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Brassinosteroid a Potential Plant Growth Regulator to Alleviate Drought Stress in Apple Cultivars

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Abstract
The objective of this study was to understand the effect of foliar application of brassinosteroid (BRs) to mitigate the adverse impacts of drought stress on one year old apple seedlings of two cultivars i.e. Super Chief and Red Chief. Both the cultivars were pre-treated by foliar application of brassinosteroid (0.05 and 0.10 ppm), 3 days before imposition of drought. Drought was imposed on plants withholding water for 15 and 30 days. Adequate moisture was maintained in control plants near to field capacity. Results of this study revealed that drought for 15 and 30 days led to a reduction in Physio-biochemical characteristics with a higher reduction under 30 days of drought. Pre-treatment with brassinosteroid at 0.05 and 0.10 ppm concentration prior to subjecting to drought helped in minimizing the detrimental effect of drought in both cultivars. However, 0.05 ppm was more effective in counteracting the effect of drought by maintaining vital physiological and biochemical changes. The chlorophyll fluorescence, photosynthetic rate, canopy temperature depression, stomatal conductance and transpiration rate showed significant increase under 0.05 ppm brassinosteroid treatment as compared to 15 and 30 days of stress. However, brassinosteroid at both concentrations could help in the relative accumulation of osmoregulating substances (proline) and activities of anti-oxidant enzymes namely peroxidase under different stress durations and treatments. Foliar application of brassinosteroid (0.05 ppm) prior to imposition of stress can prove the way for reversal of deleterious effects of water stress on apple plants. Therefore, it may be concluded that foliar application of BRs prior to drought has the potential to reverse the deleterious effect of drought in apple plants.

Keywords: Drought; Brassinosteroid; Stress durations; Photosynthetic rate; Canopy temperature depression; Water use efficiency; Transpiration

Introduction
Mild to severe drought stress is a common phenomenon, which adversely affects orchard performance and productivity potential throughout the world. Water is an essential component required for establishment, subsequent growth and fruit production in orchards. However, the most of apple orchards are growing under rainfaid conditions and always exposed to mild, moderate to severe drought stress at different phonological phase during entire growth period (Bolat et al., 2014). Apple is widely cultivated for commercial purpose in temperate region of world and has a major contribution in the horticultural wealth. The productivity of apple orchards is adversely affected as moisture availability declines. The loss is always greater in case of orchards raised and maintained under rainfed conditions.

Rainfall is scattered throughout the year and there may be very less or no rains during critical periods of growth and development such as seedling stage, after transplanting, fruit setting, walnut stage etc (Kumari and Thhakur, 2020). Apple plants after translating required regular and adequate water otherwise most of the grafted seedling are unable to complete their lifecycle as tree. It has been shown that water deficit influences various physiological, biochemical, metabolic and molecular processes in various plants, including apple trees (Zu et al., 2017; Mihaljević et al., 2021). Lack of water in plants induces oxidative stress (Lei et al., 2006; Noctor et al., 2014), overproduction of reactive oxygen species (ROS), including the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2), which cause lipid peroxidation and damages the membrane, proteins, chlorophyll, nucleic acids and cell death (Mihaljević et al., 2021). Drought can cause a significant reduction and damage in photosynthesis and chlorophyll degradation (Viljevac et al., 2013; Bhusal et al., 2019).

During the summer months particularly 1st fortnight of May to 1st fortnight of June generally soil moisture remains very low, which leads to development of drought. Stress induced reversible and /or irreversible changes depend on the duration of drought (Giuliani et al., 2018; Singh and Sharma 2016; Thakur and Thakur, 2018). Droughts a major threat for sustain fruit production all over the world because it alters extensive changes, ranging from physiology to phenology. Several researchers have reported adverse effects of drought on growth, physiology and fruit production in apple plants (Ali, 2017; Ahmad et al., 2018). To gain a comprehensive understanding of how plants cope with the challenges of drought stress, it is crucial to examine their responses at various levels, including biochemical, physiological, and molecular. This holistic approach helps to understand mechanisms that enable plants to tolerate and adapt to drought-induced changes in their environment, encompassing not only observable morphological traits but also the underlying molecular and physiological processes. Water is the scarce asset, so plants need to use judiciously at every phonological phase (Balaguer et al., 2002). Several phytohormones have been reported to benefit plants under conditions of drought stress. Plant growth regulators serve to mitigate the adverse effects of drought stress on plant growth while also activating mechanisms that enhance resistance to such stress.

Brassinosteroids (BRs) are a class of plant hormones shown to regulate broad spectrum of physiological, biochemical and molecular responses in plants (Bajguz and Tretyn, 2003). Similar in structure to natural brassinosteroids, several synthetic compounds have proven valuable in agricultural practices (Zullo and Adam, 2002). Few workers have shown tolerance inducing role of brassinosteroid under drought stress in annual crops (Vardhini and Anjum, 2015; Yuan et al., 2010). However, the role of brassinosteroids in ameliorating adverse impacts of water stress in fruit plants is not known. This study was, therefore, conducted in two apple cultivars under polyhouse conditions to test the hypothesis, if pre-stress application of brassinosteroid has any role in reducing or reversing deleterious effects of water stress.

Materials and methods

Experimental materials

Suitable methodologies were used to understand the responses of apple cultivars to imposed conditions of drought, 15 and 30 days and in combination with two concentrations (0.05 and 0.10 ppm) of brassinosteroid under polyhouse conditions. In this experiment, brassinosteroid was used in the form of brassinolide (BR of synthetic technical grade). Apple cultivars, Super Chief and Red Chief, were transplanted as one-year-old grafted plants into plastic pots measuring 85 × 30 cm (diameter × height). These pots were filled with a mixture of soil and farmyard manure in a 3:1 ratio. Planting, one plant in each pot was done in the first week of February, 2016 in the polyhouse of department of fruit science and all the biochemical analysis was done in the laboratory of plant physiology, University of Horticulture and Forestry, Nauni, Solan (Figure 1). Further all plants were allowed to establish and grow in the pots till the first week of June 2016 with day temperature 22.5-33°C, photosynthetically active radiation (PAR) ranging between 400-680 mol $m^{-2}s^{-1}$. Watering was done at regular intervals to maintain soil saturation in order to avoid water stress to apple cultivars during establishment and subsequent growth. Foliar applications of brassinolide (Br) of two different concentrations (0.05 and 0.10 ppm) were given three days before imposition of drought to apple plants. The treatments comprised: control (unstressed for 15 and 30 days), Drought alone for 15 days, brassinolide 0.05 ppm followed by 15 days drought , brassinolide 0.10 ppm followed by 15 days drought, brassinolide 0.05 ppm followed by 30 daysdrought, brassinolide 0.10 ppm followed by 30 days of drought stress (Figure 2).

Table 1. Effect of drought and brassinosteroid on chlorophyll fluorescence (*Fv/Fm* ratio) in apple cultivars on 15 and 30 days of stress. D (durations), T (treatments).

Treatments	Super Chief		Red Chief	
	Stress Durations			
	15 days	30 days	15 days	30 days
	Chlorophyll fluorescence (*Fv/Fm* ratio)			
Control	0.737 [a]	0.742[a]	0.713 [a]	0.730 [a]
Water stress	0.685[d]	0.612[d]	0.660[d]	0.587[d]
0.05 ppm Br followed by drought	0.731[b]	0.716[b]	0.706[b]	0.691[b]
Br 0.10 ppm followed by drought	0.711[c]	0.708[c]	0.686[c]	0.683[c]
$CD_{(0.05)}$ D	0.029**		0.025**	
T	0.020**		0.017**	
D×T	0.041**		0.035**	

* Significant at p < 0.05, ** p < 0.01; *** p value< 0.001, variation between among all treatments on drought durations were assessed by Turkey's honestly significant difference. According to Turkey's HSD mean with the same letters are not significantly different.

Drought stress conditions were subjected by withholding water for 15 and 30 days, respectively. Prior to imposition of drought soil moisture was maintained near to field capacity (26%). The other set of plants of both the cultivars was maintained near to field capacity during the entire experiment to serve as unstressed control.

Table 2. Water use efficiency changes in control, stressed, and brassinosteroid treated stressed apple cultivars on 15 and 30 days of stress. D (durations), T (treatments)

Treatments	Super Chief		Red Chief	
	Stress Durations			
	15 days	30 days	15 days	30 days
	Water use efficiency (µ mol CO_2 /m mol H_2o)			
Control	0.398	0.386	0.389	0.382
Water stress	0.324	0.294	0.377	0.360
0.05 ppm Br followed by drought	0.382	0.335	0.379	0.353
Br 0.10 ppm followed by drought	0.357	0.287	0.374	0.305
$CD_{(0.05)}$ D	0.011**		0.021**	
T	0.008**		0.011**	
D×T	0.015**		0.017**	

* Significant at p < 0.05, ** p < 0.01; *** p value< 0.001, variation between among all treatments on drought durations were assessed by Turkey's honestly significant difference. According to Turkey's HSD mean with the same letters are not significantly different.

Soil moisture Content

Soil moisture content was measured with soil moisture meter model HH-2 by putting the probe at different soil depths.

Physiological parameters

Photosynthetic rate was recorded between 9 AM and 11 AM with portable pre-calibrated LICOR-6200 photosynthesis system. Three middle leaves of all treatment per replication were selected arbitrarily from all plants, positioned carefully one after the other in the broad leaf chamber and recorded photosynthetic rate as well as transpiration rate. Chlorophyll Fluorescence Meter OS-30P was used for *Fv/Fm* record; the leaves were first dark adapted for 10 minutes with the help of dark adaption clips. The dark adapted leaves were used for estimation of chlorophyll fluorescence. The data are expressed as *Fv/Fm*, where *Fm* = maximum fluorescence and *Fv* = variable fluorescence. Three plants per replication per treatment were used to estimate canopy temperature depression (CTD) (Figure. 3). Infra-Red Thermo Meter 6110ZL was used for determination. Singh et al. (1972) method was followed for total proline content estimation. Peroxidise activity was estimated by method of Abohatem et al., (2011). Instantaneous water use efficiency (WUE) was recorded from the given formula.

$$\text{Water use efficiency} = \frac{\text{Photosynthetic rate}}{\text{Rate of transpiration}}$$

Table 3. Effect of drought and brassinosteroid on transpiration (m mol/ m²/s) rate of apple varieties

Treatments	Super Chief		Red Chief	
	Stress Durations			
	15 days	30 days	15 days	30 days
	Transpiration (m mol/ m²/s)			
Control	29.11 [a]	30.06 [a]	27.48 [a]	28.60 [a]
Water stress	25.85 [d]	22.50 [d]	24.20 [c]	20.85 [d]
0.05 ppm Br followed by water stress	27.65 [b]	25.21 [b]	26.00 [b]	23.56 [b]
Br 0.10 ppm followed by water stress	26.62 [c]	24.63 [c]	24.97 [c]	22.98 [c]
$CD_{(0.05)}$ D	0.62*		0.62*	
T	0.44*		0.40*	
D×T	0.87*		0.88*	

*Significant at p < 0.05, ** p < 0.01; *** p value< 0.001, variation between among all treatments on drought durations were assessed by Turkey's honestly significant difference. According to Turkey's HSD mean with the same letters are not significantly different.

Statistical analysis

The study was performed in Completely Randomize Block Design with four treatments, two cultivars and three replications. Data are pooled of three replicates. The effect of different treatments on both the cultivars under 15 and 30 days of drought were determined statistically using two-way ANOVA followed by Tukey's HSD Test with Statistic 10 software (p < 0.05).

Results

Soil moisture

Decline in soil moisture content was significantly higher in pots supporting apple plants without prior treatment of brassinosteroid (Figure 4). Soil moisture content decreased from 30.4% in control pots to 12.5% in pots kept under 15-day water stress, further decline in soil moisture was observed up to 30 days of stress where 7.3% and 7.1% soil moisture was recorded in pots with Red Chief and Super Chief cultivars, respectively (Figure 4).

Effect on physio-biochemical parameters

The impact of drought on chlorophyll fluorescence is quantified by the ratio of variable to maximum fluorescence in leaves, which is denoted as Fv/Fm. This ratio provides insight into how drought stress affects the photosynthetic efficiency of plants (Table 1). Chlorophyll fluorescence ratio was highest in control (unstressed), which was next followed in stressed plants having prior treatment of brassinosteroid where *Fv/Fm* ratio was significantly higher (P < 0.05) compared to that under drought stress alone; ratio being comparatively more in water-stressed plants having prior treatment of 0.05 ppm brassinosteroid than that in 0.10 ppm treated plants (Table 1). The ratio was minimal in plants of both cultivars imposed to drought for 15 and 30 days without prior treatment of brassinosteroid; with reduction being significantly higher (P < 0.05) on day 30 of stress compared to that on 15 day of drought (Table 1). For example, *Fv/Fm* ratio on 15 day of stress was 0.685 and 0.660 in Super Chief and Red Chief, respectively whereas, the ratio was significantly lower (0.612 and 0.587) on day 30 of stress in Super Chief and Red Chief, respectively. Critical differences for quantum efficiency (*Fv/Fm* ratio) between stress durations (D) and among treatments (T) as well as interactions (D x T) were highly significant (P < 0.05).

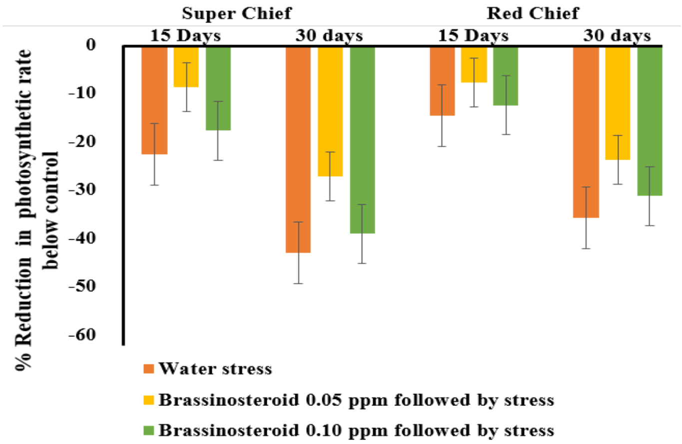

Figure 2. Depicting per cent reduction of photosynthetic rate below control in stressed and brassinosteroid treated apple cultivars on 15 and 30 days of water stress. Error bars are indicated on the histogram (n=6)

Photosynthetic rate

Photosynthetic rate in unstressed or control apple plants of both cultivars during this study was more or less same over 30 days period, however, a significant turn down in photosynthetic rate was recorded in simple drought plants with increasing stress durations (Figure 5). Drought alone in cultivars Super Chief and Red Chief reduced photosynthetic rate by 43.1% and 35.8%, respectively compared to control up to 30 days of drought. Spray of BRs (0.05 ppm) before subjecting stress helped drought plants to maintain significantly (P < 0.05) higher rate of photosynthesis; rate being significantly higher (P < 0.05) than that under water stress alone. In case of brassinosteroid (0.10 ppm) treatment, plants recorded comparatively less photosynthetic inhibition in comparison to drought alone up to day 30 of stress Figure. 6).

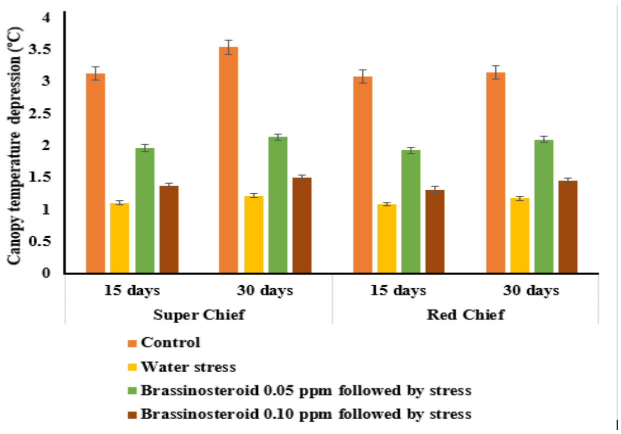

Figure 3. Canopy temperature depression in unstressed, stressed and brassinosteroid treated apple cultivars on 15 and 30 days of stress. Error bars are indicated on the histogram (n=6)

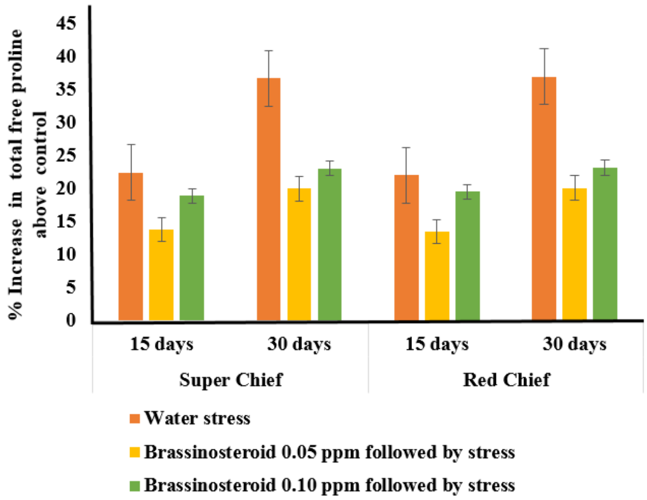

Figure 4. Per cent increase (over control) in free proline content in stressed and brassinosteroid treated apple cultivars on 15 and 30 days of stress. Error bars are indicated on the histogram (n=6)

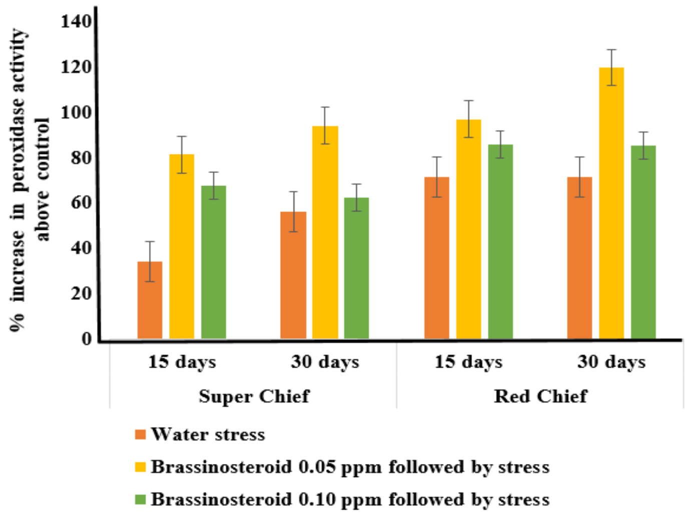

Figure 5. Per cent increase (over control) in peroxidase enzyme activity in stressed and brassinosteroid treated stressed apple cultivars on 15 and 30 days of water stress. Standard error bars are indicated on the figure (n=6)

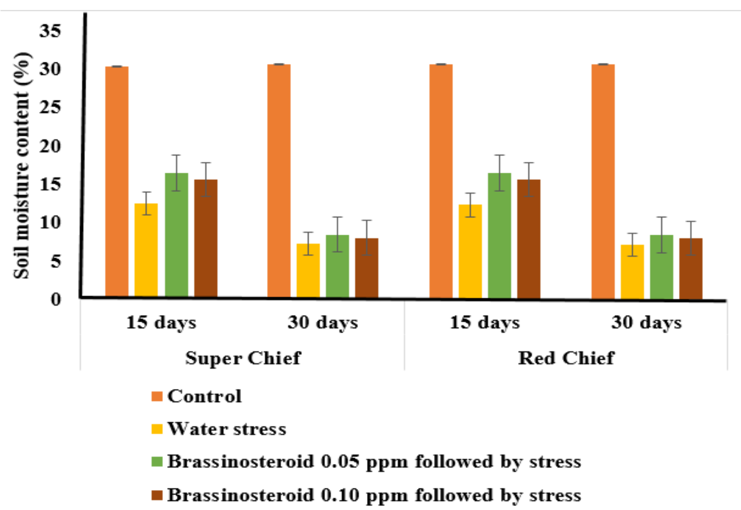

Figure 6. Soil moisture content (%) on 15 and 30 days of stress.

Canopy temperature depression

Drought conditions during this study adversely impacted canopy temperature depression index in both apple cultivars. Brassinosteroid application at 0.05 ppm and 0.10 ppm concentrations, however, improved canopy temperature depression in drought stressed apple plants (Figure 3). Highest mean canopy temperature depression was recorded by unstressed control plants of cultivar Super Chief (3.33°C) and Red Chief (3.11°C) while minimum was obtained for plants of both cultivars subjected to water stress for 30 days in absence of brassinosteroid application the average values being 1.17 and 1.13°C for Super Chief and Red Chief, respectively (Figure. 6). BRs 0.05 ppm and 0.10 ppm resulted in significantly greater canopy temperature depression in water stressed cultivars compared to stress alone (Figure. 3). Brassinosteroid at 0.05 ppm concentration resulted higher canopy temperature depression (cooler canopy) at both drought stress durations compared to 0.10 ppm concentration of brassinosteroid. Differences between stress durations and among treatments with reference to changes in canopy temperature depression were observed to be highly significant ($P < 0.05$).

Transpiration rate

In Super Chief and Red Chief apple varieties the transpiration rate was decreased with decline in soil moisture. Transpiration rate (30.06 m mol/m²/s) was significantly increased in Super Chief variety when compared with the control (22.50 m mol/m²/s). Application of BRs at 0.05 ppm& 0.10 ppm as spray before the impostion of grought stress in apple could help the plant to maintain its transpiration rate than the stressed plants. In this study, it was also recorded that Plant subjected to drought alone showed maximum percent reduction in transpiration rate i.e 25.15 %. Whereas, the transpiration was recorded minimum in plants pre-treated with BRs 0.05 ppm under 15 days drought (5.02 %). The transpiration rate of Red Chief was Significantly higher (28.60 %) under 30 days of control (Unstress) as compared to stress (20.85 %).

Water use efficiency (WUE)

Water use efficiency was maximum in unstressed control plants of both apple cultivars (0.392 and 0.386 µ mol CO_2 /m mol H_2o), whereas a significant decline was evident with increasing stress durations; decline being significantly higher ($P < 0.05$) on day 30 in comparison to 15 day of stress (Table 2). WUE values, for example, decreased from 0.386 and 0.382 µ mol CO_2 /m mol H_2o in control to 0.294 and 0.360 µ mol CO_2 /m mol H_2o, respectively in Super Chief and Red Chief up to 30 days of stress. BRs 0.05 ppm before subjecting drought was found very effective in maintaining higher water use efficiency both on 15 as well as 30 days of stress. Brassinosteroid at 0.10 ppm could improve WUE up to 15 days of stress; the effect diminished up to 30 days of stress (Table 2). Critical differences for WUE between and within stress durations (D), among treatments (T) and interactions (D x T) were statistically significant.

Stomatal conductance

The influence of drought stress and BRs on stomatal conductance of apple varieties viz., Super Chief and Red Chief has been presented in Table 3. Maximum stomtal conductance was observed in Super chief (0.772 m mol/s) under 30 days of control, whereas minimum in plants subjected 30 days of drought alone which was statistically at par with brassinolide pre-treated plant at 0.10 ppm followed 15 days of water stress (0.646 m mol/s). However, maximum stomatal conductance (0.758 m mol/s) Red Chief apple variety which was statistically at par with stomatal conductance (0.730 m mol/s) recorded for 15 days of control. The plants which are imposed to 30 days of drought showed minimum stomatal conductance (0.618 m mol/s) which was statistically at par with 15 days of drought (0.651 m mol/s).

Total proline content

In response to mild to severe drought stress, plants employ a defense mechanism by accumulating certain compounds known as osmolytes. Among these osmolytes, proline is frequently found and serves as one of the most prevalent substances in drought-stressed plant (Figure 9). In this study, it was observed that proline is significantly increased as the stress durations is increased in both the cultivars over drought alone. The critical differences between stress durations and among treatments were statistically significant ($P < 0.05$).

Peroxidase activity

Minimum peroxidase enzyme activity was recorded in 30 days of drought without brassinosteroid, whereas greater increase was evident in both cultivars given foliar spray of brassinosteroid at 0.05 and 0.10 ppm concentrations, 3 days prior to water stress (Figure. 10). Among treatments, maximum increase in peroxidase activity was resulted by brassinosteroid 0.05 ppm, where increase was 81.5 and 93.9%, on 15 and 30 days of stress, respectively in stressed Super Chief; increase being comparatively more in Red Chief (97.5 and 119.3%, respectively). Brassinosteroid 0.10 ppm during this study also resulted a significant increase in peroxidase activity in both cultivars under stress where an increase was 68.2 and 62.6%, respectively in Super Chief and 85.9 and 79.5% in Red Chief on 15 and 30 days drought. The effect of stress durations and treatments on peroxidase enzyme activity was found to be highly significant ($P < 0.05$).

Discussion

During the present investigation, brassinosteroid spray on apple plants at 0.05 and 0.10 ppm prior to drought treatments enabled plants of both cultivars to exhibit relatively higher drought tolerance, resulting in better growth as well as pace of physiological indices even up to 30 days of drought in comparison to drought alone. Out of the two brassinosteroid concentrations applied, 0.05 ppm was found to be more effective than 0.10 ppm in sustaining better leaf area in water-stressed plants up to 30 days of stress. Protection against dehydration or conditioning of vital physiological indices associated with drought tolerance appears to be the prime role of brassinosteroids under water stress. The assumption cannot be ruled out because brassinosteroid, especially 0.05 ppm during this study has been observed to lessen detrimental effects of water stress on key physiological indices like, *Fv/Fm* ratio photosynthetic rate, transpiration rate, CTD, stomatal conductance, WUE, osmolite such as proline and peroxidase enzyme activity in apple plants even up to 30 days of drought stress. This is important since higher photosynthetic rate, higher *Fv/Fm* ratio, higher canopy temperature depression (cooler canopies), higher proline, higher peroxidase enzyme and higher WUE are all desirable physiological attributes which impart drought tolerance under conditions of water stress.

Higher photosynthetic rate, for example, helps plants to maintain carbohydrate supply, which in turn helps in maintaining osmotic potential (Ψ_s) and water relations of plants (Thakur and Thakur, 2018). Likewise, Fv/Fm is an important index which reflects quantum yield / quantum efficiency and functional status of PS-II in plant species under stress. Higher the ratio better would be light harvesting and electron transport efficiency of PSII. Other workers have also reported adverse effect of water stress on the efficiency of PSII (Balaguer et al., 2002; Misra et al., 2012). So brassinosteroid treatment helping plants to maintain a higher *Fv/F*m ratio under stress conditions shall help apple plants to continue photosynthetic rate at a higher pace. Exogenous BRs mitigate the negative effects of this type of stress on growth, gas exchange parameters (increasing P_N, g_s, and water use efficiency (WUE)), photosynthetic pigment contents, or chlorophyll fluorescence measurements such as F_v/F_m in cultivated plants (Khamsuk et al., 2018; Lima and Lobato, 2017; Talaat, 2020). Foliar applications of BRs help plants to cope with drought stress by improving water relations and plant growth, leaf gas exchange parameters (P_N, g_s, and *E*), photosynthetic pigment contents (TChl and Cx + x), and chlorophyll fluorescence parameters such as F_v/F_m, qP and NPQ in chili pepper [*Capsicum annuum* L. var. *frutescens* (L.)

Our findings regarding the photosynthetic rate (as shown in Figure 2) support the idea that using brassinosteroid pre-treatment at both concentrations (0.05 and 0.10 ppm) before subjecting the plants to drought can lead to a higher photosynthetic rate compared to what is typically observed in drought-stressed apple cultivars without the application of Brassinoloid. Brassinosteroid application appears to benefit Super Chief and Red Chief apple cultivars by maintaining cooler canopies (higher temperature depression), thus protecting photosynthetic apparatus against dehydration. Canopy temperature depression is a widely employed indicator for evaluating a plant's water status and its reaction to both water stress and elevated temperatures (Balaguer et al., 2002; Helyes et al., 2006). Previous studies have consistently indicated that plants experiencing water stress typically exhibit higher canopy temperatures, resulting in reduced canopy temperature depression (Pratima et al., 2016).

The transpiration rate and stomatal conductance decreased as soil moisture content decreased in both plant varieties, as indicated by Table 3 and Figure 7. The reduced transpiration rate can be attributed to a decrease in available soil moisture content under water stress conditions. This reduction in soil moisture signals the accumulation of substances similar to abscisic acid in the leaves, leading to a reduction in stomatal pore size and a subsequent decrease in transpiration rate. These findings align with previous studies by Bhardwaj (2010) and Chandel (1989), which observed varying stomatal conductance in apple plants under different soil moisture levels. Higher transpiration rates at the -0.5 bar irrigation level can be attributed to the fact that more frequent irrigation leads to an increase in stomatal pore size, as noted by Misger and Kumar (2008). Water stress resulted in a significant accumulation of total free proline, with the accumulation being notably higher in plants subjected to water stress alone compared to those that were pre-treated with brassinosteroids, as shown in Figure 4.

Proline accumulation in plant tissues under any abiotic stress *per se* is a general response of plants to stress (Jie et al., 2010; Yuan et al., 2010; Surendar et al., 2013). Proline is mainly a cytoplasmic osmotic substance highly soluble in water; probably should help plants under stress by affecting osmotic potential (Ψs), however, the role of accumulated proline in protecting plants against dehydration is still not clear because at various occasions proline bears poor correlation with drought tolerance (Hanson and Hitz, 1982; Mali and Mehta, 1977; Mihaljević et al., 2021). The higher accumulation of proline under water stress during this study does not seem to benefit apple cultivars since the adverse impact of water stress is greater on growth and physiological indices of both apple cultivars in stressed plants up to 30 days without brassinosteroid. The higher activity of peroxidase (antioxidant) enzyme observed in brassinosteroid treated stressed plants is desirable as this seems to help apple plants to cope with the stress situation by getting rid of harmful free radicals. The drought can be mitigated by brassinosteroid by maintaining the level of ROS by efficient quenching of oxidants by increased enzymatic and non-enzymatic antioxidant activity (Bajguz and Hayat 2009; Mihaljević et al., 2021).

Few earlier workers have observed that exogenous application of brassinosteroid resulted in enhanced tolerance to abiotic stresses in crop plants (Fariduddin et al., 2009). Recent studies reported that some secondary metabolites synthesized in plant organs such as volatile compounds terpens [Mahdavi et al., 2020] and some phytohormones such as brassinolide (Naservafaei et al., 2021). alleviated the effect of drought stress on plants by improving the plant's defense system. Water use efficiency (WUE) was comparatively greater in plants of both cultivars pre-treated with brassinosteroid at 0.05 and 0.10 ppm, the former concentration being more effective. This is a good signal because this is indicative of the judicious use of available water for growth and physiological processes. Brassinosteroid-treated stressed apple plants, which used water more economically have shown better growth and physiological activities during this study than stressed plants using water less economically. It is possible that brassinosteroid application in the present study might have maintained higher metabolism by readjustment of stomatal aperture. Lui et al. (2012) earlier reported that WUE was significantly and positively correlated with stomatal conductance under water stress in different cultivars of apple. Close correlation between stomatal conductance, assimilation rate and WUE in tomato plants was established earlier (Giuliani et al., 2018). The decline in soil moisture content with increasing stress duration is well established. The less depletion of soil moisture in pots containing apple plants with prior treatment of brassinosteroid than stress only is difficult to elucidate how brassinosteroid is doing that but their role mediated through water use efficiency cannot be ruled out because comparatively higher water use efficiency was observed in stressed plants of both apple cultivars pre-treated with brassresteroid.

Conclusion

The better performance in terms of growth and key physiological parameters obtained for water-stressed apple cultivars pre-treated with brassinosteroid is of great relevance because brassinosteroid application in this experiment has benefited apple cultivars by reversal of water stress induced adverse changes. The outcome of this study is that foliar spray of brassinosteroid at low concentrations (0.05 and 0.10 ppm), before subjecting drought, minimizes deleterious effects of water stress in two apple cultivars. The finding is encouraging, however, more information with regard to mechanism of action (site of action) and the critical role of brassinosteroid in mitigating water stress effects and / or inducing drought tolerance, especially in fruit crops needs to be collected through in-depth experimentations.

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SK and AT conceived the concept, wrote and approved the manuscript.

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