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Effects of chemicals on water stress alleviation in black pepper (*Piper nigrum* L.) rooted cuttings

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ABSTRACT

In the era of climate change, water stress is a major limiting factor in black pepper, as it is highly sensitive to water deficit stress especially during summer, which can lead to greater production losses.Therefore, strategies aimed at enhancing water deficit tolerance in black pepper is crucial to stabilize production and productivity. Water deficit alleviating chemicals improves tolerance in plants; however, its mechanism in black pepper rooted cuttings under water deficit stress is yet to be explored. Therefore, we conducted a two pot culture experiment during March and April, 2022 using the variety Panniyur-1 to investigate the effects of alleviating chemicals with six treatments viz., T_1 - Well-Watered (WW), T_2 - Water stress (WS), T_3 -WS + foliar spray chitosan (25 ppm), T_4 - WS + foliar spray of lime (1%), T_5 – foliar spray of Potassium silicate (0.5%) + Calcium chloride (0.5%), T_6 - WS + foliar spray of Melatonin (50 µM) on the recovery per cent, leaf relative water content, photosynthetic gas exchange, chlorophyll fluorescence, canopy temperature and proline accumulation in black pepper leaves under water deficit stress. In general, the application of exogenous stress alleviating chemicals significantly relieved the inhibitory effects of water deficit stress on leaves. Especially, the melatonin enhanced tolerance attributed to improved leaf water content, photosynthetic activity, reduced canopy temperature, and ultimately improved the recovery percent of rooted cuttings under water deficit stress. After rehydration, melatonin-treated plants recovered more quickly than untreated plants. In addition, melatonin counteracted the water stress induced accumulation in proline content. Overall, the results of this study demonstrated that melatonin at 50 µM L^{-1} (T6) significantly alleviated the adverse effects of water deficit stress on the black pepper plants compared to other stress alleviating chemicals.

Key words: *Black pepper, Water stress-alleviating chemicals, Melatonin*

Introduction

Among the various abiotic stresses encountered by plants, water deficit is considered to be the major one, limiting crop productivity to a great extent (Fedoroff *et al*., 2010). In several plant species, moisture stress is found to disturb the photosynthetic rate by damaging the photosynthetic apparatus and significantly reducing the chlorophyll pigments. Plants have various mechanisms to combat the water deficit tolerance, few of the physiological ways are i) accumulation of proline, ii) maintaining water content in leaves, iii) stable photosystem and so on (George *et al*., 2017). Black pepper (*Piper nigrum* L) in general is grown as rainfed crop which needs a well distributed rainfall of 2000-3000 mm for attaining

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optimum productivity. Black pepper productivity is severely affected by water deficit stress especially during summer (Prasada Rao, 2016). Changes in rainfall pattern and intensity severely affects the black pepper plantations in vast majority areas. Lack of physiological interventions in water stress management is a limiting factor in black pepper which remains to be one of the most important constraint in black pepper productivity. Therefore, physiological interventions is the need of the hour in enhancing water deficit tolerance of black pepper. There are several water stress alleviating chemicals tested in horticultural crops like lime, potassium silicates, calcium chloride, chitosan and melatonin which is found to improve the stress tolerance in plants (Ye *et al*., 2016); however, the effect of these chemicals in black pepper rooted cuttings and the mechanism thereof remains unknown. This study was aimed to identify the effective chemicals for alleviating water stress tolerance in black pepper and to explore the physiological traits conferring tolerance.

Materials and Methods

Experimental design and treatment

The experiment was conducted with 4 months old rooted black pepper cuttings by using variety Panniyur-1 under pot culture during March, 2022 at ICAR-Indian Institute of Spices Research, Experimental farm. Peruvannamuzhi, Kerala, India. The experimental pots containing potting mixture (top soil, sand and FYM 2:1:1, enriched with bio-control agents) and plants were placed in a protected condition during the entire period of experiment.

The water stress alleviating chemicals were sprayed under withheld irrigation. The experiment included six treatments: T_1 - Well-Watered (WW), T_2 - Water stress (WS), T_3-WS + foliar spray chitosan (25 ppm), T_4- WS + foliar spray of lime (1%), T_5– foliar spray of Potassium silicate (0.5%) + Calcium chloride (0.5%), T_6- WS + foliar spray of Melatonin (50 µM). Water stress was imposed by withholding irrigation until the soil moisture content reached 30-35 % field capacity (FC) for 14 days. Soil moisture content was measured using gravity method. On the 14th day of water stress treatment, the physiological parameters like relative water content, net photosynthetic rate, stomatal conductance, transpiration rate and proline accumulation were measured on the tagged leaves and after 7 days of subsequent rehydration, the recovery per cent was measured.

Determination of recovery per cent

The total plant recovery was determined by counting the total number of plants survived through the treatment by the actual number of plants per treatment. Plant recovery per cent = (No. of plants survived/Total No. of treated plants) x 100.

Estimation of Leaf and Soil water content

Leaf Relative Water Content (LRWC) was measured following the method of Weatherley (1950). After plucking leaf samples, fresh weight (FW) of the sample was recorded on an electronic scale. For the measurement of turgid weight (TW), the leaves were submerged in distilled water for 24 hours and weighed. Then, the leaf samples were oven dried at 80°C for 72 h to find out their dry weight (DW). The LRWC was determined as follows: LRWC (%) = (FW-DW)/(TW-DW)×100. Soil water content (SWC, ù %) was measured by collecting soil from all the pots in the same treatment and taken 100 g (Wet weight, Ww) from the pooled sample for analysis. Soil sample was dried at 90^{o}C for 24 h and taken the dry weight (Dw). The SWC was calculated as follows: ù(%)={(Ww-Dw)/Dw} × 100%.

Measurement of leaf gas exchange and chlorophyll fluorescence

The net photosynthesis rate (A, ìmol m^{-2} s^{-1}) was measured in the control and treated plantsleaves with a portable photosynthetic system (LCpro-SD Advanced Photosynthesis Measurement System, England) at from 9:00-10:30 AM. Chlorophyll fluorescence was measured using chlorophyll fluorometer (Os-30p) in 10-15 minutes dark adapted leaves. The maximum PS II quantum yield (F_v/F_m) was determined in dark-adapted leaves 9:00-10:30 AM local time according to Strasser *et al.* (2000). All measurements were performed on the attached fully expanded leaves with five replicates.

Observation of canopy temperature (*CT*) and proline content

Leaf canopy temperature was measured using infrared thermometer (Fluke 568 IR Thermometer) between 9.00 AM to 10.30 AM. The IR thermometer was placed about an inch from the select adaxial leaf surface and the temperature (in $^{\circ}$C) was recorded. Proline content of the leaf sample was estimated by the method of Bates *et al*. (1973) and expressed as µg

g^{-1} of fresh weight.

Statistical analysis

The experiments were performed in a completely randomized design with six replicates. The data were analysed using the WASP-Web Agri Stat Package 2.0 program. Statistical variance analysis was performed using ANOVA and compared with least significant differences (LSD) at 5% and 1% level.

Results and Discussion

The foliar application of water stress alleviating chemicals significantly relieved the inhibitory effects of water deficit stress on black pepperleaves in various significant levels. After rehydration for 7 days, the treatments (T5) Potassium silicate (0.5%) + Calcium chloride (0.5%) foliar sprayed plants had recovered to the normal levels up to 60 % and also the treatment (T3) WS+Chitosan foliar spray (25 ppm) showed the similar recovery per cent as T5. But, the treatment (T6) WS + Melatonin (50 µM) foliar sprayed plants showed 80 % recovery and also exhibited faster recovery (Fig. 1A). Water stressed plants without chemical treatment (T2) showed very less recovery per cent (33%) due to death of plants at the end of the experiment even after the recovery irrigation. Meng *et al.*, 2014 reported that melatonin application results in the reduction of leaf osmotic potential, thereby preventing water loss through/ from leaves by improving the thickness of cuticle and spongy tissues. Ye *et al.*, 2015 and Cui *et al.*, 2017 demonstrated the role of exogenous application of melatonin in maintaining the leaf water content, resulting in the regulation of the plant water status under water stress conditio thus improving the plant stress tolerance and recovery ability.In present study, plants treated with melatonin (T6) showed a significant improvement (84.9%) in relative water contentcompared to water stressed plants (61.8%) (Fig. 1B).

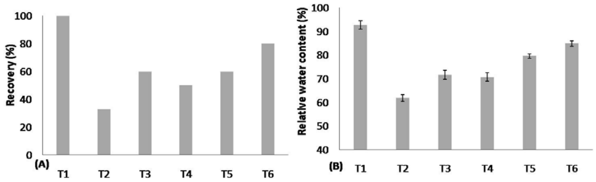

Fig. 1. Effect of foliar spray of antitranspirants on (A) Recovery per cent and (B) Relative water content in black pepper rooted cuttings

Water stress directly affects the structure and functional activity of chloroplast in plant leaves as well as affects the efficiency of photosystem II photochemical reactions (Fracheboud and Leipner, 2003). In present study, waterdeficit stress significantly reduced the net photosynthetic rate (*Pn*) in water stressed plants (0.337 µmol m^{-2} s^{-1}) compared to well-watered plants (2.22 µmol m^{-2} s^{-1}). However, the application of melatonin (T6) partly reversed the reduction in net photosynthetic rate (1.629 µmol m^{-2} s^{-1}) compared to other treatments (Fig. 2 A). Thus, exogenous melatonin application could alleviate the influence of water deficit stress on photosynthesis in black pepper by improving the net photosynthetic rate to ensure the normal growth and development of black pepper. Canopy temperature depression was found to be robustly associated with photosynthetic gas exchange (Mason *et al.*, 2013; Rebetzke *et al.*, 2012). In present study, also explained that melatonin treated plants maintained low CT led to a higher photosynthetic rate under water stressed condition.

Water deficit affected chlorophyll fluorescence (Fv/Fm) water stressed plants, causing the curve to significantly decline (Fig. 2B). In contrast, antitranspirants treatments ameliorated the water deficit stress effect, resulting in increasing the Fv/ Fm value. Melatonin showed a positive effect on chlorophyll florescence in water-stressed coffee plants as evidenced by a markedly high F_V/F_M ratio (Liu *et al.,* 2016; Cherono *et al.*, 2021). In our study, a significant increase in the chlorophyll fluorescence suggests that melatonin ameliorates the adverse effect of water deficit in black pepper plants.

Canopy temperature among all treatments ranged from a minimum of 31.2 °C to a maximum of 32.4 °C. The plants treated with melatonin maintained significantly lowest canopy temperature compared to other treatments, while water stressed plants (T2) showed the highest canopy temperature (32.4 °C) (Fig. 2), which indicate melatonin could reflect their ability to maintain the balance between the water loss through transpiration and water absorption through roots.

Moreover, antitranspirants improved water stress tolerance by enhancing the accumulation of compatible osmolytes like proline (Alagupalamuthirsolai *et al.*, 2023), as well as reducing chlorophyll degradation which leads to maintaining net photosynthetic activity (Huang *et al.*, 2019). In the present study, proline content was significantly decreased in all the

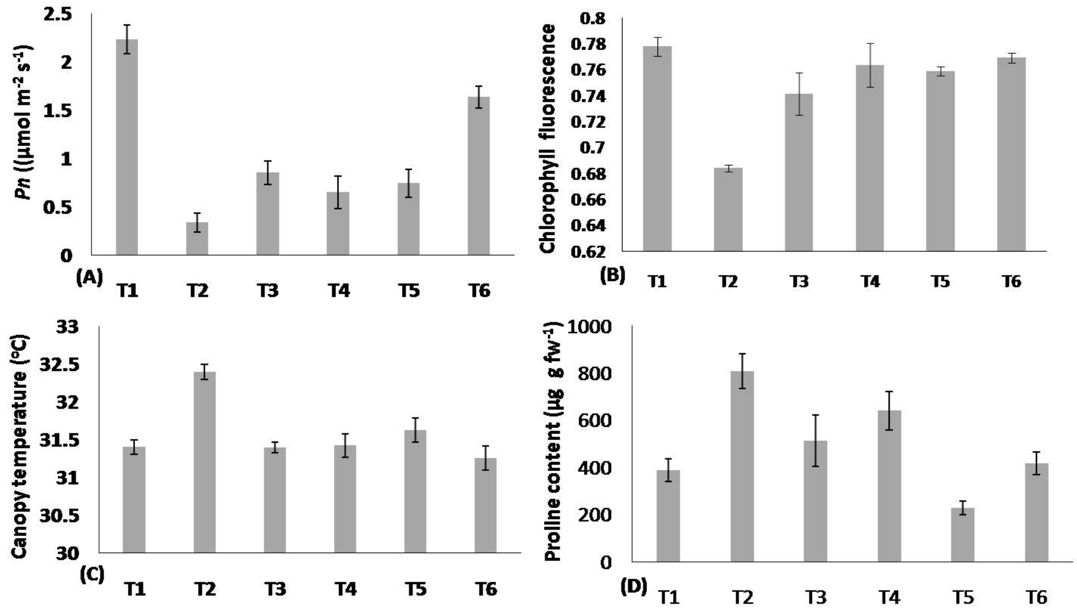

Fig. 2. Effect of foliar spray of antitranspirants on (A) Net photosynthetic rate, (B) Chlorophyll fluorescence, (C) Canopy temperature and (D) Proline content in black pepper rooted cuttings.

antitranspirants treated plants compared to water stressed plants (Fig. 2D). Moreover, the plants treated with melatonin (T6) showed highly significant decrease in proline content (415.5 µg g fw^{-1}) compared to water stressed plants (389.15 µg g fw^{-1}) and on par with the well watered plants (T1). These results showed that antitranspirants, especially melatonin treated plants counteracted the water stress induced accumulation in proline content.

Conclusion

The data presented in this study demonstrated that melatonin at 50 µM L^{-1} concentrations significantly alleviated the adverse effects of water deficit stress on the black pepper plants compared to other antitranspirants chemicals. The results revealed that exogenous melatonin application could improve water stress tolerance by maintaining the accumulation of compatible osmolytes like proline and reducing chlorophyll degradation which leads to maintaining higher net photosynthetic activityand efficiency of photosystem II under water stress. However, further studies are necessary to standardise the concentration of melatonin for better recovery of black pepper plants under water deficit stress and conferring its tolerance mechanism.

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