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Plant Growth Regulators Mediated Changes in the Growth, Photosynthesis, Nutrient Acquisition and Productivity of Mustard

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Abstract: Plant growth regulators (PGRs) are naturally occurring signaling molecules that modulate numerous phenological traits and physicochemical features of plants throughout their life cycles. Exogenous supplementation of PGRs is an effective strategy for improving the productivity of important agricultural crops. This research was planned to evaluate the effects of six PGRs, namely indole-3-acetic acid (IAA), 24-epibrassinolide (EBL), gibberellic acid (GA3), putrescine (put), salicylic acid (SA) and triacontanol (Tria), on morphology, photosynthesis, nutrient acquisition, and the yield and quality characteristics of three mustard cultivars, i.e., Chutki, Nath Sona, and Rohini. Two foliar sprays each of water, IAA (10−6 M), EBL (10−6 M), GA3 (10−5 M), put (10−3 M), 10−5 M SA, and Tria (10−6 M) were applied to plants at fifty and seventy days after sowing (DAS). The crops’ phenological, physicochemical and microscopic parameters were evaluated at ninety DAS, and yield characteristics were evaluated at harvest (120 DAS). The observations of this study indicated that foliar feeding with PGRs increased all studied parameters, relative to water-spray treatment. The Nath Sona cultivar displayed a stronger response than Rohini and Chutki. Among the leaf-applied PGRs, 24-EBL, followed by IAA and GA3, proved the most effective and improved all the studied parameters. Moreover, the exogenous application of PGRs, especially EBL, significantly enhanced stomatal dimensions and root cell longevity. Treatment with EBL enhanced plant dry weight by 34.7, 35.4, and 37.6%, the net photosynthetic rate by 65.3, 64.7, and 60.2%, seed yield per plant by 67.1, 65.2, and 67.3%, and oil yield per plant by 42.6, 48.2, and 41.1%, in the Chutki, Nath Sona, and Rohini cultivars, respectively, relative to the water-spray treatment. It may be concluded that of the tested PGRs, 24-EBL proved most effective at enhancing the morphological, physicochemical, and yield features of the mustard cultivars.

Keywords: growth; microscopy; mustard; photosynthesis; plant growth regulators; yield

1. Introduction

Oilseed crops are extensively grown throughout the world for the production of edible oils, and are ranked fourth place in terms of their importance as food commodities, relative to other crops. The demand for oilseed crops regularly increases due to population surges, dietary choices, and biodiesel product requirements. Oilseed crops are the foremost source of vegetable oil due to their use for culinary purposes. Moreover, oilseed crops are a substantial component of human nutrition and feed for animals [1]. Mustard (Brassica juncea L.) is a crucial agricultural crop, grown in arid and/or semi-arid regions, and is the predominant rapeseed-mustard crop cultivated in India [1], where it is ranked second
among edible oilseed crops because of its uses for culinary purposes and in biodiesel production [2]. Mustard oil contains omega fatty acids, saturated fats, thiamine, ascorbic acid, iron, potassium, calcium, riboflavin, and β-carotene, which make it more valuable than other edible oils [3]. The green leaves of this herb are commonly used as a vegetable. The seeds of mustard are used for making pickles, soups, stews, and hair oil throughout the world. Due to the commercial importance of mustard, increasing its production is highly desirable. In this regard, mineral nutrients and plant hormones/plant-growth promoters may have roles in enhancing mustard growth and oil production [4]. Mustard cultivation is profitable, but the productivity is currently insufficient to meet the needs of the people. Efforts are currently being made by scientists to enhance the cultivation and production of edible oilseed crops, including mustard. Of these efforts, supplementation of plant growth regulators (PGRs) has been recognized as an efficient approach that has been shown to improve several crop plants growth, physicochemical characteristics, yield, and quality [4,5].

PGRs are small signaling molecules that profoundly modulate the growth and development of crop plants, affecting cell division and enlargement, growth traits, metabolic processes, vascular patterning, flowering, and fruit and seed development [5,6]. PGRs are well recognized for enhancing photoassimilates partitioning, nutrient acquisition, stress tolerance, membrane permeability, and stability, etc. [5]. The foliar feeding of phytohormones is an efficient method for providing growth stimulants at crucial times in order to trigger developmental phases and obtain higher production. The use of PGRs, such as indole-3-acetic acid (IAA), 24-epibrassinolide (EBL), gibberellic acid (GA₃), putrescine (put), salicylic acid (SA), and triacontanol (Tria), is a well-known strategy for ensuring efficient production of crops, including oilseed [5]. The PGR, IAA stimulates cell elongation, apical dominance, embryogenesis, organogenesis, root initiation, and flowering [7]. The PGR, 24-EBL is a vital analog of brassinosteroids, recognized to modulate many morpho-physiological processes of crop plants, including seed germination, cell elongation, vascular tissue differentiation, pollen and flower development, and fruit maturation [8]. GA₃ is an essential PGR that controls many biological functions, such as seed germination, stem elongation, enzyme induction, flowering, and fruit senescence, throughout the life cycle of plants [9]. The PGR, put is a polyamine that is involved in growth and several other physiological processes of plants [10]. SA is a key PGR that regulates numerous plant morphological and physio-biochemical processes [11]. Tria is known for enhancing photosynthetic efficiency, carbon and nitrogen metabolism, secondary metabolite production, essential oil biosynthesis, and abiotic stress tolerance [12]. In this context, there are many previous studies in which the exogenous application of PGRs has improved growth, physiological processes, and production of various crop plants [13–16]. However, there is still a considerable need to research the comparative effects of leaf-applied PGRs on the growth and productivity of oilseed crops, including mustard.

Taking into consideration the importance of mustard crops and the eco-friendly and beneficial role of PGRs, the present experiment was conducted with a factorial randomized design, to assess the effects of six selected leaf-applied PGRs (IAA, 24-EBL, GA₃, put, SA, and Tria) on the phenological traits, physio-biochemistry, yield, and quality of three mustard cultivars.

2. Material and Methods

2.1. Plant Material

Based on our previous experiment [1], the seeds of three high-yielding cultivars of *Brassica juncea* L., i.e., Chutki, Nath Sona, and Rohini, were purchased from the Aligarh market. Care was taken to select seeds of high quality for the experiment. Before sowing, seeds were decontaminated with HgCl₂, and then thoroughly cleaned with distilled water to eliminate any HgCl₂ adhering to the seeds.
2.2. Experimental Setup and Treatment Plan

The present experimental work was conducted during the rabi (winter) season under natural environmental conditions (27°88′ N latitude, 78°08′ E longitude, and 184 m altitude above sea level; the annual average temperature is between 28 and 33 °C, the average temperature of winter varies from 12 to 16 °C and the relative humidity ranges from 56 to 77%) in the Botany Department, Aligarh Muslim University, Aligarh, India. Earthen pots (25 × 25 cm) were packed with sandy loamy soil and organic manure at a 4:1 ratio. A basal recommended dose of nutrients, such as nitrogen (N) in the form of urea, phosphorus (P) in the form of superphosphate, and potassium (K) in the form of muriate of potash, at 80 kg N, 40 kg P₂O₅ and 40 kg K₂O/ha, i.e., 36 mg N, 17.9 mg P₂O₅ and 17.9 mg K₂O/kg, were applied at the time of sowing [1]. Later, seeds were uniformly sown in the pots at a depth of 4–5 cm. On the basis of previous finding from Shah et al. [1], the two time-points i.e., fifty and seventy days after sowing were selected as the appropriate times for foliar feeding. The plants were sprayed twice with water, 10⁻⁶ M IAA, 10⁻⁶ M EBL, 10⁻⁵ M GA₃, 10⁻³ M put, 10⁻⁵ M SA and 10⁻⁶ M Tria on 20-days interval. The selected PGRs and their concentrations were decided based on studies [17–22]. Sampling was performed at ninety days after sowing, in order to study morphological and physicochemical parameters, and conduct microscopic analysis. The yield features were studied at 120 DAS (harvest). The mustard plant growth stages are mentioned in Figure 1.

![Figure 1](image_url)

**Figure 1.** A diagrammatic overview of the growth stages of mustard (BBCH staging system).

2.3. Morphological Traits

Plants from each specified treatment were taken and washed with running tap water, and then the shoot lengths (SL) of the plants were determined in cm with the aid of a measuring scale. The branch and leaf numbers of the plants were counted in terms of number. The fresh weight (FW) of the plants was determined in g using a digital balance, and then the plants were oven-dried to calculate the dry weight (DW) in g. The areas of leaves were estimated in cm² with the help of graph paper, and then the leaf areas of the plants were obtained by multiplying the leaf number by the area per leaf, calculated in cm².

2.4. Chlorophyll Content and Photosynthetic Parameters

A chlorophyll-measuring tool (SPAD-502, Konica Minolta, Inc., Osaka, Japan) was used to assess the chlorophyll (Chl) content of green leaves. Leaf gas exchange attributes were assessed at 11:30 am on a sunny day, using expanded leaves of treated plants, as well as those of control plants, with an Infrared Gas Analyzer (LI-COR-6400, Lincoln, NE, USA) under suitable environmental conditions: 23 °C temperature, 58% average relative humidity, 690 µmol m⁻² s⁻¹ photosynthetic active radiation, and 374 µmol mol⁻¹ atmospheric CO₂ concentration.

2.5. Relative Water Content (RWC)

Fresh leaves were taken from experimental plants and cut into fragments, eliminating the midrib. These fragments were weighed to determine the fresh mass, and then soaked in distilled water for twenty hours in the dark. Water droplets adhering to the leaf discs were removed and the turgor mass was noted. The leaf discs were then dehydrated at 80
\[ \text{Relative water content} = \frac{\text{Fresh mass} - \text{Dry mass}}{\text{Turgor mass} - \text{Dry mass}} \times 100 \]

2.6. Leaf Carbonic Anhydrase (CA) Activity

Leaf CA activity was determined using the protocol defined by Dwivedi and Randhawa [24]. Leaves were carved into sections, transferred to test tubes containing cysteine hydrochloride solution (10 mL, 0.2 M), and left for 20 min. The leaf pieces were soaked and put into a test tube containing sodium bicarbonate solution, phosphate buffer (pH 6.8), and bromothymol blue. The test tube was shaken gently and left for 20 min at 4 °C. The product of the reaction was titrated in the presence of 0.05 N HCl, using methyl red as an indicator. The activity of CA was recorded as mol CO\(_2\) kg\(^{-1}\) (leaf FM) s\(^{-1}\).

2.7. Leaf Nitrate Reductase (NR) Activity

The NR activity of leaves was assessed using the method of Jaworski [25]. Leaves taken from experimental plants were carved into sections and placed into test tubes containing phosphate buffer (pH 7.5), then \(\text{KNO}_3\) and isopropanol solutions were added. The test tubes were then incubated at 30 °C for two hours, and N-1-naphthylethylenediamine dihydrochloride and sulfanilamide solution were then added. The optical density of the color was measured at 540 nm. The activity of NR was recorded as nmol CO\(_2\) kg\(^{-1}\) (leaf FM) s\(^{-1}\).

2.8. Leaf Nitrogen, Phosphorous, and Potassium contents

Fresh leaves were plucked and then dried in a hot air oven at 80 °C for twenty-four h. The dried leaves were ground into a powder, and then approximately 100 mg of the leaf powder was put into a test tube, to which was added 2 mL of concentrated H\(_2\)SO\(_4\). The test tube contents were heated on a Kjeldahl assembly (temperature-controlled) for approximately two hours and then cooled for approximately 15 min at room temperature. To this solution was added 0.5 mL of 30% H\(_2\)O\(_2\), in a drop-by-drop fashion, and then heating and cooling was repeated until the color of the solution had become clear. The processed leaf material was then transferred into test tubes, and the required volume was reached by adding distilled water. Leaf N and P content was analyzed following the standard protocols of Lindner [26] and Fiske and Subba Row [27], respectively. Leaf K content was assessed with the aid of a flame-photometer (Model-381, ESICO, Parwanoo, India) by following the method of Hald [28].

2.9. Scanning Electron Microscopy (SEM)

Experimental plant leaves were collected and the samples were prepared for SEM according to the procedure adopted in our previous study [1]. Finally, stomatal opening images were taken using SEM (JSM-6510 LV, JEOL, Tokyo, Japan).

2.10. Confocal Laser Scanning Microscopy (CLSM)

The viability of root cells was visualized using the procedure of Rattan et al. [29]. Thin, longitudinal sections of mustard roots were placed in propidium iodide (PI) solution (25 \(\mu\)M) for 30 min. Roots stained with PI were then arranged on microscopic slides, and observations were made using a CLSM (LSM 780, ZEISS, Germany).

2.11. Yield and Quality Features

Yield and quality traits such as pod number/plant, seeds/pod, 1000-seed weight, seed yield/plant, oil yield/plant, and oil content were evaluated at the harvest. The oil yield/plant was estimated on the basis of the oil percentage and seed yield/plant. For oil content (crude fat) estimation, collected seeds were dried and ground into a powder.
The seed powder (10 g) was transferred to the Soxhlet apparatus, the required quantity of petroleum ether was added, and then the Soxhlet apparatus was kept running for six hours. When the extraction process was complete, the seed extract was left in the open air to allow evaporation of the petroleum ether. After this process, the resulting oil was weighed and used to calculate the oil content using the following formula:

\[
\text{Oil content (\%) = } \frac{m_o}{m_s} \times 100.
\]

where \(m_o\) = mass of oil and \(m_s\) = seed mass, in g.

2.12. Statistical Analysis

Two-way analysis of variance (ANOVA) was used for data analysis at \(p \leq 0.05\), using OPSTAT statistical software (CCS HAU, Hisar, India). The differences between various treatments were investigated using Duncan’s multiple range test, using SPSS 16.0 software (Windows 11). The principal component analysis (PCA) was performed with OriginPro. The Pearson correlation matrix of various parameters was analyzed using R software.

3. Results

The observations of the current research study revealed that the effects of spraying PGRs on the mustard cultivars were significant for all characteristics studied, except for the leaves and branches/plant, RWC, and seeds/pod. The cultivar Nath Sona, relative to Rohini and Chutki, proved more responsive to PGR treatment.

3.1. Morphological Traits

Of the PGRs tested, EBL proved to be the most effective, and significantly improved all growth attributes of mustard. The effects of EBL were followed by SA. The foliar application of EBL increased SL by 31.2, 35.3, and 28.4%, branches per plant by 30, 36.3, and 33.3%, leaves per plant by 11.4, 10.8, and 9.2%, area per leaf by 8.4, 8.7 and 7.4%, leaf area per plant by 20.7, 27.8, and 22.9%, FW by 9.9, 12.6, and 10.4%, and DW by 34.7, 35.4, and 37.6%, in the Chutki, Nath Sona and Rohini cultivars, respectively, relative to water-spray treatment (Figures 2A–F and 3A).

3.2. Physio-Biochemical Attributes

3.2.1. Chlorophyll Content and Gas Exchange Parameters

The data (Figure 3B–F) showed that PGR treatment significantly enhanced chlorophyll content and gas exchange parameters, relative to the water-spray treatment. The exogenous application of EBL, followed by GA\(_3\), IAA, and SA, proved best for photosynthetic pigment and gas exchange parameters. Foliar feeding of EBL enhanced the leaf Chl content by 15.4, 20.4, and 18.2%, the net photosynthetic rate (\(P_N\)) by 65.3, 64.7, and 60.2%, the stomatal conductance (\(g_s\)) by 86.6, 79.2, and 71%, the intercellular CO\(_2\) concentration (\(C_i\)) by 3.9, 11.5, and 5.4%, and the transpiration rate (\(E\)) by 4.9, 7.2, and 6.6%, in the Chutki, Nath Sona and Rohini cultivars, respectively, relative to the water-spray treatment.
Figure 2. Effect of foliar spray of PGRs on (A) Shoot length per plant, (B) Branches per plant, (C) Leaves per plant, (D) Area per leaf, (E) Leaf area per plant and (F) Fresh weight per plant of the mustard cultivars Nath Sona, Rohini and Chutki. Data are treatment means with the standard error (±SE) of four replicates. The different small and capital letters represent a significant difference in three cultivars among various treatments confirmed through Duncan’s Multiple Range test at p ≤ 0.05.
Figure 3. Effect of foliar spray of PGRs on (A) Dry weight per plant, (B) Chlorophyll content, (C) Net photosynthetic rate, (D) Stomatal conductance, (E) Inter-cellular CO$_2$ concentration and (F) Transpiration rate of the mustard cultivars Nath Sona, Rohini and Chutki. Data are treatment means with the standard error (±SE) of four replicates. The different small and capital letters represent a significant difference in three cultivars among various treatments confirmed through Duncan’s Multiple Range test at $p \leq 0.05$. 
3.2.2. Relative Water Content (RWC)

Among the leaf-applied PGRs, EBL proved to be the most effective, and resulted in a higher value for leaf RWC; following EBL in effectiveness were Tria, SA, and GA3. Spray treatment with EBL improved RWC by 12.8, 11.5, and 12.5% in the Chutki, Nath Sona, and Rohini cultivars, respectively, relative to the control plants (Figure 4A).

Figure 4. Effect of foliar spray of PGRs on (A) Relative water content, (B) Carbonic anhydrase activity, (C) Nitrate reductase activity, (D) Nitrogen content, (E) Phosphorous content and (F) Potassium content of the mustard cultivars Nath Sona, Rohini and Chutki. Data are treatment means with the standard error (±SE) of four replicates. The different small and capital letters represent a significant difference in three cultivars among various treatments confirmed through Duncan’s Multiple Range test at \( p \leq 0.05 \).
3.2.3. Carbonic Anhydrase (CA) Activity

Spray treatment with EBL, followed by SA, resulted in a higher value for leaf CA activity. The EBL spray increased CA activity by 48.9, 60.8, and 53.5%, in the Chutki, Nath Sona, and Rohini cultivars, respectively, relative to the control plants (Figure 4B).

3.2.4. Nitrate Reductase (NR) Activity

The data (Figure 4C) indicated that spray treatment with EBL, followed by GA\textsubscript{3} and IAA, resulted in the highest value for leaf NR activity. The leaf-applied EBL increased leaf NR activity by 21.3, 24.9, and 25.9% in the Chutki, Nath Sona, and Rohini cultivars, respectively, relative to the water-sprayed plants.

3.2.5. Mineral Nutrient Content

Spray treatment with PGRs, especially EBL, followed by SA and Tria, significantly enhanced leaf mineral nutrient content. The leaf-applied EBL increased N content by 10.9, 15.4, and 13.3%, P content by 7.9, 15.2, and 10.7%, and K content by 11.8, 15.7, and 13.4% in the Chutki, Nath Sona, and Rohini cultivars, respectively, relative to the water-sprayed plants (Figure 4D–F).

3.2.6. Yield and Quality Characteristics

The PGRs not only enhanced growth and the photosynthetic parameters of mustard plants, but also improved the yield characteristics. Among leaf-applied PGRs, EBL was the most effective, resulting in the highest yield characteristics values. IAA and GA\textsubscript{3} followed EBL in effectiveness. The foliar application of EBL increased the number of pods per plant by 8.9, 11.2, and 10%, seeds per pod by 69, 52, and 57.4%, 1000-seed weight by 15.7, 24.7, and 19.8%, seed yield per plant by 67.1, 65.2, and 67.3%, oil content by 46.3, 45.4, and 46.3%, and oil yield per plant by 42.6, 48.2, and 41.1% in the Chutki, Nath Sona, and Rohini cultivars, respectively, relative to the water-sprayed plants (Figure 5A–F).

3.3. Microscopic Studies

Based on growth and physio-biochemical analysis, the best-performing cultivar, Nath Sona, was selected for microscopic studies.

3.3.1. Scanning Electron Microscopy (SEM)

SEM showed that the leaves of plants treated with PGRs had higher stomatal aperture widths relative to the control plants, with EBL treatment having the greatest impact, followed by GA\textsubscript{3}, IAA, and SA. The stomatal aperture width was greater in plants treated with EBL than in the water-treated plants (Figure 6A–G).
Figure 5. Effect of foliar spray of PGRs on (A) Pods per plant, (B) Seeds per pod, (C) 1000-seed weight, (D) Seed yield per plant, (E) Oil content, and (F) Oil yield per plant of the mustard cultivars Nath Sona, Rohini, and Chutki. Data are treatment means with the standard error (±SE) of four replicates. The different small and capital letters represent a significant difference in three cultivars among various treatments confirmed through Duncan’s Multiple Range test at \( p \leq 0.05 \).
followed by GA3, IAA, and SA. The stomatal aperture width was greater in plants treated with EBL than in the water-treated plants (Figure 6A–G).

3.3.2. Confocal Laser Scanning Microscopy (CLSM)

Propidium iodide, a red-fluorescent dye, enters through the plasma membrane of dead cells, intercalates into DNA, resulting in red, illuminated spots. In the current study, treatment with PGRs improved root cell viability relative to water-treated plants. Treatment with EBL was more effective than with the other PGRs and resulted in a higher number of viable cells relative to the water-spray treatment (Figure 7A–G).

Figure 6. Scanning electron microscope images (at 4000 × magnification) of the stomatal response of leaves of the Nath Sona mustard cultivar at 90 DAS. Results for the (A) Control and (B–G) plants treated with EBL, GA3, IAA, SA, put and Tria.

Figure 7. Confocal microscopic images, assessing the viability of root cells of the Nath Sona mustard cultivar at 90 DAS. Results for the (A) Control and (B–G) plants treated with EBL, IAA, GA3, SA, put and Tria.
3.3.3. Principal Component Analysis (PCA)

Figure 8 shows a PCA biplot encompassing two components (PC1 and PC2) that cover 87.3% and 6.2% of the total variation in the studied parameters among the three cultivars. Positive correlations were observed between several traits, such as seed yield/plant, CA and NR activity, 1000-seed weight, NPK content, oil yield and content, Chl content, and gas exchange parameters. The correlation matrix (Figure 9) showed significant positive correlations between different parameters studied ($p \leq 0.05$).

Figure 8. The PCA biplot of PGR treatments and the various parameters studied for the three cultivars.
Figure 9. Pearson correlation matrix for the three cultivars and the various studied parameters. The symbol (*) represent significant level at $p \leq 0.05$. The narrow ellipses showed greater correlation between the variables and the wider and more rounded ellipses represented that variables are uncorrelated.

4. Discussion

Plant growth regulators are the chemical messengers that control many phenological, physiological, and metabolic features of plants. They are produced by cells and regulate cellular processes in other cells by interacting with receptor proteins through the signal transduction pathways within cells [5,15]. The exogenous application of PGRs is an emerging eco-friendly approach to influencing the morphogenesis, physicochemical attributes, and productivity of crop plants in a range of environmental conditions. Plant growth regulators are involved in enhancing the biological functioning of plants, for example by maintaining water availability, essential nutrient content, and enzymatic activity. The foliar application of PGRs for the purposes of boosting the productivity of agricultural crops has been widely explored [15,30,31].

In this study, different PGRs, such as IAA, EBL, GA$_3$, put, SA, and Tria, were investigated for their effects on morphological characteristics, photosynthesis, enzymatic activities, mineral nutrient contents, and the yields of three mustard cultivars. The results (Figures 2A–F and 3A) indicated that spraying plants with PGRs resulted in improvements in the SL of the plants, increased the numbers of branches and leaves of the plants, increased the area/leaf, leaf area/plant, FW, and DW/plant, relative to the controls. The enhancements in the growth characteristics of the plants following foliar application of PGRs, are
due to the diverse roles of PGRs in increasing cell division, cell enlargement/expansion, tissue differentiation, organ formation, vascular development, and biomass accumulation [5]. The increase in plant biomass caused by applying PGRs demonstrated their positive influences on other developmental stages that lead to higher productivity. The effects of PGRs on the growth traits of oilseed crops have been observed by many researchers, for instance, Peng et al. [32] and Mshelmbula et al. [33] sprayed IAA on groundnut and sesame, respectively, EBL was tested on mustard by Yusuf et al. [34] and Hussain et al. [35], GA$_3$ was tested on mustard by Kumari et al. [36], SA was tested on mustard by Yusuf et al. [37] and Islam and Mohammad [38], PGRs were also tested on mustard by Hussain et al. [20], and Tria was tested on wheat by Perveen et al. [39].

This experiment demonstrated that the supplementation of PGRs influences the leaf Chl content, $P_N$, $g_s$, $E$ (Figure 3B–F), RWC (Figure 4A), CA activity (Figure 4B), and stomatal width (Figure 6A–G) of treated plants, relative to the water-sprayed plants. The enhancement in Chl content might be due to the participation of plant hormones in the biosynthesis of chlorophyll during the plant life cycle. Moreover, PGRs might also be involved in increasing the activity of the δ-aminolevulinic acid dehydratase (ALAD) enzyme, decreasing the concentration of chlorophyll-degrading enzymes, and improving leaf mineral nutrient content, including that of magnesium, which leads to enhanced chlorophyll content in plants [1,5]. In the present work, the improvements in leaf photosynthetic parameters and CA activity may be due to PGR-mediated enhancement of chlorophyll, CA enzyme, and rubisco enzyme biosynthesis, ultimately influencing the photosynthetic efficiency of plants. CA is a key enzyme that participates in photosynthesis through its role in carbon fixation, i.e., conversion of CO$_2$ to HCO$_3^-$, and is also involved in regulating stomatal behavior in plant leaves. The rise in CA activity would enhance photosynthetic efficiency. Moreover, application of PGRs improved leaf RWC; this could be due to the prominent role of PGRs in enhancing the levels of osmoprotectants in leaves, which maintain turgidity, water balance, and ionic adjustments in plant cells. RWC is an important indicator of water status/water amount present in leaf tissues, which plants require for the performance of many metabolic activities. PGRs are essential for the biosynthesis of coenzymes, amino acids, and various other metabolites that play central roles in redox homeostasis, membrane stability, ionic balance and the metabolic balance of cells. Leaf RWC is also related to the leaf tissue growth rate, $g_s$, and $E$, which may have contributed to the improvements in overall metabolic processes [40]. In this study, the observed enlargement in the stomatal aperture width could be due to the PGR-regulated influx of K$^+$ into the guard cells and effects on the activity of H$^+$-ATPase, which regulates stomatal movement. The improvements in stomatal width and increased activity of CA caused by PGRs led to an enhanced photosynthetic rate. Our observations resemble the findings from the study on the effects of EBL on soybean by Ramesh and Prasad [41], of IAA and GA$_3$ on tomato plants by Siddiqui et al. [42] and Siddiqui et al. [43], of SA and put on soybean and mustard by Ghassemi-Golezani and Farhangi-Abriz [44], and Ghassemi-Golezani et al. [45], of EBL and put on mustard plants by Hussain et al. [20], of IAA on groundnut by Peng et al. [32], and of Tria on cucumber plants by Sarwar et al. [46].

The present study also found that root cell viability (Figure 7A–G) was increased by treatment with PGRs, relative to the water-spray treatment, as confirmed by confocal microscopic studies. This could be due to the triggering of cell signal transduction pathways by PGRs, resulting in cell proliferation, elongation, and differentiation, effects which are strongly associated with improved root growth and development [47]. Our study corroborates the findings of Islam and Mohammad [38] on the effects of PGRs on mustard. Furthermore, in our study, foliar-applied PGRs enhanced NR activity (Figure 4C) relative to water-spray treatment. For nitrogen accumulation by plants, NR is an important enzyme for reducing nitrate to nitrite, which is a key step in protein production for many crop plants. The enhancement in NR activity may be due to PGR-mediated influences on protein synthesis and enzymatic activities that increase the rate of reduction of nitrate. Similar effects have also been reported by Siddiqui et al. [48], Fariduddin et al. [49], and Nazar
et al. [50] regarding mustard. Furthermore, in this study, PGR application improved leaf N, P, and K content, relative to water-spray treatment (Figure 4D–F). Plants require certain mineral nutrients for their growth, development, and productivity. Such mineral nutrients are called essential nutrients. Optimum concentrations of these nutrients are required to regulate plant growth, mediating changes at the morpho-physiological, biochemical and molecular levels. Nitrogen is an essential micronutrient that participates in protein synthesis, enzymatic activities, and chlorophyll synthesis, enhancing photosynthesis and ultimately increasing the growth and yield of crop plants. Phosphorus is a major element that plays a fundamental role in physiological processes, particularly photosynthesis and respiration. Phosphorus is a component of many metabolic compounds, such as phosphonucleotides (ATP, UTP, CTP, GTP, ADP, and AMP), nucleic acids (DNA and RNA), enzymes (e.g., RuBisCO), cofactors (NADP and NADPH), and membrane phospholipids. Phosphorus is found in orthophosphate ions (H$_2$PO$_4^-$ and HPO$_4^{2-}$) in the soil. The phosphorous necessary for optimal growth is in the range of 0.3 to 0.5% of the dry weight of plants. Potassium is a key element for plant growth and development. Potassium is crucial for the movement of water, carbohydrates, and nutrients through plant tissue. Potassium is an activator of many enzymes, and is involved in protein synthesis, the transport of sugars, both N and C metabolism, and photosynthesis. The enhancement in mineral nutrient content could be due to PGR-mediated increases in their uptake, assimilation, and remobilization, through the stimulation of transporter activities, increased membrane permeability, and root growth. These nutrients are involved in many growth and developmental processes, including pigment synthesis, photosynthesis, enzyme activities, and the synthesis of proteins and other biomolecules. These results are also supported by the work of Islam and Mohammad [38] on the effects of PGRs on mustard.

Treatment with PGRs increased pods/plant, seeds/pod, 1000-seed weight, seed yield/plant, oil content, and oil yield/plant (Figure 5A–F), relative to the control. This may be due to PGR-mediated improvements in growth characteristics and physio-biochemical processes that result in higher productivity. Moreover, the enhancements in plant biomass accumulation, enzymatic activities, photosynthesis, and mineral nutrient content would positively impact yield traits and oil production. PGRs aid the synthesis of amino acids, chlorophyll, vitamins, protein, and oils, and also affect carbohydrate metabolism. PGR application augments oil synthesis and acetyl CoA content. Foliar treatment with PGRs, showed increases in seed yield, weight, oil percentage, and biological yield in oilseed crops had been investigated. The changes in yield features might be due to the higher values for morphological and photosynthetic traits, and the nutrient content of the plants. Our results are supported by those of Vekaria et al. [51] on the effects of IAA and GA$_3$ on sesame, Zafari et al. [52] on 24-EBL and safflower, Hasan and Ismail [53] on GA$_3$ and sunflower, Meena et al. [54] on SA and mustard, Mabudi Bilasvar et al. [55] on the effects of SA and put on mustard, Alamri et al. [56] on IAA and brinjal, and Ahmad et al. [57] on GA$_3$ and pea plants. Among the various PGRs, the stronger effects of 24-EBL could be due to its influences on gas exchange parameters, photochemical efficiency, activation of proton pumps, stimulation of protein and nucleic acid synthesis, and modulation of cell division and expansion, effects which would lead to better growth and productivity of plants.

5. Conclusions

It is well established that PGRs regulate a broad range of growth and developmental aspects of plants throughout their life cycles. PGRs are messenger molecules that control a number of characteristics related to stages of the plant life cycle, such as cell division and elongation, growth traits, metabolic processes, vascular patterning, flowering, and fruit and seed development. The findings of the present work indicate that foliar supplementation with PGRs proved beneficial in enhancing morphological, physio-biochemical, and yield characteristics of three cultivars of mustard. Moreover, microscopic analysis confirmed that PGR application influenced the stomatal dimensions and root cell longevity of the mustard plants. PGRs proved effective on growth parameters in the following order: EBL > IAA >
GA₃ > SA > put > Tria, and on gas exchange and yield attributes in the following order: EBL > IAA > GA₃ > SA > put > Tria. In summary, two foliar sprays of PGRs, particularly of EBL, proved effective in enhancing the growth and productivity of mustard cultivars, particularly for the Nath Sona cultivar. Foliar application of PGRs is an impressive strategy for enhancing the oil yield of edible oilseed crops.

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**References**


20. Hussain, A.; Nazir, F.; Fariduddin, Q. Polyamines (sperrmidine and putrescine) mitigate the adverse effects of manganese induced toxicity through improved antioxidant system and photosynthetic attributes in *Brassica juncea*. *Chemosphere* 2019, 236, 124830. [CrossRef]
26. Lindner, R.C. Rapid analytical methods for some of the more common inorganic constituents of plant tissues. *Plant Physiol.* 1944, 19, 76. [CrossRef]
42. Siddiqui, M.H.; Alamri, S.A.; Al-Khaishany, M.Y.; Al-Qutami, M.A.; Ali, H.M.; Khan, M.N. Sodium nitroprusside and indole acetic acid improve the tolerance of tomato plants to heat stress by protecting against DNA damage. *J. Plant Interact.* 2017, 12, 177–186. [CrossRef]


55. Mabudi Bilasvar, H.; Ghassemi-Golezani, K.; Mohammadi Nassab, A.D. Seed development, oil accumulation and fatty acid composition of drought stressed rapeseed plants affected by salicylic acid and putrescine. *Gesunde Pflanzen.* 2022, 74, 333–345. [CrossRef]


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