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Physiological studies on effect of chitosan nanoemulsion in pearl millet under drought condition

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Abstract
An experiment was conducted to study the effect of chitosan nanoemulsion in pearl millet under water deficit condition. Foliar spray of 0.1% chitosan nanoemulsion along with control was done at flowering stage under moisture stress condition. Physiological traits on gas exchange parameters (photosynthetic rate, stomatal conductance, transpiration rate and leaf temperature), chlorophyll index, relative water content and yield attributes were recorded after foliar spraying of chitosan. The results showed that the treatment with chitosan causes reduction in stomatal conductance thereby limiting the photosynthesis, transpiration rate and raise in leaf temperature than unsprayed plants. The yield was also significantly increased in treated plants than control under drought condition. Hence, foliar spray of chitosan plays an important role in alleviating the harmful effects of water stress by improving the plant water status and yield.

Keywords: Pearl millet, chitosan, gas exchange parameters, yield, water deficit

Introduction

Millets are one of the most important agricultural crops due to the presence of higher nutritional content than other cereals like wheat, rice or corn. Millets are called as "Nutri-Cereals" because of its rich iron, protein content, antioxidants, gluten-free nature and low glycaemic index, which helps to prevent or manage diabetes (Rao *et al*., 2017) [18]. India is one of the leading producers of millets in the world and about 50% of the global millets production is contributed by pearl millet (ICRISAT, 2016) [8]. Pearl millet also known as poor man's crop is one of the most important cereal crop grown largely in India next to rice, wheat, maize, and sorghum (Lata *et al*., 2013) [12]. It is cultivated in arid and semi-arid tropics were the rainfall is about 150-700 mm (Patil, 2010) [16]. In India, pearl millet is grown in 7.45 million hectares with production of 9.73 million tonnes and average productivity of 1305 kg ha^{-1} (Indiastat, 2016-17) [7]. The pearl millet growing states in India are Rajasthan, Gujarat, Uttra Pradesh, Harayana and Maharastra (Yadav and Rai, 2013) [21].

Pearl millet has 11.6% crude protein, 5.0% fat, 67.5% carbohydrate, 1.2% crude fiber, and 2.3% minerals (Rai *et al*., 2008) [17]. Pearl millet production in India declined by about 6.2% during 2016 – 2018 due to adverse environmental factors like drought. Drought stress is one of the major abiotic stresses that affect the crop production worldwide, which is characterized by reduction of water content, decline in leaf water potential and turgor loss, stomatal closure and decrease in cell enlargement and growth and other physiological process like activation of reactive oxygen species and lipid peroxidation (Ghatak *et al*., 2016) [6]. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel *et al*., 2008) [10]. In pearl millet under drought condition, all the above mentioned plant process gets affected so in order to maintain the water status adaptive traits like osmotic regulation helps the plant to maintain turgidity of cell under severe moisture stress (Shao *et al*., 2008) [20]. For mitigating the drought that occurs during crop growth of pearl millet, many compounds, formulations and other nutrients are applied for the plants to thrive under dehydration stress condition. Chitosan is a natural polymer, that can be used to mitigate the water stress in pearl millet which is derived from chitin by alkaline deacetylation process, obtained from exoskeleton of insects, crustaceans shells such as prawns, lobster, fish, crab, shrimp and fungal cell walls. Chitosan is biocompatible, eco-friendly and non-hazardous polymers. Chitosan has a wide variety of applications in biotic and abiotic stress management strategies. Foliar application of chitosan reduces the stomatal conductance, decreases the transpiration and improves the water use efficiency by acting as an antitranspirant compound by promoting the synthesis of jasmonic acid by influencing the water use of plants as abscisic acid results in stomatal closure has been reported (Iriti *et al*., 2009, Bittelli *et al*., 2001) [9, 4]. The uncertainty of rainfall generally leads to poor grain filling or complete failure of the pearl millet. Grain filling is a major problem in pearl millet during terminal drought stress condition. Due to this constraint, the farmers are facing more yield loss. With this background, the objective was framed to study the effect of chitosan nanoemulsion in pearl millet under drought condition.

Materials and methods

The pot culture experiment was conducted in the glass house of the Department of Crop Physiology 11.01°N latitude, 76.39°E longitude and 426.7 MSL, Tamil Nadu Agricultural University, Coimbatore during 2018-2019. The pearl millet (ICMR 102187) seeds for the experiment were obtained from International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad. Chitosan nanoemulsion was obtained from Department of Nano Science and Technology, TNAU, Coimbatore. The treatments include control and spray of 0.1% chitosan nanoemulsion under drought condition. Drought was imposed by withholding irrigation at flowering stage. The treatment was imposed at flowering stage and every day the soil moisture data were recorded by using Delta devices Theta Probe moisture meter. When the percent reduction of soil moisture reaches above 20%, spraying of chitosan nanoemulsion was done and soil moisture data were recorded up to rewatering. The observations were recorded on 1, 3, 5 and 7 days after foliar spray of nanoemulsion with percent reduction in moisture content of 26.4, 35.8, 45.3 and 55.7 respectively.

Gas exchange parameters was measured from young and fully expanded leaves using a portable photosynthetic system (PPS) (LI-6400 XT; LI-COR Inc. Lincoln, Nebraska, USA) and the units were expressed as: photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}) and leaf temperature (°C). The relative water content (RWC) was estimated according to Barrs and Weatherley (1962) [3] and calculated by using the following formula and expressed in percentage. RWC (%) = (Fresh weight - dry weight) / (Turgid weight - dry weight) x 100. Chlorophyll index in leaves was measured using chlorophyll meter from Minolta (Model SPAD 502 of Minolta Co., Japan) was used to measure SPAD values. After reaching the physiological maturity, ear heads were harvested. The length of earhead, earhead weight, grain yield/plant and 1000 grain weight were measured. The data were analysed statistically by adopting Factorial Completely Randomized Design (FCRD) in which mean and standard error were computed.

Results and Discussion

Results on gas exchange parameters showed significant difference between control and chitosan spray (Fig.1). On first day after foliar application of chitosan under water deficit condition, the photosynthetic rate, stomatal conductance and transpiration rate were decreased by 4.89%, 6.75% and 7.97% over untreated plants, respectively. Under low moisture stress, there was a slight decline in transpiration rate and stomatal conductance up to five days, after which rapid reduction were observed. The photosynthetic rate decreased and the decline in rate of photosynthesis was initially more rapid, but persisted significantly higher than control, from about five days to till the completion of the moisture stress period. Leaf temperature was increased under drought over the days in both the treatments, but the increment was higher in treated plants up to five days, afterwards, there was a decline in temperature was noticed.

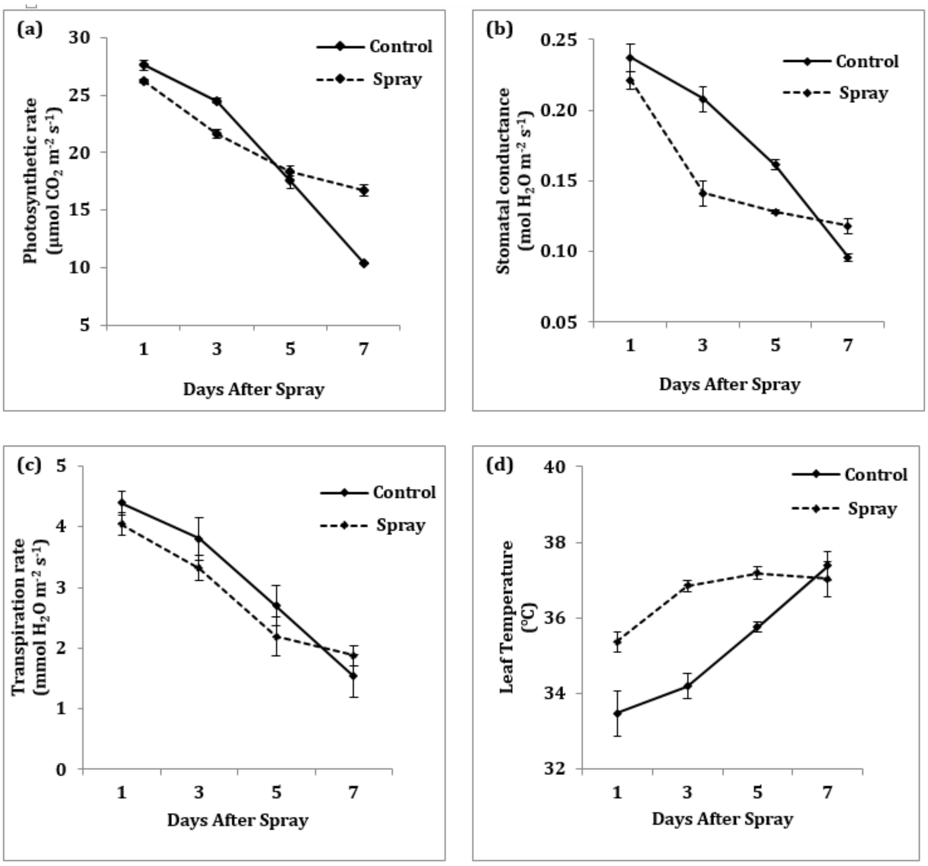

Fig 1: Effect of foliar application of chitosan nanoemulsion on (a) photosynthetic rate, (b) Stomatal conductance, (c) transpiration rate and (d) leaf temperature in pearl millet under drought condition

Chitosan treatment significantly decreased the water loss from plants by regulating the stomatal resistance to water vapour diffusion. Since, stomata play a major role in carbon dioxide intake and loss of water vapour, thereby causing reduction in photosynthetic rate was observed. The closure of stomata through ABA is up regulated by chitosan. Decrease in transpiration leads to increase in the leaf temperature due to stomatal closure (Khan *et al*., 2002 [11]; Abdullah *et al*., 2015 [1]; Del Amor *et al*., 2010 [5]; Ludwig *et al*., 2010 [14]).

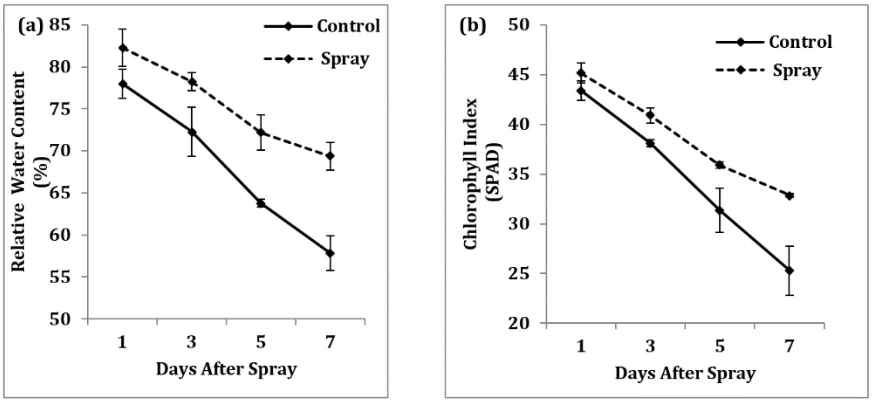

Fig 2: Effect of foliar application of chitosan nanoemulsion on (a) relative water content and (b) chlorophyll index in pearl millet under drought condition

Relative water content was an important parameter which reflects the leaf water status of the plants (Fig.2a). Under stress, there was a drastic reduction in relative water content. The RWC of control and treated plants was about 78% and 82.27% on first day and 57.87% and 69.35% on seventh day after imposition of treatments. Chitosan sprayed plants have higher leaf water status than unsprayed plants under drought condition. Similar results were obtained by Del Amor *et al*. (2010) [5] who found that the RWC was improved by 18.6 per cent over unsprayed capsicum plants under drought condition.

Table 1: Effect of foliar application of chitosan nanoemulsion on yield attributes in pearl millet under drought condition

Treatments	Earhead Length (cm)	Earhead Weight (g)	Grain Yield (g/plant)	1000 Grain Weight (g)
Control	12.8 ± 0.27	3.6 ± 0.29	1.9 ± 0.26	4.4 ± 0.12
Spray	13.1 ± 0.31	9.3 ± 0.40	7.2 ± 0.40	11.9 ± 0.23

Values in the table are denoted by mean and error bars are ±SD were made using Excel.

The results on chlorophyll index showed that there was a significant difference among the treatments (Fig.2b). The decrease in SPAD values has been observed in both control and chitosan sprayed plants. But the reduction was higher in control plants than treated plants. The percentage increase was about 14.5% and 30% on fifth and seventh days after spray. Salachna and Zawadzinska (2014) [19] reported that application of 0.5% chitosan to freesia plants increased the chlorophyll index in treated plants than control plants.

Among the treatments, the maximum yield was recorded in chitosan treated plants than control plants under drought condition (Table 1). There was no significant difference in earhead length in both the treatments, but the data on earhead weight, grain yield and 1000 grain weight showed significant difference. The grain yield per plant decreased when the plants are exposed to drought during flowering stage. The plants which are sprayed with chitosan recorded maximum grain yield (7.2 g) under drought condition when compared with control (1.9 g) plants. It was observed that flowering stage were severely affected by drought. Our results confirmed by the earlier findings of the Mondal *et al*. (2012) [15], Abu-Muriefah (2013) [2] and Zeng and Luo (2012) [22] in okra, common bean and wheat plants respectively. Hence, in the present study, foliar spray of chitosan had significant effect on physiological traits, particularly; it reduced the transpiration rate and thereby improving the plant water status as well as yield attributes under drought condition.

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