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Foliar Application of Signal Molecules can Augment Quality in *Andrographis paniculata* under Prolonged Water Deficit Stress

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The quality of a medicinal plant is decided by the content of specific secondary metabolite respective to the plant species. Therefore, an attempt was made to assess the effect of water deficit (WD) stress and signal molecules on physiological and biochemical responses of *Andrographis paniculata* under polyhouse condition. To keep the soil at field capacity (FC) in control (well-watered) treatment, a pre-measured quantity of water was applied to the pots once in a week. The signal molecules (i) ascorbic acid @200 mg l^{-1}, (ii) glutamic acid @150 mg l^{-1} and salicylic acid @200 mg l^{-1} applied foliar at 10, 25, 40 and 55 days after WD treatment. Total chlorophyll, total carotenoid and proline content increased due to WD stress at 50 days after treatment (DAT) whereas, total chlorophyll and total carotenoid content decreased but, proline content increased at 80 DAT. Ascorbic acid enhanced the ascorbate peroxidase activity and glutamic acid enhanced guiacol peroxidase activity of leaves under WD stress. WD stress reduced actual efficiency of photosynthesis *(Fv'/Fm')* and energy utilized for photochemistry ($!_{PSII}$) at 80 DAT. WD stress during 20-50 DAT did not improve the andrographolide content in leaves of *A. paniculata*. However, a prolonged WD stress till 80 DAT hastened andrographolide content in leaves. Foliar application of salicylic acid under polyhouse condition indicated its scope to augment the quality of *Andrographis paniculata* under prolonged WD stress.

Key words: *Andrographis paniculata*, andrographolide, antioxidant enzymes activity, chlorophyll fluorescence yield, relative water content, water deficit stress

Andrographis paniculata Wall ex Nees, is an important medicinal plant belonging to Acanthaceae family. Traditionally it is also known as the "king of bitter". Many ailments and diseases like a hepatoprotective drug, anti-inflammatory agent and against stomach ulcers in India and many South-Eastern countries are being cured by this medicinal plant (1). The main active principal component in *A. paniculata is* a diterpene lactone called as the andrographolide. It is mainly found and cultivated in tropical and sub-tropical Asia, South-East Asia and India (2). Indian farmers grow it as a rain-fed medicinal crop with supplemented irrigation as per the availability. The erratic rainfall pattern along with shortage of irrigation water creates intermittent periods of WD stress to this crop. Along with the primary metabolism, the synthesis and accumulation of secondary metabolites is also affected by WD stress. Formation of reactive oxygen species (ROS) such as superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), and singlet oxygen (O^2) is enhanced through osmotic stress due to which mitochondria and chloroplasts cellular structures get disrupted (3). In a plant cellular system, there exist many protection mechanisms along with repair systems to minimize the occurrence of oxidative damage caused by ROS (4). Through its multiple roles in plant growth, ascorbate has been shown to have impact on cell division, cell wall expansion, and other develop-mental processes (5). Being a very strong antioxidant it acts in osmotolerance mechanism. Chlorophyll *a*, proline and ã-amino butyric acid (GABA) are formed from glutamic acid. The proline and GABA have been documented to be responsible for imparting protection under various environmental stresses (6). According to Al-Gabbiesh (7) and Kleinwächter and Selmer (8), the phytohormone application is proposed as an auxiliary strategy to enhance accumulation of secondary metabolite and thereby to influence product quality in medicinal plants. However, the information on impacts of some stress tolerance imparting signal molecules on physiology of this important medicinal plant is lacking. Hence, the objective of this study was to determine the effects of foliar application of stress tolerance imparting signal molecules on physiological and biochemical performance with special focus on

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andrographolide content in *A. paniculata* under WD stress.

Materials and Methods

Plant material, field plot design and treatment allocation: A pot experiment was carried out *(Kharif,* 2015*)* in polyhouse at the ICAR- Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand (latitude 07° 15'N, longitude 78° 14'E.) in Gujarat, India in *Kharif,* 2015. Dry sandy loam soil from the experimental fields having nearly 23% water holding at field capacity and neutral pH (pH 6.5 - 7.5) was collected and 5.0 kg soil (80:20 soil: manure ration) was filled in pots with 30 cm height and 30 cm diameter. The seeds of a local cultivar were sown in nursery in the month of May 2015 and 45-days seedling were transplanted in pots as completely randomized design with five treatments in four replications. All the treatments were sufficiently and equally irrigated to keep the soil moisture at field capacity (FC) on every 5th day till 20 DAT and henceforth, in order to generate WD stress condition, quantity of water was reduced to create WD stress. The FC was calculated as described by Singh *et al.* (9). To keep the soil moisture content of the soil below 50% of the FC in WD treatment, a pre-measured quantity of water was applied to the pots so as to maintain SMC below 10% on weight basis. The treatment combinations were T_1: Control- well watered as per to fulfill demands to reach field capacity once in a week, T_2: water deficit stress (WD) – to keep soil moisture content below 50% of the field capacity (<12.5% on weight basis), T_3: WD + 200 mg l^{-1} ascorbic acid, T_4: WD + 150 mg l^{-1} glutamic acid and T_5: WD+ 200 mg l^{-1} salicylic acid. Signal molecules were applied foliar at 30, 45, 60 and 75 DAT and various observations were recorded at 50 and 80 DAT.

Soil moisture content and relative water content: Gravimetric method was used to measure percent soil moisture content (SMC %) on weight basis from the upper (0–5 cm) and lower layer (05–10 cm). The relative water content (RWC) was calculated as: RWC = (FW-DW)/TW x 100 where, FW was fresh weight, DW was dry weight and the TW was turgid weight. To measure the specific leaf area (SLA) the fifth fully opened leaf was selected in each treatment in the morning [illegible] 10:00 hours. After measuring leaf area with [illegible] area meter (LI-COR Inc., Lincoln, NE, USA) [illegible] then oven-dried at 60^{0}C for 72 h and weighed. Specific leaf area (SLA; cm^2g^{-1}) = leaf area/ leaf dry weight.

Metabolite content and membrane [illegible] chlorophyll pigments (chlorophyll *a* and chlorophyll *b*) and total carotenoid content were estimated by method described by Arnon (10). Through the method described by Bates *et al.* (11). the leaf proline content was estimated using ninhydrin as reagent. To extract andrographolide content the powdered material prepared from shade dried leaves was used. By cold maceration method given by Rajani *et al.* (12). To prepare stock solutions of andrographolide the standard andrographolide used was from M/s Sigma Aldrich, USA. To produce the working standards, different dilution of these solutions. The LC-10AD pumps and SPD-10A UV-VIS detector along with Aimil chromatographic data station together formed the complete HPLC system (Shimadzu, Japan) in which the RP-18 column (250 mm × 4.6 mm, 5 ìm, Merck) were used. Methanol and water (65:35) as mobile phase at a flow rate of 1ml min^{-1} was used to cause separation as described by Gajbhiye *et al.* (13) and the absorbance was read at 229 nm for detection on UV-VIS detector. Using standard andrographolide, the calibration curve for andrographolide was prepared. Membrane stability index (MSI), a measure of all membrane leakage was determined by Sairam (14) where, MSI(%)= (EC1/EC2) x 100

Antioxidant enzymatic activities assays: Antioxidant enzymes i.e. SOD, CAT, APX and GPX enzymes were extracted with minor modifications (15). To a properly homogenized fresh leaf tissues (500 mg) with the help of a pre-chilled mortar and pestle, 3 ml extraction buffer containing 1 mM EDTA and 1% (W/V) polyvinylpyrolidone (PVP) in 50 mM sodium phosphate (pH 7.4) filtered and centrifuged (10,000xg, 20 min), supernatant was used for the assay of enzymatic activities. Inhibition in the photochemical reduction of nitrobluetetrazoilum (NBT), the total SOD (EC 1.15.1.1) was considered to measured activity of SOD spectrophotometrically (A560nm). The reaction mixture contained 13 mM methionine, 75 iM

NBT, 2 ìM riboflavin, 0.1 mM EDTA in 50 mM sodium phosphate buffer (pH 7.8). To 2.9 ml of reaction mixture 0.1 ml of the enzyme was added to start the reaction under photochemical light. The quantity of enzyme required to inhibit the reduction of NBT by 50 % in a reaction mixture was considered as one unit of SOD. According to Constantine and Stanley (16), enzyme unit of SOD was calculated. According to the method of Aabi (17) by measuring the decrease in absorption at 240 nm as H_2O_2 (e = 39.4 mM^{-1} cm^{-1}) consumed by the 0.1 ml extract in 3 ml reaction mixture, total catalase (EC 1.11.1.6) enzyme activity was expressed as ì mol H_2O_2 oxidized min^{-1} g^{-1} protein. Immediately in fresh extract, the APX (EC 1.11.1.11) activity was measured as described by Nakano and Asada (18). The method given by Lowry *et al.* (19) using folin-phenol reagent, the protein concentration of enzymes extract was determined.

Chlorophyll fluorescence parameters: Chlorophyll fluorescence parameters were measured with fully extended leaves (5th position from apax). In a properly dark adapted (30 minutes) leaf the maximum efficiency of PSII (*Fv/Fm*) were recorded. After a minimum 20 minute, the actual quantum yield of PSII (*Fv'/Fm'*) were recorded. The process of light and dark adaptation followed by the measurement of chlorophyll fluorescence parameters were completed between 08:00-10:00 am. Theses parameters were recorded using the portable photosynthesis system (LI-COR 6400, Inc. Lincon, Nebraska, USA). To calculate the quantum yield of PSII ($Ö_{PSII}$), and non photochemical quenching (*NPQ*) formulas given by Maxwell and Johnson (20) were used.

Statistical analysis: The data were subjected to analysis of variance appropriate to the experimental design using OSAASTAT (21) in MS Excel and the least significant differences were calculated to assess the significance of treatment means where the "F" test was found significant P Å 0.05. The Duncan's multiple test was performed to compare the means using the same Microsoft excel based add-in module.

Results and Discussion

Soil water plant relations: Soil moisture content (SMC %) on weight basis ranged between 13.2 % and 18.5 % in control pots whereas in WD treatment pots it ranged between 6.1% and 9.2% after 20 DAT (Table1). The relative water content (RWC %) decreased due to WD stress. It was 91.5% in control and less than 82.2% in WD treatment at 50 DAT. A significant reduction from 92.2% in control to less than 76.1% in WD plants was observed at 85 DAT. Foliar applications of none of the signal molecules helped in retaining high RWC at any stage under WD stress (Table 2). Based on the experiments, researchers have established that the leaf RWC is a more convenient measure of plant water balance than the leaf water potential in plants (22). The water status of the leaf and ultimately the whole plant is governed by the discrepancy between water absorbed by the root system and transpired by leaves. The soil water availability was governing force of the lowered RWC in this study. The reason behind the lowered RWC seems to be that under limited water availability the insufficient absorption of water by the roots would have failed to compensate for the transpiration losses (23). To overcome such deviations, a plant need to have either better root growth so as to absorb more quantity of water or needs to have better stomatal regulation to check water loss (24).

The specific leaf area was 267 cm^2 g^{-1} at 50 DAT and reduced to 234 cm^2 g^{-1} (12.4%) at 80 DAT. WD stress

Table 1: Soil moisture content (%) of the experimental pots

Treatment	Days after transplanting									
	20		30		40		60		85	
	5 cm	10 cm	5 cm	10 cm	5 cm	10 cm	5 cm	10 cm	5 cm	10 cm
Well watered	17.4	16.0	18.5	18.3	13.2	15.2	17.0	17.6	16.0	18.5
WD*	16.3	19.5	8.5	8.1	9.0	15.0	5.9	5.2	8.1	9.8
WD*+Ascorbic acid @200 mg l^{-1}**	15.9	19.1	9.4	12.2	6.8	8.6	6.7	6.6	7.6	9.2
WD*+ Gutamic acid @150 mg l^{-1}**	20.2	19.5	7.8	7.7	7.7	6.6	6.7	6.7	6.8	9.8
WD*+ Salicylic acid @200 mg l^{-1} **	15.8	16.7	7.8	8.6	6.1	7.8	5.3	6.0	4.6	6.9
Mean WD	17.1	18.7	8.4	9.2	7.4	9.5	6.2	6.1	6.8	8.9

* Water deficit stress imposed from 20 days after transplanting ** Foliar application at 10, 25, 40 and 55 days after WD stress

Table 2: Effect of signal molecules application on relative water content, specific leaf area, membrane stability index and leaf proline conten in *Andrographis paniculata* under water deficit stress

Treatment	RWC (%)		SLA (cm^2g^{-1})		MSI (%)		Proline (mg g^{-1} FW)	
	50DAT	80DAT	50DAT	80DAT	50DAT	80DAT	50DAT	80DAT
Well watered	91.5±2.6a	92.2±1.0a	284±38a	274±22a	78.4±7.9a	73.1±1.6a	0.100±0.003d	0.134±0.150c
WD*	80.1±9.2b	73.2±3.0b	249±25a	224±13b	58.5±1.6c	39.8±3.6c	0.258±0.015b	0.398±0.107b
WD*+Ascorbic acid @200 mg l^{-1} **	81.1±6.2b	73.7±1.9b	260±13a	221±11b	68.1±6.3b	40.0±2.1c	0.223±0.0017bc	0.464±0.179ab
WD*+ Gutamic acid @150 mg l^{-1} **	80.2±3.8b	76.1±6.0b	267±23a	234±15b	63.4±4.1bc	42.3±6.4c	0.218±0.040c	0.495±0.062ab
WD*+ Salicylic acid @200 mg l^{-1} **	82.2±6.8b	74.3±4.1b	276±17a	219±33b	60.1±5.9c	51.7±3.9b	0.595±0.042a	0.5960.084a
Mean	83.0	77.9	267	234	65.7	49.4	0.279	0.417
SEM	3.02	1.81	12.35	9.75	2.8	1.96	0.014	0.062
LSD $(_{0.05})$	NS	5.48	NS	29.4	8.4	5.9	0.042	0.187

Mean values ± standard deviation

*Water deficit stress (WD) imposed from 20 days after transplanting (DAT) ** Foliar application at 10, 25, 40 and 55 days after WD stress

had non-significant effect on SLA at 50 DAT but, had significantly reduced the SLA from 274 cm^2 g^{-1} in control to less than 234cm^2 g^{-1} in WD stress at 80 DAT. The MSI reduced from 78.4 in control to the lowest of 58.5 in WD treatment at 50 DAT. It is prominent to mention here that the ascorbic acid and glutamic acid helped to maintain higher MSI under WD stress. Nearly 60% reduction in MSI as compared to that of the control was reported in WD stress at 80 DAT. Among the signal molecules tested salicylic acid had a positive and promising impact on preventing membrane damage due to WD stress at this stage (Table 2). When plants are not able to utilize all the absorbed photons for photochemistry, the unutilized photons are diverted towards non photochemical quenching through heat generation. Reduction in SLA is a kind of protective mechanism of the photosynthetic pigments under stress condition as lesser surface area for harvesting photosynthetic light is made available when the SLA reduced. In addition, reduced SLA exposes lesser surface area in direct contact with the ambient air circulation. This in turn prevents loss of water from leaves making the plants to withstand the adverse impacts of WD condition in plants (25).

Among the first targets during stress full conditions come the cellular membranes (26). The level of tolerance to WD stress depends upon the ability of a plant to maintain membrane integrity (27). Reduction in plant water content seriously impairs both membrane structure and thereby its function in plants (28). MSI has been advocated as a reliable trait to evaluate drought tolerance of plants under osmotic stress condition (29). WD stress induced disruption of cell membranes is manifested by lower values of MSI (23). Damage to cellular membranes and chlorophylls are reliable indicators for determination of the extent of damage to the plants due to oxidative stress (30). In wheat, integrity of cell membrane is significantly affected by water deficit stress and a correlation between MSI, growth and water use efficiency (31, 32). High MSI is indication of low ion leakage and greater stability (33). High MSI under salicylic acid application seems to be because salicylic acid would have increased the accumulation of Ca^{+2} which can maintain membrane integrity as a protection mechanism (34).

Metabolite accumulation pattern: There was an increase in chlorophyll *a*, chlorophyll b, total carotenoids and proline content due to WD stress at 50 DAT (Table 3). It is interested to note that, chlorophyll *a* (2.3 mg g^{-1} FW) and chlorophyll b (1.2 mg g^{-1} FW) content were highest in WD stressed plants sprayed with glutamic acid. The total carotenoids content was highest (14.5 mg g^{-1} FW) in WD stress plants without any signal molecules application at this stage. The total carotenoids content was not affected by WD stress at 80 DAT. Chlorophyll *a* (1.9 mg g^{-1} FW) and chlorophyll b (0.82 mg g^{-1} FW) content significantly reduced due to WD stress at 80 DAT however, sprayed with glutamic acid helped maintain higher content of chlorophyll under the WD stress. The proline content was 0.100 mg g^{-1} FW in control and more than two fold increase in proline was noted due to WD treatment at 50 DAT. Foliar spray of salicylic acid under WD stress increased six times in leaf proline content as compared to control and more

Table 3: Effect of signal molecules application on chlorophyll a, chlorophyll b, total ccarotenoid and andrographolide content in leaf *Andrographis paniculata* under water deficit stress

Treatment	chlorophyll a (mg g^{-1} FW)		chlorophyll b (mg g^{-1} FW)		carotenoid (mg g^{-1} FW)		andrographolide (mg g^{-1} DW)	
	50 DAT	80 DAT	50 DAT	80 DAT	50 DAT	80 DAT	50 DAT	80 DAT
Well watered	1.6±0.30b	2.07±0.02a	0.45±0.06b	1.05±0.04a	7.4±1.9c	8.16±0.1a	2.63±0.43a	1.77±0.30c
WD*	2.1±0.20a	1.80±0.05c	1.10±0.28a	0.77±0.13c	14.5±1.2a	6.02±1.5ab	3.05±0.45a	2.37±0.13b
WD*+Ascorbic acid @200 mg l^{-1} **	2.2±0.05a	1.94±0.03b	1.17±0.23a	0.85±0.03bc	11.6±1.3b	5.84±1.5b	2.86±0.45a	2.29±0.34b
WD*+ Gutamic acid @150 mg l^{-1} **	2.3±0.15a	2.13±0.13a	1.20±0.24a	0.94±0.15b	13.3±2.8ab	5.62±2.1b	2.92±0.48a	2.57±0.32a
WD*+ Salicylic acid @200 mg l^{-1} **	1.9±0.18b	1.75±0.12c	1.2±0.48a	0.74±0.20c	12.4±1.2ab	7.03±1.0ab	2.94±0.40a	2.62±0.25a
Mean	2.0	1.94	1.02	0.87	11.9	6.54	2.88	2.32
SEM	9.75	0.042	0.15	0.065	0.90	0.71	0.22	7.51
LSD $(_{0.05})$	0.29	0.13	0.21	0.197	2.68	NS	NS	0.22

Mean values ± standard deviation

* Water deficit stress (WD) imposed from 20 days after transplanting (DAT) ** Foliar application at 10, 25, 40 and 55 days after WD stress

than 2.5 times that of other WD treatments at this stage. The magnitude of increase in proline content due to WD stress was higher due to application of salicylic acid which was statistically with ascorbic acid and glutamic acid treatment at 80 DAT. WD stress increased the leaf andrographolide content from 2.63 mg g^{-1} DW in control to 3.05 mg g^{-1}DW at 50 DAT. With the advancement of growth stage, the constitutive content of leaf andrographolide decreased from 2.63 mg g^{-1} DW at 50 DAT to 1.77 mg g^{-1} DW at 80 DAT. WD stress increased the leaf andrographolide content significantly at 80 DAT. Application of glutamic acid and salicylic acid increased leaf andrographolide content in *A. paniculata* under prolonged WD stress.

Being one of the major components of the chloroplast, there is a positive relationship between relative chlorophyll content, photosynthetic rate and plant growth. Limited water supply usually causes a reduction in chlorophyll content (35) but in this study WD stress during 20 and 50 DAT had increased the total chlorophylls and total carotenoid content. Increase in chlorophyll content help better harvest solar energy thus, it seems to be the adaption mechanism of *Andrographis paniculata* to perform well under initial phase of WD stress. A typical symptoms of oxidative stress characterized by decrease in chlorophyll content under WD stress during later growth phase seems to be the result of pigment photo-oxidation and chlorophyll degradation. Decreased chlorophyll level during WD stress has been reported in many species (36, 37). Increase in total chlorophyll content due to foliar application of glutamic acid seems to be because the more availability of glutamic acid which is the starting point of the chlorophyll biosynthetic pathway in plants. Application of glutamic acid had increased chlorophyll content in wheat and watermelon seedlings (38,39). A large class of isoprenoids molecules called the carotenoids is *de novo* synthesized by all photosynthetic and many non-photosynthetic organisms (40). They not only act as an accessory pigment but also as an effective antioxidant and play a unique role in protecting photochemical processes and sustaining them (41). The ²-carotene in photosynthetic tissue quenches the triplet chlorophyll directly and thus prevents the generation of singlet oxygen and protects from oxidative damage (42). Increase in carotenoid content under WD stress help plants to hardess the solar energy in an efficient way for photosynthesis and a high carotenoid content also act as antioxidant and thus, would have imparted osmotic stress tolerance in *Andrographis* under WD stress for initial 30 days. However, a persistent WD condition had adversely affected the chlorophyll and carotenoids content at 80 DAT showing a negative effects on these metabolites. Accumulation of free proline is among the most common responses to stress in plants, specially in medicinal plants, it is important to note that proline synthesis generates $NADP^+$ which can be utilized for the synthesis of other secondary metabolites. Additionally role of proline in quenching singlet oxygen, scavenging OH^- radicals, stabilizing proteins, DNA and acting as osmolyte have been reported (43). Increased proline content seems to be one of the adaptive mechanisms to cope up the WD stress in *Andrographis paniculata*. The results of the present investigation indicated that feeding precursor of proline the L-glutamic acid in *Andrographis paniculata* under WD stress did not enhance the proline content however, it enhanced

the chlorophyll and carotenoid content. A competition between chlorophyll and proline biosynthesis pathway under WD stress at 50 DAT. A marginal but, non-significant increase in andrographolide content at 50 DAT seems to be because the reductive powers generated through the activity of light reaction of PS II would have been diversified towards the formation of chlorophyll and carotenoid hence, very short fluxes of these reductive powers would have been utilized for andrographolide formation. At 80 DAT, increased andrographolide content showed that under a prolonged WD stress condition biosynthetic pathways shifted for the production of secondary metabolites as an adaptation mechanism. Positive responses of signal molecules under prolonged WD stress condition indicated stage specific responses of these signaling molecules. Thus, glutamic and salicylic acid can alter the functioning of any or all of the DXP, MAV and MEP proposed pathways responsible for andrographolide biosynthesis under prolonged water deficit. At the same time, WD stress would have a positive and profound effect on these pathways leading to a higher production of andrographolide in *A. paniculata.*

ROS scavenging efficiency: WD stress had increased activities of antioxidant enzymes namely, SOD, CAT, APX and GPX at both growth stages (Table 4).The constitutive level of SOD remained almost same with mean SOD activity of 44.0 Units g^{-1} protein and 41.3 Units g^{-1} protein at 50 and 80 DAT, respectively. The SOD increased from 30.2 units g^{-1} protein in control to more than 44units g^{-1} protein in WD treatment with the highest SOD activity (55.6 units g^{-1} protein) in salicylic acid treatment under WD stress at 50 DAT. Applicati of salicylic acid enhanced the SOD activity which w statistically at par with ascorbic acid and glutan treatment 80 DAT under water stress. Constitutive C activity increased with advancement of growth with me value of 49.0 µ mol H_2O_2 min^{-1} g^{-1} protein at 50 DAT a 64.0 µ mol H_2O_2 min^{-1} g^{-1} protein at 80 DAT. WD stre resulted in increased CAT production. The GPX activ increased from 64.9 µ mol guiacol min^{-1} g^{-1} protein at DAT to 84.4µ mol ascorbate min^{-1} g^{-1} protein at 80 DA Application of ascorbic and salicylic acid helped mainta high GPX activity under WD stress at 50 and 90 DA thus found suitable signal molecules to enhan antioxidant defense system. The mean APX activi enhanced due to progression of growth and it was 16 mol ascorbate min^{-1} g^{-1}protein at 50 DAT and 192 µ m ascorbate min^{-1} g^{-1} protein at 80 DAT.

To maintain normal cellular function through avoidin injuries caused by active oxygen species even unde challenging stress full condition, plants have evolved a internal antioxidative system (44).

The induction of ROS-scavenging enzymes, such a SOD, CAT, POX, and APX is the most commo mecha-nism for detoxifying ROS synthesized durin stress response (Gressel and Galun 1994). Being on of the ubiquitous enzymes in aerobic organisms, th SOD plays a key role in cellular defense mechanism against ROS. Increased SOD activity in *Andrographi paniculata* leaves indicated that SOD activity was allie to better protection against water stress. CAT is the chief enzyme that scavenges harmful oxygen species in plants (45) and thus increased CAT activities unde

Table 4: Effect of signal molecules application on antioxidant enzymes activity of superoxide dismutase (SOD), guiacol peroxidise (GPX), catalase (CAT) and ascorbate peroxidise (APX) in leaf of *Andrographis paniculata* under water deficit stress

Treatment combination	SOD (Units g^{-1} protein)		GPX (µmolguaiacol min^{-1} g^{-1} protein)		CAT (µ mol H_2O_2 min^{-1} g^{-1} protein)		APX (µ molascorbate min^{-1} g^{-1} protein)	
	50 DAT	80 DAT	50 DAT	80 DAT	50 DAT	80 DAT	50 DAT	80 DAT
Well watered	30.2±7.6c	17.3±4.9d	30.0±12.1c	49.6±8.9c	30.9±3.4c	43.6±6.5b	122±17.3c	135±35.8b
WD*	43.4±7.2b	38.8±7.1c	61.9±9.2b	84.1±13.1b	46.2±12.1b	69.6±6.7a	163±12.3b	221±21.7a
WD*+Ascorbic acid @200 mg l^{-1} **	45.6±4.1b	39.6±5.6c	77.0±4.5ab	87.0±16.6ab	44.2±4.9b	64.6±7.3a	192±15.8a	201±11.8a
WD*+ Gutamic acid @150 mg l^{-1} **	45.3±5.0b	50.6±5.8b	73.9±14.2ab	96.9±14.3ab	45.9±5.0b	65.9±8.7a	164±16.9b	204±25.2a
WD*+ Salicylic acid @200 mg l^{-1} **	55.6±7.6a	60.0±4.0a	81.7±6.2a	106.2±8.6a	77.5±6.6a	76.0±10.2a	171±13.6ab	200±25.0a
Mean	44.0	41.3±	64.9	84.8	49.0	64.0	162.0	192.0
SEM	3.23	2.78	4.94	6.33	3.53	3.99	7.65	12.5
LSD $(_{0.05})$	9.73	8.39	14.91	19.1	10.7	12.1	23.0	38.0

Mean values ± standard deviation

*Water deficit stress (WD) imposed from 20 days after transplanting (DAT) **Foliar application at 10, 25, 40 and 55 days after WD stress

WD stress suggested that it may be responsible for protection against oxidative damage. Many important reactions such as ascorbate oxidation, indoleacetic acid oxidation, lignification, phenol oxidation, pathogen defense, cell wall elongation, etc. are found to be associated with the activity of large family of important plant enzymes; the peroxidases (46, 47). APX scavenges H_2O_2 and uses ascorbate as an electron donor in plants. The increase in APX activity in leaves may probably a response to the enhanced production of ROS, and particularly H_2O_2 under WD stress. Local as well as systemic defense responses are conveyed by salicylic acid through a regulatory signal mediating plant response to WD stress (48) and an osmotic stress (49). Salicylic acid improved antioxidant system necessary to reduce oxidative damage and ion leakage from membranes and ameliorates the impact of abiotic stress (50). Action of salicylic acid is closely associated with the generation of various ROS (51, 52). Treatment of wheat seedlings with salicylic acid caused a transitory enhancement of $O_2^{\cdot}$ and H_2O_2 production by plants and simultaneous increase in the activity of SOD whereas the enhancement of the generation of H_2O_2 in salicylic acid treated seedlings was accompanied by the activation of peroxidase (53). Exogenous application of ascorbic acid in plants resulted in increased resistance to salt stress through reduced oxidative stresses (54). It is a primary substrate in the cy-clical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen (55). Glutamic acid is the common precursor of ³-amino butyric acid (GABA) and proline. Proline and GABA are frequently accumulated in stressed plants and several protective roles have been assigned to these molecules. At 50 DAT, foliar application of glutamic acid did not affect the proline content in plants under stress which indicated that GABA biosynthesis may be the dominant pathway from glutamic acid metabolism under water stress in *Andrographis*. Such dominant pathway had already been demonstrated in tobacco leaves and GABA had been proved as an effective osmolyte to reduce the production of reactive oxygen species under water stress by (6).

Chlorophyll fluorescence yield: There was non-significant variation in maximum quantum yield of PSII (F_v/F_m) across the treatments (Table 5).The response of signal molecules was also not effective for *Fv/Fm* at 50 DAT under WD stress. But at 80 DAT, the *Fv/Fm* reduced from 0.836 in control to less than 0.792 in WD plants with no positive effects of any signal molecules under WD stress. The actual efficiency of photosynthesis (F_v'/F_m') reduced from 0.573 in control to less than 0.551 in WD plants. Under WD condition highest F_v'/F_m' was reported with ascorbic acid application at 50 DAT but none of the signal molecules had prevented WD stress induced reduction in F_v'/F_m' at 80 DAT. Proportion of absorbed energy utilized for photochemistry also known as the quantum yield of PSII (ϕ_{PSII}) reduced at 80 DAT as compared to 50 DAT. Minimum reduction in ϕ_{PSII} under WD stress was due to ascorbic acid spray at this stage whereas at 80 DAT none of the signal molecules helped enhanced the ϕ_{PSII}. The non-photochemical quenching (*NPQ*) increased from 0.71 at 50 DAT to 1.41 at 80 DAT. WD stress enhanced the *NPQ* at both the growth stages. At 80 DAT, the highest *NPQ* was due to salicylic acid application (Table 5). The open PS II centers (qP) were highest under well watered condition followed by ascorbic acid treatment under WD stress at 50 DAT. At later stage, the all tested signaling molecules gave positive results for maintaining high qP in order of salicylic acid > glutamic acid > ascorbic acid under water stress.

The chlorophyll fluorescence can be of immense use in defining the physiological fitness under varied environmental conditions (56). Chlorophyll fluorescence study was used to evaluate photosynthetic efficiencies of various crops during dry season (57). Recently, chlorophyll fluorescence parameters were studied in peanut genotypes with different irrigation regimes during summer season and found to be significantly varied due to different irrigation regimes (58). Recombination of the charge separated state between P_{680} and its primary electron accepter the pheophytin creates triplet reaction center chlorophyll (Chl; $3P_{680}$) through which singlet oxygen are produced which are responsible for the damage to the D1 protein and inactivation of the PS II (59). An efficient drainage of electrons from the primary quinone acceptor QA to an exogenous electron acceptor, cytochrome c can alleviate this acceptor-side damage (60). and such a warranted rapid electron transfer from

Table 5: Effect of signal molecules application on maximum efficiency of photosynthesis *(Fv/Fm)*, actual efficiency of photosynthesis (*Fv'/Fm'*), quantum yield of PSII ($Ö_{PSII}$), non photochemical quenching (*NPQ*) and open PS II centers (*qP*) in *Andrographis paniculata* under water deficit stress

Treatment combination	*Fv/Fm*		*Fv'/Fm'*		PSII		*NPQ*		*qP*	
	50 DAT	80 DAT	50 DAT	80 DAT	50 DAT	80 DAT	50 DAT	80 DAT	50 DAT	80
Well watered	0.807 ±0.009a	0.836 ±0.015a	0.573 ±0.003a	0.545 ±0.020a	0.428 ±0.145a	0.309 ±0.070a	0.634 ±0.007c	0.86 ±0.067d	0.746 ±0.250a	0.5 ±0.(
WD	0.803 ±0.003a	0.786 ±0.011b	0.497 ±0.005d	0.370 ±0.070b	0.199 ±0.041c	0.104 ±0.006b	0.851 ±0.051a	1.49 ±0.0207ab	0.401 ±0.084b	0.3 ±0.(
WD*+Ascorbic acid @200 ppm**	0.802 ±0.004a	0.792 ±0.006b	0.551 ±0.011b	0.421 ±0.039b	0.316 ±0.020b	0.164 ±0.042b	0.616 ±0.044c	1.30 ±0.158bc	0.573 ±0.025ab	0.4 ±0.(
WD*+ Gutamic acid @150 ppm**	0.808 ±0.005a	0.775 ±0.017b	0.527 ±0.002c	0.374 ±0.080b	0.228 ±0.015bc	0.126 ±0.013b	0.692 ±0.013b	1.18 ±0.194c	0.433 ±0.028b	0.3 ±0.(
WD*+ Salicylic acid @200 ppm **	0.802 ±0.005a	0.790 ±0.005b	0.522 ±0.017c	0.362 ±0.077b	0.233 ±0.036bc	0.150 ±0.060b	0.680 ±0.033b	1.68 ±0.235a	0.445 ±0.053b	0.4 ±0.(
Mean	0.804	0.786	0.524	0.382	0.244	0.136	0.710	1.41	0.520	0.4
SEM	0.0027	0.0058	0.0047	0.0301	0.0351	0.0227	0.017	0.091	0.0607	0.0
LSD $_{(0.05)}$	NS	0.0175	0.014	0.0932	0.049	0.0686	0.052	0.274	0.182	0.0
CV	0.67	1.46	1.78	14.9	25.0	26.8	5.0	14.0	23.0	14

Mean values ± standard deviation

*Water deficit stress (WD) imposed from 20 days after transplanting (DAT) **Foliar application at 10, 25, 40 and 55 days after WD st

the acceptor side of PSII can prevent photo-inhibition (61). Ascorbic acid affected some nutritional cycle activities in higher plants and played an important role in the electron transport system (62). In vitro data strongly suggested that a continuous electron flow to PSII from alternative donors, such as diphenylcarbazide (DPC) or ascorbate can alleviate the photo-inactivation of the reaction centers (63). The study indicated that 30 days long water stress did not cause photo-inhibitory action in *A. paniculata*. Further, high $!_{PSII}$ and *Fv'/Fm* due to the application of ascorbic acid even under WD stress seems to be because of the provision of a better alternative electron acceptor whereas increase in *NPQ* due to salicylic acid application indicated enhancement of efficiency to dissipate heat through *NPQ*. The *qP* showed the measure of efficient transport of electrons to the sink. It was highest under well watered condition which was decreased to the lowest in water stress at both the stages. High *qP* due to signaling molecules indicated their role in efficient drainage of electrons and making the PS II centers open ready to accept the electrons from the light harvesting complexes.

The WD stress during 20 and 50 DAT did not improve the andrographolide content in leaves of *A. paniculata* however a prolonged WD stress period from 20 to 80 DAT hastened andrographolide content in leaves in polyhouse condition. Application of tested signaling molecules improved photosynthetic pigments production and ROS scavenging enzymes system ther increased WD stress tolerance. The foliar applicatio salicylic acid under prolonged WD stress indicatec scope to augment the quality of *Andrographis panicul*

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