

PGR-Mediated Metabolic and Oxidative Stress Responses in *Simarouba glauca* under Water Deficit Conditions

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Abstract

Simarouba glauca DC is known for its varied range of bioactive substances and its ability for resilience under abiotic stress circumstances, especially drought, which is still a major limit to crop yield. Understanding the physiological and biochemical mechanisms underlying drought tolerance in *S. glauca* can provide valuable insights into its adaptive strategies and inform broader applications in stress biology and crop improvement. The present study investigated the role of plant growth regulators (PGRs) including salicylic acid (SA), γ -aminobutyric acid (GABA), abscisic acid (ABA), and putrescine—in modulating enzymatic and non-enzymatic antioxidant defense systems under water stress. Enzyme activities (invertase, dehydrogenase, ATPase, acid phosphatase, and nitrate reductase) were assayed alongside oxidative stress markers and antioxidant capacities (DPPH, FRAP, H_2O_2 scavenging, hydroxyl radical scavenging). Key findings shown that SA and GABA substantially boosted the activities of invertase and dehydrogenase during water stress, while PGR application restored ATPase activity that had previously decreased under stress. Under stress, acid phosphatase activity elevated and was further boosted by GABA and SA. Under stress, antioxidant activities as determined by DPPH, FRAP, and TAC were increased, and

PGR treatments further increased these levels. The effects of SA, ABA, GABA, and putrescine were especially potent. These results are consistent with mounting evidence that PGRs are essential for improving drought tolerance through the regulation of antioxidant defense mechanisms. Few studies have investigated perennial, oilseed-bearing trees like *S. glauca*, despite the fact that numerous studies have documented the function of PGRs in annual crops. By showing how PGRs alter both enzymatic and non-enzymatic antioxidant pathways in a woody perennial species during drought, this study closes that gap. The findings show possible directions for creating PGR-based treatments to increase stress tolerance in *S. glauca* as well as other perennial crops that are significant to the economy.

Keywords: *Simarouba glauca*, non-enzymatic antioxidants, plant growth regulators, metabolic changes, water stress

Introduction

Simarouba glauca, a member of the *Simaroubaceae* family, is an evergreen edible oil tree. With a pan-tropical range, this family counts thirty-two genera and more than 170 species of trees and plants (1). The *Simaroubaceae* family is Known for its wide range of bioactive chemicals with great research poten-

tial, the *Simaroubaceae* family (3). *S. glauca* itself is valued not only for its rich unsaturated fatty acid high-quality edible oil, but also for its medicinally significant secondary metabolites including quassinoids, flavonoids, and others with antibacterial, anticancer, and antimalarial characteristics, terpenoids. Little is known about its adaptive systems to drought stress notwithstanding its pharmaceutical and commercial value.

One of the most significant environmental elements influencing plant growth and development and constraining plant production is drought. Decreased water availability instantly impacts water status and inhibits plant development by means of harmful effects on water absorption, photosynthesis, and the transport of water and solutes to growing organs like fruit. By changing their cellular metabolism and activating several defensive mechanisms, plants can react and adapt to water stress (7). Drought causes higher electron leakage toward O_2 during photosynthesis and respiration processes causes oxidative stress in the plant cell, therefore increasing reactive oxygen species (ROS) creation (2). Although ROS are essential for typical plant development and play significant functions in signal transduction, they can also trigger cell damage. Therefore, maintaining oxidative equilibrium is essential for plant stress adaptation. Secondary metabolites, enzymatic as well as non-enzymatic antioxidants have a significant impact on plant development and growth under such difficult circumstances.

Plant growth regulators (PGRs), also known as plant hormones are chemical messengers that control most cellular and physiological activities including cell division, cell expansion, flowering, fruit ripening, seed dormancy, bud dormancy, seed germination, and leaf abscission (28). A combination of two or more hormones often orchestrates plant responses, especially under abiotic stresses. ABA, SA, putrescine (a polyamine), and GABA have individually been implicated in drought tolerance by modulating ROS metabolism, osmotic adjust-

ment, and stress signalling, yet their combined comparative influence on perennial oil-yielding trees like *S. glauca* remains poorly understood.

Antioxidants derived from plants, such as carotenes, phenolic acids, vitamin C, and vitamin E, have been shown to lower the risk of disease. The first method for assessing antioxidant capacity of compounds, extracts, or other biological sources is the DPPH free radical scavenging assay (21). Foods, some beverages, and plant extracts are increasingly acknowledged as important nutritional antioxidant sources that favourably affect human health and the aging process (12). Herbs and spices have been connected to defense against cardiovascular disorders, cancer, inflammation, and atherosclerosis (32). Numerous substances, including vitamins, terpenoids, polyphenols, and flavonoids, contribute to these protective effects (33).

While antioxidant regulation by PGRs has been reported in annual crops, there is a paucity of information on perennial, medicinally important oilseed trees like *S. glauca*. Given its dual significance as a source of edible oil and bioactive medicinal compounds, understanding its antioxidant defense under drought stress is both economically and pharmacologically relevant. This study uniquely evaluates the effect of foliar applications of ABA, SA, putrescine, and GABA individually and comparatively on enzymatic and non-enzymatic antioxidant responses in *S. glauca* under water stress. By bridging the gap between PGR-mediated stress adaptation and the antioxidant potential of this underexplored species, the work provides novel insights into strategies for enhancing abiotic stress resilience in perennial oilseed crops.

Particularly under drought, PGRs are instrumental in modifying plant responses under abiotic stressors. Exogenous use of PGRs has been shown to increase drought tolerance by controlling physiological and biochemical processes like osmotic adaptation, antioxidant protection, and stress-related gene expression.

Recent studies emphasize the integrative capability of PGRs as well as microbial treatments to enhance drought resilience in crops (25,36). The complex interactions among different classes of PGRs, including auxins, cytokinins, gibberellins, abscisic acid, and ethylene, are central to mitigating water deficit effects and maintaining growth and productivity under stress conditions (30).

Simarouba glauca seeds are rich in oil, with reported yields ranging from 32–34% using solvent extraction and up to 52% via cold pressing, while kernels ($\approx 40\%$ of seed) contain 55–65% oil, highlighting the species' high oil potential (19). Fatty acid composition is influenced by presowing PGR treatments: cysteine, salicylic acid, and methionine increase saturated fatty acids, 6-benzylaminopurine elevates mono-unsaturated fatty acids, and gibberellic acid or chlormequat chloride enhances polyunsaturated fatty acids such as linolenic acid (38). These shifts likely result from modulation of desaturase and elongate enzyme activities, affecting chain elongation and desaturation during lipid biosynthesis.

The predominance of oleic and linoleic acids contributes both to nutritional value and industrial utility, while saturated fatty acids improve stability and shelf life. Variability in oil yield and fatty acid composition underscores the importance of optimizing cultivation practices, PGR treatments, and extraction methods to maximize oil quality and quantity. Such integrative approaches position *S. glauca* as a sustainable source for nutritional oils and bio industrial applications. Previous work by Awate and Gaikwad (39) demonstrated that foliar-applied PGRs significantly increased secondary metabolite content including polyphenols, flavonoids, tannins, and alkaloids in *S. glauca* under water stress. Similar to these findings, SA, GABA, and ABA treatments were particularly effective in elevating these metabolites, suggesting that PGR-mediated biochemical modulation is a conserved response in this species. These metabolites likely contribute to enhanced antioxi-

dant capacity, osmotic adjustment, and overall stress mitigation, thereby reinforcing the medicinal and nutraceutical potential of *S. glauca*.

Simarouba glauca DC. Often referred as paradise tree, is a tropical evergreen genus of the *Simaroubaceae* family that has been traditionally utilized to cure several of illnesses including malaria, cancer, and gastrointestinal disorders. The pharmacological potential of *S. glauca* is attributed to its varied phytochemical make-up, which includes quassinoids, alkaloids, flavonoids, and phenolic compounds, which show a broad spectrum of biological processes including anticancer, antimicrobial, anti-inflammatory, antioxidant, and hepatoprotective action (10,18,20). Using sophisticated analytical methods including HPLC and LC-MS, recent research have further defined these bioactive chemicals and confirmed their therapeutic value and drug potential development (20). These findings highlight *S. glauca* as a promising candidate for pharmacological applications and justify further investigation into its bioactive constituents and mechanisms of action.

Materials and Methods

One-year-old seedlings of *Simarouba glauca* were transplanted singly into 5 L earthen pots (18–20 cm diameter, 18–20 cm height) containing a homogenized potting mixture of garden soil, river sand and well-decomposed farmyard manure (2:1:1, v/v/v). Pots were provided with drainage holes and placed on raised benches under a naturally ventilated polyhouse at Shivaji University, Kolhapur. Environmental conditions averaged 25 ± 3 °C temperature and $65 \pm 5\%$ relative humidity, with natural photoperiod (11–13 h). Plants were acclimated for one month under these conditions. No additional fertilizers were applied during the experiment to avoid confounding effects on drought stress responses.

The experiment was conducted in a completely randomized design with six biological replicates per treatment ($n=6$ plants). For biochemical assays, each replicate was one

independent plant; assays were performed in triplicate (technical replicates) and averaged.

Water stress was imposed by withholding irrigation for fixed durations of 4, 8, 12 or 16 days. Separate groups of plants were used for each stress duration; plants were not measured sequentially. Control plants were irrigated every two days to maintain soil moisture at approximately 70–75% of field capacity (FC). Soil moisture was monitored gravimetrically by determining pot weights relative to dry weight and field capacity; irrigation volumes (≈ 400 –500 mL per pot) were adjusted to restore control pots to 70–75% FC.

Prior to stress treatments, foliar applications of salicylic acid (SA, 50 mg L⁻¹), abscisic acid (ABA, 10 mg L⁻¹), putrescine (10 mg L⁻¹) and γ -aminobutyric acid (GABA, 10 mg L⁻¹) were administered. All compounds were of analytical grade (Sigma-Aldrich, USA). Solutions were prepared in distilled water; ABA stocks contained $\leq 0.1\%$ ethanol for solubility. A non-ionic surfactant (Tween-20, 0.05% v/v) was added to improve wetting. Each plant received 10 mL of spray solution applied uniformly until leaf surfaces were wetted but without runoff. Sprays were applied once, 24 h prior to the initiation of water withholding for each treatment group.

At the end of each stress period (day 4, 8, 12 or 16), fully expanded young leaves were sampled between 09:00–11:00 h, immediately frozen in liquid nitrogen and stored at -80°C until analysis. Enzymatic antioxidants (superoxide dismutase [SOD], catalase [CAT], ascorbate peroxidase [APX], peroxidase [POD]) and non-enzymatic antioxidants (ascorbate, glutathione, proline, phenolics) were quantified using standard protocols. Data are expressed as mean \pm standard error (SE $n = 6$). Statistical significance among treatments was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test ($p < 0.05$).

Enzymatic changes

1. Invertase, E.C. 3.2.1.26

A slightly modified version of Jaborova methodology (11) was used to investigate invertase activity. 500mg of leaves derived from unstressed control, stressed control, and PGR-treated stressed plants were homogenized in 10 mL of cold 0.1 M phosphate buffer (pH-7.5), the obtained solution was filtered. The filtrate was centrifuged at 10,000 rpm for 20 min and supernatant was taken. To that, 4 mL of 1% sucrose solution, 1 mL of phosphate buffer were added and then allowed to react. The mixture was boiled and 2 mL of Dinitro salicylic acid reagent was added to it and absorbance was measured at 530 nm. The soluble proteins in the enzyme extract were determined using the Lowry method (26).

2. Dehydrogenase (E.C. 1.1.1.4.)

Dehydrogenase activity measured by triphenyl tetrazolium chloride (TTC) reduction. Using tetrazolium method, (8) the action of the broad-spectrum enzyme dehydrogenase was studied using 500 mg of fresh leaves from stressed, unstressed, and PGR-treated stressed. Plants were cut into little pieces, incubated for one hour in a dark environment with 4 mL of 0.2% T.T.C. (2-3-5 triphenyl tetrazolium chloride). Later on, the cultured tissue was rinsed two to three times with distilled water, then the surface was patted dry and treated with 5 mL of methoxy ethanol to extract the red formazon that results from the activity of dehydrogenase. Furthermore, the optical density of colored formazon was measured at 470 nm.

3. ATPase (E.C.3.6.1.3)

Enzyme was extracted from 0.5 g fresh leaves of stressed control, stressed control, PGR-sprayed leaves; they were homogenized in 10 mL ice-cold 0.1 M Tris-HCl buffer (pH-8.0) with 1 M KCl, 0.01 M EDTA, and 0.4 mL 0.2 M β -mercaptoethanol. The homogenate was filtered and centrifuged at 10,000 rpm for 20 minutes. The supernatant was added to 0.5 mL of 0.01 M CaCl₂ and 0.5 mL 0.003 M ATP.

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The reaction was carried out at 38°C for 1 h and stopped by adding 1 mL of 0.1 M NaOH. The liberated inorganic phosphorus was estimated by the method of Fiske and Subba Rao (14,37).

4. Acid phosphate (E.C. 3.1.3.2)

Acid phosphatase activity was measured using the Besford approach (6). Fresh leaves weighing 0.5 g each of unstressed, stressed, and PGR-sprayed controls were homogenized in 10 mL of ice-cold water. The resulting homogenate was centrifuged for 20 minutes at 10,000 rpm. Solution containing 3mL of p-nitrophenyl phosphate, 1 mL of enzyme and 2 mL of pH-5 acetate buffer was kept at room temperature for 30 min. Further, 1.5 mL of 1 N NaOH was added to a solution to stop the reaction. The assay medium and the enzyme were added to another test tube in a similar manner, and the reaction was seen right away (0 min) immediately terminated. The optical density of the developed pale-yellow color complex was read at 420 nm.

Non-enzymatic antioxidant assays

Methods for methanolic extracts

Fresh roots, stems, and leaves from control, stressed, and PGR-treated stressed plants were collected separately, washed thoroughly, shade-dried, and powdered. For extraction, 10 g of powdered material was macerated in 100 mL of 80% methanol (plant material: solvent ratio 1:10, w/v) and incubated at room temperature ($25 \pm 2^\circ\text{C}$) with occasional shaking for 48 h. Extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40°C using a rotary evaporator. The residues were dried to constant weight, re-dissolved in 80% methanol to obtain a stock solution (10 mg/mL), and stored at -20°C . Working concentrations were freshly prepared by dilution with methanol prior to assays. All assays were conducted in triplicate for each biological replicate. Antioxidant activity was expressed as % radical scavenging or reducing activity, and IC_{50} values (concentration required

to scavenge 50% of radicals) were calculated from dose-response curves plotted with extract concentrations ranging from 20–200 $\mu\text{g/mL}$, unless otherwise specified.

DPPH radical scavenging test

The free radical scavenging activity was assessed according to Koleva (23) with modifications. Equal volumes of DPPH solution (100 μM in methanol) and plant extract (20–200 $\mu\text{g/mL}$) were mixed and incubated at room temperature for 30 min in the dark. Absorbance was measured at 516 nm against methanol blank using a double-beam UV-Vis Spectrophotometer. L-ascorbic acid (100 $\mu\text{g/mL}$) served as standard. The scavenging activity was calculated as:

$$\% \text{ scavenging} = (\text{Ac} - \text{Ae}) / \text{Ac} \times 100$$

Where Ac = absorbance of control (DPPH only), Ae = absorbance with plant extract.

IC_{50} values were determined from regression analysis of scavenging percentage versus concentration.

Ferric reducing antioxidant power (FRAP)

FRAP activity was estimated according to Benzie and Strain (5). The reaction mixture contained acetate buffer (300 mM, pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl_3 in a 10:1:1 ratio. Plant extracts (20–200 $\mu\text{g/mL}$) were added, and absorbance of the Fe^{2+} -TPTZ complex was measured at 593 nm after 10 min incubation at 37°C . Ascorbic acid (200 $\mu\text{g/mL}$) was used as standard. Results were expressed in milligram equivalents of FeSO_4 per milligram. The calibration line was established using the following concentrations of FeSO_4 : 0.0025, 0.005, 0.01, and 0.02 mg/mL.

Results and Discussion

Enzymatic modulations.

Under water stress condition, foliar sprays of abscisic acid (ABA), salicylic acid (SA), putrescine, and gamma-aminobutyric

acid (GABA) differentially modulated invertase activity (μmol sucrose hydrolysed g^{-1} fresh weight h^{-1}) over 4, 8, 12, and 16 days. The stressed control showed low, stable activity (0.018–0.024) (Fig. 1). ABA slightly increased activity (0.022–0.028), with minimal impact. SA significantly enhanced activity, peaking at 0.040 by day 16, supporting osmotic adjustment. Putrescine had a delayed effect, reaching 0.036 by day 16. GABA showed the strongest effect, rising to 0.044 by day 16, likely via the GABA shunt. Statistical differences (a–d, $p < 0.05$) confirm SA and GABA as most effective in boosting sugar metabolism for drought tolerance. Further growth and photosynthetic data are needed to confirm broader impacts.

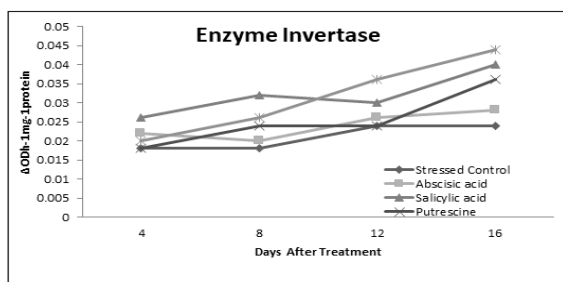


Fig.1. Effect of foliar sprays of ABA, SA, Putrescine and GABA on the activity of enzyme invertase in the leaves of *S. glauca* grown under waterstress

The foliar applications of abscisic acid (ABA), salicylic acid (SA), putrescine, and gamma-aminobutyric acid (GABA) influenced dehydrogenase activity (μmol NADH oxidized g^{-1} fresh weight h^{-1}) over 4, 8, 12, and 16 days. The stressed control exhibited a decrease in activity (0.471 to 0.412). ABA and putrescine boosted activity initially (0.622 and 0.608 at day 4) but declined by day 16 (0.500 and 0.491). SA and GABA maintained elevated activity, peaking at 0.688 and 0.723 on day 4 and stabilizing at 0.526 and 0.531 by day 16, aiding energy homeostasis. Statistical differences (a–c, $p < 0.05$) confirm SA and GABA as most effective in enhancing drought resilience, though additional physiological data are required for validation (Fig 2).

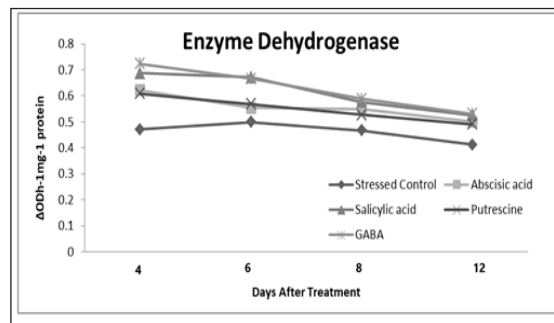


Fig.2. Effect of foliar sprays of ABA, SA, Putrescine and GABA on the activity of enzyme Dehydrogenase in the leaves of *S. glauca* grown under waterstress

The influenced ATPase activity of enzyme ATPase (μmol Pi released g^{-1} fresh weight h^{-1}) was noticed across 4, 8, 12, and 16 days (Fig 3). The stressed control exhibited a steady decrease in activity (0.0148 to 0.0093). ABA elevated activity on days 8 (0.0162) and 12 (0.0156) but fell to 0.0098 by day 16. SA and GABA markedly boosted activity, reaching peaks of 0.0177 and 0.0180 on day 8, and maintaining 0.0126 and 0.0144 by day 16. Putrescine showed a slight increase (0.0150–0.0135) until day 12, dropping to 0.0098 by day 16. Statistical differences (a–b, $p < 0.05$) highlight SA and GABA as most effective in enhancing ion transport and stress resilience, though additional physiological data are required for validation.

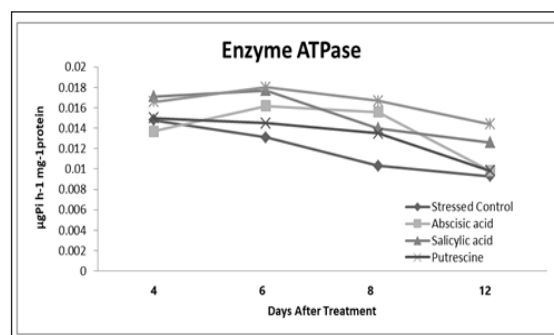


Fig.3. Effect of foliar sprays of ABA, SA, Putrescine and GABA on the activity of enzyme ATPase in the leaves of *S. glauca* grown under waterstress

Acid phosphatase (ACP) activity ($\mu\text{mol p-nitrophenol released g}^{-1}$ fresh weight h^{-1}) in leaves of *Simarouba glauca* under water stress was affected by foliar treatments of putrescine, gamma-aminobutyric acid (GABA), salicylic acid (SA), and abscisic acid (ABA) during 4, 8, 12, and 16 days (Fig. 4). A baseline stress response for phosphorus remobilization was seen in the progressive rise in activity (0.113 to 0.301) in the stressed control. In support of metabolic changes, ABA and SA markedly increased activity, peaking at 0.367 and 0.324 by days 16 and 12, respectively. Early effects of putrescine were negligible (0.118–0.286), but by day 16, they had increased to 0.333. The greatest effect was shown by GABA, which peaked at 0.399 on day 16 and showed a significant phosphorus turnover.

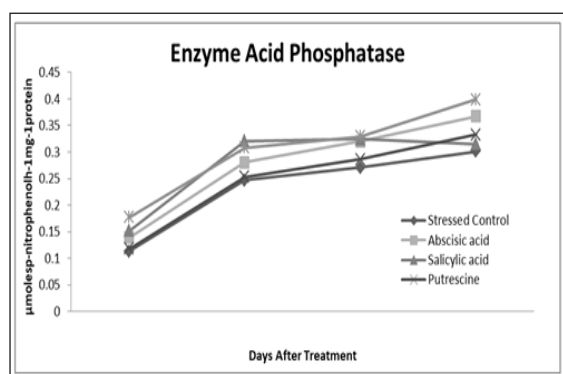


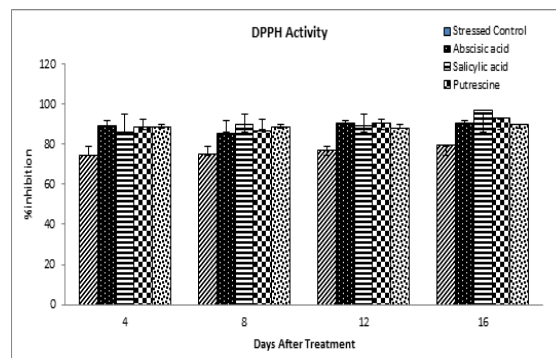
Fig.4. Effect of foliar sprays of ABA, SA, Putrescine and GABA on the activity of enzyme Acid Phosphatase in the leaves of *S. glauca* grown under waterstress

Non-enzymatic antioxidants

DPPH radical scavenging activity

Foliar treatments of putrescine, gamma-aminobutyric acid (GABA), salicylic acid (SA), and abscisic acid (ABA) increased the DPPH radical scavenging activity (% inhibition) in leaves of *Simarouba glauca* under water stress during 4, 8, 12, and 16 days. Antioxidant activity in the stressed control increased somewhat (74.56–79.5%), suggesting a baseline reaction to oxidative stress (Fig 5). Activity was

markedly increased by ABA, SA, putrescine, and GABA; ABA peaked at 90.74% on day 12, SA peaked at 97.24% on day 16, putrescine reached 93% by day 16, while GABA remained constant at 88–89.91%. SA is most effective at increasing antioxidant capacity, followed by



ABA and putrescine, which probably helps with ROS detoxification and drought tolerance, according to statistical differences (a–d, $p < 0.05$).

Fig. 5. Effect of foliar sprays of ABA, SA, Putrescine and GABA on DPPH radical scavenging activity of the leaves of *S. glauca* grown under waterstress

Ferric reducing antioxidant power (FRAP) activity ($\mu\text{mol Fe}^{2+} \text{g}^{-1}$ fresh weight) in leaves of *Simarouba glauca* under water stress was affected by foliar sprays of putrescine, gamma-aminobutyric acid (GABA), salicylic acid (SA), and abscisic acid (ABA) over 4, 8, 12, and 16 days (Fig. 6). The baseline antioxidant response was shown by the small increase in FRAP activity (1.05–1.31) in the stressed control. Activity increased steadily with ABA (1.31–1.45), reaching its maximum on day 16. On day 12, putrescine dramatically raised activity to 1.52, while SA climbed somewhat (1.08–1.24) before dropping to 1.09 on day 16. GABA's impact was negligible, staying near control values (1.05–1.17). The statistical differences (a–d, $p < 0.05$) show that putrescine and ABA are the most effective in increasing antioxidant capacity, which probably helps with ROS detoxification under drought.

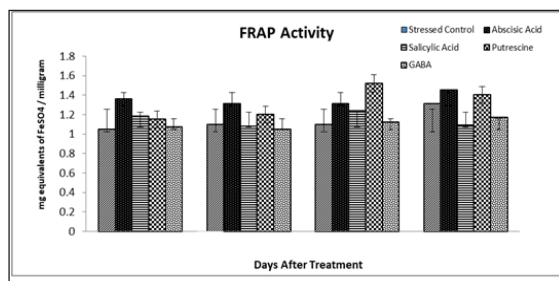


Fig. 6. Effect of foliar sprays of ABA, SA, Putrescine and GABA on FRAP radical scavenging activity of the leaves of *S. glauca* grown under water stress.

Discussion.

Saccharide hydrolysis into glucose and fructose is facilitated by invertase, which can act as osmolytes and signal molecules during drought conditions (34). SA modulates carbohydrate metabolism by controlling sugar transporters and vacuolar invertases, which supports osmotic adjustment and ROS detoxification, as evidenced by recent studies (35). GABA, in turn, influences carbon flux through the GABA shunt, linking sugar metabolism to energy supply under drought stress. The enhanced invertase activity observed here is consistent with the role of PGRs in stabilizing energy supply and osmolyte balance during stress, although further work integrating growth and photosynthetic measurements is needed. (22).

Dehydrogenases are central to redox balance and energy metabolism (glycolysis, TCA cycle). Exogenous SA has been shown to sustain mitochondrial activity and antioxidant enzyme expression under drought. Similarly, GABA enhances NADH/NAD⁺ homeostasis through its catabolism, thereby supporting dehydrogenase activity (16).

Ion gradients, cytoplasmic pH, and nutrients uptake during stress are maintained by plasma membrane H⁺-ATPases, which are significant (15,17). Cucumber and grapevine have been shown to exhibit enhanced nutrient transport and ROS homeostasis through H⁺-ATP activity promoted by SA (36). GABA may aid in

maintaining membrane stability and producing ATP during drought conditions. Potentially improved stress signalling and ion transport are suggested by increased activity of the ATPase under PGR treatments (8).

In response to stress, phosphorus is mobilized and altered through the action of Phosphatases (4,17). Similar increases in phosphatase activity have been observed in other species under treatments such as the AABA-SA complex (31). An adaptive response that facilitates phosphorus turnover and maintains the appropriate energy balance during drought may be represented by an increase in ACP activity here.

DPPH scavenging activity increased under drought, with further enhancement by PGR treatments, especially in roots. This suggests improved hydrogen-donating capacity of metabolites. SA and ABA are known to upregulate phenolic biosynthesis, contributing to DPPH activity (22,31). GABA treatment has also been associated with elevated phenolic and flavonoid accumulation under drought (16). FRAP activity was elevated in water-stressed tissues and further enhanced by PGR application, indicating stronger reducing power. This is consistent with studies showing SA and ABA increase ferric-reducing antioxidant capacity through modulation of ascorbate-glutathione cycling (13).

Conclusion

Overall, metabolic and antioxidant stress responses in *S. glauca* indicate that SA and GABA were particularly effective in enhancing drought-associated biochemical adjustments. These responses likely contribute to improved redox homeostasis and nutrient transport. However, since physiological traits (e.g., relative water content, photosynthesis, biomass) were not measured, claims of "enhanced stress tolerance" must be interpreted with caution. Future studies integrating biochemical, physiological, and yield-related traits are recommended to establish a direct link between PGR-mediated biochemical changes and functional drought tolerance in *S. glauca*.

PGR-mediated metabolic and oxidative stress responses in *Simarouba Glauca* under water deficit conditions

The study reveals the important effects of many plant growth regulators (PGRs) on the enzymatic and non-enzymatic antioxidant activities in *Simarouba glauca* under water stress, including ABA, SA, putrescine, and GABA. PGR treatments resulted in increased activity of key enzymes like acid phosphatase, dehydrogenase, ATPase, and invertase, indicating better metabolic and stress tolerance mechanisms in the plant. Furthermore, there was a notable increase in non-enzymatic antioxidant activities, suggesting a heightened capacity to reduce oxidative damage. These results indicate that PGRs may improve the medicinal value of *S. glauca* by encouraging the production of bioactive compounds with potential therapeutic uses, in addition to facilitating the species adapt to water stress.

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Conflicts of interests

No conflicts of interest have been identified by the author(s) concerning the research, authorship, and/or publication of this article.

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