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Research article

Abscisic acid and glycine betaine-mediated seed and root priming enhance seedling growth and antioxidative defense in wheat under drought

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ABSTRACT

The extent of drought tolerance in the seedlings of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) was investigated by seed and root priming using abscisic acid (ABA) and glycine betaine (GB). The seeds were primed with ABA (10 and 20 μM) and GB (50 and 100 mM) and grown in pots maintaining control (0 % PEG) and drought (10 % PEG) conditions. Under drought, the root and shoot length, root and shoot biomass were significantly increased in ABA and GB primed seedlings than non-primed seedlings in all cultivars. Among the priming agents, either 20 μM ABA or 50 mM GB triggered better seedling growth in all wheat cultivars. These two levels were then applied with the nutrient solution in the hydroponics following four treatments: Control, Drought, Drought + ABA and Drought + GB. The seedling growth significantly declined in drought, while an improved seedling growth was observed in ABA and GB-treated plants in all cultivars. A considerable increase in lipid peroxidation, proline content, total antioxidant capacity and total flavonoid content in roots and leaves were recorded in all drought conditions, while these values were considerably reduced in ABA and GB treatments. Hierarchical clustering heatmap using stress tolerance index (STI) values showed that Drought + ABA and Drought + GB secured higher STI scores suggesting a greater degree of drought tolerance in all cultivars. In conclusion, seed and root priming of ABA and GB enhanced drought tolerance in the wheat seedlings by improving seedling growth and antioxidative defense suggesting a declined state of oxidative damage.

1. Introduction

Wheat (*Triticum aestivum* L.) is a key cereal worldwide and is Bangladesh's second-most vital grain after rice. It is a cereal crop that has a high yield and the potential to satisfy the needs of the world's population as its cultivation continues to expand. Wheat growth and grain yield are significantly impacted in most of the world's wheat-growing areas due to water scarcity, including Bangladesh [1,2]. The ever-dwindling water resources and sporadic droughts caused by constantly shifting environmental regimes are among the biggest obstacles to future agricultural research [3,4]. In Bangladesh, particularly in the northwestern part, drought increasingly poses

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a serious threat to wheat production. The drought's severity is worsening, reducing wheat production and endangering food security. The morphological and physiological responses of plants to drought stress include the reduction in plant biomass, closure of stomata, decrease in the rate of transpiration and photosynthesis as well as the lowering of water use efficiency [5–8]. Drought alters the cell membrane structure, antioxidant capacity, proline buildup, and chlorophyll levels, which causes plant senescence and shortens the time of active photosynthesis [9,10]. Lack of water also causes salts to build up in the top layers of soil, which makes it harder for plants to get water and nutrients from the soils [11,12]. Additionally, the disruption of water and mineral balance disturbs redox homeostasis and stimulates the excessive generation of reactive oxygen species (ROS). The overproduction of ROS enhances lipid peroxidation and hampers the functions of proteins and membranes which is a fatal threat when plants experience severe drought [13–15]. Numerous anti-drought agents, such as plant growth regulators and suitable solutes, are being studied [16,17]. Abscisic acid (ABA) and glycine betaine (GB) may increase drought resistance to varied degrees depending on the plant species, developmental stages, duration and extent of the stress [18]. There is an urgent need to search for strategies to lessen the severe effects of drought in the event of abrupt climatic changes. We need to implement practical and affordable solutions to deal with unfavorable changing climate circumstances. This requires a better understanding of the features that adapt to climate change. Several strategies are employed to deal with the difficulties of drought-related crop losses, including the exogenous delivery of plant growth regulators [19]. Numerous studies revealed that the exogenous application of a stress hormone ABA and an osmolyte GB could significantly improve crop plants' ability to withstand droughts [16,20–23]. ABA and GB enable plants to better tolerate abiotic challenges by improving the hydration status of tissues and protecting biological membranes from ROS while the plant is under drought stress [24].

ABA is a crucial stress hormone generated in response to drought and functions as an early warning system [25] with the expansion of roots, and stimulation of ABA-dependent pathways [26]. The ABA signal regulates the rates of water loss and CO_2 intake and functions as the initial line of defence against drought-induced stomatal closure [27,28]. Exogenous ABA has been shown to boost the rate of photosynthesis and stomatal conductance resulting in an increase in primary and secondary metabolites production in plants under drought [29,30]. Similarly, it has been observed that the use of ABA under drought can increase the enzymatic and non-enzymatic antioxidant defense capacity [31]. Moreover, seed priming with ABA performs as a potential plant growth regulator under limited water situations by accumulating osmoprotectants [32]. Priming with ABA affects rapeseed (*Brassica napus* L.) production both in low temperature and drought [33]). Exogenous ABA application increased proline accumulation and the activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) in maize and wheat [31,34]. Exogenous ABA treatment to wheat improved leaf water relations and desiccant tolerance while increasing ABA production during soil drying but did not boost yield [21]. However, exogenous ABA may increase wheat grain formation even when there is just a moderate shortage of water [35]. Exogenous ABA application increased the water use efficiency and grain yield in wheat, primarily due to a decrease in water use [21]. One of the most effective compatible solutes, glycine betaine (GB), is required for the maintenance of cell organelles, the cellular osmotic adjustment, and the efficient use of water in plants in water scarcity conditions [36]. Seed priming with GB, is a promising approach to reduce the antagonistic effects of drought on seed germination as well as early seedling establishment of wheat [37]. Improved boll weight, leaf greenness, and membrane stability were observed in cotton (*Gossypium hirsutum* L.) primed with GB under drought conditions [38]. Previous studies reported that exogenous application of GB in wheat increased grain yield and grain numbers per spike [39]; improved stress tolerance index, enhanced osmolytes accumulation, and increased relative water content [40]; increased net photosynthesis rate and maximum photochemical efficiency of PSII and higher antioxidative enzymes activities [11]; stabilized thylakoid membrane functions, suppressed chlorophyll degradation and enhanced Ca^{2+}-ATPase and Hill reaction activities, improved growth and enhanced activities of SOD, CAT, and APX [41]. GB causes a rise in the soluble sugars and free amino acids accumulation, both of which serve to protect plant cells from the osmotic stress that is caused by drought [42]. Exogenous GB improved the height, leaf area, and total dry weight of drought-stressed maize plants [43]. Meanwhile, tolerant wheat genotypes accumulate more GB than sensitive wheat genotypes subjected to drought [44].

Novel and dynamic strategies should be developed to deal with drought stress, and phytohormone engineering may be a way to increase productivity. Little information is available regarding the exogenous use of ABA and GB alone and/or in combination to improve drought tolerance in wheat cultivars produced in Bangladesh under field and control conditions. Therefore, thorough research of the physio-chemical responses to exogenous ABA and GB administration under drought, including synthesis of secondary metabolites, antioxidant activities, and photosynthetic apparatus, is required. This study aimed to investigate the efficacy of seed and root priming with ABA and GB on drought tolerance in wheat and to better understand the roles of ABA and GB on morphological and biochemical responses under drought in wheat at the seedling stage.

2. Materials and methods

2.1. Plant materials and growth conditions

Two experiments were set at the laboratory of the Department of Crop Botany from October 2021 to February 2022 at Bangladesh Agricultural University, Mymensingh, Bangladesh. Firstly, a germination test was carried out to examine the effect of seed priming with different concentrations of ABA and GB at early growth stages of three wheat cultivars under control and polyethylene glycol (PEG-6000)-induced drought conditions. The three wheat cultivars, such as WMRI-1, BARI GOM-33 and BARI GOM-21 were provided from Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur, Bangladesh. The collected seeds of three wheat cultivars were surface sterilized with Vitavax-200 @ 2 g kg^{-1} seed. The seeds were primed with two concentrations of each of ABA (10 and 20 μM) and GB (50 and 100 mM). The priming was executed by soaking the seeds into respective priming solutions in 50 mL glass beakers maintaining the seed weight to solution volume ratio (*w*/*v*) as 1:6. The seed priming was done for 24 h at 25 °C in dark conditions. The primed seeds were then rinsed properly using distilled water for 2 min and left overnight at room temperature for drying to obtain the original moisture level. The primed seeds were sown in plastic pots (15 cm diameter) with sterilized sand under different growth conditions. For each cultivar, six treatment combinations were followed such as i) Control (0 % PEG-6000, 0 MPa osmotic potential, Ψ_s), ii) Drought (PEG 10 %, −0.54 MPa Ψ_s), iii) Drought and ABA 10 μM, iv) Drought and ABA 20 μM, v) Drought and GB 50 mM and vi) Drought and GB 100 mM. PEG-6000 was used to induce drought stress as it cannot pass through the cell wall due to its higher molecular weight. PEG decreases the water potential of the solution and 10 % PEG-6000 solution maintaining an osmotic potential level of −0.54 MPa [45]. For control and drought conditions, the seeds were primed with distilled water only. The drought was imposed in all conditions except control by applying PEG solutions into the sands seven days after sowing. The experiment was set following a completely randomized design (CRD) with four replications. In each pot, 15 seeds of each cultivar (a total of 45 seeds pot^{-1} and separated by tiny sticks) were sown maintaining a single growth condition and treated as a single replicate (Figure A1a). The pots were irrigated every second day with 10 mL of distilled water and PEG-10 % solutions for control and drought conditions, respectively. The germination and seedling growth were facilitated at 25 °C temperature providing 500 μmol m^{-2} s^{-1} artificial photosynthetic photon flux density (PPFD) with fluorescent tubes maintaining a photoperiod of 12 h. The second experiment was established to examine the exogenous application of ABA and GB to enhance drought tolerance in the same wheat cultivars under a hydroponic system at the seedling stage. The seeds were allowed to germinate by placing them in a net, which was then maintained in a pail of water with a light mist of water to help the seeds sprout (Figure A1b). Square plastic tanks (25 cm × 25 cm × 21 cm; L × W × H) were used to maintain the hydroponic system. In each tank, perforated cork sheets with 9 holes (3 × 3) were served as trays. To secure each plant, five-day-old pregerminated seedlings of all cultivars were inserted into the openings of the plastic containers using a piece of Styrofoam (Figure A1c). Initially, each tank was filled with 4 L of distilled water and after five days of transplanting, the tanks water was replaced by a modified full-strength nutrient solution [46] maintaining pH 6.0–6.5 with the following composition: $Ca(NO_3)_2.4H_2O$ (2.5 mM), K_2SO_4 (1 mM), KH_2PO_4 (2 mM), $MgSO_4.$ $7H_2O$(5 mM), $CaCl_2.2HO$ (2 mM), H_3BO_3 (1 μM), $MnSO_4.6H_2O$ (2 μM), $ZnSO_4.$ $7H_2O$ (5 μM), $CuSO_4.5H_2O$ (3 μM), $(NH_4)6Mo_7O_{24}$(01 μM), Fe-EDTA (200 μM). All cultivars' seedlings were assessed under the following four growth conditions: i) Control (PEG 0 %), ii) drought (PEG10 %) iii) drought and ABA 20 μM, and iv) drought and GB 50 mM. The drought, drought and ABA and drought and GB were simulated to the respective tanks after two days of nutrient addition. The control plants were treated only with the nutrient solution. The solutions were replaced in five days intervals and continuously aerated using an individual air pump for each tank. In a tank, three holes were used for a single cultivar with two seedlings per hole and therefore, nine holes comprised of three cultivars with 18 seedlings (6 seedlings of each cultivar). Each tank represented a single growth condition considered as a single replicate. The CRD was followed with three replications and therefore, a total of 12 tanks were set (treatment × replication:4 × 3). The plants were artificially illuminated by LED tubes (60W) with a PPFD of approximately 650 μmol m^{-2} s^{-1} maintaining a 12 h photoperiod. An optimum growing temperature (25±2 °C day and 16±2 °C night) was controlled for proper seedling growth.

2.2. *Morphological measurements*

For the priming experiment, the morphological data were collected from 15 days old seedlings (eight days stressed plants). The seedlings were carefully pulled out from the sand and the roots and shoots were separated. Six seedlings were randomly selected from each pot (single replicate) for morphological measurements and the average values were used for data analysis. The total plant height (TPH), root length (RL) and shoot length (SL) were measured in cm using a 1-m ruler. Root and shoot fresh weight (RFW and SFW in mg) were determined using a digital weighing balance. The dry weight of root (RDW) and shoot (SDW) were obtained by oven drying the samples at 70 °C for 72 h. The total fresh (RFW) and dry weight (TDW) of the seedlings were calculated in mg with the aggregate of RFW and SFW and RDW and SFW, respectively. The root-shoot ratio (RSR) was assessed as RDW over SDW [47,48]. In the hydroponic experiment, 28-day-old seedlings of all cultivars grown under four growth conditions were harvested for morphological data collection and biochemical analysis. Three seedlings from each tank (single replicate) were used for data recording. Root and Shoot length and their biomass were measured as per the priming experiment.

2.3. *Determination of lipid peroxidation (TBARS assay)*

The lipid peroxidation of root and leaf samples from the hydroponic experiment was determined by TBARS assay [49]. Approximately 0.1 g of leaf and root samples of 28 days old seedlings were ground using mortar and pestle and mixed with 0.1 % Trichloroacetic acid (TCA) in a centrifuge tube. The homogenized samples were centrifuged at 12000 rpm for 15 min. The supernatants were collected and transferred to a new tube and mixed with 0.5 % Thiobarbituric Acid (TBA) containing 20 % TCA. The tubes were then placed in a water bath for 15 min at 90 °C and stopped the reaction immediately by putting the tubes in ice for 10 min. The solution mixture was centrifuged at 10,000 rpm for 5 min and the optical density of the supernatants was measured at 532 and 600 nm against a blank (containing all reagents except biological sample) using a UV-VIS spectrophotometer (Multiskan Go, Thermo Fisher Scientific, Ratastie, Finland). The absorbance values at 600 nm were subtracted from the 532 nm absorbance and the Malondialdehyde (MDA) contents were determined using the extinction coefficient of 155 mM^{-1} cm^{-1} [50].

2.4. *Proline estimation*

The proline contents in roots and leaves were calculated using the approach described by Ref. [51] with minor modifications. A homogenate of freshly harvested plant leaves and root samples (0.05 g) was filtered after being blended in 80 % ethanol. The reaction mixture containing 1 % Ninhydrin (*w/v*) in 60%acetic acid (*v/v*) and 20 % ethanol (*v/v*) was added to the filtrate in a 2 mL Eppendorf tube. The mixture tubes were sealed and kept in a water bath at 95 °C for 90 min. The tubes were cooled in room temperature and the absorbance reading was taken at 520 nm using the same spectrophotometer. The quantity of proline in the extracts was calculated from the standard curve of L-proline at different levels ranging from 0 to 80 μg ml^{-1} ($y = 0.0461x – 0.0874$, $r^2 = 0.997$).

2.5. Total antioxidant capacity and total flavonoid contents determination in root and leaves

Total antioxidant capacity (TAC) in roots and leaves was measured following the method described by Ref. [52] with minor changes. The TAC was extracted in 80 % methanol (v/v) and an assay of 1.5 mL contained the methanolic extracts and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After incubating the samples in a water bath at 95 °C for one and a half hours, the reaction was stopped by bringing the temperature down to room temperature, and an absorbance measurement was obtained at 695 nm in contrast to a blank using a spectrophotometer (Multiskan Go, Thermo Fisher Scientific, Ratastie, Finland). The TAC content was expressed as Ascorbic acid (AA) equivalent (mg AA g^{-1}FW) and AA (prepared in 95 % methanol) was used as the reference standard ($y = 0.00458493x + 0.07094$, $r^2 = 0.99$). Total flavonoid contents were estimated following the $AlCl_3$ colorimetric method [53]. Quercetin was used as the reference standard ($y = 0.002x – 0.0125$, $r^2 = 0.99$). The amount of TFC was calculated as μg of quercetin equivalent per gram of fresh extract.

2.6. Pigment analysis

Leaf samples from plants cultivated under normal and stressful conditions were collected and analyzed for pigments. Fresh leaf samples weighing around 50 mg were dissolved in 10 mL of 80 % acetone and allowed to adequately extract for seven days in the dark. Following the method of [54], the contents of chlorophylls (Chl *a* and Chl *b*) and carotenoids (carotene and xanthophyll) were calculated from the absorbance readings at 470, 648.6, and 663.2 nm by a UV–vis spectrophotometer (Multiskan Go, Thermo Fisher Scientific, Ratastie, Finland).

2.7. Statistical data analysis

The data analysis was performed using an open-source statistical software R [55] version 4.0.5 accessed in April 2021. Two-way (cultivars and growth conditions) analysis of variance (ANOVA) was done to compare the means among the cultivars and growth conditions and their interactions. The stress tolerance index (STI) for each trait was computed as (stress values/control values) × 100. The multiple comparisons of treatment means were performed by the Tukey HSD test of the R software. The normalized STI scores were considered to create the two-way hierarchical clustering heatmap following the *ComplexHeatmap* package of the R program.

Table 1
Shoot length (SL), Shoot Fresh Weight (SFW), Root length (RL), Root Fresh Weight (RFW) and Plant Height (PH) of three wheat cultivars grown under six growth conditions.

Cultivar	Treatments	SL (cm)	SFW (mg $plant^{-1}$)	RL (cm)	RFW (mg $plant^{-1}$)	PH (cm)
WMRI-1	Control	17.08 ± 0.65 a	107.08 ± 5.20 a	19.70 ± 0.87 ab	110.3 ± 5.6 ab	36.8 ± 0.8 a
	Drought	12.13 ± 0.23 ef	41.01 ± 5.35 g	15.29 ± 0.76 def	86.0 ± 3.6 d-g	27.4 ± 0.3 g
	Drought + ABA_{10}	14.31 ± 0.81 d	66.34 ± 3.11b-f	14.02 ± 0.56 f	86.7 ± 1.7 c-g	28.3 ± 0.2 g
	Drought + ABA_{20}	14.24 ± 0.42 d	70.32 ± 1.54 b-e	19.08 ± 0.33 abc	102.5 ± 1.4 a-e	33.3 ± 0.4 b-e
	Drought + GB_{50}	16.56 ± 0.24 ab	91.00 ± 3.86 ab	18.73 ± 0.33 abc	103.4 ± 1.5 a-d	35.3 ± 0.3 a-d
	Drought + GB_{100}	16.34 ± 0.53 abc	88.25 ± 3.73 a-c	14.00 ± 0.32 f	75.6 ± 2.6 g	30.3 ± 0.7 efg
BARI GOM-33	Control	15.22 ± 0.37 a-d	107.19 ± 1.41 a	20.20 ± 0.42 a	91.0 ± 3.4 c-g	35.4 ± 0.7 abc
	Drought	11.81 ± 0.20 f	42.07 ± 2.87 fg	17.30 ± 0.19 b-e	78.4 ± 4.9 fg	29.1 ± 0. 6g
	Drought + ABA_{10}	11.88 ± 0.35 f	53.26 ± 1.41 efg	16.94 ± 0.41 cde	112.0 ± 1.4 ab	28.8 ± 0.4 g
	Drought + ABA_{20}	14.35 ± 0.28 cd	79.58 ± 13.29 bcd	18.63 ± 0.45 abc	116.1 ± 1.7 a	33.0 ± 0.3 cde
	Drought + GB_{50}	15.01 ± 0.22 bcd	78.73 ± 3.91 bcd	18.80 ± 0.24 abc	105.2 ± 3.4 abc	33.8 ± 0.6 a-d
	Drought + GB_{100}	15.01 ± 0.33 bcd	80.26 ± 3.92 bcd	17.51 ± 0.32 bcd	104.5 ± 6.5 a-d	32.5 ± 0.5 c-f
BARI GOM-21	Control	16.55 ± 0.26 ab	108.60 ± 2.99 a	19.73 ± 0.37 ab	95.7 ± 3.7 b-f	36.3 ± 1.3 ab
	Drought	14.18 ± 0.26 d	53.13 ± 5.89 efg	15.50 ± 0.60 def	86.1 ± 2.6 c-g	29.7 ± 0.8 fg
	Drought + ABA_{10}	14.11 ± 0.21 de	57.06 ± 5.97 d-g	15.17 ± 0.56 def	84.0 ± 1.7 efg	29.3 ± 0.2 g
	Drought + ABA_{20}	14.83 ± 0.37 bcd	65.22 ± 4.57 c-g	14.99 ± 0.50 ef	104.5 ± 3.8 a-d	29.8 ± 0.1 fg
	Drought + GB_{50}	15.94 ± 0.28 a-d	68.38 ± 2.10 b-e	19.13 ± 0.54 abc	94.2 ± 6.8 b-g	35.1 ± 0.4 a-d
	Drought + GB_{100}	16.38 ± 0.43 ab	59.45 ± 2.92 d-g	16.02 ± 0.21 def	86.8 ± 3.2 c-g	32.4 ± 0.6 def

Values are mean ± SEM. In a column, treatment means with dissimilar letters are significant at 5 % levels of probability. Drought = PEG 10 %; Drought + ABA_{10} = PEG10 % + ABA 10 μM; Drought + ABA_{20} = PEG10 % + ABA 20 μM; Drought + GB_{50}= PEG10 % + GB 50 mM; Drought + GB_{100}= PEG10 % + GB 100 mM.

3. Results

3.1. Morphological traits for priming experiment

Drought stress induced by PEG had a substantial negative effect on the seedling growth in all three wheat cultivars (Table A1). However, seed priming with ABA and GB improved the seedling growth in all cultivars while growing under drought conditions (Table 1, Fig. 1 and A2). Moreover, different levels of ABA and GB caused variably to seedling growth. In the case of ABA seed priming under drought, the longest root, shoot and maximum plant height were recorded in Drought + ABA_{20} treatment than in Drought treatment in all cultivars (Table 1, Fig. 1). In contrast with GB priming, the highest root and shoot length and tallest plant were observed in Drought + GB_{50} treatment in comparison to Drought and Drought + GB_{100} treatments in the studied cultivars (Table 2, Fig. 1 and A2). Root and shoot fresh weight were significantly reduced under drought conditions (Drought) compared to control but the primed seeds with ABA and GB grown under drought increased the root and shoot fresh weight (Table 1). The seed priming with 20 μM ABA and 50 mM GB ameliorated root and shoot fresh weight compared to other priming treatments (Table 1). Drought stress significantly reduced the shoot, root and total dry weight in three wheat cultivars and considerable variations in biomass were also detected among the six treatments (Table 2). The shoot, root and total dry weight ranged from 11.18 to 17.36 mg; 21.6–50.68 mg and 33.7–63.9 mg in all cultivars, respectively (Table 2). Similar to the other traits, the shoot, root and total dry weight were found considerably higher in Drought + ABA_{20} and Drought + GB_{50} treatments in comparison to the other treatments except for control in all three wheat cultivars (Table 2). Total fresh weight exhibited a similar pattern as total dry weight. An increased fresh and dry weight of root in all drought + priming treatments was observed in BARI GOM-33 compared to the other two cultivars (Tables 1 and 2).

3.2. Stress tolerance index (STI) of priming traits

To better view the extent of drought tolerance, the stress tolerance index (STI) values of all traits against treatment combinations are presented in a hierarchical clustering heatmap (Fig. 2). Considering the existing variations in the traits, the treatment combinations were categorized into two distinct row-clusters, while the traits were grouped into three column clusters (Fig. 2). Cluster-1 consisted of six interventions, while Cluster-2 had nine. The heatmap clearly reflects that the seed treatment with 20 μM ABA and 50 mM GB in all cultivars (Cluster-1) exhibited greater drought tolerance showing more greenish (higher STI values) in the studied traits. The sole treatments of drought in all cultivars perceived a more pinkish color exhibiting drought susceptibility. Comparing the cultivars while growing individual drought conditions, BARI GOM-21 performed better in comparison to the other two cultivars. Furthermore, the heatmap refined that seed priming with 20 μM ABA and 50 mM GB was more effective than 10 μM ABA and 100 mM GB, respectively under drought conditions in all cultivars. The traits were clustered into three closely related groups, such as Group-I, Group-II and Group-III and comprised of one (RSR), three (SL, SFW and SDW) and six (RL, PH, RDW, TDW, RFW and TFW) traits, respectively (Fig. 2).

3.3. Seedling growth under a hydroponic system

The lengths of root (RL) and shoot (SL) of the plants grown in a hydroponic system were significantly reduced in drought conditions compared to the control (Table A1, Fig. 3). The RL in Drought + ABA and Drought + GB treatments were significantly higher than Drought in all cultivars except BARI GOM-33. A similar pattern was followed to SL in all cultivars (Fig. 3). The root length showed higher sensitivity to drought stress compared to shoot length. The plants grown under Drought + ABA performed better than Drought + GB in relation to root and shoot length (Fig. 3). Root Fresh Weight (RFW) and Shoot Fresh Weight (SFW) significantly differed among the treatments (Table A1, Fig. 4). The maximum RFW and SFW were recorded in control plants followed by Drought + ABA, Drought + GB and Drought treatments. The plants grown with ABA had significantly higher RFW than with GB under drought conditions in all

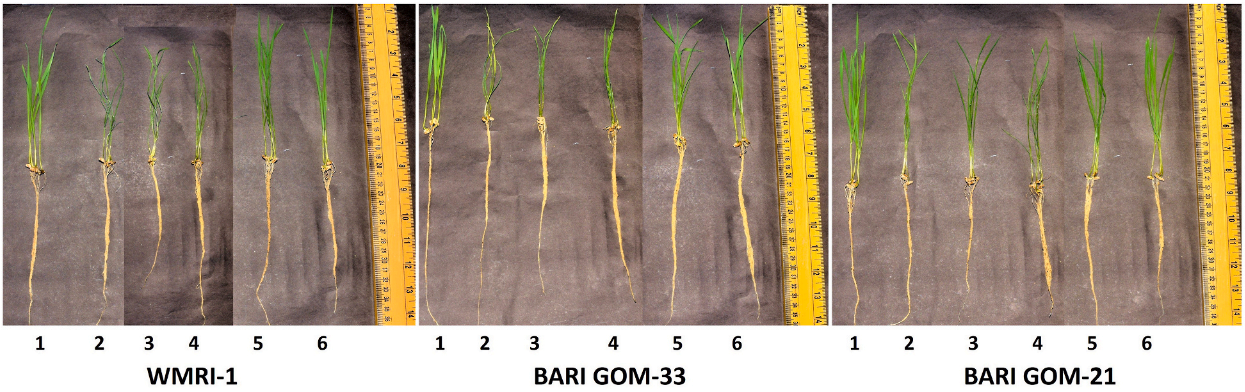

Fig. 1. Seedlings of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) established under six growth conditions– (1) Control; (2) Drought (PEG 10 %); (3) Drought + ABA_{10}; (4) Drought + ABA_{20}; (5) Drought + GB_{50} and (6) Drought + GB_{100}.

Table 2
Shoot Dry Weight (SDW), Root Dry Weight (RDW), Total Fresh Weight (TFW) and Root Shoot Ratio (RSR) of three wheat cultivars grown under six growth conditions.

Cultivar	Treatments	SDW (mg plant^{-1})	RDW (mg plant^{-1})	TFW (mg plant^{-1})	TDW (mg plant^{-1})	RSR
WMRI-1	Control	17.36 ± 1.43 a	38.99 ± 1.04 b	217 ± 9. 6 a	56.4 ± 0.4 ab	0.26 ± 0.01 ab
	Drought	11.52 ± 0.19 de	28.05 ± 1.95 fg	127 ± 1.3 jk	39.6 ± 1.8 ef	0.31 ± 0.01 ab
	Drought + ABA$_{10}$	14.05 ± 0.98 a-e	30.72 ± 0.78 def	153 ± 3.2 g-j	44.8 ± 1.4 cde	0.29 ± 0.01 ab
	Drought + ABA$_{20}$	14.53 ± 0.72 a-e	39.31 ± 1.18 b	173 ± 13.7 c-h	53.9 ± 1.9 b	0.32 ± 0.01 a
	Drought + GB$_{50}$	16.510.46 ab	37.77 ± 0.69 bcd	194 ± 4.6 a-e	54.3 ± 1.6 b	0.28 ± 0.01 ab
	Drought + GB$_{100}$	14.90 ± 0.75 a-d	25.16 ± 0.58 fg	164 ± 8.2 e-i	40.1 ± 1.3 ef	0.25 ± 0.02 ab
BARI GOM-33	Control	16.0 ± 0.17 abc	42.11 ± 1.59 b	198 ± 3.5 abc	58.1 ± 1.9 ab	0.29 ± 0.01 ab
	Drought	12.09 ± 0.12 de	21.60 ± 1.19 g	121 ± 2.5 k	33.7 ± 1.0 f	0.28 ± 0.01 ab
	Drought + ABA$_{10}$	11.18 ± 0.30 e	41.93 ± 0.45 b	165 ± 2.0 d-i	53.1 ± 1.2 bc	0.32 ± 0.00 a
	Drought + ABA$_{20}$	13.16 ± 0.58 b-e	50.68 ± 1.78 a	196 ± 3.9 a-d	63.9 ± 2.5 a	0.33 ± 0.03 a
	Drought + GB$_{50}$	14.70 ± 0.25 a-d	40.78 ± 2.34 b	184 ± 7.6 b-g	55.5 ± 0.5 b	0.30 ± 0.01 ab
	Drought + GB$_{100}$	14.34 ± 1.00 a-e	36.18 ± 1.81 bcd	185 ± 8.2 b-f	50.5 ± 1.3 bcd	0.28 ± 0.01 ab
BARI GOM-21	Control	17.31 ± 0.78 a	35.67 ± 0.95 b-e	204 ± 3.4 ab	53.0 ± 1.3 bc	0.26 ± 0.01 ab
	Drought	11.24 ± 0.43 e	31.42 ± 0.55 c-f	139 ± 4.9 ijk	42.7 ± 1.0 de	0.31 ± 0.01 ab
	Drought + ABA$_{10}$	12.69 ± 0.67 cde	25.62 ± 1.46 fg	141 ± 0.8 ijk	38.3 ± 0.5 ef	0.27 ± 0.01 ab
	Drought + ABA$_{20}$	13.51 ± 0.74 b-e	36.38 ± 0.73 bcd	170 ± 6.7 c-i	49.9 ± 2.3 bcd	0.29 ± 0.01 ab
	Drought + GB$_{50}$	14.21 ± 0.51 a-e	38.53 ± 1.34 bc	163 ± 5.2 f-i	52.7 ± 1.9 bc	0.32 ± 0.02 a
	Drought + GB$_{100}$	14.89 ± 0.36 a-d	28.73 ± 2.68 efg	146 ± 3.0 h-k	43.6 ± 2.7 de	0.30 ± 0.02 ab

Values are mean ± SEM. In a column, treatment means with dissimilar letters are significant at 5 % levels of probability. Drought = (PEG 10 %); Drought + ABA$_{10}$ = PEG 10 % + ABA 10 μM; Drought + ABA$_{20}$ = PEG 10 % + ABA 20 μM; Drought + GB$_{50}$= PEG 10 % + GB 50 mM; Drought + GB$_{100}$= PEG 10 % + GB 100 mM.

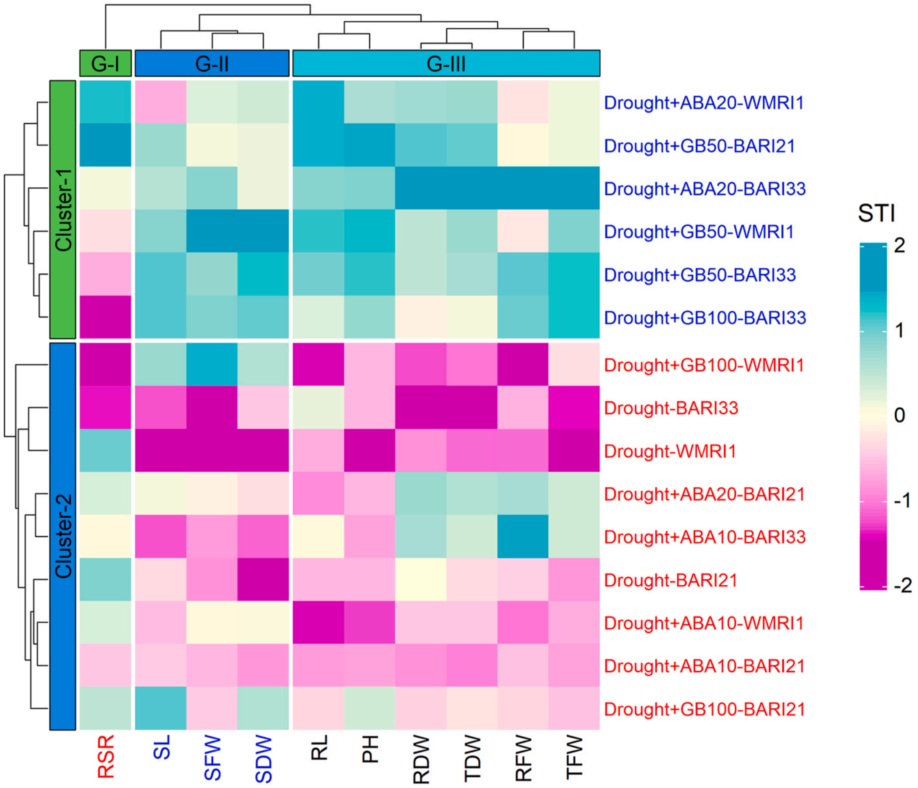

Fig. 2. The heatmap illustrates the hierarchical clustering of traits (column-wise) and treatments (row-wise) based on the stress tolerance index (STI) values. The rows and columns represent the traits and treatments, respectively. Colors are representative of a relative scale of −2 to 2 which is derived from the data normalization of the STI values. The darker pink indicates lower STI, while the darker green indicates higher STI values. The traits were clustered into three groups whereas, the treatments were grouped into two distinct clusters. BARI21 = BARI GOM-21, BARI33 = BARI GOM-33 and WMRI1= WMRI-1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

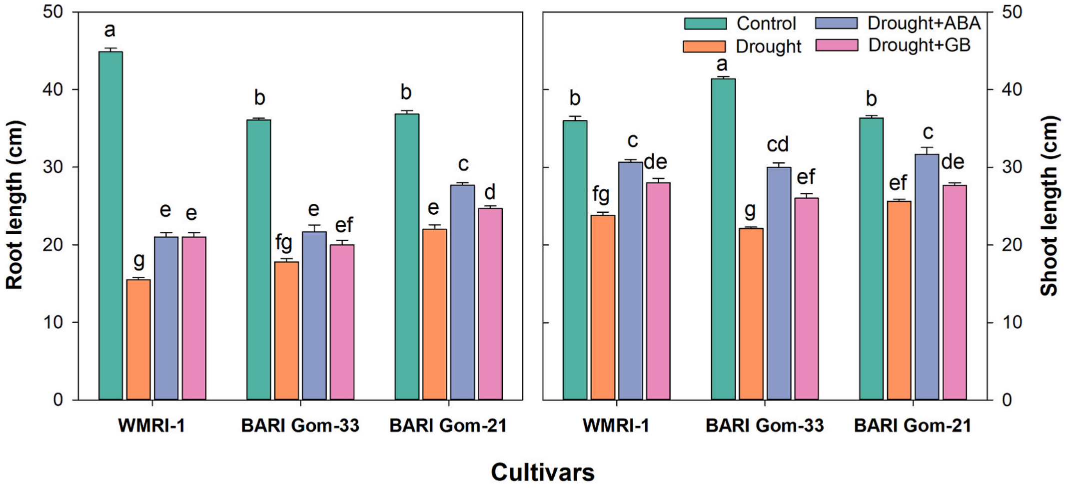

Fig. 3. Root length (cm) and shoot length (cm) of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions under the hydroponic system. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

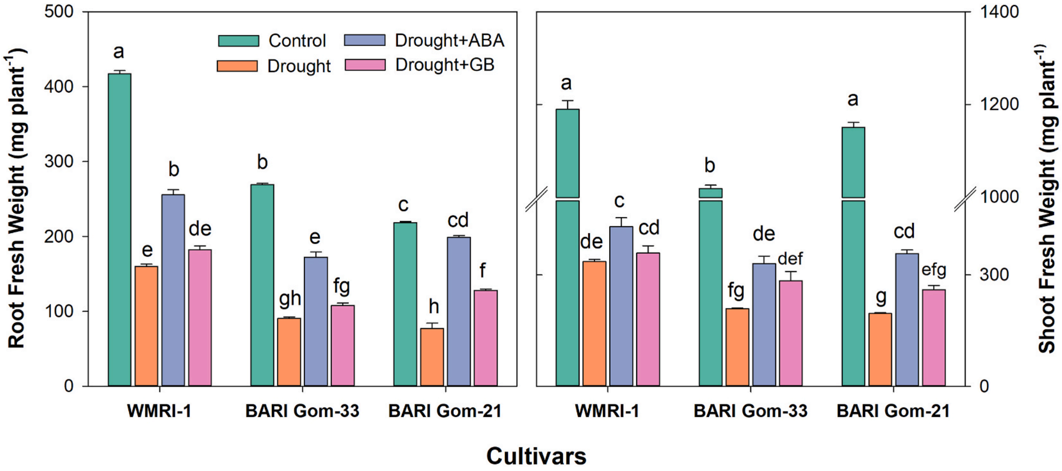

Fig. 4. Root fresh weight (mg) and shoot fresh weight (mg) of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions under the hydroponic system. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

cultivars, but the differences in SFW between the two treatments were statistically significant only in BARI GOM-21 (Fig. 4). A substantial variation in root and shoot dry weight was found in control and drought alone and with Drought + ABA and Drought + GB treatments (Table A1, Fig. 5). Highest RDW and SDW were observed in the controlled or normal condition except the RDW in BARI GOM-33. Plants grown in drought alone had the poorest root dry weight and compared to this treatment, significantly higher values were found in PEG with ABA and GB treatments. The SDW was affected more severely than RDW in drought with or without ABA and GB applications and the differences in RDW among the drought treatments were statistically insignificant (Fig. 5).

3.4. *Lipid peroxidation (LPO) in root and leaf*

The plants of all wheat cultivars grown in PEG-induced drought conditions experienced oxidative stress revealing significantly raised lipid peroxidation (Table A1, Fig. 6). The LPO in root and leaf ranged from 14.4 to 61.2 and from 10.9 to 61.5 nmol MDA g^{-1} FW, respectively. The LPO in both root and leaf in all cultivars were substantially increased in Drought treatments while reduced leaf and root LPO was found in Drought + ABA and Drought + GB treatments followed by control (Fig. 6). The LPO in both root and leaf in Drought + ABA and Drought + GB was closer to control and showed an insignificant difference in some of the treatment combinations. Among the cultivars, the lowest LPO in root and leaf was found in WMRI-1 and BARI GOM-21, respectively in drought-alone growth conditions (Fig. 6).

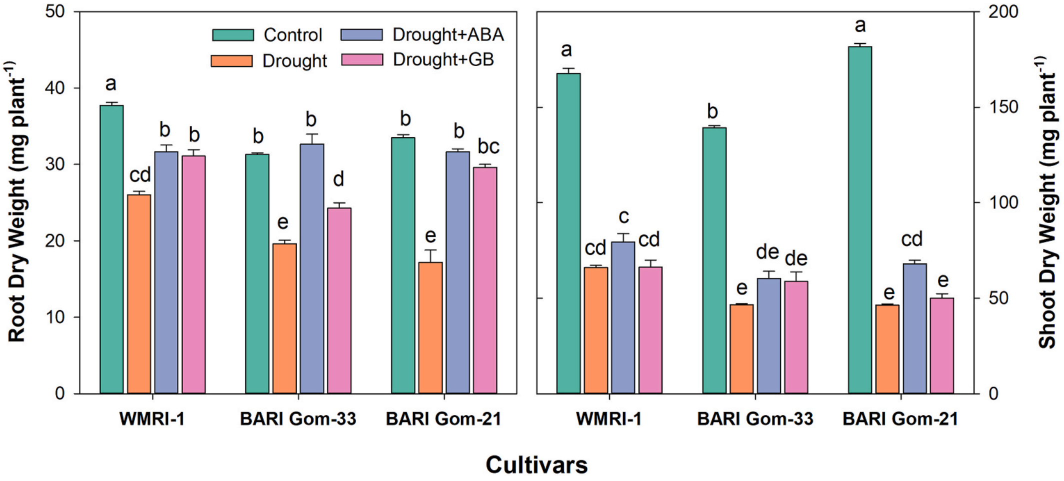

Fig. 5. Root dry weight (mg) and shoot dry weight (mg) of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions under the hydroponic system. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

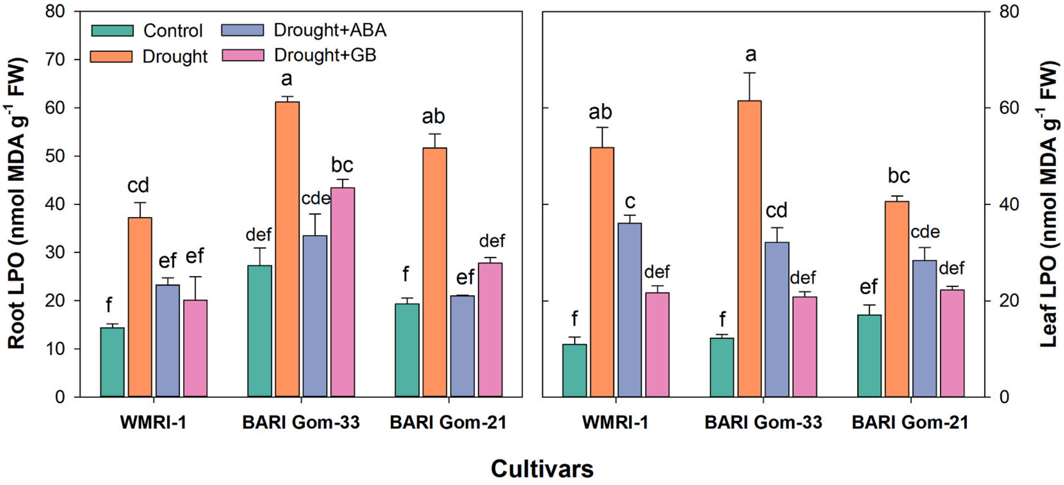

Fig. 6. Lipid Peroxidation (LPO) in root and leaf of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

3.5. Proline content in root and leaf

The proline contents in the root and leaf of all three wheat cultivars altered notably but the extent of variations was greater in the leaf than in the root (Table A1, Fig. 7). In the root, the highest proline accumulation was recorded in Drought treatments in all cultivars except BARI GOM-33 while the lowest in Control condition. In the leaf, the proline contents remarkably enhanced in Drought conditions of all cultivars compared to other growth conditions. Among the drought conditions, Drought + ABA and Drought + GB had higher leaf proline contents compared to Drought treatment, but the differences were statistically insignificant in all cultivars except BARI GOM-33 (Fig. 7).

3.6. Total antioxidant capacity (TAC) and total flavonoid contents (TFC) in root and leaf

The root and leaf total antioxidant capacity (TAC) were significantly increased in three wheat cultivars in drought conditions compared to the control (Table A1, Fig. 8). Among drought treatments, the fluctuations in root TAC were insignificant and the drought values showed significant variations with control in all cultivars (Fig. 8). In leaf, Drought got maximum TAC followed by Drought + GB, Drought + ABA and control in WMRI-1 and BARI GOM-33 whereas the TAC found significantly higher in Drought + ABA than Drought + GB in BARI GOM-21 (Fig. 8). Data showed a higher TAC in leaf than root in both control and individual drought conditions

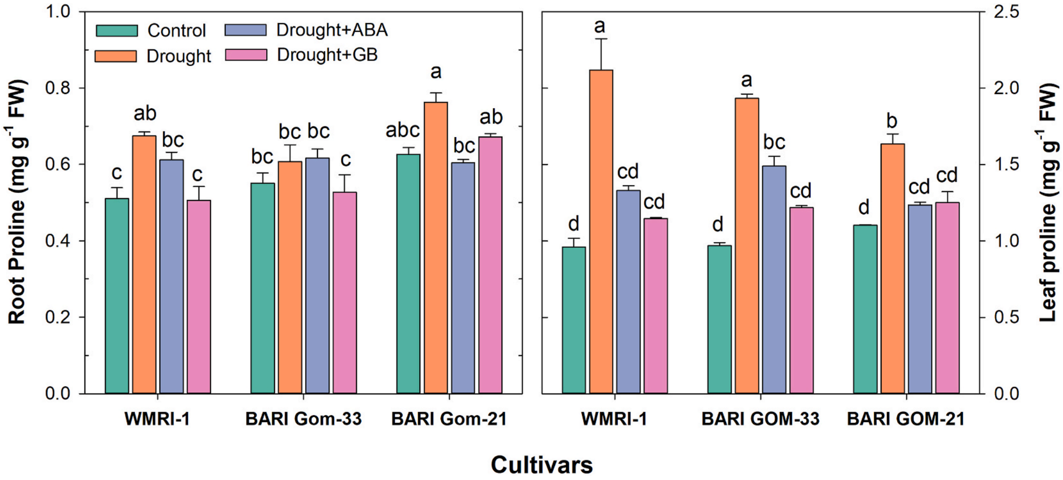

Fig. 7. Proline content in root and leaf of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

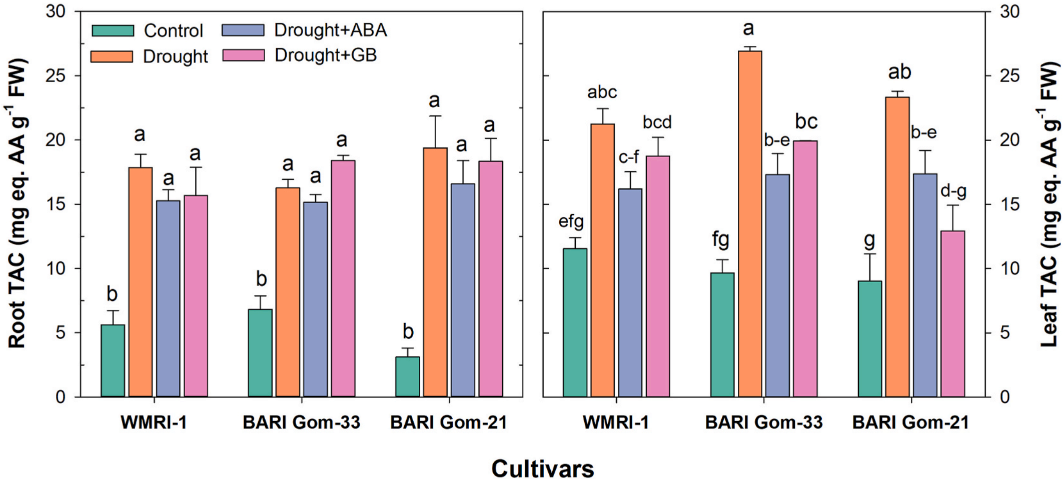

Fig. 8. Total Antioxidant Capacity (TAC) in root and leaf of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

in all cultivars (Fig. 8). Similar to proline contents and TAC, the alteration among the treatments in total flavonoid contents (TFC) were greater in leaf than root in all wheat cultivars (Fig. 9). The lowest and highest leaf TFC was observed in Control (0.28 mg eq. Quercetin g^{-1} FW) and Drought + ABA (0.57 mg eq. Quercetin g^{-1} FW), respectively in BARI GOM-33 (Fig. 9).

3.7. Leaf pigment contents

The pigments contents in leaves of all wheat cultivars showed substantial variations among the treatments (Table A1, Fig. 10). The leaf chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Total Chl) and total carotenoids contents of all cultivars were considerably greater in Drought + GB in comparison to the other treatments (Fig. 10). All cultivars suffered drastically in relation to pigment contents under Drought conditions. Chl *a* was highest in Drought + GB and lowest in Drought of WMRI-1. Chl *b* in contrast was highest in Drought + GB of WMRI-1 and lowest in Drought of BARI GOM-33. Total Chl contents followed a similar pattern as Chl *a*. In all wheat cultivars, the total carotenoid contents were significantly higher in Drought + GB while the individual drought-treated leaves noticeably lost total carotenoid contents (Fig. 10).

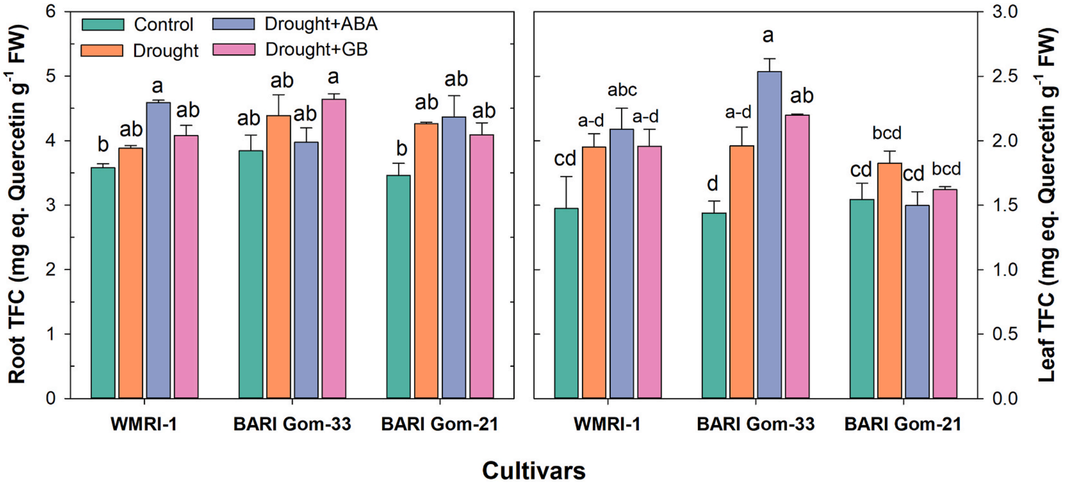

Fig. 9. Total Flavonoid Contents (TFC) in root and leaf of three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

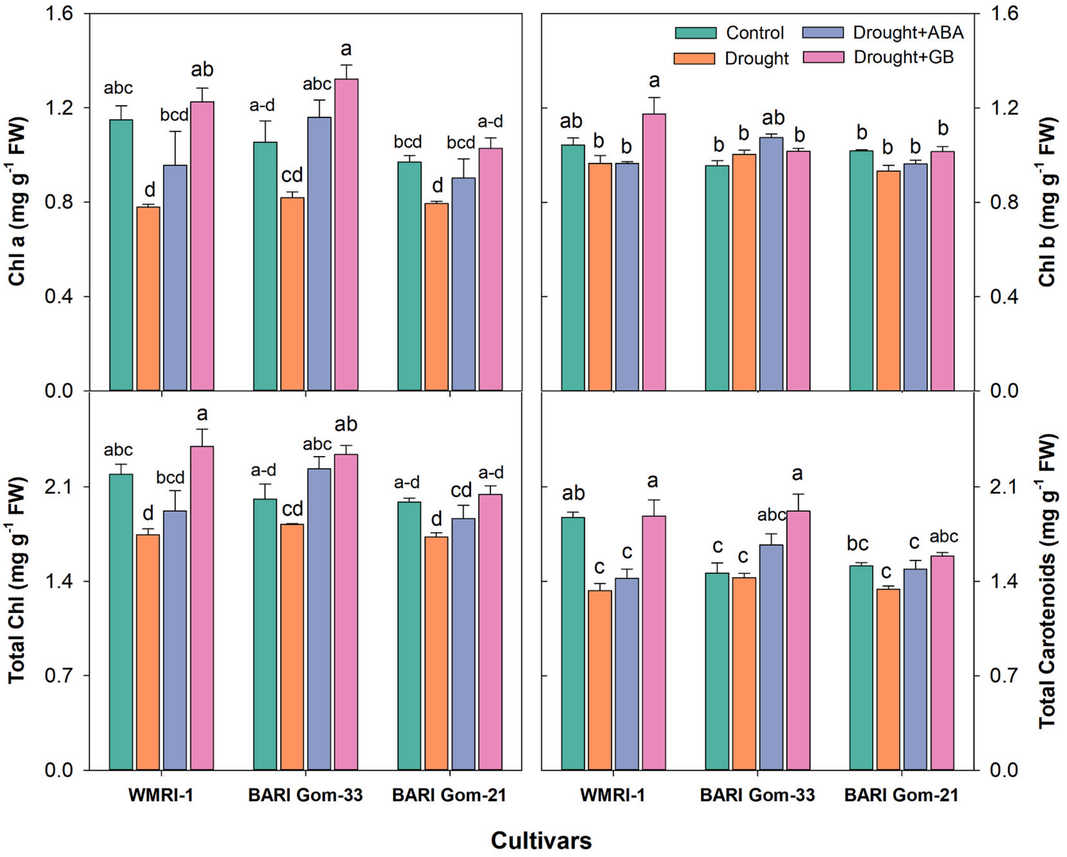

Fig. 10. Leaf Chlorophyll *a*, Chlorophyll *b*, Total chlorophyll and Total Carotenoids contents in three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) grown in Control, Drought, Drought + ABA and Drought + GB conditions. The vertical bars represent SEM (n = 3). Treatment means with dissimilar letters within the cultivars denote significant at 5 % levels of probability.

3.8. *Stress tolerance index (STI) and heatmap*

The degree of drought tolerance is visualized by the hierarchical clustering heatmap considering the STI values of the 20 studied parameters recorded in the hydroponic System (Fig. 11). The treatment combinations were classified into three clusters, as shown in rows. Cluster-I comprised of individual drought treatments of three cultivars, Cluster-II with drought with ABA and GB of BARI GOM-21 and Cluster-II with the rest of the treatments (Fig. 11). The studied 20 traits were categorized into three groups as shown in columns. The traits that were decreased under drought conditions got lower STI (more pinkish) and were placed in Group 1 and 3. On the other hand, those who were increased in drought got higher STI (more greenish) and were placed in Group 2. Thus, the treatments scoring higher STI values of Groups 1 and 3 traits and lower STI of Group 2 traits were considered as greater drought tolerance. Considering these criteria, the individual drought (Cluster-I) had a detrimental effect on the parameters in all cultivars, while the drought with ABA and GB treatments (Cluster-II and III) exhibited enhanced drought tolerance in all cultivars (Fig. 11). Among the cultivars, BARI GOM-21 responded better in relation to the enhancement of drought tolerance with the application of ABA and GB.

4. Discussion

Drought is a severe abiotic stress that can become extreme due to global climate change and thus affects plant growth and yield [56]. Wheat, an important staple food worldwide, is widely grown in drought-prone areas and the increasing water scarcity condition causes considerable yield losses in agriculture. Therefore, to cut yield losses and ensure food security, it is very crucial to enhance drought tolerance in wheat. The exogenous application of phytohormones or osmolytes is one of the cutting-edge and essential techniques for drought tolerance. ABA and GB aid in reducing the detrimental effects of water stress on plants [18,57]. ABA is an important stress phytohormone that provides an adaptive physiological response for plants conferring abiotic stress tolerance [58]. GB, on the other hand, is a compatible solute that protects cellular structures by adjusting osmotic balance under abiotic stresses [59]. Considering this viewpoint, we investigated the influence of seed priming and exogenous application of abscisic acid and glycine betaine for the enhancement of drought tolerance in wheat at the seedling stage. The results in this study revealed that the root and shoot growth significantly declined in the plants grown in individual drought (10 % PEG) compared to well-watered conditionsand. The water scarcity condition could severely decline the turgidity and cell expansion at root meristem causing the shrinkage of root length and proliferation [60]. As a result, uptake of essential mineral nutrients might be limited and thus, restricted proper growth and reduced seedling biomass [61]. In contrast, seed priming with ABA and GB in all three wheat cultivars (WMRI-1, BARI GOM-33 and BARI GOM-21) in the present study showed an increased root and shoot growth (length and biomass) under drought conditions compared to the non-primed seeds. Although both levels of ABA and GB increased the root and shoot growth, 20 μM ABA and 50 mM GB performed better among the drought treatments with or without priming. An increased root and shoot growth were also revealed when 20 μM ABA and 50 mM GB were exogenously applied with a nutrient solution in PEG-induced drought conditions. The results suggest that exogenous ABA decreased the growth inhibition caused by 10 % PEG-stimulated drought stress exhibiting enhanced drought tolerance of wheat seedlings, similar to the previous reports in wheat [34], rice [62], maize [63], barley [64] and sesame [65] under osmotic stresses.

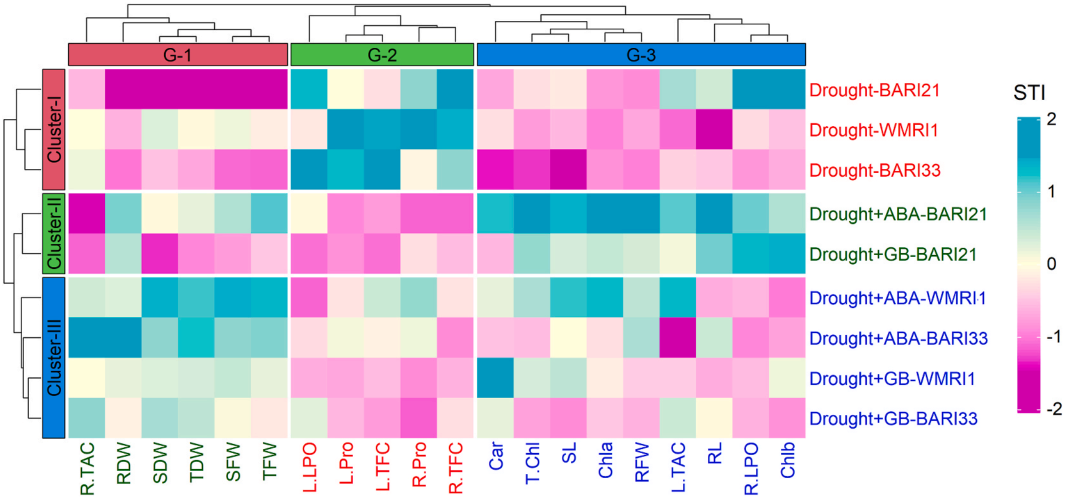

Fig. 11. The heatmap with dendrogram represents the clustering of traits (column-wise) and treatments (row-wise) based on the stress tolerance index (STI) values. BARI21 = BARI GOM-21, BARI33 = BARI GOM-33 and WMRI1= WMRI-1. R.TAC = Root Total Antioxidant Capacity; RDW = Root Dry Weight; SDW= Shoot Dry Weight; TDW = Total Dry Weight; SFW= Shoot Fresh Weight; TFW = Total Fresh Weight; L. LPO = Leaf Lipid Peroxidation; L. Pro = Leaf Proline; L.TFC = Leaf Total Flavonoid Content; R. Pro = Root Proline; R. TFC= Root Total Flavonoid Content; R. LPO= Root Lipid Peroxidation; Chlb = Chlorophyll *b*; RL = Root Length; L. TAC = Leaf Total Antioxidant Capacity; RLWC= Relative Leaf Water Content; Car = Total Carotenoids; T. Chl = Total Chlorophyll; SL= Shoot Length; Chla = Chlorophyll *a*; RFW= Root Fresh Weight.

Several reports suggested that the pre-treatment of ABA and GB increased seedling growth in many crop plants under different abiotic stress conditions [58,6658,66–6858,66–68]. ABA has been reported to improve seed dormancy, germination, dormancy as well as vegetative growth in plants [69,70]. In limited water condition, ABA changes root morphology and alters the growth pattern and dormancy in roots [71]. Water flow and ion flux in root tissues can be stimulated by the exogenous ABA regulating turgor pressure through reducing transpiration as well as by enhancing water flow into roots [72]. It has been reported that ABA maintains primary root growth under lower water potential in maize by limiting ethylene production, which acts as an inhibitor in root elongation [73]. Another evidence suggests that ABA may perform in the maintenance of root meristem and stem cells by promoting quiescence center (QC) and suppressing the stem cell differentiation and thus, modulating root architecture to adopt the abiotic stressed situations [74,75]. However, other protective roles of ABA such as stomatal closing, decreasing transpiration and solute uptake might also be related to the ABA-induced drought tolerance in plants [76]. On the other hand, GB is an important compatible solute that might improve seedling growth in all wheat cultivars by improving the tissue water status and maintaining the osmotic balance for proper root and shoot growth [24]. Previous reports also highlighted the enhancement of drought tolerance with the exogenous application of GB in wheat and other crop plants [37,68,77].

The findings from this investigation revealed that the lipid peroxidation (MDA content) in roots and leaves of all wheat cultivars were significantly increased in Drought treatments compared to Control, Drought + ABA and Drought + GB treatments. This might be due to the excess buildup of ROS (singlet oxygen, hydrogen peroxide, superoxide radicals or hydroxyl radicals) under drought condition leading to serious oxidative damage and causes exaggerated lipid peroxidation of cellular membranes [78–8078–8078–80]. In contrast, a reduction in lipid peroxidation was observed in ABA and GB treatments in both root and leaves of all cultivars suggesting that the exogenous ABA and GB in roots quenched the excess ROS and thereby, alleviated oxidative damage. These findings indicated that the application of ABA and GB could regulate ROS homeostasis increasing membrane stability, and might protect cell organelles by lowering lipid peroxidation escaping the lethal effects of drought stress. Exogenous ABA and GB applications have been reported to decline ROS production and MDA contents in different plants [81,82]. Plants regulate a complex ROS production-detoxification system, and the scavenging of ROS is performed by the combined activities of low-molecular non-enzymatic (glutathione, ascorbate, carotenoids) and enzymatic antioxidants (SOD, CAT, POD, APX etc.) [83]. The increased activities of these enzymatic and other non-enzymatic antioxidants under ABA and GB treatment could play a crucial role in ROS scavenging in the present investigation. However, we were not able to measure the activities of these enzymes which is a major limitation of this study. Previous reports confirmed the increased production of enzymatic antioxidants under drought conditions combined with the exogenous application of ABA and GB in plants [21,22,37,57,68,84].

The total antioxidant capacity (TAC) and total flavonoids contents in the root of all three wheat cultivars in the present study showed no significant differences among the drought treatments (Drought, Drought + ABA and Drought + GB) whereas lipid peroxidation was significantly higher in individual Drought treatments. It indicates a relatively higher production of antioxidants in Drought + ABA and Drought + GB treatments than in Drought and thereby, reduced the oxidative damage showing significantly lower MDA contents. On the other hand, leaf TFC followed a similar pattern, but leaf TAC contents were significantly higher in Drought compared to the other treatments. Phenolic compounds having aromatic ring in their structures help to prevenet cellular membrane by scanvenging ROS under abiotic stresses [85]. The shoot development (length and biomass) was affected more under drought conditions than root growth and it might be due to the higher ROS production and subsequently lower antioxidant activities and proline contents in the leaf than root. The roots might be benefited from the direct absorption of ABA and GB from the soil solution leading to increased endogenous ABA and GB in roots exhibiting enhanced drought tolerance.

Plants tend to accumulate low-molecular osmolytes such as proline and soluble sugars to maintain osmotic adjustment and quench the ROS in limited water conditions [86,87]. In this study, PEG-induced drought treatments accumulated significantly higher proline contents in root and leaves, particularly in the non-primed plants of Drought treatment. Proline and soluble sugars accumulation in plants have been reported to aid in improving membrane stability attaining better water status in the tissues by the osmotic balance and reducing ROS [24,88]. Proline and soluble sugars as well as other osmoregulatory substances can be significantly increased by exogenous ABA signaling resulting in improved osmotic adjustment in plants [89]. The findings in this study are supported with other studies in relation to the accumulation of proline under ABA and GB application for the enhancement of drought tolerance in wheat and other plants [57,68,90,91]. The plants with ABA and GB application enhanced drought tolerance might be due to the increased expressions of genes at the transcription level. *TaNCED* and *ABA8'OH2* are considered to be the most prominent genes incovled in ABA biosynthesis and regulating endogenous levels of ABA in plants [92,93]. The ABA and GB-primed wheat roots in our study might be attributed to greatly higher expression of these genes as compared to the non-priming roots under drought conditions.

Under drought stress, reduction in leaf pigments has been considered a characteristic sign of pigment photooxidation and chlorophyll degradation [56]. Both chlorophyll *a* and *b* are sensitive to soil dryness [86]. In this research, the leaf chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Total Chl), and total carotenoids of all cultivars were substantially declined in the Drought (PEG 10 %) condition than in the Drought + ABA or GB conditions. It is also noticeable that cultivars with Drought + GB performed better than Drought + ABA. The higher contents of total chlorophyll and total carotenoids in ABA and GB-treated plants supported the phenomena of enhanced drought tolerance and exhibited the capability of greater photosynthesis under drought conditions. A similar observation was also reported by Ref. [57]. Stress tolerance index (STI) is regarded as an important indicator for estimating drought tolerance in plants because it is derived from the relative changes in stress interventions compared to control values [47,94]. In this study, seed priming with ABA (20 μM) and GB (50 mM) outperform having higher STI scores compared to individual drought treatment exhibiting a greater degree of drought tolerance in all three studied wheat cultivars. The vital traits reflected in this study were lipid peroxidation, proline content, total flavonoid content, root andand shoot dry weight suggesting that these traits would be considered for the screening of drought tolerance or further breeding program in wheat. All the cultivars responded similarly in relation to the priming and exogenous application of ABA and GB, but the cultivar BARI GOM-21 performed slightly better under drought conditions with or without ABA and GB priming.

5. Conclusions

Drought stress induced by 10 % PEG-6000 considerably impaired the seedling growth in three wheat cultivars. Seed priming with ABA and GB improved the seedling growth under drought conditions and among them 20 μM ABA and 50 mM GB outperformed. Seedling growth was also enhanced as well with the application of these two levels of ABA and GB in roots under PEG-induced drought conditions in hydroponic culture. In drought conditions, the lipid peroxidation, proline content, total antioxidant capacity and total flavonoid contents in roots and leaves were remarkably increased whereas reduced levels of these components were recorded in Drought + ABA and Drought + GB treatments. From the study, it can be concluded that the seed and root priming with ABA and GB simultaneously triggered drought tolerance in wheat seedlings by maintaining osmotic adjustment for proper seedling growth and effective detoxification of ROS minimizing the oxidative damage under drought.

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Table A1

Analysis of Variance (ANOVA) for several measured traits in three wheat cultivars grown in different growth conditions.

Experiment	Traits	Sources of variation			LSD of interactions @ 5 % LOS
		Cultivar (C)	Treatment (T)	C × T	
Germination	Shoot length (SL)	***	***	*	2.02
	Shoot Fresh Weight (SFW)	*	***	**	**25.29**
	Root Length (RL)	***	***	***	2.47
	Root Fresh Weight (RFW)	***	***	**	19.16
	Plant Height (PH)	NS	***	***	2.99
	Shoot Dry Weight (SDW)	*	***	NS	3.46
	Root Dry Weight (RDW)	***	***	***	7.39
	Total Fresh Weight (TFW)	***	***	***	31.32
	Total Dry Weight (TDW)	***	***	***	8.36
	Root Shoot Ratio (RSR)	NS	**	*	0.08
Hydroponic	Root Length (RL)	***	***	***	2.57
	Shoot length (SL)	NS	***	***	2.48
	Root Fresh Weight (RFW)	***	***	***	22.35
	Shoot Fresh Weight (SFW)	***	***	***	78.02
	Root Dry Weight (RDW)	***	***	***	4.01
	Shoot Dry Weight (SDW)	***	***	***	14.30
	Total Fresh Weight (TFW)	***	***	***	91.49
	Total Dry Weight (TDW)	***	***	***	16.81
	Root Lipid Peroxidation	***	***	*	13.67
	Leaf Lipid Peroxidation	NS	***	**	13.42
	Root Proline Content	***	***	*	1.59
	Leaf Proline Content	NS	***	**	3.96
	Root Total Antioxidant Capacity	NS	***	NS	7.07
	Leaf Total Antioxidant Capacity	*	***	*	6.89
	Root Total Flavonoid Contents	***	***	**	0.13
	Leaf Total Flavonoid Contents	NS	***	NS	0.19
	Chlorophyll *a*	**	***	NS	0.34
	Chlorophyll *b*	*	**	**	0.15
	Total Chlorophyll	**	***	NS	0.44
	Total Carotenoids	*	***	**	0.36

* = significant at 5 % level.

** = significant at 1 % level.

*** = significant at 0.1 % level, NS= Non-significant.

Fig. A1. Photographs showing experimental setup: a) seed germination and seedling growth of wheat cultivars in plastic pots filled with sterilized sands; b) Sprouting of wheat seeds in the plastic pots for seedling establishment in hydroponic system; and c) hydroponic system using plastic tanks with the seedlings of three wheat cultivars.

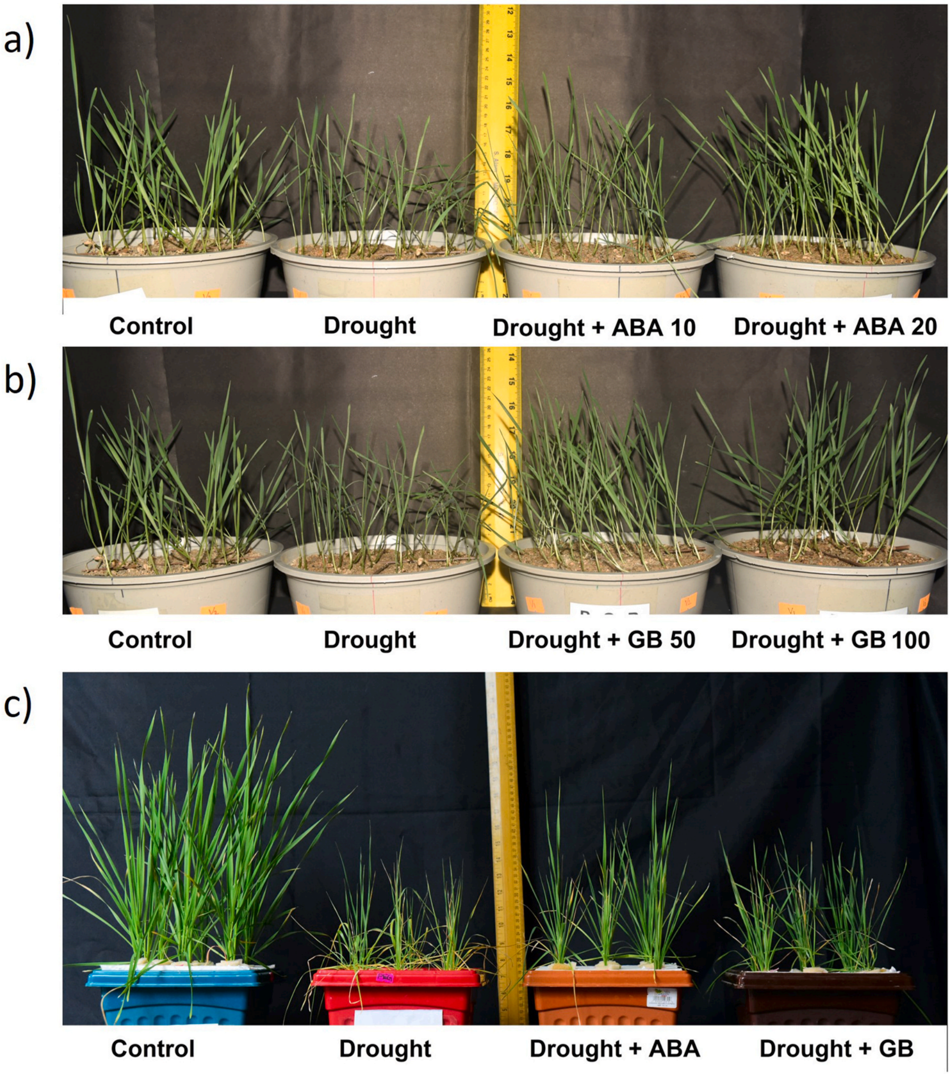

Fig. A2. a) 15 days old seedlings of three wheat cultivars grown in different growth conditions with ABA seed priming (Control, Drought, Drought + ABA_{10} and Drought + ABA_{20}); b) 15 days old seedlings of three wheat cultivars grown in different growth conditions with GB seed priming (Control, Drought, Drought + GB_{50} and Drought + GB_{100}); c) 28 days old seedlings of three wheat cultivars grown in different growth conditions (Control, Drought, Drought + ABA and Drought + GB) in hydroponic system.

CRediT authorship contribution statement

Artho Baroi: Writing – original draft, Methodology, Investigation, Data curation. **Sadia Afroz Ritu:** Methodology, Investigation. **Md Shihab Uddine Khan:** Methodology, Investigation. **Md Nesar Uddin:** Writing – review & editing, Supervision. **Md Alamgir Hossain:** Writing – review & editing, Supervision. **Md Sabibul Haque:** Writing – review & editing, Visualization, Supervision, Project administration, Formal analysis, Data curation, Conceptualization, Funding acquisition.

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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