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Research article

Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes

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ABSTRACT

Salt stress affects all the stages of plant growth however seed germination and early seedling growth phases are more sensitive and can be used for screening of crop germplasm. In this study, we aimed to find the most effective indicators of salt tolerance for screening ten genotypes of soybean (SL-295, Gujosoya-2, PS-1042, PK-1029, ADT-1, RKS-18, KDS-344, MAUS-47, Bragg and PK-416). The principal component analysis (PCA) resulted in the formation of three different clusters, salt sensitive (SL-295, Gujosoya-2, PS-1042 and ADT-1), salt tolerant (MAUS-47, Bragg and PK-416) and moderately tolerant/sensitive (RKS-18, PK-1029 and KDS-344) suggesting that there was considerable genetic variability for salt tolerance in the soybean genotypes. Subsequently, genotypes contrasting in salt tolerance were analyzed for their physiological traits, photosynthetic efficiency and mitochondrial respiration at seedling and early germination stages under different salt (NaCl) treatments. It was found that salt mediated increase in AOX-respiration, root and shoot K^+/Na^+ ratio, improved leaf area and water use efficiency were the key determinants of salinity tolerance, which could modulate the net photosynthesis (carbon assimilation) and growth parameters (carbon allocation). The results suggest that these biomarkers could be can be useful for screening soybean genotypes for salt tolerance.

1. Introduction

Soil salinity is becoming a major threat to realizing crop yield of agricultural crops. About 20% cultivated land and 33% of irrigated agricultural land is affected worldwide by salinity and average yields for most major crop plants have dropped by more than 50% (Gupta and Huang, 2014). Thus out of the 25% irrigated area, 17% area is salt affected in India and this is increasing every year (Parihar et al., 2015). To address this threat, plant based solutions have assumed significance for improving salt tolerance in crop germplasm. This requires screening of salt tolerant genotypes which can survive and produce higher yield in salt affected soils. In a given species, it is known that different genotypes show contrasting response to salinity (Ali et al., 2014; Sharma, 2015; Kan et al., 2015) and it is thus desirable to, screen for salt tolerant genotypes which will be good candidates for cultivation in saline soils.

It is well established that salinity imposes osmotic (physiological drought or water limitation) and ionic stress (ion toxicity) (Munns and Tester, 2008). In the early phase, salinity inhibits water uptake, cell elongation, root development, formation of new leaves while in the later phase, salt ions accumulates and causes premature senescence, disruption in enzyme functionality and inhibition of photosynthesis (Munns, 2005; Roy et al., 2014). It affects most of the developmental stages of plants from seed germination to reproductive stage. The seed germination and early seedling growth are the most susceptible stages of plant growth to salt stress and can be used to screen genotypes for their tolerance or sensitivity (Pandey and Suprasanna, 2016). Seed germination and early seedling stages are the important stages in the life cycle of a plant, as they regulate the seed vigor and consequently plant adaptation to salt stress (Carpici et al., 2009). Genotype screening at seedling

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Abbreviations

G%	Germination percentage
RL	Root length
SL	Shoot length
SFW	Shoot fresh weight
RFW	Root fresh weight
SDW	Shoot dry weight
RDW	Root dry weight
STWC%	Shoot tissue water content percentage
RTWC%	Root tissue water content percentage
GFW	Gain in Fresh weight
SR	Secondary root
Chl A	Chlorophyll- A
Chl B	Chlorophyll-B
T Chl	Total Chlorophyll
Car	Carotenoid
R K^+/Na^+	Root Potassium/Sodium
R Ca^+	Root Calcium
S K^+/Na^+	Shoot Potassium/Sodium
S Ca^+	Shoot Calcium
LA	Leaf area
PN	Photosynthetic rate
WUS	Water use efficiency
gS	Stomatal conductance
E	Transpiration rate

and germination are most preferred because it is rapid, less laborious and inexpensive (Bafeel, 2014).

Salt tolerant genotypes may have reduced ionic toxicity, osmotic balance by synthesis of compatible solutes (Munns and Tester, 2008), enhanced antioxidant mechanisms and maintenance of photosynthetic rate for efficient scavenging of salt induced excess reactive oxygen species (ROS) scavenging (Miller et al., 2011; Nikalje et al., 2017). Development of biomarkers is important for screening a large number of genotypes. These include, antioxidant enzymes, osmolytes (proline, glycine betaine, total soluble sugars etc.), physiological markers like photosynthetic efficiency, relative water content, malondialdehyde content. Alternative oxidase (AOX) is a key enzyme in the mitochondrial electron transport chain of plants. The differential behavior of AOX under salinity stress has shown potential to be used as an indicator of salt tolerance; particularly at early germination stage (Pandey and Suprasanna, 2016). The mechanisms by which AOX is involved in the tolerance of plants to abiotic stress is by maintaining the redox balance in plant cells (Cvetkovska and Vanlerberghe, 2012; Feng et al., 2013), repair the photosynthetic machinery (Gandin et al., 2012; Feng et al., 2013) and balance of carbon and nitrogen ratio by modulating carbon-use efficiency (Kornfeld et al., 2013; Feng et al., 2013). Soybean (*Glycine* max (L.) Merr.) is the leading economic oil seed crop worldwide, and is also an attractive crop for biodiesel production and source of protein for human and animal diet. Soybean is also economically important because of its high oil content (20%) and protein (40%) (Amirjani, 2010). It is classified as a moderately salt-sensitive glycophyte (Munns and Tester, 2008) and exhibits high degree of variation in the salt response among genotypes. Therefore it is essential to select highly tolerant genotype for cultivation under saline soils. Different strategies have been applied to screen tolerant genotypes by using physiological (Hakeem et al., 2012; Wu et al., 2014) biochemical (Hakeem et al., 2012; Wu et al., 2014) and molecular attributes (Fan et al., 2013; Guan et al., 2014). Most of these strategies are time consuming and cost effective. Hence there is a need for development of quick and reliable methods of screening genotypes for salt tolerance. Negrao et al. (2017) outlined that studies on effects of salinity on plants should not be based on whole-plant or total plant biomass, but rather on traits that may contribute to salinity tolerance. The Principal component analysis (PCA) has been suggested to provide simultaneous analysis of the most important traits contributing to salinity tolerance (Su et al., 2013). This method has also been shown as an accurate and easy tool to screen germplasm at germination and early seedling level in melon landraces (Sarabi et al., 2016) and rice (Chunthaburee et al., 2016). There is a need for development of rapid and consistent means for screening of soybean genotypes, The present study was aimed to examine the physiological and biochemical indicators at germination and early seedling stage in soybean under salt stress, and to apply the principal component analysis to discriminate soybean genotypes for salinity tolerance.

2. Materials and methods

2.1. Plant material, growth and treatment conditions

The study included seven soybean genotypes: MAUS-47, PK-416, ADT-1, SL-295, Gujosoya-2, PS-1042 and KDS-344 collected from the National Institute of Soybean Research, Indore, MP, India and three genotypes: Bragg, PK-1029 and RKS-18 from Agharkar Research Institute, Pune, MS, India.

The seeds of all genotypes were surface sterilized with 0.1% mercuric chloride for 3 min and subsequently washed five times with sterile distilled water. The seeds were further allowed to germinate in separate petri plates (90 mm), containing germination paper supplemented with 15 ml of 0, 50, 100, 150 and 200 mM NaCl solution. The experiment was performed in triplicates and each replicate had 10 seeds. Seeds were incubated in dark at 25 ± 2 °C temperature for 10 days. After 10 days, growth parameters, such as germination percentage (G %), shoot length (SL), root length (RL), number of secondary roots (SR), Fresh weight (FW), Dry weight (DW) and tissue water content (TWC) as per Muchate et al. (2016) were measured.

A hydroponic set up was maintained to assess the impact of ionic stress in salinity. In short, evenly germinated seeds (5 No.) were transferred to hydroponic system after 3 days of germination and allowed to grow in controlled condition for 12 more days (total 15 days). The nutrient medium for plant growth was ½ strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Seeds of Bragg and Gujosoya-2 genotypes were grown under control (0), 50, 100 and 150 mM salt treatment and on the basis of lipid peroxidation rate (MDA content), 100 mM NaCl concentration was selected for further experimentation (Supplementary Fig. S1). At the end of 15 days, fresh weight of the seedlings was recorded and salt treatment was given with 100 mM NaCl for a period of 10 days. After the completion of salt stress treatment, fresh weight of the seedlings was recorded and, relative gain in fresh weight (GFW) was calculated as per Xu et al. (2011). The effect of stress treatment over dry matter partitioning was also analyzed, by calculating DW of the sample. The seedlings were blot dried and then were kept at 60 °C until a constant weight was achieved. Other parameter like RL, SL, SR, were manually measured, while tissue water content (TWC) was assayed as per Muchate et al. (2016).