



RESEARCH ARTICLE

# Exogenous foliar application of abscisic acid on polyethylene glycol induced drought by improving the morphological and biochemical characters of four rice (*Oryza sativa* L.) varieties

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## Abstract

Climate change is one of the critical defining concerns today's world, altering earth ecosystem. Drought stress management is a major issue in agricultural and crop research, mainly negatively impacting rice growth and yield. Abscisic acid (ABA) is a plant stress hormone that plays a crucial role in regulating, and mitigating drought stress. The objective of this study was to evaluate the effect of exogenously applied ABA and adaptation strategies responding to concrete challenges in four rice varieties (ASD-16, ADT-45, TKM-13 and CO-50) and analysed growth characteristics, physiological, biochemical and antioxidative enzyme activities induced by polyethylene glycol (2 % and 4 %) drought stress and exogenous application of ABA (100 µM). The present findings help to predict the degree of drought resistance variety of rice. The experiment was designed by six different treatments, such as control plants, control + ABA, polyethylene glycol induced drought (2 % and 4 %), and combination treatment of 2 % and 4 % PEG with exogenous application of ABA (100 µM). Exogenous ABA treatment significantly increased in morphological characteristics compared to control. The chlorophyll pigments, RWC, biochemical parameters such as reducing sugar, starch, protein and antioxidant activities of CAT, POD were increased and proline content was decreased at PEG 2 % and 100 µM ABA-treated rice in TKM-13 compared to ASD-16, ADT-45, CO-50. There were statistically significant morphological, physiological and biochemical parameters between treatments. The present findings depict that four rice varieties under drought imposition decrease the growth characteristics, physiological and biochemical content. However, the PEG induced drought (2 %) and foliar application of ABA (100 µM) were found to increase morphology, physiological and biochemical contents and can provide valuable insights into plants' drought responses and may help identify novel drought tolerance traits.

## Keywords

abscisic acid, antioxidant, biochemical analysis, drought, *Oryza sativa*, PEG

## Introduction

The global population is constantly growing, and by 2050, it is expected to reach 9.7 billion people (1). Climate change affects food security and the challenges of changing in increasing temperature and hence, to need the demand of food, and production increased by 50 % by 2050 (2, 3). Rice is the vital source of food crops for millions of people worldwide; hence, increased production is the only way to feed the rapidly growing population (4). Polyethylene glycol (PEG-6000) is a polyether substance that is an effective com-

pound for induced drought in plants, and the less absorbed water content is not toxic to plants (5). Drought stress is one of the significant challenges in agriculture, leading to a massive loss of crop productivity and the scarcity of water is responsible for significant losses in rice production (6, 7). ABA is a stress hormone; that plays a vital role in the perceiving and responding to stress (8). Mainly, drought stress-induced plants enhanced ABA levels which promotes the biochemical and physiological responses that enhance the drought tolerance mechanism (9). Exogenous ABA application controls osmotic adjustment and helps maintain a balance between excessive water loss and CO<sub>2</sub> uptake for photosynthesis through stomatal closure. (10). On the other hand, the growth characteristics, RWC, photosynthetic pigments, and enzymatic antioxidant activities of *Pennisetum glaucum* are improved under PEG-induced drought due to exogenous application of jasmonic acid and ABA (6). The evaluated wheat cultivar, improved relative water status under drought-stressed and ABA applied plants by providing drought tolerance (11).

In Tamil Nadu, many rice varieties such as ASD-16, ADT-45, TKM-13 and CO-50 are cultivated in various regions. It is mainly affected by drought stress and growth and yield loss, so we selected this variety, and a comparative analysis was carried out. Hence, the current study was carried out to evaluate morphometric parameters, physiological, biochemical contents and antioxidative enzyme activities of four rice varieties under PEG-induced drought stress and ABA treated plants. This finding could help researchers' better understand the drought tolerant mechanism of rice.

## Materials and Methods

### Plant materials and experimental site

Four varieties of rice (*Oryza sativa* L.) seeds such as (Ambasamudram (ASD)-16, Aduthurai (ADT)-45, Tirukuppam (TKM)-13 and Coimbatore (CO)-50 collected from Tamil Nadu Agricultural University, Coimbatore (TNAU), India. The experiment was performed at Department of Botany, Periyar University, Salem, Tamil Nadu, India. Seeds were sterilized with 0.1 % HgCl<sub>2</sub> for 5 min and cleaned with deionized water. Sterilized seeds were soaked in deionized water for 48 hrs in dark condition at 25 ± 2 °C, after that seeds transferred in pots (15 X 12 cm) at culture rack with light source 14:10 hrs light/dark with triplicates (soil mixture containing coir pith, sieved soil, farm yard manure at 1:3:1 ratio).

### In-vivo studies

In this study, the average atmosphere temperature was observed at 33°C. Five weeks (31<sup>st</sup> day) old plants were subjected to drought stress induced for five days by Polyethylene glycol (PEG-6000) at the concentration of 2 and 4 %. After the drought test, the plants were foliar sprayed with a concentration of ABA (100 µM) for three days. Thereafter, recover the period for seven days. Then, the triplicate samples of the treated plants on the 46<sup>th</sup> day were

harvested for analyzing the different growth characteristics, physiological and biochemical analysis and enzymatic antioxidant activities.

### Plant growth parameters

The randomly three plants were collected at 46<sup>th</sup> day of control, PEG induced drought and ABA treated rice plants of four varieties and measured the shoot and root length were expressed in cm/plant.

### Fresh weight and dry weight (g)

Plants collected from triplicates of the shoot and root length recorded. Three plants of each replicate were taken into consideration of observation in control, PEG-induced drought and the control. The fresh weight was recorded and the plant samples were dried in hot air oven at 60°C for 48 hrs. and weighted as dry weight.

### Physiological analysis

Drought-induced and control shoots and roots were weighted and dried at 70°C for 48 hours. The dry weight (DW) was recorded, and the water content (%) was calculated and expressed as the mean standard deviation (SD). The relative water content of the soil was calculated (12). The approach was used to estimate the concentration of photosynthetic pigments such as chlorophyll (a and b) and carotenoids (13, 14).

### Determination of reducing sugar (mg/g fr wt)

Two hundred milligram of fresh shoot and root tissue macerated with ten ml of ethanol (80%) and centrifuged for 15 min at 8000 rpm. The upper phase transferred in a fresh tube. A soluble sugar estimated (15) using glucose as standard.

### Determination of soluble starch (mg/g fr wt)

For starch extraction, to the residue, 5 ml of distilled water was added and 6.5 ml of 52 % of perchloric acid also added and thoroughly mixed before being heated in a water bath at 80 °C for 30 min. The extract was then preserved after adding twenty ml of distilled water and centrifuged at 8000 rpm for 15 min and the extract was saved. The re-extracted residue was pooled together and a final volume of ten ml distilled water. The filtrated extract through the Whatmann No.1 filter paper and estimated (16).

### Determination of protein content (mg/g fr wt)

Fresh plant tissue (500 mg) ground with 5 ml trichloroacetic acid (10 %) in a pestle and mortar. The macerated sample was centrifuged for 15 min at 8000 rpm. The upper phase discarded and the residue was suspended in 0.1 N sodium hydroxide (NaOH) to solubilize the protein, and the aliquot was estimated for a protein [17].

### Determination of (osmoprotectant) proline content (mg/g fr wt)

500 mg of fresh rice plants samples (shoot and root) were taken and grind with 10 ml of sulphosalicylic acid (3 %). Ninhydrin reagent was added to 2 ml of extract in a test tube and boiling for 1 hr at 100 °C. After that, 4 ml of toluene was added and vigorously, then proline content was calculated (18).

### Enzymatic antioxidant activity assays (U mg<sup>-1</sup> protein)

500 mg of fresh frozen plant materials was homogenized with five ml of 50 mM phosphate buffer (pH 7.0) and centrifugation for 5000 rpm at 20 min. The extract supernatant was used for enzyme assay.

### Determination of catalase activity (CAT)

The 40 µl of enzyme extract samples was taken and added 50 mM concentration of phosphate buffer (2.6 ml). Reaction was initiated by 0.4 ml of 15 mM H<sub>2</sub>O<sub>2</sub>. The catalase activity was read at 240 nm and computed (19).

### Determination of peroxidase activity (POX)

500 µl of enzyme extract were added with two ml of 50 mM K<sub>3</sub>PO<sub>4</sub> buffer (pH-7.0), 0.01 percent pyrogallol, and 50 mM of H<sub>2</sub>O<sub>2</sub>. The reaction mixture was held at 25 °C for 5 minutes, then one ml of 2.5 N sulphuric acid was added to terminate the activity, then read at 420 nm (20).

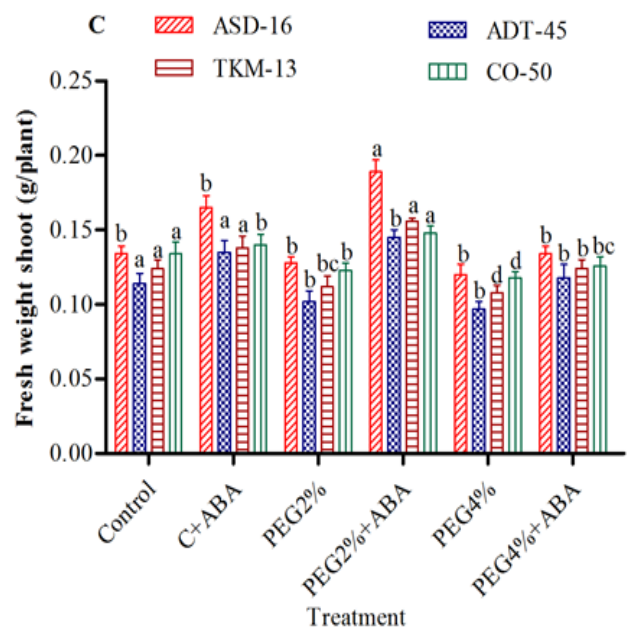
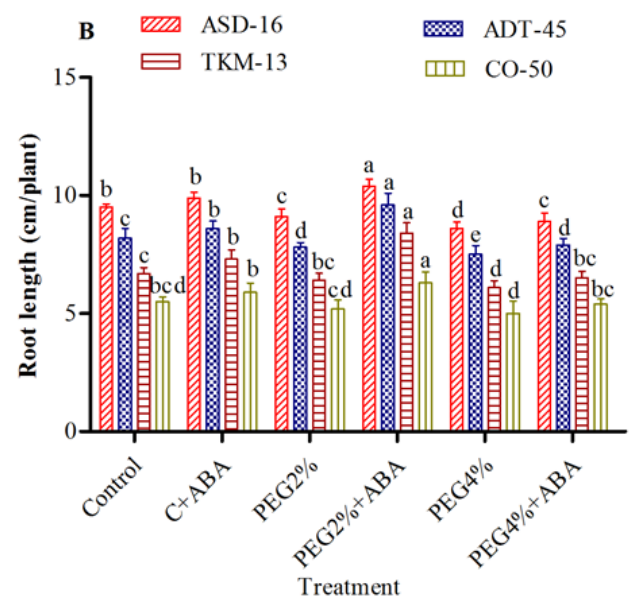
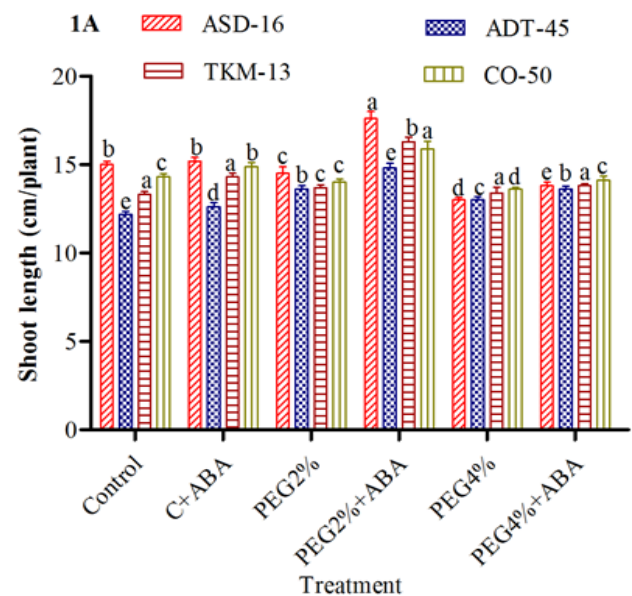
### Statistical analysis

Pearson correlations and ANOVA for four rice varieties were employed in SPSS software version 21.0. The turkey test at  $p < 0.05$  was used to determine the data and significant difference between the means. All the growth, physiological, biochemical and antioxidant parameters were calculated for Standard Error Means (SEM), and statistical investigation analysis was carried out in Graph Pad Prism using Triplicates (n=3).

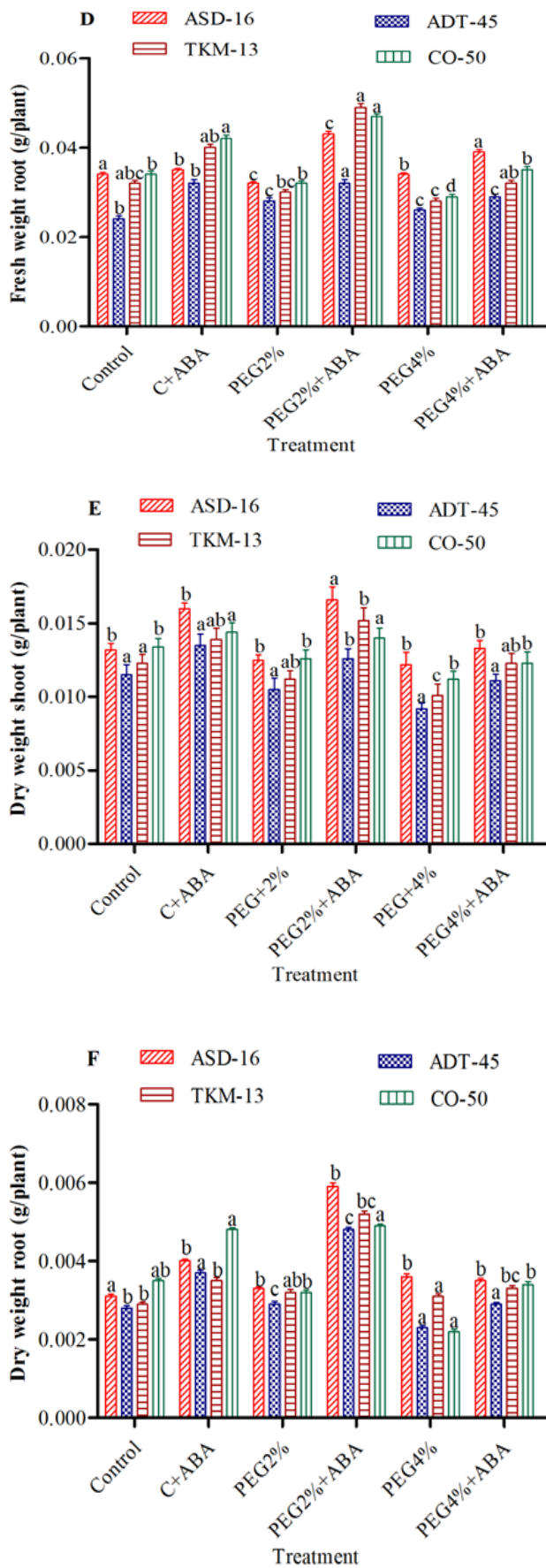
## Results and Discussion

### Effect of PEG-induced drought and ABA on growth characteristics

The growth characteristics such as shoot, root length, fresh and dry weight were reduced by PEG-induced drought stress (2 % and 4 %) in all rice varieties than well-watered plants. In this study, we observed that exogenous ABA improved plant growth and mitigated the adverse effects of drought. Therefore, among the variety, TKM-13 achieved the highest shoot length was showed (16.3 cm). In contrast, the root length of drought-induced (PEG 2 % + ABA (100 µM) treated plants showed an elongation rate (8.4 cm) than the other varieties and respective control (Fig. 1A, B). In the present results was also demonstrated by the significantly increased plant fresh and dry weights. Moreover, 2 % PEG + ABA (100 µM) treated rice plants were shown significantly higher in fresh weight of shoot (0.158 g) and dry weight of shoot (0.0151 g) (Fig. 1C, E). Similarly, (root) fresh and dry weight also (0.049; 0.0052 g) in TKM-13 rice variety than other varieties and non stressed plants respectively (Fig. 1D, F). The fresh weight of shoot was positively most significantly correlated to reducing sugar and protein content of root ( $p < 0.01$ ). Similarly, it reported that the plant shoot and root length, fresh and dry weight were significantly enhanced in water stress-induced and exogenous application of paclobutrazol and ABA treated of peanut plants respectively compared with the control plants (21). The present results showed changes in the growth characters of 2 % PEG + ABA (100 µM) because the ABA plays a crucial role in mediated stomatal regulation in drought-stressed plants.







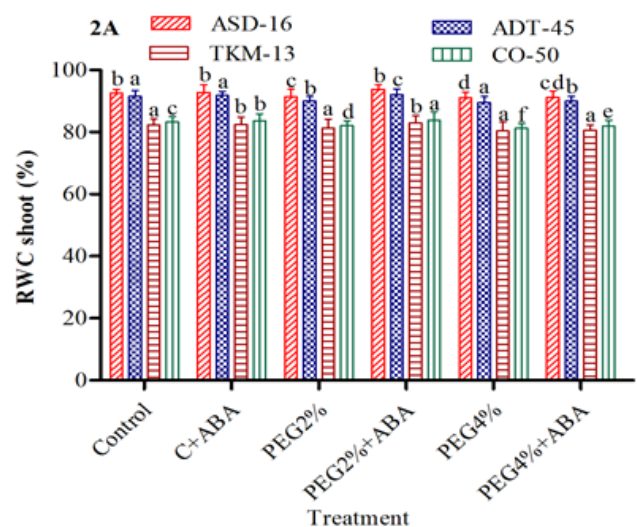
**Fig. 1.** Effect of PEG-induced drought stress and exogenous application of ABA on four varieties of rice (ASD-16, ADT-45, TKM-13 and CO-50). A) Plant shoot length B) Plant root length C) Fresh weight shoot D) Fresh weight root E) Dry weight shoot F) Dry weight root. The values were calculated the Mean $\pm$ SEM for three replicates in each treatments. Different letters are significance difference to turkey's post hoc test (\* $p$ <0.05; \*\* $p$ <0.01).

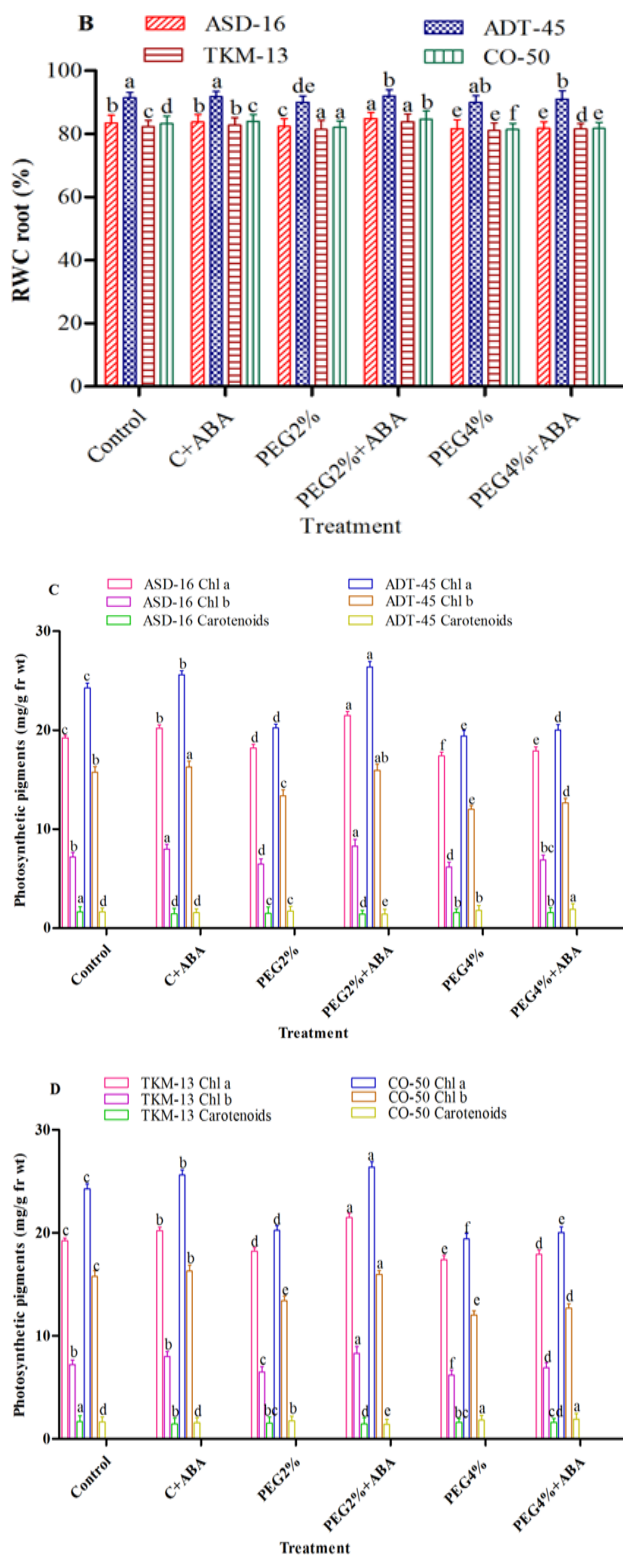
This effect is suggested to be important for rapid improvement of morphological characteristics, then recovery of water substance and up-regulation of stress-responsive genes, changes of the root system and other stress-related proteins in cellular water stress resistance in plants (22).

#### Physiological changes of rice under PEG induced drought

Plant water relations in rice were affected by PEG-induced drought stress. It certainly evidences that drought stress affects the relative water content compared to the non-treated plants. Relative water content was increased in all the rice varieties (ASD-16, ADT-45, TKM-13 and CO-50), which response to ABA than control. However, the RWC was comparatively more in drought stress (PEG 2 %) treated with ABA (100  $\mu$ M) concentration of shoot (82.98 %). Likewise, the RWC of root (83.90 %) in TKM-13 rice variety (Fig. 2A, B). The present data determined a significant negative correlation to the RWC and proline content ( $p$ <0.01). The previous findings, reported that the RWC was increased in response to water stress and ABA-treated wheat plants (23). In crops, RWC is a relevant attribute for understanding the drought tolerance mechanism, which shows the regulation of osmotic adjustment and regulation of stomatal closure and considered to measure water status in a plant (24). The exogenous application of ABA reduced the loss of water content due to trigger stomatal closure via transport to guard cells (25).

Photosynthetic pigments such as chl-a and chl-b changed in the PEG-induced drought plants in all rice varieties. Chl a and chl b were higher levels (17.92; 9.74 mg/g fr wt) noted in PEG 2 % + ABA (100  $\mu$ M) treated plants observed in TKM-13 rice variety compared to other varieties and control. PEG-induced drought stress increased the level of carotenoids content respectively, compared to control. A particularly TKM-13 variety exogenous application of ABA under (PEG 2 %) induced drought-stressed plant resulted in a level of carotenoids content (1.423 mg/g fr wt) was lower, respectively, compared to well-watered and other varieties of treated plants (Fig. 2C, D). The carotenoids differed in all treated plants showed a negative correlation of plant dry mass of shoot ( $p$ <0.01) and starch content shoot significantly correlated ( $p$ <0.05). ANOVA indicated





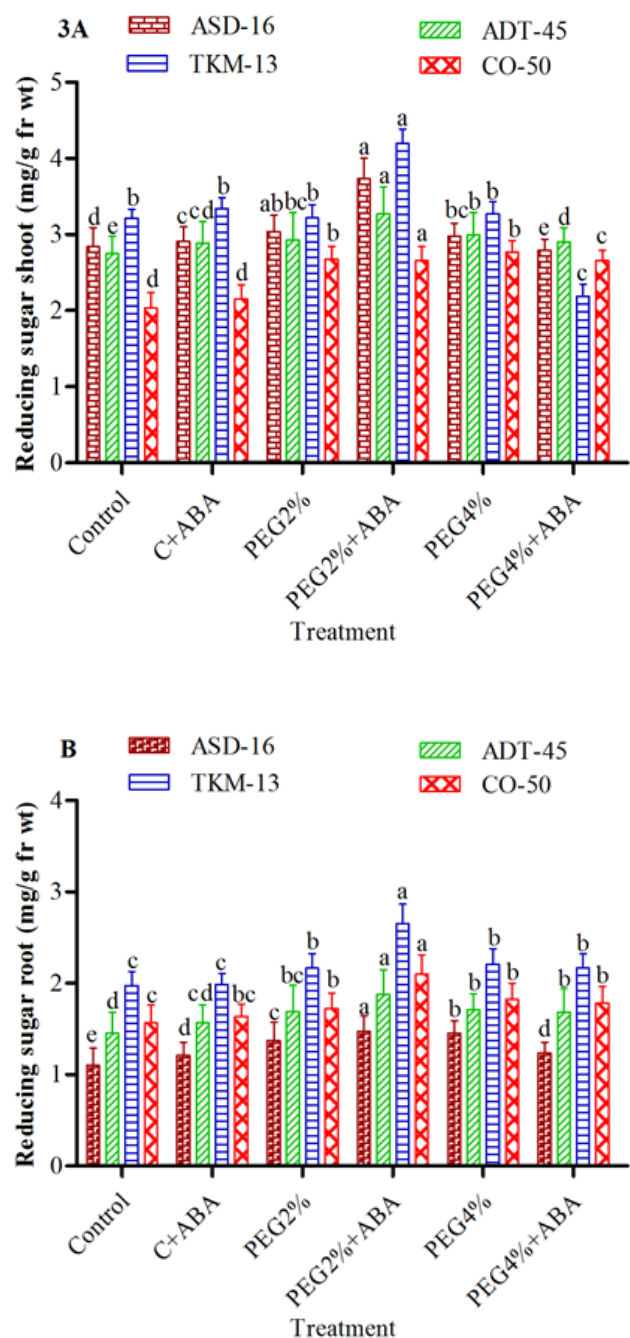
**Fig. 2.** Effect of PEG-induced drought stress and exogenous application of ABA on four varieties of rice (ASD-16, ADT-45, TKM-13 and CO-50). A) RWC shoot B) RWC root C) Photosynthetic pigments (ASD-16 and ADT-45) D) Photosynthetic pigments (TKM-13 and CO-50). The values are calculated the Mean $\pm$ SEM for three replicates in each treatments. Different letters are significance difference to turkey's post hoc test (Single letter - $p < 0.05$ ; different letters - $p < 0.01$ ).

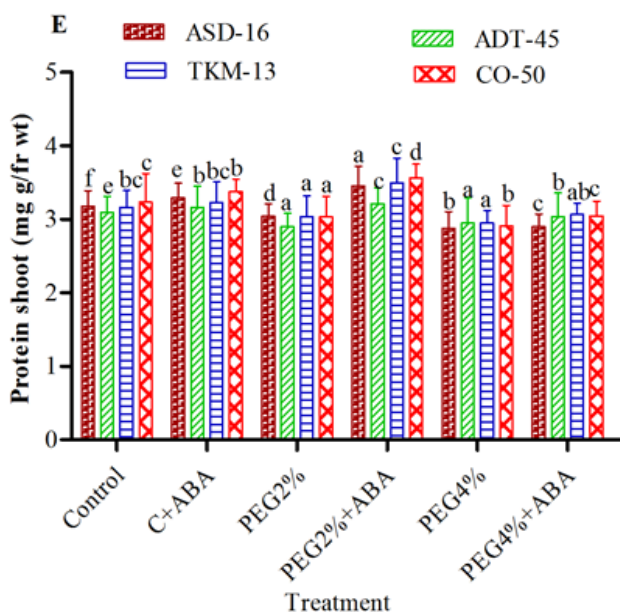
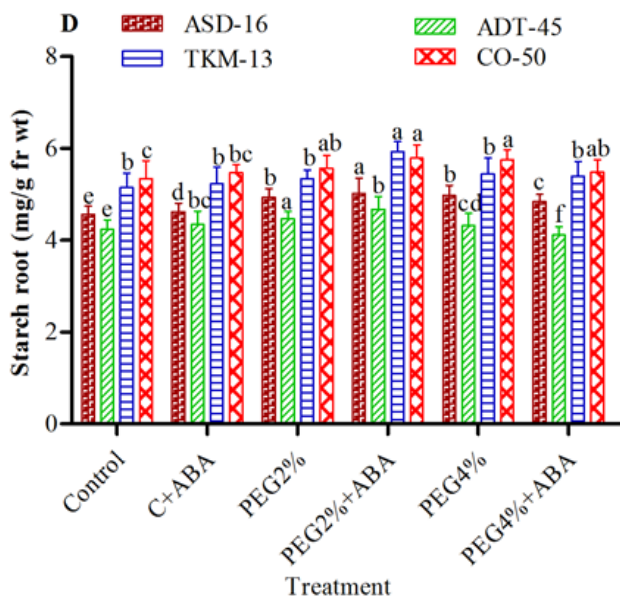
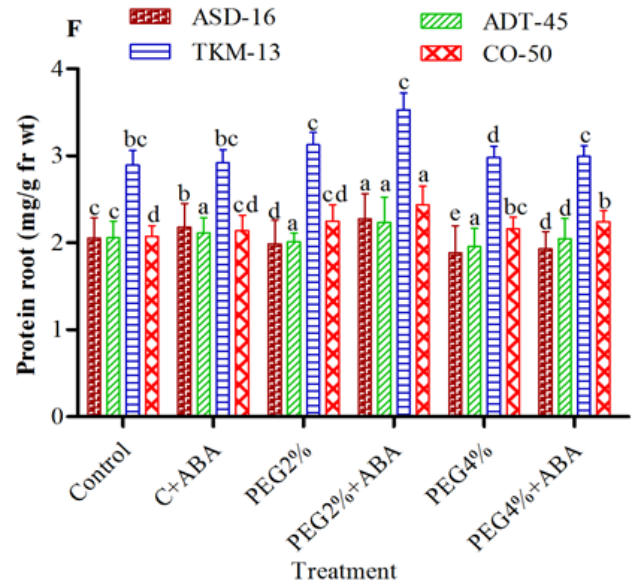
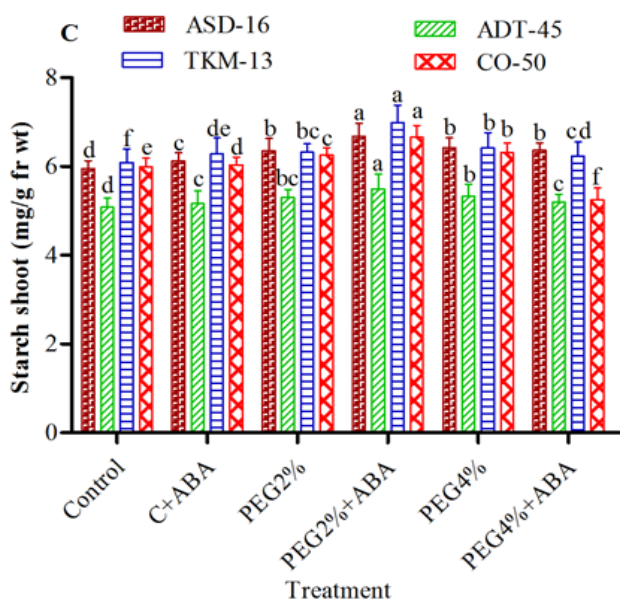
significant variation in the concentration of carotenoid ( $F=3.541$ ) content in all varieties of drought and ABA-treated plants. Similar results were reported that exogenous ABA increases chlorophyll contents in two rice cultivars under drought conditions (26). Carotenoids are the precursor for ABA, which sustain high photosynthetic pig-

ments, and these might be the reason for the increased stability of pigments by ABA (27). In the present investigation, the photosynthetic pigments were higher because of the induction of synthesis or modification of pigments biosynthesis pathway to develop drought-resistant varieties.

#### **Influence of drought stress on reducing sugar and starch content**

The two percent (2 %) PEG-induced drought stress and application of ABA (100  $\mu$ M) significantly increased the reducing sugar content of shoot (4.198 mg/g fr wt). However, the maximum value was recorded in the root (2.654 mg/g fr wt) of the TKM-13 rice variety, compared to non-treated plants and other rice varieties (Fig. 3A, B). The present study showed that reducing the sugar content of root was negatively correlated significant to the relative water content of root ( $p < 0.01$ ).





**Fig. 3.** Effect of PEG-induced drought stress and exogenous application of ABA on four varieties of rice (ASD-16, ADT-45, TKM-13 and CO-50). A) Reducing sugar shoot B) Reducing sugar root C) Starch shoot D) Starch root E) Protein shoot F) Protein root. The values are calculated the Mean $\pm$ SEM for three replicates in each treatments. Different letters are significance difference to turkey's post hoc test (Single letter - $*p<0.05$ ; different letters-  $**p<0.01$ )

The soluble starch content of all treatments and varieties differed significantly (Fig. 3C, D). The maximum starch content was noted in 2 % PEG with ABA (100  $\mu$ M) treated plants in all rice varieties. TKM-13 showed a higher level starch content of shoot (6.991 mg/g fr wt), and we detected a maximum starch content (5.932 mg/g fr wt) in root than other varieties (ASD-16, ADT-45, and CO-50) of treated plants. Similarly, sucrose and fructose were also increased; exogenous treated ABA resulted in enhanced sugar and starch accumulation than control rice plants (28). Exogenous ABA plays a vital role under drought conditions that hydrolysis to synthesize sucrose and sugar accumulation on osmotic adjustment, improve plant water balance, and better survive under drought stress conditions (29). Present results warrant that ABA is responsible for altering carbohydrate metabolism because of multiple genes involved (30, 31) in the metabolic pathway.

#### PEG induced drought and ABA effect on protein content

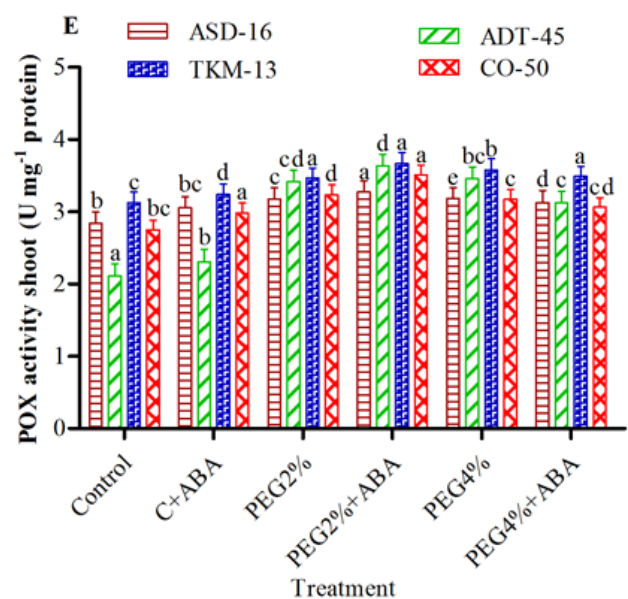
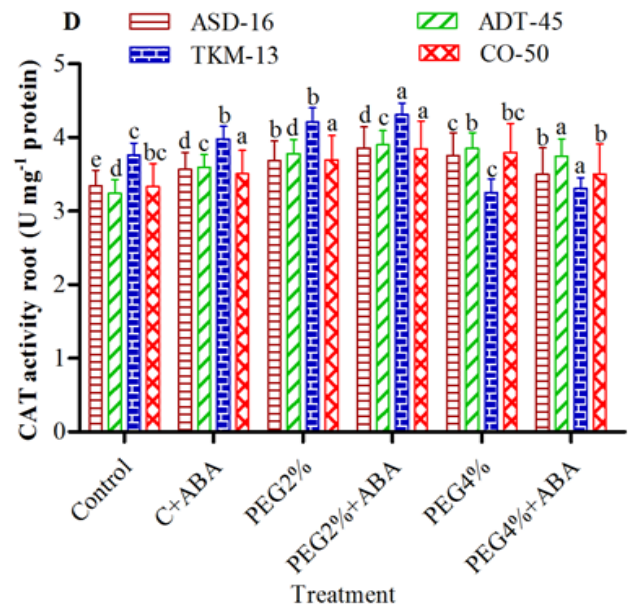
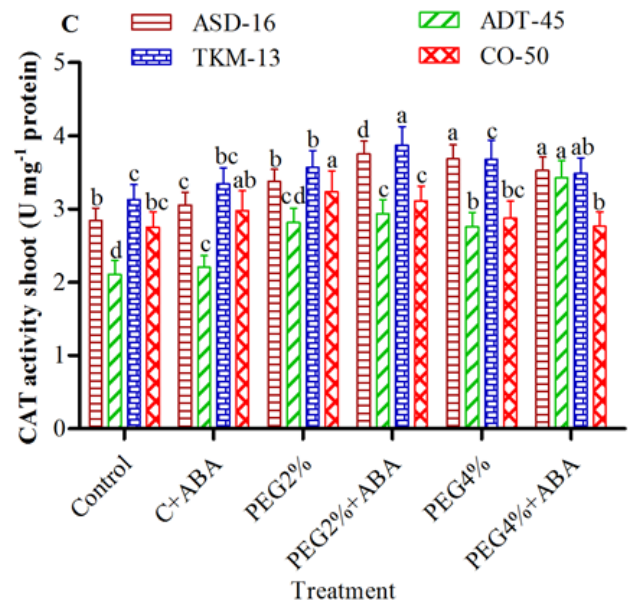
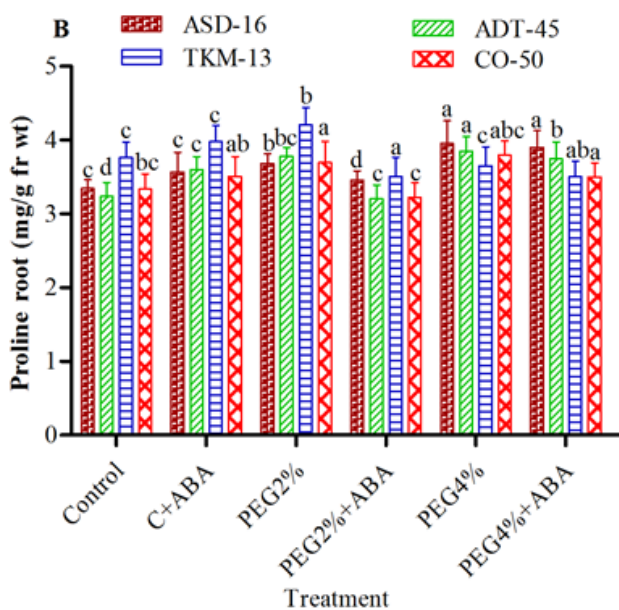
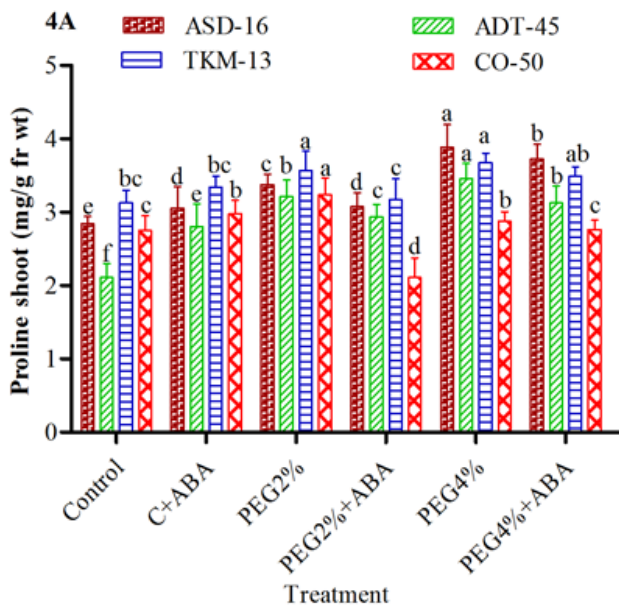
The polyethylene glycol-induced drought stress affected rice protein content compared to control plants of all rice varieties. The PEG-induced drought with ABA treatment resulted in significant changes in shoot and root protein content in all the rice varieties. Nevertheless, a particularly exposed two percent (2 %) PEG with ABA (100  $\mu$ M) treated plants noted higher protein content (shoot- 3.499 mg/g fr wt). Likewise, it was increased in the root (3.530 mg/g fr wt) of TKM-13 rice variety, respectively than other treated plants (Fig. 3E, F). A significant positive correlation was observed in protein root content ( $p<0.01$ ). The present results were confirmed by the results of authors (32), who stated that drought stress and application of ABA leads to protein content in the *Crocus sativus*. Besides, the ABA also promoted protein synthesis, carbon metabolism and the expression of resistant proteins in drought conditions (33). Exoge-

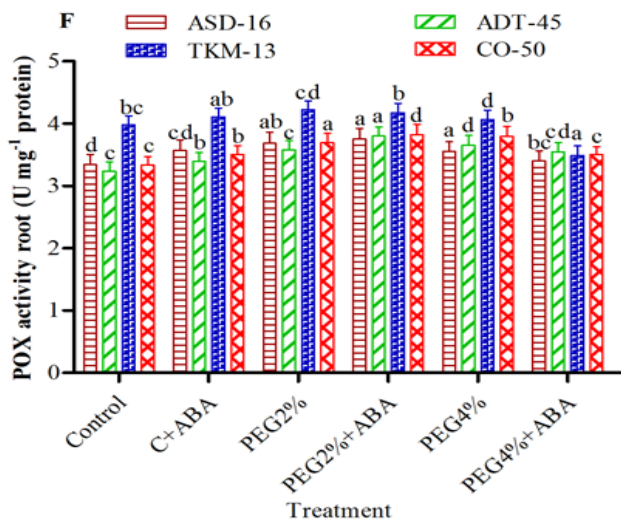


nous application of ABA plays an important role in stress-responsive genes, biosynthesis of osmolytes, prevention for stress cell damage and help to crop stress resistance mechanism (34).

#### Effect of drought induced stress and ABA on osmoprotectant (proline)

The proline contents increased in all the rice varieties (ASD-16, ADT-45, TKM-13, and CO-50) by PEG-induced drought stress imposed compared to control, whereas the exogenously foliar application of ABA, after the PEG treatment showed a significant reduction of proline content. However, in 2 % PEG + ABA (100  $\mu$ M) treatment, TKM-13 had shown significant lower proline content (shoot: 3.167 mg/g fr wt). The lower proline content was recorded in the root (3.509 mg/g fr wt) compared to other rice varieties along with control (Fig. 4A, B). The results showed that proline root content was negatively correlated with the dry weight of the shoot ( $p < 0.05$ ). The proline content was increased in drought stress conditions, and ABA treated plants showed





**Fig. 4.** Effect of PEG-induced drought stress and exogenous application of ABA on four varieties of rice (ASD-16, ADT-45, TKM-13 and CO-50). A) Proline shoot B) Proline root C) Catalase shoot D) catalase root E) eroxidise shoot F) peroxidise root. The values are calculated the Mean $\pm$ SEM for three replicates in each treatments. Different letters are significance difference to turkey's post hoc test (Single letter- $*p < 0.05$ ; different letters- $**p < 0.01$ ).

decreased proline content compared to control in sunflower (35). Proline acts as a concentration level of reactive oxygen species (ROS), thus preventing oxidative burst of plants, osmolyte balance, and better performance of drought tolerance (36). Accumulation of proline results from regulating of mainly two pathways such as biosynthesis of enzymes and degradation of enzyme activities, protecting cellular structures during drought stress (37).

#### Enzymatic antioxidant activities

The production rate of reactive oxygen species significantly increased under drought stress conditions. The enzymatic antioxidant activities of CAT, POX were remarkably affected in the four rice varieties of shoot and root under polyethylene glycol drought stress induced whereas, ABA treated plants. The PEG 2 % induced drought and ABA treated rice plants increased the activities of CAT and POX by 3.867 U mg<sup>-1</sup> protein and 3.667 U mg<sup>-1</sup> protein in a shoot at TKM-13 rice variety compared to control (Fig. 4C, E) and other treated plants respectively. A significant improvement in antioxidant enzyme activities such as CAT and POX was observed in ABA-treated roots, which showed 4.309 U mg<sup>-1</sup> protein and 4.229 U mg<sup>-1</sup> protein enhanced at TKM-13 rice variety compared to control and other treated plants (Fig. 4D, F). The CAT and POX antioxidant activities were increased and significantly negative correlation ( $p < 0.05$ ) with proline content. These findings are similar to our previous studies that showed catalase and peroxidase activities were higher level observed stress induced and ABA foliar applied rice plants compared to control (38). ABA enhanced the enzymatic antioxidants activities by regulating genes related to encoding antioxidant while protecting the stressed plant under various environmental conditions (39).

#### Conclusion

In this present study, PEG-induced drought stress negatively impacted the all the rice varieties in all parameters. However, ABA improved the plant morphological characteristics

physiological, biochemical contents and enzymatic antioxidants activities under PEG-induced (2 %) with 100  $\mu$ M of ABA treated plants in all the rice varieties. Furthermore, the specific TKM-13 rice variety showed more tolerance to PEG-induced stress as compared to ABA and other rice varieties compared to control. The protective role of ABA and it proved, moderating the physiological activities, biochemical metabolites (osmoprotectants) to sustain drought conditions for better rice variety. ABA provides a comprehending understanding of drought, and ABA metabolism could be helpful for the identification of drought-stress rice varieties.

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#### Authors contributions

Concept and experimental design: DAB; Data collection and investigation: MR, SR & DAB; Manuscript preparation and Statistical analysis: MR, SR & DAB; Review and editing: MR & DAB.

#### Compliance with ethical standards

**Conflict of interest:** The authors declare that there is no Conflict of interest.

**Ethical issues:** None.

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