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GR24 Application Alleviates Drought Stress in *Eruca sativa* Through Antioxidant Enzyme Activation and Improved Stomatal Conductance

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Abstract

Drought stress is a significant challenge for growth of plants and agricultural productivity, impacting global food security. Strigolactones (SLs) are a class of plant hormones recognized for enhancing tolerance to various abiotic stresses; however, their specific function in mitigating drought stress in *Eruca sativa* (rocket) plant remains unclear. This work investigated the effect of various levels of the synthetic SL analogue GR24 (0, 3 μM, 5 μM and 7 μM) on two rocket plant accessions (Eruca-34961 and Eruca-34936) exposed to drought stress (50% water holding capacity) for four weeks. Drought stress negatively affected growth (45%), chlorophyll content (35%), gas exchange parameters (32%) and relative water content (37%-RWC), while increasing reactive oxygen species (30%-ROS), lipid peroxidation (23%-MDA) and membrane permeability (22%). The application of GR24 mitigated these effects by improving chlorophyll content (64%), photosynthetic efficiency (23%), water status (89%) and accumulation of osmolytes (113%). GR24 also increased levels of proline (8%), phenolics (27%), flavonoids (67%), and ascorbic acid (27%), especially in Eruca-34961, and improved the antioxidant enzyme activities (51%) for ROS detoxification. The optimal level for improving drought tolerance was found to be 5 μM GR24. These results suggest that applying GR24 as a foliar spray may serve as an active strategy to enhance the drought tolerance in rocket plants (*Eruca sariva)* and promote sustainable agricultural practices in regions facing water scarcity.

Keywords: *Eruca sativa;* Oxidative damage; ROS homeostasis; Osmolytes; Photosynthetic efficiency; Secondary metabolism

Introduction

The global increase in average temperatures is leading to more unpredictable environmental conditions and frequent, severe droughts (Sheoran *et al.* 2022). Abiotic stresses like salt stress, metals stress, water scarcity, and heat stress significantly restrict growth of plants and survival in the face of climate change (Chaudhry and Sidhu 2022). Drought poses a significant threat to agricultural productivity, driven by temperature fluctuations, variable light intensity, and insufficient rainfall, ultimately affecting crop quality and global food security (Seleiman *et al.* 2021). By 2050, drought stress may influence about 2.5 billion people in South Asia, with agriculture as the most at-risk sector (HDR 2006). Human activities are altering natural ecosystems, depleting water resources, and intensifying drought stress, especially in rain-fed agricultural systems (Hegerl *et al.* 2019).

Global freshwater availability has declined by 20%, affecting around 3.2 billion people living in areas facing high to very high-water stress (FAO 2020). In rain-fed regions, yield potential has dropped by 40–60% due to reduced water availability. FAO estimates suggest that drought-induced agricultural losses in developing countries totalled USD 29 billion between 2005 and 2015 (FAO 2018). Severe droughts continue to cause significant annual crop production losses worldwide. Drought stress affects plant morphology, physiology, and biochemistry, reducing photosynthetic capacity and overall plant productivity (Bijalwan *et al.* 2022).

Plants respond to drought stress through complex physiological and biochemical adaptations (Sharma *et al.* 2022). One early response involves stomatal closure to reduce water loss, which also decreases CO_2 uptake and photosynthesis. The regulation of guard cell turgor pressure and the synthesis of abscisic acid (ABA) are central to this process. In *Arabidopsis*, drought stress triggers the enzyme NCED3, a key player in ABA biosynthesis (Behnam *et al.* 2013). Drought stress also leads to an overproduction of ROS like singlet oxygen ($^1O_2$), superoxide radicals (O_2^-), hydroxyl radicals (•OH) and hydrogen peroxide (H_2O_2). While ROS act as signalling molecules at low levels, their excessive buildup triggers oxidative stress, resulting in lipid

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peroxidation, genetic damage, and cell death (Liu *et al.* 2025). The imbalance in ROS production disrupts the Calvin cycle, reducing NADPH and ATP consumption and impairing photosynthesis (Rahimi *et al.* 2022). Plants counteract ROS through antioxidant enzymes, but drought often alters their activity, reducing their effectiveness (Ilyas *et al.* 2021).

Strigolactones (SLs) are a class of carotenoid-derived phytohormones first recognized for their role in inhibiting shoot branching, a function that was discovered in 2008 (Banerjee and Bhadra 2020). Earlier research showed that SL-deficient mutants exhibit excessive shoot branching, while the application of synthetic SL analog GR24 suppresses axillary bud outgrowth (Kelly *et al.* 2023). The term "strigolactones" was originally introduced by Butler (1995) to describe lactone-containing compounds involved in plant–microbe interactions. These signaling molecules are primarily exuded from plant roots into the rhizosphere, where they mediate various developmental and ecological processes (Mitra *et al.* 2021; Yuan *et al.* 2024).

Plants, being immobile, must develop efficient mechanisms to survive environmental stresses. Understanding these mechanisms is crucial for enhancing plant resilience and agricultural productivity. Despite progress in water-saving technologies, efficient water management systems, and conservation practices, more efforts are required to achieve zero hunger under growing climate change pressures. SLs are key regulators involved in plant growth, development, and adaptation to various stress conditions. SLs are multifunctional and influence shoot branching, root architecture, leaf senescence, and nutrient acquisition, particularly under phosphorus-deficient conditions, by enhancing arbuscular mycorrhizal associations (Ameen *et al.* 2025). Among synthetic analogues of SLs, GR24 is widely used in research due to its consistent effects. Studies in rice mutants indicate that reduced SL levels lead to shorter plant stature, reduced biomass, and increased tillering, highlighting a significant regulatory role in plant architecture and growth (Ling *et al.* 2020). Although, the role of SLs, especially GR24, in drought tolerance remains underexplored, particularly in crops like rocket plants (*Eruca sativa* L.). Foliar application of GR24 presents a promising and eco-friendly approach to enhance drought tolerance, but limited data exist on its mechanisms in rocket plants under water-deficit conditions.

Rocket plant *is* a valuable oilseed crop grown for fodder, forage, and biodiesel production. Its oil exhibits excellent thermal and mechanical properties, making it suitable for renewable energy applications (Hussain and Biradar 2021). The crop residue also enhances soil fertility (Yadav *et al.* 2021). However, its productivity in tropical regions is significantly lower than in temperate zones, mainly due to its drought sensitivity. A 40% reduction in water availability can lead to a 60% decrease in grain yield (Nezhadahmadi *et al.* 2013). Drought-induced ROS accumulation negatively impacts gas exchange and physiological parameters, resulting in impaired growth. Therefore, in improving drought tolerance in rocket plants is essential for developing effective stress mitigation strategies. This study aimed to investigate the impacts of foliar-applied GR24 on growth performance, gas exchange parameters and osmolyte accumulation in rocket plants under drought stress. We hypothesized that GR24 may increase the chlorophyll content, enhance photosynthetic performance, and promote osmolyte accumulation, thus mitigating the negative impacts of water stress in rocket plants.

Materials and Methods

Experimental site, growth condition and design

Seeds of two accessions of rocket plants, Eruca-34961 and Eruca-34936, were collected from the National Agricultural Research Centre (NARC) in Islamabad, Pakistan. Strigolactones GR24 (Molecular weight: 298.2) are artificially synthesized plant hormones obtained from the Plant Nutrition Molecular Biology Laboratory, Northwest A&F University, China. The study was carried out in the Botanica Garden of Government College University, Faisalabad, from October 2022 to February 2023. The experiment was arranged using a completely randomized design (CRD) with three replicates. During the experimental period, the maximum temperatures fluctuated between 26°C and 30°C, while the minimum temperatures ranged from 11°C to 15°C. Before sowing, the soil's physicochemical characteristics were assessed following the methodology described by Jackson (1962). The soil was identified as sandy loam, containing approximately 70–80% sand and 15–19% clay, with 0.45% organic matter and 20% moisture content. It had an electrical conductivity (ECe) of 1.61 dSm^{-1} and a pH of 7.60. Nutrient analysis indicated nitrogen at 3.26 $mgkg^{-1}$, potassium at 128 $mgkg^{-1}$, and phosphorus at 2.59 $mgkg^{-1}$. In terms of heavy metals and micronutrients, lead was recorded at 0.90 $mgkg^{-1}$, calcium at 105 $mgkg^{-1}$, and zinc at 0.73 $mgkg^{-1}$.

Before sowing, seeds were surface sterilized with 10% H_2O_2 to minimize microbial contamination and subsequently rinsed thoroughly with distilled water. Ten seeds were sown in each plastic pot (28 cm in height, with a top circumference of 79 cm and a bottom circumference of 63 cm) filled with sandy loam soil. After germination and thinning, three uniform plants were maintained per pot. Drought stress was imposed at the vegetative stage for four weeks by maintaining soil moisture at 50% of the water-holding capacity (WHC). The experiment consisted of two groups: one subjected to drought stress (50% WHC) and the other maintained under control saturated conditions. All plants received full-strength Hoagland nutrient solution, following the protocol described by Hoagland and Arnon (1950). After drought stress exposure, plants were foliar-sprayed for three consecutive weeks with different concentrations of the synthetic strigolactone analogue GR24 (3 μM, 5 μM and 7 μM) prepared with 0.1% Tween-80 as a

surfactant. Control plants obtained only 0.1% Tween-80. Each plant was treated with 300 mL of the solution, applied daily at 9:00 A.M. After spray, plants were harvested to assess various attributes, including growth, physiological attributes, electrolyte leakage, malondialdehyde, hydrogen peroxide content, osmolytes, ascorbic acid and antioxidants.

Measurements

Plant growth and physiological attributes: Root and shoot lengths were measured with a meter rod, from the root tip to the apex of the main stem. Harvested plants were washed with distilled water to remove adhering soil particles and then separated into root and shoot portions. Excess surface moisture was removed, and the biomasses of both root and shoot were noted. These components were rinsed and then oven-dried at 80°C for 48 h. Dry weights were subsequently measured and recorded.

The estimation of chlorophyll and carotenoid pigments was carried out following the method described by Arnon (1949). Fresh leaf tissues were grinded in 80% acetone and then centrifuged to obtain a clear supernatant. Absorbance readings were taken at 663 nm and 645 nm to quantify chlorophyll a and chlorophyll b, respectively and at 470 nm for carotenoid content. The concentrations of these chlorophyll and carotenoid were computed using established equations.

Leaf gas exchange parameters were recorded between 10:00 and 11:00 a.m. using a portable open-system infrared gas analyzer (IRGA; model LCA-4, ADC, Hoddesdon, UK) on fully expanded leaves of rocket plants. These observations were recorded after spraying the leaves with SLs, and the leaf chamber conditions were as follows: the gas flow rate in the leaf chamber was maintained at 261 μmol s^{-1}, with an ambient CO_2 concentration (*Ca*) of 410.2 $_{\mu}$mol mol^{-1}. The leaf chamber temperature ranged between 25°C and 27°C and the photosynthetically active radiation (*PAR*) was measured up to a maximum of 731-933 μmol (photon) m^{-2} s^{-1} throughout data collection. Gas exchange parameters measured included net photosynthetic rate (*Pn*), stomatal conductance (*gs*), transpiration rate (*E*), intercellular CO_2 concentration (*Ci*), the *Ci/Ca* ratio and water use efficiency (*WUE*). These parameters were measured following the procedure described by Maqsood *et al*. (2021).

RWC was recorded for each treatment based on the method of González and González-Vilar (2001), using the following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \times 100 \qquad (1)$$

Nitrogenous compounds

TFAA were quantified following the method of Hamilton and Slyke (1943). A 0.15 g sample of fresh leaf tissue was grinded in 10 mL of potassium phosphate buffer (50 m*M*; pH 7.5). After centrifugation at 12,000 rpm for 10 min, 1 mL of the resulting supernatant was mixed with 1 mL pyridine (10%) and 1 mL acid ninhydrin solution (2%). After heating at 100 °C for 30 min, the mixture was cooled to 25 °C and absorbance was measured at 570 nm using a spectrophotometer.

Total soluble protein content was measured using the Bradford method (1976). Leaf tissues (0.15 g) were grinded in chilled 50 m*M* phosphate buffer (pH 7.5) and centrifuged at 12,000 rpm for 20 min. The supernatant was reacted with Bradford reagent and optical density of mixture was recorded at 595 nm using a spectrophotometer.

Free proline levels were determined using a modified Bates assay (Bates *et al.* 1973). A 0.15 g sample of fresh leaf tissue was grinded in 3% (w/v) sulfosalicylic acid under ice-cold conditions, followed by centrifugation for clarification. The supernatant was then incubated with glacial acetic acid and acidified ninhydrin reagent (1.25 g ninhydrin + 30 mL glacial acetic acid + 20 mL 6 *M* phosphoric acid) at 95°C for 60 min. The reaction was stopped by rapid chilling, and the toluene-solubilized adduct was measured spectrophotometrically at 520 nm.

Primary metabolites: Total soluble sugars were quantified following the protocol of Yemm and Willis (1954). For this, 0.15 g of leaf tissue was extracted with 80% ethanol. The extract was then reacted with anthrone reagent, vortexed, and incubated in a water bath at 97 °C for 10 min. After cooling to 25°C, the absorbance was measured at 570 nm using a spectrophotometer.

Ascorbic acid contents were measured using the Mukherjee and Choudhuri method (1983). A 0.15 g fresh leaf tissue was ground in 6% trichloroacetic acid (TCA) and subsequently centrifuged to obtain the supernatant. To 1 mL of this extract, 0.5 mL dinitrophenylhydrazine (DNPH) solution was added, along with one drop of thiourea (10%) in TCA (10%). The mixture was incubated in a water bath for 15 minutes, then immediately cooled in an ice bath. Subsequently, 80% H_2SO_4 (1 mL) was added gradually with continuous stirring. The absorbance of the final solution was recorded spectrophotometrically at 530 nm.

Secondary metabolites: Anthocyanin content was determined following Hodges and Nozzolillo (1996). Leaf tissues (0.15 g) were grinded in 2 mL acidic methanol (methanol + 1% HCl). The mixture was heated at 50 °C for 1 hour, then centrifuged at 12,000 rpm for 15 min. The optical density of mixture was recorded at 530 nm and 657 nm by using a spectrophotometer.

$$\text{Anthocyanin} = A_{530} - 0.25 \times A_{657} \qquad (2)$$

Phenolic compounds were quantified by extracting 0.15 g of leaf tissue in 80% acetone using the method of Julkunen-Tiitto (1985). The extract was then centrifuged at 1000 × g for 15 min, and supernatant (0.1 mL) was combined with distilled water (2 mL) and Folin–Ciocalteu's phenol reagent (1 mL). After mixing, 5 mL of Na_2CO_3 (20%) was added and diluted with 10 mL of distilled water. The mixture was warmed at 25 °C for 30 min and optical density was recorded at 760 nm using a spectrophotometer.

Total flavonoid content was measured following the method of Zhishen *et al.* (1999). Leaf extract (1 mL) was mixed with 80% ethanol (1 mL) and 300 μL of 5% sodium nitrite. The mixture was then warmed at 25°C for 5 min. Then, 0.3 mL $AlCl_3$ (10%) and 2 mL NaOH (1 *M*) were added. The absorbance of the solution was determined at 510 nm by using a spectrophotometer.

Oxidative stress and membrane integrity assays

For analysis of H_2O_2 content, 0.5 mL phosphate buffer (10 m*M*; pH 7) and 1 mL potassium iodide (1 *M*) were mixed with 10 mL sample extract, which was obtained using 5% TCA using the methodology of Velikova *et al.* (2000). The solution was placed in darkness at 25°C for 30 min, and the optical density of mixture was noted at 390 nm by using a spectrophotometer.

Lipid peroxidation, indicated by MDA content, was assessed according to Dhindsa *et al*. (1981). Fresh plant leaves were extracted in 20% TCA and centrifuged at 15,000 × g for 15 min. The supernatant was mixed with 20% TCA and 0.5% thiobarbituric acid at a 1:4 ratio, incubated in a 95°C water bath for 30 min and then cooled in ice water. After centrifugation at 10,000 rpm for 15 min, optical density was noted at 532 nm and 600 nm using a spectrophotometer. MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and stated as nmol g^{-1} fresh weight.

$$\text{MDA (nmol/ mL FW)} = [A_{532} - A_{600})/155000 \times 10^6] \quad (3)$$

Electrolyte leakage (EL) was measured by placing ten 1.2 cm diameter leaf discs in a test tube having 10 mL of dH_2O. Initial electrical conductivity (EC_1) was recorded, followed by heating the tubes at 120°C for 15 min to release total electrolytes. The samples were then cooled to 25 °C. The final conductivity (EC_2) was recorded, and EL was measured as a percentage using the formula by Dionisio-Sese and Tobita (1998).

$$\text{EL (\%)} = \frac{EC_1}{EC_2} \times 100 \quad (4)$$

Antioxidant enzyme activities

Leaf samples, each weighing 0.5 g, were subjected to extraction using an ice-cold potassium phosphate buffer (50 m*M*; pH 7.0). Polyvinylpyrrolidone (PVP) was added to the buffer to remove phenolic compounds that might inhibit enzyme activity. The extract was centrifuged at 10,000 g for 15 min at 4°C to separate cellular components.

Superoxide dismutase (SOD) activity was determined following the protocol of Giannopolitis and Ries (1977). A mixture of 50 m*M* phosphate buffer (pH 7.8), methionine (13 m*M*), riboflavin (2 μM), NBT (75 μM), EDTA (0.1 m*M*), and Triton X-100 (0.1%), was added to enzyme extract (50 μL) to fluorescent light for 15 min and then illuminated under a 60-watt luminous light source for a further 20 min to complete the reaction. Optical density was observed at 290 nm using a spectrophotometer.

Peroxidase (POD) activity was assessed using the guaiacol oxidation method by Chance and Maehly (1954). The reaction mixture contained 50 m*M* phosphate buffer (3 mL), 20 m*M* guaiacol (50 μL), H_2O_2 (20 m*M*) and enzyme extract. The change in absorbance was monitored at 470 nm for 2 min using a spectrophotometer, with measurements recorded at 30s intervals.

Catalase (CAT) activity was measured following the method of Chance and Maehly (1954). A mixture of phosphate buffer (50 m*M*; pH 7.0), H_2O_2 (15 m*M*) and the enzyme extract was prepared. The breakdown of H_2O_2 was monitored by measuring the decrease in absorbance at 240 nm over a 120 s period, with measurements recorded at 30s intervals.

Statistical analysis

Data was analyzed using Statistix software (version 8.0) through three-way ANOVA to assess the effects of the factors and their interactions. Treatment means were compared using Tukey's HSD test at a significant level of $P < 0.05$.

Results

Impact of GR24 foliar application on growth parameters of rocket plants under drought stress

Drought stress (maintained at 50% WHC) had a significant impact ($P < 0.001$) on all assessed growth parameters in both rocket accessions, Eruca-34961 and Eruca-34936. Under water-limited conditions, notable reductions were observed in shoot and root lengths, as well as in fresh and dry biomass, when compared to control plants. However, foliar applied GR24 (3.0 μM, 5.0 μM and 7.0 μM) significantly improved these growth parameters under drought stress. Among the treatments, 5.0 μM GR24 consistently resulted in the greatest enhancement, significantly increasing shoot and root lengths, along with biomass, in both accessions relative to controlled plants. Overall, exogenous GR24, particularly at 5.0 μM, effectively lessened the harmful effects of drought. The order of effectiveness among treatments was: 5.0 μM GR24 > 7.0 μM GR24 > 3.0 μM GR24 > NS (Table 1-2).

Impact of GR24 foliar application on photosynthetic pigments of rocket plants under drought stress

Drought stress led to a substantial ($P < 0.001$) reduced in photosynthetic pigment levels in both rocket plant accessions, Eruca-34961 and Eruca-34936. The chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were notably abridged under drought conditions compared to the well-watered control. However, the exogenous GR24 (3.0 μM, 5.0 μM, and 7.0 μM) effectively alleviated these negative effects, enhancing photosynthetic pigment content under water stress.

Table 1: Summary of ANOVA for growth and physio-biochemical traits in rocket plants treated with strigolactones under drought stress

Source of variance	Accession (A), df = 1	Drought (D), df = 1	Foliar Spray, (FS), df = 3	A × D, df = 1	A × FS, df = 3	D × FS, df = 3	A × D × FS, df = 3	Error, df = 32	CV
Shoot length	417.13***	497.297***	202.70***	32.505***	1.186ns	1.797ns	3.172ns	1.828	3.75
Root length	221.021***	825.021***	216.576***	1.687ns	4.021*	1.354ns	4.188**	0.964	6.88
Shoot fresh weight	54.827***	455.717***	69.434***	10.360***	0.355ns	13.515***	0.996*	0.431	6.88
Shoot dry weight	4.813***	64.403***	528.671***	2.430***	0.341ns	3.504***	0.491**	0.163	13.08
Root fresh weight	10.184***	87.399***	13.247***	1.798***	0.094ns	2.410***	0.234*	0.086	7.16
Root dry weight	0.207***	1.781***	0.269***	0.037***	0.0018ns	0.049***	0.0048*	0.0017	7.11
Chl *a*	3.018***	2.859***	2.067***	0.057**	0.549***	0.477**	0.052**	0.0120	7.82
Chl *b*	0.059***	0.693***	0.261***	0.0344**	0.0017ns	0.0022ns	0.0049ns	0.0060	13.44
Total Chl	3.925***	6.367***	3.767***	0.003ns	0.046*	0.050**	0.0837**	0.01624	6.44
Total Car	0.0038ns	0.1162***	0.0245**	0.00011ns	0.00230ns	0.01280ns	0.00230ns	0.00736	30.97
Pn	88.021***	526.688***	45.910***	7.521**	0.021ns	3.910**	0.076ns	1.042	6.72
E	48.340***	73.433***	6.017***	2.818***	0.093ns	0.515**	0.0035ns	0.1360	6.59
Ci	83834***	126075***	9986***	2977***	183ns	1238***	111ns	172	5.26
gs	0.1281***	0.0901***	0.0073***	0.0048***	0.00029ns	0.0008***	0.00007ns	0.00015	5.68
WUE	3.116***	0.0038ns	0.0148***	0.0025ns	0.0049ns	0.0002ns	0.0033ns	0.00287	1.94
Ci/Ca	0.4920***	0.7550***	0.5869***	0.0192***	0.0010ns	0.0072***	0.00087ns	0.00096	5.09
RWC	816.750***	24.083***	338.917***	10.083**	6.250**	148.694***	13.361***	1.542	3.94
H_2O_2	1299.99***	139.82***	73.32***	85.14***	3.17ns	3.95ns	0.56ns	2.35	9.46
MDA	41.285***	71.493***	32.641***	4.045n*	5.540**	9.612***	3.148*	1.109	4.75
Proline	113.324***	44.738***	12.005***	29.828***	0.511ns	2.499ns	0.191ns	2.185	14.85
Flavonoid	11.826***	5.271***	5.162***	0.616***	0.287***	0.279***	0.366***	0.055	8.94
Ascorbic acid	19.456***	2.824***	1.981***	0.047ns	0.124ns	0.184*	0.265**	0.067	8.88
TSS	29.146***	20.703***	38.292***	0.477ns	0.042ns	0.060ns	0.407*	0.175	7.45
TFAA	0.414**	0.544***	2.977***	0.0297ns	0.4183***	0.1834**	0.0803ns	0.0552	13.80
TSP	5.590***	8.372***	13.041***	0.773***	0.029ns	0.042ns	0.219***	0.0274	4.78
Phenolics	34.686***	87.550***	26.011***	1.252**	0.554**	0.281ns	1.244***	0.155	6.18
Anthocyanin	6.740***	8.810***	4.411***	0.182**	1.443***	1.480***	2.103***	0.037	5.96
SOD	181.36***	1281.14***	1919.07***	6.76ns	638.96***	181.94***	28.95*	11.80	10.00
POD	5376.92***	7534.91***	3846.09***	974.68***	352.53**	347.25**	550.38***	91.51	10.65
CAT	344.03ns	5148.78***	1707.01***	188.21ns	153.09ns	200.49ns	204.60ns	131.14	12.18

Asterisks indicate significance levels: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; ns = non-significant. *Abbreviations*: df, degree of freedom; CV, coefficient of variation; FS, foliar spray of strigolactones; A – accession; D – drought; Chl – chlorophyll; Car – carotenoids; Pn – net photosynthetic rate; E – transpiration rate; gs – stomatal conductance; Ci – sub-stomatal CO_2 concentration; WUE – water use efficiency; Ca – ambient CO_2 concentration; RWC – relative water content; H_2O_2 – hydrogen peroxide; MDA – malondialdehyde; TSS – total soluble sugars; TSP – total soluble protein; SOD – superoxide dismutase; POD – peroxidase; CAT – catalase

Table 2: Impact of strigolactones (GR24) on growth characteristics of two rocket plant accessions under drought stress

Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)
			Eruca-34961			
NS	$36.67 \pm 0.88^{c\text{-}f}$	15.83 ± 0.60^{de}	9.03 ± 0.20^{cd}	2.23 ± 0.15^{de}	3.90 ± 0.11^{cd}	0.56 ± 0.02^{cd}
3 μM SLs	$39.33 \pm 0.88^{c\text{-}e}$	17.17 ± 0.60^{cd}	12.10 ± 0.38^{b}	3.87 ± 0.19^{c}	5.23 ± 0.15^{b}	0.75 ± 0.02^{b}
5 μM SLs	45.33 ± 0.88^{a}	24.33 ± 0.60^{a}	16.37 ± 0.63^{a}	6.90 ± 0.21^{a}	7.08 ± 0.27^{a}	1.01 ± 0.04^{a}
7 μM SLs	44.33 ± 0.33^{ab}	24.17 ± 0.44^{a}	15.40 ± 0.38^{a}	6.17 ± 0.09^{ab}	6.63 ± 0.18^{a}	0.95 ± 0.03^{a}
Drought (50% WHC)	31.33 ± 0.88^{g}	7.17 ± 0.73^{hi}	$5.73 \pm 0.51^{e\text{-}g}$	1.13 ± 0.09^{ef}	$2.40 \pm 0.26^{e\text{-}g}$	$0.34 \pm 0.04^{e\text{-}g}$
Drought + 3 μM SLs	$35.67 \pm 0.88^{d\text{-}f}$	9.17 ± 0.44^{gh}	7.43 ± 0.33^{de}	1.90 ± 0.21^{de}	$3.07 \pm 0.12^{d\text{-}f}$	$0.44 \pm 0.02^{d\text{-}f}$
Drought + 5 μM SLs	40.00 ± 0.58^{c}	17.17 ± 0.44^{cd}	9.80 ± 0.42^{c}	2.77 ± 0.18^{cd}	4.23 ± 0.19^{c}	0.60 ± 0.03^{c}
Drought + 7 μM SLs	39.50 ± 0.29^{cd}	16.33 ± 0.60^{de}	9.00 ± 0.12^{cd}	2.30 ± 0.15^{de}	3.90 ± 0.06^{cd}	0.56 ± 0.01^{cd}
			Eruca -34936			
NS	33.33 ± 0.88^{fg}	11.17 ± 0.73^{fg}	7.47 ± 0.15^{de}	1.23 ± 0.15^{ef}	3.21 ± 0.05^{de}	0.46 ± 0.01^{de}
3 μM SLs	$35.33 \pm 0.88^{e\text{-}g}$	14.00 ± 0.58^{ef}	10.83 ± 0.44^{bc}	2.87 ± 0.19^{cd}	4.67 ± 0.20^{bc}	0.67 ± 0.03^{bc}
5 μM SLs	40.33 ± 0.88^{bc}	20.83 ± 0.44^{b}	15.17 ± 0.52^{a}	5.57 ± 0.35^{b}	6.53 ± 0.23^{a}	0.93 ± 0.03^{a}
7 μM SLs	39.67 ± 0.88^{cd}	19.83 ± 0.44^{bc}	14.60 ± 0.26^{a}	5.17 ± 0.09^{b}	6.30 ± 0.12^{a}	0.90 ± 0.02^{a}
Drought (50% WHC)	23.67 ± 0.88^{h}	4.67 ± 0.44^{i}	3.83 ± 0.38^{g}	0.30 ± 0.12^{f}	1.63 ± 0.19^{g}	0.23 ± 0.03^{g}
Drought + 3 μM SLs	26.00 ± 0.58^{h}	6.17 ± 0.44^{i}	4.57 ± 0.29^{fg}	1.13 ± 0.07^{ef}	1.93 ± 0.15^{g}	0.28 ± 0.02^{g}
Drought + 5 μM SLs	33.67 ± 0.88^{fg}	10.83 ± 0.93^{g}	5.87 ± 0.29^{ef}	3.03 ± 0.50^{cd}	$2.50 \pm 0.10^{e\text{-}g}$	$0.36 \pm 0.01^{e\text{-}g}$
Drought + 7 μM SLs	33.00 ± 0.58^{fg}	9.50 ± 0.29^{gh}	5.43 ± 0.38^{fg}	2.90 ± 0.46^{cd}	2.30 ± 0.15^{fg}	0.33 ± 0.02^{fg}

Each column presents the mean ± standard error based on three biological replicates. Different lowercase letters show statistically substantial differences among treatments, as determined by Tukey's HSD test at $P < 0.05$. *Abbreviations*: NS – no spray; SLs – strigolactones; WHC – water holding capacity

Among the treatments, 5.0 μM GR24 exhibited the highest efficacy, significantly increasing total chlorophyll content in both accessions. Furthermore, carotenoid levels were significantly enhanced by GR24 application, with the 3.0 μM GR24 showing the most robust response under drought conditions. The overall effectiveness of GR24 treatments in improving photosynthetic pigments followed the order: 5.0 μM GR24 > 3.0 μM GR24 > 7.0 μM GR24, under both control and drought-stressed conditions in both accessions (Table 1-3).

Table 3: Impact of strigolactones (GR24) on photosynthetic pigments of two rocket plant accessions under drought stress

Treatments	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Total Chl (mg/g FW)	Total Car (mg/g FW)	Relative water contents (%)
		Eruca-34961			
NS	1.47 ± 0.01^{de}	$0.66 \pm 0.07^{b-e}$	2.13 ± 0.09^{de}	$0.24 \pm 0.01^{b-e}$	$36.33 \pm 0.88^{b-d}$
3 μM SLs	1.66 ± 0.18^{cd}	$0.71 \pm 0.03^{a-d}$	2.38 ± 0.15^{cd}	$0.33 \pm 0.03^{a-d}$	37.00 ± 0.58^{bc}
5 μM SLs	2.23 ± 0.07^{a}	0.93 ± 0.04^{a}	3.16 ± 0.04^{a}	0.40 ± 0.02^{a}	37.33 ± 0.88^{bc}
7 μM SLs	2.08 ± 0.02^{ab}	$0.74 \pm 0.05^{a-c}$	2.82 ± 0.07^{ab}	0.37 ± 0.03^{ab}	$36.66 \pm 0.67^{b-d}$
Drought (50% WHC)	0.91 ± 0.08^{f}	0.28 ± 0.04^{g}	1.19 ± 0.11^{g}	$0.21 \pm 0.07^{c-e}$	21.00 ± 0.58^{g}
Drought + 3 μM SLs	1.14 ± 0.03^{ef}	0.40 ± 0.04^{fg}	1.54 ± 0.06^{fg}	$0.21 \pm 0.01^{c-e}$	35.00 ± 0.58^{cd}
Drought + 5 μM SLs	2.03 ± 0.02^{ab}	$0.69 \pm 0.03^{b-e}$	2.72 ± 0.02^{bc}	$0.33 \pm 0.02^{a-c}$	42.00 ± 1.16^{a}
Drought + 7 μM SLs	1.69 ± 0.10^{cd}	$0.50 \pm 0.05^{d-g}$	2.18 ± 0.12^{de}	$0.20 \pm 0.05^{c-e}$	40.00 ± 0.58^{ab}
		Eruca -34936			
NS	0.86 ± 0.01^{f}	$0.46 \pm 0.02^{e-g}$	1.33 ± 0.01^{g}	$0.28 \pm 0.07^{a-e}$	23.70 ± 0.88^{fg}
3 μM SLs	1.33 ± 0.04^{e}	$0.57 \pm 0.03^{c-f}$	1.90 ± 0.02^{ef}	$0.31 \pm 0.09^{a-e}$	26.00 ± 0.58^{f}
5 μM SLs	1.84 ± 0.04^{bc}	0.84 ± 0.07^{ab}	2.68 ± 0.05^{bc}	0.36 ± 0.07^{ab}	31.00 ± 0.58^{e}
7 μM SLs	1.68 ± 0.03^{cd}	$0.67 \pm 0.08^{b-e}$	2.35 ± 0.10^{cd}	$0.32 \pm 0.04^{a-d}$	30.00 ± 0.58^{e}
Drought (50% WHC)	0.41 ± 0.02^{g}	0.30 ± 0.01^{g}	0.71 ± 0.02^{h}	0.19 ± 0.07^{de}	16.00 ± 0.58^{h}
Drought + 3 μM SLs	0.85 ± 0.06^{f}	0.38 ± 0.02^{fg}	1.23 ± 0.04^{g}	0.19 ± 0.02^{de}	26.00 ± 0.58^{f}
Drought + 5 μM SLs	1.40 ± 0.02^{de}	$0.63 \pm 0.03^{b-e}$	2.03 ± 0.05^{de}	$0.31 \pm 0.01^{a-e}$	$33.66 \pm 0.88^{c-e}$
Drought + 7 μM SLs	0.82 ± 0.05^{f}	$0.48 \pm 0.03^{d-g}$	1.31 ± 0.04^{g}	0.18 ± 0.05^{e}	33.00 ± 0.58^{de}

Each column presents the mean ± standard error based on three biological replicates. Different lowercase letters show statistically substantial differences among treatments, as determined by Tukey's HSD test at $P < 0.05$. *Abbreviations*: NS – no spray; SLs – strigolactones; WHC – water holding capacity; Chl– chlorophyll; Car– carotenoids

Table 4: Impact of strigolactones (GR24) on biochemical characteristics of two rocket plant accessions under drought stress

Treatments	TFAA (mg/g FW)	TSP (mg/g FW)	Anthocyanin (mg/g FW)	Flavonoids (mg/g FW)	Phenolics (mg/g FW)	TSS (mg/g FW)	Proline (μmol/g FW)	Ascorbic acid (mg/g FW)
				Eruca-34961				
NS	$1.33 \pm 0.02^{c-e}$	1.83 ± 0.01^{e}	2.05 ± 0.02^{d}	$2.61 \pm 0.24^{c-f}$	3.72 ± 0.31^{ij}	3.35 ± 0.15^{hi}	6.63 ± 0.93^{de}	$3.13 \pm 0.15^{c-f}$
3 μM SLs	$2.02 \pm 0.34^{a-c}$	3.81 ± 0.11^{c}	3.89 ± 0.07^{b}	3.82 ± 0.12^{b}	5.71 ± 0.19^{fg}	$5.38 \pm 0.10^{d-f}$	8.12 ± 0.57^{de}	4.13 ± 0.17^{ab}
5 μM SLs	2.49 ± 0.13^{a}	4.13 ± 0.04^{bc}	2.78 ± 0.11^{c}	4.80 ± 0.16^{a}	$7.02 \pm 0.10^{c-e}$	7.84 ± 0.13^{ab}	$10.05 \pm 0.77^{b-d}$	4.35 ± 0.16^{a}
7 μM SLs	$1.66 \pm 0.16^{b-d}$	4.27 ± 0.12^{bc}	4.24 ± 0.14^{ab}	3.01 ± 0.17^{cd}	$6.37 \pm 0.23^{e-g}$	6.79 ± 0.37^{bc}	8.98 ± 0.74^{de}	$3.42 \pm 0.17^{b-d}$
Drought (50% WHC)	0.86 ± 0.13^{ef}	2.82 ± 0.11^{d}	4.67 ± 0.15^{a}	1.78 ± 0.06^{h}	$7.33 \pm 0.25^{c-e}$	$4.88 \pm 0.18^{e-g}$	$13.90 \pm 0.93^{a-c}$	$2.78 \pm 0.24^{d-g}$
Drought + 3 μM SLs	$1.70 \pm 0.13^{b-d}$	4.21 ± 0.06^{bc}	4.22 ± 0.16^{ab}	$2.66 \pm 0.12^{c-f}$	7.70 ± 0.17^{cd}	6.80 ± 0.37^{bc}	$13.90 \pm 1.13^{a-c}$	$3.37 \pm 0.12^{b-e}$
Drought + 5 μM SLs	2.24 ± 0.10^{ab}	4.87 ± 0.12^{a}	3.03 ± 0.08^{c}	3.30 ± 0.10^{bc}	10.97 ± 0.49^{a}	8.90 ± 0.38^{a}	16.03 ± 0.74^{a}	$3.71 \pm 0.14^{a-c}$
Drought + 7 μM SLs	2.06 ± 0.13^{ab}	4.47 ± 0.12^{ab}	3.97 ± 0.15^{b}	2.95 ± 0.18^{cd}	8.92 ± 0.36^{b}	7.24 ± 0.17^{bc}	14.32 ± 1.13^{ab}	$3.47 \pm 0.07^{b-d}$
				Eruca -34936				
NS	$1.08 \pm 0.13^{d-f}$	1.14 ± 0.06^{f}	1.41 ± 0.16^{e}	1.78 ± 0.12^{h}	2.59 ± 0.12^{j}	1.93 ± 0.17^{j}	5.13 ± 0.74^{e}	2.32 ± 0.25^{g}
3 μM SLs	$1.99 \pm 0.13^{a-c}$	2.85 ± 0.12^{d}	2.05 ± 0.02^{d}	$2.21 \pm 0.12^{e-h}$	3.78 ± 0.16^{i}	$3.99 \pm 0.22^{g-i}$	7.05 ± 0.74^{de}	2.53 ± 0.18^{fg}
5 μM SLs	$1.77 \pm 0.10^{b-d}$	3.25 ± 0.09^{d}	2.78 ± 0.10^{c}	$2.88 \pm 0.12^{c-e}$	5.74 ± 0.10^{fg}	$5.98 \pm 0.08^{c-e}$	8.34 ± 0.74^{d}	$2.71 \pm 0.05^{d-g}$
7 μM SLs	2.13 ± 0.10^{ab}	3.05 ± 0.09^{d}	3.22 ± 0.06^{cd}	$2.50 \pm 0.10^{d-g}$	5.20 ± 0.15^{gh}	$4.43 \pm 0.30^{f-h}$	7.27 ± 0.93^{de}	$2.62 \pm 0.11^{e-g}$
Drought (50% WHC)	0.57 ± 0.10^{f}	1.90 ± 0.09^{e}	2.05 ± 0.02^{d}	1.00 ± 0.12^{i}	4.43 ± 0.10^{hi}	3.13 ± 0.23^{ij}	9.62 ± 0.74^{cd}	1.15 ± 0.14^{h}
Drought + 3 μM SLs	$1.77 \pm 0.04^{b-d}$	4.01 ± 0.06^{bc}	4.27 ± 0.12^{ab}	1.87 ± 0.12^{gh}	$6.68 \pm 0.19^{d-f}$	$5.36 \pm 0.29^{d-f}$	9.62 ± 0.74^{cd}	2.15 ± 0.12^{g}
Drought + 5 μM SLs	$1.62 \pm 0.13^{b-d}$	4.47 ± 0.09^{ab}	3.06 ± 0.12^{c}	$2.61 \pm 0.14^{c-f}$	8.04 ± 0.12^{bc}	7.70 ± 0.18^{ab}	$10.48 \pm 0.93^{b-d}$	2.44 ± 0.09^{fg}
Drought + 7 μM SLs	$1.95 \pm 0.06^{a-c}$	4.27 ± 0.09^{bc}	4.00 ± 0.14^{b}	$2.14 \pm 0.10^{f-h}$	7.67 ± 0.20^{cd}	6.19 ± 0.25^{cd}	9.83 ± 0.93^{cd}	2.25 ± 0.03^{g}

Each column presents the mean ± standard error based on three biological replicates. Different lowercase letters show statistically substantial differences among treatments, as determined by Tukey's HSD test at $P < 0.05$. *Abbreviations*: NS – no spray; SLs – strigolactones; WHC – water holding capacity; TFAA– total free amino acid; TSP– total soluble protein; TSS– total soluble sugar

Impact of GR24 foliar application on gas exchange attributes of rocket plants under drought stress

Gas exchange parameters namely net photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*) and sub-stomatal CO_2 concentration (*Ci)* were all significantly reduced ($P < 0.001$) in both rocket plant accessions when subjected to drought. Foliar applications of GR24 at 3.0, 5.0 and 7.0 μM effectively mitigated these negative effects, with the 5.0 and 7.0 μM concentrations showing the most pronounced improvements in *Pn*, *E* and *gs* (Fig. 1a-c). In Eruca-34936, GR24 treatment also led to a notable recovery in the *Ci/Ca* ratio. Additionally, water use efficiency (*WUE*) significantly improved in Eruca-34936 (Fig. 2a-c), but remained unchanged in Eruca-34961, indicating stronger drought adaptation in Eruca-34936. These results suggest that GR24 enhances drought tolerance by improving gas exchange performance and promoting osmolyte accumulation. Overall, the order of effectiveness across treatments for gas exchange attributes was 5.0 μM GR24 ≈ 7.0 μM GR24 > 3.0 μM GR24 > NS.

Impact of GR24 foliar application on secondary metabolites, osmolytes and ascorbic acid of rocket plants under drought stress

Both rocket plant accessions, Eruca-34961 and Eruca-34936, showed a substantial reduction in TFAA under drought compared to non-stressed controls. Foliar application of GR24 at 3.0, 5.0, and 7.0 μM markedly enhanced TFAA levels in both accessions. In Eruca-34961, the highest TFAA content was recorded under 5.0 μM GR24 during drought, whereas in Eruca-34936, the maximum was observed with 7.0 μM GR24 (Table 4).

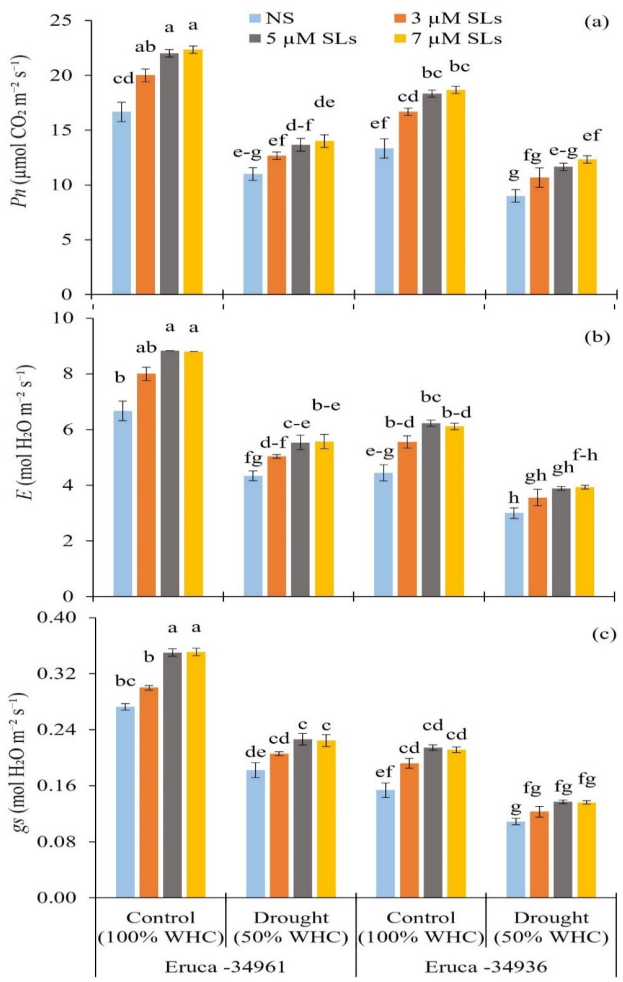

Fig. 1: Impact of strigolactones (GR24) on gas exchange attributes: (**a**) net photosynthetic rate (*Pn*), (**b**) transpiration rate (*E*) and (**c**) stomatal conductance (*gs*) in two rocket plant accessions, Eruca-34961 and Eruca-34936 under drought stress. Different lowercase letters show statistically substantial differences among treatments, as determined by Tukey's HSD test at $P < 0.05$. Abbreviations: NS = no spray; SLs = strigolactones; WHC = water holding capacity

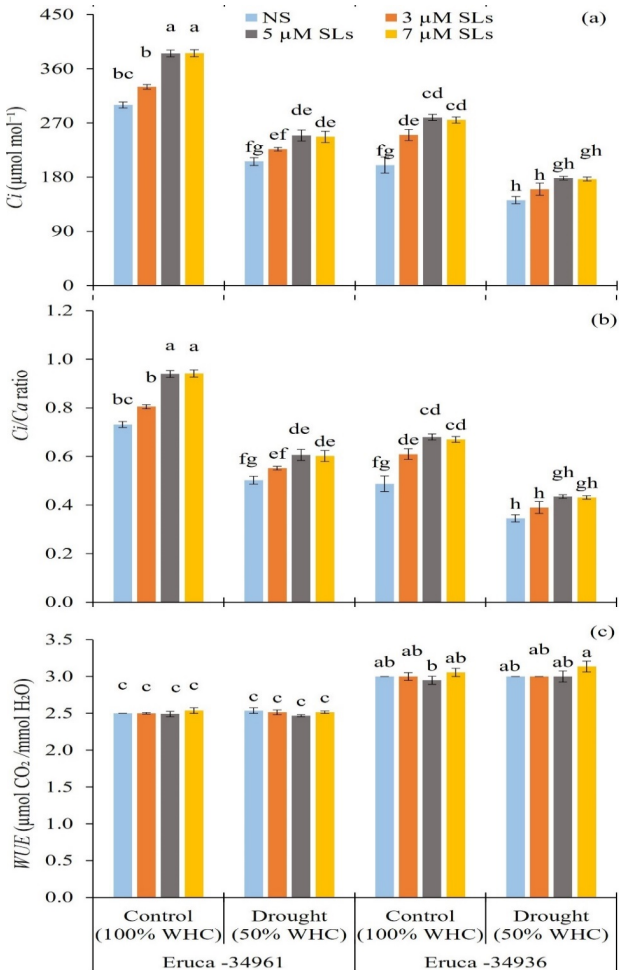

Fig. 2: Impact of strigolactones (GR24) on gas exchange attributes: (**a**) internal carbon dioxide concentration (*Ci*), (**b**) internal carbon dioxide concentration and ambient concentration of carbon dioxide ratio (*Ci/Ca*) and (**c**) water use efficiency (*A/E*) in two rocket plant accessions, Eruca-34961 and Eruca-34936 under drought stress. Different lowercase letters show statistically substantial differences among treatments, as determined by Tukey's HSD test at $P < 0.05$. Abbreviations: NS = no spray; SLs = strigolactones; WHC = water holding capacity

Total soluble sugars (TSS) substantially increased in both accessions in response to drought, and GR24 application (3.0, 5.0 and 7.0 μM) further elevated TSS levels. The 5.0 μM GR24 treatment led to the highest TSS accumulation under drought in both Eruca-34961 and Eruca-34936 as shown in Table 4.

Proline accumulation significantly increased in both Eruca-34961 and Eruca-34936 accessions in response to drought stress. Foliar application of GR24 at 3.0, 5.0 and 7.0 μM further elevated proline levels, with the 5.0 μM concentration inducing the highest accumulation under both normal and drought conditions. Notably, 5.0 μM GR24 markedly enhanced proline content in both accessions, indicating its potential role in stress mitigation. In contrast, the 3.0 μM GR24 treatment showed a comparatively lower stimulatory effect and was associated with a reduction in proline under drought stress (Table 1-4). These results suggest that GR24 modulates osmoprotectant accumulation in a concentration-dependent manner, with 5.0 μM being the most effective (Table 1-4).

Ascorbic acid concentrations also reduced in both rocket plant accessions under drought stress but GR24 treatment at 3.0, 5.0 and 7.0 μM more increased the ascorbic acid content. The highest ascorbic acid levels under drought were observed with 5.0 μM GR24 in Eruca-34961 and Eruca-34936 as shown in Table 1-4.

Drought stress led to a noticeable upsurge in TSP content in both Eruca-34961 and Eruca-34936 accessions. Exogenous GR24 (3.0, 5.0 and 7.0 μM) further enhanced TSP accumulation under drought conditions. Among these,

the 5.0 μM GR24 treatment was the most effective, resulting in a significant improvement in TSP levels in both accessions compared to the untreated drought-stressed and control plants. These findings suggest that GR24 application can enhance protein synthesis or stability under water-deficit conditions, thereby contributing to improved stress tolerance. Overall, the order of effectiveness across treatments for osmolytes and secondary metabolites was 5 μM > 7 μM > 3 μM > NS in both accessions (Table 1-4).

Impact of GR24 foliar application on non-enzymatic antioxidants of rocket plants under drought stress

Drought stress triggered a substantial increase in non-enzymatic antioxidants, including total phenolics, flavonoids, and anthocyanins, in both Eruca-34961 and Eruca-34936 accessions. The application of GR24 at 3.0, 5.0 and 7.0 μM further elevated the levels of these metabolites, with 5.0 μM GR24 producing the most pronounced effects. Under drought conditions, Eruca-34961 exhibited the highest accumulation of total phenolics, flavonoids, and anthocyanins with 5.0 μM GR24 treatment, and similar enhancement patterns were observed in Eruca-34936. These findings indicate that GR24 augments the plant's non-enzymatic antioxidant defense system, thereby offering protection against oxidative stress induced by drought. The order of effectiveness in increasing non-enzymatic antioxidants was 5 μM GR24 > 7 μM GR24 > 3 μM GR24 > NS in both Eruca accessions (Table 4).

Impact of GR24 foliar application on oxidative stress markers in rocket plants under drought stress

Drought caused a significant rise in oxidative stress indicators, such as H_2O_2, MDA and EL in both Eruca-34961 and Eruca-34936. Notably, drought-treated Eruca-34961 plants exhibited significantly elevated levels of H_2O_2 and MDA compared to non-stressed controls. Foliar application of GR24 at concentrations of 3.0, 5.0 and 7.0 μM effectively reduced these stress markers under drought conditions. Among the treatments, 5.0 μM GR24 demonstrated the greatest efficacy, bringing the levels of H_2O_2, MDA and EL close to or even below those observed in untreated control plants (Fig. 3a–c). These results underscore the potential of GR24 in alleviating oxidative damage induced by drought stress. The order of effectiveness in increasing oxidative stress markers was 3.0 μM GR24 > 7.0 μM GR24 > 5.0 μM GR24 > NS in both Eruca accessions (Fig. 3).

Impact of GR24 foliar application on antioxidant enzyme activities of rocket plants under drought stress

Table 1 shows that both drought and GR24 application had highly substantial effects ($P < 0.001$) on key antioxidant enzymes activities, particularly POD and SOD.

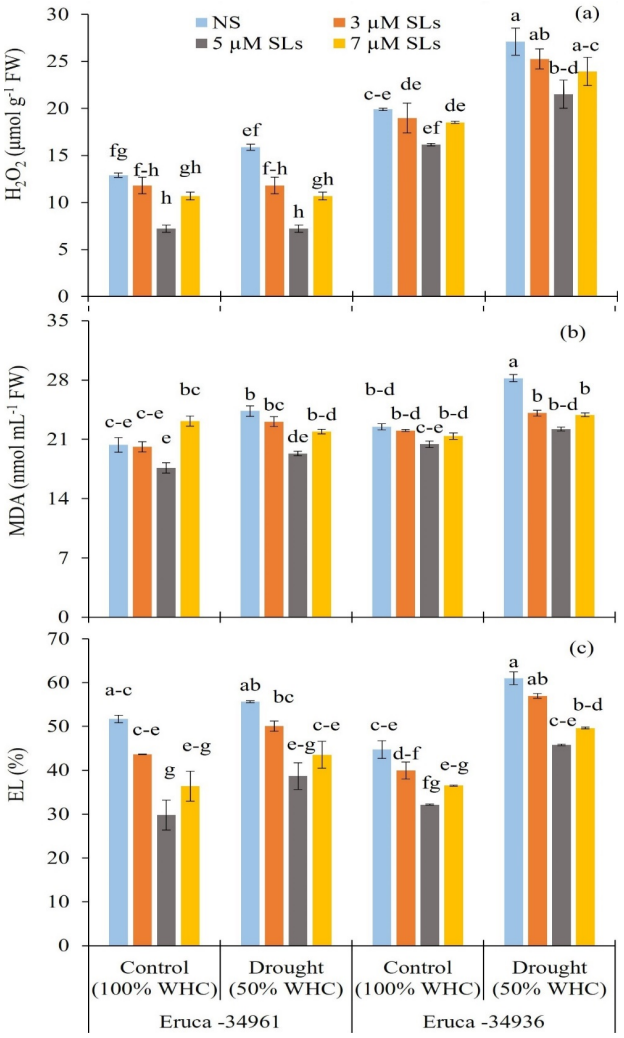

Fig. 3: Impact of strigolactones (GR24) on oxidative stress indicators: (**a**) hydrogen peroxide (H_2O_2), (**b**) malondialdehyde (MDA) and (**c**) electrolytes leakage (EL) in two rocket plant accessions, Eruca-34961 and Eruca-34936 under drought stress. Different lowercase letters show statistically substantial differences among treatments, as determined by Tukey's HSD test at $P < 0.05$. Abbreviations: NS = no spray; SLs = strigolactones; WHC = water holding capacity

Under drought, antioxidant enzyme activities (CAT, SOD and POD) were generally suppressed, with the decline more evident in Eruca-34936. However, GR24 foliar application, especially at 5.0 μM, noticeably improved the activities of these enzymes under stress conditions. In both accessions, 5.0 μM GR24 led to a substantial upsurge in POD and SOD activites, with Eruca-34961 showing nearly a twofold rise in SOD activity compared to drought-stressed plants without GR24 treatment (Fig. 4a–c). Although catalase activity also improved, the response was comparatively moderate. The order of effectiveness in increasing enzymatic antioxidants was 5 μM GR24 > 7 μM GR24 > 3 μM GR24 > NS in both Eruca accessions (Fig. 4).

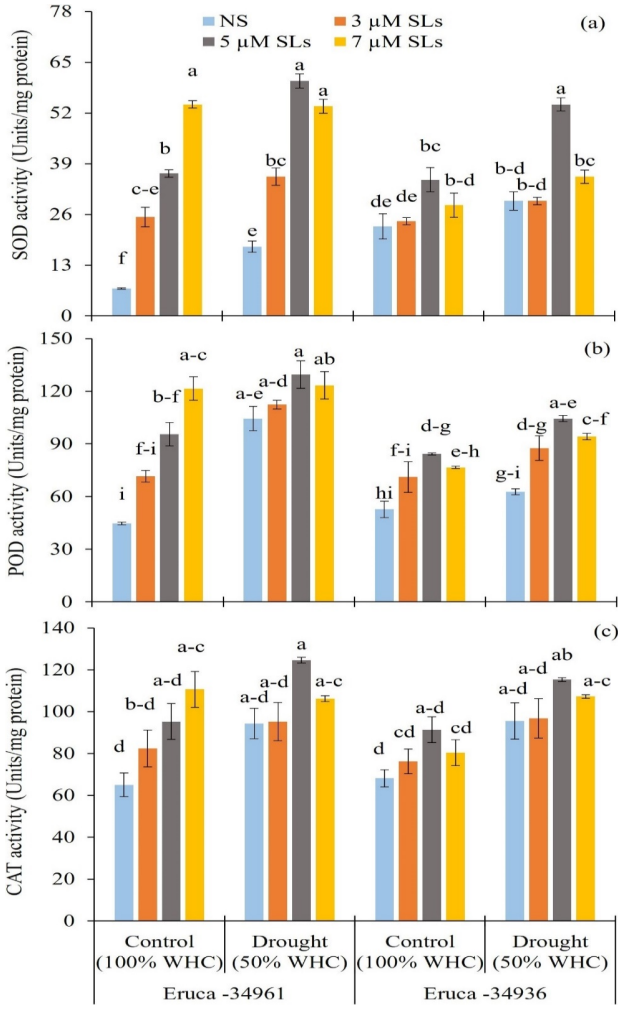

Fig. 4: Impact of strigolactones (GR24) on antioxidants enzymes: (**a**) superoxide dismutase (SOD), (**b**) peroxidase (POD), and (**c**) catalase (CAT) activities in two rocket plant accessions, Eruca-34961 and Eruca-34936 under drought stress. Different lowercase letters show statistically substantial differences among treatments, as determined by Tukey's HSD test at $P < 0.05$. Abbreviations: NS = no spray; SLs = strigolactones; WHC = water holding capacity

Multivariate analysis

To assess the physio-biochemical responses of Eruca accessions to GR24 treatments under drought, Pearson correlation analysis and principal component analysis (PCA) were executed. Both positive and negative correlations were identified among various measured traits in the two rocket plant accessions subjected to drought and three concentrations of GR24. The correlation matrix showed positive correlations among growth parameters, pigment content, antioxidant activity, and protein content, and negative correlations with oxidative stress markers. This analysis confirmed that GR24 application alleviated drought-induced oxidative stress and promoted overall plant performance through coordinated enhancement of physiological and biochemical traits (Fig. 5).

The correlation pattern among various physiological and biochemical attributes of the two Eruca accessions was further elucidated through PCA. As illustrated in Fig. 6, the PCA biplot represents the distribution of treatments under drought conditions combined with GR24 foliar applications. The analysis presented that the first two principal components, Dim 1 and Dim 2, accounted for 92.4% of the total variance among the treatments. Dim 1, contributing 52% of the variation, was strongly and positively associated with growth parameters, photosynthetic pigments, osmolyte accumulation, and antioxidant enzyme activities highlighting the substantial impact of GR24 in promoting drought resilience. In contrast, Dim 2, explaining 40.4% of the variation, was negatively associated with oxidative stress indicators, suggesting a decline in oxidative damage with GR24 application. These findings emphasize the efficacy of GR24, particularly at 5.0 µM, in improving physiological performance and stress tolerance under drought conditions (Fig. 6).

Discussion

This study demonstrated that applying GR_{24} on rocket plant leaves can mitigate drought damage by increasing chlorophyll levels, improving gas exchange efficiency, and enhancing osmolyte accumulation. These benefits highlight GR24's protective role against water deficit stress, as it plays vital role in regulating developmental processes and signaling networks to help plants cope with various stresses and enhance their survival rates (Nian *et al.* 2022). Since the discovery of SL as plant hormones in 2008, considerable advances have been achieved in elucidating their biosynthetic pathways, modes of transport, and mechanisms of perception (Wang *et al.* 2020). Key components such as DWARF14 (D14), MORE AXILLARY GROWTH 2 (MAX2) and SUPPRESSOR OF MAX2-LIKE (SMXL6, SMXL7, SMXL8) proteins have been identified in model plants like *Arabidopsis thaliana* and rice to mediate SL responses at the molecular level, integrating developmental cues with environmental stress responses (Li *et al.* 2019). Our findings suggest that SLs interact synergistically with other phytohormones, especially abscisic acid (ABA) and cytokinins (CKs), to strengthen drought tolerance mechanisms in plants. GR24 treatment likely increased ABA sensitivity, leading to improved *WUE* by reducing stomatal conductance (Korek and Marzec 2023). Strigolactones also play a role in hormonal crosstalk involving auxin, ethylene, and CKs to optimize plant adaptation to stress (Ameen *et al.* 2025). Drought stress negatively impacted rocket plant growth and physiological functions by causing oxidative damage and disrupting photosynthesis and cellular balance (Ma *et al.* 2017). Our findings clearly demonstrate that drought stress significantly impairs rocket plant growth by inducing oxidative stress,

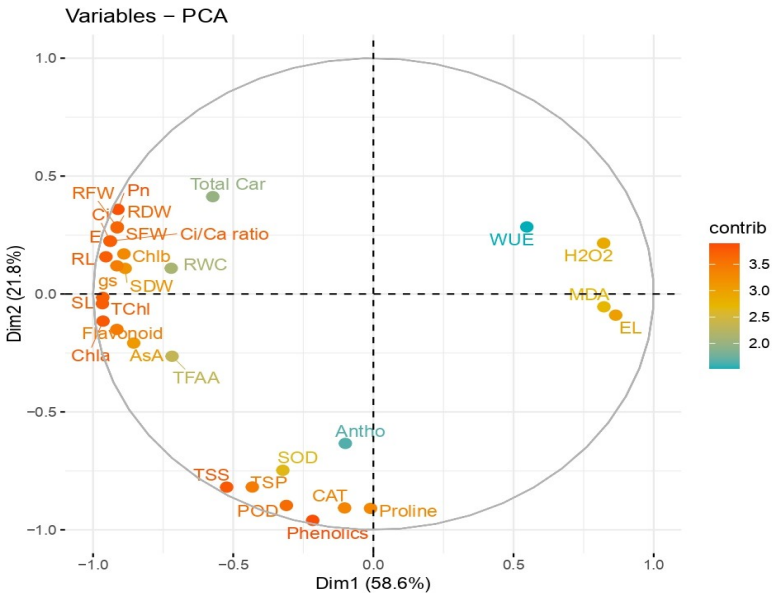

Fig. 5: Principal component analysis (PCA) of physio-biochemical attributes in rocket plant accessions under drought stress. Abbreviations: SL-shoot length; RL-root length; SFW-shoot fresh weight; SL-shoot length; RL-root length; SFW-shoot fresh weight; RFW-root fresh weight; SDW-shoot dry weight; RDW-root dry weight; Chla-chlorophyll a; Chlb-chlorophyll b; TChl-total chlorophyll; Car-carotenoids; RWC-relative water content; EL-electrolyte leakage; MDA-malondialdehyde; H_2O_2-hydrogen peroxide; ASA-ascorbic acid; TFAA-total free amino acids; TSP-total soluble protein; TSS-total soluble sugar; SOD-superoxide dismutase; POD-peroxidase; CAT-catalase; Antho-anthocyanin; *Pn*-net photosynthetic rate; *gs*-stomatal conductance; *E*-transpiration rate, *Ci*-intercellular CO_2 concentration, *Ci/Ca*-ratio-ambient CO_2 concentration/intercellular CO_2 concentration and *WUE*-water use efficiency

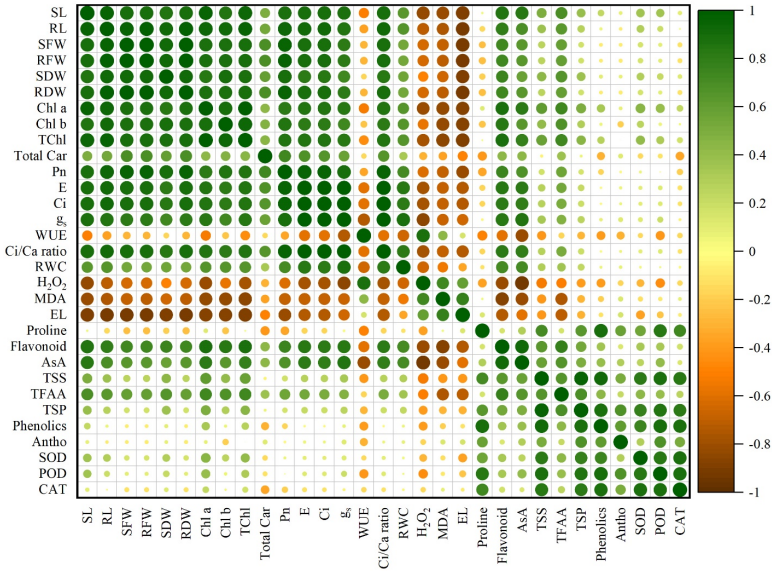

Fig. 6: Correlation matrix of physio-biochemical attributes in rocket plant accessions under drought stress. Abbreviations: SL-shoot length; RL-root length; SFW-shoot fresh weight; SL-shoot length; RL-root length; SFW-shoot fresh weight; RFW-root fresh weight; SDW-shoot dry weight; RDW-root dry weight; Chla-chlorophyll a; Chlb-chlorophyll b; TChl-total chlorophyll; Car-carotenoids; RWC-relative water content; EL-electrolyte leakage; MDA-malondialdehyde; H_2O_2-hydrogen peroxide; ASA-ascorbic acid; TFAA-total free amino acids; TSP-total soluble protein; TSS-total soluble sugar; SOD-superoxide dismutase; POD-peroxidase; CAT-catalase; Antho-anthocyanin; *Pn*-net photosynthetic rate; *gs*-stomatal conductance; *E*-transpiration rate, *Ci*-intercellular CO_2 concentration, *Ci/Ca*-ratio-ambient CO_2 concentration/intercellular CO_2 concentration and *WUE*-water use efficiency

which disrupts key physiological processes such as photosynthesis and turgor maintenance. This stress was primarily attributed to ultrastructural alterations in root cells, consistent with previous reports (Luqman *et al.* 2023). However, foliar application of GR24 successfully alleviated these negative impacts and supported improved growth

performance in rocket plants (Table 2). In agreement with Luqman *et al.* (2023), the observed improvement is likely due to GR24's ability to decrease oxidative damage, thereby improving physiological performance under drought conditions. Furthermore, Pearson's correlation analysis showed a substantial negative association between growth-related traits and oxidative stress indicators under drought conditions, reinforcing the protective role of GR24 in alleviating drought-induced oxidative damage (Fig. 6).

Drought stress negatively affects plant processes at molecular, physicochemical, and morphological levels (Naeem *et al.* 2012). Chlorophyll pigments are crucial for photosynthesis, playing a significant role in light absorption and the production of reducing agents, reflecting the plant's photosynthetic capacity (Sarwar and Shahbaz 2020). SL regulate genes related to light absorption in tomatoes and modify the physiology of photosynthetic pigments in *Arabidopsis* leaves to enhance energy capture (Wahab *et al.* 2022). Under stress conditions, SL can enhance physiological functions such as *Pn*, *E*, *gs*, and *Ci*, ultimately leading to improved photosynthetic efficiency (Ling *et al.* 2020). SL also help maintain photosystem II stability, enhance electron transport, increase ATP and NADPH demand, improve photosystem efficiency, and promote plant growth. Studies on *Arabidopsis* mutants suggest that SL can enhance drought tolerance alone or in combination with ABA. Reduced chlorophyll levels may result from chloroplast membrane disorganization and lipid droplet formation, while limited CO_2 access can lead to stomatal closure and reduced photosynthesis (Bhoi *et al.* 2021). SL enhance gas exchange in grapevines by upregulating F-box Max2 proteins involved in SL biosynthesis and ABA signaling, crucial for regulating stomatal function and gas exchange during drought (Wu *et al.* 2022). Our findings demonstrated a notable decline in chlorophyll content under drought stress, aligning with the observations reported by Luqman *et al.* (2023). However, the application of GR24 enhanced chlorophyll content in both rocket plant accession (Table 3). The positive association between GR24 application and chlorophyll levels underscores its role in counteracting the detrimental influences of drought on the photosynthetic apparatus. Similar effects have been reported in maize seedlings treated with growth promoters previously (Luqman *et al.* 2023).

Drought stress increases oxidative stress markers like MDA, indicating elevated lipid peroxidation in plant cell membranes. This damage is caused by drought-induced oxidative stress, affecting unsaturated fatty acids and leading to membrane instability and impaired cellular function (Ashraf *et al.* 2023). Applying GR24 to leaves during drought conditions effectively reduces MDA levels, suggesting that GR24 enhances antioxidant defenses and improves membrane stability (Ameen *et al.* 2025). Our study confirms that exogenous GR24 significantly declines the harmful effects of drought in rocket plant. Similar results have been reported in *Salvia nemorosa*, where GR24 treatment resulted in lower levels of H_2O_2 and MDA, indicating reduced oxidative stress (Fig. 3). Additionally, Pearson's correlation analysis showed a strong inverse relationship between GR24 application and oxidative stress markers, highlighting its protective role under drought conditions (Fig. 5).

Research suggests that SL mutant plants are associated with stress tolerance mechanisms in various plant species. The exogenous GR24 has been shown to increase the drought tolerance in maize by enhancing physiological and biochemical responses (Luqman *et al.* 2023). Non-enzymatic antioxidants such as leaf ascorbic acid, anthocyanin, flavonoids, TSS, TFAA and phenolics play a crucial role in helping plants combat stress by neutralizing free radicals (Velderrain-Rodríguez *et al.* 2014). These compounds are produced as secondary metabolites in plants and aid in mitigating oxidative stress. In our study, external application of GR24 significantly increased the content of leaf ascorbic acid, anthocyanin, flavonoids, phenolics, TSP, TSS and TFAA under drought conditions (Table 4). This is consistent with previous research in maize seedlings treated with GR24 (Luqman *et al.* 2023). This study indicates that the application of SL exerted a stronger beneficial effect on plants experiencing drought stress. The precise mechanism by which GR24 influences these secondary metabolites in rocket plant leaves remains unclear, however, due to their redox-active nature, phenolics and flavonoids are widely recognized for their strong antioxidant properties (Velderrain-Rodríguez *et al.* 2014). They can disrupt chain reactions by donating electrons, making them effective antioxidants.

Organic osmolytes and antioxidant properties are essential for protecting plants from drought stress by enhancing water and osmotic stress tolerance (Sharma *et al.* 2022). The buildup of osmoprotective compounds in cells is a key indicator of stress tolerance in plants (Blum 2017). Our study found that the application of GR24 increased free proline levels, consistent with previous research in maize (Luqman *et al.* 2023). External sources of growth promoters can improve the osmotic adjustment capacity of plants under water-deficient conditions by regulating osmo-regulators (Gupta *et al.* 2014). Proline accumulation under drought stress helps shield plants from oxidative damage by stabilizing membranes and enzymes, especially in young tissues (Khan *et al.* 2015). Proline acts as an osmoprotectant and stabilizes the photosynthetic machinery of plants against ROS (Khan *et al.* 2015). Elevated proline levels under drought stress are crucial for protecting plants from oxidative stress and maintaining cellular structures (Liu *et al.* 2020).

The evidence suggests that the SL application had a stronger positive impact on both rocket plant accessions when water was reduced in the experimental pots. However, further trials on different crops are needed to make field-level recommendations. More extensive experimentation, including studies on hormone biosynthesis, crosstalk, and gene expression in various crops, could provide valuable insights.

Investigating the molecular interactions of this phytohormone could offer a deeper understanding of its effects.

Conclusion

This study showed that drought stress negatively affects rocket plants by increasing oxidative stress, disrupting physiological functions, and reducing biomass and chlorophyll content. The application of GR24 on leaves significantly mitigated these effects by improving photosynthesis, osmotic adjustment, and antioxidant defenses. GR24 enhanced cellular protection by increasing compatible solute levels and boosting antioxidant enzymes, maintaining membrane integrity and promoting growth during drought stress. These results indicate that GR24 could be a valuable tool for enhancing drought tolerance in rocket plants. Future research should focus on optimizing GR24 application methods and dosages, testing its effectiveness in other crops, and understanding its molecular mechanisms. Field trials and environmental impact assessments are necessary to support its sustainable use in agriculture. Overall, GR24 shows promise for improving drought resilience and ensuring food security in water-limited conditions.

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Author Contributions

FA, IH, MI and MAA contributed equally to this research. FA conceptualized the study, performed data analysis, and prepared the initial draft of the manuscript. IH contributed significantly to the study design and assisted in the development and revision of the manuscript. MI and MAA supported data collection and critically revised the manuscript. All authors reviewed and approved the final version for submission.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this study.

Data Availability

The data presented in this study will be available upon request to the corresponding author.

Ethical Approval

This study did not involve any experiments on human participants or animals.

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