in the cropping patterns of several developing countries in Asia, Africa, and Latin America. They occupy about 5.8% of the world's arable land area and are grown mainly in less fertile and marginal lands as sole or intercrops with cereals and oilseeds (Joshi and Rao and California and Rao and California and Rao and Latin and Rao and Latin and Rao and Latin and Rao and Latin and Rao and 2017). However, the pulse crops are overly sensitive to salinity stress and therefore, the growth, development and yield are severely reduced. The excess concentration of Ca, Na, K and Mg ions causes  $\Big|$ <sub>25</sub> degradation of the soil and leads to induction of leaf chlorosis and stunted growth due to high uptake of Na+ and low uptake of nutrients (Millar et al. 2007). The effect, however, varies from one genotype to  $\frac{1}{10}$ another due to its adaptive mechanisms.

The Indo-Gangatic plain where the pulses are grown on large scale, has about 2.8 mha area affected by alkaline stress. Gradually, the area under the pulse crops in the traditional pulse growing areas is decreasing over the years partly due to increased soil salinity and enhanced use of brackish water for irrigation (Gowda et al. 2009). Salinity is thus a major constraint limiting plant growth and development, and severely affecting the reproductive processes resulting in lowered pulse productivity and thus threatening sustenance of global production of pulses. The imperative is therefore, to mitigate the salinity stress and sustaining pulses production by stress management approaches and breeding tolerant cultivars of the pulse crops. In this review we have elucidated a few strategic points to provide holistic view of recent advances in breeding for salinity tolerance in pulse crops.

# **Global scenario: Area, production, and per capita availability of pulses**

The pulse crops grown in different parts of the world include the pulse family consists of a number of pulse crops and the FAO broadly classifies them into 10 pulse types in addition to a category including many minor pulses as a group whose data are not given separately in FAOSTAT (e.g., lablab, sword bean, gaur bean). As a combined group, pulses include dry beans, broad bean, cowpea, chickpea, pigeon pea, lentil, dry peas (field pea), bambara bean, lupin, vetches, and other minor pulses. As per the classification given by FAO, the dry bean category comprises common bean, mung bean, black gram, and adzuki bean. All of these beans belong to the genus Phaseoulus, but they are grown in different regions and environments. The farmers in Asian countries grow common bean/dry bean mainly green gram, black gram, and moth bean. Another category includes broad bean or faba bean, which further constitutes broad bean, horse bean, and field bean etc. Global share of the major pulses in terms of area and production is shown in Fig. 1. The



**Fig. 1. Per cent area under cultivation and per cent production of different pulse crops**

pulses accounts for 80.3 mha of global crop area with 72.3 million metric tons (mt) produce. The top 20 pulseproducing countries account for about 82% of pulse area and production in which top seven countries contribute for 60%. India is the world's largest producer of pulses, accounting for 29.28 mha (34% of area) and 22.40mt (24% of production) followed by Myanmar, Canada, China, Nigeria, Brazil, and Australia (Joshi and Rao 2017). Among the pulse growing countries of the world, the most significant expansion of pulses in terms of area and production is observed in Africa.

The yield variations among the pulse producing countries of the world is very high. Usually, the countries with higher yields found to have lesser area under pulses as compared to other countries (Fig. 2).



**Fig. 2. Pie diagram showing per hectare production of pulse crops in different countries of the world**

For example,countries such as Niger, Nigeria, Myanmar etc.have more area under pulses but total production and productivity is low.

In global scenario, India stands twelve in production despite having the largest share of area under pulses. Production of pulses in India found to increase by 1.3% during 1980-2013; however, import remains at around 15 per cent of the domestic production, and the per capita availability remained stagnant at around 6.5 kg/capita/year, which is far below the per capita requirement of 43g/day. To meet the local demand, India has now become the largest importer of pulses in the world. Akibode et al. (2011) reported that more than 75% of the pulse crop in developing countries is grown in rain-fed and low-input production systems. In India and other countries in Southern Asia, only 10-15%area of pulses is under irrigation. In India, Madhya Pradesh is the largest producer of pulses followed by Maharashtra, Rajasthan, Uttar Pradesh, Andhra Pradesh and Karnataka. Being the largest producer (18-20mt), India is also the largest consumer (20-24mt) and importer (4-6mt) of pulses resulting in a loss of \$ 2.3 billion foreign exchange each year (Business line, 2015). Estimates indicate that India needs an annual growth rate of 4.2% in pulse production to ensure projected demand of 30 mt by 2030 (Rana et al. 2016). These crops being grown as rainfed (87%) on marginal and sub-marginal lands are frequently prone to biotic and abiotic stresses (Rana et al. 2016) which are the major barriers in realizing the yield potential of the pulses.

# **The effects of salinity in pulse crops**

Soil salinity, in general, creates a soil condition unfavorable for crop growth and development. Maas and Hoffman (1977) observed the relationship between response of the crop yield against salinity and quantified crop's relative growth or yield as a continuous function of gradually higher levels of soil saline condition, which follows a sigmoid curve. It indicated that at low salt concentration yield is either unaffected or decrease slowly, then decrease in yield at a greater but relatively constant rate at moderate concentrations and lastly the yield start to decline more slowly towards zero at high concentrations.

De Pascale and Barbieri (1994) studied the effects of soil salinity and removal of top soil in faba bean by using two different models viz., Maas and Hoffman (1977) model Yr = 100 – S(EC<sub>e</sub>) – T) and the per van Genuchten (1980) model Yr =  $100/[1 +$ 

(ECeECe50)p] and concluded that shoot and leaf dry mass, leaf area, leaf number and shoot length declines with increase in salt stress level. The models depicted that in terms of relative yield per unit area, the threshold value was 1.7 dS m<sup>-</sup> and yield was reduced at the rate of 15% compared to 4.7 dS m<sup>-</sup> of the van Genuchten model. Broadbean was more sensitive two weeks after flowering i.e. at higher EC level, leaf area reduction was 60% at flowering and 73% after two weeks and leaf dry mass reduction was 50% and 55% respectively. Rameshwaran et al. (2015) studied the effect of salinity to the response of faba bean, chickpea and lentil by using the threshold-slope linear response function and modified discount function. Comparison was also done by SALTMED model predictions. It was concluded that the variation in response is dependent on the accession of these three crops and a wide range of response was found for each crop. The study showed similar range of variation in relative yield for faba bean and chickpea while lentil has small range of variation in yield compared to faba bean and chickpea.

Under condition of salt stress, the plants suffer from disruption of ion homeostasis; however, in the salt stress tolerant plant, the inherent mechanisms of exclusion and compartmentalization and self-inducible Na<sup>+</sup>/H<sup>+</sup> antiporter enzyme overcome the damage (Apse et al. 1999). The disruption of normal homeostasis under salt stress also causes abnormal photosynthesis and increased photorespiration generating high level of reactive oxygen species such as super oxide radical, hydrogen peroxide and hydroxyl radical which disturb cells and damage normal pathways (Mayak et al. 2004). A fully developed leaf maintains high Na<sup>+</sup> and CI<sup>-</sup> ion concentration in the reproductive organs during pod filling stage resulting in restricted ion accumulation in flowers and the developing ovules. The salinity effect in chickpea is minimized by compartmentalization of toxic ions in the leaf's vacuoles (Munns 2008; Mullan et al. 2007; Reginato et al. 2014). The high ion accumulation in leaves might result in decreased photosynthesis efficiency which ultimately affects the seed yield. A very few studies have been done relating salinity, seed germination and yield, however, Manchanda and Sharma (1990) found a good agreement between seedling stage evaluation and yield-based assessment in chickpea.

Ebbisa and Getachew (2015) evaluated the effects of different salinity levels on growth performance and number of nodulation in chickpea (Cicer arietinum L.) under green house condition at five levels of NaCl concentrations (0, 3, 6, 12 and 18 g of NaCl) which were mixed in 200ml of water for each NaCl levels. The study showed that the number of germinated plants, root length, root number, number of nodulation and harvested fresh and dry seedling weights were significantly reduced as compared to control. Only plant height was not reduced at 3g NaCl and the number of nodules, number of root also showed positive response. This gradual reduction in growth parameters and nodulation as NaCl levels increases (highest at 18g) indicated that chickpea is extremely

sensitivity to NaCl out of the legume crops.

Legumes are comparatively hardy crops generally known for tolerance against various abiotic stresses. Hence, they are often considered as crops for the stress affected regions of the world. However, the physiological effects of salt stress in many legume crops are less investigated. Genotype dependent responses in salt affected soils are mostly observed in chickpea, lentil and to some extent in pigeonpea cultivars in the form of accumulation of osmolytes like proline, glycine betaine, starch and soluble sugars during salt stress (Joshi 1984). However, a little is known about the salinity tolerance in pigeonpea. In an attempt to understand the effect of salinity in pigeonpea plants, Tayyab et al. (2016) found that the plants survived up to 3.5 ECe dS.m-1 in sea salt salinity. An increase of chlorophyll contents and carotenoids in leaf was recorded at 1.6 dS.m-1 but subsequently decreased in higher salinity. Low moisture content and succulence along with more accumulation of soluble sugars and proteins may be attributed to leaf osmotic adjustments at low salinity. Salinity adversely affects reproductive growth where production of flowers, pods, number of seeds and seed weight are significantly reduced.

Under salinity stress, phosphorus and potassium contents get reduced while those of calcium, magnesium, sodium, chloride, and sulphate get enhanced. As a result, regulation on the uptake of latter elements under saline conditions is highly affected and, the photosynthetic efficiency of the plant is decreased due to degradation of essential pigments like chlorophyll and carotenoids. Salinity reduces plant growth chiefly in three mechanism- osmotically induced water stress, specific ion toxicity due to high concentration of sodium and chloride ion and nutrient imbalances which hampers the uptake of water by plants (Greenway and Munns 1980; Manchanda and Sharma 1990). Salinity stress also limits physiological response and photosynthetic capacity. Gama et al (2007) studied the effect salt stress in common bean (Phaseolus vulgaris L.) and found that high concentration of NaCl is associated with a reduction in leaf chlorophyll fluorescence and photosynthesis (Fig. 3). It is envisaged that blocking of the electron



**Fig. 3. Schematic representation of effect of salinity stress in crop plants**

transport might reduce the chlorophyll content in the plants under salt stress.

Since pulses are more sensitive to saline soil as compared to cereals and oilseeds, the damage caused in yield is enormous. This might be due to the fact that, pulses accumulate excess salts that quickens the anthocyanin pigmention in leaves and stems which ultimately reduce germination and seedling establishment (Kumar et al. 2016). Thus, growth and development of the pulse crops are more prone to salinity-induced moisture stress. In mungbean, salt stress along with other pests (stemand pod-borer) and yellow mosaic disease caused 80- 100% yield loss particularly during rainy season (Sehrawat et al. 2015).

#### **Mitigation of salinity stress**

Management of salt stress may be rewarding to the farmers who face the serious challenge by the type of soils they have. Several strategies are being followed to reclaim the sick soil and bring it back under cultivable condition. Sharma and Minhas (2005) suggested the modification of the saline environment to suit the plant or to modify plant to suit the environment for improving and sustaining the production under salinity. The gypsum-based technology has been quite successful for reclamation

of the alkaline land. In India, approximately, >1.8 mha a of alkaline soils has been reclaimed and the same has been used to cultivate rice and wheat for uplifting socio-economic condition of the people (CSSRI 2015). Experiments on removal of top soil have been conducted to grow legume crops. In one such study, Pascale and Barbieri (1997) found the response of faba bean genotypes on top soil removal during irrigation free season with irrigation treatment ranging from 0% to 1% commercial NaCl and observed that soil salinity at low level affected higher early production indicating more efficient of assimilation to growing pods. At low soil salinity the only effect of top removal was higher early production, indicating a more efficient partition of assimilates to growing pods and, therefore, rapid pod enlargement and higher yield at the first harvest was recorded.

In arid or semi-arid regions with salt affected soils, farmers are forced to use saline water for irrigation in areas with poor water quality or less available water for irrigation. Legumes are very sensitive crops towards soil salinity, and secondary salinization mainly through irrigation water is the hardest challenge for survival of legume crops in arid regions (Mann et al. 2020).

For saline soils, the amendment is to lower the salt concentration through leaching with good quality water and disposal of drainage effluent through well laid out horizontal sub-surface drainage system (Minhas and Sharma 2002). The sub-surface drainage technology has successfully been used in Gujarat, Haryana and Rajasthan reclaiming waterlogged soils (Kamra 2015). However, the technology requires high initial investments and need continuous operational and maintenances. Cuevas et al. (2019) analysed a case specific optimization of irrigation and drainage management including the residue management and reported that these amendments can significantly reduce the soil salinity and help increase the productivity. Although, it is difficult to manage salt affected soils by chemical amendments, the only precautionary measure is to use quality water and salt tolerant pulse varieties. The other alternative may be the change in cropping pattern, green manuring, use of drainage and mulching technologies. The conservation agriculture can also achieve the higher yields required for upscaling and sustaining crop production (Yadav et al. 2017). It is suggested that the desi types of chickpea are more tolerant than kabuli types (Vadez et al. 2007). In view of the cost effectiveness, agro-forestry is now emerging as a potential tool in not only arresting salinity but to

mitigate climate change, sequestering carbon, and restoration of biodiversity (Minhas and Dagar 2016). Zhou and Li (2013) studied the effects of oasis ecosystem hydrological processes on soil salinization in the lower reaches of the Tarim River, China and found that the methodology is useful in reclaiming the saline soils.

Plant growth promoting bacteria are associated with several plant species and improve plant performance under stress environments (Ruiz-Lozano 1996). Kasotia et al. (2015) emphasized the role of plant growth promoting bacterium to characterize activities of stress-associated enzymes, proline a c c u m u l a t i o c h o p h y l e s t i m a n d  $\mathcal{L}$   $^+/\mathsf{K}^+$  ratio

in soybean (Glycine max L.) under salt stress and showed that inoculation of plants with Psedomonas koreensis strain AK-1 grown under salt treatment induced growth promotion, reduced Na<sup>+</sup> levels but increased K<sup>+</sup> levels in leaves and roots in comparison to non-inoculated plants. To improve the growth of faba bean cultivars in saline soil, Metwali et al (2015) investigated the effect of bacterial isolates, Pseudomonas putida, P. fluorescens and Bacillus subtilis of plant growth promoting rhizobacteria under two levels of salinity stress (S1=4000 and S2=8000 ppm). They found high percentage of germination (96%) in the tested cultivars (Wadi 1 and Line 1) in presence of P. fluorescens. Futher, the plants treated with P. fluorescens showed significant increase in plant length (10.66%), plant shoot fresh weight (9.52%), and plant leaf area (61.86%). This positive effect was highest in P fluorescens followed by B. subtilis then P. putida. Bio-fertilization with soil yeast, Rhodotorula glutinis also reduces the deleterious effect of salinity stress. Increasing salinity level was accompanied by decreasing dry weight particularly at higher concentration (6000 ppm) of salt. Gaballah and Gomaa (2004) found a few faba bean varieties (Giza Blanka and Giza 674) performing better at the above level of salinity. Exploring genetic variation for salinity tolerance, Asif (2017) reported that most tolerant varieties of faba bean possessed better ability to maintain higher leaf K<sup>+</sup>/Na<sup>+</sup> and Cl<sup>-</sup> accumulation, lower root K<sup>+</sup>/Na<sup>+</sup> and leaf chlorosis resulting in greater salt tolerance at 100 mM NaCl concentration. The potential role of nicotinamide in alleviating the deleterious effects of salinity on some physiological and biochemical traits of faba bean has also been envisaged by Abdelhamid et al. (2013). Common adaptation mechanisms of plants exposed to environmental stresses such as drought, temperature and salinity changes the root

morphology (Potters 2007). Modulation of some plant hormones could also improve crop salt tolerance by reducing the toxic effects of salinity (Bianco and Defez 2009).

# **Genetic variations for salinity stress tolerance**

Salt tolerance is genetically variable, and the degree of salt tolerance varies with plant species and varieties within a species. In case of cereals barley is more tolerant than rice and wheat. However, the degree of variation is more pronounced among dicotyledonous plants; Arabidopsis thaliana is highly sensitive to salinity than Mesembryanthemum crystallinum, Atriplex spp., Thellungiella salsuginea a close relative of Arabidopsis (Pang et al. 2010; Abraham et al. 2011). Some legumes are very sensitive e.g., threshold salinity level of beans (Phaseolus vulgaris) is 1 dS/m (Table3) as compared to rice (3 dS/m), wheat (6dS/m) and barley (7dS/m). Alfalfa or lucerne (Medicago sativa) is very tolerant, and halophytes such as salt bush (Atriplex spp.) continue to grow well at salinity level greater than that of seawater.

The knowledge of genetic variation in different pulse crops is essential to develop genotypes, which have the capacity to withstand the salinity stress. Most of the pulse crops are sensitive to salinity, which is reflected in poor rate of germination, vegetative growth, reproduction (Maliro et al. 2004), reduction in nodule formation and yield (Hailesilasse et al. 2012). In chickpea a large genotypic variation has been reported in landraces and core collection based on shoot mass under saline conditions at vegetative and maturity stage in glasshouse conditions (Turner et al. 2013). Differences in tissue-ion regulation in different parts of the plants of various pulse crops due to  $\text{Na}^+\text{/Cl}^-\text{/K}^+$  a ion concentration has also been observed. Lo'pez (1999) studied the role and effect of  $Na<sup>+</sup>$  and Cl ions in salt-tolerance mechanism in plants and found that salt tolerance is associated with the ability to limit the uptake and/or transport of ions from root to aerial parts, while Singh et al. (2017) found that Na<sup>+</sup> concentration flov in shoot was lower than the roots of Lens culinaris but it was reverse for CI<sup>-</sup>. The tolerant genotypes develop inherent capacity in terms of regulation of Na<sup>+</sup> and  $CI^-$  f to the aerial parts in order to avoid the deleterious effect of salt on plant metabolism in Vigna unguiculata (Fernandes de Mello et al. 1994). Samineni et al. (2011) observed salt sensitivity at vegetative and reproductive stages in chickpea (Cicer arietinum) podding in particular. Genetic variation in different morphological features under salinity stress was also recorded in Vicia faba L. (Tavakkoli et al. 2012).

Significant genetic variation for salinity tolerance also prevails among the genotypes of lentil (Singh et al. 2017a). They endorsed that seed germination and seedling growth are the major limiting factors for normal crop growth under salt stress may be due to water imbibition and toxic effects during seed germination. Salt injury and yellowing of leaves leading to necrosis at leaf margins resulting in wilting and poor survival of the plants occurs in lentil. Variation for seedling survivability in saline soils has been considered a useful parameter to evaluate salt tolerance. The contrasting differences in root and shoot anatomical features at 120mM NaCl treatment, such as vascular arrangement in stele region in tolerant accession (PDL-1) and susceptible/sensitive cultigens L-4076 were observed (Figs. 4a and 4b). Murillo-Amador et al. (1986) evaluated seedling emergence of cowpea and found that salt tolerant genotypes survived at 120 mM NaCl concentration. Kökten (2010) studied germination percentage, shoot and root length and shoot/root ratio, shoot and root weight and shoot/root ratio and salt tolerance index salt tolerance in lentil at 50 mg/l, 100 mg/l, 150 mg/l and 200 mg/l– NaCl concentrations and reported significant differences in the salt tolerance of lentil genotypes and their different responses to increasing salt concentrations. Although genotypes responded similarly during the first 3 salt treatments, significant differences among the genotypes were obvious with the 150 and 200 mg  $I^{-1}$  treatments, concerning the salt tolerance index of genotypes.

 Dry matter production is also a potent indicator of plant's performance under salinity stress associated with yield (Maas 1986). As compared to the gathering of root and shoot mass by the plant, normal flowering and flower development in a plant is an indication of tolerance during salinity stress (Vadez et al. 2012a). The observations on number of flowers on plants under salt stress recorded by Vadez et al. (2012b) in chickpea were well supported by Kotula et al. (2015). Physiological studies have also shown delayed flowering, failure of fertilization even after the development of pollen tube resulting into poor pollen viability affecting pod filling and is also affected resulting in empty pods (Kotula et al. 2015). The leaf area, dry weight, and chemical analysis by atomic absorption spectrophotometer and chloride content by Mohr's volumetric method, relative water content (Subbarao et al. 1990), membrane stability index chlorophyll content, K<sup>+</sup>/Na<sup>+</sup> estimation (Sherawat et al. 2013) and SCMR (Spad chlorophyll metre reading) and shoot sodium accumulation (Srivastava et al. 2007) can be used for screening and identifying tolerant and







**Fig. 4b. Changes in stellar region and vascular bundles in cross-sections of stem in tolerant and sensitive Lens species raised under control (0 mM) and (120mMNaCl) saline stress condition grown in hydroponics**

susceptible genotypes. Bulut and Akinci (2010) determined the influence of salinity tolerance in faba bean at 50 and 100 mM NaCl concentration and reported reduction in plant height, number of internodes and leaf dry weight in cultivars, Eresen 87 and Filiz 99 at higher level of salinity. According to the flame photometer, flame atomic absorption spectrophotometers (FAAS) and inductively coupled

plasma atomic emission spectrometry (ICP-AES) analyses of Filiz 99, Na<sup>+</sup> was found in higher amounts in roots, stems and leaves of seedlings exposed to 100 mM NaCl. In leaves, the elements  $K^+$ ,  $Ca_2^+$  and  $Mg_2$ <sup>+</sup> were decreased significantly and, sodium and potassium content was 3- and 2.4- times higher than in cv. Eresen 87 indicating that Filiz 99 was slightly more resistant to salinity.

 Sehrawat et al. (2015) reported salinity induced moisture stress and retarding of growth and development in mungbean. Similarly, nodulation, nodule size, nitrogen fixation are highly affected by sodium saline conditions and thus impact on yield and yield traits in kabuli chickpea (Swaraj and Bishnoi 1999; Flowers et al. 2010; Jha et al. 2014).

#### **Genetics of salinity tolerance**

Understanding the genetic basis of salt tolerance is vital to develop tolerant varieties. Genetics of salinity tolerance has been worked out in several crops namely, rice (Lee et al. 2007), wheat (Ma et al. 2007), pigeonpea (Subba Rao et al. 1990, 1994), tomato (Villalta et al. 2007); however, limited progress has been made in the pulse crops. In an inter-specific crosses in pigeonpea, dominant monogenic control was observed in (Subbarao et. al. 1990). Similar reports of genetic control for some attributes of salinity tolerance in soybean, and chickpea has been reported. Salt tolerance rating (STR) and ion accumulation found to determine the salinity stress tolerance in soybean (Abel 1969; Hamwieh and Xu 2008; Liu et al. 2016). However, Phang (2008) had reported that salt tolerance in soybean is a quantitative trait and controls by a few major loci. Hamwieh and Xu (2008) identified conserved salt tolerance quantitative trait locus (QTL) in wild and cultivated soybeans and later, Hamwieh et al. (2011) observed a major QTL with PVE ranging from 44.0-47.1 and 38.2-40.7 in salinity tolerance rating and chlorophyll content, respectively. Guan et al. (2014) mapped a salt tolerance gene on chromosome 3 using SSRs and INDELs markers in soybean, whereas Singh et al. (2020) identified a major QTL or salt tolerance in lentil explaining comparatively less phenotypic variance. Earlier, Singh et al. (2017b) reported that different traits such as seedling growth, biomass accumulation, seedling survival, antioxidant activities and fluorescent signals, and  $Na^+$ ,  $K^+$  and  $Cl^-$  contents could be used in lentil for salinity stress tolerance. All these parameters have been used to map the QTLs for salinity stress tolerance in other crops also (Dubcovsky et al. 1996; Farshdfar et al. 2008;

Shavrukov et al. 2010). In chickpea, dominant effects mostly controlled the seed yield in saline environment, with minor contributions from additive effects (Ashraf and Waheed 1998). Only additive gene effects have been reported to be important for seed yield in pigeonpea under saline environment (Ashraf 1998).

Attempts to study genetics of salt tolerance in other species suggested that it is highly unlikely that one gene alone determines plant salinity tolerance in the pulse crops, rather it is a complex trait (Flowers 2004). Diallel analysis for assessment of salt tolerance in terms of relative root length in different crops witnessed both additive and dominance effects of salt (Azhar and McNeilly 1988). Phang (2008) reported salt tolerance in soybean as quantitative trait dominated by a few major loci. Studies on biochemical, physiological and phenological genetic analysis in common bean (Phaseolus vulgaris L.) under salt stress conducted by Yacine et al (2018) also suggested both additive and dominant gene action in salt tolerance. Thus, salt tolerance is multi-genic features regulated by a number of biochemical, physiological and molecular processes and is involved in modulating accumulation of various solutes/osmolytes, polyamines, reactive oxygen species and proteins (Kumar et al. 2015). Therefore, for accuracy and certainty, analysis of salinity responses should be done not only at whole plant level but also at the level of traits contributing to salinity tolerance.

Singh et al. (2020a) investigated salinity stress tolerance at seedling stage in lentil to understand the genetics in a segregating population derived from sensitive (L-4147 and L-4046) and salt tolerant (PDL-1 and PSL-9) lentil genotypes. The parents,  $F_1$ ,  $F_2$ ,  $F_3$  and backcross populations were evaluated in salt solution at 120 mM NaCl concentration on seedling survivability and a Fluorescein diacetate (FDA) signal. The study revealed a dominant monogenic control of salinity stress tolerance. The test of allelism confirmed that a single dominant gene governed salt tolerance in both PDL 1 and PSL 9 genotypes. They further identified seven markers to be closely associated with seedling survival under salinity stress. A QTL was mapped on linkage group 1 (LG\_1) that explained about 65.6% of phenotypic variances.

# **Strategies to breed salinity tolerant genotypes**

The development of saline tolerant cultivars can be an appropriate approach for minimizing yield losses under salinity stress conditions. The foremost understanding of genetic determinant is essential to breed a suitable genotype for salinity tolerance. Indepth understanding of mechanism of salt tolerance and the traits which can be manipulated genetically is also a pre-requisite.

Salinty stress in plants is observed right from the beginning of germination, initial growth and development and reproductive development by imposing ion toxicity, osmotic stress, nutrient deficiency (N, Ca, K, P, Fe and Zn) and oxidative stress on plants resulting in water uptake from the soil. Therefore, identification of morphological and physiological traits and determination of different genetical parameters are essential pre-requisite to manipulate the plant mechanism for developing salt tolerant genotypes through conventional and/or molecular techniques. Osmotic adjustment is the main mechanism to conserve plant cell hydration under salt and drought stresses. The osmo-protectants accumulation realizes the prime cell tolerance response providing adaptation, improve plant growth, osmotic and anti-oxidative defense of Cajanus cajan modulated by salt stress and therefore, the osmotic pressure in cytoplasm is to be maintained (Garg and Noor 2009; Hanafy et al. 2013).

The ion channel regulation mechanism improves salt tolerance in chickpea. Effects of sodium chloride and sodium sulphate on the content of some organic and inorganic constituents in the leaves of pigeonpea (Cajanus cajan L. Var. C-11) were studied. Increased water content under saline conditions made the leaves succulent. The concentration of reducing sugars appeared to be higher while that of total sugars and starch was lower. The plants also failed to accumulate proline at higher salinity levels. Phosphorus and potassium content were lowered while those of calcium, magnesium, sodium, chloride, and sulphate were increased under salinities. This indicates that there is no regulation on the uptake of latter elements under saline conditions.

# **Genetic improvement of pulses for tolerance to salinity stress**

The response of plants to various abiotic stresses at the cellular level is often interconnected leading to morphological, physiological, biochemical and molecular changes that affect plant growth, development and productivity (Ahmad and Prasad 2012). The kind of efforts needed to improve pulse crops are limited and therefore, require special attention. The emphasis must be laid upon trait-based breeding to ensure yield stability across the locations

as well as crop seasons. Appreciable improvement in salt tolerance of important crops (barley, rice, pearl millet, maize, sorghum, alfalfa, and many grass species) have been attained in the past, but not in legumes.

The need to produce salt-tolerant crops has been realized from the days of yore (Jacobsen and Adams 1958) and different approaches and strategies were extensively rehearsed to increase tolerance against salinity. Epstein (1977) reported that germplasm display a spectrum of salt tolerance capability from high to low. Existence of a wide range of germplasm from euhalophytes to extremely sensitive glycophytes within a species provides a good scope for intensive and precise screening and identification of tolerant lines for use in breeding programs to deliver agronomically superior cultivars tolerant to salt stress. Ndakidemi and Makoi (2009) established that there is a high genetic potential into salt tolerance among Phaseolus vulgaris cultivars. In fact, several national breeding programs in Africa have been established to find dry bean genotypes with better salt tolerance, plant growth, chlorophyll levels and high seed yield.

Mungbean [Vigna radiata (L.) R. Wilczek var. radiata] is an important food and cash legume crop in Asia. Mungbean productivity is constrained by several abiotic stresses including salinity. Salt stress mainly reduces seed germination, fresh and dry biomass, shoot and root length, and yield attributes of mungbean (Promila and Kumar 2000; Rabie 2005; Ahmed 2009). It affects root growth and elongation, thereby, hampering nutrient uptake and distribution. Root growth was significantly reduced with higher Sodium Chloride concentrations. In general, salinity tolerance breeding work is limited in pulses, in general and mungbean, in particular (Ambede et al. 2012). Breeding for salt tolerance in mungbean has been critical in developing varieties with resistance to salinity however, the lack of identification of resistance source(s) is major constraint. Nevertheless, BARI Mung4 showed better performances at higher NaCl concentration considering a yield-contributing character. Nodules/plant decreased with the increase of salinity although the nodule size increased (Naher and Alam, 2010). Being polygenic in nature, salinity tolerance is genotype-dependent and growth stage-specific phenomenon, therefore, tolerance at an initial (seedling) stage may not be corroborated with tolerance at later growth (maturity) stages (Sehrawat et al. 2013). It also involves multidimensional responses at several organ levels in plants (e.g., tissue, molecular, physiological and plant





Source: Maas 1993; Subbarao et al.1991; Singh et al. 2017

canopy levels) (Hanumantha Rao et al. 2016). Because of this complexity and lack of appropriate techniques for introgression, little progress has been achieved in developing salt-tolerant mungbean varieties over years (Ambede et al. 2012; HanumanthaRao et al. 2016).

In breeding for salinity tolerance, one should not select a genotype based on overall performance; rather one should look for the traits that contribute to salinity tolerance (such as shoot  $Na<sup>+</sup>$  content or plant vigour) (Yeo et al. 1990) Wild relatives of pigeonpea from secondary and tertiary gene pools (C. scarabaeoides, C. albicans and C. platycarpus) confers resistance to salinity stress involving traits such as root retention of Na<sup>+</sup> and Cl<sup>-</sup> and their limited translocation to shoots, and maintenance of optimum rates of transpiration

Name of crop	Donor for salt tolerance
Chickpea	JG 62, ICC 5003, ICC 15610, and ICC 1431 (Vadez et al. 2007); ICC 10755 (Serraj et al. 2004); SG- 11(SG-11); CSG 8962 and CSG 8927 (Dua and Sharma 1995)
Pigeonpea	ICPL 227 (Subbarao et al. 1991); C. platycarpus, C. scarabaeoides, and C. sericeus (Srivastava et al. 2006); C. albicans (Subbarao et al. 1990)
Adzuki bean	JP107879 of V. nakashimae (strain 'Ukushima'), JP205833 of V. riukiuensis (strain 'Tojinbaka') (Yoshida et al. 2016); V. trilobata, V. vexillate, V. marina subsp. Oblonga, V. luteola, V. marina (Iseki et al. 2016)
Lentil	PDL-1, PSL-9 and ILWL-09, ILWL-137, ILWL-96 and ILWL-428 (Dharmendra et al. 2017); Cagil and Altin Toprak (Kökten et al. 2010)
	Soybean Lee, Lee 68, and S-100 (Do et al. 2016)

**Table 3.** List of donors for salt tolerances in different pulse crops

under salinity. Srivastava et al. (2006) reported that the cultivated and wild accessions of pigeonpea varies widely for salinity susceptibility index (SSI) and relative reduction per cent (RR%). Such genotypic differences for salinity provide opportunity for selecting tolerant genotypes. However, the concentration of salt in the screening experiments, need to be optimized as it varies from one species to another. The level of salt concentration optimal for discriminating sensitive and tolerant genotypes in different species is given in Table 3. Further, salinity tolerance of a species may vary between growth stages, therefore, screening needs to be performed at different growing times during the vegetative and reproductive stages. Ion exclusion (from root), tissue tolerance of toxic ions, and perhaps internal detoxification may be simultaneously operating to mitigate the effects of salinity stress. Screening of core and mini-core germplasm sets can lead to identification of genotypes with salt tolerance which can further be used in breeding programme for salt tolerance. There is a lack of direct correlation between the threshold salinity and yield decrease per unit increase in salinity which may be attributed to the differences in salt exclusion, uptake, compartmentation, and other mechanisms of salt tolerance among these crop species. Differential responses and genetic variations to salinity stress in plants have enabled researchers to identify physiological mechanisms, sets of genes, and gene products that are involved in increasing stress tolerance. Basic tolerance mechanisms involve the activation of different stress regulated genes through integrated cellular as well as molecular responses.Hence, the breeding of salt tolerant genotypes of different pulse crops need specific emphasis on the traits need to be improved so that these could contribute directly or indirectly to yield.

Plant type or ideotype of a plant need to be designed to help acclimatize it to the saline stress conditions with remarkable physiological and biochemical adjustments. Selection should be directed towards individual plant that has improved capacity of taking the nutrients from the soil. Further, emphasis should be given on both constitutive and adaptive traits while selecting the plants for salinity stress tolerance. The plants having higher number of flowers and higher number of seeds under salinity stress indicate its tolerance to salinity in chickpea (Vadez et al. 2012a). Genetic variation in different pulse genotypes has been studied by several workers (Saxena 1984; Flowers et al. 2010). Lauter and Munns (1986) evaluated a large number of chickpea genotypes using 50mM NaCl and/ or 25 mM  $Na<sub>2</sub>SO<sub>4</sub>$  salts and identified tolerant cultivar L550 which showed normal growth under saline conditions. Turner et al. (2013) tested about 50 chickpea genotypes at various levels of salinity and concluded that that high pod and seed number bearing genotype which may accumulate low concentration of salt will provide better tolerance under salinity stress. Remarkable efforts have been made to identify donors for and decipher the inheritance and genetics of tolerance to salt stress in pulses. Details of donors are presented in Table 3.

#### **Breeding approaches for salinity tolerance**

Legume crops in general are highly sensitive to salinity stress (Mass and Hoffman 1977) and therefore, breeding for salinity tolerance has been a major challenge due to complexity of various factors including the inheritance pattern of genes governing tolerance/ susceptibility, genotype, and its interaction with environment and, the level of salinity. However, genomic resources and various molecular breeding (MB) approaches including marker-assisted

backcrossing (MABC), marker-assisted recurrent selection (MARS), etc., may act as a catalyzing agent facilitating breeding for salinity stress (Greenway and Munns 1980).

Salinity tolerance, a complex phenomenon, is controlled by traits involving intrinsic morphological, physiological and biochemical responses of multiple genes under stress. Before choosing any breeding approach to develop salt tolerant genotypes, a breeder has to identify genes associated with salt tolerance. Several attempts have been made to understand the molecular and physiological basis of salt tolerance in crop species. However, very limited work has been done in pulse crops as compared to cereals and other crops. Recent advances made in biotechnology such as high-throughput next-generation sequencing, generation of functional genomics resources, microarray analysis in order to identify differentially expressed genes under high salinity, salt related transcriptome analysis of root nodules, shoots tissues, root morphogenesis, cell wall modifications, electrolyte movements and ion (Na+/K+) dynamics in soil and plant organisms etc. have paved the way to identify differentially expressed genes and their regulatory mechanisms under salinity stress (Jain 2011, Jain and Chattopadhyay 2010; Kudappa et al. 2018; Mantri et al. 2007; Kaashyap et al. 2018).

The phenotypic responses to salinity stress are mainly due to the genetic constitution of plant(s) and genotype environmental variables. Availability of genetic variation within a gene pool of a crop would enable the identification of desirable salinity tolerance related traits. Since salinity tolerance is conferred by several physiological factors, the traits giving positive response to salinity stress may help in identification of sensitive and tolerant genotypes. Following molecular approaches, Mantri et al. (2007) identified salinity tolerant (CPI 060546) and sensitive (CPI 60527) chickpea genotypes both differing in temporal gene regulation. Kaur et al. (2014) delineated on the molecular mechanism underlying salt tolerance in well characterized tolerant, ICCV 10 and JG 11 and sensitive, DCP-92-3 and Pusa 256 genotypes and studied phenological and physiological attributes, viz., seedling growth , relative water content (RWC), EL, chlorophyll content, and ionic distribution of Na<sup>+</sup> and K + . They found that sensitive genotypes had higher Na<sup>+</sup>/K<sup>+</sup> ratios than the tolerant genotypes, whereas av shoot Na<sup>+</sup>/K<sup>+</sup> ratios in the tolerant genotypes (ICCV event 10 and JG 11) was maintained under salt stress by accumulating less Na<sup>+</sup> and more  $K^+$  than the sensitive the

genotypes and the distribution of Na<sup>+</sup> and  $K^+$  in plant tissue is critical for tolerance and supported the earlier reports (Shabala and Cuin, 2008). Understanding salinity tolerance at the molecular level can facilitate the development of salt-tolerant varieties through molecular breeding approaches in chickpea.

Using comparative transcriptome analysis of the same set of contrasting genotypes taken by Kaur et al.(2014), Kumar et al. (2021) studied salt tolerance mechanism. They identified a total of 530 million reads from root samples of four genotypes, generated 21,698 differentially expressed genes (DEGs) which were upand down-regulated. These DEGs were likely to be associated with crucial metabolic pathways such as hormone signaling, photosynthesis, lipid and carbohydrate metabolism, and cell wall biogenesis. Significant up-regulation of transcripts encoding potassium transporter family of different proteins occurred under salinity stress. The tolerant lines (ICCV 10 and JG 11) engaged highly efficient machinery in response to elevated salt stress, especially for signal transduction, transport and influx of K+ ions, and osmotic homeostasis. Earlier, Kasshyap et al. (2018) reported that NACs transcription factor genes impart salt tolerance in Cicer arietinum. These studies have emphasized the role of potential candidate gene(s) and the identification of these gene(s) responding to tolerance mechanisms will support the development of salt-tolerant high-yielding chickpea varieties.

Soren et al. (2020) also identified two genotypes of chickpea, ICCV 10 showing salt-tolerance whereas, DCP 92-3 exhibiting salt-sensitivity and developed RIL population to generate data for phenotyping and genotyping analysis. Salinity stress tolerance at seedling stage in lentil has been investigated to understand the genetics in a segregating population derived from sensitive (L-4147 and L-4046) and salt tolerant (PDL- 1 and PSL-9) lentil genotypes (Singh et al. 2020). Combining field and hydrponic screening techniques two varieties of lentil, PDL- 1 and PSL-9 have been released for cultivation under saline conditions. Similarly, Manasa et al. (2017) selected five mungbean germplasm lines by canopy phenotyping assay based on seedling tolerance under 150 and 300 mM NaCl level of stress. These lines may serve as useful donor to breed salinity tolerant mungbean genotypes. The research findings clue available so far in different pulse and other crops can eventually pave the path for genetic improvement of salinity tolerance utilizing the candidate genes and their regulatory mechanisms to formulate effective

genomic-assisted breeding approaches for chickpea breeding programs.

# **Screening for identification of salinity tolerant genotypes/donors**

Differences in salt tolerance at the varietal or genotypic levels have been reported in several leguminous crops (Dua et al. 1989; Subbarao et al. 1991; Subbarao and Johansen 1994). Rapid screening methods and evaluation techniques are required to identify putative donor parents in a breeding program (Malhotra 1997; Saha et al. 2010). In a comprehensive study, Manasa et al. (2017) screened 40 mungbean lines sourced from World Vegetable Center for salinity tolerance using Salinity Induction Response (SIR) technique at the seedling as well as at whole plant levels by canopy phenotyping assay under 150 and 300 mM NaCl stress scenario. The results showed a marked reduction in growth and yield performances of both tolerant and susceptible lines, but a few lines displayed a relatively better biomass and pod yield on par with non-stressed control plants. The intrinsic ability of salt portioning to vacuole (more influx of Na+ ions) by tolerant lines during high salt concentration in the cytocol could be one of the reasons for their tolerance. Based on the extent of salt tolerance both at seedling and whole plant stages, a few salt tolerant lines viz., EC 693357, EC 693358, EC 693366, EC 693371, and ML1299 lines were identified (Manasa et al. 2017). A set of 105 pigeonpea genotypes/germplasm of different maturity groups ranging from super early to late maturing, popular varieties and wild accessions of Cajanus cajanifolius were screened in 2019 under lab conditions at different levels (0,40,80,100 and 120 mM) of sodium chloride (NaCl) concentrations for 30 days (Fig. 2) with regular maintenance of electric conductivity (Kumar Durgesh unpublished). A large variation in tolerance was recorded at 100 mM concentration.

It is well known that the degree of salt tolerance of different crops varies with their ontogeny. Salt tolerance in three varieties, Local arhar, ICPL 151 and ICPL 850014 of pigeon pea (Cajanus cajan (L.) Millsp.), was determined at three growth stages viz., germination, seedling, and adult plant by Ashraf (1994). It was also found that there is no positive correlation between tolerance at the early growth stages and at the adult stage since no clear difference in salt tolerance of the three accessions was observed at the germination and the seedling stages, whereas accessions differed considerably at the adult stage. Although increasing salt concentrations adversely



**Fig. 5. Growth of tolerant genotype (BDN708) is higher as compared to sensitive genotypes (Hy3c) under saline conditions (80 mM). C = Control, T = Salt tolerant and S = Salt sensitive**

affected the growth of all three accessions, ICPL 151 was however, superior to the other two accessions in fresh and dry biomass, yield and yield components when tested at the adult stage. It accumulated significantly lower Na<sup>+</sup> and CI<sup>-</sup> in shoots but by contrast it was higher in shoot and root K<sup>+</sup>, K/Na ratios, K vs Na selectivity, soluble sugars, free amino acids and proline compared with the other two accessions. Sharma et al. (2001) screened a few genotypes of pigeonpea and its wild relatives, Atylosia platycarpa and A. albicans and found differences in tolerance to salinity and sodicity which was attributed to regulation of uptake and distribution of Na+ and K\_ ions. Ashraf (1998) studied Some  $F_1$  hybrids derived from salt tolerant and salt sensitive parents of pigeonpea and observed that the high value of additive effect showed a



**Fig. 5. The plates depicting significant genotypic variation for salinity stress in pigeonpea. A: Genotypes growing before salt stress and B: Differential behaviour of genotypes in salt stress conditions**

significant improvement of salt tolerance is possible through mass selection and breeding.

Lentil like other pulse crops is also sensitive to salinity stress (Ashraf and Waheed 1990). Significant genetic variation for salinity tolerance prevails among the genotypes of lentil (Singh et al. 2017).An effective screening for salinity stress tolerance is required to develop salt tolerant genotypes. Screening under field conditions is generally difficult because of soil heterogeneity and other environmental factors. Therefore, laboratory-based techniques such as hydroponic may provide a suitable method for screening as it provides uniform salinity in the growing medium. However, a sensitive stage needs to be identified for accurate phenotyping.

Several reports have earlier suggested that selection of genotypes at the seedling stage is a more accurate and rapid criterion as compared to vegetative and reproductive stages for salinity stress tolerance (Gregorio 1997). Different physiological and biochemical traits such as seedling survivability salinity score, seedling growth and biomass, Na+ and Cl" accumulation, production of  $H_2O_2$ , antioxidant activities  $\qquad\qquad$ id $_{\rm f}$ etc. can be used for characterization of salinity tolerance at the seedling stage in crop plants (Singh et al. 2017; Singh et al. 2018a; Singh et al. 2018b). The hydroponic system of screening is more reliable, less time consuming and can be used to diminish the environmental variation commonly encountered in the field trials (Chen et al. 2008). Seedling survival and FDA-based florescent signals have been considered the most important parameters for accurate

phenotyping of salinity tolerance in crops. Amador et al. (2000) distinguished salt-tolerant and sensitive lines of cowpea based on seedling survival trait as criterion using the hydroponic method of screening while Singh et al. (2017) using similar parameter to distinguish between salt-tolerant and salt-sensitive lines in lentil. Further, the FDA-based fluorescent signal  $(H_2O_2)$ production) in roots is reported as a more reliable parameter for the evaluation of genotypes of different plant species, based on the intensity of fluorescent signals. These traits have been found suitable and well correlated with salinity stress tolerance (Singh et al. 2017). Physiological parameters such as stomatal conductance, evapo-transpiration and leaf area, early maturity, higher predawn water potential, maintenance of high osmotic adjustment and retention of high number of stems per plant provide indications about tolerance to salinity in chickpea (Katerji et al. 2005).

Molecular approaches have recently been developed to identify trait associated molecular markers to facilitate the understanding of involved mechanism and genetics of salt tolerance. The bulk segregant analysis approach has been used for the identification of markers linked with specific genes or QTLs of interested traits (Michelmore et al. 1991). A few researchers have reported the QTLs associated with salt tolerance in various crop plants based on morpho-physiological traits (Mano and Takeda 1997; Rivandi et al. 2011; Shavrukov et al. 2010; Tiwari et al. 2016). However, the QTLs associated with different traits for salt tolerance have been studied only in few legume crops, such as chickpea (Pushpavalli et al. 2015), cowpea (Arraouadi et al. 2012) and soybean

(Lee et al. 2004). Tuyen et al. (2010) studied two RIL populations (F<sub>6</sub> and F<sub>2</sub>) developed by crossing salt tolerant (JWS156-1, G soja) and sensitive (Jackson, G max) genotypes of soybean and mapped a significant QTL for alkaline salt tolerance on chromosome 17, which accounted for 50.2 and 13.0% of total phenotypic variations in the F<sub>6</sub> and F<sub>2</sub> population, respectively. It was further resolved that the QTL for alkaline salt tolerance and NaCl tolerance are not the same.

Transcriptomic analysis provides detailed knowledge about the gene expression at mRNA level, which is widely used to screen candidate genes involved in stress responses. Genomic approaches which play a significant role in encoding, cloning, and characterization of important genes may lead to identification and utilization of genes/QTL for improvement of pulse crops towards salinity stress. Screening can also be done through traditional and image-based approaches to quantify growth under salt stress. Genotyping could be performed using two contrasting methods such as targeted (tGBS) and transcriptome (GBS-t) genotyping-by-sequencing. tGBS may help constructing number of single-base variants (SNPs) and markers which may evenly be distributed across the genome compared to GBS-t. A genome-wide association (GWAS) needs to be conducted to pin-point the marker-trait associations on specific chromosome. The methodology could facilitate the identification of candidate gene(s) from the reference genome to find a nutrient transporter which may be involved in salt tolerance (Dissanayake et al. 2021)

#### **Mechanism of salinity tolerance**

The physiological, biological, cellular and molecular mechanisms of salt tolerance have been studied in cereals (rice, barley and wheat), Arabidopsis and other crop plants. However, limited work on salinity tolerance has been carried out in pulse crops. Various physiological and metabolic processes are affected by salinity stress, depending on severity and duration of the stress (Munns 2005; Rozema and Flowers 2008). The soil salinity represses plant growth in the form of osmotic stress which is then followed by ion toxicity, reduces water absorption capacity by roots, decrease water loss from leaves due to osmotic stress of high salt accumulation in soil and plants (Gupta and Huang 2014). The salinity stress, which is also considered as hyperosmotic stress (Munns 2005), leads to several physiological changes in the plants such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity, and decrease in stomatal aperture (Munns and Tester 2008).

Under salinity stress the ion uptake is affected and therefore, ion homeostasis plays a crucial role for normal plant growth and development. Singh et al. (2018) elucidated the role of osmotic, ionic and major salt responsive transcript components towards salinity tolerance in contrasting chickpea (Cicer arietinum L.) genotypes and found that under lower electrolyte leakage (EL) ratio in tolerant genotypes improves their ability to protect the membranous cellular network from uncontrolled EL under salt stress. Previous reports have shown plant tolerance to salt stress is closely related to the maintenance of high cytosolic K+/Na+ homeostasis under salinity stress. For example, coexpression of ZxNHX and ZxVP1-1 in transgenic alfalfa plants resulted in a higher accumulation of  $Na<sup>+</sup>$ ,  $K<sup>+</sup>$ , and  $Ca^{2+}$  in both leaves and roots (Bao et al. 2016).

Both glycophytes and halophytes do not tolerate high concentration of salts in their cytoplasm due to salt stress, hence excess salt is either transported to vacuole or sequestered in older tissues to protect the plant (Hasegawa 2013; Jhu 2003; Dietz et al. 2001). In saline soils, a major form of salt present is NaCl. The Na<sup>+</sup> ion is transported from the cytoplasm to the vacuole via Na<sup>+</sup>/H<sup>+</sup> antiporter. Two types of H<sup>+</sup> pumps are present in the vacuolar membrane: vacuolar type H + -ATPase (V-ATPase) and the vacuolar pyrophosphatase (V-PPase) (Dietz et al. 2001; OliveiraOtoch et al. 2001). Both Na<sup>+</sup> and Cl<sup>-</sup> are readily translocated in the phloem so that higher concentrations can be redistributed throughout the plant. They also accumulate salt and soluble carbohydrates in cell sap to maintain low osmotic potential. Many plants have inherent system to keep the ion concentration in the cytoplasm in a low level. The transport phenomenon is maintained by different carrier proteins and antiporters. Ma et al. (2012) have reported cellular Na<sup>+</sup>/K<sup>+</sup> homeostasis in Arabidopsis NADPH oxidases AtrbohD and AtrbohF function in ROS- dependent regulation of Na<sup>+</sup>/K<sup>+</sup> homeostasis under salt stress. Both ions, Na<sup>+</sup> and  $K^+$  have similar transport mechanism and thereby decrease the uptake of K<sup>+</sup>. Salinity tolerance in cultivated pigeonpea is perhaps facilitated by low accumulation of Na in roots and translocation of high content of K to shoots (Subbarao et. al. 1991). Oliveira Otoch et al. (2001) observed in the hypocotyls of Vigna unguiculata

seedlings that the activity of V-ATPase pump increased when exposed to salinity stress while that of V-PPase got inhibited. On the otherhand, in the case of halophyte Suaeda salsa, V-ATPase activity was upregulated and V-PPase played a minor role (Wang et al. 2001).

The role of Salt Overly Sensitive (SOS) stress signaling pathway has come in prominence recently in ion homeostasis in salt tolerance (Hasegawa et al. 2000; Shi et al. 2000a). Three major proteins namely, SOS1, SOS2 and SOS3 play a major role in encoding a plasma membrane bound  $\text{Na}^+\text{/H}^+$  antiporter, which  $\text{C}^{\text{Na}}$ is essential in regulating Na<sup>+</sup> efflux at cellular level facilitating transport of Na<sup>+</sup> from root to shoot. The  $\sum_{n=1}^{\lfloor n/9\rfloor}$ second protein, SOS2 encodes a serine/threonine kinase and that is activated by salt stress elicited Ca<sup>+</sup> signals. Viswanathan et al. (2005) advocated that Na\_ efflux and vacuolar compartmentation. putative osmosensory histidine kinase (AtHK1)-MAPK cascade probably regulates osmotic homeostasis and ROS scavenging. Os- motic stress and ABA (abscisic acid) mediated regulation of LEA (late-embryogenesisabundant)-type proteins also play important roles in plant salt tolerance. Over expression of this protein confers salt tolerance in plants (Shi et al. 2000b; Liu et al. 2000). The third type of protein SOS3 is also involved in the stress signaling pathway which is a myristoylated Ca<sup>+</sup> binding protein and contains a the myristoylation site at its N-terminus (Hasegawa et al. 2000; Shi vet al. 2000; Shi et al. 2000; Liu et al. 2000).

Compatible solutes or compatible osmolytes, include proline (Nounjan et al. 2012), glycine betaine (Wang and Nii 2000), sugar (Kerepesi and Galiba 2000; Kerepesi and Galiba 2000) and polyols (Ashraf and Foolad 2007; Ford 1985). The accumulation of proline and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and Glutathione peroxidase (GPX) are efficiently involved in scavenging of ROS (reactive oxygen spices) produced during salt stress conditions, and act as an important tolerance mechanism against oxidative stresses in plants. It is reported that soybean salt tolerance 1, named GmST1, exhibited strong tolerance to salt stress in the Arabidopsis transgenic lines. The GmST1-overexpressed Arabidopsis also increased sensitivity to ABA and decreased production of reactive oxygen species under salt stress (Ren 2016). Organic osmolytes are synthesised and accumulated in varying amounts amongst different plant species. And their major functions are to protect the structure and to maintain osmotic balance within

the cell via continuous water influx (Hasegawa et al. 2000). Glycine betaine , a non-toxic cellular osmolyte, plays an important role in stress mitigation by raising the osmolarity of cell during stress period. It also also protects the cell by osmotic adjustment (Gadallah 1999), stabilizes proteins (Makela et al. 2000), and protects the photosynthetic apparatus from stress damages (Cha-Um and Kirdmanee 2010) and reduction of reactive oxygen species (ROS) (Saxena et al. 2013).When glycine betaine is applied as a foliar spray in a plant subjected to stress, it led to pigment stabilization and increase in photosynthetic rate and growth (Cha-Um and 2010; Ahmad et al. 2013). In higher plants glycine betaine is synthesized by the enzyme choline monooxygenase (CMO), and catalysed by betaine aldehyde dehydrogenase (BADH).

A few amino acids such as cysteine, arginine, and methionine are decreased when exposed to salinity stress, whereas proline concentration rises in response to salinity stress (El-Shintinawy and El-Shourbagy 2001). Proline accumulation is a well-known measure adopted for alleviation of salinity stress (Saxena et al. 2013; Matysik et al. 2001). Intracellular proline which is accumulated during salinity stress not only provides tolerance towards stress but also serves as an organic nitrogen reserve during stress recovery. To maintain the concentration of compatible solutes and osmolarity in the cells different genes and biochemical pathways are involved are to protect the structure and to maintain osmotic balance within the cell via continuous water influx (Hasegawa et al. 2000). Similarly, polyamines also play a significant role in abiotic stress tolerance including salinity stress. They are small, low molecular weight, ubiquitous, polycationic aliphatic molecules widely distributed throughout the plant kingdom. The most common polyamines that are found within the plant system are diamine putrescine, triamine spermidine and tetra-amine spermine (Kuznetsov and Shevyakova; Hussain et val. 2011; Shu et al. 2012). Polyamine biosynthesis pathway in Arabidopsis involves six major enzymes. The exposure of plants to salinity stress increases the level of polyamines, which is regulated by polyamine catabolism. They are oxidatively catabolised by amine oxidases which include copper binding diamine oxidases and FAD binding polyamine oxidases. These enzymes play a significant role in stress tolerance (Takahashi and Kakehi 2010; Cona et al. 2006). Further, salt stress regulates polyamine biosynthesis and catabolism by acting as a cellular signal in hormonal pathways and

thereby regulating abscisic acid (ABA) in response to stress (Shevyakova et al. 2013).

War et al. (2011) studied different sensory mechanisms in chickpea to cope up with salt stress. They found that signaling mechanism is regulated at three different levels, first at the plant hormonal level, second by activation of transcription factors for gene expression and third by activation of metabolic pathways. Deinlein et al. (2014) found that plasma membrane is the first line of defense which perceives the stress through trans-membrane protein sensors. Initially, phospholipids receive the signal when high extracellular NaCl concentration occurs at ionic receptors of the root cell. The phospholipids have specific roles in regulating activation of calciumdependent protein kinase CaCDPK1 genes, which are involved in the release of signaling messengers such as calcium ions  $(Ca^{2+})$  (Zepeda-Jazo et al.2011; Syam Prakash and Jayabaskaran 2006; Geiger et al. 2011) through the control of transcription factors during the saline stress responses in chickpea (Dixit and Jayabaskaran 2012).

Transcription factors bind to the promoter regions of the genes to facilitate the RNA polymerase to start the transcription and subsequent translation of the gene products (Franco-Zorrillaa et al. 2014). They are important regulators of stress response and have been widely found to show differential expressions in saltchallenged tissues in Cicer arietinum (Hiremath et al. 2011; Jain et al. 2013).A number of transcription factors in Arabidopsis have been identified to be associated in the activation of genes responsible for osmotic adjustments (Peng et al. 2009). Transcription factors such as CAP2/AP2, CarNAC1, CaZF and CarF that are known to up-regulate the CaCDPK1 genes have been identified in chickpea (Jain et al. 2013; Jia et al. 2012).

The application a phytohormone ABA to plant ameliorates the effect of stress condition(s). This phytohormone is upregulated due to soil water deficit around the root. Salinity stress causes osmotic stress and water deficit, increasing the production of ABA in shoots and roots (Cabot et al. 2009). The accumulation of ABA can mitigate the inhibitory effect of salinity on photosynthesis, growth, and translocation of assimilates (Jeschke et al. 1997) probably due to the accumulation of compatible solutes and assimilates. The accumulation of  $K^+$ , Ca<sup>2+</sup> and compatible solutes, such as proline and sugars, in vacuoles of roots, which counteract with the uptake of Na<sup>+</sup> and Cl<sup>"</sup>and thus ch

modulates the expression of several salt and water deficit-responsive genes. Certain compounds such as salicylic acid (SA) and brassinosteroids (BR) produce plant abiotic stress responses in plants (Fragnire et al. 2011; Clause and Sasse 1998). Jayakannan et al. (2013) have shown that SA improves salinity tolerance in Arabidopsis by restoring membrane potential and preventing salt-induced  $K^+$  loss via a guard cell outward rectifying K(+) (GORK) channel and also upregulate H<sup>+</sup>-ATPase activity, thereby improving K<sup>+</sup> retention during salt stress. Nazar et al. (2011) found that SA alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in mung bean cultivars. The negative effects of salinity may also be mitigated by BR (El-Mashad and Mohamed 2012; Krishna 2003). Application of BR enhanced the activity of antioxidant enzymes (SOD, POX, APX, and GPX) and the accumulation of nonenzymatic antioxidant compounds (tocopherol, ascorbate, and reduced glutathione) (El-Mashad and Mohamed 2012). Both BRs and SA are ubiquitous in the plant kingdom, affecting plant growth and development in many ways, and are known to improve plant stress tolerance. The possible applications of BRs and SA to mitigate the harmful effects of salt stress in plants has been reviewed (Ashraf et al. 2010) and discussed the roles of exogenous applications of BRs and SA in the regulation of various biochemical and physiological processes leading to improved salt tolerance in plants.

A huge number of salt-responsive transcription factors and genes which are either upregulated or downregulated in response to salinity stress have been identified and characterized using transcriptomic and genomic approaches. To screen candidate genes involved in gene expression of stress response, transcriptomic analysis at mRNA level is essential. Transcription factors such as bZIP, WRKY, AP2, NAC, C2H2 zinc finger gene, and DREB families comprise many stress-responsive members which can control the expression of a broad range of target genes by binding to the specific cis-acting element in the promoters of these genes. Vincente and Plasencia(2011) found that transcriptomic factors and gene transcription are highly activated on up-regulated plant hormones such as ABA, Indole Acetic Acid (IAA), gibberellic acid (GA) and methyl jasmonate (MeJA), which induce expression of CarLEA genes. Accordingly, these genes viz., CarLEA genes (CarLEA1, CarLEA2 and CarLEA4) isolated from chickpea cDNA libraries, were found to impart

desiccation tolerance during seed development, thereby protecting plants against a variety of stresses, including drought, salinity and freezing (Gu et al. 2012; Romo et al. 2001).

Transcriptions factors such as OsNAC5 and ZFP179 show an upregulation under salinity stress, which may regulate the synthesis and accumulation of proline, sugar, and LEA proteins that in turn play an integral role in stress tolerance (Hu et al. 2013). A transcription factor gene, SALT-RESPONSIVE ERF1 (SERF1) has shown root-specific induction upon salt and  $H_2O_2$  treatment and the loss of SERF1impairs the salt-inducible expression of genes (Schmidt et al 2013). It has been observed that the plants deficient for SERF1 are more sensitive to salt stress compared with the wild type, while constitutive overexpression of SERF1 improves salinity tolerance in crops.

Transcription factors are considered as most important regulators that control gene expressions. They are important regulators of stress response and have been widely found to show differential expressions in salt-challenged tissues (Hiremath et al. 2011: Jain et al. 2013). Several transcription factors have been identified to be associated in the activation of genes responsible for osmotic adjustments (Peng et al. 2009). Transcription factors such as CAP2/AP2, CarNAC1, CaZF and CarF that are known to up-regulate the CaCDPK1 genes have been identified in chickpea (Jia et al. 2012).

Singh et al. (2021) conducted an extensive transcriptomic skimming to determine the molecular, morphological, physiological, and biochemical responses in salt-tolerant variety, PDL-1 and saltsensitive cultivar, L-4076 under control (0mMNaCl) and salinity stress (120mMNaCl) conditions at seedling stage. The investigation revealed that PDL-1 had no salt injury and showed higher K+/Na+ ratio, relative water content, chlorophyll, glycine betaine, and soluble sugars in leaves while lower  $H_2O_2$  induced fluorescence signals in roots as compared to L-4076. The study further indicated that putative molecular mechanism of salinity tolerance in lentil together with identification of 5643 simple sequence repeats (SSRs) and 176,433 single nucleotide polymorphisms (SNPs) would facilitate the construction of dense linkage maps along with detection of quantitative trait loci (QTLs) associated with traits of interests. They further argued that stress-related pathways can be targeted to improve salinity stress tolerance in crop species.

# **Potential strategies to develop salt stress tolerant genotypes of pulse crops**

Identification of morphological and physiological traits and determination of other genetical parameters are essential prerequisite to manipulate the plant mechanism for developing salt tolerant genotypes by conventional or molecular techniques. Viswanathan et al. (2005) suggested that genetic engineering of ion transporters and their regulators, and of the CBF (Crepeat-binding factorholds promise for future development of salt-tolerant crops.

Mielewczik et al. (2013) demonstrated a novel method of analyzing two-dimensional leaf expansion in high temporal solution based on marker tracking approach in soybean. Similarly, ion channel regulation has been found to be effective in enhancing salinity tolerance in chickpea.

Screening of the germplasm and identification of tolerant genotypes would remain to be to the most effective and quick approach of developing genotype tolerant to salinity stress. A set of specific physiological indicators may be selected while assessing the crop for salt tolerance. Vadez et al. (2012b) screened chickpea genotypes based on sensitivity at reproductive stage and reported large variation for salt tolerance. The identified genotypes could be used as potential resource for salt tolerance related traits to facilitate genet transfer of useful alleles. Molecular breeding approaches would facilitate effective translation of the genomic knowledge towards the development of salinity tolerant genotypes. With the advent of next generation phenomics platforms such as robotic field sensors, high resolution multi-spectral mapping using UAVs and laser light back scattering technology, it has become easier to study developmental stages of plant more precisely and analyses the multi-dimensional large volume of bioimaging data (Mielewczik et al. 2013; Cai et al. 2016).

# **Biotechnological approaches for improvement of pulses against salinity stress**

The attempts in the development of salt-tolerant pulse crops through genetic engineering approach are limited. One of the possible ways is to increase the plant's ability to limit the uptake of salt ions from the soil, increasing the active extrusion rate of salt ions and improving the compartmentalization of salt ions in the cell vacuole may the best strategy.

Kaashyap et al. (2018) investigated physiological

and transcriptional responses in salt tolerant and salt sensitive chickpea genotypes and created RNA-Seq reads from root tissues and identified differentially expressed genes responding to salt stress. They also reported major transcriptional reorganization in response to salt stressand significant upregulation of various genes encoding important enzymes including DREB1E and ERF in the tolerant genotype. Further, the study identified important genes involved in cell wall modification and root morphogenesis. The integration of phenotypic and genomic data may provide a better understanding of salt tolerance mechanism. The availability of genome of several pulses could offer an important opportunity in finding the candidate genes involved in salt tolerance mechanisms in the pulse crops. Soren et al. (2020) carried out an extensive analysis of the phenotypic and genotypic data generated from the RILS derived from a cross, ICCV 10 (salt-tolerant) x DCP 92-3 (saltsensitive) chickpea genotypes. The RIL population was evaluated under salt stress conditions in field and phenotypic data was assembled. Axiom®CicerSNP analysis identified 28 quantitative trait loci (QTLs) explaining up to 28.40% of the phenotypic variance in the population. Deployment of such QTL may help development of chickpea genotype with salt tolerance.

A wide range of genes encoding different structural and regulatory proteins have been reported and/or have been employed over the past decades to develop abiotic stress-tolerant transgenic plants (Bhatnagar et al. 2008). Pathogenesis-related (PR) proteins accumulate in plants upon pathogen infestations and, in many cases, in response to abiotic stresses for survival (Stratilová et al. 2020). The protein PR10 iswell known as osmotically inducible protein and elicitor and plays an important role in plant defense mechanisms of salinity stress (Abreu et al. 2013; Agarwal et al. 2016). Studies on proteomic investigation of pea under salt stress revealed significant increase in the level of several classes of PR10 proteins.

The constitutive expression of a pea PR10 gene in Brassica napus enhanced germination and growth rates under salinity (Srivastava et al. 2004). Accumulation of PR10 proteins was also observed in grass pea in response to salt stress (Chattopadhyay et al. 2011). Overexpression of the PR10a gene in faba bean (Vicia faba) plants promotes sustained growth and development under salt stress (Desouky et al. 2021).

#### **Future perspectives**

Breeding pulse crops for stressful environments is especially important for enhancing the production and productivity of the pulses. Genomic approaches and available molecular marker technology will help in the evaluation and identification of salt tolerant genotypes and donors for developing new lines and reduce dependency on the phenotypic data alone. Genome Wide Association Studies would help in better understanding the genetic basis of the phenotypes. The traits related to salinity stress needs to be quantified for strategic application of phenomics tools. As evident from the published literature, some of the traits such as high photosynthesis or quantum yields have been associated with tolerance to drought, salinity, or high temperature. Generally, it is attributed to the capacity of plants to maintain nutrient and water balance in the tissue reflected by relative water content and stress avoidance mechanism. Therefore, an efficient integration of genomics, proteomics, metabolomics, ionomics and phenomics will enrich biological understanding of salinity stress tolerance. Of late, converging various ultra-modern technologies like, infra-red thermography, automated robotics, camera images, and computational algorithms, which all make components of high throughput phenotyping facilities (phenomics and phenospex) can facilitate high throughput phenotyping for stress tolerance. Genetic engineering has been proved to be an efficient approach to the development of salinity-tolerant plants, and this approach will become more powerful as more candidate genes associated with salinity tolerance are identified and widely utilized.

#### **Declaration**

The authors declare no conflict of interest.

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