

# Root responses under water deficit stress: unraveling the impact on wheat crop and the ameliorating role of brassinolide

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## Abstract

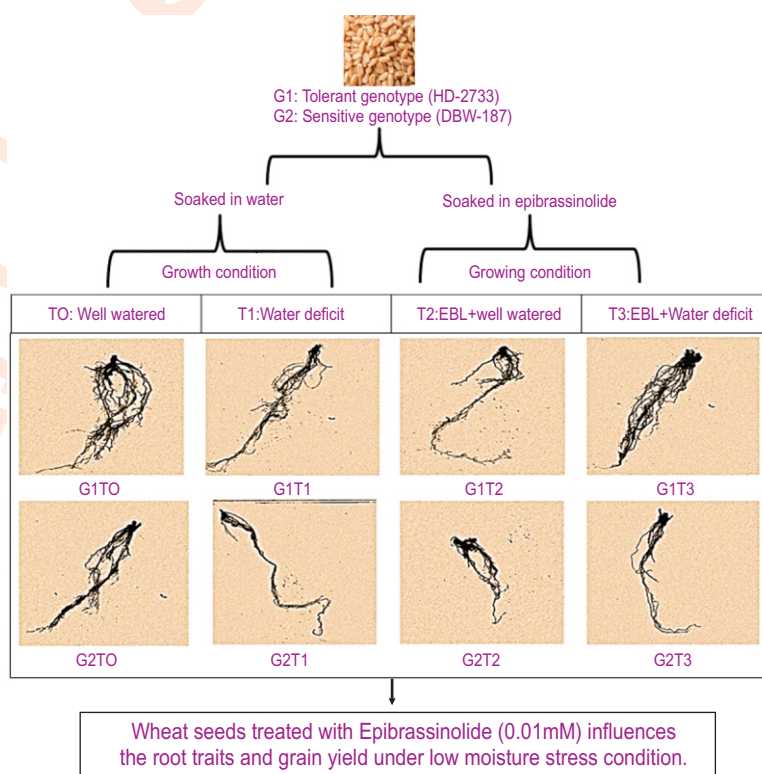
**Aim:** To identify changes in root system architecture traits of wheat due to exogenous application of epibrassinolide for the alleviation of negative impact of low moisture stress in wheat crop.

**Methodology:** On the basis of growth performances one set of contrasting wheat genotypes were identified (HD-2733, relatively stress tolerant and DBW-187 relatively stress sensitive). Similarly, brassinosteroids (BRs) concentration was selected by pilot experiments, wherein 0.01mM performed best among all. Taking all these results into consideration, four treatments (T0=well-watered, T1=water deficit, T3=EBL + well watered and T4=EBL + water deficit) were maintained for evaluation of root architectural traits, biomass and grain yield per plant.

**Results:** The tolerant genotype (HD-2733) showed better tolerance in almost all root traits and in yield as compared to the sensitive genotype (DBW-187). epibrassinolide under water deficit condition was found to be effective as the root trait values were higher for root length, root volume, root surface area and root biomass under EBL+ water deficit treatment as compared to water deficit. Shoot biomass was highly sensitive to water deficit as the biomass allocation under water deficit was more towards root as compared to shoot.

**Interpretation:** Epibrassinolide can be a potent biochemical to improve the root characteristics as well as yield per plant. Seeds of tolerant genotype treated with 0.01m M EBL even under low moisture stress can be recommended.

**Key words:** Epibrassinolide, Root system, Wheat, Water deficit



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## Introduction

As we proceed towards the year 2030 as well as beyond, the task of providing sustenance for India's expanding populace poses a significant challenge. It is projected that by 2030, the urban population of India will reach 600 million, necessitating a consistent provision of wholesome and secure food from the surrounding rural areas (United Nations, 2019). The existing challenge is exacerbated by the restricted accessibility and declining condition of natural resources, including land, water, and air. The challenge of climate change is an additional concern in India, as evidenced by the IPCC report, which highlights the increasing temperatures and more frequent and intense droughts in the western and southern regions as well as floods in the northern and north-eastern regions (Djalante, 2019). Wheat has been a staple food crop for the world population since it sustains 50% of the world population (Tubiello *et al.*, 2016). Wheat consumption is predicted to rise by 60% to feed the world's population and to accomplish, worldwide agricultural productivity must be increased and wheat yield should increase from the current 1% per year to a minimum of 1.6% (Huang *et al.*, 2023).

However, due to projected climate change, drought stress will become more frequent, which will lead to drastic changes in wheat yield potential. To mitigate stress, development of an efficient root system in wheat crop is necessary, as root is the first organ which senses drought. Roots serve as the primary organ of the plant system, facilitating anchorage in the soil and the provision of essential nutrients and water necessary for plant growth and development (Lynch, 2013). The spatial distribution of roots plays a crucial role in determining the source-sink relationship between plants and the intricate soil environment, as roots serve as the interface between the plants and soil. Several root characters such as root diameter, root length, root volume, root biomass and root surface area allow the plants to adapt and respond to various environmental factors and they might be quite useful for improving water use efficiency in wheat crop. Therefore, it is very important to understand the root system architecture regulating mechanisms for crop improvement. Different types of roots have special features such as primary root length, root diameter, root volume and root surface area which help in determining water absorption ability among various root types as an adaptation strategy under water deficit conditions. While there exist a considerable body of literatures on the phenotyping of above-ground traits, further advancements are necessary in the realm of root phenotyping research (Mann *et al.*, 2023).

The impact of abiotic stresses on crop productivity is attributed to their interference with the uptake and transportation of vital nutrients and water, which are essential for achieving optimal grain yield (Marková *et al.*, 2023). To mitigate the negative impact of stress on crop yield, one potential approach is to identify specific root phenotypes and implement treatments involving chemical compounds such as antioxidants, phytohormones, and polyethylene glycol, etc. Previous studies have explored the efficacy of these methods. Brassinosteroids (BRs) are a class of

compounds that exhibit steroid phytohormonal properties. These compounds are known to play a crucial role in regulating plant growth and development, as well as in enhancing plant defense mechanisms against various environmental stressors. BRs are particularly effective in improving water and nutrient use efficiency in plants (Marková *et al.*, 2023). Numerous studies have demonstrated that the application of BR results in increased drought tolerance through up regulation of antioxidant enzyme activities, including SOD, POD and CAT. Additionally, BR treatment leads to elevated levels of proline, which in turn results in reduced ROS production and malondialdehyde content (Castañeda *et al.*, 2022; Helaly *et al.*, 2022).

One of the BR named 24-Epibrassinolide (EBL) has been reported by scientists to have the ability to enhance plant tolerance to water deficiency through modification of various physiological processes, including the functioning of antioxidant enzymes, synthesis of osmoprotectants, levels of antioxidant molecules and H<sub>2</sub>O<sub>2</sub>, peroxidation of lipids, and membrane integrity in plants (Li *et al.*, 2021; Hafeez *et al.*, 2021). It is required for optimal plant growth and development, and it also regulates elongation of cells and division (Hafeez *et al.*, 2021). Hence, the exogenous application of EBL can lead to beneficial effects on root system architecture of wheat plant during water deficit stress. In view of the aforementioned facts, the present study was undertaken to assess the efficacy of two wheat genotypes (HD-2733 and DBW187) treated with epibrassinolides exogenously under conditions of water deficit stress with the objective to compare root system architecture traits of two wheat genotypes under water deficit stress, and to investigate the influence of epibrassinolide in relation to water deficit stress tolerance in wheat.

## Materials and Methods

The research work was carried out in the open environment, Department of Botany, Plant Physiology and Biochemistry, Dr Rajendra Prasad Central Agricultural University, Pusa (848125), Bihar, India. The experimental layout was a completely randomized design with four replications and four treatments (well-watered, water deficit, EBL + well watered, and EBL + water deficit). The root system architecture (RSA) is affected by various factors such as soil temperature, moisture, nutrients and soil pH which greatly affect crop growth and yield.

To analyse the effect of EBL on root system of wheat during water deficit stress: root length, root volume, root biomass, root surface area and average root diameter were recorded in full vegetative stage of the plants. by Win-RHIZO (Model-Epson Perfection V700 Photo) machine. To understand the effect of root architecture on shoot biomass, fully grown (peak vegetative stage) plants were selected and used for shoot biomass study. The shoot was placed in a hot air oven (Hot Point, Acme Instruments Co.) at 65±2°C for three days or until constant weight was obtained. The samples were weighed with a sophisticated electronic weighing balance (Afcoset, FX 400). The root shoot ratio of each treatment was calculated.

Experimental data were statistically analyzed by ANOVA using OPSTAT and the correlation analysis was carried out through KAUgrapes software (Gopinath *et al.*, 2020). The critical difference was calculated at 5% probability level.

## Results and Discussion

The influence of exogenously applied EBL (0.01mM) across water deficit treatments in two contrasting genotypes (HD-2733 and DBW-187) on root length is presented in Fig. 1a. Among the genotypes, HD-2733 performed better (152.12 cm) as compared to sensitive genotype (71.24 cm). Across the treatments EBL treated (132.24 cm) followed by EBL + water deficit (117.07cm) showed better performance. Root length had positive significant correlation with root volume, root biomass and root surface area. Tomar *et al.* (2016) used Win-Rhizo software to study the wheat seedling root traits targeting drought tolerance. Hussain *et al.* (2020) reported that the total root length of maize genotype; Yu13 significantly decreased at all the stress treatments, while in Yu37, chilling and drought + chilling stresses significantly decreased as compared to control. Among all treatments, the EBL + well watered and EBL + water deficit plants had superior root length which was necessary in mitigating water stress (Fig. 1a). In most crop species root length was affected to water deficit condition than shoot length (Rao *et al.*, 2021). EBL enhanced root length since it might have led to the converging of ABA signalling pathways and enhanced production of auxins and cytokinins to initiate responses to water deficit stress (González *et al.*, 2011; Gupta *et al.*, 2020).

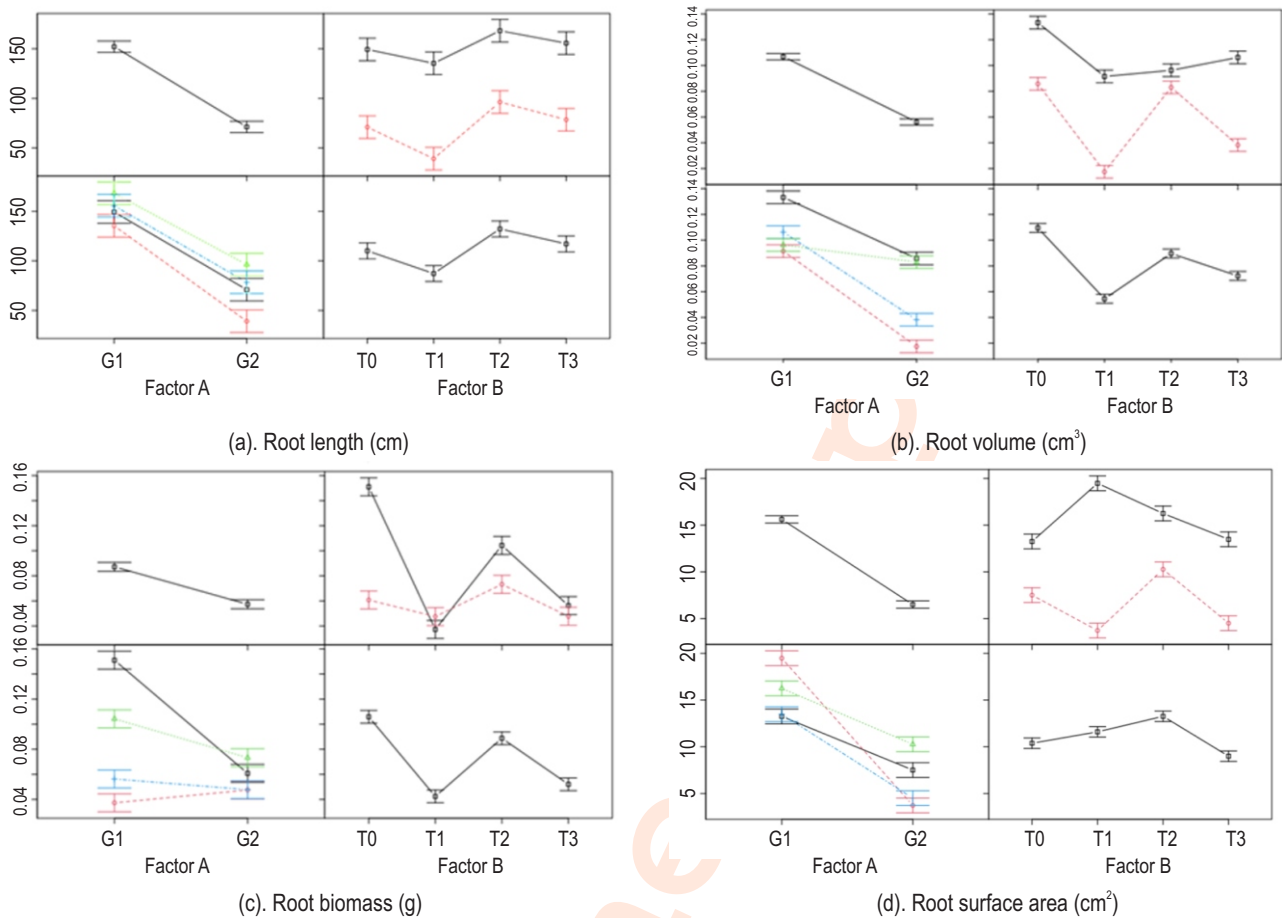
Interaction between auxins and cytokinins might have led to optimal growth of root apical meristem (González *et al.*, 2011; Sharma *et al.*, 2019). Moreover, brassinolides are also involved in cell expansion and elongation processes. Brassinolides stimulates proliferation of coleorhiza in dicots, as well as coleoptiles and mesocotyls in monocots (Cheon *et al.*, 2010; Zhiponova *et al.*, 2013). The expansion of cells induced by BRs is concomitant with the extrusion of protons and hyperpolarization of cell membrane. This phenomenon significantly enhances and expedites the progression of cell cycle growth (Bajguz and Czerpak, 1996). The influence of exogenously applied EBL (0.01mM) across water deficit treatments in two contrasting genotypes (HD-2733 and DBW-187) on root volume is shown in Fig. 1b. The tolerant genotype HD-2733 gave better response under well-watered having the value of 0.13 cm<sup>3</sup> followed by EBL + water deficit treatment of the same genotype (0.12 cm<sup>3</sup>). Similarly, the lowest response of sensitive genotype DBW-187 was recorded when treated with water deficit (0.02 cm<sup>3</sup>). Root volume had positive significant correlation with root length, root biomass, root surface area and shoot biomass. Salarpour *et al.* (2020) highlighted that longer roots might assist increased grain yield under water deficient conditions than root volume. Wang *et al.* (2022) reported significant decrease in root volume tolerant genotype (ZD958) and sensitive genotype (XD20) under water stress by 33.53% and 35.88%, respectively. Root volume as well as root elongation increased in potato crop due to the enhanced

activity of BL (Hu *et al.*, 2016). EBL played a major role in maintenance of ionic homeostasis and enzymatic activity as well as turgor pressure of root cells leads to cell elongation and expansion (Ashraf, 2004).

This leads to accumulation of more root biomass leads to increase in the root volume under stress (Tanveer *et al.*, 2019). Fig. 1c shows the influence of exogenous EBL (0.01mM) across water deficit treatments in two contrasting genotypes (HD-2733 and DBW-187) on root biomass. The tolerant genotype (HD-2733) showed better response under control treatment (0.15g) followed by EBL treatment of same genotype (0.10g) (0.04g). Similarly, the lowest response of tolerant genotype was recorded under water deficit conditions. Root biomass had positive significant correlation with root length, root volume, shoot biomass and root shoot ratio. In a study it was reported that when roots of wheat plants were subjected to deficient irrigations with 35% and 75% water holding capacity on application with 24-epibrassinolide and timber waste biochar, the root fresh and dry weights showed reduction in former (Lalarukh *et al.*, 2022).

The abovementioned growth parameter improved significantly owing to BRs applications versus control (Lalarukh *et al.*, 2022). The increase in root biomass might be due to the effect of EBL in the enhancement of Ca and K uptake which enhanced the Ca:Na ratio. Furthermore, Ca is an integral component of cell wall and plays an important role as secondary messenger in cell osmoregulation (Engels *et al.*, 2012). The tolerant genotype (HD-2733) showed better response under water deficit treatment (19.47 cm<sup>2</sup>) followed by EBL treatment of the same genotype (16.25 cm<sup>2</sup>) whereas DBW-187, the susceptible genotype, had minimum root surface area (3.72 cm<sup>2</sup>) (Fig. 1d). Our findings corroborate with the reports of (Urbanavičiūtė *et al.*, 2022) which revealed that water deficit condition affected the root surface area in different genotypes differently (Zulkarami *et al.* (2014) observed rice root surface area increased by 59.2% following application of epibrassinolide in comparison to control. Brassinosteroid signalling in *Arabidopsis thaliana* showed that BRs and auxin interact in an antagonistic way which determines the developmental gradient of stem cells in plant root tips by the opposing gradients of these antagonistic signals (Chaiwanon and Wang, 2015). Shifting of meristematic cell division to cell elongation might have increased the root surface area. Similar type of mechanism, as in *Arabidopsis thaliana*, might have worked in wheat for increasing the root surface area. The lowest response of sensitive genotype (DBW-187) was recorded under water deficit (3.73 cm<sup>2</sup>). Root surface area showed positive significant correlation with root length and root volume.

The influence of exogenously applied EBL (0.01mM) across water deficit treatments in two contrasting genotypes (HD-2733 and DBW-187) on average root diameter is shown in Fig. 1e. Among the treatments (water deficit, EBL treated + well water and EBL + water deficit), water deficit showed better performance (0.32 mm) while EBL treatment showed lower performance (0.30 mm). The perusal of data showed among all the treatments



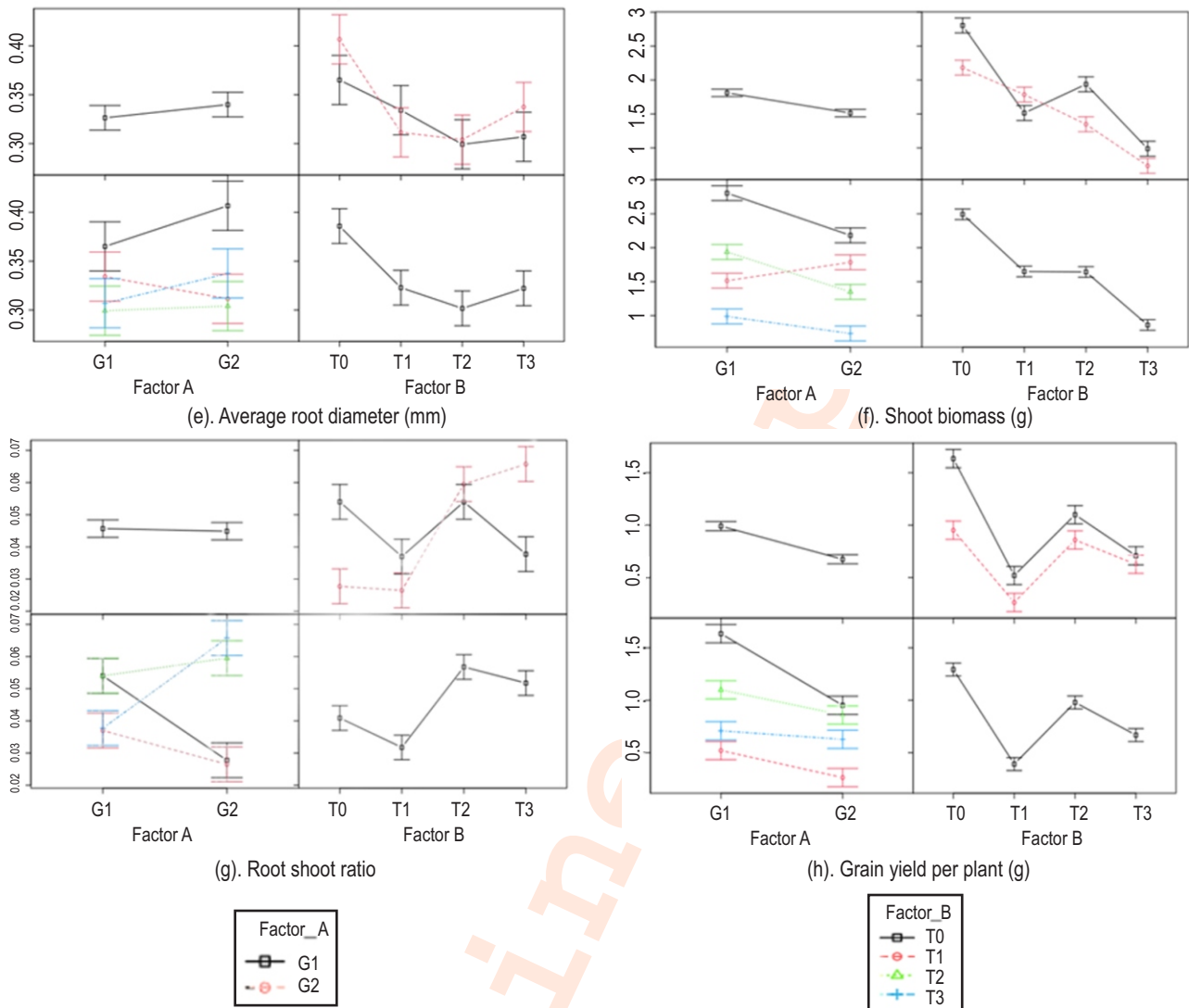
**Fig. 1 (a-b):** Influence of exogenously applied epibrassinolide on wheat genotypes across water deficit treatments; Factor A: Genotypes; G1: Tolerant genotype (HD-2733), G2: Sensitive genotype (DBW-187) Factor B: Treatments; T0: Well watered, T1: Water deficit, T2: EBL + well watered, T3: EBL + Water deficit.

(control, water deficit, EBL treated & EBL + water deficit) control performed better (0.39 mm). No significant correlations were observed for average root diameter. Similar research finding showed that the abiotic stress (heat stress) on tomato crop had severe deleterious effect on the total root volume as well as root diameter which might be due to the allocation of photosynthates more towards shoots as compared to roots under EBL treatment with or without stress (Khan *et al.*, 2014).

Fig. 1f shown the influence of exogenously applied EBL (0.01mM) across water deficit treatments in two contrasting genotypes (HD-2733 and DBW-187) on shoot biomass. The tolerant genotype (HD-2733) performed better (2.80g) under control condition having the value of followed by control treatment (2.18g) of the sensitive genotype (DBW-187). Similarly, the lowest response of sensitive genotype was recorded when treated with EBL + water deficit (0.74g). As compared to other treatments, the control plants had higher shoot biomass. The perusal of data showed significant lower shoot (fresh and dry) weights of wheat under 35% water holding capacity as compared

to 75% water holding capacity which improved significantly owing to BRs treatment (Lalarukh *et al.*, 2022).

The total plant biomass was affected due change the source-sink relationships due to allocation of available carbon to the root system rather than to the shoot under limited water supply (Khadka *et al.*, 2020). Brassinosteroid application enhances the photosynthetic efficiency in plants resulting in increased shoot biomass. The prevailing theory claims that BRs promote the efficacy of photosynthetic carbon reduction cycle through up regulation of associated enzymes (Yu *et al.*, 2004). Previous studies have reported that the application of 24-epibrassinolide on the leaves resulted in enhanced net CO<sub>2</sub> assimilation rate and rubisco carboxylation rate (Yu *et al.*, 2004). Furthermore, (Yusuf *et al.*, 2017) demonstrated that the application of EBL via foliar spray resulted in an increase in the rate of rubisco carboxylation, RuBP renewal, and quantum yield of PSII. Shoot biomass showed positive significant correlation with root volume and root biomass. The influence of exogenously applied EBL (0.01mM) across water deficit treatments in two contrasting genotypes (HD-

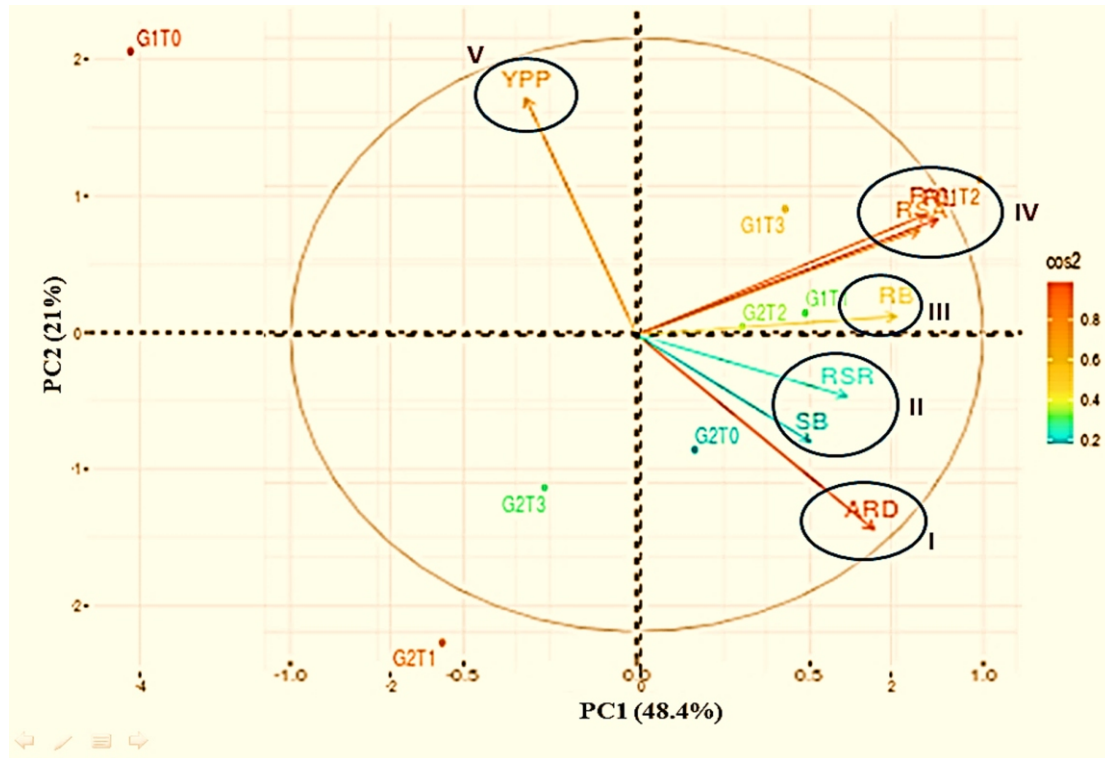


**Fig. 1 (e-h):** Influence of exogenously applied epibrassinolide on wheat genotypes across water deficit treatments; Factor A: Genotypes; G1: Tolerant genotype (HD-2733), G2: Sensitive genotype (DBW-187) Factor B: Treatments; T0: Well watered, T1: Water deficit, T2: EBL + well watered, T3: EBL + Water deficit.

2733 and DBW-187) in Fig. 1g is on root to shoot ratio. The sensitive genotype (DBW-187) performed better under EBL+water deficit treatment (0.07 followed by EBL treatment (0.06). Similarly, the lowest response was recorded in sensitive genotype (DBW-187) under water deficit (0.03) condition. Root to shoot ratio had significant positive correlation with root biomass. This result is in accordance with Khan *et al.* (2010) who observed that the suppression of both shoot length and root length might be due to under water stress conditions. Therefore, the selection for higher root shoot ratio will be beneficial for selecting drought resistant genotypes. The reason for increase in the root-to-shoot ratio under water deficit condition may be due to limited supply of water and nutrients to the shoot and some hormonal messages induced in roots when they encounter water deficit condition.

Among all treatments, the EBL + water deficit treated plants had better root to shoot ratio and water deficit had the lowest root to shoot ratio (Fig. 1g). Even in case of EBL treated plants, the root to shoot ratio was superior when compared with all treatments. Wang *et al.* (2022) reported that under water deficit condition, BRs promoted the root shoot ratio in maize seedling which indicates that BRs are involved in root growth, through enhanced expression of auxin.

The findings on *Arabidopsis thaliana* indicate that the *dwf4-1* mutants exhibited an elevated root/shoot ratio, while the AOD4-51 mutants displayed a decreased ratio due to the restricted availability of endogenous brassinosteroids (Li *et al.*, 2005). The regulation of endogenous brassinosteroid levels is



**Fig. 2:** Principal component analysis of root and yield variables RL: Root length, RV: Root volume, RSA: Root surface area, ARD: Average root diameter, SB: Shoot biomass, RB: Root biomass, RSR: Root to shoot ratio, YPP: Grain yield per plant.

crucial in fulfilling the growth and developmental requirements of both shoots and roots (Choe *et al.*, 2001). Investigation of the mechanisms that regulate the endogenous levels of brassinosteroid in terms of their temporal and spatial distribution, with a focus on the contribution of tissue-specific synthesis and degradation, is an important area of research that requires prioritization in the future (Sahni *et al.*, 2016).

Variation in yield per plant among the contrasting genotypes and treatments is shown in Fig. 1h. The yield per plant was significantly different among the genotypes, where apparently the tolerant genotype performed well with 46% increase in yield per plant. The difference among the treatment was significant in tolerant genotype, whereas it was non-significant in susceptible genotype, which indicated that the impact of brassinolide is noteworthy in susceptible genotype, that it can ameliorate stress (Groszyk and Szechyńska-Hebda, 2021). Amongst different treatment water deficit noticeably performed lesser. The performance of T3 *i.e.*, brassinolide treated plants under water deficit was restored than plants under water deficit. On comparing the T3 in both the genotypes, it was observed that they were non-significantly different. Brassinolide showed similar

impact on root biomass which corroborated with the findings of Khan *et al.* (2021) in wheat. The significant improvement in yield was due to exogenous application of EBL, which play active role in various physiological processes through translocation to different plant parts and conferred plant stress tolerance. Reports also revealed the role of brassinosteroids in unveiling of genes and metabolic pathways that confer drought resistance to Indian mustard (Fariduddin *et al.*, 2009). Tolerance provided by brassinosteroid treatment is mediated via provoked expression of genes involved in defense and regulation (Xia *et al.*, 2009).

The pattern of variations in root response under water deficit conditions was investigated by Principal Components. The factors serve to condense the correlations present in the observed correlation matrix and possess the ability to accurately replicate the observed correlation matrix (Jixi *et al.*, 2006). Out of seven Principal Components, only three were deemed significant because of eigenvalues greater than 1, which contributed 83.26% to the total variations. The remaining non-significant Principal Components (eigenvalue <1) were not sufficient for investigation (Table 1). Eight traits were dispersed in various ordinates and the length of vectors indicated primary component contribution of the

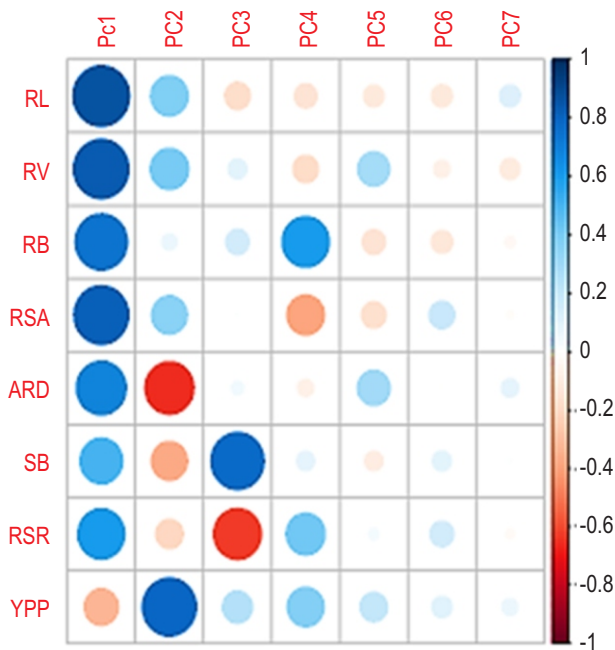


Fig. 3: Correlation between root and yield traits with Principal Components.

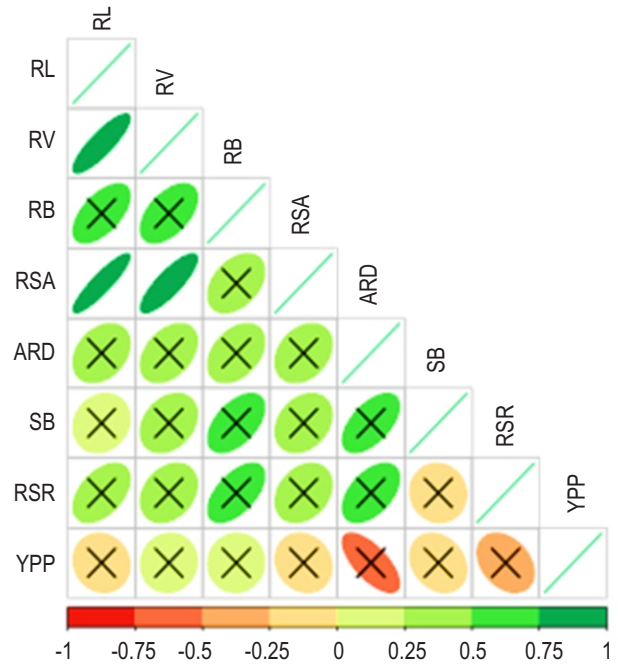


Fig. 4: Correlation between root traits and grain yield.

Table 1: Extracted Eigenvalues and % contribution of variables on the first three principal components

Variables	Principle Components						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Extracted Eigenvalues	3.87	1.68	1.11	0.90	0.29	0.12	0.04
Explained variance (%)	48.37	21.04	13.86	11.25	3.58	1.44	0.48
Cumulative variance (%)	48.37	69.40	83.26	94.51	98.09	99.52	100
% contribution of variables on Pcs							
(Root Length)	19.52	9.03	2.91	2.19	4.59	12.48	35.26
(Root Volume)	17.98	9.75	0.82	3.62	28.41	4.58	30.86
(Root Biomass)	14.52	0.23	2.18	41.27	7.76	13.52	2.44
(Root Surface Area)	17.22	7.41	0.00	16.42	9.78	28.84	0.34
(Average Root Diameter)	12.08	25.55	0.17	0.57	30.97	0.00	17.52
(Shoot Biomass)	6.48	7.74	53.51	0.88	2.87	8.35	0.02
(Root to Shoot Ratio)	9.53	2.58	34.84	19.49	0.34	21.75	1.24
(Yield per plant)	2.67	37.71	5.57	15.56	15.27	10.49	12.32

traits and the quality of depiction. With the help of a PCA biplot analysis, traits were divided into main groups and subgroups based on homogeneity and dissimilarity. Five sets of traits were found in the data set using the PCA biplot, which concurrently took into account PC1 and PC2. The ARD was grouped in I, RSR and SB in Group II, RB in Group III, RL, RSA and RV are clustered in Group IV, while YPP in Group V (Fig. 2). The length and colour intensity of a vector in a biplot show, the representation of qualities and their contribution to the principal components. Remarkably, the PCA biplot showed that Group III and IV, which

contributed significantly to PC1 were highly interactive with G1T1 (tolerant genotype *i.e.* HD- 2733 under water deficiency) and G1T2 (EBL treated seeds of HD-2733), respectively whereas the traits associated with Group I, II and IV contributed more to PC2 and most positively correlated to G2T0 (Sensitive genotype: DBW-187 and well-watered) and G1T0 (Tolerant genotype: HD-2733 and well-watered) (Table 1; Fig. 2, 3).

Independent correlation was observed between traits Groups I & IV and IV & V for the qualities being researched based

on the angles between the vectors derived from the middle point of biplots, which also showed positive correlation between trait groups I to III and II to IV (Fig. 2). From the correlation analysis (Table 1), it can be concluded that except for root diameter significant correlations were observed for other root architectural traits. The positively correlated traits depict that selection of plant for improvement of any of the particular root architectural trait (except root diameter) may lead to simultaneous indirect improvement in other correlated characters through proper breeding efforts (Nayak *et al.*, 2022). Hence, from the Principal Component Analysis, it can be deduced that plant selection for most of the root architectural traits along with the Principal Component will be effective for developing better root system architecture for drought stress, since all of the root architectural traits showed positive correlation with each other. Person correlation analysis between the studied traits revealed maximum significant correlation between root length, root volume and root surface area (Fig. 4). The study also witnessed non-significant positive correlation of root length, root volume and root surface area with all other root traits. There was non-significant negative correlation between grain yield per plant with all root traits (Fig. 4).

This study showed that seed treatment with 0.01m Epibrassinolide had positive impact on various root architectural traits and grain yield in wheat. The wheat genotypes *viz.*, HD-2733 showed high degree drought tolerance with better performance in almost all root traits and grain yield as compared to the sensitive genotype *viz.*, DBW-187. Hence, findings of this study will be beneficial to the farming community and referred to research personnel working on drought mitigation in wheat.

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**Authors' contribution:** **A.K. Sharma:** Contribution of experimental materials, Execution of field/laboratory experiments and data collection, Preparation of manuscript; **J. Pradhan:** Conceptualization of research, Designing of experiments, Contribution of experimental materials, Execution of field/laboratory experiments and data collection, Preparation of the manuscript; **S. Kumar:** Conceptualization of research, Designing of the experiments; **K. Pramanik:** Analysis of data and interpretation, Preparation of the manuscript; **C. Kastury, G. Kumari, A. Jaiswal:** Designing of the experiments and **C. Jena:** Analysis of data and interpretation, Preparation of the manuscript.

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**Research content:** The search content of manuscript is original and has not been published elsewhere.

**Ethical approval:** The research design, data collection, and analysis procedures were guided by established ethical principles. No ethical concerns arose during the course of this study.

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

**Data availability:** The data used to support the findings of the study are included in the article.

**Consent to publish:** All authors agree to publish the paper in *Journal of Environmental Biology*.

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