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Research Article

Anti-transpirant Application Improves the Drought Tolerance of Fig (*Ficus carica* L.) Under Optimization of Brassinolide

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Abstract

Background and Objective: Possible role of exogenously applied anti-transpirant in alleviating the detrimental effects of drought in fig under brassinolide optimization were evaluated in a greenhouse to discover the role of anti-transpirants and BLs in improving drought tolerance in fig. **Materials and Methods:** Fig planting materials (Improved Brown Turkey cultivar) were propagated using cuttings taken from mature 2-3 years old figs and transferred into substrate containing 3:2:1 mixed soil (top soil:organic matters:sand). The experiment was arranged as RCBD factorials with 3 replications. Fig was subjected into 2 water stress levels (well watered and drought stressed) followed by foliar spray of anti-transpirant (control and 2 kg ha⁻¹) to assess the changes in water status, leaf gas exchange, photosynthetic pigments and biochemical responses. **Results:** Drought substantially reduced the water status on relative leaf water content, photosynthetic pigments, leaf gas exchange but increased water status on leaf water content. Moreover, substantial increased in biochemical responses attributes to proline content, malondialdehyde, soluble sugar content, peroxidase, catalase but decreased on starch and protein content. However, exogenous application of anti-transpirant remarkably improved water status on LWC, the gas exchange and photosynthetic pigments both under drought and well-watered conditions. **Conclusion:** The results indicate that the application of anti-transpirant can ameliorate the effects of water stress and enhance drought resistance of fig by adjust water loss using stomatal control.

Key words: Anti-transpirant, brassinolide, drought stressed, fig, photosynthesis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil water deficit and excessively high temperature are two of the most common yield-limiting factors in crops of the world. The main objectives in many studies have been to improve growth and yields by reducing the effects of drought and making agricultural water use more efficient^{1,2}. In arid and semi-arid areas actively growing plants would transpire water equal to its weight each hour if water is supplied adequately³. Plant tolerance to drought results from both morphological adaptation and responses at biochemical and physiological levels⁴⁻⁶. Different mechanisms contribute to drought resistance in plants such as avoidance of water deficits by drought escape, water conservation and more efficient water 75% uptake⁷. Thus, plants close their stomata apparatus and modulate their leaf area and thereby adjust the loss of water from the canopy⁸.

Stomatal control is the first and most important step in response to drought, as stomatal conductance reduces the rate of water loss and slows the rate of water stress development and minimizes its severity⁹. Stomatal closure not only allows plants to limit transpiration but also restricts the CO₂ intake, which leads to a decline in photosynthetic rate¹⁰.

Anti-transpirants are the materials or chemicals which decrease the water loss from plant leaves by reducing the size and number of stomata. Nearly 99% of the water absorbed by the plant is lost in transpiration. Anti-transpirant containing magnesium and calcium as a main component is developed to increase photosynthesis and plant growth. Anti-transpirants and was any natural applied to transpiring plant surfaces for reducing water loss from the plant. The beneficial effects of anti-transpirants on counteracting the adverse effects of very hot climates on growth and production of horticultural and other crops were reviewed by many authors such as Ahmed *et al.*¹¹ and Ebrahiem-Asmaa¹².

Upon exposure to environmental stresses, proline accumulation usually increases¹³. Proline was not only involved in osmotic adjustment but also helps in scavenging free radicals, buffering cellular redox potential and stabilizing sub-cellular structures under stress conditions¹⁴.

Fig, being third most important crop worldwide is considered as one of the most drought sensitive crops. It requires 1 to 1 1/2 inches of water each week during life cycle and can grow yearly¹⁵. In order to improve the agricultural productivity under limiting water supply, it is imperative to improve tolerance against drought stress. Various agronomic and physiological practices were being applied to minimize the detrimental effects of water stress on crop plants, exogenous application of growth regulators was one of the pragmatic approach in this regard^{14,16,17}.

Plant growth regulators, both natural and synthetic, were widely applied to agricultural crops for induction of drought tolerance.

Out of the various compounds exploited to alleviate the moisture stress, the brassinolide (BL) was recognized to regulate the plant growth and productivity under water deficit conditions^{18,19}. The BL was known to alleviate various biotic and abiotic stress effects^{20,21}. It has unique growth promoting action when applied exogenously²².

Nonetheless, to best of our knowledge, a few studies have been undertaken to unravel the potential of anti-transpirants and BLs in improving the drought tolerance in fig. This study was undertaken to explore the possible role of anti-transpirants and BLs in improving drought tolerance in fig, based on changes in enzymatic antioxidants, gas exchange traits and growth.

MATERIALS AND METHODS

Plant material and growth conditions: The 2-3 years mature fig trees cultivar of Improved Brown Turkey (IBT) were propagated using cutting methods and transferred into media containing 3:2:1 mixed soil (top soil:organic matters:sand) in a greenhouse experimental field at Ladang 10, Faculty of Agriculture, Universiti Putra Malaysia situated at 2°58"N and 101°44'04" E in Serdang, Selangor, Malaysia from August, 2018 to January 2019. The greenhouse was a Poly house-type with double spans, oriented north and south, covered with transparent UV stabilized polyethylene film, 200 micron thickness was used for covering the poly house roof and surrounding by net and has one door as entering access. Provided natural ventilation from the roof and side windows, which were operated manually based on the air temperature inside the greenhouse. During the experiment, the daytime mean air temperature was 24-30°C, the night-time mean air temperature was 17-22°C and the daily mean relative humidity was maintained above 60%. Although the CO₂ concentration in the canopy was not measured, it was assumed to be close to the outside level, based on measurements in the same season in another year (data not shown).

Experimental design: The experiment was arranged as Randomized Complete Block Design (RCBD) factorials with 3 replications. First factor was two levels of anti-transpirant concentrations (0 and 2 kg ha⁻¹) and second factor was 2 level of water stress (well-watered [100% FC] and drought-stressed [25% FC]). There were 4 plants as destructive samples observed monthly for each replication. Data were recorded monthly.

Application of treatments

Optimum brassinolide: Zulkarnaini *et al.*²³ reported that the best concentration of brassinolide application to promote growth and physiological changes of fig was 200 mL L⁻¹ therefore, the suggested concentration in this study was used. One month old fig tree seedlings were sprayed monthly with a solution of brassinolide 200 mL L⁻¹ (100 mL tetrahydroxy-methyl-B-homo-oxa-cholestan-lactone+ 26 mL multi purpose cultivation [MPC]+20 L water) and applied directly onto leaves at 0900-1100.

Anti-transpirant: Anti-transpirant treatments (control and 2 kg ha⁻¹ = 40 g anti-transpirant+4 L water) were applied in the form of a fine spray onto the leaf surface and sprayed onto the leaf surface in the form of a fine mist. We used spraying nozzles with a diameter between 150 and 300 µm, at pressures ranging from 3-4 bars. While filling the tank of the sprayer with water, add anti-transpirant slowly and evenly. Apply anti-transpirant when weather was right, as anti-transpirant takes about 2-4 h to be absorbed by the plant. Apply a fine layer to the fig plants leaves in the morning (0900-1100). After used, wash the spraying equipment thoroughly after used to prevent any sedimentation of the material. Apply anti-transpirant every 14 days during the vegetative period. To prevent settlement of the materials after an extended standstill, the stirrer should not be deactivated; otherwise the material must be properly mixed again before commencement of spraying.

Water stress

Field capacity (FC) determination: Field capacity was the ability of soil particles to hold as much water as possible against gravity. The field capacity (FC) determination was conducted to determine the watering volume. Seven pieces of 250 g pot were filled with 100 g of planting media each. All the pots were watered 100 mL until they got saturated then was left for 3 × 24 h until the water stopped dripping. The result was then weighed as the wet weight (WW). The planting media were put into the oven at 100°C. After 24 h, the planting media were taken out from the oven to be cooled in a desiccator and then were weighed as the dry weight (DW). To get the average result, the experiment was replicated 5 times. After that, the field capacity (FC) of the land was calculated using the following equation²⁴:

$$FC = (WW-DW) \times 100\% / DW \quad (1)$$

The result of the calculation from the observation of field capacity determination was as follows:

$$FC = 0.295 = 29.5\% = 30 \text{ mL}$$

where, FC is 30 mL in 100 g of planting media of top soil+sand+organic matters, so 8 kg of planting media required water volume of 2400 mL and severe drought stress (25% FC) required a watering volume of 600 mL. The average water flow/polybag = 404 mL min⁻¹. Therefore, for treatment well watered (W0) or FC 100% need 2400 mL water and irrigated for 6 min/day. Whilst for treatment severe drought stress (W1) or FC 25% need 600 mL water and irrigated for 1.5 min/day.

Soil water content determination: Soil sample was collected as much as 5 g in each potted plant then was put into the oven at 105°C. After 24 h, the sample was weighed. Water content was obtained from the sample weight before oven drying subtracted by the sample weight after oven drying divided by the sample weight after oven drying multiplied by 100%:

$$\text{Soil water content (\%)} = \frac{W_0 - W}{W_0} \times 100 \quad (2)$$

where, W₀ is weight of the soil sample before oven drying and W is weight of the soil sample after oven drying. Based on the observation results, the soil water content in the well watered treatment (100% FC) was 50.40% and the soil water content in the severe drought stress was 36.90%.

Measurements

Determination of leaf water content (LWC): Three compound leaves were collected from each seedling. After leaf fresh weight was measured immediately, the leaves were dried at 80°C for 24 h and weighed again²⁵:

$$LWC = \frac{\text{Fresh weight} - \text{Dry weight (g)}}{\text{Dry weight (g)}} \quad (3)$$

Determination of relative leaf water content (RLWC):

Leaf relative water content (LRWC) was estimated according to the method of Ekanayake *et al.*²⁶. The leaf material was weighed to determine its fresh weight and then placed in distilled water at 4°C for 19 h and its turgid weight was recorded. Finally, the samples were dried in an oven at 80°C for 24 h and their dry weights were recorded. The RWC was calculated as:

$$RLWC (\%) = \frac{\text{Fresh weight} - \text{Dry weight (g)}}{\text{Turgid weight} - \text{Dry weight (g)}} \times 100 \quad (4)$$

Determination of photosynthesis rate, stomatal conductance and transpiration rate: Photosynthetic rate, stomatal conductance and transpiration rate of fully expanded leaf was measured by using a portable photosynthesis system (LICOR-6400, LI-COR Inc., USA).

Determination of chlorophyll content: The leaves of *Ficus carica* L. with different greenness (pale yellow, light green and dark green) were selected for analysis and total leaf chlorophyll content was analyzed. After that, all samples collection carried back to laboratory. Leaf discs 3 mm in diameter were obtained from the leaf sample using a hole puncher. The leaf disks were immediately immersed in 20 mL of 80% acetone in an aluminium foil covered glass bottle and kept in the dark for approximately 7 days until all the green color had bleached out. Finally, 3.5 mL of the solution was transferred to measure absorbance at 2 wavelengths using a light spectrophotometer (UV-3101P, Labomed Inc, USA). The 2 wavelengths of 664 and 647 nm were used as the peak absorbance of chlorophyll-a and chlorophyll-b. The total amount of chlorophyll-a and chlorophyll-b were then calculated according to the method of Coombs *et al.*²⁷:

$$\text{Chlorophyll-a content (mg cm}^{-2} \text{ fresh leaf)} = 13.19 (A_{664}) - 2.57 (A_{647}) \quad (5)$$

$$\text{Chlorophyll-b content (mg cm}^{-2} \text{ fresh leaf)} = 22.1 (A_{647}) - 5.26 (A_{664}) \quad (6)$$

$$\text{Total chlorophyll content (mg cm}^{-2} \text{ fresh leaf)} = \frac{3.5 \times (\text{Chl-a} + \text{Chl-b})}{4} \quad (7)$$

where, A_{647} and A_{664} represent absorbance of the solution at 647 and 664 nm, respectively, while 13.19, 2.57, 22.1 and 5.26 were the absorption coefficients, 3.5 was the total volume used in the analysis taken from the original solution (mL) and 4 was the total discs area (cm²).

Determination of proline content: Proline was determined in fully expanded leaves according to Pesci and Beffagna²⁸. Reagents Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid, with agitation, until dissolved. Kept cool (stored at 4°C) the reagent remains stable 24 h. The sample (0.5 g fresh weight) was homogenized in 10 mL of 3% aqueous sulfo-salicylic acid and the homogenate filtered through Whatman No. 2 filter paper. About 2 mL of filtrate was reacted with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid in a test tube and incubated in boiling water (100°C) for 1 h and

the reaction terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene, shaken vigorously with a test tube stirrer for 15-20 sec. The toluene layer at the top (pink-red in color) was collected with a pipette. The absorbance read at 520 nm using proline as standard. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

$$\text{Proline (}\mu\text{moles g}^{-1} \text{ FW)} = \frac{[(\text{Proline } \mu\text{g mL}^{-1} \times 4 \text{ mL toluene}) / 115.5 \mu\text{g } \mu\text{mole}^{-1}]}{0.5 \text{ g sample} / 5} \quad (8)$$

Determination of starch: Starch content was determined spectrophotometrically using the method by Thayumanavan and Sadasivam²⁹. In this method, about 250 mg of dry sample was homogenized in 80% ethanol to remove the sugar. The sample was then centrifuged at 5000 rpm for 5 min and then the residue was retained. After that, add 5 mL of cold distilled water and 6.5 mL of 52% perchloric acid were added to the residue. Then the solution was centrifuged and the supernatant separated and then filtered with filter paper No. 5 (Whatman). The processes were repeated until the supernatant was made up to 100 mL. A 100 μ L of the supernatant was added to distilled water until the volume became 1 mL.

After that, 4 mL of anthrone reagent (dissolve 200 mg anthrone in 100 mL of 95% sulphuric acid) was added to a tube. The mixed solution was placed in the water bath at 100°C for 8 min and then cooled to the temperature room and then the sample was read at absorbance of 630 nm to determine the sample starch content. Glucose was used as a standard and starch content was expressed as glucose equivalent mg g⁻¹ dry sample. The mean value of four representative plants was used to represent each treatment.

Determination of malondialdehyde and soluble sugar content: Fig seedlings were assessed for malondialdehyde (MDA) and soluble sugar contents that were determined using the methods of Zou³⁰. Briefly, a 100 mg sample of fresh leaf tissue was ground in a mortar with 10 mL of 10% tri-chloroacetic acid (C₂HCl₃O₂) and a small quantity of quartz. The homogenate was centrifuged at 4,000 rpm for 10 min, then a 2 mL aliquot was removed and mixed with 2 mL 0.6% thiobarbituric acid (TBA) solution. The solution was incubated at 100°C for 15 min, allowed to cool and then centrifuged again at 4,000 rpm. Absorbance values of the supernatant

were recorded at 532, 600 and 450 nm and TBA acid uses as a standard. The MDA and soluble sugar contents were calculated as follows:

$$\text{MDA} \left(\mu\text{mol g}^{-1} \text{FW} \right) = \frac{(6.45 (A_{532} - A_{600}) - 0.56A_{450})}{1000} \times \frac{\text{Vol. extract solution (mL)}}{\text{Fresh weight (g)}} \quad (9)$$

$$\text{SSC} \left(\text{mmol g}^{-1} \text{FW} \right) = \frac{(11.74 A_{450})}{1000 \text{ mL}} \times \frac{\text{Vol. extract solution (mL)}}{\text{Fresh weight (g)}} \quad (10)$$

Determination of protein: The protein contents of the crude extract was determined using bovine serum albumin (BSA) as a standard, according to the method of Bradford³¹. Leaf sample (0.5 g) was grinded with liquid nitrogen in a mortar. The homogenate powder was mixed with 10 mL of 50 mM or 7.1 mL sodium phosphate buffer (pH 7.0) containing 1 mM or 0.3 g EDTA- Na_2 and 2% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 11000 g for 15 min at 4°C. About 1 mL of Bradford solution was added to 100 μL crude extract and absorbance recorded at 595 nm for estimate of total protein content. The protein concentration was determined monthly and calculated from a BSA standard curve.

Determination of POD and CAT: Enzyme activity assays were based on the methods of Zou³⁰. Fig leaves were harvested in the light, frozen in liquid N and stored at -80°C until assay. For determine enzyme activities, leaf samples (0.5 g fresh weight) was grinded with liquid nitrogen in a mortar. The homogenate powder was mixed with 10 mL of 50 mM or 7.1 mL sodium phosphate buffer (pH 7.0) containing 1 mM or 0.3 g EDTA- Na_2 and 2% (w/v) polyvinylpyrrolidone (PVP). The extract was centrifuged at 16,000 g for 4 min at 4°C and the supernatant was used for following enzyme assays.

Peroxidase (POD) activity was assayed by measuring the ability of the enzyme extract to increase absorption at 470 nm due to the oxidation of guaiacol. A reaction mixture consisting of 3 mL of 0.1 M sodium phosphate buffer (pH 7.0), 28 μL guaiacol and 30 μL of 30% H_2O_2 was prepared and incubated at 32°C. We added 100 μL enzyme extract to the mixture and then measured the increase in absorbance at 420 nm at least 2 min per 30 sec read the absorbance and used the absorbance change 0.01 as a POD activity.

Catalase (CAT) activity was assayed by measuring the ability of the enzyme extract to decompose H_2O_2 . The reaction mixture consisted of 2 mL of 0.1 M sodium phosphate buffer (pH 7.0), 1 mL 0.08% H_2O_2 and 0.2 mL enzyme extract. One unit of CAT activity was defined as 1 mg tissue proteins consumed 1 μmol H_2O_2 at 405 nm sec^{-1} . The CAT activity was expressed as enzyme units/mg of protein.

Statistical analysis: All the data obtained were analyzed using Statistic Analysis System (SAS) version 9.4, Microsoft Excel 2013 and SPSS 23. Significant difference of mean values were determined and analyzed using two-way ANOVA and the mean differences were compared using least significant different test (LSD) at 1 and 5% level of significance.

RESULTS

Water status: The results of data analysis (data not be shown) showed that leaf water status of fig was affected by anti-transpirant levels and water stress treatment under optimization of brassinolide. Treatment anti-transpirant alone was significant on leaf water content (LWC) at third month after treatment (MAT). Treatment of different concentrations of anti-transpirant (0 and 2 kg ha^{-1}) caused an increment in LWC at every month observations and anti-transpirant concentration 2 kg ha^{-1} resulted higher LWC than 0 kg ha^{-1} (Fig. 1a).

Treatment water stress alone (Fig. 1b) was significant on relative leaf water content (RLWC) at 1st and 2nd MAT. Treatment of different concentrations of anti-transpirant (0 and 2 kg ha^{-1}) caused a decrement in RLWC at every month observations and well-watered treatment resulted higher RLWC than drought-stressed.

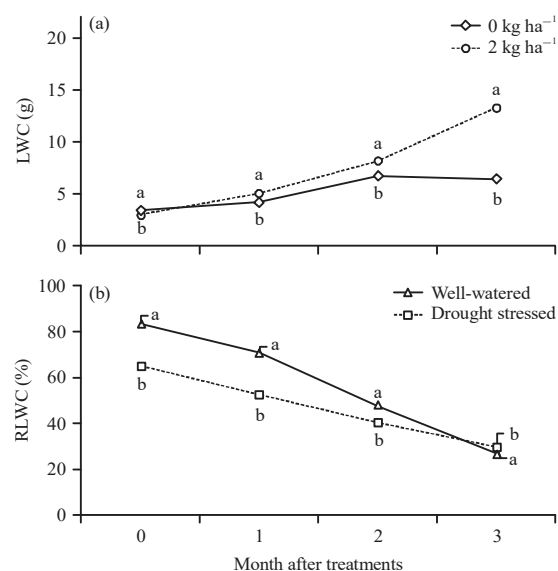


Fig. 1(a-b): Leaf water status of fig on parameters (a) Leaf water content as main effect of anti transpirant and (b) Relative leaf water content as main effect of water stress under optimization of brassinolide. Curves represent means followed by the different small letters were significant at $p < 0.05$

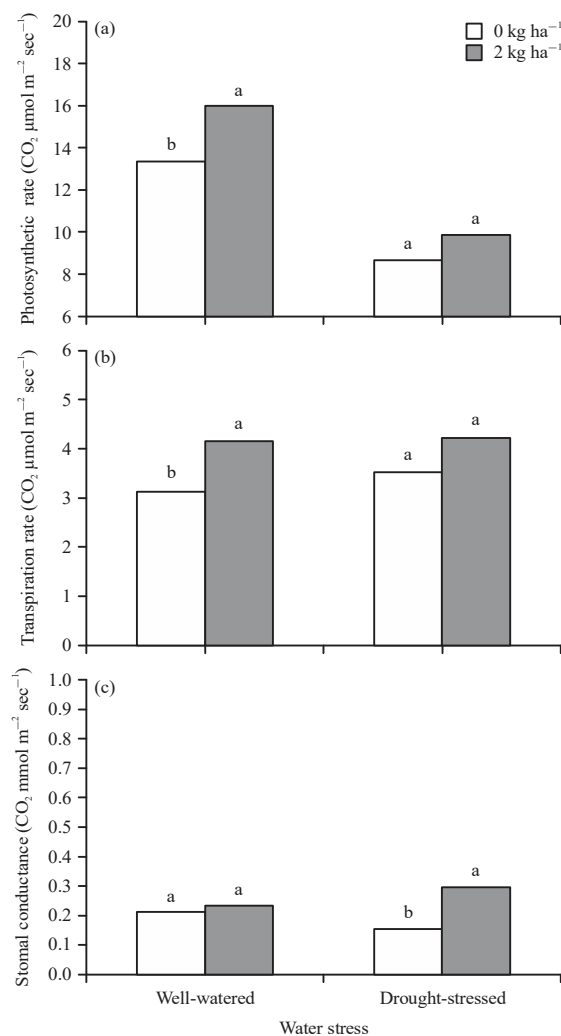


Fig. 2(a-c): Significant interaction between anti-transpirant and water stress of fig under optimization of brassinolide according to parameters (a) Photosynthetic rate at 3rd MAT, (b) Stomatal conductance at 3rd MAT and (c) Transpiration rate at 3rd MAT

Bars represent means followed by the different small letters were significant at $p < 0.05$

Leaf gas exchange: Leaf gas exchange of fig (Fig. 2) was affected by interaction between anti-transpirant and water stress of fig under optimization of brassinolide. Drought stress led to decline in photosynthesis (Fig. 2a), transpiration rate (Fig. 2b) and stomatal conductance (Fig. 2c) against well-watered. Exogenous application of anti-transpirant 2 kg ha⁻¹ resulted higher A, E and g_s than anti-transpirant 0 kg ha⁻¹ under drought and well-watered conditions in fig. Anti-transpirant-treatment enhanced photosynthesis (9.71%), transpiration rate (11.51%), stomatal conductance (8.80%) in

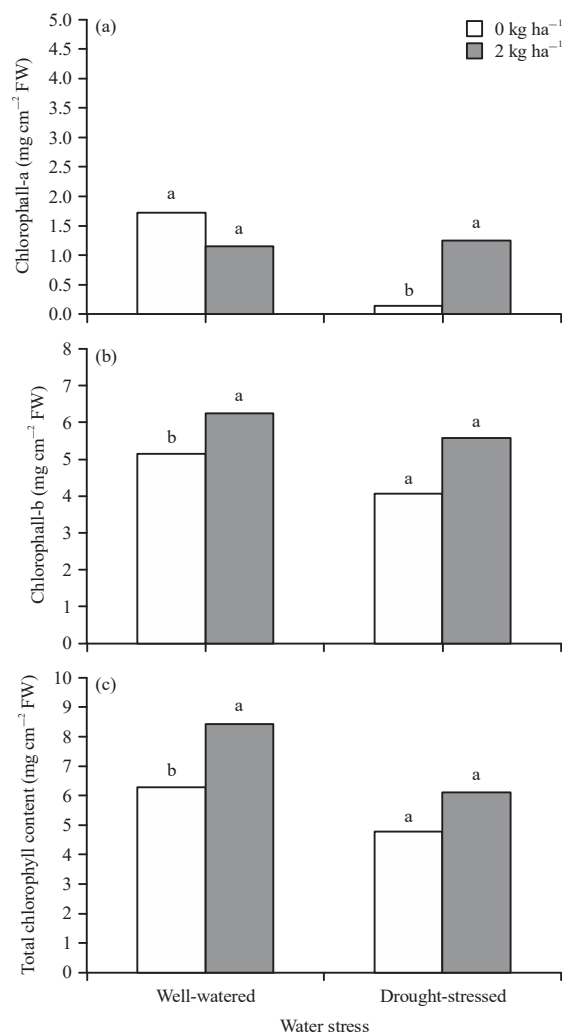


Fig. 3(a-c): Significant interaction between anti-transpirant and water stress of fig under optimization of brassinolide according to parameters (a) Chlorophyll-a at 2nd MAT, (b) Chlorophyll-b at 1st MAT and (c) Total chlorophyll content at 1st MAT

Bars represent means followed by the different small letters were significant at $p < 0.05$

well-watered, while improved the photosynthesis (12.05%), transpiration rate (14.95%) and stomatal conductance (7.48%) under water-deficit conditions.

Photosynthetic pigments: The contents of photosynthetic pigments (Chl a, Chl-b and Chl-a+b) (Fig. 3a-c) were noticeably decreased with the progression of drought stress and the extent of this decrease was obviously less in the anti-transpirant-treated plants than in the non anti-transpirant-treated plants. Under well-watered

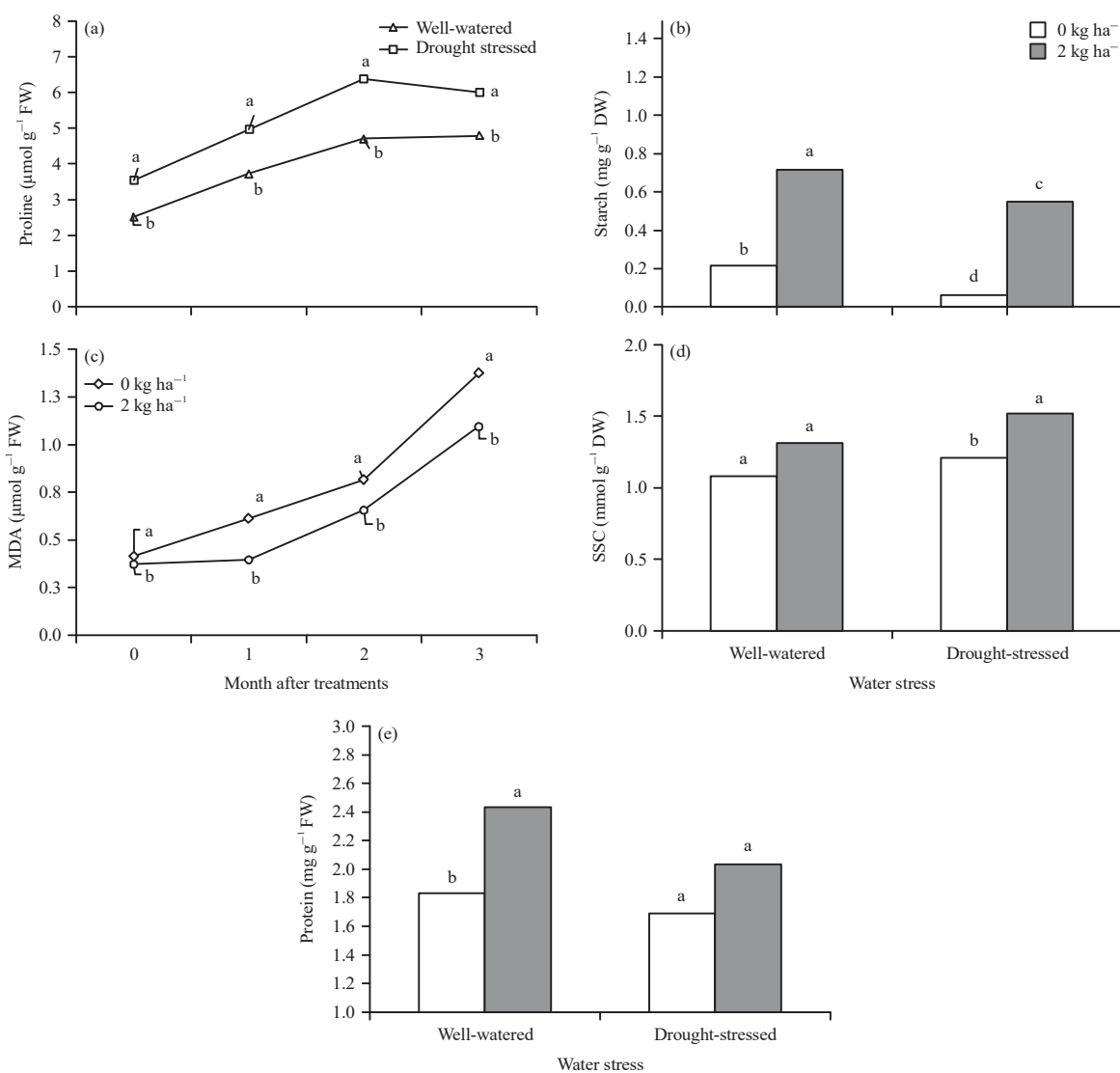


Fig. 4(a-e): Biochemical responses of fig under optimization of brassinolide according to parameters (a) Proline content as main effect of water stress, (b) Starch as interaction between anti-transpirant and water stress at 3rd MAT, (c) MDA as main effect of anti-transpirant, (d) SSC as interaction between anti-transpirant and water stress at 3rd MAT and (e) Protein content as interaction between anti-transpirant and water stress at 1st MAT
 Bars and curves represent means followed by the different small letters were significant at $p < 0.05$

conditions, the treatment of plants with anti-transpirant also enhanced the chlorophyll contents in the fig plants. Photosynthetic pigments were affected by interaction between anti-transpirant and water stress of fig under optimization of brassinolide. Treatment interaction between anti-transpirant and water stress was significant on chlorophyll-a at 2nd MAT, on chlorophyll b at 1st MAT and on chlorophyll (a+b) or total chlorophyll content at 1st MAT too.

Protein, proline content, starch, malondialdehyde and soluble sugar content: Proline accumulation (Fig. 4a), starch (Fig. 4b) and malondialdehyde (MDA) (Fig. 4c), soluble sugar content (SSC) (Fig. 4d) were also improved, whereas, protein content (Fig. 4e) were decreased with anti-transpirant application. The decreased protein contents under drought were noticeably increased by the foliar application of anti-transpirant and reached a peak on 1st MAT of anti-transpirant and then decreased. Application of

Table 1: Pearson correlation between all measured parameters in the experiment

Parameters	A	gs	E	Chl-a	Chl-b	T-Chl	Starch	LWC	RLWC	POD	CAT	MDA	Protein	Proline	SSC
A	1														
gs	0.391*	1													
E	0.358*	0.542**	1												
Chl-a	0.256	0.429**	0.214	1											
Chl-b	0.285	0.265	0.376*	0.427**	1										
T-Chl	0.318	0.365*	0.375*	0.699**	0.945**	1									
Starch	0.473**	0.672**	0.667**	0.610**	0.743**	0.808**	1								
LWC	-0.056	0.059	0.090	-0.085	-0.227	-0.210	-0.080	1							
RLWC	0.343*	0.575**	0.558**	0.600**	0.553**	0.655**	0.765**	-0.191	1						
POD	-0.211	-0.344*	-0.268	-0.249	-0.397*	-0.404*	-0.463**	0.180	-0.465**	1					
CAT	-0.477**	-0.499**	-0.550**	-0.165	-0.390*	-0.368*	-0.676**	-0.102	-0.468**	0.260	1				
MDA	-0.468**	-0.400*	-0.349*	-0.274	-0.459**	-0.463**	-0.613**	0.094	-0.512**	0.376*	0.521**	1			
Protein	0.321	0.523**	0.214	0.546**	0.213	0.366*	0.544**	0.117	0.384*	-0.013	-0.520**	-0.349*	1		
Proline	-0.239	-0.286	-0.068	-0.303	-0.216	-0.280	-0.416*	0.082	-0.168	0.224	0.133	0.410*	-0.374*	1	
SSC	-0.138	-0.379*	-0.252	-0.328	-0.485**	-0.502**	-0.443**	0.360*	-0.653**	0.603**	0.069	0.350*	-0.031	0.068	1

A: Photosynthetic rate, gs: Stomatal conductance, E: Transpiration, Chl-a: Chlorophyll-a, Chl-b: Chlorophyll-b, T-Chl: Total chlorophyll content, LWC: Leaf water content, RLWC: Relative water content, POD: Peroxidase, CAT: Catalase, MDA: Malondialdehyde, SSC: Soluble sugar content, *p<0.05, **p<0.01

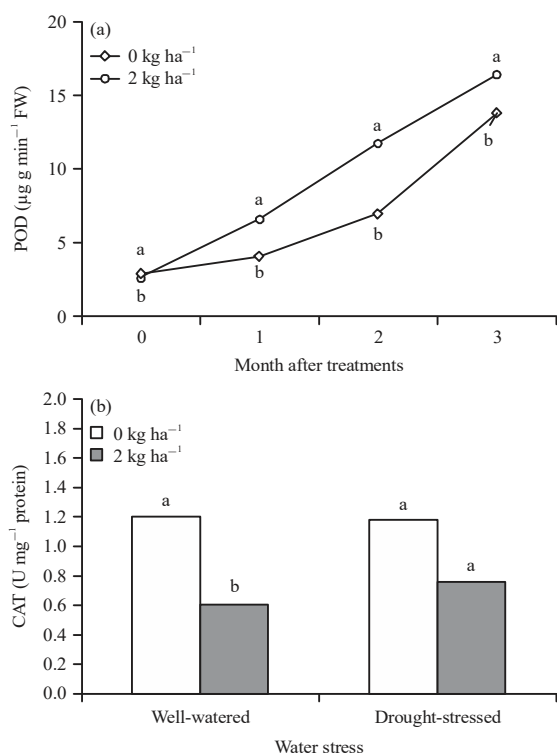


Fig.5(a-b): Enzyme activities of fig under optimization of brassinolide according to parameters (a) POD as main effect of anti-transpirant and (b) CAT as interaction between anti-transpirant and water stress at 1st MAT

Bars and curves represent means followed by the different small letters were significant at p<0.05

anti-transpirant led to increase in proline, SSC and MDA 16.40, 9.15 and 11.61% under well-watered and 12.35, 7.73 and 9.42% in water-deficit conditions after

3 MAT of anti-transpirant-treatment, respectively. The proline contents were subsequently increased by the foliar application of anti-transpirant and reached at peak on 2nd MAT of anti-transpirant and then found to be slightly decreased on 3rd MAT application of anti-transpirant. Spray of anti-transpirant significantly improved the proline accumulation under drought and well-watered conditions. MDA content was subsequently increased with prolonged drought stress, reached a peak at 3rd MAT of anti-transpirant-treatment.

Enzyme activities: Drought led to significant modulation of antioxidant defence in leaves of fig plants. Activities of antioxidant enzymes (POD and CAT) (Fig. 5a, b) increased in fig plants due to the imposition of drought than those in non-stressed plants. However, exogenously applied anti-transpirant caused a further increase in antioxidant enzymes activity of stressed plants. The POD activity was affected by treatment anti-transpirant alone and CAT activity was affected by interaction between anti-transpirant and water stress under optimization of brassinolide. Exogenous application of anti-transpirant enhanced the activities of antioxidant enzymes in well-watered plants as well.

Correlation analysis: Correlation analysis was carried out to establish the relationship between the parameters. Table 1 shows that a significant positive inter-correlation among parameters such as A with gs, A with E, gs with E, gs with Chl-a, E with Chl-b, Chl-a with Chl-b, gs with T-Chl, E with T-Chl, Chl-a with T-Chl, Chl-b with T-Chl, A with starch, gs with starch, E with starch, Chl-a with starch, Chl-b with starch, T-Chl with starch, A with RLWC, gs with RLWC, E with RLWC, Chl-a with RLWC,

Chl-b with RLWC, T-Chl with RLWC, starch with RLWC, POD with MDA, CAT with MDA, Chl-a with protein, T-Chl with protein, starch with protein, RLWC with protein, MDA with proline, gs with SSC, LWC with SSC, POD with SSC and MDA with SSC. Increasing in A, gs, E, Chl-a, T-Chl, Chl-b, starch, POD, CAT, RLWC, MDA and LWC was associated with an increment in gs, E, Chl-a, Chl-b, T-Chl, starch, RLWC, MDA, protein, proline and SSC.

Significant negative correlation was noted between gs with POD, Chl-b with POD, T-Chl with POD, RLWC with POD, A with CAT, gs with CAT, E with CAT, Chl-b with CAT, T-Chl with CAT, starch with CAT, RLWC with CAT, A with MDA, gs with MDA, E with MDA, Chl-b with MDA, T-Chl with MDA, starch with MDA, RLWC with MDA, CAT with protein, MDA with protein, starch with proline, protein with proline, Chl-b with SSC, T-Chl with SSC, starch with SSC and RLWC with SSC. Increasing in gs, Chl-b, T-Chl, RLWC, A, E, Chl-b, starch, CAT, MDA and protein was associated with a decrement in POD, CAT, MDA, protein, proline and SSC.

DISCUSSION

In this study, effect of exogenously applied anti-transpirant on physiological biochemical changes traits of fig under optimization of brassinolide were investigated under water-deficit and well-watered conditions. Drought imposed at growth stage of fig severely disrupting water relations and leaf gas exchange properties. Alteration in LWC and RLWC serves as the first indicator to identify the stress status of stressed plants at a specific time point^{32,33}.

Photosynthesis was one of the most vital physiological processes contributing to growth and productivity of crop plants for food. Although under drought, gas exchange anti-transpirant-treatment considerably improved the gas exchange attributes of fig in drought as well as in well-watered conditions. Stomata were the entrance of water loss and CO₂ absorbability. Upon exposure to drought, stomatal closure was one of the first responses, which reduces the rate of photosynthesis^{16,34}. Shanan and Shalaby³⁵ stated magnesium carbonate (MgCO₃) was considered to be an anti-transpirant that closes stomata and thus affects metabolic processes in leaf tissues. The anti-transpirant-induced increase in photosynthesis could be due to improvements in leaf water balance as indicated by increased water potential under water-deficit³⁶ and improved chlorophyll content (Fig. 3).

Cornic³⁷ stated that photosynthesis was strongly affected by water shortage as a decrease in stomatal assimilation. Water was also lost to the atmosphere through transpiration and the potential for reducing transpiration water loss without significantly reducing photosynthetic rate was based on the

premise that resistance to the movement of carbon dioxide in the mesophyll was greater than the stomatal resistance that limits water loss to the atmosphere. Application methods using anti-transpirants have been proposed to reduce water loss conductance reduces the CO₂ and enhance the water status of plants.

Plants accumulate various proteins and osmolytes, which help in osmoregulation and maintenance of cell turgor³⁸. In this study, foliar application of anti-transpirant noticeably reduced the total soluble proteins (Fig. 4e), enhanced proline accumulation (Fig. 4a), which helped in maintenance of tissue water contents (Fig. 1b). Proline is one of the organic compounds that accumulate in leaves of a great number of species subjected to drought. Proline accumulation is used to evaluate the tolerance or sensitivity of plants to water stresses. Garcia-Sanchez and Syvertsen³⁹ reported that an increase in proline causes protection against oxidative damage during dehydration. In the current investigation, proline increased in both under well-watered and drought stress conditions, its accumulation could be considered a symptom of drought stress and an indicator of tolerance to stress.

Plants have an internal protective enzyme-catalyzed clean up system, the defence system, which scavenges the plants from reactive oxygen species, thus guaranteeing normal cellular function⁴⁰. When the plants suffered from drought, the whole defensive system needs to be activated in order to resist the active oxygen injury.

Water stress resulted in oxidative damage to plant cell membranes. Malondialdehyde (MDA), the end product of lipid peroxidation, showed greater accumulation in plants under stress condition (Fig. 4c). The rise in MDA content under stress conditions suggests that drought could induce membrane lipid peroxidation by means of ROS^{41,42}. Nonetheless anti-transpirant-treatment considerably lowered the MDA contents (Fig. 1b).

Likewise anti-transpirant application substantially enhanced the activities of enzymatic antioxidants (Fig. 5). Furthermore, CAT activity was substantially enhanced later (Fig. 5b). This regulation of enzymatic antioxidants seems the result of anti-transpirant-induced regulation of transcription and translation⁴³, which led to improvement in the level of SSC and enzymatic antioxidants^{44,45} and increment in MDA and proline content⁴⁶.

CONCLUSION

In conclusion, anti-transpirant application partially alleviated the detrimental effects of drought by adjusting the activity of enzymatic antioxidants and gas exchange traits, which helped in sustaining plant growth of fig.

SIGNIFICANCE STATEMENTS

This study investigated the utilization of anti-transpirant and brassinolide can be beneficial to reduce drought condition that always faced by local farmers especially in tropical and sub tropical zone which degrade the farmers productivity. This study will help the researchers to uncover the critical areas of anti-transpirant and brassinolide concentration that many researchers were not able to explore.

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