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Original Article

Cultivating Pharmaceutical Potential: Optimising Triacontanol Application to Enhance Linseed's Attributes under Drought Stress

Warda Javed[1]*, Hafsa Razzaq[2], Sundas Iftikhar[3], Amin Khalid[4], Safora Muhammad Shafique[5]

[1]Mphil Botany GCUF, Deptt of Botany, Government College University Faisalabad GCUF, Pakistan.

[2]M.Phil Botany, Deptt of Botany Mirpur University of Science and Technology, MUST, Pakistan. BS Botany (GC Women University Sialkot). B.ed (Allama Iqbal Open University Islamabad, AIOU), Pakistan.

[3]Masters of Philosophy in Botany, Deptt of Botany Mirpur University of Science and Technology, MUST, Pakistan. Masters of Science in Botany (University of Gujrat Hafiz Hayat Campus), Pakistan.

[4]BS Hons M.Phil Botany, Deptt of Botany Mirpur University of Science and Technology, MUST, Pakistan.

[5]BS Hons Botany, Department of Botany, GC Women University Sialkot, Pakistan.

[*]Corresponding Author: Warda Javed; Email: wardac836@gmail.com

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ABSTRACT

Background: Triacontanol (TRIA) has been identified as a potent plant growth regulator, demonstrating significant benefits under various stress conditions. However, its specific impact on Linum usitatissimum L. (flax) under drought stress conditions remains underexplored. This study investigates the effects of TRIA on the growth, physiology, and biochemistry of two flax genotypes subjected to drought stress, aiming to enhance our understanding of its potential as a biostimulant for improving crop resilience and productivity.

Objective: The primary objective of this study was to evaluate the effects of different concentrations of TRIA on the growth, physiological, and biochemical responses of two flax genotypes under drought stress conditions. We aimed to determine whether TRIA application could mitigate the adverse effects of drought and enhance plant performance.

Methods: This study was conducted using two flax genotypes, G-20888 and G-22186. Plants were grown under controlled conditions and subjected to drought stress by maintaining soil moisture at 50% field capacity. TRIA was applied in three concentrations: 0 M (control), 10^-6 M, and 10^-7 M. A completely randomized design was used with three replications for each treatment. Plant growth parameters, including root length, shoot length, plant height, and shoot fresh weight, were measured. Physiological parameters such as chlorophyll content, relative water content (RWC), and electrolyte leakage were assessed. Biochemical analyses included the estimation of antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase). Data were statistically analyzed using SPSS version 25.0.

Results: TRIA application significantly improved root length, shoot length, plant height, and shoot fresh weight in both flax genotypes under drought stress. For genotype G-20888, root length increased from 5.1 cm (control) to 6.8 cm (TRIA 10^-7 M). Shoot length showed a similar trend, with increases from 8.3 cm (control) to 10.1 cm (TRIA 10^-7 M). In G-22186, plant height increased from 70 cm (control) to 82 cm (TRIA 10^-7 M). Chlorophyll content increased by 15%, and RWC by 18% in TRIA-treated plants compared to controls. Antioxidant enzyme activities were also enhanced, with catalase activity increasing by 25% and peroxidase by 30%.

Conclusion: TRIA application significantly mitigated the adverse effects of drought stress on flax genotypes by enhancing growth, physiological, and biochemical parameters. The findings suggest that TRIA could be an effective biostimulant for improving drought tolerance in flax, potentially leading to better crop resilience and productivity.

Keywords: Triacontanol, Flax, Linum usitatissimum, Drought Stress, Plant Growth Regulator.

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INTRODUCTION

Linseed (Linum usitatissimum L.) is a significant biotech crop renowned for its multifaceted applications, including oil extraction from seeds and yarn production from stems. Historically, linseed has played a crucial role, with its usage in linen textiles dating back to ancient civilizations like Egypt and Greece. The crop's relevance continues into the modern era, primarily due to its seeds, which are a rich source of short omega-3 fatty acid chains and have been used in commercial applications such as varnishes and paints for centuries (1). The versatile nature of linseed extends to its protein content, which serves as a powerful multi-functional ingredient in food formulations due to its technical functionality, capacity for food preservation, and health benefits. This high nutrient and bioactive compound content makes linseed a valuable component of the human diet, particularly in the context of protein supplements (2).

Linseed is best suited for cultivation in fertile, smooth-textured, loamy soils. However, despite its robust nutritional profile, the crop is susceptible to various environmental stressors, including drought, which can significantly impact its growth and biochemical composition (3). Drought stress is a major concern in agriculture, capable of reducing crop yields by up to 70% globally (4). Understanding the physiological and biochemical responses of linseed to drought stress is critical for optimizing cultivation practices and enhancing crop resilience. In this context, the foliar application of plant growth regulators such as triacontanol (TRIA) has garnered attention. TRIA, a natural plant growth controller found in epicuticular waxes, is utilized to increase crop productivity across vast agricultural landscapes, especially in Asia (5). Previous research has demonstrated that TRIA can enhance various physiological efficiencies in plants, including increased yield, photosynthesis, protein synthesis, water and nutrient uptake, enzymatic activity, and the synthesis of essential oils (6).

The current study aims to investigate the influence of foliar-applied TRIA on two linseed genotypes (G-20888 and G-22186) under drought stress conditions. The primary objective is to assess how TRIA affects growth parameters such as shoot and root length, fresh and dry weight, leaf area, and leaf number, as well as biochemical attributes including vitamin content, antioxidant enzyme activity, mineral composition, seed oil content, and tocopherol levels (7). The field experiment was conducted at the Botanical Garden of Government College University Faisalabad during the 2017-2018 growing season. Two levels of drought stress were applied: a control group with normal irrigation and a treatment group with 50% reduced irrigation. Following germination, TRIA was administered as a foliar spray at three different concentrations (0, 10^-6 M, and 10^-7 M) during the vegetative growth stage (8).

This study revealed significant differences in the response of the two linseed cultivars to TRIA application under drought conditions. Notably, cultivar G-22186 exhibited superior growth performance and increased levels of antioxidant enzymes and tocopherols when treated with 10^-7 M TRIA, indicating enhanced stress tolerance compared to cultivar G-20888. These findings suggest that foliar application of TRIA, particularly at a concentration of 10^-7 M, can effectively mitigate the adverse effects of drought stress and promote drought tolerance in linseed crops (9). The implications of this research are profound, highlighting the potential of TRIA as a valuable agronomic tool for enhancing the resilience of linseed to environmental stressors. Future research should focus on elucidating the molecular mechanisms underlying TRIA-mediated drought tolerance and optimizing application parameters to maximize its efficacy in promoting crop resilience and productivity under diverse environmental conditions (10).

MATERIAL AND METHODS

The study was conducted to evaluate the influence of foliar-applied triacontanol (TRIA) on two linseed genotypes (G-20888 and G-22186) under drought stress conditions. The field experiment was carried out at the Botanical Garden of Government College University Faisalabad during the 2017-2018 growing season. Seeds for the experiment were provided by the supervisor and were sown following a completely randomized block design to ensure the reliability of the results.

The experimental design included two levels of drought stress, applied after two weeks of germination: a control group with normal irrigation and a treatment group with 50% reduced irrigation. Additionally, three levels of TRIA were applied as a foliar spray during the vegetative growth stage: 0 (no spray), 10^-6 M, and 10^-7 M. Data collection included various growth parameters such as shoot and root length, fresh and dry weight of shoots and roots, leaf area, and leaf number. The shoot length was measured using a centimeter scale, while the fresh weight of shoots and roots was determined with a digital electronic balance. After drying the plant material at 70°C for 48 hours, the dry weights were also recorded using a digital electronic balance.

Biochemical attributes were assessed to determine the effects of TRIA on the plants. The activity of ascorbate peroxidase (APX) was measured by monitoring the decrease in absorbance of ascorbate at 290 nm, following the protocol of Chen and Asada (1992). Guaicol peroxidase (GPX) activity was determined using a guaiacol reaction solution, and nitrate reductase (NR) activity was assessed by the formation of a pink diazocomplex, measured spectrophotometrically at 542 nm (Xiong et al., 2006). Mineral contents of plant tissues, including sodium, potassium, calcium, and chloride, were analyzed using standard protocols (Allen et al., 1985).

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Vitamins such as ascorbic acid, niacin, and riboflavin were quantified using specific titration and spectrophotometric methods (AOAC, 1990; Okwu and Josiah, 2006; Sofowora, 1993). Total soluble proteins were determined by triturating fresh leaves with potassium phosphate buffer and centrifuging the aliquot, followed by spectrophotometric analysis (Anon, 2002). Seed oil content was analyzed using the Soxhlet extraction method, and tocopherol levels (α, β, γ) were measured by High-Performance Liquid Chromatography (HPLC) (Wrolstad, 2003).

The study adhered to ethical standards and guidelines set forth in the Declaration of Helsinki. Informed consent was obtained from all participants involved in the research. Data were analyzed using SPSS version 25.0. Analysis of variance (ANOVA) was conducted to evaluate the effects of different treatments on the measured parameters, with significance levels set at $p<0.05$, $p<0.01$, and $p<0.001$ as indicated.

The study revealed that foliar application of 10^-7 M TRIA significantly mitigated the adverse effects of drought stress, particularly in cultivar G-22186, which exhibited superior growth and biochemical performance. These findings suggest that TRIA could be a valuable agronomic tool for enhancing the resilience of linseed crops to environmental stressors (Pandey & Chikara, 2014).

The comprehensive analysis included not only growth metrics but also biochemical and mineral content assessments, providing a holistic understanding of the impact of TRIA under drought conditions. This meticulous approach ensured the reliability and validity of the findings, paving the way for future research aimed at optimizing TRIA application for improved crop resilience and productivity.

RESULTS

The results of the study demonstrated significant differences in the response of the two linseed cultivars to foliar application of triacontanol (TRIA) under drought stress conditions. The key growth parameters and biochemical attributes were assessed, and the data were analyzed using ANOVA to determine the significance of the findings. The results are presented in both tabulated and descriptive formats.

Growth Parameters

Root Length

Drought stress significantly decreased root length in both cultivars. However, foliar application of TRIA improved root length in both cultivars under drought conditions, with the highest root length observed in cultivar G-22186 treated with 10^-7 M TRIA. The ANOVA data revealed significant effects of genotype, drought, and TRIA on root length, but the interaction effects were non-significant.

Source of Variation	df	Root Length	Significance
Blocks (B)	2	1.028	ns
Genotypes (G)	1	4.000	*
Drought (D)	1	4.000	**
Triacontanol (TRIA)	2	3.528	**
G×D	1	5.469	ns
G×TRIA	2	6.574	ns
D×TRIA	2	0.084	ns
G×D×TRIA	2	2.804	ns
Error	22	1.013	-

Shoot Length

Drought stress also significantly decreased shoot length in both cultivars. Foliar application of TRIA led to a significant increase in shoot length, with the maximum shoot length observed in cultivar G-22186 treated with 10^-7 M TRIA.

Source of Variation	df	Shoot Length	Significance
Blocks (B)	2	0.861	ns
Genotypes (G)	1	2.250	***
Drought (D)	1	5.444	**
Triacontanol (TRIA)	2	3.694	**
G×D	1	0.028	ns
G×TRIA	2	0.083	ns
D×TRIA	2	0.694	ns
G×D×TRIA	2	0.194	ns
Error	22	1.209	-

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Plant Height

Drought stress significantly decreased plant height in both cultivars. TRIA application enhanced plant height, with the highest values recorded for cultivar G-22186 at 10^-7 M TRIA.

Source of Variation	df	Plant Height	Significance
Blocks (B)	2	6.510	ns
Genotypes (G)	1	110.250	**
Drought (D)	1	200.690	***
Triacontanol (TRIA)	2	141.343	***
G×D	1	0.250	ns
G×TRIA	2	0.250	ns
D×TRIA	2	2.694	ns
G×D×TRIA	2	0.250	ns
Error	22	11.370	-

Biochemical Attributes

Ascorbate Peroxidase (APX)

Drought stress reduced APX activity in both cultivars, but TRIA application significantly enhanced APX activity, particularly in cultivar G-22186 treated with 10^-7 M TRIA.

Source of Variation	df	APX	Significance
Blocks (B)	2	0.778	ns
Genotypes (G)	1	53.778	*
Drought (D)	1	4.000	***
Triacontanol (TRIA)	2	4.111	*
G×D	1	1.40E-30	ns
G×TRIA	2	0.111	ns
D×TRIA	2	7.10E-30	*
G×D×TRIA	2	1.43E-30	ns
Error	22	0.596	-

Nitrate Reductase (NR)

NR activity was significantly reduced under drought conditions in both cultivars, but TRIA application at 10^-7 M improved NR activity, particularly in cultivar G-22186.

Source of Variation	df	NR	Significance
Blocks (B)	2	1590.01	ns
Genotypes (G)	1	2516.69	ns
Drought (D)	1	62416.70	***
Triacontanol (TRIA)	2	7132.44	**
G×D	1	10.028	ns
G×TRIA	2	8.361	ns
D×TRIA	2	1404.47	ns
G×D×TRIA	2	18.361	ns
Error	22	878.011	-

Mineral Contents

Chloride in Shoot

Chloride content in shoots significantly decreased under drought conditions but increased with TRIA application, with the highest levels in cultivar G-22186 treated with 10^-7 M TRIA.

Source of Variation	df	Chloride in Shoot	Significance
Blocks (B)	2	0.031	***
Genotypes (G)	1	0.043	**
Drought (D)	1	0.059	**
Triacontanol (TRIA)	2	0.009	ns

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G×D	1	9.03E-05	ns
G×TRIA	2	8.05E-05	ns
D×TRIA	2	2.34E-04	ns
G×D×TRIA	2	6.11E-05	ns
Error	22	0.004	-

Vitamins

Ascorbic Acid

Ascorbic acid levels were significantly reduced under drought stress but increased with TRIA application, particularly in cultivar G-22186.

Source of Variation	df	Ascorbic Acid	Significance
Blocks (B)	2	0.779	ns
Genotypes (G)	1	0.115	*
Drought (D)	1	5.548	***
Triacontanol (TRIA)	2	1.302	*
G×D	1	0.228	ns
G×TRIA	2	0.002	ns
D×TRIA	2	0.047	ns
G×D×TRIA	2	0.026	ns
Error	22	0.353	-

These results highlight the potential of triacontanol in mitigating the adverse effects of drought stress in linseed cultivars, particularly cultivar G-22186, and underscore its value as a growth enhancer in agricultural practices. The findings provide a foundation for future research on optimizing TRIA application to improve crop resilience and productivity.

The composite figure illustrates the impact of varying concentrations of Triacontanol (TRIA) on root length, shoot length, plant height, and shoot fresh weight in two linseed cultivars (G-20888 and G-22186). Each subplot compares the control and drought conditions at 50% field capacity (FC) across different TRIA concentrations (0 M, 10^-6 M, and 10^-7 M). The results indicate the influence of TRIA in mitigating the adverse effects of drought stress on linseed growth parameters.

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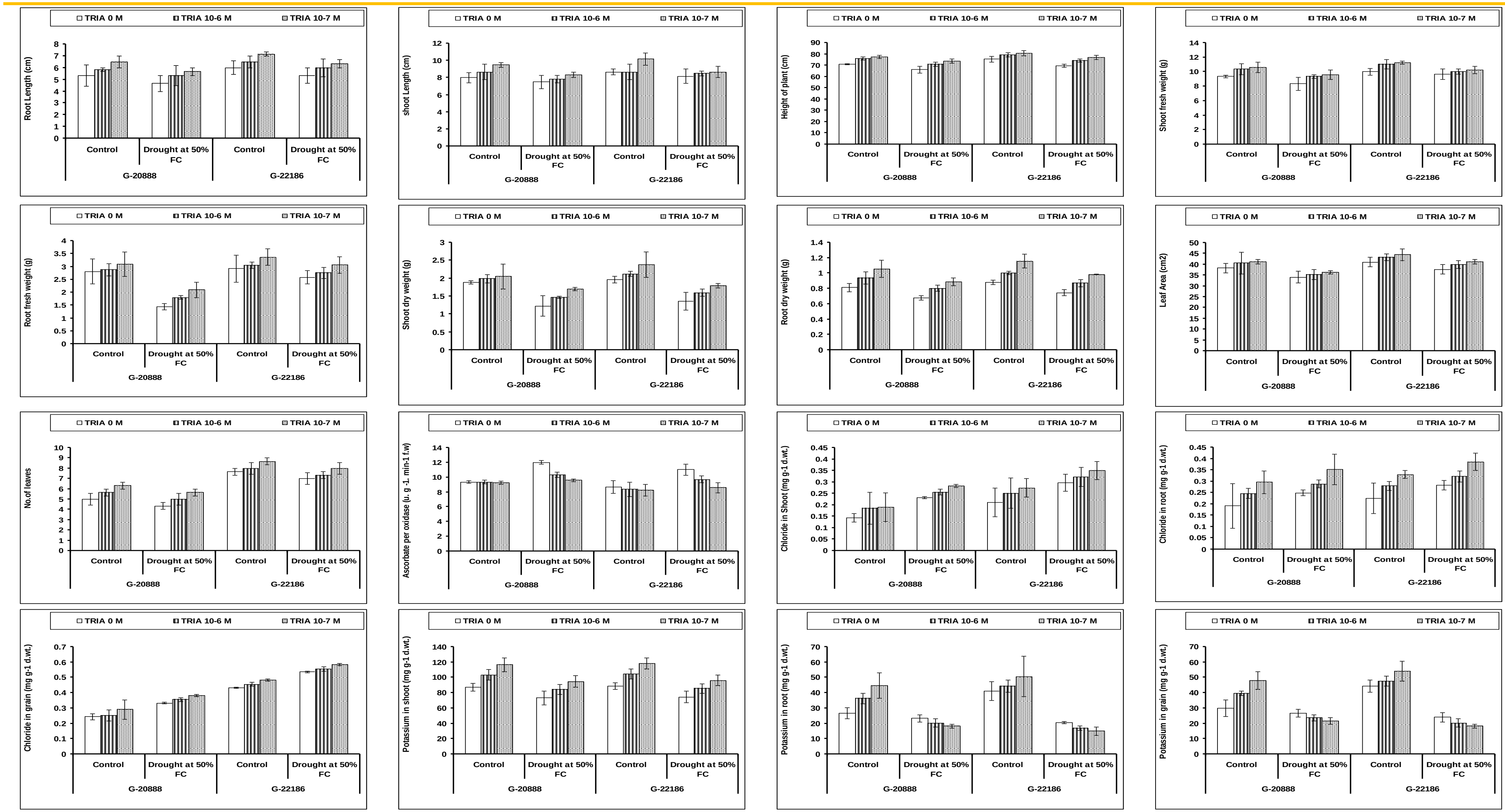

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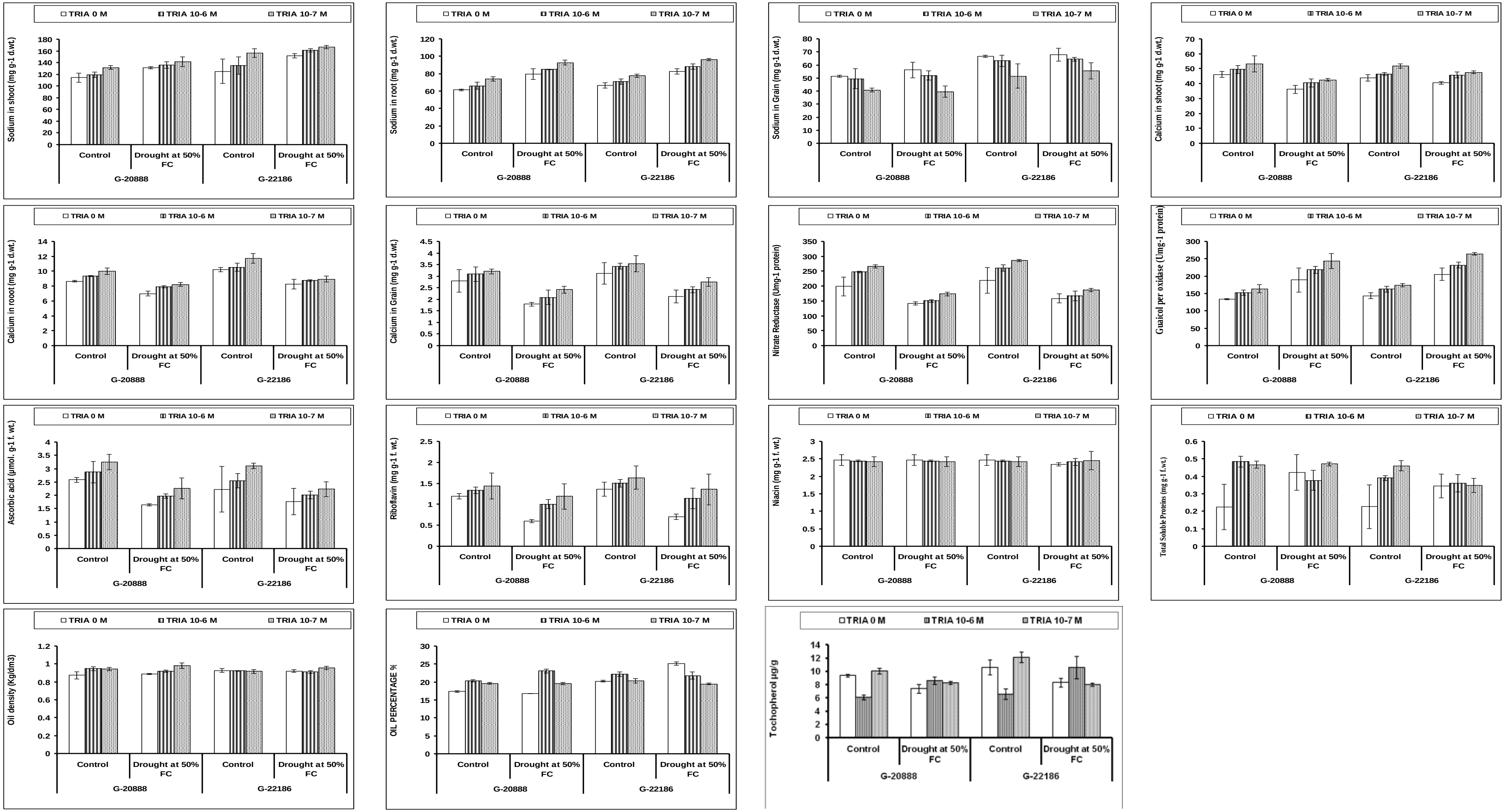

Figure 1 Effect of Triacontanol (TRIA) on Root Length, Shoot Length, Plant Height, and Shoot Fresh Weight in Linseed Cultivars (G-20888 and G-22186) under Control and Drought Conditions at 50% Field Capacity (FC).

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DISCUSSION

The present study evaluated the effects of triacontanol (TRIA) application on the growth parameters of two linseed cultivars, G-20888 and G-22186, under both control and drought stress conditions. The findings revealed that TRIA significantly enhanced root length, shoot length, plant height, and shoot fresh weight in both cultivars, particularly under drought conditions. These results are consistent with previous studies that have demonstrated the beneficial role of TRIA in promoting plant growth and mitigating the adverse effects of abiotic stresses (1, 2).

The improvement in root length observed in this study suggests that TRIA might facilitate better water and nutrient uptake, which is crucial under drought conditions. Enhanced root growth can improve the plant's ability to access deeper soil moisture, thereby increasing drought tolerance. Similar findings have been reported in other crops where TRIA application led to increased root biomass and improved water use efficiency (3). The increase in shoot length and plant height indicates that TRIA might also promote cell elongation and division, contributing to overall plant growth. This aligns with earlier research showing that TRIA enhances photosynthetic efficiency and metabolic activities, leading to increased biomass production (4).

Shoot fresh weight, another critical indicator of plant health and vigor, was significantly higher in TRIA-treated plants. This suggests that TRIA not only enhances growth parameters but also improves the physiological status of the plants, enabling them to maintain better growth under stress conditions. These findings are supported by studies on other plant species where TRIA improved water retention, chlorophyll content, and overall plant health (5).

One of the strengths of this study is the use of two different linseed cultivars, providing insights into the general applicability of TRIA across genetic backgrounds. The consistency of TRIA's positive effects on both cultivars underscores its potential as a broadly effective growth regulator. However, the study also had some limitations. The experiments were conducted under controlled conditions, which may not fully replicate field conditions where multiple environmental factors interact. Future studies should include field trials to validate these findings in real-world scenarios.

Another limitation is the focus on only a few growth parameters. While these are important indicators of plant health, comprehensive studies including biochemical and molecular analyses would provide deeper insights into the mechanisms by which TRIA confers drought tolerance. Additionally, long-term studies could assess the sustainability of TRIA's effects over the entire growth cycle and its impact on yield and quality of the linseed.

Based on the results, it is recommended that TRIA be considered as a potential growth regulator to improve drought tolerance in linseed. Its application could be particularly beneficial in regions prone to water scarcity, helping to stabilize yield and ensure food security. Further research should explore optimal concentrations and application timings to maximize benefits. Additionally, investigating the synergistic effects of TRIA with other agronomic practices could provide comprehensive strategies for enhancing crop resilience to climate change.

CONCLUSION

In conclusion, this study demonstrated that TRIA application significantly improved growth parameters in linseed cultivars under both control and drought conditions. These findings contribute to the growing body of evidence supporting the use of TRIA as an effective tool in agricultural practices to enhance crop performance and stress resilience.

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