



RESEARCH ARTICLE

# Modulation of antioxidant enzymes and growth traits by exogenous application of auxin and cytokinin in wheat under induced drought stress

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## Abstract

An experiment was conducted to study the effect of phytohormones (auxin and cytokinin) in mitigation of drought stress on pairs of contrasting genotypes of wheat i.e. tolerant (DBW 110 and HI 1655) and susceptible (BW Local 1 and BW Local 2) at seedling stage under four conditions. Drought was induced by PEG 6000. It was observed that phytohormones had a significant role in mitigating stress by adjusting growth parameters, physiological parameters, reactive oxygen species level and antioxidants activity. Application of auxin and cytokinin leads to desirable changes in shoot length (2.36 and 3.26), root length (1.21 and 1.17), seedling length (2.36 and 2.74), fresh shoot weight (1.24 and 1.28), fresh root weight (1.41 and 1.21), fresh seedling weight (3.37 and 4.19), total chlorophyll (1.72 and 1.63), maximal quantum yield of photosystem II (1.51 and 1.70), stomatal conductance (1.38 and 1.19), catalase activity (4.75 and 5.68), ascorbate peroxidase activity (14.63 and 18.05) and proline content (1.37 and 1.28) and hydrogen peroxide content (1.22 and 1.31) represented in average folds as compared to control. Fresh seedling weight showed positively significant correlation with fresh shoot weight ( $r=1.00^{**}$ ), shoot length ( $r=1.00^{**}$ ), catalase ( $r=0.99^*$ ), ascorbate peroxidase ( $r=0.99^*$ ), seedling length ( $r=0.98^*$ ), total chlorophyll ( $r=0.97^*$ ) and negative and significant correlation with hydrogen peroxide ( $r=-1.00^{**}$ ) under effect of auxin whereas it showed positively significant correlation with fresh shoot weight ( $r=1.00^*$ ), chlorophyll b ( $r=0.98^*$ ), stomatal conductance ( $r=0.98^*$ ), maximal quantum yield of photosystem II ( $r=0.97^*$ ), total chlorophyll ( $r=0.96^*$ ), chlorophyll a ( $r=0.95^*$ ), fresh root weight ( $r=0.95^*$ ) and significant and negative correlation with hydrogen peroxide ( $r=-1.00^*$ ) under effect of cytokinin indicating that these are the important traits targeted by the phytohormones to mitigate the stress. Biplot analysis revealed that traits were grouped as positively correlated (all traits except  $H_2O_2$ ) and negatively correlated ( $H_2O_2$ ) to the drought tolerance. Study concluded that application of auxin and cytokine can minimize yield reduction of susceptible high yielding genotypes under drought stress. Further study under natural stress is required to understand the mechanism of phytohormones in mitigation of drought.

**Keywords:** antioxidants; drought; PEG; phytohormone; ROS; seedling; wheat

## Introduction

Bread wheat, scientifically known as *Triticum aestivum* L. is one amongst top three cereal crops, providing a significant portion of day-to-day calories and protein consumption, with an annual production of about 780 million tonnes globally (1). Heat is a common source of energy, however significant number of proteins, fibre, lipids, vitamins, minerals and other phytochemicals which are also integral part of diet (2–4). As per an estimate, wheat is second most cultivated crop globally (5,6). However, with the growing worldwide demand for wheat expected to rise by up to 60 % by 2050, production is expected to decrease by 29 % owing to climate change and environmental stresses it imposes (7). Worldwide, drought reduces more than 50–60 % yield of wheat crop (8). This poses a serious challenge for global resources as the average surface temperature continues to rise. Consequently, drought has

emerged as a critical obstacle in wheat research, as it is one of the key worldwide challenges driven by climate change, adversely impacting wheat growth, development and yield (9). Drought stress negatively impacts the plant biomass, photosynthetic apparatus and their function, antioxidant activity and on the other hand it promotes synthesis of ROS which ultimately cause the reduction in yield (10). Under water deficit environment, plants become unable to translocate photosynthates from source to sink which leads to the deprivation of energy in the system and reduce its efficiency (11). Increase in osmotic pressure trigger the burst of ROS (singlet oxygen, superoxide radical and hydrogen peroxide) and in response enzymatic (catalase, superoxide dismutase, ascorbate peroxidase, peroxidase) and non-enzymatic (proline, glycine betaine, ascorbic acid, glutathione and phenolic compounds) antioxidant defence get activated to cope the stress (12).

Exogenous application of auxins and cytokinin at appropriate concentrations enhances the dry matter yield and stability of plants, while also reducing disease occurrence (13). These hormones contribute to improved tillering, foliage growth and the induction of mass and intensity of flowering. Foliar applications of auxins and cytokinin impact the chemical composition of plant dry matter in various ways, typically increasing potassium and calcium content without affecting phosphorus levels (14). Consequently, they play a vital role in cell growth and development. Indole-3-acetic acid (IAA), an auxin produced from tryptophan, plays a significant role in drought stress. This has been studied through TLD1/OsGH3.13, a gene encoding IAA-amido synthase, which upregulates the expression of Late Embryogenesis Abundant (LEA) genes, thereby increasing drought tolerance in plants (15,16). Cytokinin are also vital in numerous plant processes, including seed germination, vascular development, photomorphogenesis, shoot apical meristem development, floral development and leaf senescence. Their multifaceted roles underscore their importance in plant growth and adaptation to environmental stresses (17). Drought tolerance is attained through the accumulation of enzymatic and non-enzymatic antioxidants in different tissues of plants (18,19). Accumulation of proline, amino acids, catalase, ascorbate peroxidase, superoxide dismutase, peroxidase etc., has been observed in different studies (20,21). Crop growers always worry about susceptible high yielding well adapted genotypes. Considering above fact, current study aimed to test hormonal rescue of the susceptible genotypes with auxin and cytokinin phyto-hormones under drought stress by studying response of seedling growth parameters, physiological traits and biochemical traits.

## Materials and Methods

### Experimental site and plant material

The present experiment was conducted at Banda University of Agriculture and Technology, Banda during winter season of 2023-24. In this study, four wheat genotypes namely DBW 110, HI 1655, BW Local-1 and BW Local-2 were evaluated under drought stress at seedling stage. Genotypes DBW 110 and HI 1655 are drought tolerant procured from AICRP on wheat whereas genotype BW Local-1 and BW Local-2 are sensitive to drought collected from neighboring region of Banda district of Uttar Pradesh.

### Plant growth and drought treatment

The wheat genotypes were grown in pro-trays filled with 50 % compost and 50 % sand by placing 10 seeds. After fifteen days of sowing, the different treatments namely control (without PEG application), drought (PEG application), auxin applied in drought (PEG + auxin) condition and cytokinin applied in drought (PEG + cytokinin) condition, were created in three replications each. The genotypes were evaluated for seedling drought in completely randomized design. Auxin and cytokinin phyto-hormones were used in chemical composition IAA and BAP (6-Benzylaminopurine), respectively at fifteen days old seedling. A preliminary screening was conducted using varying concentrations of IAA (25, 50, 100 and 200  $\mu$ M) and BAP (40, 80, 160 and 320  $\mu$ M). Among these, 50  $\mu$ M IAA and 40  $\mu$ M BAP consistently produced the most desirable responses without inducing any phytotoxic effects. After 24 hr of phytohormone application, drought stress was imposed by applying PEG-6000 solution with 20 % concentration. After 72 hr of PEG application, the observations were

recorded on different growth parameters, physiological and biochemical traits across all treatments.

### Observations recorded

#### Growth parameters

From each genotype in different treatment conditions, five sampled seedlings were selected for noting down observations on root length, shoot length, seedling length, fresh root weight, fresh shoot weight, fresh seedling weight. The observations are recorded on seedling Root Length (RL), measured from the root-shoot connection to the root tip; Shoot Length (ShL) of seedling, measured from the soil surface node to the tip of the seedling; Seedling Length (SL), referred to the measurement from root tip to shoot tip. The RL, ShL and SL were denoted in centimeters (cm). Fresh Root Weight (FRW), Fresh Shoot Weight (FShW) and Fresh Seedling Weight (FSW) were measured in gram (g).

#### Physiological traits

The study focused on physiological traits, including photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll), chlorophyll fluorescence, stomatal conductance and proline levels.

#### Photosynthetic pigments

To assess chlorophyll in wheat, a 100 mg of leaf sample was homogenized in 80 % acetone and then centrifuged for 5 min at 8000 rpm at room temperature. The absorbance of the resulting 2 mL supernatant was measured using a spectrophotometer at wavelengths of 663 nm and 645 nm. Estimated chlorophyll content was  $\frac{V}{1000 \times W}$  performed using formula provided by Arnon and expressed in mg/g FW (22).

$$\text{Chl-a} = 12.7 \times A_{663} - 2.69 \times A_{645} \times \frac{V}{1000 \times W}$$

$$\text{Chl-b} = 22.9 \times A_{645} - 4.68 \times A_{663} \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = (20.2 \times A_{645}) + (8.02 \times A_{663}) \times \frac{V}{1000 \times W}$$

Where,

A=Absorbance

V= Final volume of 80 % acetone (mL)

Chl-a= chlorophyll 'a'

Chl-b= chlorophyll 'b'

W= weight of sample (g)

#### Chlorophyll fluorescence

Chlorophyll fluorescence was recorded under various treatments using a fluorescence meter. PS II activity was assessed as chlorophyll fluorescence between 10:00 and 11:00 AM on a fully extended leaf, employing the dark-adapted test with a modulated chlorophyll fluorometer (OS1-FL, OptiSciences, OS300p+, Tyngsboro, MA, United States).

#### Stomatal conductance

Stomatal conductance was assessed on a fully extended leaf using a portable leaf porometer (Model AP4, Delta-T Devices, Cambridge, UK) at 11:00 AM, with results expressed in  $\text{mmol m}^{-2}/\text{s}$ .

#### Biochemical traits

Various parameters such as catalase, ascorbate peroxidase and hydrogen peroxide were determined for four wheat genotypes under all treatment conditions.

**Catalase (CAT) activity:** The activity of CAT was measured by making a reaction mixture comprising 50 mM sodium phosphate buffer (pH 7.5) with addition of the enzyme extract. The reaction began with the addition of 1 mL  $H_2O_2$  and the decline in  $H_2O_2$  concentration was tracked by measuring the reduction in absorbance at 240 nm every 30 sec for 3 min (23). The extinction coefficient of  $H_2O_2$  was calculated as  $0.0394 \text{ mM}^{-1} \text{ cm}^{-1}$ . The activity of CAT was expressed as the micromoles of  $H_2O_2$  decayed per minute per gram of fresh weight ( $\mu\text{moles min}^{-1} \text{ g}^{-1} \text{ FW}$ ).

**Ascorbate peroxidase (APX) activity:** The APX assay was conducted using a mixture comprising 50 mM sodium phosphate buffer (pH 7), 0.5 mM ascorbic acid and the enzyme extract (24). Absorbance was measured at 290 nm using a spectrophotometer, with readings taken every 30 sec for 3 min. The extinction coefficient for monodehydroascorbic acid (MDA) was determined to be  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ . APX activity was expressed as the amount of MDA formed per minute per gram of fresh weight ( $\text{nmoles min}^{-1} \text{ g}^{-1} \text{ FW}$ ).

**Hydrogen peroxide ( $H_2O_2$ ):** The method for estimating  $H_2O_2$  activity was based on a method described in a previous study, with some modifications (25). A 200 mg sample of fresh leaves was crushed with 2 mL of phosphate buffer (pH 7), then transferred into 2 mL tubes and centrifuged for 15 min at 12000 rpm at room temperature. The pure supernatant was utilized for  $H_2O_2$  measurement. For the assay, 1 mL of the supernatant was mixed with 1 mL of 10 mM phosphate buffer (pH 7). The reaction was initiated by adding 2 mL of 5% potassium dichromate ( $K_2Cr_2O_7$ ) in glacial acetic acid. Absorbance was obtained at 570 nm using a UV spectrophotometer.

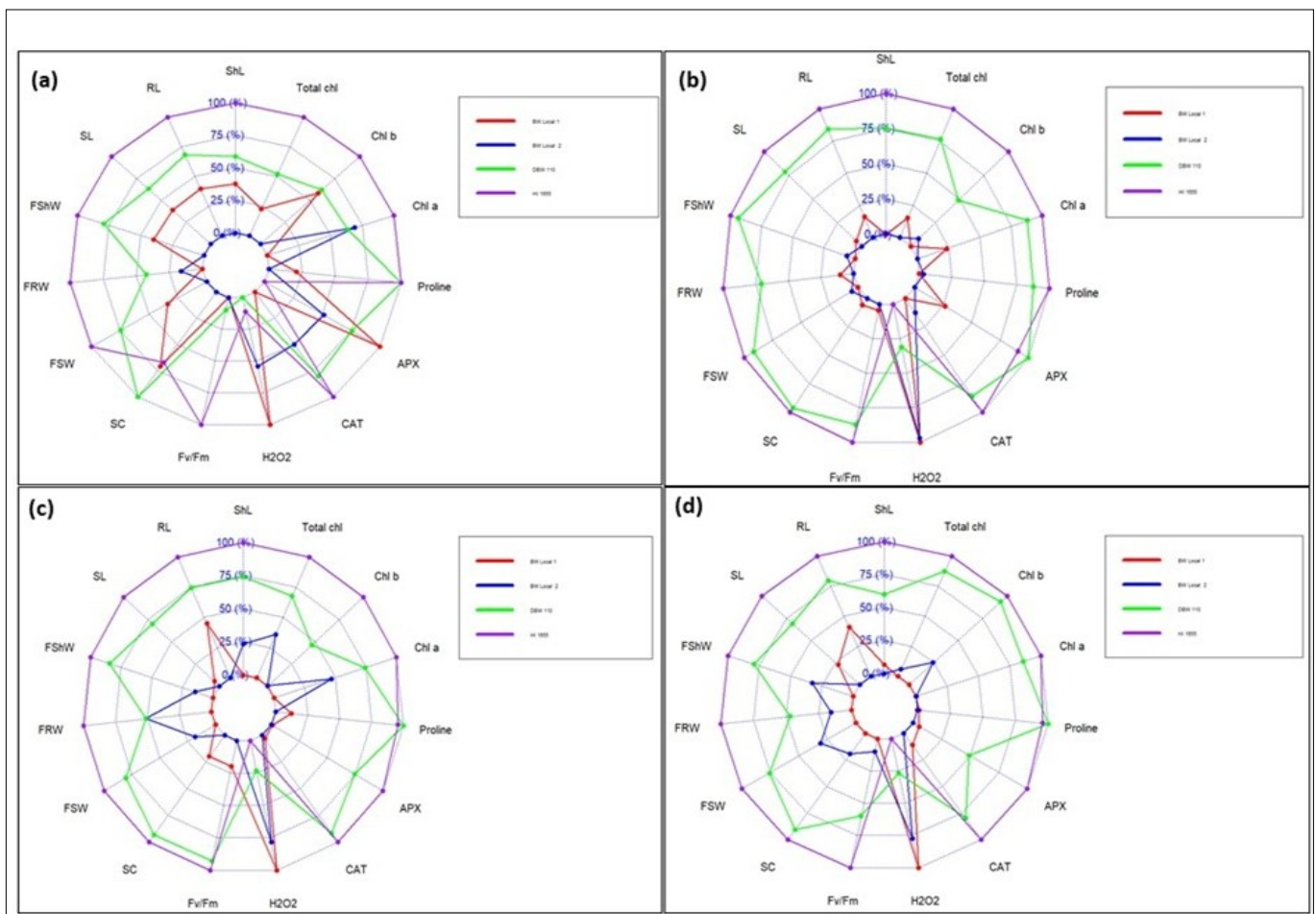
**Proline:** Content of proline was quantified using the colorimetric method (26) based on the reaction between proline and ninhydrin. Fresh leaf tissue (0.5 g) was homogenized on ice in 10 mL of 3% sulfosalicylic acid. The homogenized material was centrifuged for 15 min at  $11500 \times g$ . After centrifugation, 2 mL of the supernatant was mixed with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. This mixture was incubated for 1 hr at  $100^\circ\text{C}$ , then cooled, followed by the addition of 4 mL of toluene. The absorbance was measured at 520 nm, using toluene as the blank.

**Statistical analysis:** Replicates-wise means of four wheat genotype for various growth parameters, physiological and biochemical traits under all treatment conditions were used for analysis of the observed data. All graphs were created using MS Excel 2016. The radar graph was created using R program (version 4.1.3) using package 'fmsb' version 0.7.6. The estimates of correlation coefficients and PCA biplot were calculated using R (version 4.1.3) program using package 'Metan' and 'Factoextra'.

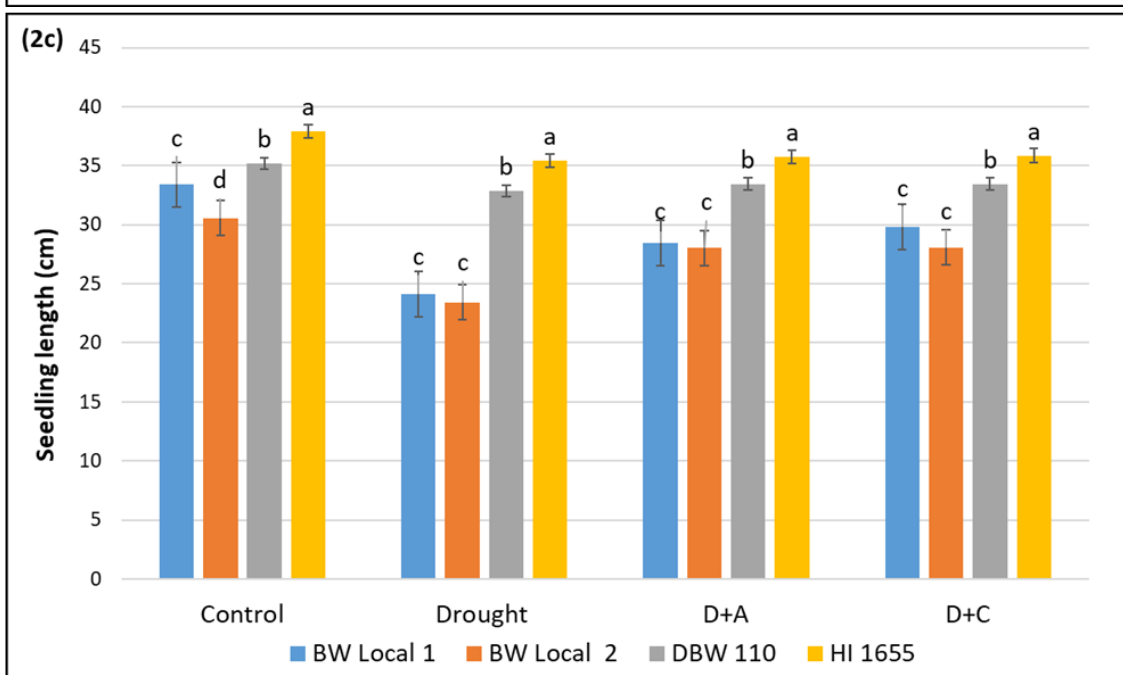
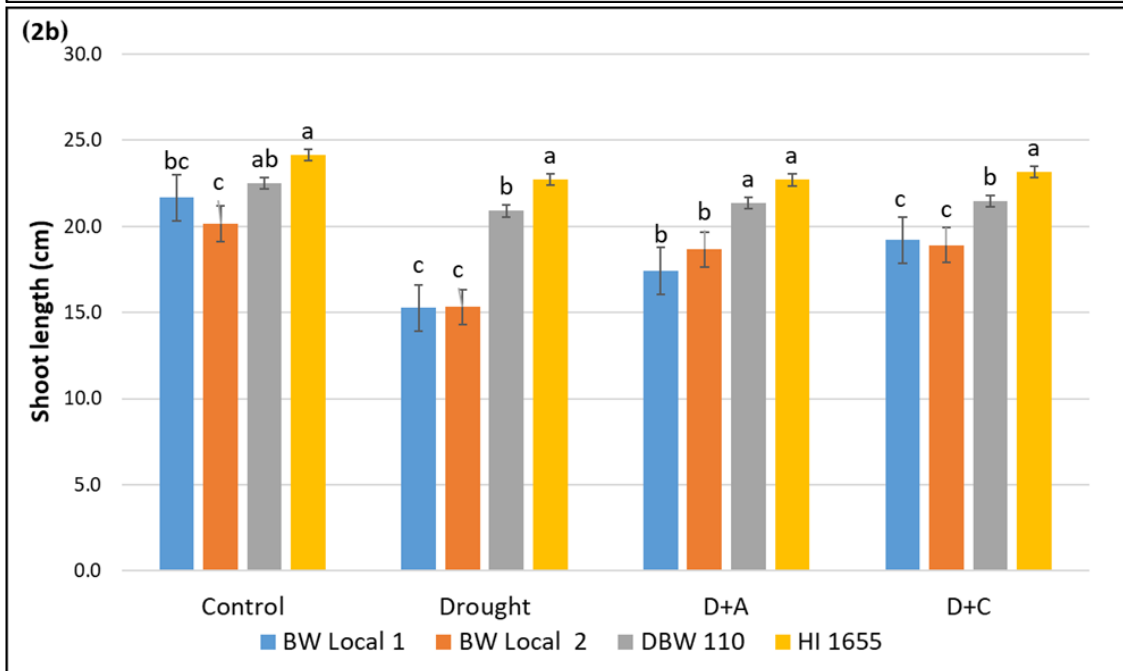
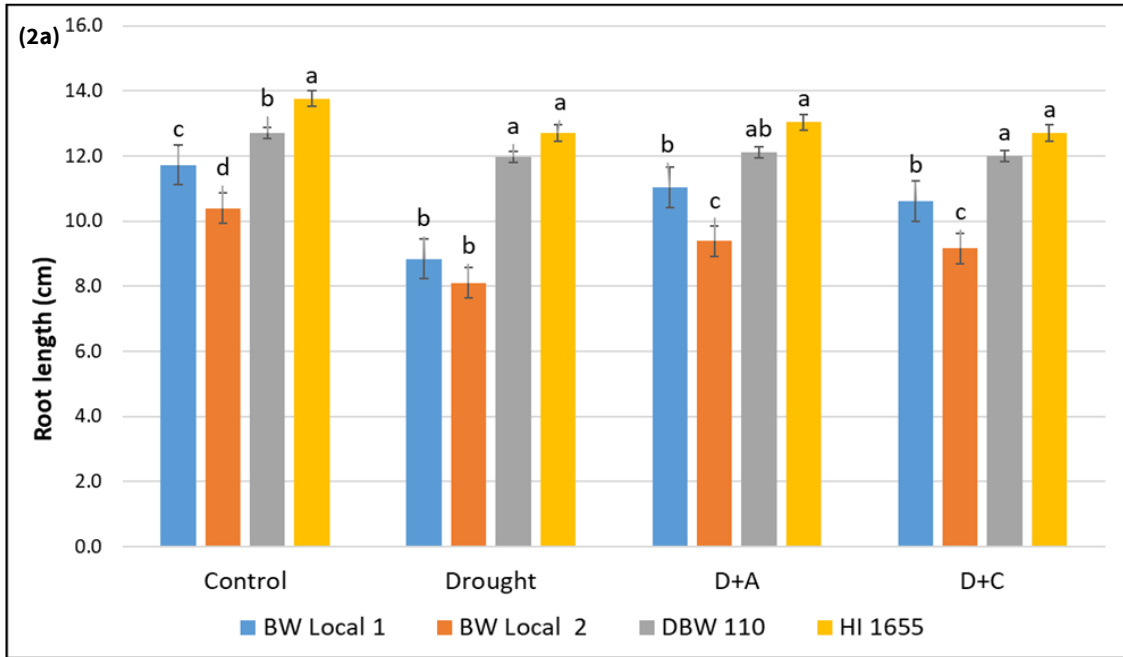
## Results and Discussion

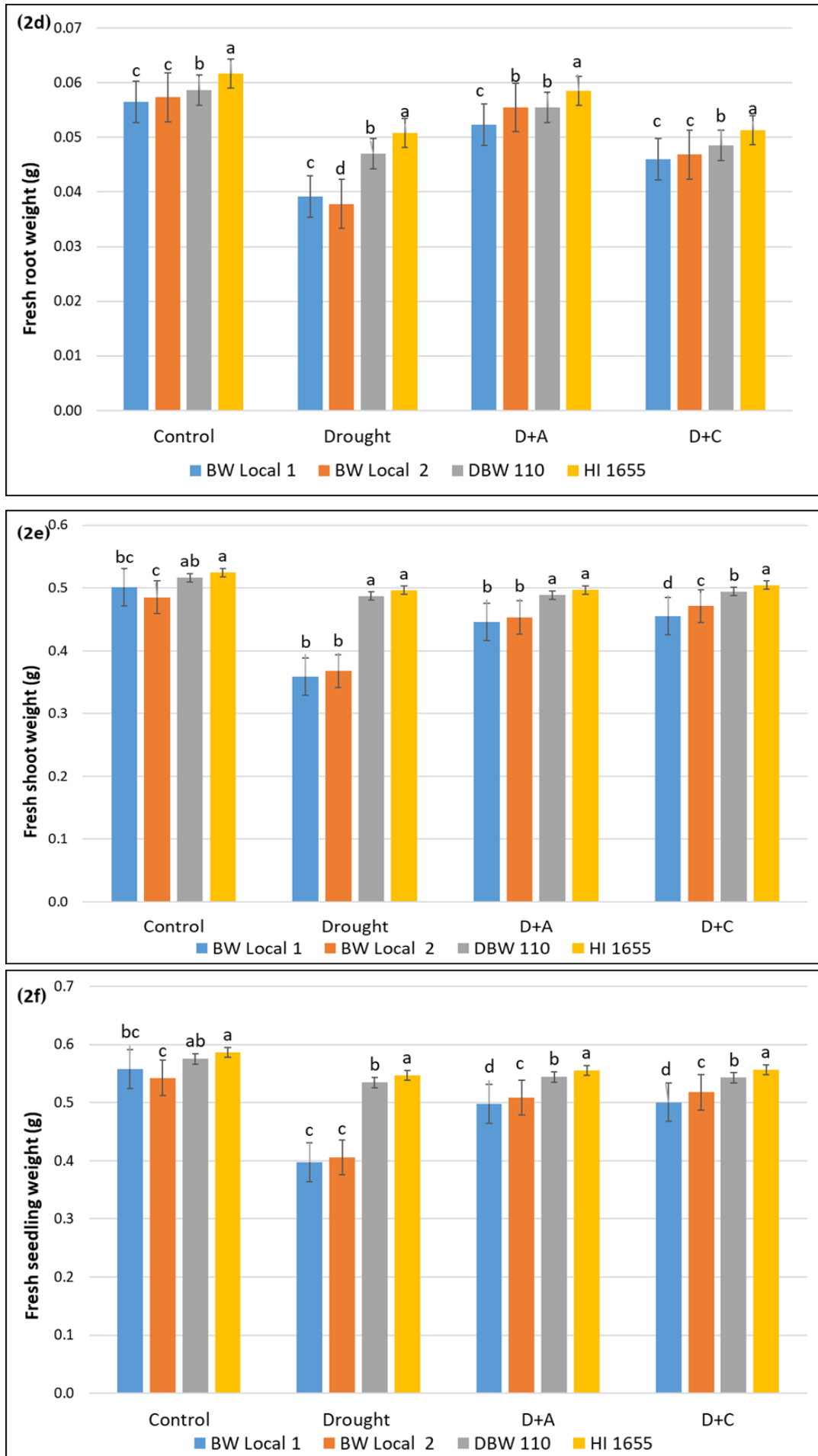
The average performance of four wheat genotypes for growth parameters, physiological and biochemical traits varied across different treatment conditions (control, drought, drought with auxin and drought with cytokinin) are summarized using radar plot in Fig. 1. All the genotypes showed variation in their performance under different treatment conditions.

### Effect of auxin and cytokinin on seedling growth parameters under drought stress



**Fig. 1.** Radar plot showing the average performance of wheat genotypes for different studied traits. (a) control; (b) drought; (c) drought with auxin; (d) drought with cytokinin





**Fig. 2.** Variation in wheat seedling growth traits in control, drought, drought with auxin (D+A) and drought with cytokinin (D+C) treatment conditions. (a) root length; (b) shoot length; (c) seedling length; (d) fresh root weight; (e) fresh shoot weight; (f) fresh seedling weight. Distinct letters indicate statistically significant differences among genotypes at  $p \leq 0.05$ , as determined by Fisher LSD test

In current study, effect of phyto-hormones like auxin and cytokinin was studied on RL, ShL, SL, FRW, FShW and FSW under induced drought stress at seedling stage as represented in Fig. 2.

#### Root Length (RL)

Roots serve as the primary site for water and nutrient uptake, which are essential for photosynthesis and plant growth and the study of root provides insight to understand the mechanism of drought below ground. In this study, among the genotypes studied RL ranged from 10.39 to 13.77 cm under control condition. Under drought conditions, drastic fall in length of root was observed for susceptible genotypes BW Local 1 (24.62 %) and BW Local 2 (22.03 %), while tolerant genotypes DBW 110 (5.74 %) and HI 1655 (7.74 %) was affected to a very low extent. Effect of auxin was positive in mitigating the drought stress by increasing RL by 1.25 folds and 1.16 folds in susceptible genotypes BW Local 1 and BW Local 2, respectively than that of the drought condition, while tolerant genotypes DBW 110 (1.01 folds) and HI 1655 (1.03 folds) did not respond to a greater extent. Same pattern was also observed for cytokinin where susceptible genotypes achieved considerable increase in RL which was 1.20 and 1.13 folds for BW Local 1 and BW Local 2, respectively and tolerant genotypes showed almost no changes.

#### Shoot Length (ShL)

Photosynthetic capacity of plants is confined to the shoot of plant because it has leaves on it makes photosynthates which is crucial for growth and development. It was observed in this study that genotypes under control condition showing normal ShL ranged from 20.16 to 24.14 cm. When these genotypes were exposed to drought conditions, a steep reduction in ShL of susceptible genotypes i.e. BW Local 1 (29.54 %) and BW Local 2 (24.04 %) was observed as compared to control. Tolerant genotypes DBW 110 (11.54 %) and HI 1655 (10.04 %) also showed reduction, but it was lower than that of the susceptible genotypes. Application of auxin under drought stress had substantial effect on susceptible genotypes by minimizing yield reduction by 1.50 and 3.21 folds for BW Local 1 and BW Local 2, respectively. Tolerant genotypes also experienced same pattern and showed 1.39 (DBW 110) and 0.99 (HI 1655) folds less reduction than that of the drought condition. The effect of cytokinin on ShL was significant, it allowed very low extent of reduction than the drought condition, the reduction was 2.61 (BW Local 1) and 3.91 (BW Local 2) folds less for susceptible genotypes and 1.54 (DBW 110) and 1.45 (HI 1655) folds less for tolerant genotypes as compared to drought condition.

#### Seedling Length (SL)

SL shows vigor of the plant and a good indicator of plant health. In the control condition, variation in the SL was lower as compared to other conditions. SL ranged from 30.55 to 37.91 cm add for both tolerant and susceptible genotypes whereas severe reduction was detected in SL of susceptible genotypes BW Local 1 (27.81 %) and BW Local 2 (23.36 %) as compared to the control condition while tolerant genotypes DBW 110 (6.60 %) and HI 1655 (6.56 %) showed minimal reduction. After application of auxin susceptible genotypes BW Local 1 (1.87 folds) and BW Local 2 (2.84 folds) whereas tolerant genotypes DBW 110 (1.34 folds) and HI 1655 (1.15 folds) showed increase in SL as compared to the drought condition. Cytokinin enhanced SL to 2.59 folds (BW Local 1), 2.88 folds (BW Local 2) and

1.34 folds (DBW 110), 1.22 folds (HI 1655) for susceptible and tolerant genotypes, respectively.

#### Fresh Root Weight (FRW)

A plant's root system is key for its growth and development and a well-developed root system with a higher cell mass can withstand stress longer. Under the control condition, all the genotypes performed almost in similar way and ranged between 0.057 g and 0.062 g. Susceptible genotypes BW Local 1 and BW Local 2 were severely affected by the drought and showed 30.68 % and 34.01 %, respectively. Moreover, tolerant genotypes DBW 110 (19.87 %) and HI 1655 (17.57 %) were also affected by drought and showed a rather large reduction in root weight. Mitigating effect of auxin tolerated this severe reduction and increased root weight up to 1.34 and 1.47 folds in susceptible genotypes (BW Local 1 and BW Local 2) and 1.18 and 1.15 folds in tolerant genotypes (DBW 110 and HI 1655), respectively as compared to drought condition. Cytokinin application led to 1.17- and 1.24-folds increase in root weight of susceptible genotypes BW Local 1 and BW Local 2, respectively whereas tolerant genotypes showed only 1.03 (DBW 110) and 1.01 (HI 1655) folds increase in root weight.

#### Fresh Shoot Weight (FShW)

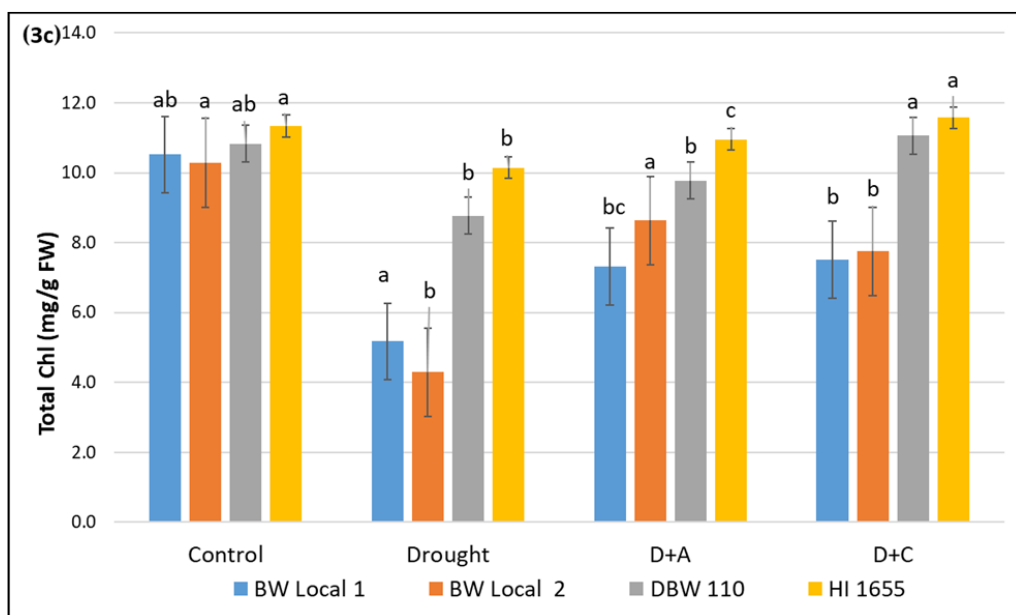
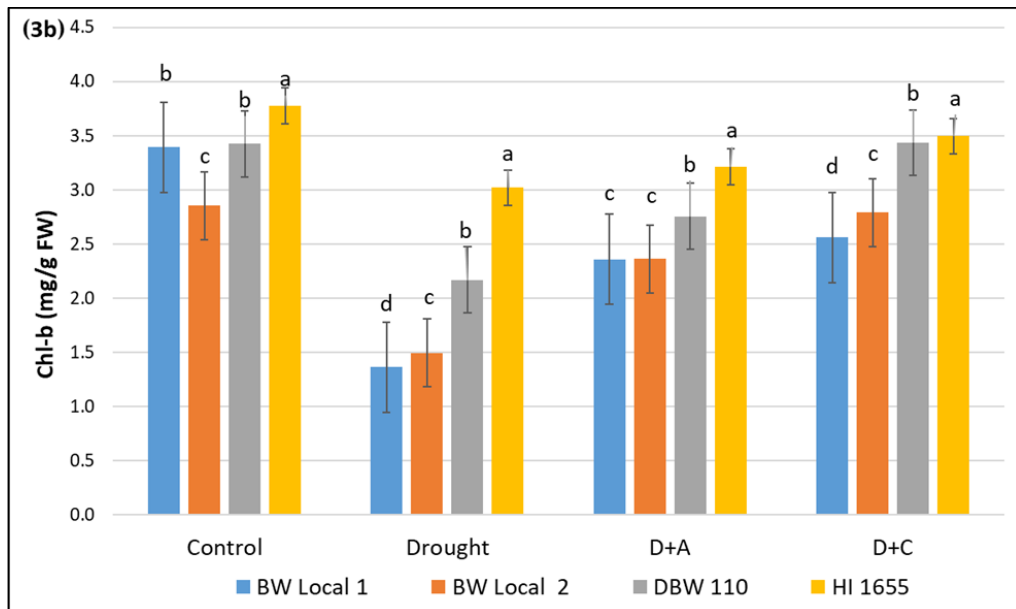
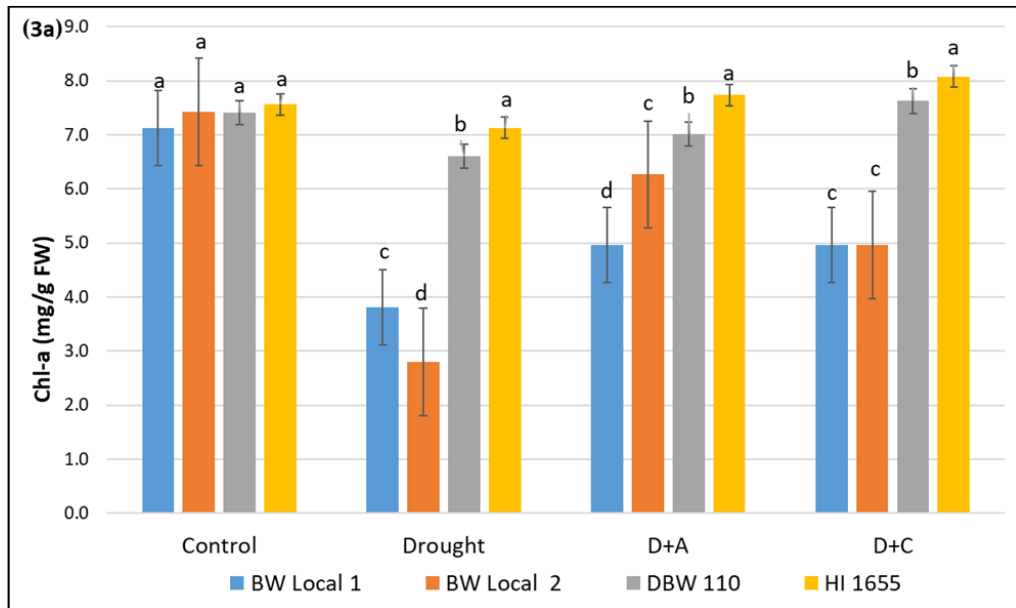
FShW is a major attribute that helps in conferring the drought response through efficient photosynthate production and translocation. In control condition both susceptible and tolerant genotypes showed normal shoot weight ranging from 0.49 to 0.52 g whereas severe reduction in susceptible genotypes BW Local 1 (28.45 %) and BW Local 2 (24.22 %) was observed under drought condition. However, tolerant genotypes DBW 110 and HI 1655 were able to maintain their shoot weight with very low reduction i.e. 5.60 % and 5.38 %, respectively, as compared to control condition. Under effect of auxin, susceptible genotypes BW Local 1 and BW Local 2 showed 1.24- and 1.23-folds increase in shoot weight, respectively. Cytokinin application had increased shoot weight to 1.27 and 1.28 folds for susceptible genotypes BW Local 1 and BW Local 2, respectively. The effect of both auxin and cytokinin on tolerant genotypes for shoot weight was negligible.

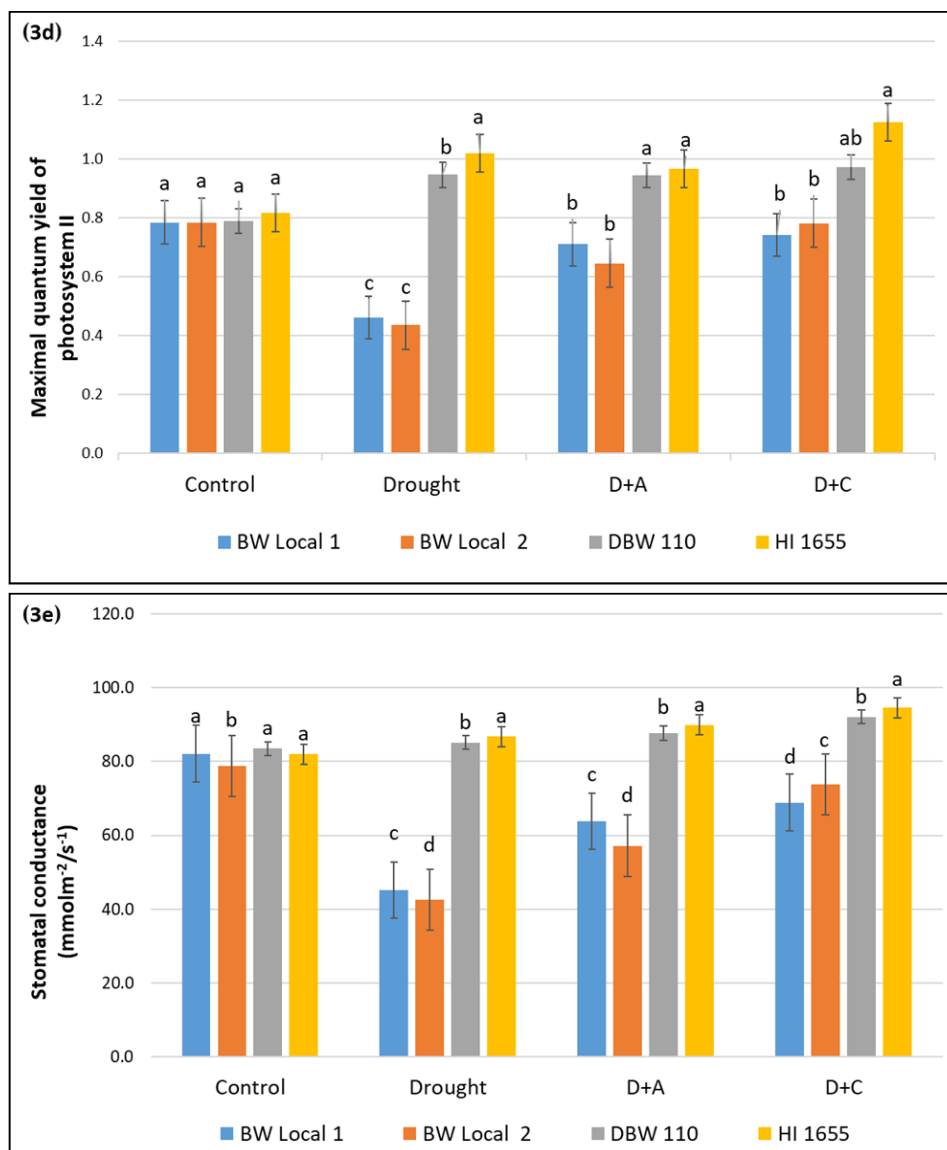
#### Fresh Seedling Weight (FSW)

Biomass of a plant is a major attribute which helps in conferring the drought response, reduced water availability hit it at differential rate depending on severity of the stress. In control condition, seedling weight ranged from 0.54 to 0.59 g and all the genotypes showed comparable seedling weight. Under drought stress, susceptible genotypes BW Local 1 (28.68 %) and BW Local 2 (25.25 %) showed more reduction than tolerant genotypes DBW 110 (7.06 %) and HI 1655 (6.66 %) as compared to drought condition. Auxin played a significant role in mitigation of stress by increasing FSW to 2.69 folds (BW Local 1), 4.05 folds (BW Local 2) and 1.32 folds (DBW 110), 1.27 folds (HI 1655) for susceptible and tolerant genotypes, respectively. Under the effect of cytokinin, susceptible genotype showed 2.82 folds (BW Local 1) and 5.55 folds (BW Local 2) increase whereas tolerant genotypes showed 1.26 folds (DBW 110) and 1.29 folds (HI 1655) increase in FSW.

#### Effect of auxin and cytokinin on physiological traits under drought stress

As revealed in Fig. 3, variation was observed in photosynthetic pigment (chlorophyll content), chlorophyll fluorescence and stomatal conductance effect in different treatment conditions drought and phytohormones.





**Fig. 3.** Variation in physiological traits of wheat seedling in control, drought, drought with auxin (D+A) and drought with cytokinin (D+C) treatment conditions. (a) chlorophyll a; (b) chlorophyll b; (c) total chlorophyll; (d) chlorophyll fluorescence; (e) stomatal conductance. Distinct letters indicate statistically significant differences among genotypes at  $p \leq 0.05$ , as determined by Fisher LSD test

#### Chlorophyll a (Chl-a)

Chlorophyll a is the main photosynthetic pigment in higher plants. It absorbs light to provide energy for photosynthesis. Under control it ranged from 7.12 to 7.56 mg/g FW. When plants were exposed to drought conditions, sudden decline observed in the level of chlorophyll a in susceptible genotypes BW Local 1 (46.54%) and BW Local 2 (62.37%) whereas tolerant genotypes DBW 110 (10.84%) and HI 1655 (5.79%) showed a minimal reduction as compared to the control. Auxin application under drought condition increased the level of chlorophyll a by 1.30 folds and 2.24 folds for susceptible genotypes (BW Local 1 and BW Local 2) and by 1.06 and 1.09 folds for tolerant genotypes (DBW 110 and HI 1655). Increase under effect of cytokinin for susceptible genotypes was 1.30 and 1.78 folds for susceptible genotypes (BW Local 1 and BW Local 2) and for tolerant genotypes, it was 1.16 and 1.13 folds than that of the drought condition.

#### Chlorophyll b (Chl-b)

Chlorophyll b complements the efficiency of chlorophyll and enhances photosynthetic activities of the plants. Under control condition, the level of chlorophyll b ranged between 2.85 to 3.78 mg/g FW. Due to drought, susceptible genotypes BW Local 1 (59.82

%) and BW Local 2 (47.64%) showed a drastic decline in level of chlorophyll b content and tolerant genotypes DBW 110 (36.64%) and HI 1655 (20.03%) also showed rather decline as compared to the control condition. Auxin augmented chlorophyll b level by 1.73 and 1.58 folds for susceptible genotypes (BW Local 1 and BW Local 2) and by 1.27 and 1.07 folds for tolerant genotypes (DBW 110 and HI 1655). Increase in level of chlorophyll b was observed after application of cytokinin, an increment of 1.88 and 1.87 folds had been seen for susceptible genotypes BW Local 1 and BW Local 2, respectively and increase of 1.58 and 1.16 folds was observed for tolerant genotypes DBW 110 and HI 1655, compared to the drought condition.

#### Total chlorophyll

In this study, total chlorophyll content varied from 10.28 to 11.34 mg/g FW under controlled conditions. Steep reduction was observed under drought conditions in the level of total chlorophyll for susceptible genotypes BW Local 1 (50.83%) and BW Local 2 (58.28%) whereas reduction for tolerant genotypes was 18.20% and 10.53% for DBW 110 and HI 1655, respectively as compared to control. Application of auxin enhanced total chlorophyll content by 1.42 and 2.01 for susceptible genotypes BW Local 1 and BW Local 2,

respectively and tolerant genotypes showed increase by 1.11 and 1.08 folds for DBW 110 and HI 1655, respectively than that of the drought condition. When cytokinin was applied to the plants under drought conditions it enhanced chlorophyll content by 1.45 and 1.81 folds for susceptible genotypes (BW Local 1 and BW Local 2) and by 1.26 and 1.14 folds for tolerant genotypes (DBW 110 and HI 1655). Maximal quantum yield of photosystem II was reduced under drought conditions in susceptible genotypes manifesting the loss of the photosynthetic membrane function of PSII. Moreover, it was regained by the application of auxin and cytokinin indicating the mitigating effect of these plant hormones. Susceptible genotypes showed very low stomatal conductance as compared to the tolerant genotypes under drought conditions due to reduced activity of guard cells of stomata. Application of auxin and cytokinin enhanced the function and activity of guard cells of stomata and improved stomatal conductance in both susceptible and tolerant genotypes.

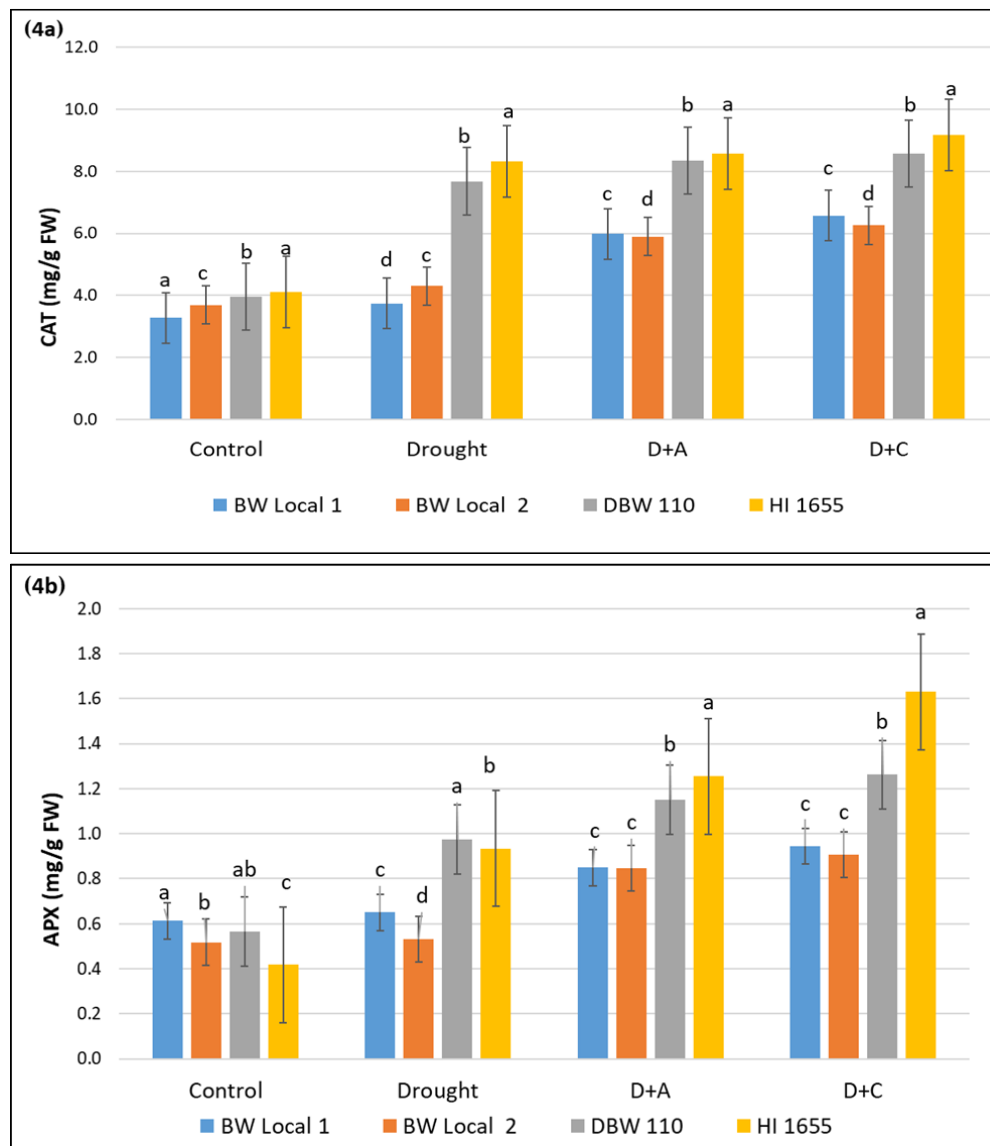
### Chlorophyll fluorescence

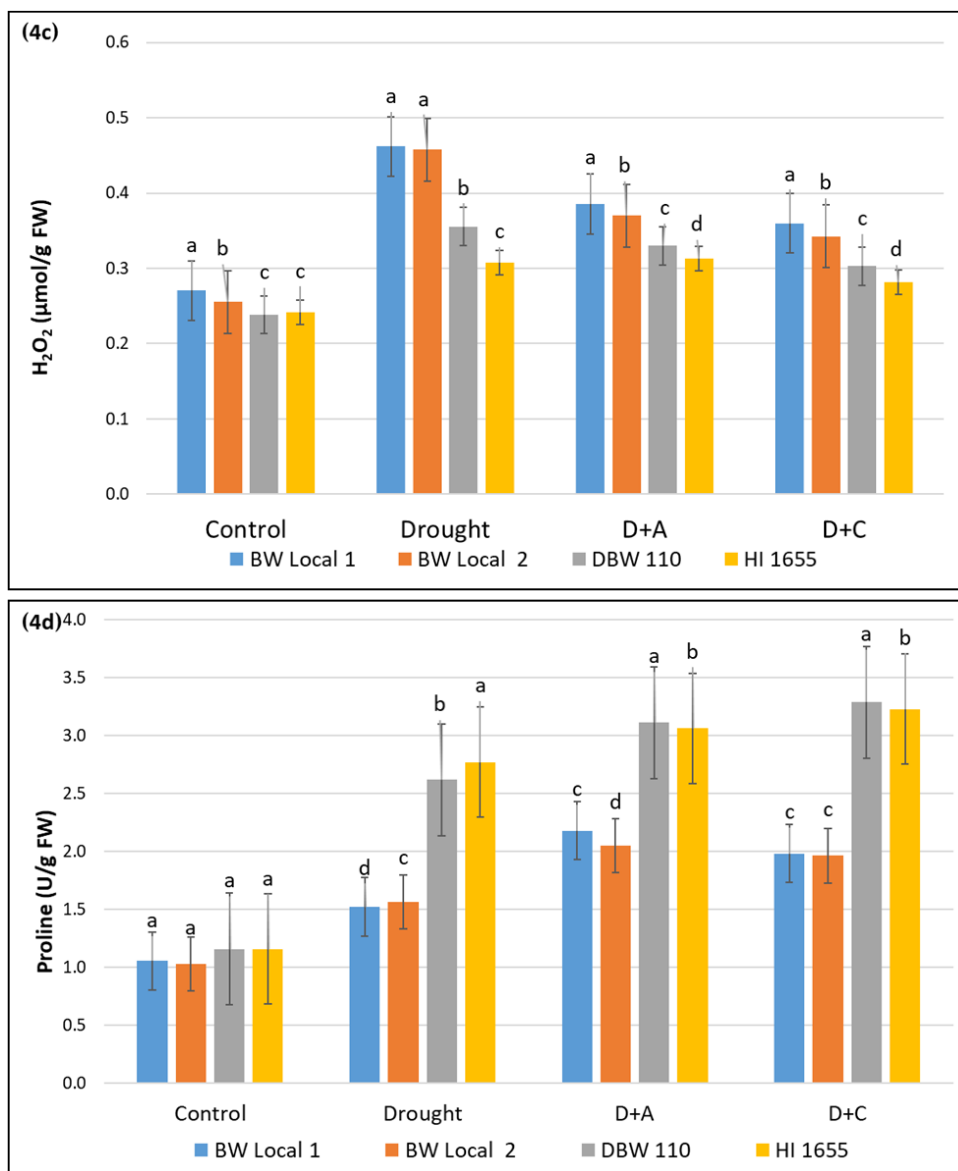
Maximal quantum yield of photosystem II is the proportion of variable Fluorescence (Fv) to the maximum Fluorescence (Fm). In controlled condition, the range of fluorescence varied between 0.78 to 0.82. Due to exposure of drought, this ratio showed a sudden decline in susceptible genotypes BW Local 1 (41.09 %) and BW Local 2 (44.45 %) however, tolerant genotypes DBW 110 and HI 1655 showed increment in the ratio by 20.06 % and 24.65 %, respectively

as compared to the control. After application of auxin, susceptible genotypes showed increased ratio of 1.54 folds (BW Local 1) and 1.48 folds (BW Local 2) and tolerant genotypes showed increment by 0.99 and 0.95 folds for DBW 110 and HI 1655, respectively. Under effect of cytokinin the ratio was increased by 1.61 and 1.79 folds for susceptible genotypes BW Local 1 and BW Local 2, respectively whereas tolerant genotypes showed the increment by 1.03 (DBW 110) and 1.10 (HI 1655) folds than that of the drought condition.

### Stomatal Conductance (SC)

SC is the rate at which water vapour and gases pass through the stomata. In the control condition, it ranged from 78.75 to 83.47  $\text{m mol m}^{-2}/\text{s}^{-1}$ . Under drought condition it reduced to 44.95 % and 46.03 % for susceptible genotypes BW Local 1 and BW Local 2, respectively however pattern for tolerant genotypes was opposite to it since they showed increment in SC by 1.94 % (DBW 110) and 5.86 % (HI 1655) as compared to the control condition which reflected their mitigation efficiency. When auxin was applied to the plants under drought condition it increased SC 1.41 and 1.34 folds for susceptible genotypes BW Local 1 and BW Local 2, respectively whereas tolerant genotypes DBW 110 and HI 1655 showed only 1.03 and 1.04-folds increment, respectively than that of the drought condition. Application of cytokinin also increased the SC 1.21 and 1.17 folds for susceptible genotypes BW Local 1 and BW Local 2, respectively and contribution of both tolerant genotypes DBW 110 and HI 1655 was equal i.e. 1.02 folds, an increase of conductance.





**Fig. 4.** Variation in biochemical traits of wheat seedling in control, drought, drought with auxin (D+A) and drought with cytokinin (D+C) treatment conditions. (a) Catalase; (b) Ascorbate peroxidase; (c) Hydrogen peroxide; (d) Proline. Distinct letters indicate statistically significant differences among genotypes at  $p \leq 0.05$ , as determined by Fisher LSD test

#### Effect of auxin and cytokinin on biochemical traits under drought stress

In the present study, effect of auxin and cytokinin under induced drought stress at seedling stage was studied on CAT, APX, hydrogen peroxide and proline as shown in Fig. 4.

##### Catalase (CAT)

CAT is an enzymatic antioxidant that helps plants to mitigate the stress by breaking down H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O. In control condition, CAT concentration ranged from 3.28 to 4.12 mg/FW. Under drought condition its concentration was increased by 13.99 and 16.51 % for the susceptible genotypes BW Local 1 and BW Local 2 respectively while tolerant genotypes DBW 110 and HI 1655 showed increment by 94.37 and 102.16 %, respectively, as compared to the control condition. Because of auxin, susceptible genotypes could be able to increase CAT level in their system in order to mitigate the drought which was 5.89 and 3.60 folds for BW Local 1 and BW Local 2 than that of the drought condition and tolerant genotypes also showed increment in CAT level to 1.18 to 1.06 folds for DBW 110 and HI 1655, respectively. Mitigation effect of cytokinin led to 7.17- and 4.19-folds increment in level of CAT for susceptible genotypes BW Local 1 and

BW Local 2, respectively whereas for tolerant genotypes DBW 110 and HI 1655 it was 1.24 and 1.20 folds, respectively.

##### Ascorbate peroxidase (APX)

APX is involved in detoxification of H<sub>2</sub>O<sub>2</sub> at lower concentrations with the help of ascorbate. Level of APX was very low (0.41 to 0.61 mg/FW) in control condition as compared to drought condition. Under drought condition, tolerant genotypes DBW 110 (72.57 %) and HI 1655 (123.67 %) showed dramatic increment in the level of APX as compared to control, however susceptible genotypes BW Local 1 (6.17 %) and BW Local 2 (2.76 %) could not respond that much. Under the effect of auxin, level of APX was found to be increased in both tolerant and susceptible genotypes but increase was higher in susceptible genotypes BW Local 1 (6.26 folds) and BW Local 2 (22.99 folds) than that of the tolerant genotypes DBW 110 (1.43 folds) and HI 1655 (1.63 folds) when compared to drought condition. Similar pattern was also observed in the case where cytokinin was applied. In response to the application of cytokinin, susceptible genotype BW Local 1 and BW Local 2 showed 8.80- and 27.21 folds increase in APX level, respectively whereas tolerant

genotypes DBW 110 and HI 1655 showed 1.70- and 2.35-folds increment in APX level, respectively.

**Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**

Hydrogen peroxide is an oxidative molecule which generally acts as signalling molecule under stress condition. It was observed in this study that under normal condition, level of H<sub>2</sub>O<sub>2</sub> ranged between 0.72 and 0.81. Under drought condition, susceptible genotypes BW Local 1 (70.89 %) and BW Local 2 (79.03 %) showed a very high increment in level of H<sub>2</sub>O<sub>2</sub> whereas tolerant genotypes showed 49.27 and 27.30 % increment in the level of H<sub>2</sub>O<sub>2</sub> for DBW 110 and HI 1655, respectively. When auxin was applied to drought stressed plants, they showed fall in level of H<sub>2</sub>O<sub>2</sub> by 1.20 folds (BW Local 1) and 1.24 folds (BW Local 2) in susceptible genotypes and by 1.08 folds (DBW 110) and 0.98 folds (HI 1655) for tolerant genotypes. Cytokinin application led to 1.28- and 1.34-folds fall in level of H<sub>2</sub>O<sub>2</sub> for susceptible genotypes BW Local 1 and BW Local 2, respectively, while tolerant genotypes showed fall of 1.17 and 1.09 folds for DBW 110 and HI 1655, respectively.

**Proline**

Accumulation of proline is a response of plant under stress; it increases with increase in severity of stress. In control condition its level was very low in both susceptible and tolerant genotypes but in drought condition slight increment was witnessed in susceptible genotypes BW Local 1 (44.61 %) and BW Local 2 (52.65 %), tolerant genotypes were also experienced this rise to a very higher extent i.e., 126.50 and 139.71 % for DBW 110 and HI 1655, respectively as compared to control condition. After application of auxin, 1.43- and 1.31-folds increment in the level of proline was observed for susceptible genotypes BW Local 1 and BW Local 2, respectively whereas increment was 1.19 folds and 1.11 folds for tolerant genotypes DBW 110 and HI 1655 than drought condition. In case of

cytokinin application, the increase for susceptible genotypes were 1.30 (BW Local 1) and 1.25 (BW Local 2) folds whereas tolerant genotypes showed increment to 1.26 (DBW 110) and 1.17 (HI 1655) folds than the drought condition.

**Correlation analysis**

A correlation study enables understanding of the nature and magnitude of association between characters. The estimates of correlation between all possible pairs are represented in Fig. 5-8. Under control condition (Fig. 5), RL had shown significantly positive correlation with ShL (r=0.99\*\*), FSW (r=0.99\*\*), FShW (r=0.99\*), total chlorophyll (r=0.97\*) and chlorophyll b (r=0.97\*). SL showed significantly positive correlation with ShL (r=1.00\*\*), RL (r=1.00\*\*), FSW (r=0.99\*\*), FShW (r=0.98\*), total chlorophyll (r=0.98\*) and chlorophyll b (r=0.97\*). ShL had significantly positive correlation with RL (r=0.99), FSW (r=0.98\*), FShW (r=0.97\*), total chlorophyll (r=0.99\*\*) and chlorophyll b (r=0.97\*). FSW showed positively significant correlation with FShW (r=1.00\*\*), total chlorophyll (r=0.97\*) and proline (r=0.96\*). The rest of the trait combinations showed non-significant correlation between them. FShW had significantly positive correlation with total chlorophyll (r=0.95\*) and proline (r=0.96\*). CAT showed negative and significant correlation with H<sub>2</sub>O<sub>2</sub> (r=-0.96\*).

Under drought condition (Fig. 6), among all the growth parameters, physiological and biochemical traits were significant and positive correlation were observed for all combinations except between Chl-b with total chlorophyll (r=0.94), SL (r=0.95), Chl-a (r=0.90), RL (r=0.92), FSW (r=0.92), FShW (r=0.91), proline (r=0.93), CAT (r=0.94), Fv/Fm (r=0.92), SC (r=0.90) and APX (r=0.81). H<sub>2</sub>O<sub>2</sub> showed negative correlation with all the growth parameters, physiological and biochemical traits. APX also had positive and non-significant association with ShL (r=0.94) and CAT (r=0.93).

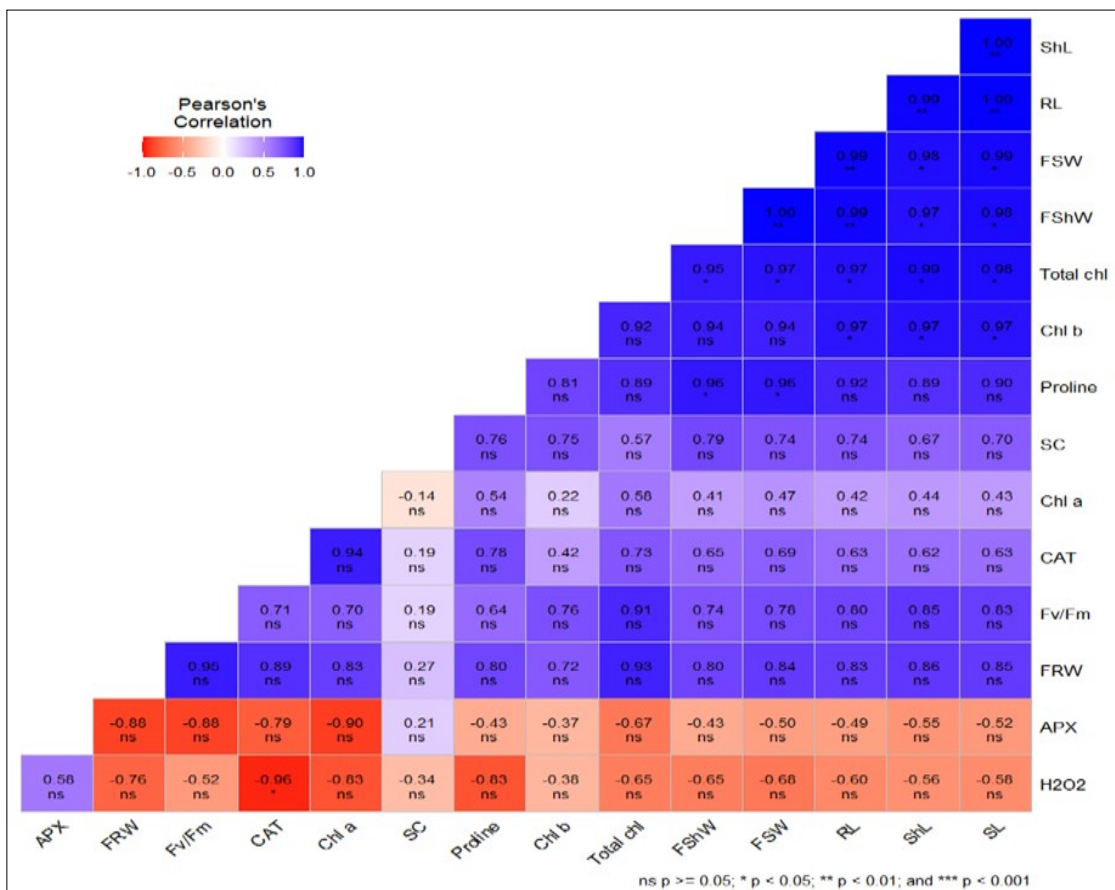


Fig. 5. Estimates of Pearson correlation between different traits of wheat under control condition.

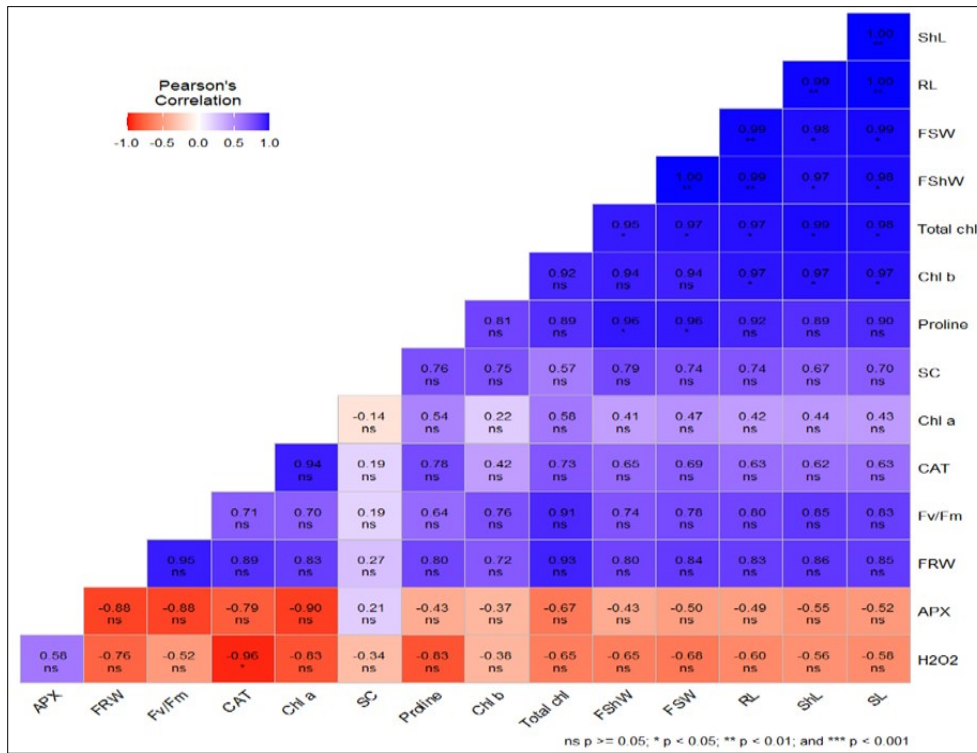


Fig. 6. Estimates of Pearson correlation between different traits of wheat under drought condition.

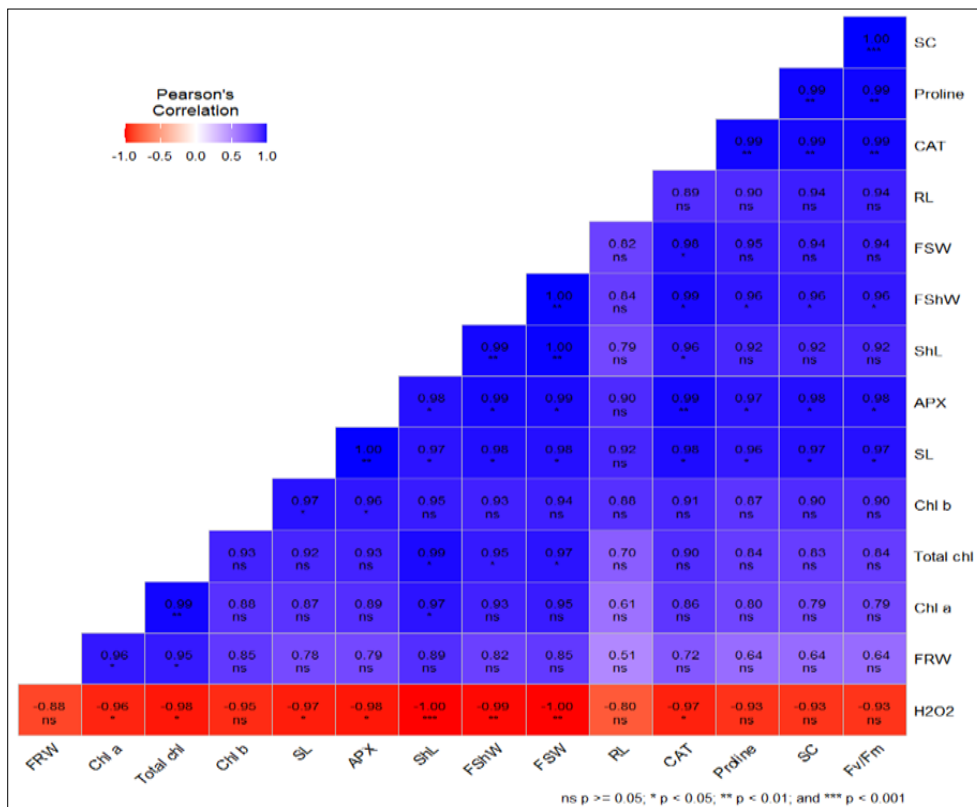
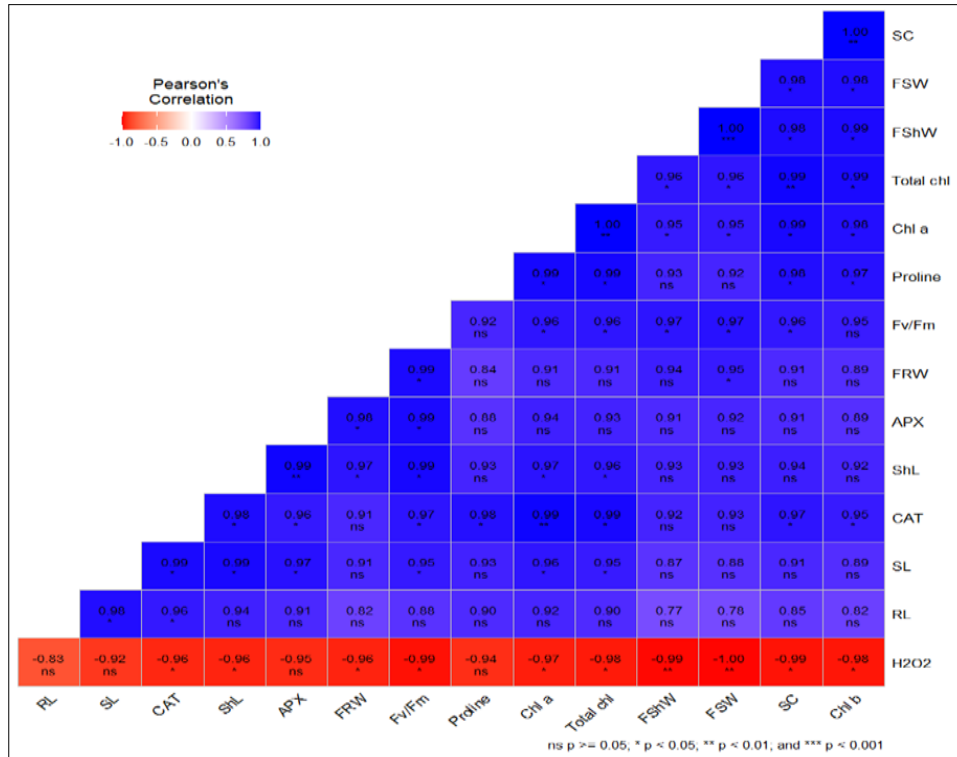


Fig. 7. Estimates of Pearson correlation between different traits of wheat under drought with auxin (D+A) condition.

Under effect of auxin (Fig. 7), Fv/Fm had significantly positive correlation with SC (r=1.00), proline (r=0.99\*\*), CAT (r=0.99\*\*), FShW (r=0.96\*), APX (r=0.98\*) and SL (r=0.97\*). SC had significantly positive correlation with proline (r=0.99\*\*), CAT (r=0.99\*\*), FShW (r=0.96\*), APX (r=0.98\*) and SL (r=0.97\*). Proline had significantly positive correlation with CAT (r=0.99\*\*), FShW (r=0.96\*), APX (r=0.97\*) and SL (r=0.96\*). CAT had significantly positive correlation with FSW (r=0.98\*), FShW (0.99\*\*), ShL (r=0.96\*), APX (r=0.99\*\*) and SL (r=0.98\*). FSW had significantly positive correlation with FShW (r=1.00\*\*), ShL (r=1.00\*\*), APX (r=0.99\*\*), SL (r=0.98\*) and total

chlorophyll (r=0.97\*). FShW had significantly positive correlation with ShL (r=0.99\*\*), APX (r=0.99\*\*), SL (r=0.98\*) and total chlorophyll (r=0.95\*). ShL had significantly positive correlation with APX (r=0.98\*), SL (r=0.97\*), total chlorophyll (r=0.99\*\*) and chlorophyll a (r=0.97\*). The significantly positive association were also found between APX and SL (1.00\*\*), APX and chlorophyll b (r=0.96\*), SL and chlorophyll b (r=0.97\*), total chlorophyll and chlorophyll a (r=0.99\*\*), total chlorophyll and FRW (r=0.95\*), chlorophyll a and FRW (r=0.96\*). H<sub>2</sub>O<sub>2</sub> showed negative association with all the studied traits.



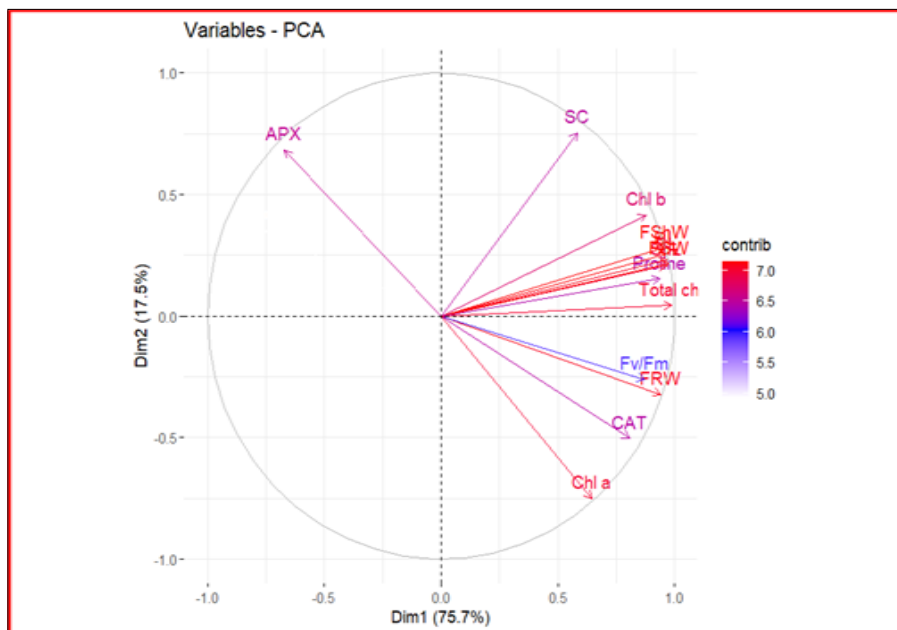
**Fig. 8.** Estimates of Pearson correlation between different traits of wheat under drought with cytokinin (D+C) condition.

Under effect of cytokinin (Fig. 8), drought stress was mitigated to a desired extent and direction. Chlorophyll b showed significantly positive association with SC ( $r=1.00^{**}$ ), FSW ( $r=0.98^*$ ), FShW ( $r=0.99^{**}$ ), total chlorophyll ( $r=0.99^{**}$ ), chlorophyll a ( $r=0.98^*$ ), proline ( $r=0.97^*$ ) and CAT ( $r=0.95^*$ ). SC showed significantly positive association with FSW ( $r=0.98^*$ ), FShW ( $r=0.98^*$ ), total chlorophyll ( $r=0.99^{**}$ ), chlorophyll a ( $r=0.99^{**}$ ), proline ( $r=0.98^*$ ), Fv/Fm ( $r=0.96^*$ ) and CAT ( $r=0.97^*$ ). FSW showed significantly positive association with FShW ( $r=1.00^{**}$ ), total chlorophyll ( $r=0.96^*$ ), chlorophyll a ( $r=0.95^*$ ), Fv/Fm ( $r=0.97^*$ ) and FRW ( $r=0.95^*$ ). FShW showed significantly positive association with total chlorophyll ( $r=0.96^*$ ), chlorophyll a ( $r=0.95^*$ ) and Fv/Fm ( $r=0.97^*$ ). Total chlorophyll showed significantly positive association with chlorophyll a ( $r=1.00^{**}$ ), proline ( $r=0.99^{**}$ ), Fv/Fm ( $r=0.96^*$ ), ShL ( $r=0.96^*$ ), CAT ( $r=0.99^{**}$ ) and SL ( $r=0.95^*$ ). Chlorophyll a showed significantly

positive association with proline ( $r=0.99^{**}$ ), Fv/Fm ( $r=0.96^*$ ), ShL ( $r=0.97^*$ ), CAT ( $r=0.99^{**}$ ) and SL ( $r=0.96^*$ ). Fv/Fm showed significantly positive association with FRW ( $r=0.99^{**}$ ), APX ( $r=0.99^{**}$ ), ShL ( $r=0.99^{**}$ ), CAT ( $r=0.97^*$ ) and SL ( $r=0.95^*$ ). The significantly positive association were also found between FRW and APX ( $r=0.98^*$ ), FRW and ShL ( $r=0.97^*$ ), APX and ShL ( $r=0.99^{**}$ ), APX and CAT ( $r=0.96^*$ ), APX and SL ( $r=0.97^*$ ), ShL and CAT ( $r=0.98^*$ ), ShL and SL ( $r=0.99^*$ ), CAT and SL ( $r=0.99^{**}$ ), CAT and RL ( $r=0.96^*$ ), SL and RL ( $r=0.98^*$ ). It was also observed that  $H_2O_2$  had negative association with all the studied traits.

**Biplot analysis**

Principal component analysis was performed to know the distribution of total variability in different uncorrelated principal components. First three PCs in control condition, first in drought condition, first two in drought with auxin (D+A) condition and first in



**Fig. 9.** Biplot analysis of variables in control condition.

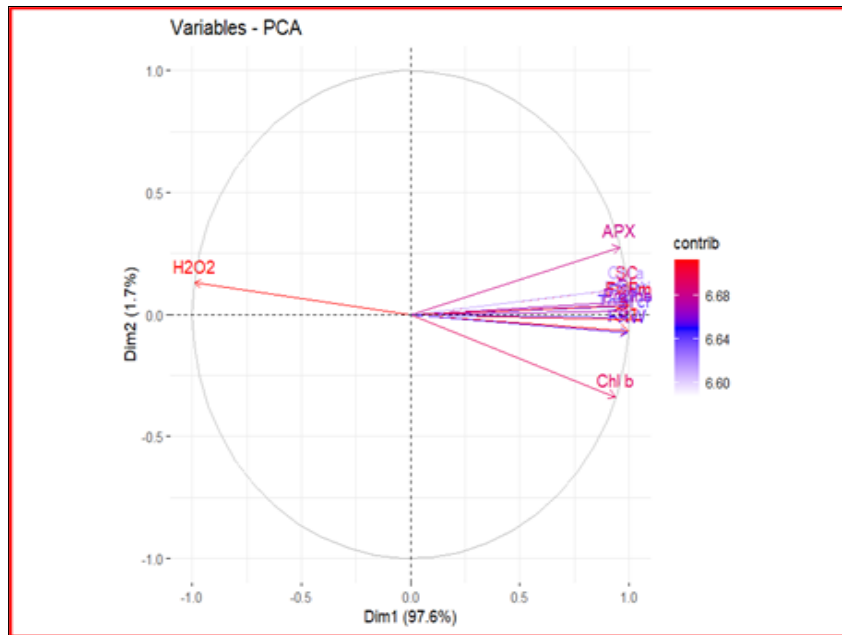


Fig. 10. Biplot analysis of variables in drought stress condition.

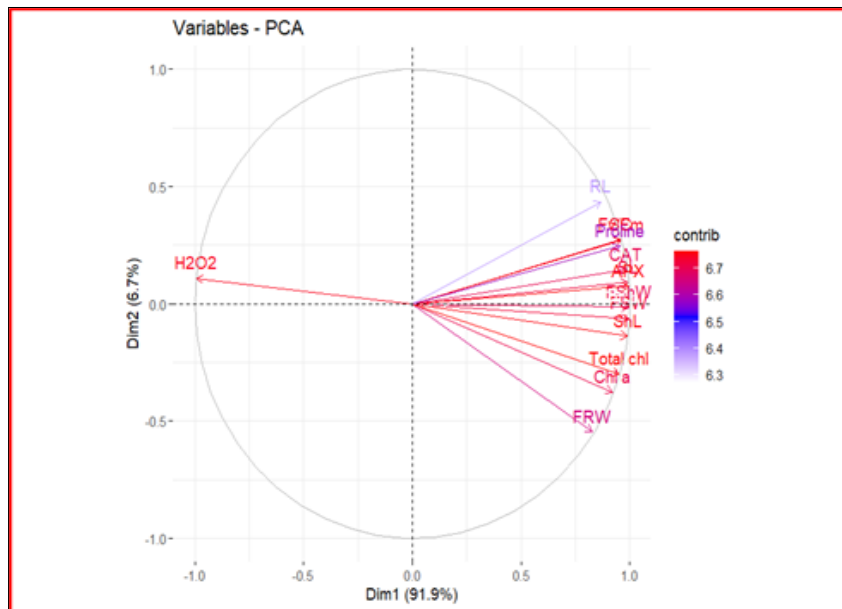


Fig. 11. Biplot analysis of variables under (D+A) condition.

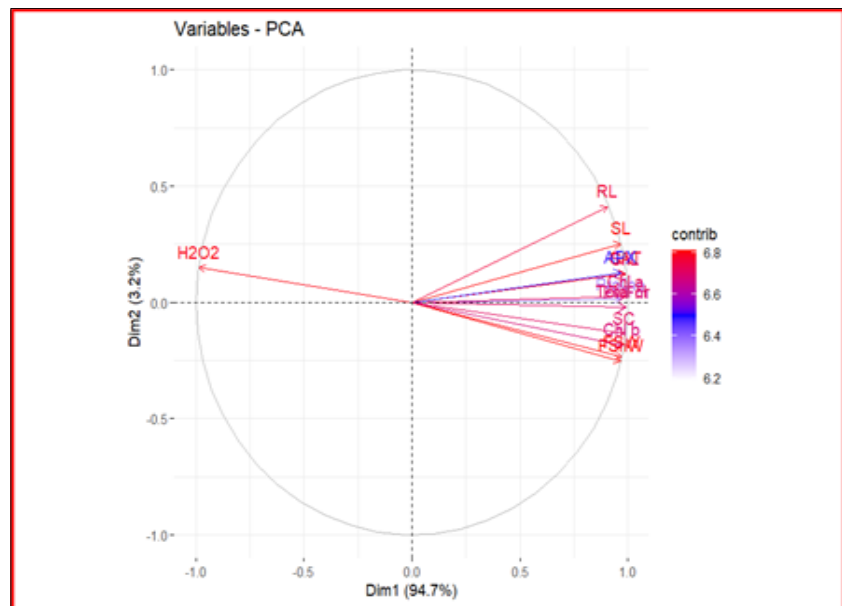
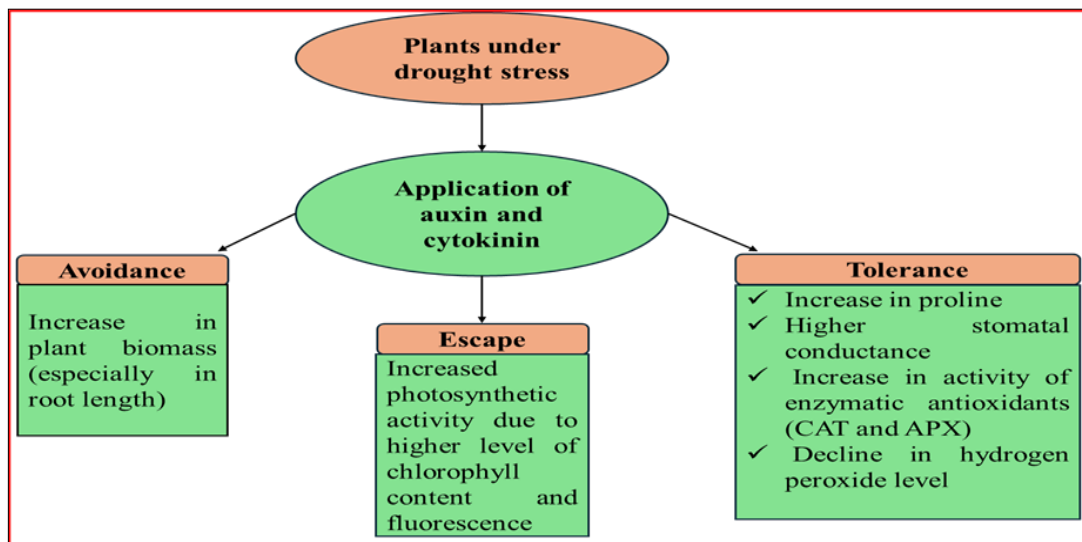


Fig. 12. Biplot analysis of variables under (D+C) condition.

drought with cytokinin (D+C) condition were considered dominant PCs as their eigen values were more than or equal to one. Only first PCs were enough to explain most of variation in all the conditions (Supplementary Table 1 and Supplementary Fig. 1). Biplot Analysis (BA) revealed that under the control condition (Fig. 9) all the studied parameters showed their expression independently. However under drought stress condition (Fig. 10), drought with auxin (D+A) condition (Fig. 11) and drought with cytokinin (D+C) condition (Fig. 12), parameters were grouped as positive contributors (all studied parameters except  $H_2O_2$ ) and negative contributors ( $H_2O_2$ ).

Drought stress is a major problem owing to the consistent occurrence of climate change phenomenon. It unfavorably affects crop growth, development and reproductive activities which eventually leads to a severe drop in crop yield (27–30). The use of tolerant variety is one of the best ways to mitigate stress but there are high yielding susceptible varieties where it is necessary to address the problem. Considering the fact, an experiment was carried out to study the effect of phytohormones (auxin and cytokinin) in mitigation of drought stress on pairs of contrasting genotypes i.e. tolerant (DBW 110 and HI 1655) and susceptible (BW Local 1 and BW Local 2) under four conditions namely control (without PEG application), drought, auxin applied in drought condition and cytokinin applied in drought. Development of drought tolerant varieties rely on mechanistic basis of adaptation to the stressed environment by the plant. It is dependent on the different attributes of the plant which make these genotype able to cope the stress. In this study, those attributes were studied under drought conditions along with exogenous application of auxin and cytokinin plant hormone to study their stress mitigating potential. Improvement in all characters had been observed under drought conditions when plant hormones were applied however susceptible genotypes responded much better than that of the tolerant genotypes it may be due to more stress load in previous than in the later (31,32). The results revealed that ShL and weight are the major attributes of the plant which decide its photosynthetic ability, it was observed higher in tolerant genotypes in all treatment conditions whereas susceptible genotypes under effect of the plant hormones showed better response under drought condition. It implies that the auxin and cytokine have potential part in the growth and development by controlling expression of different photosynthetic enzyme. RL and weight were higher under auxin applied drought condition which indicated that the hormone had potential to mitigate drought by affecting root morphology. An appreciable increment was also observed

in RL and weight under effect of cytokinin, may be due to its role in cell division. Similar patterns were also observed for the SL and FSW in each condition. These finding are in accordance with a previous study (33). Massive increase in ROS content was evident in different studies (34,35). Hydrogen peroxide was found to be increased in this study enormously under drought condition in susceptible genotypes, but tolerant genotypes suppressed this decline (36). Application of auxin and cytokinin made susceptible genotypes able to tolerate drought by reducing the rate of increase in the level of hydrogen peroxide under drought condition. Lower quantities of  $H_2O_2$ , which is an indicator of stress, in tolerant genotypes may be attributed to their enhanced antioxidant enzyme activity. This suggests that more tolerant genotypes likely have a more efficient antioxidant defence system at the cellular level, which guard them from oxidative stress. Proline content was higher in tolerant genotypes as compared to the susceptible under drought stress condition (37,38). Proline accumulation plays a defensive role against oxidative stress by aiding in osmotic regulation, helping cells cope with dehydration and scavenging ROS (39,40). Application of auxin and cytokinin cause decrease in the level of proline in susceptible genotypes under drought condition. It had been observed in previous studies in maize that increase in hydrogen peroxide level led to the activation of proline synthesis pathway by the enzymes  $\Delta^1$ -pyrroline-5-carboxylic acid synthase and glutamate dehydrogenase (41). Enzymatic antioxidants CAT and APX showed increased activity under drought condition especially in tolerant genotypes whereas susceptible genotypes experienced minute changes (42,43). However, after application of auxin and cytokinin the activity of these antioxidants was increased appreciably both in susceptible and tolerant genotypes, increase was greater in susceptible genotypes. For the wheat suffering from drought, it was observed that the accumulation of CAT was higher than that of the APX indicated short duration response mechanism of later. Similar results were witnessed in other studies in rice and wheat (44–46). Photosynthetic pigments are the key to adaptation and survival of plants under stress condition because they make photosynthates which is the substrate for all other metabolic activities. In current study, it was detected that under drought condition level of chl a, chl b and total chlorophyll was reduced very drastically in susceptible genotypes as compared to the tolerant genotypes (47). Increase in level of these photosynthetic pigments was experienced under effect of auxin and cytokinin in both tolerant and susceptible genotypes however it was higher in susceptible genotypes. Stress indicator molecules i.e. hydrogen



**Fig. 13.** Mechanism of drought tolerance witnessed under effect of exogenous auxin and cytokinin in wheat.

peroxide showed significantly negative correlation with root shoot length and weight, antioxidant enzymes and photosynthetic components under stress condition showing complex interaction among them to tolerate the stress (48). In rest conditions, most of the characters showed non-significant correlation between them indicating less struggle in plant system due to proper mitigation of stress.

Taking mechanism under consideration, in drought condition, all the studied traits showed downregulated expression except hydrogen peroxide (Fig. 13). Application of auxin considerably affected root related traits whereas cytokinin gave observable effect on shoot and its related traits, proving antagonistic role of these two hormones. To mitigate the stress, auxin and cytokinin upregulated the expression of all physiological and biochemical traits except hydrogen peroxide. CAT and APX act as reducing agents and change  $H_2O_2$  into  $H_2O$  (49).

## Conclusion

Phyto-hormones auxin and cytokinin found to be helpful in eliminating the adverse effect of drought stress by increasing level of antioxidant enzymes, improving RL and weight and enhancing photosynthetic machinery i.e. SC, inflorescence and chlorophyll content in susceptible genotypes. The correlation and BA also revealed the positive impact of auxin and cytokinin in mitigating stress by reducing struggle of complex interaction among characters when compared to the control condition. Morpho-physiological and biochemical markers like RL and weight, SC, chlorophyll content and inflorescence, level of different antioxidants can be utilized to select and breed drought adaptive genotypes.

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## Authors' contributions

VS, VG, MK and PKP contributed to conceptualization. Data curation was done by PKP. Methodology was developed by VS, VG, PKP and K. Validation was carried out by VG and PKP. Investigation was undertaken by VS, PKP and HJ, while resources were provided by VG and MK. Formal analysis was performed by PKP and PS. Writing-original draft preparation was done by VS, PKP and PS, whereas writing-review and editing and supervision were provided by MK and K. Visualization was carried out by HJ, M and NK. Software was performed by PKP, M and NK. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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