Asian Journal of Advances in Research

Volume 7, Issue 1, Page 444-451, 2024; Article no.AJOAIR.3960

Response of Tobacco (*Nicotiana tabacum* L.) Plants to Foliar Application of Brassinosteroids (BRs) under Conditions of Drought-induced Oxidative Stress

Ahmed Soufi [a*]

[a] *Department of Field Crops, Faculty of Agriculture, Tishreen University, Lattakia, Syria.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3960

Original Research Article

Received: 23/06/2024
Accepted: 26/08/2024
Published: 10/09/2024

ABSTRACT

In this work, we studied the effect of treatment with Brassinosteroid at concentrations (0.01, 0.05 and 0.1 mM) on some biochemical and production characteristics of tobacco plants under conditions of applied drought stress (15%, 30% and 45%).

Chlorophyll content in leaves decreased under conditions of drought stress, and H_2O_2, proline, MDA and Protein increased steadily with increasing applied stress, while Chlorophyll content in leaves increased when sprayed with Brassinosteroid, especially at low concentrations (0.01 mM).

The treatment with the reduced concentration of Brassinosteroid and the applied stress outperformed all treatments and the control for all indicators studied. Therefore, it is recommended to use Brassinosteroid, especially at a concentration of 0.01 mM, on tobacco plants because of its role in improving chemical traits under conditions of drought stress.

**Corresponding author: Email: 7mada.movo9@gmail.com;*

Cite as: *Soufi, Ahmed. 2024. "Response of Tobacco (Nicotiana Tabacum L.) Plants to Foliar Application of Brassinosteroids (BRs) under Conditions of Drought-Induced Oxidative Stress". Asian Journal of Advances in Research 7 (1):444-51. https://jasianresearch.com/index.php/AJOAIR/article/view/473.*

Keywords: Tobacco; brassinosteroid; drought stress; oxidative stress.

1. INTRODUCTION

The tobacco plant is one of the model plants for scientific studies due to its ease of handling and its many varieties with high commercial value [1].

"Drought stress is one of the most serious abiotic stresses and negatively affects crop growth and development. Given global climate change, it is important to identify effective methods of alleviating drought stress effects. Brassinosteroids (2,4-epibrassinolide-EBR) play an important role in mitigating the negative effects of drought stress on plants [2]. High concentration with PEG, caused an increase in the malondialdehyde, poly phenols, total soluble sugars, Nicotine Content" [3].

"Plants are vulnerable to many abiotic stresses, resulting in reduced plant productivity. Its adaptation to unfavorable environments relies on transmitting external stress signals into internal signaling pathways. A series of stress response mechanisms have been developed. Among them, brassinosteroids (BRs) are a class of steroid hormones that are widely involved in plant growth, development, and stress response" [4].

"Brassinostreoids (BRs) possess a large array of growth and development-regulated functions in plants ranging from morphology to cellular metabolic activity regulation and, at molecular level, change in gene expression as well as modulating the metabolism of nucleic acids and proteins. Because of the number of regulating potentials that BRs have shown in the life cycle of plants, they are considered a sixth class of plant growth regulators (PGRs)" [5].

"Several studies have shown that BR application confers drought tolerance via higher antioxidant enzyme activities such as SOD, POD, and CAT, along with a higher accumulation of proline, and consequently lowered the ROS production and MDA content" [6].

The aim of this study is to determine phytotoxic changes formed in tobacco plant due to application of drought stress and to investigate the effect of exogenous BR_S application on these changes.

2. MATERIALS AND METHODS

The experiment was carried out during the 2024 season. The field experiment was conducted in the village of Kassab - Lattakia- Syria.

Tobacco seeds were grown on an agricultural medium containing compost. The seedlings were transferred to plastic bags (60 ✕ 40) cm forty days after germination.

Growth of plants and experimental design tobacco plants: Plants were grown from seeds in trays of compost until the seeds germinated. After germination, when the second leaf appeared, the seedlings were transferred to plastic pots with a 11-cm diameter containing sand, loam and peat (2:1:1) in a greenhouse. Each seedling was placed in one pot. The seedlings were irrigated with water once a day. At the same time, seedlings were also irrigated with Hoagland's solution (pH 6.7) once a week (on soil media around the root) to prevent mineral deficiency. Then the tobacco plants with 3 fully expanded leaves (about 15 days after growing in the pot), were left to grow in a growth chamber at a day/night temperature of 26/18°C, 16/8 hour (light/dark) photoperiod and 6000 lux light intensity for 5 days. After the adaptation period in the growth chamber, 24-epibrassinolide (Sigma chemicals, USA) dissolved in ethanol was sprayed on the leaves at 0.01, 0.05 and 0.1 mM concentrations for 3 days (Tween20 (0.01%) used as surfactant). Then three levels of water stress (control, 3 days, 5 days and 7 days withholding water) were applied. Four replicates were assigned for each treatment. After treatment, the third leaf of plants was harvested. The harvested leaves were rapidly frozen in liquid nitrogen and stored at -80°C for biochemical analysis [7].

- **Determination of Chlorophyll content in leaves:**

"Determination of total chlorophyll levels 1 g of leaf tissue was homogenized with 50 mL acetone (100%) and then centrifuged. Absorbance values of the samples were measured at 662, 645 and 470nm (Pelkin Elmer/Lambda 25)" [8,9].

- **Determination of H_2O_2 content in leaves:**

"For H_2O_2 estimation, fresh leaves (500 mg) were homogenized with 0.1% (w/v) trichloroacetic acid. The absorbance was recorded at 390 nm, and calculation was done using a H_2O_2 standard" [10].

- **Determination of proline content in leaves:**

Determination of proline and GB contents Proline content was estimated following the method of

Bates et al. [11]. After extraction with sulphosalicylic acid, a known volume of extract was reacted with ninhydrin reagent, and the absorbance was recorded at 520 nm using a spectrophotometer (Beckman 640 D, USA), with toluene as a blank. The method of Grieve and Grattan (1983) was employed for the estimation of GB. Absorbance was recorded at 365 nm using a spectrophotometer and calculations were done using the reference standard of GB (50–200 mg mL−1).

- **Determination of malondialdehyde content in leaves:**

"Determination of MDA content 0.5 g of leaf was homogenized in 5mL of 0.1% mL trichloroacetic acid (TCA) and then centrifuged at 10,000 rpm. 2 mL of this solution and 2 mL of 0.5% thiobarbituric acid (TBA) were boiled in a 95 °C boiling water bath for 30 min (TBA was prepared in 20% TCA). The samples were cooled in an ice-bath. The final mixture was centrifuged at 10,000 rpm for 15 min. We measured the absorbances of the supernatants at 532 and 600 nm. The measurements made at 600nm were deduced from those at 532 nm, and the levels of MDA were calculated with a 155 mM 1cm 1extinction coefficient" [12].

- **Determination of Total Protein Content of Leaves (%):**

Proteins and total nitrogen were estimated by the Kjeldahl method, considering that proteins contain one-sixth of their weight in nitrogen. The protein was digested by long boiling with 98% concentrated sulfuric acid, so that the nitrogen of the amino acids was transformed into ammonium sulphate. After completion of digestion, a distillation process was performed to expel ammonia from the ammonium sulphate. By adding NaOH with heating, where ammonia combines with boric acid to form ammonium borate, ammonium borate titration was performed as a final stage using standard hydrochloric acid and with appropriate evidence to determine the end point of the titration [13].

Statistical analysis: Statistical analysis of the results from experiments with three or more mean values used a one-way analysis of variance (ANOVA) as dictated by the number of main effects, followed by Tukey's HSD post hoc test or Dunnett's HSD. The difference was considered to be statistically significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

Effect of BR_S and drought stress on chlorophyll content in leaves: Data in Fig. 1. indicate that there are significant differences ($P<0.05$) between the studied treatments in terms of the chlorophyll content in leaves.

Drought stress led to a decrease in chlorophyll content in leaves, While treatment with BR_S increased chlorophyll content in leaves compared to the control.

Treatment with BR_S at a concentration of 0.01 mM under drought conditions also outperformed all other parameters and the control.

Piotrowska et al. [14] reported that chlorophyll content of Wolffia arrhiza exposed to stress decreased compared to control; but low concentration exogenous JA treatment increased chlorophyll content.

"A low dose of BR (0.1 µM EBR) facilitates stomatal opening and a high dose of BR (1.0 µM EBR) causes stomatal closure (Xia et al. 2014). Analysis of a number of studies on BR suggests that the responses of plants to BR concentrations are largely dependent on the specific application method, plant species, plant growth stage, and growth conditions" [15].

While drought stress induces excessive ROS accumulation, BR treatment can remarkably reduce the levels of ROS and lipid peroxidation under drought stress [16].

Effect of BR_S and drought stress on H_2O_2 content in leaves: Data in Fig. 2. indicate that there are significant differences ($P<0.05$) between the studied treatments in terms of the H_2O_2 content in leaves.

Drought stress led to an increase in H_2O_2 content in leaves, While treatment with BR_S decreased H_2O_2 content in leaves compared to the control.

Treatment with BR_S under drought conditions at low concentration (0.01 mM) also led to an decrease in the H_2O_2 compared to the remaining treatments and the control.

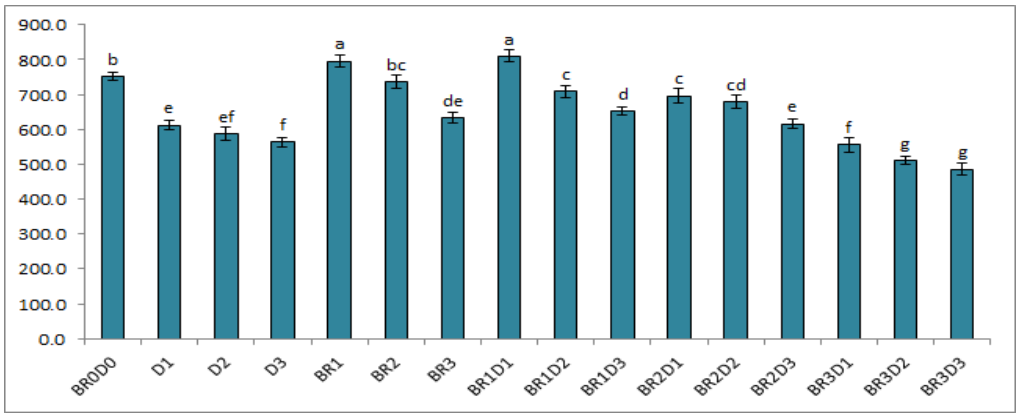

Fig. 1. Effect of BR_S on the chlorophyll content in tobacco leaves under drought stress

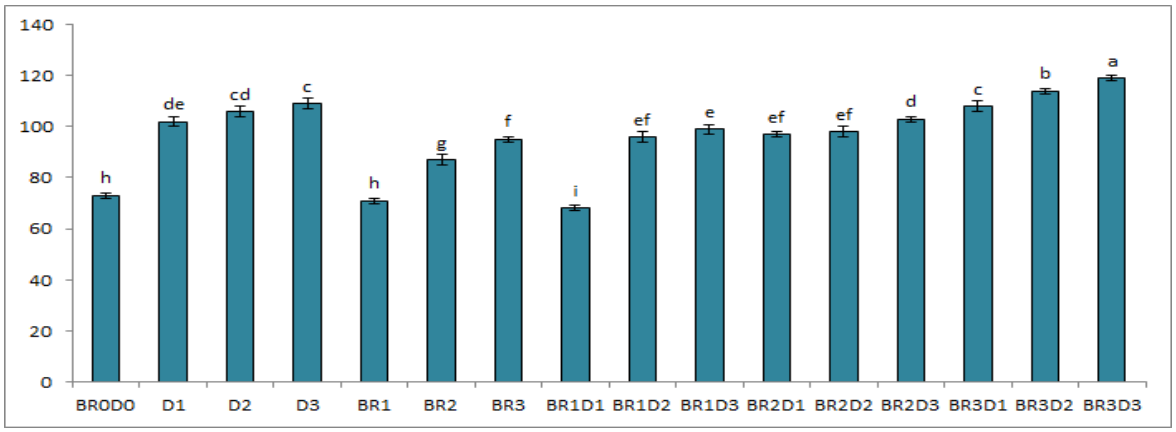

Fig. 2. Effect of BR_S on the H_2O_2 content in tobacco leaves under drought stress

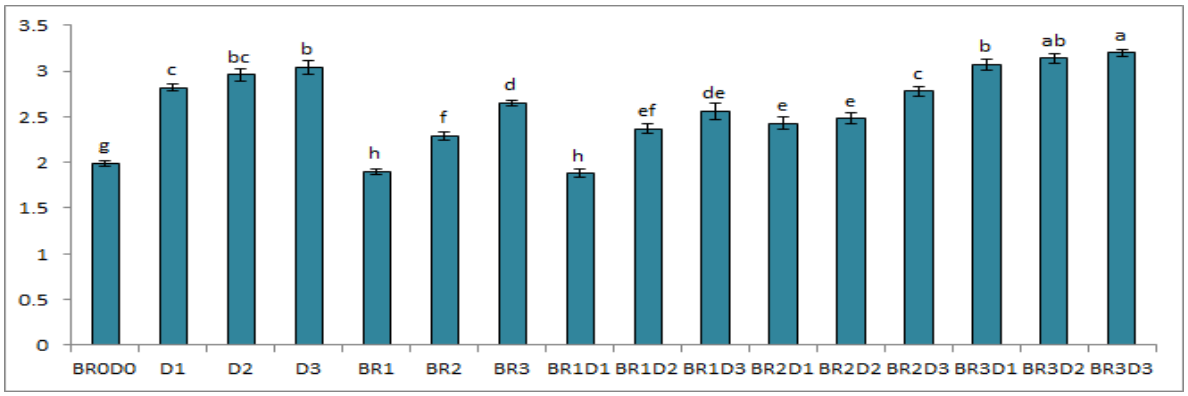

Fig. 3. Effect of BR_S on the proline content in tobacco leaves under drought stress

Drought stress caused an increase in the formation of reactive oxygen species (ROS) that are responsible for various damages to macromolecules [17]. In the present study, the H_2O_2 content increased by increasing the drought stress, Pretreatment with BRs decreased the accumulation of H_2O_2 contents under drought stress.

"BRs have anti-stress effects on plants, helping them to overcome low and high temperature stress, drought and pathogen infection" [18].

Effect of BR_S and drought stress on proline content in leaves: Data in Fig. 3. indicate that there are significant differences ($P<0.05$)

between the studied treatments in terms of the proline content in leaves.

drought stress led to an increase in proline content in leaves, While treatment with BRs decreased proline content in leaves compared to the control.

Treatment with BRs under drought conditions at low concentration (0.01 mM) also led to an decrease in the proline compared to the remaining treatments and the control.

Brassinosteroids (BR) are plant hormones that regulate plant growth and development by modulating and regulating cell division, cell elongation, and differentiation, and help mitigate the harmful effects of abiotic stresses. (Hafeez *et al*., 2021).

Hafeez, M.B.; Zahra, N.; Zahra, K.; Raza, A.; Khan, A.; Shaukat, K.; Khan, S. Brassinosteroids: Molecular and physiological responses in plant growth and abiotic stresses. *Plant Stress* 2021, *2*, 100029.

Effect of BRs and drought stress on malondialdehyde content in leaves: Data in Fig. 4. indicate that there are significant differences (P<0.05) between the studied treatments in terms of the malondialdehyde content in leaves.

drought stress led to an increase in malondialdehyde content in leaves, While treatment with BRs decreased malondialdehyde content in leaves compared to the control.
Treatment with BRs under drought conditions at low concentration also led to an decrease in the malondialdehyde compared to the remaining treatments and the control.

Zhang et al. [19] and Li et al. [20] stated that BR treatment declined MDA content under drought stress in soybean and *Robinia pseudoacacia* plants, respectively .

"DS hinders plant growth and development by decreasing plant biomass and chlorophyll content and increasing levels of reactive oxygen species (ROS) and malondialdehyde (MDA) content" [21]. "Stressful conditions unbalance the equilibrium between ROS production and the antioxidant defense system, leading to overproduction of ROS and causing oxidative damage and, ultimately, cell death" [22].

Effect of BRs and drought stress on Protein Contents in leaves: Data in Fig. 5. indicate that there are significant differences (P<0.05) between the studied treatments in terms of the Protein content in leaves.

drought stress led to an increase in Protein content in leaves, While treatment with BRs decreased Protein content in leaves compared to the control.

Treatment with BRs under drought conditions at low concentration also led to an decrease in the Protein compared to the remaining treatments and the control.

The accumulation of proteins in tobacco leaves is one of the indicators that negatively affect the quality of sporulation and its technological properties. It hinders the ignition of sporulation, and high concentrations of it cause an unpleasant odor when smoked [23,24]. The protein content of the leaves increases with the increase in applied drought stress, as growth processes become slow as a result, nitrogenous substances are transferred to The upper parts of the plant [25].

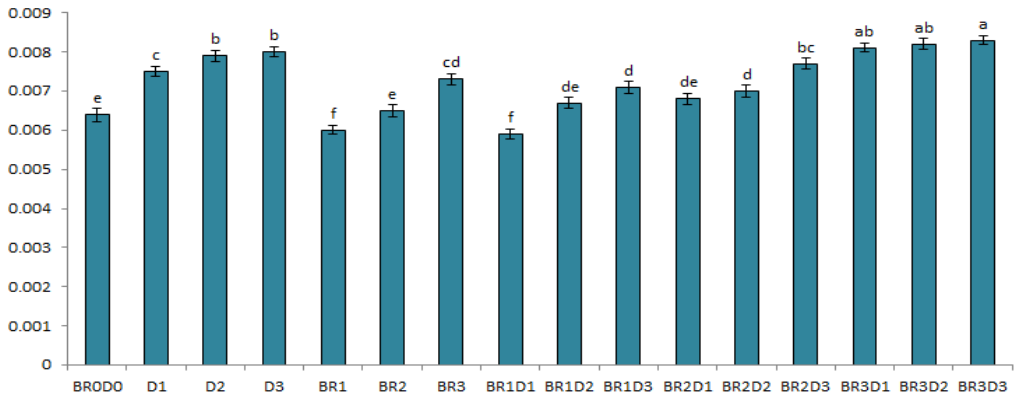

Fig. 4. Effect of BRs on the malondialdehyde content in tobacco leaves under drought stress

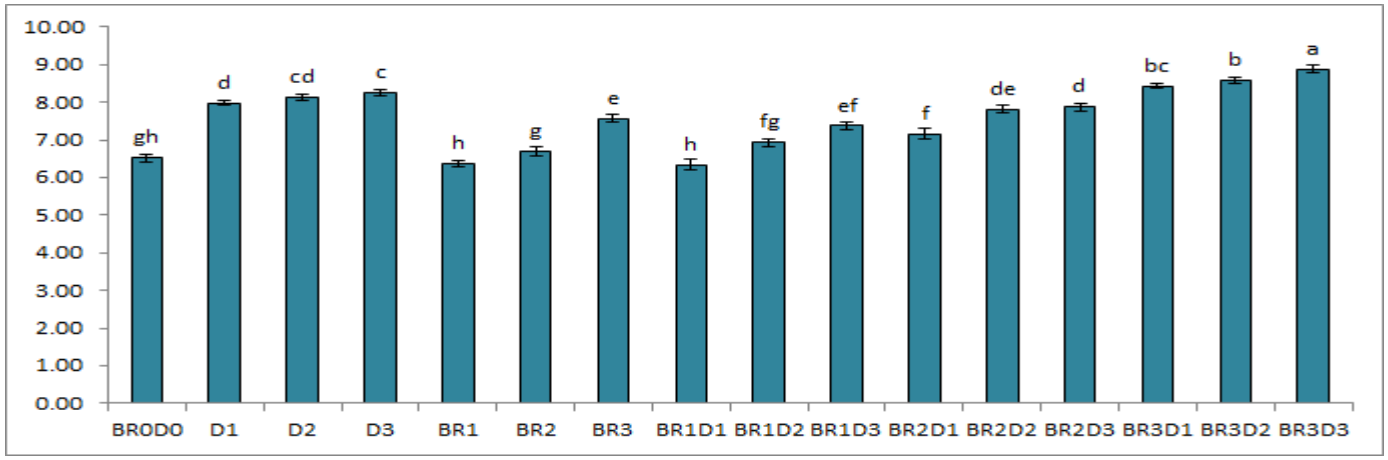

Fig. 5. Effect of BR_S on the Protein content in tobacco leaves under drought stress

"Our results found that EBR application improved antioxidant enzyme activities and scavenged ROS under drought stress" [26].

4. CONCLUSIONS

BRs treatment stimulated antioxidant defenses in stressed plants, including increasing chlorophyll content. In contrast, treatment with brassinolide resulted in decreased MDA, H2O2, and protein content. While drought stress led to negative effects on all traits. The most effective dose of 24-epipressinolide under stress conditions was found to be 0.01 mM. It is recommended to continue the study on other characteristics of the same plant and other varieties and species

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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