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Original article

Taifi rose extract improves the growth and physiology of cowpea seedling stage under drought stress

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ABSTRACT

Drought stress is the most significant environmental stress factor affecting the growth of crops and farming sector in the present era. This study was carried out mainly in the rose water extracted from the essential oil of Taif rose (*Rosa damascena* "Trigintipetala"), which was explored for drought stress tolerance from industry. The aim of this study was to investigate how Taif rose water (RW) pretreatment affected cowpea (*Vigna unguiculata* var. California blackeye NO.46) seedlings under drought stress (65% and 35% of filed capacity). The cowpea seeds are immersed in rose water with 15%, 25% and 35% for a couple of hours before being implanted. Drought stress dramatically reduces morphological traits of root and shoot length, fresh and dry biomass and leaf area. However, pretreatment of RW seed dramatically improves cowpea growth conditions, relative water content, photosynthetic rate, and transpiration rate. However, under drought stress, RW pretreated seedlings showed a decreased levels in malondialdehyde (MDA), proline, total soluble proteins and carbohydrates. However, drought stress increases the quality of antioxidant enzyme activities like superoxide dismutase and catalase in pretreated RW cowpea seedlings. This study concludes that pretreating with RW could increase the drought tolerance in cowpea seedlings by stretching their antioxidant defense mechanisms. In addition, this study holds the potential to play a vital role in ensuring food security in the near future, as it provides valuable insights into improving crop resilience and productivity under drought conditions.

1. Introduction

Cowpea (*Vigna unguiculata L. Walp*) is a very famous legume crop which is formed initially in Africa and furtherly extended to global world for using as one of the diets by humans. It takes up a limited area for cultivation and has a much lower yield than cereals. The prediction for cowpea production assumed a global coverage of around 14 million hectares by 2025. It belongs to Fabaceae family and it is well-known for warm season, as a vascular annual pulse crop (Carvoalho et al 2019; Alexandre et al 2016). Cowpeas are essential in the nourishment of the impoverished since they are a reasonably cheap source of substantial protein (Carvoalho et al 2019). Thus, it is a critical issue for research in Saudi Arabia due to its financial and dietary value.

\Environmental variables known as abiotic stressors may have negative effects on agricultural yields (Jahan et al., 2021a). Agricultural plants alter their morphology, cellular structure, physiological function, biochemical and molecular level responses in response to abiotic stressors (Jahan et al., 2021b). Among abiotic stressors, drought is a significant constraint on vegetable crops, impairing its efficiency and threatening its yield. Oxidative stress, characterized by the excessive production of activated atmospheric oxygen (O_2), also known as reactive oxygen species (ROS), can be brought on by both climate change and the resulting drought stress (Choudhury et al., 2017; Alabdallah et al., 2021; Hasan et al., 2021a; Hasan et al., 2021b). Possible side effects of ROS generation include oxidative stress and lipid peroxidation, both of which can cause cell death (Alabdallah and Hasan, 2021; Jumrani and Bhatia, 2019). Antioxidants play an important part in keeping ROS production and removal in cells at a steady equilibrium (Hasan et al., 2021c; Hasan et al., 2023). In plants, the removal of excess ROS via a variety of antioxidant defense after systems plays a crucial role

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in the evolution of drought tolerance, and it also aids in maintaining the cell's full metabolic function during drought (Rahman et al., 2022). Increase in proline and sugar levels during the adaptation process is possible because these compounds contribute significantly in the osmoregulation, the redox balance, and the scavenging of ROS under stressful conditions (Krasensky and Jonak, 2012). It has been demonstrated that drought stress causes changes in the morpho-physiological features of plants as well as the composition of their chlorophyll content (Jaleel et al., 2009). As a result, it appears that drought stress is implicated in the loss in photosynthetic efficiency in crops (Ashraf and Harris, 2013).

Several studies have demonstrated that extracts from leaves and flowers can help plants recover from stressful situations by resetting their physiological systems to normal (Basra et al., 2011; Khan et al., 2020). In light of rose's usefulness as a natural chemical and effective growth stimulant, it has been the subject of numerous studies recently (Nadeak et al., 2021; Tatke et al., 2015). The use of RW has the potential to improve plant tolerance to drought stress by lowering oxidative stress. The impacts of phytohormones and other plant extracts on plants have been extensively researched; However, pretreatment RW's impacts on plants under drought stress have not been adequately explored. The objective of this study was to examine the impact of RW on antioxidant enzymes and biochemical responses in cowpea plants exposed to various levels of drought stress. By understanding how RW affects antioxidant enzymes and biochemical responses in cowpea plants subjected to varying degrees of drought stress, we could develop an innovative agricultural practices and sustainable farming methods to boost cowpea yields and mitigate the impacts of drought stress on food production, thereby securing adequate food supplies for an ever-growing global population. Therefore, in our research, we investigated the following: cowpea's physiological reactions to drought, including its relative water content (RWC), proline content, total soluble carbohydrate, and antioxidant enzymes, and its morphological responses to RW treatment.

2. Materials and procedures

2.1. Extract by the traditional technology and phytochemical analysis of waste rose water

Roses from the Al Qurashi Rose Industry in Al Hada, Taif, Saudi Arabia (Rosa damascina var. trigintipetala) were gathered. The traditional technology as depicted in Fig. 1, includes a copper distillation system with a volume of 70–100 L. Cooling process is achieved by a single inclined pipe moving inside a separated water bath and then reaching outside connected with 20–25 L glass bottles. The copper system consumes 8–10 Kg of rose flowers (24000–30,000 flowers), which are first pressed by hands and then rose water added to them. The system is tightly closed by sticky tape. The mix is heated by a gas source (propane and butane) for 12–16 h at the boiling stage. The first period of heating is about 40 min for 100 °C after that the temperature gradually decreases to become 50–70 °C. Limited rose oil factories in the region utilize firewood as a source for heating the distillation system. The arising water vapor passes through the pipe and then condenses forming the distillate. Finally, the resultant products gather in the glass bottle, in which the oil floats at the top. Samples from rose water residue in the copper container have been collected and cooled in a refrigerator for the study. Phytochemical analysis of obtained samples of Taif rose water waste presented in Table 1.

2.2. Plants and experimental treatments

Research was conducted in a greenhouse on the campus of Imam Abdulrahman Bin Faisal University in Saudi Arabia. Seeds of cowpea (*Vigna unguiculata* var. California blackeye NO.46) were obtained in Altuajri, Saudi Arabia. Seeds were immersed in 0%, 15%, 25%, and 35% RW solutions for 2 h under optimum temperature (22 °C) conditions for the rose water (RW) treatments before planting. For the purpose of germination and subsequent growth, cowpea seeds were planted in equal amounts of mixture of soil in plastic containers (2 kg). Seedlings were watered twice weekly until saturation before receiving drought remedies. To simulate drought conditions, cowpea seedlings were exposed to several watering schedules (control at 85% FC, 65% FC, and 35% FC). Constant weighing of the pot ensured that the field capacity (FC) remained constant. RW was applied two times to both drought-stressed and control plants before conducting morphological and physiological measurements. The plants had been grown for a period of 35 days before they were exposed to drought conditions. Following 35 days, morphological and physiological measurements (such as photosynthetic and transpiration rate) were taken, and after harvesting period (45 days), biochemical parameters were analyzed. While the daytime and overnight temperatures were always around 22°/16° C, the relative humidity (RH) ranged from 60% to 70%. After being washed in double-distilled water to remove any remaining traces of sand, the cowpea seedlings' branches and roots were separated. The analytical balance (HR-200) was used to calculate the fresh and dry weights of the shoots and roots, and the results are shown in grams (gm). A portable leaf area meter was used to calculate the total leaf area of cowpea seedlings (LI-3000C).

2.3. Measurements of relative water content

Randomly chosen leaves were employed to obtain three measurements from each treatment. Fresh weight (FW) was determined by weighing leaf discs that were taken from cowpea seedlings. After 4 h, the turgid weight (TW) of the leaf discs was measured. Afterwards, the discs of leaves were dried in an oven at 75 °C (DW). The RWC % was determined using the following formula.

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

2.4. Determination of gas exchange parameters

Gas exchange measurements (photosynthesis (Pn) and transpiration (E) were taken solely from mature, healthy cowpea leaves. The leaf was measured under ambient conditions between 12:00 and 14:00. A CIRAS 3 portable photosynthesis system (PP Systems, Amesbury, Massachusetts, USA) was used to measure photosynthesis (Pn) and transpiration (E). The relative humidity in the leaf chamber was 62.8%, and the light intensity was 1200 mol m^{-2} s^{-1} 400 ppm CO_2 was used as the condition in the CIRAS-3 leaf chamber.

2.5. Determination of proline

In this study Proline determination was studied completely using spectrophotometer as described by the documented study (Bates et al 1973).

2.6. Determination of total soluble proteins (TSP)

The concentration of TSP was completely followed by the old method of Bradford (1976).

2.7. Total soluble carbohydrates (TSC) determination

TSC concentration was determined by crushing 500 mg of leaf sample with 5 mL of 95% ethanol. Three milliliters of an anthrone

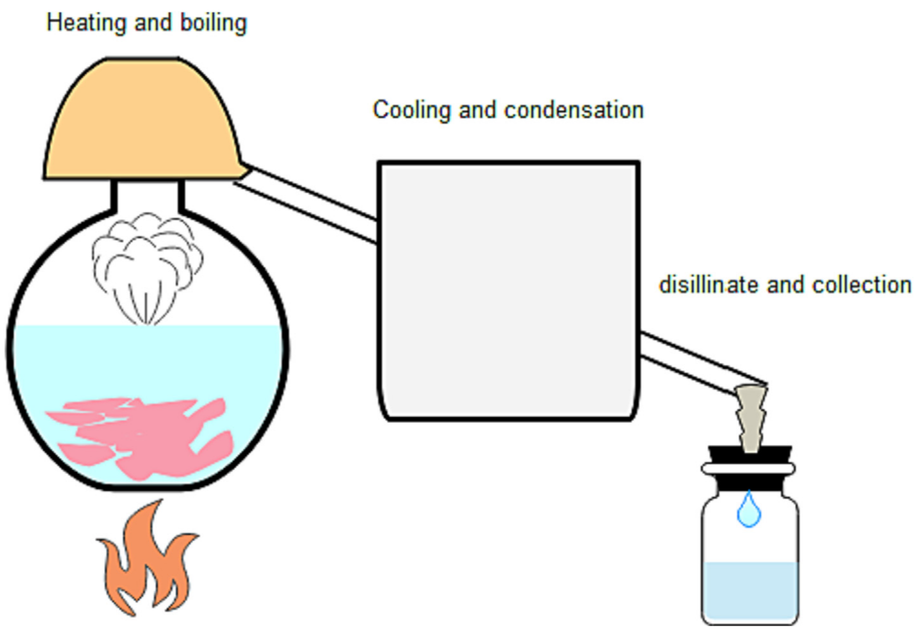

Fig. 1. Schematic view of Taif rose traditional distillation technology.

Table 1
Phytochemical analysis of taif rose water.

Chemicals	Concentration
Phenol	0.58 ppm
Styrene	0.36 ppm
Toluene	0. 12 ppm
Mesitylene	0.15 ppm
Volatile Organic Compounds	0.23 ppm

mixture (72% sulphuric acid (100 ml) and 150 mg of anthrone) were added to 100 ml of the alcoholic extract. After that, the samples spent 15 min in a hot water bath. The TSC was determined by measuring the absorbance of the samples at 625 nm against a glucose standard curve (Wardlaw and Willenbrink,1994).

2.8. MDA concentration determination

Determination of MDA concentration was estimated in this study based on Health and Packers et al. (1968).

2.9. Estimation of antioxidant enzyme activity

In this study, this technique was completely following the previous published study (Mukherjee and Chaudhary et al., 1983).

2.9.1. Analysis of SOD activity

The complete protocol for SOD activity was followed from Hasanuzzaman et al. studies (2011) using a spectrophotometer.

2.9.2. Catalase (CAT) activity measurement

CAT activity was evaluated using the Aebi method (1984). Forty milliliters of the enzyme extract were mixed with three milliliters of hydrogen peroxide (30%) and ten milliliters of a phosphate buffer solution (10 mM) (pH 7). When all else failed, a spectrophotometer was used to measure the absorbance at 240 nm (LKB-Biochrom 4050).

2.10. Statistical analysis

One-way Anova analysis was studies using Minitab statistical software (Version 17.0). All the obtained results were presented in categorical variables. In this study, LSD test confirms bars with dissimilar letters, which differ significantly associated ($p < 0.05$).

3. Result

We investigated the favorable effects of pretreatment of RW on cowpea seedlings across a range of morphological and physiological parameters. According to the current study, increasing RW dose not only benefited plants subjected to drought stress, but also increased the growth and physiology of control plants. Drought stress significantly reduced shoot and root length, shoot fresh and dry weight, root fresh and dry weight, and leaf area in cowpea plants (Table 2). These growth metrics, however, were significantly enhanced in cowpea plants that had been pretreated with RW after experiencing drought stress (Table 2). Significant reductions in RWC of 4% and 4% were observed in cowpea seedlings subjected to drought treatments (65% FC and 35% FC, respectively) compared to control seedlings. However, relative to the RWC in the drought-stressed cowpea plants alone, RWC was raised by 4%, 8%, and 13% after being pretreated with RW (15%, 25%, and 35% RW), and by 4%, 12%, and 15% after being pretreated with RW (35% FC) (Fig. 2). Both the net photosynthetic rate (Pn) and transpiration rate (E) were significantly reduced by the drought treatments (65% and 35% FC), with Pn being reduced by 37% and E by 63%, respectively. Nevertheless, cowpea seedlings exposed to 35% FC after being pretreated with RW (15%, 25%, or 35% RW) saw increases in Pn of 7%, 41%, and 67% and E of 23%, 45%, and 69% (Fig. 2).

The proline content was 24% and 46% higher under drought stress, respectively, compared to the controls. Total soluble protein (TSP) and total soluble carbohydrate (TSC) concentrations were also raised in drought-stressed (65% and 35% FC) cowpea plants by 25%, 45%, and 41%, 53%, respectively, compared to those in untreated plants. A reduction in proline, total soluble protein, and total soluble carbohydrate was seen in RW pretreated cowpea plants compared to drought-stressed (65% and 35% FC) seedlings (Fig. 3).

Table 2
The effects of pre-treatment with rose water (RW) on the length and weight of shoots and roots, the fresh and dry weight of shoots and roots, and the leaf area of cowpea seedlings under drought. P < 0.05 indicates a significant difference between RW with letters that aren't the same as each other in the data, which are the mean from three independent experiments.

Growth parameters	Treatment	Rose water (%)			
		0% RW	15% RW	25% RW	35% RW
Shoot Length (cm)	Control	46.8 ± 3.3d	49.1 ± 3.2c	59.2 ± 4b	70.3 ± 4.6a
	65% FC	30.2 ± 1.3d	38.5 ± 1c	36.8 ± 1.8b	47.1 ± 2.1a
	35% FC	11.3 ± 1.9d	13.49 ± 1.4c	19.3 ± 1.2b	25.4 ± 1a
Root Length (cm)	Control	30.3 ± 1.2d	35.2 ± 1.1c	41.4 ± 1.1b	53.7 ± 0.92a
	65% FC	25.1 ± 1.52d	27.6 ± 1.4c	32.3 ± 1.32b	39.4 ± 1.13a
	35% FC	10.7 ± 0.6d	13.6 ± 0.72c	17.5 ± 0.81b	20.5 ± 0.9a
Shoot Fresh Weight, SFW (g plant^{-1})	Control	3.3 ± 0.23d	4.35 ± 0.19c	9.2 ± 0.18b	15.1 ± 0.09a
	65% FC	2 ± 0.04d	2.51 ± 0.06c	7.3 ± 0.08b	10.6 ± 1.1a
	35% FC	1.7 ± 0.05d	2.1 ± 0.06c	4.4 ± 0.07b	7.4 ± 0.77a
Shoot Dry Weight, SDW (g plant^{-1})	Control	1.7 ± 0.04d	2.2 ± 0.05c	4.8 ± 0.08b	7.5 ± 0.05a
	65% FC	1.4 ± 0.02d	1.9 ± 0.03c	2.1 ± 0.06b	4.1 ± 0.08a
	35% FC	1.1 ± 0.04d	1.2 ± 0.033c	1.9 ± 0.06b	2.4 ± 0.07a
Root fresh weight, RFW (g plant^{-1})	Control	2.4 ± 0.07d	2.6 ± 0.08c	3.2 ± 0.08b	4.5 ± 0.09a
	65% FC	1.2 ± 0.02d	1.4 ± 0.02c	1.7 ± 0.03b	3.1 ± 0.07a
	35% FC	0.8 ± 0.03d	1.04 ± 0.04c	1.47 ± 0.04b	2.1 ± 0.06a
Root dry weight, RDW (g plant^{-1})	Control	0.8 ± 0.07d	0.99 ± 0.06c	1.2 ± 0.05b	1.65 ± 0.05a
	65% FC	0.52 ± 0.02d	0.66 ± 0.02c	0.84 ± 0.01b	1.06 ± 0.018a
	35% FC	0.32 ± 0.04d	0.48 ± 0.04c	0.73 ± 0.038b	0.9 ± 0.03a
Leaf area (cm^2)	Control	17.1 ± 0.8d	18.7 ± 0.9c	19.07 ± 0.95b	26.8 ± 1.1a
	65% FC	10.8 ± 0.7d	12.6 ± 0.83c	13.1 ± 1.67b	19.5 ± 1.54a
	35% FC	9.7 ± 2.6d	10.8 ± 2.4c	14.6 ± 2b	18.2 ± 1.8a

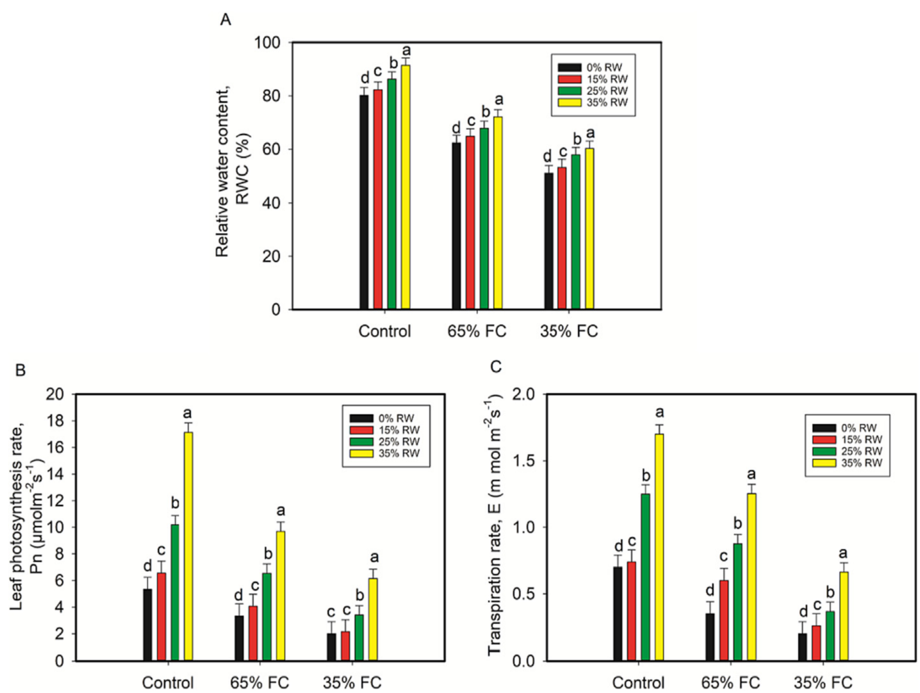

Fig. 2. Pretreatment of cowpea seedling leaves with rose water (RW) affects relative water content (A), leaf photosynthetic rate (B), and transpiration rate (C) A $p < 0.05$ indicates a significant difference between RW with letters that aren't the same as each other in the data, which are the mean from three independent experiments.

Drought treatments of 65% FC and 35% FC significantly enhanced the malondialdehyde (MDA) concentration in cowpea seedlings that had been subjected to salt stress without first being pretreated with RW. Nonetheless, pretreatment of RW (15%, 25%, and 35% RW) decreased the MDA content by 14%,32% and 50% at 65% FC and 24%,35%,48%, respectively, at 35% FC. Cow pea plants subjected to drought conditions of 65% and 35% FC showed significant increases in antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), as compared to control. Moreover, RW pretreated drought stressed seedlings showed increased SOD and CAT activity (Fig. 4) (See Fig. 5.).

4. Discussion

We evaluated morphological and physiological characteristics of cowpea to see how drought stress affects growth. Cowpea growth was considerably reduced by drought stress (Table 1). Past studies reported that cowpea showed similar findings when subjected to abiotic stressors (Matsui and Singh, 2003).

Due to drought conditions, the relative water content of cowpea leaves reduced significantly. Earlier studies have also documented that drought leads to a reduction in relative water content (RWC) in cowpea plants (Zegaoui et al., 2017). Cowpea plants pretreated

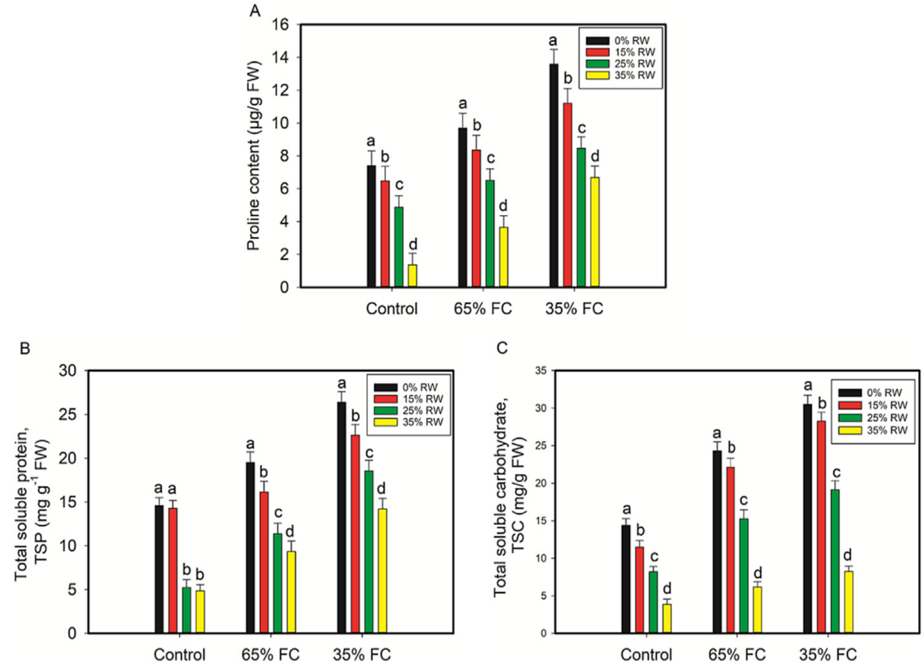

Fig. 3. For drought stress, pretreatment with rose water (RW) had an effect on the levels of proline and total soluble protein in the leaves of cowpea seedlings, as well as the levels of total soluble carbohydrate. A $p < 0.05$ indicates a significant difference between RW with letters that aren't the same as each other in the data, which are the mean from three independent experiments.

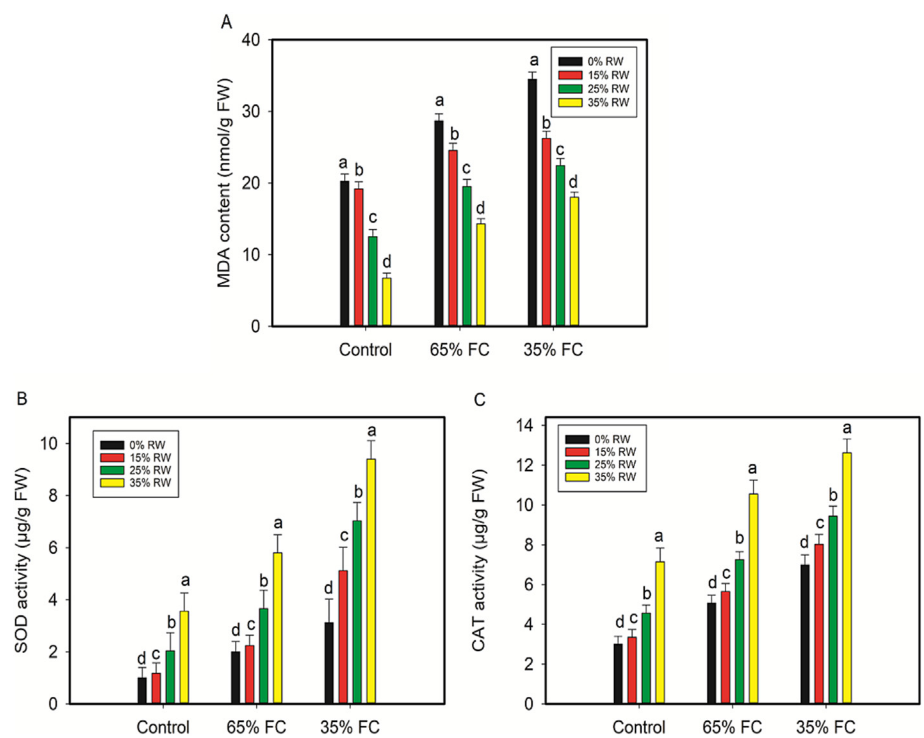

Fig. 4. MDA concentration, SOD activity, and CAT activity in the leaves of drought-stressed cowpea seedlings were all affected by RW pretreatment (Fig. 3). $P < 0.05$ indicates a significant difference between RW with letters that aren't the same as each other in the data, which are the averages (±SE) from three independent experiments.

with RW had higher water content after being subjected to drought, suggesting that this may have boosted the plants' ability to absorb water. The rate of carbon dioxide assimilation is affected by drought stress and other environmental conditions, which has a negative effect on biomass accumulation (Hasan et al., 2020). Photosynthetic and transpiration rates of cowpea were shown to be decreased in our study as a result of drought stress. These decreases in gas exchange characteristics might be a result of a decreased rate of water and nutrient intake. Pretreatment with RW enhanced photosynthesis and transpiration rates, indicating

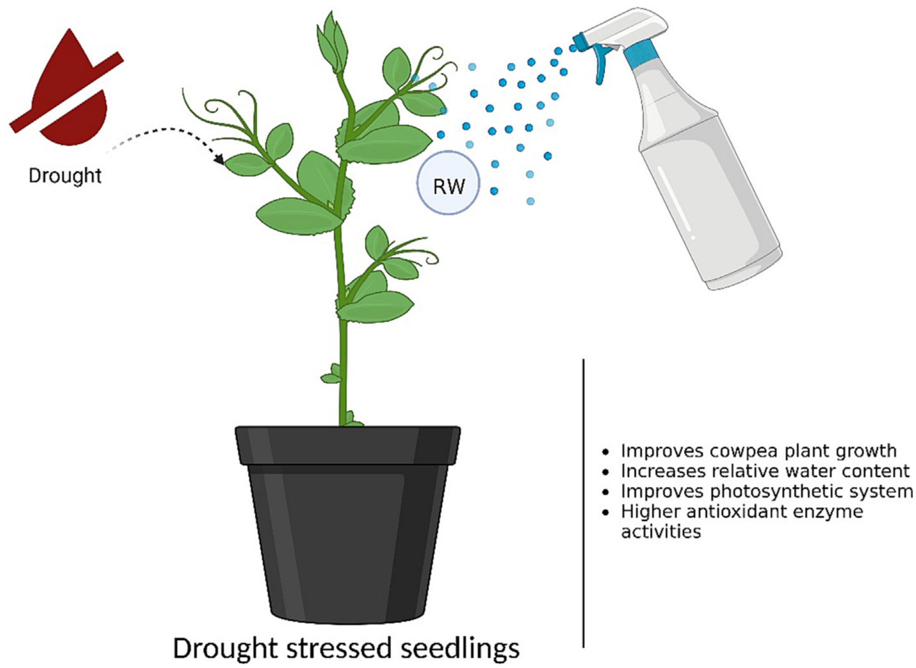

Fig. 5. A schematic depicts how rose water improves the growth and physiology of cowpea seedlings under drought stress.

that RW helped mitigate the adverse impacts of drought on gas exchange-related parameters, most likely by decreasing hydrogen peroxide formation (H_2O_2) (Kumari et al., 2018).

Proline is just one of several organic molecules that plants either create or store (Hasan et al., 2018; Hasan et al., 2020). Protecting plant cells from osmotic and other environmental stresses, proline is an osmoprotectant. Cowpea seedlings have a higher concentration of the amino acid proline after being subjected to drought stress, as discovered by our research. Under drought stress, RW pretreatment decreased proline content, most likely because of a decrease in osmotic stress. RW may be able to maintain the proline concentration that protects plant cells under drought. The impact of drought stress on plants' soluble proteins has been investigated in a number of different ways. Some research has shown that drought stress reduces the amount of soluble protein in plants (Zahoor et al., 2017), whereas other research has shown that a lack of water actually causes an increase in the amount of soluble protein in plants (Aziz et al., 2018; Zhang et al., 2019). Cowpea seedlings' soluble protein content increased during the drought, which is consistent with previous studies. Similar to other osomo-protectants like proline, RW lowers the amount of soluble protein in cowpea seedlings under drought stress, which may be due to a reduction in protein biosynthesis. Plants use carbohydrates as their main source of energy for their metabolic processes. During environmental stresses, carbohydrates accumulate in large amounts, and photosynthesis is inhibited, leading to a decrease in plant growth (Rook et al., 2010; Richter et al., 2015). Based on our findings, cowpea leaves accumulate more soluble carbohydrates when subjected to drought stress. Nevertheless, RW reduces the buildup of total soluble carbohydrates in cowpea leaves in response to drought stress, suggesting that it improves soluble sugar transport across cell membranes. Such carbohydrate contents may aid in the osmoregulation of plants and help in their water intake during times of stress (Farhangiabriz and Torabian, 2017; Abdellaoui et al., 2018).

Most research has focused on malondialdehyde (MDA), an aldehydic lipid peroxidation product. Similar to our findings, Hasan et al. (2018) reported that Moringa species experiencing drought stress had a higher total MDA content. The MDA concentration in cowpea was reduced, however, after being treated with RW, most likely because of the antioxidants' ability to scavenge free radicals (Hasan et al., 2018). Despite the fact that environmental influences have a major effect on the activity of antioxidant enzymes, these enzymes are crucial for maintaining health (Abdulmajeed et al., 2021, Alharbi et al., 2021). Our research showed that the effectiveness of RW varies with the amount administered. Antioxidant enzyme activity was boosted when RW-pretreated seed was used. Our results demonstrated that pretreatment of cowpea seed with RW at varying doses increased SOD and CAT activity when the seeds were subjected to drought stress.

5. Conclusion

Our findings offer novel insights into the physiological response to drought stress and the role of RW regulation in this process. These results lead us to propose that pretreating cowpea plants with RW is a highly effective approach for enhancing their drought tolerance. When cowpea plants were treated with different concentrations of RW (15%, 25%, 35%), their biomass, relative water content, gas exchange parameters, and antioxidant levels all increased. The highest RW concentration of 35% exhibited the most significant enhancement in cowpea plants' ability to withstand drought stress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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