

Original Research Article

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Physiological Role of Exogenous Spermine in Wheat (*Triticum aestivum* em. Thell) under Salinity

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ABSTRACT

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The elevated concentration of salts in soils impact plant growth and development by disturbing the capacity of roots to extract water and high levels of salts within the plant suppress the physiological and biochemical processes such as nutrient uptake and assimilation. Salinity induced wheat leaves were analysed for various physiological parameters viz. relative water content, leaf membrane injury, osmotic potential and chlorophyll fluorescence at five days after treatment of spermine at 1.0 mM concentration. Spermine treatment showed higher relative water content and improved efficiency of photosystem II (F_v/F_m) indicating increase water retention capacity in control and salt stressed condition. Further, spermine treatment alleviated the salinity induced leaf membrane injury with marked decrease in ion leakage and played protective role.

Introduction

Wheat and rice are two major cereal crops that occupy about 50-55% of the total cropped area of India. Wheat is consumed in various forms by more than one thousand million populations and provides more than 50 percent of human energy and protein needs. Wheat is the most widely cultivated food crop and well adapted to grow in semi-arid regions. Salinity is the main factor impeding sustainable crop production, especially in irrigated arid and semi-arid regions of the world, where the rainfall is too low to prevent accumulations of ions in the soil and where

irrigation is the cause of secondary salinization (Calanca, 2017). Salt stress is one of the major environmental constraints affecting and limiting the crop productivity and quality of economically important crops.

Plants growing in saline media may reduce internal water deficits by the absorption of inorganic ions and the synthesis of organic solutes for osmotic adjustment (Kafi *et al.*, 2003). Inorganic ions are accumulated in the vacuole and organic osmolytes in the cytoplasm to balance vacuolar osmotic potential (Mansour, 2000).

Salinity induces accumulation of a range of nitrogenous compounds, the most frequent being amino acids, amides, proteins, ammonium quaternary compounds and polyamines. These compounds functions in osmotic adjustment, cellular macromolecule protection, nitrogen storage, cellular pH maintenance, a decrease of cellular toxicity and elimination of free radicals (Zapata *et al.*, 2008).

Application of exogenous polyamines also have been reported, could alleviate salinity-induced damages in rice (Roychoudhury *et al.*, 2011), citrus (Shi *et al.*, 2010), mung bean (Nahar *et al.*, 2016), Kentucky grass (Puyang *et al.*, 2016) and cucumber (Sang *et al.*, 2016). Salt stress along with osmotic stress influences the photosynthesis, water relations, ion homeostasis, yield component, senescence, growth and development of crop plants. As a result, there is a decrease in dry mass, chlorophyll content and soluble proteins which are reflected in qualitative and quantitative grain yield and yield components (Anjum *et al.*, 2008). Tolerance to abiotic stresses is very complex due to intricate interactions between stress factors and various molecular, biochemical, agronomic and physiological phenomenon affecting plant growth and development. To present work is planned to understand the physiological functions of spermine in wheat under saline stress and their responses.

Materials and Methods

The present experiments were conducted in the Chaudhary Charan Singh Haryana Agricultural University, Hisar. The caryopses of the wheat cultivars (*Triticum aestivum* L.) DBW 88 and KRL 210 differing in tolerance to salinity were obtained from Wheat and Barley Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar. Seeds of wheat varieties were surface sterilized and

grown in earthen pots lined with polyethylene bags filled with 6 kg dune sand in a screen house under naturally lit conditions. The nutrient solution was used to irrigate the soil supplemented with nutrients in the form of N, P and K in the ratio of 10:3:3.

Wheat plants were grown at two levels of salinity (8 and 12 dSm⁻¹) and control. Artificial saline waters of 8 and 12 dSm⁻¹ levels were prepared for irrigation of pots by maintaining Na: Ca + Mg ratio as 1:1; Ca: Mg as 1:3 and that of Cl: SO₄ as 7:3. Plants were irrigated with distilled water to maintain the salinity levels. Spermine at 1.0 mM concentration was sprayed over the plants at 21 days after sowing. Plants were tagged at the time of application of spermine and leaf samples were analysed for physiological traits on 5th day after the spermine treatment.

Relative water content

Relative water content was measured by the method of Barrs and Weatherley (1962) using the following formula.

$$\text{Relative water content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{Dry weight}} \times 100$$

Turgid mass was determined after saturation of leaf blades in distilled water in sealed glass tubes for 6 h at room temperature followed by overnight storage at 5^oC. The dry weight was determined after 48 h at 75^oC.

Leaf membrane injury

Leaf membrane injury in terms of relative stress injury was measured by Sullivan and Ross, 1979 by using the following formula.

$$\text{Membrane Injury} = \frac{\text{EC1}}{\text{EC2}} \times 100$$

The EC1 was measured after soaking the 200 mg leaf segment in 8 ml deionized water at

27⁰C for 5 h and EC₂ was measured after the same sample was kept in boiling water bath at 100⁰C for 30 min. The membrane injury index was calculated as follows:

Osmotic potential

The osmotic potential of the leaf was measured with a psychrometric technique using VAPRO 5520 vapor pressure osmometer (Wescor INC., Lorganan, Utah, USA) as described by Morgan, 1980. The excised leaf was sealed in Eppendorf tube and quickly frozen at -20 ⁰C and the sap was collected after thawing for 60 min at 25⁰C. About 10 µl of sap is used to record the readings of osmometer in mmol kg⁻¹ were expressed in MPa.

Chlorophyll fluorescence

The chlorophyll fluorescence was measured by method of Zribi *et al.*, (2009), where measurements of F_v/F_m were taken from on the surface of flag leaf using a portable handy analyser (Hansatech, UK). The data was analysed, which provides parameters indicating the efficiency of photosystem II.

Results and Discussion

The mean relative water content (RWC) decreased with increase in the salinity at 21 DAS (from 86.1 % in control to 68.0, 64.6% at 8 dSm⁻¹ and 12 dSm⁻¹ respectively). Spermine treatment of 1.0 mM concentration showed higher values for RWC in stressed leaves (75.4 and 76.2 % at 8 dSm⁻¹ and 12 dSm⁻¹ respectively). RWC in KRL 210 was found higher when compared with DBW 88 (Fig. 1). Leaf membrane injury in terms of relative stress injury was measured as percent proportions of ion leakage into the external aqueous medium to the total ion concentration of the stressed tissue as measured by the EC of the external medium. There was a progressive increase in the mean leaf membrane injury with increasing levels of salinity i.e., from 37.2 % (control) to 40.0 % (8 dSm⁻¹) and 50.3 % (12 dSm⁻¹) at 21 DAS (Fig. 2). The application of spermine gradually decreased the leaf membrane injury in both the cultivars DBW88 and KRL 210. Maximum leaf membrane injury was found in DBW 88 (55.9 %) at 12 dSm⁻¹ without spermine treatment and minimum was recorded in KRL 210 with spermine treatment in control (35.7 %).

Fig.1 Effect of spermine at 21 DAS on relative water content in wheat flag leaf under salt stress

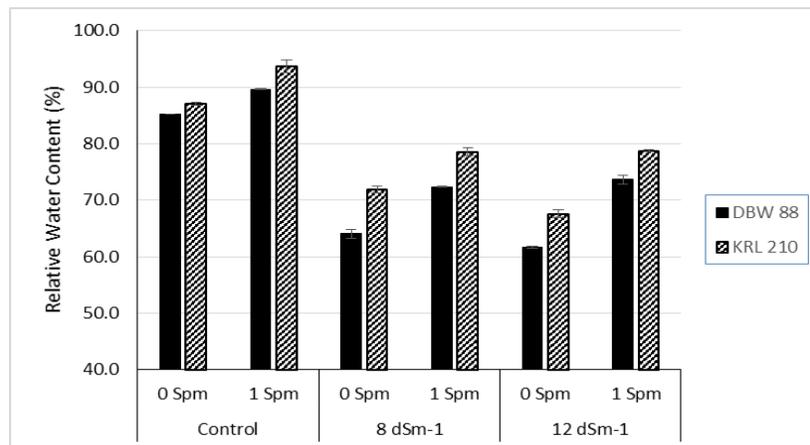


Fig.2 Effect of spermine at 21 DAS on leaf membrane injury in wheat flag leaf under salt stress

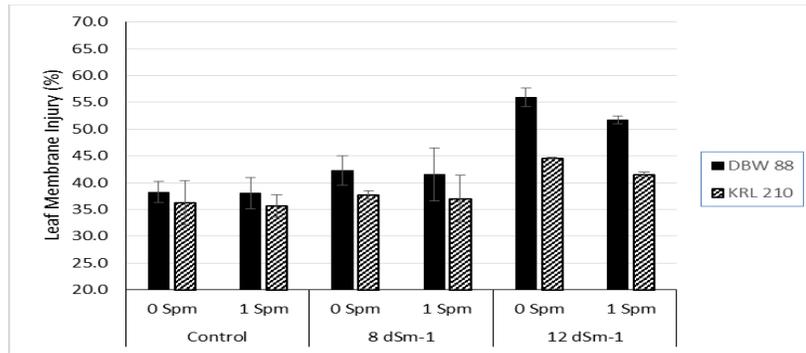


Fig.3 Effect of spermine at 21 DAS on osmotic potential in wheat flag leaf under salt stress

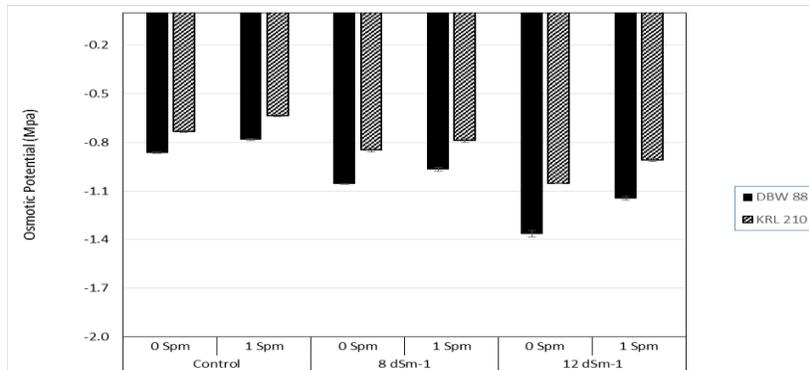
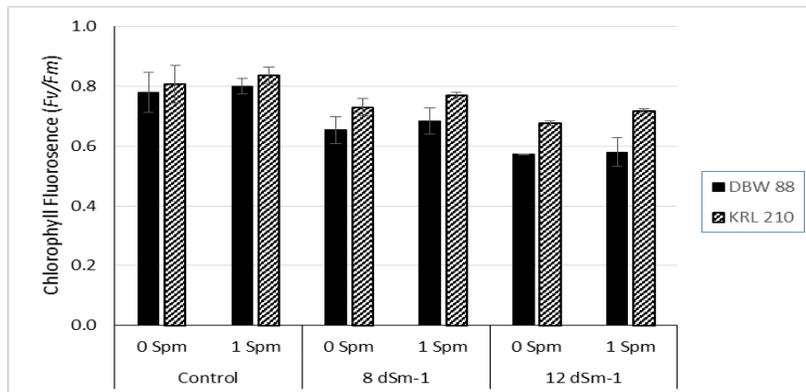


Fig.4 Effect of spermine at 21 DAS on chlorophyll fluorescence in wheat flag leaf under salt stress



The osmotic potential in wheat leaves decreased (more negative) by the influence of salinity stress at 21 DAS (Fig. 3). The highest mean osmotic potential was obtained in plants treated with spermine in control (-0.71 MPa). The saline treatment decreased the osmotic

potential in both DBW 88 and KRL 210 but was more negative in DBW 88 under 12 dSm⁻¹ (-1.36 MPa). The application of spermine in control and salt stressed plants maintained higher levels of osmotic potential in all the varieties. The maximum

photochemical efficiency of photosystem II (F_v/F_m) in wheat plants exposed to various levels of salinity treatments at 21 DAS is represented (Fig. 4). The mean values of F_v/F_m was reduced under salt stress from 0.794 to 0.692, 0.624 in 8 and 12 dSm⁻¹ respectively. The maximum efficiency of photosystem II was observed in KRL 210 treated with spermine under control conditions and more damage in photosystem II under salt stress was measured in DBW 88 at 12 dSm⁻¹ ($F_v/F_m = 0.573$). Spermine application was found effective to increase the F_v/F_m values when compared with non-spermine under salt stressed wheat varieties.

The relative water content of leaves is regarded as an important indicator of plant physiological activities and a better measure of plant water status. In this study, KRL 210 was found to maintain its RWC relatively higher than DBW 88 (Fig. 1). However, RWC in leaves decreased with increasing levels of salinity and more reduction in RWC was observed in DBW 88. Results on the decline in RWC with salt stress are in accordance with those of rice (Khan and Panda, 2008) and maize (Rohanipoor *et al.*, 2013). Further, Nahar *et al.*, (2016) also showed that exogenous PAs application to salt-stressed mung bean resulted in an increase in leaf RWC. The increase in the leaf membrane injury can disrupt water, ion, and organic solute movement across plant membranes, thus affecting carbon production, transport and accumulation (Mohammed and Tarpley, 2010). The leaf membrane injury increased with increasing levels of salinity and pronounced more in salt sensitive varieties *i.e.*, DBW 88 as compared with tolerant varieties KRL 210 (Fig. 2). However, Spm treated leaves maintained lower levels of leaf membrane injury when compared with untreated leaves. Similarly, polyamines application alleviated drought effects by substantially reducing the membrane

permeability (Farooq *et al.*, 2009). The decrease in the plant growth and development under salinity is explained by the decrease of osmotic potential in plant cells due to increasing salt concentration in the soil and consequently decreasing water potential (Akça and Samsunlu, 2012). As is evident from the results obtained during the present investigation, the osmotic potential of wheat leaves decreased (became more negative) by the influence of salinity. Leaf osmotic potential was more negative in DBW 88 than KRL 210. The reduction in osmotic potential in salt-stressed plants mainly occurs due to the accumulation of inorganic ions (Hasegawa *et al.*, 2000) limiting the availability of water to plants. Farooq *et al.*, (2009) showed that exogenously applied PAs substantially improved the drought-induced decrease of RWC and osmotic potential in rice. Results presented in Fig. 4 show that the maximum photochemical efficiency of photosystem II (F_v/F_m) in wheat plants decreased with increasing levels of salinity at 21 DAS. Similarly, a decrease in maximum photochemical efficiency of photosystem II under salt stress was reported in wheat cultivars (Khan *et al.*, 2012). However, higher values for F_v/F_m was maintained in KRL 210 under salinity when compared with salt susceptible varieties.

The results obtained in the present investigation indicate that salt tolerance in KRL 210 may be due to improved RWC, Leaf membrane injury, osmotic potential and chlorophyll fluorescence. In addition, the exogenous spermine treatment may be associated with the capacity to tolerate under salt stress in wheat.

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