

EFFECT OF DIFFERENT CONCENTRATIONS OF GR24 AS SEED PRIMING TREATMENT ON PHYSIO-CHEMICAL AND YIELD RELATED ATTRIBUTES OF MAIZE (*ZEA MAYS*) HYBRIDS UNDER DROUGHT STRESS

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Abstract

Climate change increases the intensity of drought stress throughout the world. Drought stress poses serious threat to global food security by effecting the yield of field crops. In current experiment, maize hybrids (HY-1898, FH-1046) were used to analyze the effectiveness of GR24 as seed priming strategy under drought stress (60% field capacity). Two levels of drought stress (control and 60% field capacity) were used in this experiment. Seeds of maize hybrids were soaked for 16 h in four concentrations of GR24 (control, 0.001, 0.01 and 0.1 mg L⁻¹). After 48 days of sowing data were collected which showed that net CO₂ assimilation rate and stomatal conductance were increased by GR24 priming in both maize hybrids while transpiration rate (*E*), sub-stomatal carbon dioxide concentration (*C_i*), water use efficiency (*A/E*) and *C_i/C_a* ratio remained unresponsive. Chlorophyll *b*, total chlorophyll, chlorophyll ratio and carotenoids concentrations considerably improved by seed priming with GR24 except chlorophyll *a*. Leaf ascorbic acid and total phenolics were also increased by seed priming with synthetic analogue of strigolactones (GR24). Glycinebetaine (GB) showed significant results while leaf free proline showed non-significant result in both maize hybrids by GR24. Yield parameters of both maize hybrids revealed that GR24 has positive effect on number of seeds per cob and number of seeds per plant under both regimes. In conclusion, seed priming with GR24 improved the drought tolerance in both maize hybrids.

Key words: Abiotic stress, Drought, GR24, Proline, Glycinebetaine, Yield.

Introduction

Drought is a constant threat to world food security and sustainable agriculture development. The fifth report from the Intergovernmental Panel on Climate Change (IPCC) depicts that drought intervals might be frequent in frequency and intensity in near future due to adverse climate change (Anon., 2013). Over the past few years, drought has disturbed all aspects of life more vigorously than any other natural disaster, by creating hurdles to agriculture, livestock and food industries (Anon., 2015). In plants, abiotic stress affects the morphology, physiology and biochemical processes of plants. Frequent drought intervals and their intensity accompanied by heat stress are the key abiotic factors that have significant negative impact on maize production (Hatfield *et al.*, 2011). Imposition of individual or combined abiotic stresses on the maize crop limited its yield (Lesk *et al.*, 2016). Previous literature has revealed that loss in maize yield under drought stress is specifically related to the severity of the drought level and at which growth stage, the drought stress was applied. Drought drastically reduced the plant biomass at the seedling stage and affects morphology such as plant height, leaf area, and yield at the vegetative and milk stage. In addition to these attributes, photosynthetic processes at the same level of drought stress showed more drastic effects at the tasseling stage as compared to the booting stage (Lobell *et al.*, 2014; Abid *et al.*, 2016; Mi *et al.*, 2017; Myer *et al.*, 2014; Jin *et al.*, 2017). Drought interacts effectively with salt stress and affects leaf cell size, cell division, rate of photosynthesis, stomatal closure, delaying flowering and crop productivity (Bita & Gerats, 2013; Shrivastava & Kumar, 2015; Ghatak *et al.*, 2017). Limited supply of water disturbed the plant metabolism at the molecular level by changing biochemical processes and physiology of crop nutrition which is

responsible to decrease plant growth, photosynthetic rates and consequently disturb the electron transport chain (Krasensky & Jonak, 2012; Zivcak *et al.*, 2014). At present different conventional and non-conventional approaches are used to develop drought tolerance in various crops, one of them is seed priming with different plant growth regulators and phytohormones (Ullah *et al.*, 2018). Seed priming with different plant growth regulators induced tolerance against various abiotic stresses through well seedling establishment. It provides help to seeds in mitigating the detrimental effects of drought by adopting various processes such as signaling in late embryogenesis abundant proteins (LEA), food reserves mobilization, up-regulation of transcription factors, endosperm metabolism and elongation in embryonic cells (Chen & Arora, 2011; Marthandan *et al.*, 2020; Singhal *et al.*, 2021). Plant hormones act as signaling molecules and have complex mechanisms that help plants to cope with harsh environment. Strigolactones (SLs) based research with many synthetic analogues are used to define their roles under various abiotic stresses. Among them GR24 has been extensively used because of its potential role to promote seed germination of many parasitic weeds (Screpanti *et al.*, 2016). Worldwide, GR24 is used as a synthetic analogue of strigolactones to investigate the role of strigolactones in plants (de Saint Germain *et al.*, 2019). Strigolactones trigger stomatal closure by the up-regulation of hydrogen peroxide (H₂O₂) and production of nitric oxide and anion channel activation (Zhang *et al.*, 2018). Strigolactones regulate the development of different plant parts. In root development, it controls the root architect, length of root hairs and density (Kapulnik & Koltai, 2014). It also increases grain yield, endosperm size under different phosphorus concentrations (Yamada *et al.*, 2019). SLs up-regulated anthocyanin biosynthesis, regulate root-to-shoot ratio, leaf senescence and cuticle formation (Li *et al.*, 2019).

Study on SL-deficient and SL-response max mutants explore that these mutants showed sensitivity to drought and salt stress. This ensure that strigolactones play some positive role in stress management (Ha *et al.*, 2014; Bu *et al.*, 2014; Cardinale *et al.*, 2018). Strigolactones play roles in the modulation of plant defense responses via their collaboration with other phytohormones signaling pathways particularly with jasmonic acid, salicylic acid and abscisic acid-related pathway in carotenoid cleavage dioxygenase 8 (Torres-Vera *et al.*, 2014). SLs have a positive role in gene regulation mechanism which is related to signaling in plants due to light stimulus (Mayzlish-Gati *et al.*, 2010). In vitro application of different concentrations of GR24 increased callus fresh and dry weight, protein level and ionic contents i.e. Ca^{2+} and K^{+} contents in normal conditions (Zulfiqar *et al.*, 2020). The goal of current study was to navigate the role of GR24 as seed priming on maize plants under drought stress. We examined the chlorophyll pigments, gaseous exchange parameters, osmolytes and yield of maize plants during growth season under drought stress. Following hypothesis was tested: Seed priming with GR24 diminish drought stress by improving physico-chemical processes and drought-induced yield loss in respective maize hybrids.

Materials and Methods

This research trial was conducted in wire house of old botanical garden, University of Agriculture Faisalabad (30°35'45'' N, 72°44'38'' E). Plastic pots having 25 cm diameter and 28 cm length were used for experimentation. Each pot was filled with mixture of clay loam sandy soil having 9 kg weight. Seeds of both maize hybrids (FH-1046 and HY-1898) were obtained from Yousafwala Maize Research Center, District Sahiwal. Strigolactones synthetic analogue GR24 (M. wt. 298.2) was purchased from Organic Chemistry Department, Radboud University Nijmegen, Heyendaalseweg, The Netherland. Healthy 100 seeds of each hybrid were selected for each concentration of GR24. Four concentrations of GR24 i.e. water soaked, 0.001, 0.01 and 0.1 mg L⁻¹ were prepared and seeds of each hybrid were soaked in respective concentrations for 16 h for seed priming treatment. Eight seeds of two maize hybrids primed with GR24 were sown in pots. Thinning was done after 15 days of sowing and four plants in each pot were maintained at harvesting stage. Experiment was grouped in two parts (control and 60% field capacity as drought stress). After 28 days of sowing drought stress was applied at 60% FC.

Statistical analysis

Each experimental unit had three replications and experiment was laid out in a completely randomized design (CRD). Data for analysis of variance of each

attribute was performed by using the COSTAT Computer Program and mean values of each parameter was tested by using Tukey's test at 5% level of probability.

Gas exchange parameters: Photosynthetic parameters i.e. net carbon dioxide assimilation rates (A), stomatal conductance (g_s), transpiration rate (E), intracellular carbon dioxide concentration (C_i), water use efficiency (A/E) and C_i/C_a were examined by using Infrared gas analyzer (IRGA) LCA-4 ADC (Analytical Development Company, Hoddesdon, England) system for maize leaves during 11 am-2 pm. During data collection, Gas flow in leaf chamber was 267 μmol , ambient concentration of carbon dioxide was 344 $\mu\text{mol mol}^{-1}$, leaf chamber temperature was fluctuated between 24-30°C and maximum PAR was displayed upto 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Chlorophyll contents: Arnon (1949) method was used to calculate the chlorophyll contents of maize leaves. Quantitative estimation of chlorophyll content was determined by following formulae:

$$\begin{aligned} \text{Chl. a} &= [12.7 (OD663) - 2.69 (OD645)] \times V/1000 \times W \\ \text{Chl. b} &= [22.9 (OD645) - 4.68 (OD663)] \times V/1000 \times W \end{aligned}$$

V = Volume of the extract (mL)

W = weight of the fresh leaf tissue (g)

Leaf total phenolics: Julkenen-Titto (1985) protocol was used to determine the leaf total phenolics. Maize leaf sample was homogenized in 80% acetone, centrifuged the aliquot for 10 min at 10,000 x g and supernatant was separated. 100 μl from aliquot of the supernatant was mixed with 1:2 ratio of Folin-Ciocalteu's phenol reagent and distilled water. 5.0 mL of 20% Na_2CO_3 solution was added to the reaction mixture and diluted the solution upto 10 mL by adding distilled H_2O . Absorbance was taken at 750 nm, on UV-Visible spectrophotometer (IRMECO U2020) (GmbH, Germany) after vortex.

Leaf ascorbic acid contents: Ascorbic acid (AsA) concentration of maize leaves was calculated by adopting the procedure of Mukherjee & Choudhuri (1983). 10 mL solution of 6% TCA was used to grind fresh leaf sample. 4 mL extract of lysed tissue was mixed with 2 mL of acidic solution of 2% dinitrophenyl hydrazine solution. One drop of 10% thiourea solution prepared in 70% ethanol was added in reaction mixture and placed it in water bath for 20 min at boiling temperature. Mixture was cooled at room temperature and mixed with 5 mL of 80% H_2SO_4 (v/v) at 0 °C. Readings were taken at 530 nm.

Proline determination: Bates *et al.*, (1973) protocol was used to calculate the leaf free proline in maize hybrids. Proline concentration was determined from a standard curve and calculated on fresh weight basis as follows;

$$\mu\text{mole proline g}^{-1} \text{ fresh weight} = \frac{\mu\text{g proline mL}^{-1} \times \text{mL of toluene}/115.5}{(\text{g of sample})}$$

Glycinebetaine determination: Glycine betaine of maize leaves were calculated by Grieve & Grattan (1983) with partial modifications. Fresh leaf sample was ground in 10 mL of distilled water (dH₂O). After filtration 1 mL of 2N HCl was added in filtrate and mixed with 0.2 mL of potassium tri-iodide solution of 0.5 mL of reaction mixture in a glass tube. Cooled the mixture in an ice bath for 90 min. with occasional shaking. Ice cooled dH₂O and 1-2 dichloromethane (cooled at -10°C) were added to the mixture. Two layers were formed from which upper aqueous layer was discarded and optical density of the lower organic layer was measured at 365 nm.

Yield attributes: At maturity, the cobs were separated from the plants and weighed. The seeds were segregated from the cobs and 100-seeds weight were measured with weighing balance. Cob length was measured with inchi tape in cm while cob diameter was measured with the help of vernier caliper. Number of seeds per plant and number of seeds per cob were calculated manually.

Results

Imposition of drought stress at 60% FC considerably decreased the net CO₂ assimilation rate (*A*) of both maize hybrids. Application of different concentrations of GR24 as seed priming significantly increased the net carbon dioxide assimilation rate of maize plants under drought stress. Hybrids significantly differed with each other however, FH-1046 performed better in both conditions. Hybrids, drought and GR24 interaction showed highly significant correlation. Maize plants under drought stress showed slightly significant decline in transpiration rate (*E*) of FH-1046 while seed priming with GR24 exhibited non-significant results regarding transpiration rate. Gas exchange parameter like stomatal conductance (*g_s*) remarkably decreased under drought in maize hybrids. Pre-sowing seed treatment with various concentrations of GR24 moderately increased the stomatal conductance in both hybrids. Hybrids varied significantly while FH-1046 gave more positive response under both regimes. Drought stress and GR24 application as seed priming treatment showed non-significant effect on sub-stomatal carbon dioxide concentration (*C_i*) of maize plants, while drought and GR24 interaction showed highly significant relationship. Water use efficiency (*A/E*) of maize plants decreased significantly in HY-1898 under drought stress. Exogenously applied GR24 as priming and hybrids did not cause change in water use efficiency in both regimes. Hybrids and GR24 showed highly significant interaction (14.901***) for water use efficiency. Experimental results of *C_i/C_a* ratio did not show any change upon drought stress and GR24 application. HY-1898 gave best performance under both growth medium. Drought stress and GR24 application showed highly significant interaction with respect to *C_i/C_a* ratio (Fig. 1; Table 1).

Chlorophyll *a* contents showed no effect of drought and pre-sowing treatment with GR24 in maize hybrids. Hybrids and all type of interaction showed non-significant results on chlorophyll *a* concentration. Impositions of drought stress showed non-significant

effect on chlorophyll *b* and total chlorophyll contents. Seed priming with different concentrations of GR24 showed significant increase in chlorophyll *b* and total chlorophyll concentration in both hybrids. FH-1046 performed better response in both regimes. Hybrids, drought and GR24 interaction showed significant (0.036**) interaction with respect to chlorophyll *b* contents. Chlorophyll ratio slightly decreased under drought stress. GR24 application increased the chlorophyll ratio under drought stress in maize plants. HY-1898 gave more positive results. Interaction between hybrid and GR24 showed highly significant (3.010***) results. Drought stress did not affect the carotenoids contents whereas seed priming with GR24 slightly enhanced the carotenoids concentration under drought stress in maize hybrids. Cumulative effect of hybrid, drought and GR24 showed slightly significant (0.001*) interaction for carotenoids contents (Fig. 2; Table 1).

Leaf ascorbic acid considerably increased under drought stress (60% FC) in maize hybrids. Seed priming with different concentrations of GR24 considerably enhanced the leaf ascorbic acid under both controlled as well as drought stressed mediums. FH-1046 showed better performance in both regimes. Imposition of drought stress revealed that total phenolics were slightly increased in both hybrids by the application of drought stress. GR24 as seed priming treatment slightly improved the total phenolics contents in maize plants. Hybrids considerably differed in response to total phenolics under drought stress and HY-1898 showed more increase in total phenolics than FH-1046. Significant interaction was observed between hybrids and GR24 with respect to this parameter (Fig. 3; Table 1). Glycinebetaine (GB) was prominently increased under drought stress in both maize hybrids. Maize plants observed considerable increase in GB concentration by exogenous application of different levels of synthetic analogue GR24 as seed priming under controlled and water stressed regimes. HY-1898 performed better by increasing GB under both regimes. Hybrid, drought and GR24 revealed slightly significant interaction (95.295**) for GB. Imposition of drought stress considerably increased the free proline contents in maize plants while GR24 application did not affect the free proline concentration. HY-1898 expressed overall higher production of free proline as compared to FH-1046 (Fig. 3; Table 1).

Data on yield parameters revealed that drought stress (60% FC) did not affect the cob weight, cob diameter and number of seeds per cob of maize hybrids while considerable reduction was observed in cob length, 100-seeds weight and number of seeds per plant. Application of GR24 as seed priming did not affect the cob length, cob weight and cob diameter. However seed priming with different concentration of GR24 enhanced the 100-seeds weight, seeds per cob and number of seeds per plant in both regimes. Hybrids differed significantly and FH-1046 showed better results in both growth medium. Our results displayed highly significant interactions between hybrid, drought and GR24 for 100-seeds weight (16.085**), seeds per cob (4911.36***), and number of seeds per plant (58862.743***) (Fig. 4; Table 1).

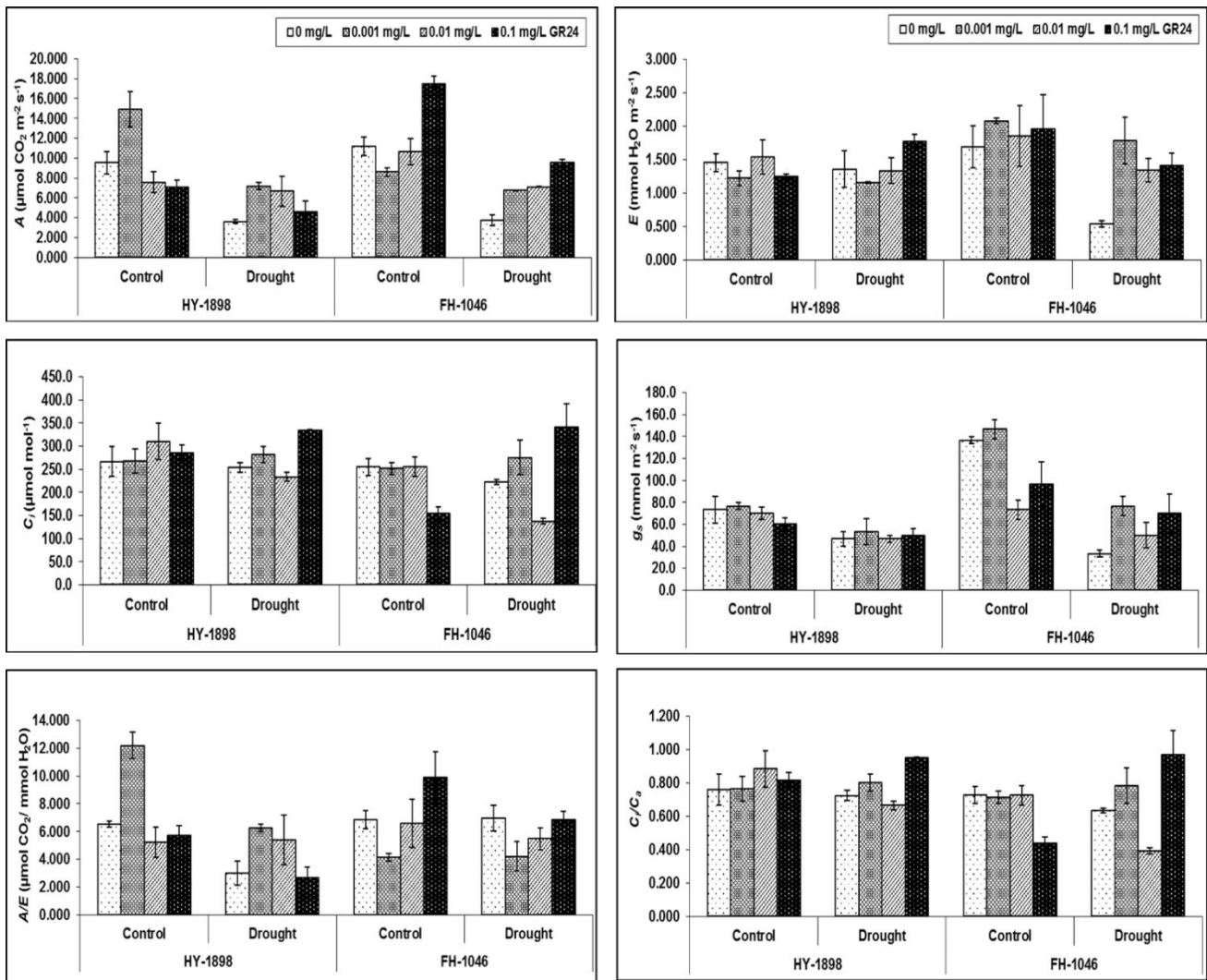


Fig. 1. Gaseous exchange attributes of hybrids of maize (*Zea mays* L.) when different concentrations of GR24 was given for 16 h as seed priming treatment under controlled and water stressed regimes.

Discussion

Plants being immobile in nature have adopted different physiological and biochemical mechanisms to mitigate the harmful effects of drought stress. Drought stress has negative impacts on biochemical and physiological processes of plants like net CO₂ assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and photosynthetic pigments (Myer *et al.*, 2014; Song *et al.*, 2018; Kosar *et al.*, 2018). Data of the current experiment exhibited that net CO₂ assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and water use efficiency (A/E) considerably decreased under drought stress while sub-stomatal carbon dioxide concentration (C_i) and C_i/C_a ratio remained without change upon the drought stress. Under drought stress diffusion rate of CO₂ to mesophyll and stomata decreased (Moriani *et al.*, 2002). This may disturb the electron transport chain as a result reactive oxygen species are produced that cause oxidative damage in cells (Das & Roychoudhury, 2014). Statistical results of seed priming with synthetic analogue GR24 revealed that net CO₂ assimilation rate (A) and stomatal

conductance (g_s) increased under drought stress however transpiration rate (E), water use efficiency, sub-stomatal carbon dioxide concentration (C_i) and C_i/C_a did not show any regulation. These results were related to previous finding in which foliar application of GR24 mitigates the drought stress by increasing the net CO₂ assimilation rate (A) transpiration rate (E) and stomatal conductance (g_s) in grapewine (Min *et al.*, 2019) and sunflower (Sarwar & Shahbaz, 2020) under salt stress. This might be due to up regulation of F-box *Max2* proteins which involved in biosynthetic pathway of SL and ABA signaling, hence ABA signaling is important indicator of drought stress that regulates the stomatal regulation which control the gas exchange through stomata (Bu *et al.*, 2014; Ha *et al.*, 2014; Visentin *et al.*, 2016; Mostofa *et al.*, 2018). Photosynthetic pigments of maize plants of current experiment showed that chlorophyll *a*, *b*, total chlorophyll and carotenoids remained intact under drought stress while sharp reduction was observed in chlorophyll ratio. These findings were contradictory to Romdhane *et al.*, (2019) in maize in which chlorophyll *a*, *b* and total chlorophyll severely decreased under extreme drought stress.

Table 1. Mean squares from three-way analysis of variance of different physiological, biochemical and yield attributes of maize hybrids (HY-1898 and FH-1046) as seed primed treatment with different GR24 concentration under normal and drought stressed conditions at vegetative growth stage.

Source of variation	df	A	E	g_s	C_i	A/E	C_i/C_a
Hybrid (HB)	1	36.192***	0.476ns	8802.083***	22345.659**	0.679ns	0.180**
Drought (D)	1	268.475***	1.027*	16502.083***	206.836ns	17.553***	0.002ns
GR 24	3	18.316***	0.280ns	1374.306**	4751.704ns	0.991ns	0.038ns
HB x D	1	2.717ns	1.320*	4218.75***	1403.571ns	3.642ns	0.011ns
HB x GR24	3	61.947***	0.536*	1174.306*	2867.54ns	14.901***	0.023ns
D x GR24	3	10.351*	0.209ns	1340.972*	24093.766***	1.361ns	0.194***
HB x D x GR24	3	17.585***	0.161ns	935.417*	4802.888ns	2.543ns	0.039ns
Error	32	2.512	0.183	304.167	1741.112	3.088	0.014
Source of variation	df	Chl a	Chl b	Total Chl.	Chl a/b ratio	Carotenoids	Leaf AsA
Hybrid (HB)	1	0.070ns	0.061**	0.003ns	1.533*	0.008ns	0.001***
Drought (D)	1	0.032ns	0.005ns	0.011ns	1.294*	0.005ns	0.003***
GR 24	3	0.038ns	0.071***	0.211**	1.803**	0.009 *	0.001***
HB x D	1	0.004ns	0.044**	0.074ns	2.651**	0.006ns	0.003ns
HB x GR24	3	0.030ns	0.787***	0.170*	3.010***	0.007ns	0.009ns
D x GR24	3	0.022ns	0.037**	0.104ns	0.460ns	0.002ns	0.001ns
HB x D x GR24	3	0.049ns	0.036**	0.121ns	0.800ns	0.001*	0.007ns
Error	32	0.027	0.005	0.046	0.298	0.002	0.005
Source of variation	df	Total phenolics	Glycine betaine	Proline	Cob length	Cob weight	Cob diameter
Hybrid (HB)	1	0.245***	1249.873***	0.143*	0.083ns	1414.515ns	5.952***
Drought (D)	1	0.080*	298.510***	10.612***	4.747*	1504.944ns	0.542ns
GR 24	3	0.044*	137.256***	0.035ns	9.055ns	1271.936ns	1.034ns
HB x D	1	0.003ns	158.888**	0.006ns	14.083ns	2469.492ns	0.002ns
HB x GR24	3	0.052*	303.368***	0.028ns	7.472ns	396.699ns	0.184ns
D x GR24	3	0.040ns	93.637**	0.068ns	2.388ns	890.447ns	1.323*
HB x D x GR24	3	0.022ns	95.295**	0.034ns	51.583***	1485.468ns	0.857ns
Error	32	0.014	17.910	0.033	5.687	897.707ns	0.41
Source of variation	df	100-seed wt.	Number of seeds per Cob	Number of seeds per plant			
Hybrid (HB)	1	104.135***	2296.333ns	357.520ns			
Drought (D)	1	132.335***	588ns	30754.688*			
GR 24	3	68.636***	5491.027***	194646.47***			
HB x D	1	9.992ns	52.083ns	36575.521*			
HB x GR24	3	68.692***	747.055ns	6449.187ns			
D x GR24	3	86.848***	6312.166***	36371.576**			
HB x D x GR24	3	16.085**	4911.361***	58862.743***			
Error	32	3.217	562.625	6896.708			

*, ** and *** = Significant at 0.05, 0.01 and 0.001 levels, ns = Non-significant respectively A = Net photosynthetic rate; E = Transpiration rate; C_i = Internal CO₂ concentration; g_s = Stomatal conductance; A/E = Water use efficiency; C_i/C_a = Ratio of leaf intrinsic CO₂ concentration to ambient CO₂ concentration, Chl a = Chlorophyll a; Chl b = Chlorophyll b; Total chl = Total chlorophyll; Chl a/b ratio = Chlorophyll a/b ratio; Leaf AsA = Leaf ascorbic acid

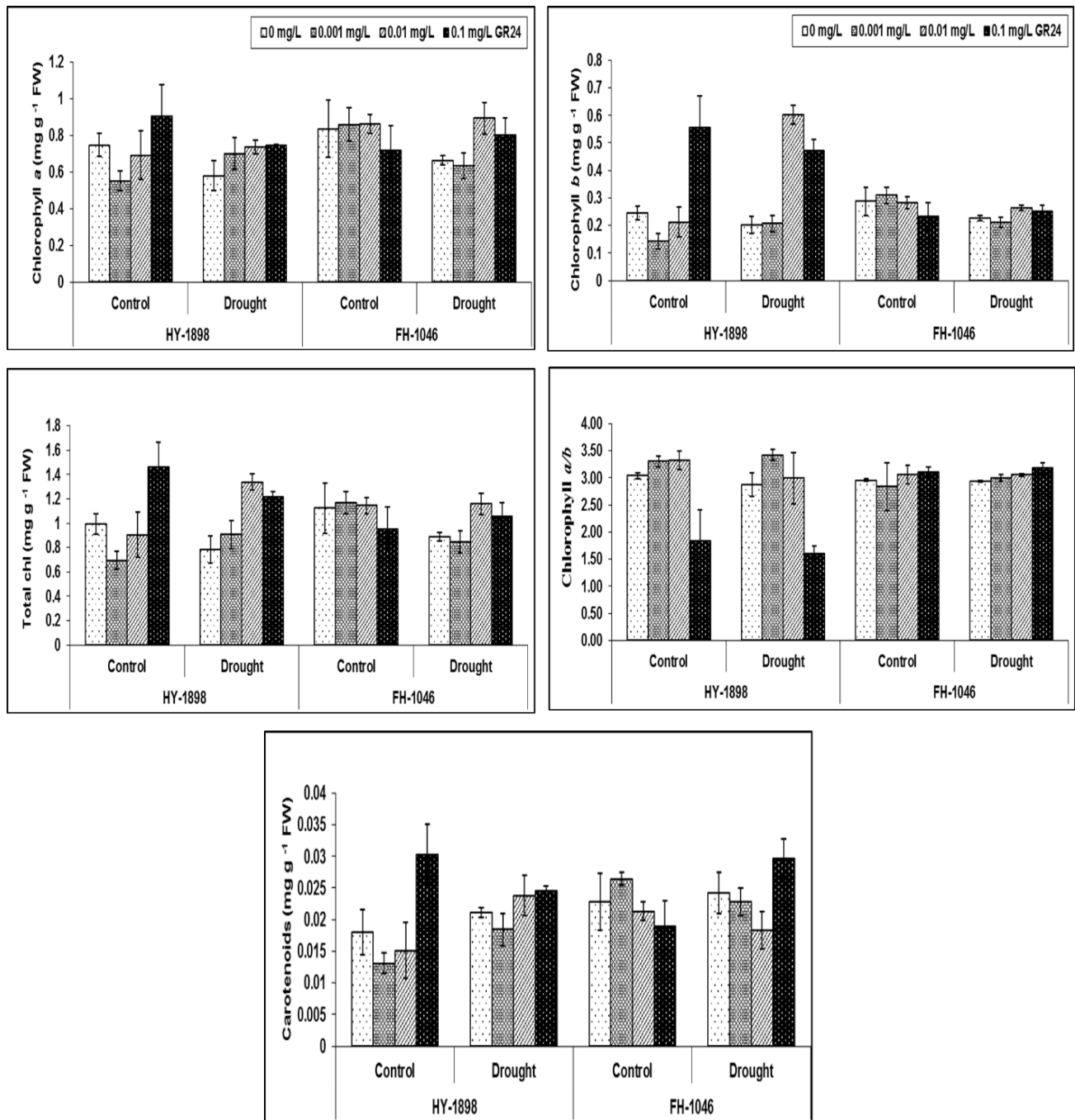


Fig. 2. Chlorophyll pigments of maize (*Zea mays* L.) hybrids when different concentrations of GR24 was given for 16 h as seed priming treatments under controlled and water stressed regimes.

Hence, exogenously applied GR24 considerably improved the Chlorophyll *a* and chlorophyll ratio under drought stress that help plants to cope with detrimental stress. Min *et al.*, (2019) and Sedaghat *et al.*, (2017) experiment on grapevine and rice respectively revealed similar results by foliar application of different levels of GR24 under drought stress. The reason behind was that strigolactone might have been involved in regulation of light harvesting genes (Mayzlish-Gati *et al.*, 2010) in tomato and changing the physiology of leaf in *arabidopsis* (Waldie *et al.*, 2014; Li *et al.*, 2019). In rice mutants of SL signaling and synthesis determined that ABA accumulation increased in shoot

under drought stress (Haider *et al.*, 2018). Research on *Max1* and *Max2* mutants of *Arabidopsis* of SL signaling with ABA signaling *aba2* mutant demonstrated that strigolactone may either separately or in relation with ABA produced drought tolerance (Ha *et al.*, 2014; Kalliola *et al.*, 2019). Phenolics and ascorbic acid are important non-enzymatic antioxidants that produced in plants as secondary metabolites (Wang *et al.*, 2011) and have significant role in scavenging reactive oxygen species to avoid from detrimental effects of oxidative stress (Bharti *et al.*, 2013). Current investigation confirmed that a significant increase in ascorbic acid and total phenolics were observed in maize hybrids under control as well

as in drought stress regimes. These investigations were correlated with Wu *et al.* (2017) in sorghum and contradictory to Puente-Garza *et al.*, (2017) in *Agave salmiana* under drought stress. Application of different levels of GR24 considerably improved the ascorbic acid and total phenolics contents under drought stress. These findings were consistent to Min *et al.*, (2019) that demonstrated that ascorbic acid value increased by foliar application of GR24 in maize seedlings (Ye *et al.*, 2016). The exact mechanism is not known that how GR24 regulate the ascorbic acid and total phenolics. Phenolics are correlated with stress tolerant antioxidants because these compounds are redox sensitive (Velderrain-Rodríguez *et al.*, 2014). They break the chain reaction due to their high donor ability of electron pair and make these phenolics composites perfect for antioxidants activity (Lin *et al.*, 2016). Among organic osmolytes glycinebetaine and proline are important osmoprotectants that mitigate the harmful effects of drought stress (Nazar *et al.*, 2011; Shabbir *et al.*, 2016). Osmoprotectants accumulation in cell is an important indicator of drought stress tolerance (Khan *et al.*, 2012) by the capability to overwhelmed water stress, osmotic stress and ion compartmentalization (Iqbal *et al.*, 2014). Current investigation revealed that concentration of glycinebetaine and free proline were also increased by exogenously applied GR24. These results coincided with Zheng *et al.*, (2021) in apple. Previous studies of Vardharajula *et al.*, (2011), Shan & Wang (2017), and Nikolaeva *et al.*, (2017) in maize exhibited that exogenous application of growth promoter substances improved the osmotic adjustment capacity by

increasing the osmoprotectants level under drought stress (Gupta *et al.*, 2014). This might be due to the up-regulation of *P5CS* and *P5CR* genes in proline biosynthetic enzyme under drought stress (Meng *et al.*, 2014; Adamipour *et al.*, 2020). Similarly increased level of GB has a protective role against oxidative stress by stabilizing membranes and enzymes under drought stress (Khan *et al.*, 2015). Drought stress has detrimental effects on maize yield and it became worse when plant meets it at the reproductive stage (Oury *et al.*, 2016; Liu *et al.*, 2020). Current experimental data demonstrated that yield related attributes of maize like cob length, weight and diameter were not affected by drought stress while 100-seeds weight and number of seeds per cob and seeds per plant were decreased on the exposure to drought stress. Water shortage in rhizosphere decreases the water content of the leaf which will lead to the closing of stomata to reduce water loss (Mutava *et al.*, 2011). Yield loss under severe drought might be due to the reduced leaf area and impairment of the chlorophyll pigments (Farooq *et al.*, 2015). Similar results were observed by Borrás & Vitantonio-Mazzini (2018) in maize of yield loss under limited water supply. Current experiment showed that GR24 did not affect the cob length and weight and cob diameter while 100-seeds weight, number of seeds per cob and per plant considerably increased under both normal as well as drought stress conditions. The exact mechanism behind this is unknown but this might be due to the seed priming with GR24 that could help to maintain water contents by improving osmoprotectants and increasing the efficiency of photosynthetic pigments of maize plants.

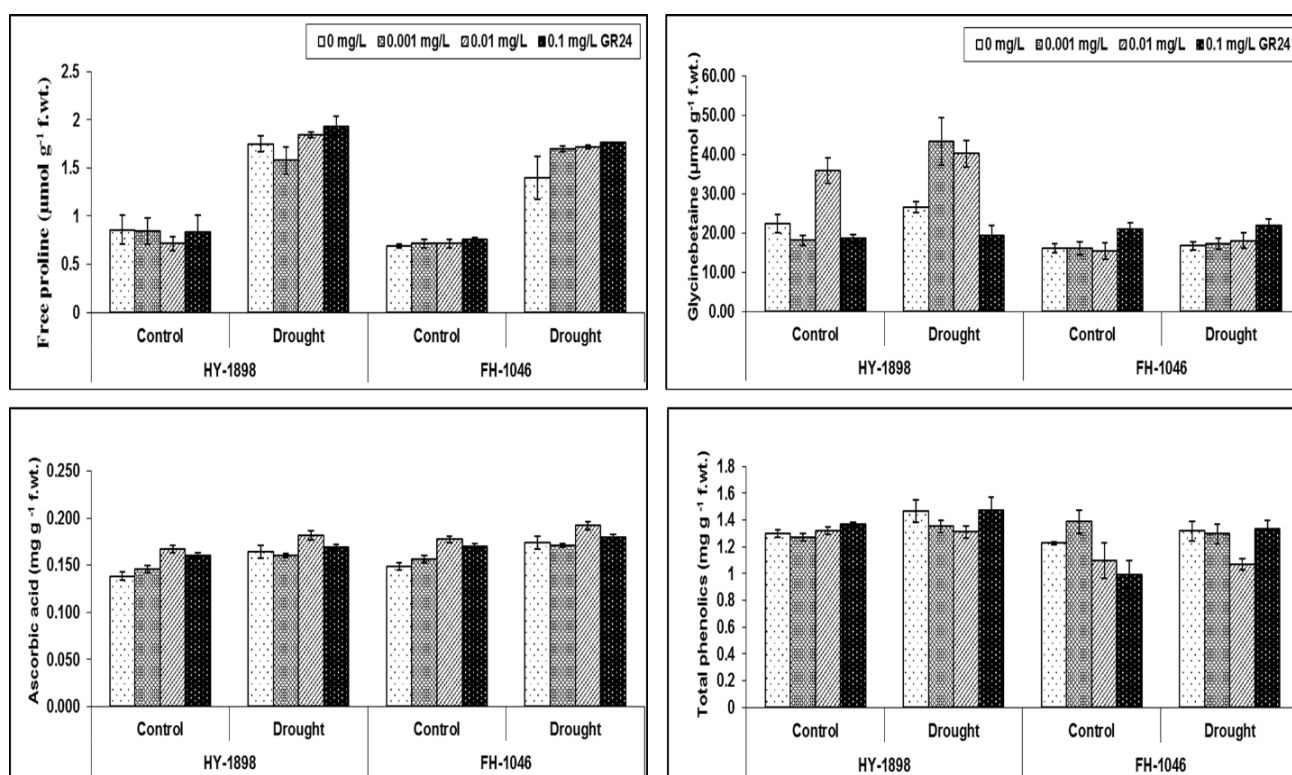


Fig. 3. Osmolytes accumulation and antioxidant response of maize (*Zea mays* L.) hybrids when different concentrations of GR24 was given for 16 h as seed priming treatments under controlled and water stressed regimes.

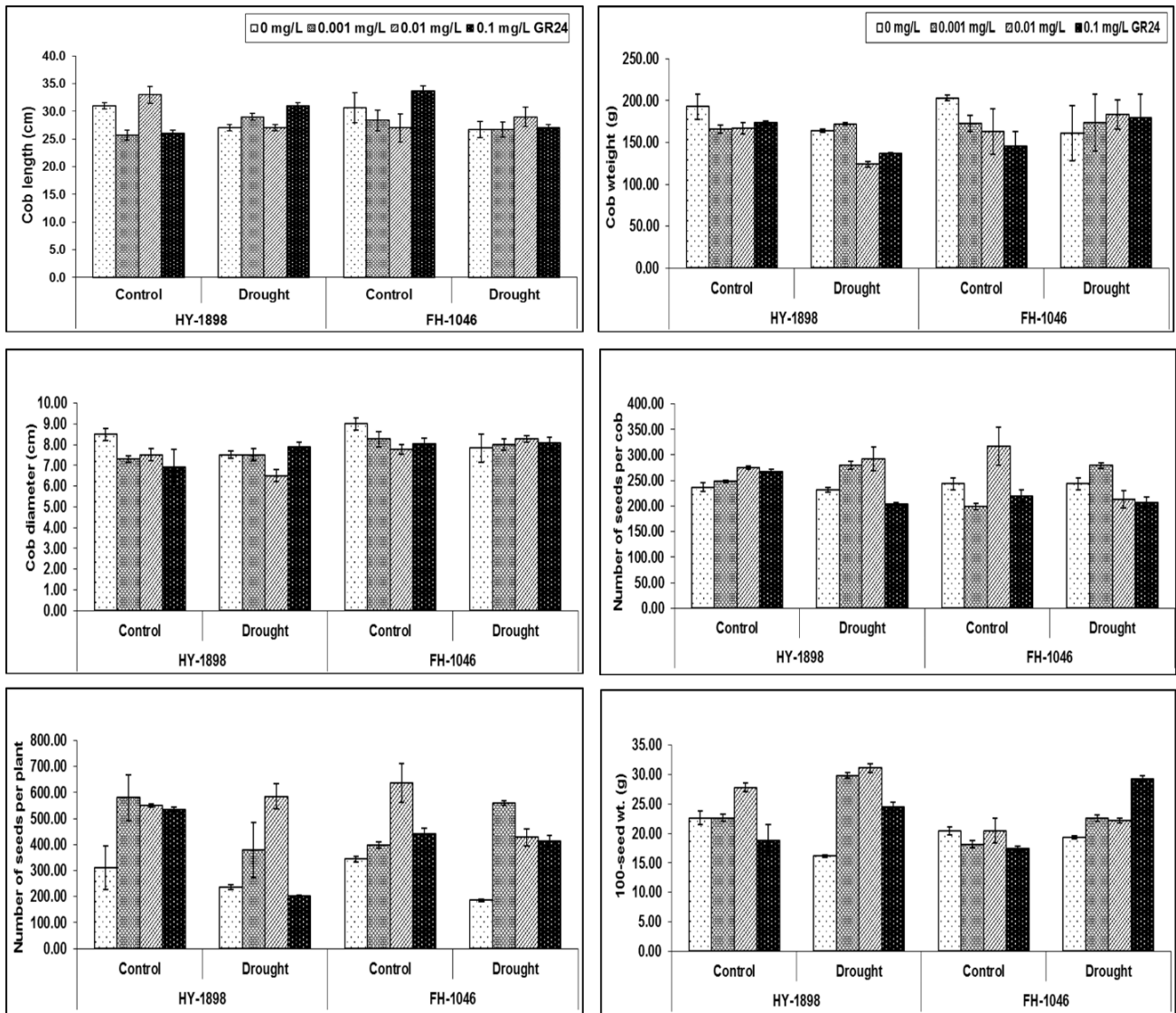


Fig. 4. Yields attributes of hybrids of maize (*Zea mays* L.) when different concentrations of GR24 was given for 16 h as seed priming treatment under controlled and water stressed regimes.

Conclusion

GR24 application as seed priming treatment on maize hybrids had a positive effect on gas exchange characteristics under both controlled as well as drought stress conditions. It enhanced the level of GB and proline contents to combat the harmful effects of drought. Number of seeds, number of seeds per cob and 100 seed weight were also improved in maize hybrids under drought stressed conditions. Implication of drought stress showed remarkable reduction in gas exchange abilities and yields properties of maize hybrids. Statistical results of studied attributes of the experiment strongly supported the hypothesis.

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