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Research article

Proteomics unravel the regulating role of salicylic acid in soybean under yield limiting drought stress

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ABSTRACT

Drought is a major concern for sustainable yield under changing environment. Soybean, an economically important oil and protein crop, is prone to drought resulting in yield instability. Salicylic acid (SA), a multifaceted growth hormone, modulates a series of parallel processes to confer drought tolerance thereby relieving yield limitations. The present study was performed in soybean plants treated with SA (0.5 mM) through seed pretreatment under drought regimes: severe stress (50% RWC) and moderate stress (75% RWC), and rehydration. Differential leaf proteome profiling with morphological, physiological and antioxidative metabolism studies were performed at two developmental stages (vegetative and flowering). This explained the tolerance attribution to soybean throughout the development attaining yield stability. Abundance of proteins involved in photosynthesis and ATP synthesis generated energy driving metabolic processes towards plant growth, development and stress acclimation. Carbon (C) metabolism proteins involved in growth, osmoregulation and C partition relieved drought-induced C impairment under SA. Defensive mechanisms against redox imbalance and protein misfolding and degradation under stress were enhanced as depicted by the abundance of proteins involved in redox balance and protein synthesis, assembly and degradation at vegetative stage. Redox signaling in chloroplast and its interplay with SA signaling triggered different defense responses as shown through thioredoxin protein abundance. Amino acid metabolism proteins abundance resulted in increased osmoprotectants accumulation like proline at initial stage which contributed later towards N (nitrogen) remobilization to developing sink. At later stage, abundance of these proteins maintained redox homeostasis and N remobilization for improved sink strength. The redox homeostasis was supported by the increased antioxidative metabolism in SA treated plants. The downregulation of proteins at flowering also contributed towards N remobilization. Yield potential was improved by SA under drought through acclimation with enhanced N and C remobilization to sink as demonstrated by increased yield parameters like seed number and weight per plant, thousand seed weight and harvest index. The potential of SA in conferring drought tolerance to plants to maintain sustainable yield possess future research interests.

1. Introduction

About 80% of the world's agricultural land is rain fed and under the threat of drought. The drought related yield reductions for the major crops in world will reach more than 50 per cent by 2050 (Li et al., 2009). The negative impact of water stress on agricultural productivity will make it challenging to meet the food demands of growing global population. In India, 64% of the population depends on agriculture for their livelihood. The country faces major challenges to increase its food production to attain 50% more grain by 2020 for its ever-growing population (Kumar and Gautam, 2014). In India, about 68% area out of net sown 140 million hectares is vulnerable to drought conditions where about 50% area has frequent droughts. India has experienced large scale droughts and the frequency is increasing posing a great threat to agriculture and food security. Climate change will also impact water resources thereby posing more risks to agriculture in India as water is the most critical agricultural input.

Soybean [*Glycine max* (L.) Merr.] is the most important leguminous crop worldwide for essential source of oil, protein, macronutrients and minerals (Clemente and Cahoon, 2009). The predicted climate change

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with persistent droughts is a great threat to sustainability of soybean yields (Foyer et al., 2016). India has experienced increase of about 24% in drought with statistically significant trends in the spatial extent of droughts in Central Northeast and West Central regions and about 55% increase in the risk of severe drought (Mishra and Liu, 2014; Sharma and Mujumdar, 2017). India has about 56% of the net cultivated area as rain-fed accounting for 44% of food production. Rainfall is crucial for Kharif crops that accounts for about 70% oilseed production of the country (Crisis Management Plan Drought (National), 2017). Drought can cause 40–60% soybean yield loss worldwide (Valliyodan and Nguyen, 2006). Soybean production in India accounts for 4% of global production. Soybean is the source of vegetable seed oil (20%) and protein (40%) for human and also used in animal feed. Major soybean producing states are part of west central region of India which is facing severe drought. Bhatia et al. (2008) indicated 28% yield reduction in soybean under adverse soil moisture conditions in India. Between 26 and 34% of the yield variability in Indian soybean yields was explained by climate variability including drought (Ray et al., 2015).

The negative impact of drought predominates at all developmental stages, starting from germination to seed maturation (Valliyodan and Nguyen, 2006). Drought avoidance, drought tolerance, drought escape, and drought recovery are the mechanisms facilitating the plants to overcome stress (Cruz De Carvalho, 2008). Salicylic acid (SA), a plant growth hormone and important signaling molecule, has great agronomic potential to improve the drought tolerance of plants. SA modulates the plant responses to environmental stresses by regulating plant growth, development, ripening, and defense responses. Water deficit condition increased the level of endogenous SA upto fivefold in *Phillyrea angustifolia* (Munne-Bosch and Penuelas, 2003) and approximately twofold in barley roots (Bandurska and Stroinski, 2005). The role of SA in regulation of drought was also supported by the induction of SA-inducible genes *PR1* and *PR2* by drought stress (Miura et al., 2013). The *Arabidopsis* mutants *adr1, myb96-1d, siz1, acd6*, and *cpr5* accumulating endogenous SA exhibit SA-dependent drought tolerance (Chini et al., 2004; Seo et al., 2009; Miura et al., 2013). Lee et al. (2006) reported conferred drought tolerance in Arabidopsis on introduction of the pepper pathogen-induced gene *CAPIP2* accompanied by the expression of *Arabidopsis PR1* gene involved in SA induced defense responses. The application of low concentration of SA enhanced the plant growth and drought tolerance in wheat (Kang et al., 2012) and muskmelon (Korkmaz et al., 2007) under water stress. Senaratna et al. (2000) reported increased plant tolerance to drought, heat and chilling stress in tomato and beans by imbibition of seeds in 0.1–0.5 mM SA. Loutfy et al. (2012) reported increase in biomass, inorganic and organic solute contents of wheat under interactive effect of SA and drought. SA treatment increased the membrane stability and levels of proline and ABA in water stressed barley conferring plants with stress tolerance (Bandurska and Stroinski, 2005). SA positively influenced the ascorbate–glutathione cycle in pretreated wheat leading to enhancement in tolerance to stress and alleviating substantial water loss (Kang et al., 2013). The detrimental effects of water stress on photosynthesis were alleviated by SA pretreatment in wheat along with increased antioxidative metabolism (Singh and Usha, 2003). SA strengthened antioxidant defense system in *Zea mays* under drought stress (Saruhan et al., 2012). Molecular studies on SA induced genes under water stress demonstrated 9 highly expressed genes in guard cells including *LTI30* (Miura et al., 2013). The over expression of *LT130* enhanced the expression of dehydrins involved in improving the drought tolerance. Proteomics revealed several different functionally characterized proteins to be upregulated by pretreatment with SA under drought stress in wheat (Kang et al., 2012). Several defence proteins such as glutathione S-transferase, ascorbate peroxidase and peroxiredoxin were upregulated suggesting the role of SA in protecting the plants from oxidative stress by enhancing the antioxidant defense system. SA increased the expression of ATP synthase to maintain the energy requirement for growth and coping with stress. Photosynthesis related proteins RuBisCO and related enzymes were upregulated in wheat under treatment of SA and drought (Sharma et al., 2017).

SA is involved in the response to abiotic stress however the actual role of SA in abiotic stress remains unresolved. The present study will give an insight on the relationship between the SA and drought tolerance in soybean plants through physiological, biochemical and proteomics analyses. Subsequently, a network of different drought adaptive/resistant responses induced by SA in plants is proposed. We hypothesize that SA will improve the photosynthetic performance of soybean driving metabolic processes for stress acclimation to maintain growth and development under water limiting environment. Interplay of redox signaling and SA signaling is substantiated triggering defense mechanisms against drought. Yield limitations relieving role of SA under water stress will be revealed by analysis of different parameters related to nitrogen use efficiency (NUE) and yield.

2. Materials and methods

2.1. Biological material

The widely adaptable soybean (*Glycine max* (L.) Merr.) variety JS335 (JAWAHAR SOYBEAN 335) was selected for the experiment due to its good germinability and longevity. JS335 variety selected due to highest germination rate and adaptability to experiment site. The seeds were obtained from Indore, Madhya Pradesh, India.

2.2. SA application and plant growth conditions

SA application was performed through seed priming by soaking the seeds in 0.5 mM SA solution for 6 h before sowing and for control seeds were soaked in water. SA concentration was selected on the basis of highest seed germinability under SA pretreatment (results not shown). The experiment was conducted at CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh (26° 55′ N latitude, 80° 59′ E longitude and at an altitude of 113 m in subtropical climate). Recommended dose of NPKS (Nitrogen: Phosphorus: Potassium: Sulphur) at 20:60:20:20 kg per hectare was applied at the time of seed bed preparation. Seeds were sown at a depth of 2–3 cm adopting a spacing of 30 × 5 cm. Irrigation was maintained regularly till the seedling establishment.

Drought was maintained by controlling the irrigation after seedling establishment (45 days of germination) till the harvesting stage. The monitoring of the soil moisture level was performed on regular basis by Soil Moisture Meter (ICT International Pvt Ltd. Australia). The two stress levels were maintained on the basis of relative water content (RWC): 50% RWC (severe stress) and 75% RWC (moderate stress) with rehydrated plants after 50% RWC stress. The well watered, stressed and rehydrated control plants were also maintained for SA treatment.

CON = Control well watered; 50% = Control 50% stressed; 75% = Control 75% stressed; RH = Control Rehydrated.

CON + SA = SA treated well watered; 50 + SA = SA treated 50% stressed; 75 + SA = SA treated 75% stressed; RH + SA = SA treated Rehydrated.

RWC of the leaves was determined by following formula:

$$\mathrm{RWC\%} = \frac{(\mathrm{FW}-\mathrm{DW})}{(\mathrm{TW}-\mathrm{DW})} \times 100$$

Here, FW = Fresh weight; DW = Dry weight; TW = Turgid weight of the leaf after equilibration in distilled water for 24 h.

2.3. Growth and yield

Plants were harvested in five replicates for biomass analysis at vegetative phase (8–9 weeks of growth), flowering stage (after heading initiation) and final harvest (full maturity). Leaf samples at both stages were freezed in Liquid N_2 and store at −80 °C for further analysis. Root