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Promoting growth and morpho-physiological attributes in linseed (*Linum usitatissimum* L.) using indole acetic acid and gibberellic acid applied individually or jointly as foliar spray

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Abstract
Gibberellic acid (GA_3) and indole-3-acetic acid (IAA) are commercially and scientifically important due to their promising impacts on growth, quantity and quality of most crops. The current work intended to observe the effect of foliar applications of plant growth regulators (IAA and GA_3) on linseed morpho-biochemical attributes and key antioxidants. Indole-3-acetic acid showed more beneficial effects on growth and antioxidant activities than those by GA_3. Root length, shoot length, photosynthetic rate, root fresh weight, and N were respectively 38%, 25%, 38%, 60%, and 1.26% higher with combined treatments of IAA+GA_3 than those of the control plants at the vegetative stage. The effect of IAA was highly significant on N percentage, total protein contents and fibre percentage in linseed at both seedling and vegetative stages. Overall, the nutritional value of linseed was boosted by both PGRs, i.e., GA_3 and IAA. Furthermore, it is recommended that IAA, GA_3 and their combined applications can be used to enhance the quality of cereal crops.

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Introduction

Linseed (*Linum usitatissimum* L.) is a potential source of quality oil and fibres. Humans have been growing this plant for years (Vaisey-Genser and Morris, 2003). The overall production of linseed in Pakistan is 2,779 tonnes utilizing 4,018 ha for its cultivation (Anon, 2011). Linseed usually contains 5% mucilage, also called viscous fibre and lignin (Muir and Westcott, 2003). Linseed normally contains 40-50% oil and meal, 23-34% protein and 4% ash, but this composition may vary from cultivar to cultivar as well as under varying environmental conditions (Muir and Westcott, 2003). Linseed crop capacity is not adequately utilized due to various limiting factors for its cultivation, such as an inadequate supply of nutrients and accumulation of nutrients (Oad et al., 2004).

Plant growth, development and differentiation are regulated by growth hormones either by inhibition or improvement in a variety of metabolic processes (Naeem et al., 2004). Of different plant hormones reported in the literature, indole acetic acid plays a critical role in cell division and elongation, as well as in several other physiological functions involved in plant growth and development (Aloni et al., 2006; Tian et al., 2014; Zhang et al., 2022). It is believed that it plays a significant role in the regulation of stress tolerance in plants subjected to various stressful cues. Similarly, gibberellic acid is known to boost cell division and elongation, promoting plant height (Shan et al., 2021). However, like IAA, gibberellic acid can also play an essential role in improving stress tolerance in plants by triggering a myriad of metabolic processes in stressed plants (Tuna et al., 2008; Emamverdian et al., 2020). Plant growth regulators such

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as IAA and GA can effectively improve the elongation and quality of crops by promoting fibre production (Gokani and Thaker, 2002). Likewise, yield and other corresponding characteristics of fibre crops, including linseed, are influenced by plant growth regulators to a considerable extent (Siddiqui et al., 2014). For example, in cotton, elongation and output of fibre was found to be enhanced by the application of IAA (Zhao and Oosterhuis, 1998; Gialvalis and Seagull, 2001; Gokani and Thaker, 2002). In linseed, IAA and gibberellic acid were reported to improve fibre quality (Ayala-Silva et al., 2005). Thus, the premier purpose of carrying out the current experimentation was to examine that how and up to what extent IAA and gibberellic acid affect key morpho-physiological attributes and oxidative defense mechanism involved in regulating growth of linseed.

Materials and Methods

A field experiment was conducted to examine the influence of foliar spray of gibberellic acid (GA_3) and indole-3-acetic acid (IAA) on linseed at the University of Gujrat, Gujrat, Pakistan, during the growing season 2018-19. Preliminary laboratory experiments were performed at the Pakistan Council of Scientific and Industrial Research (PCSIR), Plant Biotechnology and Organic Food Lab, Lahore, Pakistan.

Growth conditions and treatments

The field experiment was conducted using sandy loam soil having a good drainage system. Seeds of linseed variety LS-29 were sown in mid-November. Varying treatments of PGRs were applied after 10 days of germination as T_0=Control T_1= 50 mg L^{-1} IAA, T_2= 50 mg L^{-1} GA_3, and T_4= 50 mg L^{-1} IAA + 50 mg L^{-1} GA_3. Plants were irrigated with tap water with an interval of one week. Agronomic conditions were noted on daily basis. The minimum and the maximum temperatures were 26 ^{o}C and 36 ºC, respectively.

Data was collected at the seedling stage (21 days after germination) and at the vegetative stage (42 days after germination).

Morpho-physiological parameters

Morphological attributes such as root and shoot lengths were measured from the base to the tip of a root. Roots were uprooted carefully, and washed well with distilled water. Root and shoot fresh weights (g) were also measured. Root and shoot samples were oven-dried at 65 °C for 5-6 days and then dry weights recorded.

Chlorophyll a and carotenoids were determined using the Arnon method (1949). For this purpose, 0.1 g of fresh leaves was macerated in a pestle-mortar. After that, 80% acetone was added to make the volume 10 mL. All samples were filtered and then their absorbance was read at 663, 645, 510, and 480 nm using a spectrophotometer (IREMCO U2020). Following formulae were used to calculate the amount of chlorophyll a, and carotenoids:

$$\text{Chl. a (mg g}^{-1}\text{ f.wt)} = (12.7OD663 - 2.69OD645) \times V/(1000 \times W)$$
$$\text{Carotenoids (mg g}^{-1}\text{ f.wt)} = 7.6(OD480 - 1.49 \times OD510) \times V/1000 \times W$$

Gas exchange parameters were noted using the ADC portable open system Infrared Gas Analyzer (IRGA). Following leaf gas exchange parameters were measured for 2 hours at 12:30 p.m. (at full sunlight).

C_i = sub-stomatal CO_2 (µmol mol^{-1})
E = transpiration rate (mmol H_2O m^{-2} s^{-1})
g_s = stomatal conductance (mol m^{-2} s^{-1})
A = photosynthetic rate (µmol CO_2 m^{-2} s^{-1})

Biochemical attributes

The Kjeldahl method was employed to determine nitrogen content in plant samples. To one gram tissue sample, one g of selenium oxide and 20 mL of H_2SO_4 were added and allowed the sample for complete digestion. An aliquot of 5 mL of the digested sample was transferred into a distillation flask with sodium hydroxide solution. The distillation was started until the purple color of boric acid solution was changed. Then it was titrated with 0.01 N HCl.

To estimate total fibre contents, 0.5 g of the sample was taken in a round bottom flask and 100 mL of H_2SO_4 (1.25%) were added to it. Each flask was placed on an electro-mantle machine for half an hour. All samples were filtered and to the filtered samples 100 mL of NaOH (1.25%) were added. Then the flasks were again fitted on the electro-mantle machines for half an hour. After that all samples were filtered again and rinsed again and again with hot water. The final rinsing was with 10-20 mL of alcohol. The weight of that filter paper was noted before and after charring.

Evaluation of free radical scavenging activity by DPPH method

Free radical scavenging activity of the extract was determined using the stable DPPH (0.004%). Dilution concentrations were 20 µl, 40 µl, 60 µl, 80 µl, and 100 µl of the ethanolic and acetonic extractions (0.5 g/5 mL) mixed with 3 mL of 0.1 µM DPPH ethanolic solution. The mixture was placed at room temperature for 30 minutes. The absorbance of the resulting solution was then read at 517 nm against DPPH as a blank in a spectrophotometer after 30 minutes. BHA was taken as a standard to compare the antioxidant activity with the sample.

The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = Abbs of blank − abbs of sample ÷ Abbs of blank × 100

Where: Abbs of blank = Absorbance of DPPH

Statistical analysis

Experimental design used was completely randomized (CRD) with three replicates. The presence or absence of significant differences between different factors was ascertained with analysis of variance (ANOVA) at $P \leq 0.05$. Means were compared to find significant differences among them using the Duncan's New Multiple Range test (DMR) at a probability level of 5% (Steel and Torrie, 1997).

Results and Discussion

Hormonal effects on growth and chlorophyll attributes

The results regarding the influence of foliar-applied GA_3, IAA, and GA_3 + IAA demonstrated that all variables related to growth and chlorophyll contents (**Figures 1 &2**) increased significantly with PGRs that resulted in higher growth and ultimately enhanced crop productivity.

It was noted that IAA enhanced the root length by 50% higher than that in the control plants at the vegetative stage, while GA_3 increased root length by 30% at the seedling stage (**Figure 2**). Analysis of variance also showed significant effects of all IAA and GA_3 treatments (**Tables 1 and 2**). Higher shoot length (35 cm) was noted with IAA compared to that of the control plants at the vegetative stage. Similarly, GA_3 increased the shoot length up to 33 cm at the seedling stage (**Figure 2**). Moreover, with the application of hormones in linseed, root and shoot fresh weights also increased significantly (**Figure 1**). Application of GA_3 increased root fresh weight by 60% at the seedling stage and 173% at the vegetative stage (**Figure 1**). Shoot fresh weight was also increased significantly with the applications of GA_3. It increased shoot fresh weight by 60% at the vegetative stage and by 36% at the seedling stage. IAA also increased the shoot fresh weight of linseed (**Figure 1**). All the treatments of PGRs increased the root fresh weight. GA_3 increased the root dry weight up to 98% and IAA showed 22% increase in root dry weight (**Figure 1**). Shoot dry weight was the most promising attribute that had shown a marked influence of PGRs (**Figure 1**). Higher shoot dry weight (0.6 g) was noted at the vegetative stage with the applications of GA_3.

Table 1. Mean squares from analysis of variance (ANOVA) of data for morphological attributes of linseed plants under foliage applications of IAA and GA3 and their interaction at the seedling stage

Source	df	RL	SL	RFW	SFW	RDW	SDW
IAA	2	6.715**	62.722***	0.002*	0.005**	1.886**	0.005*
GA3	2	0.568***	8.0**	5.555*	0.011**	3.755***	0.001**
Interaction IAA×GA_3	2	0.568**	8.0**	5.555ns	0.011**	3.755**	0.001**
Error	13	17.595	11.638	8.166	0.037	1.845	0.001
Total	19						

RL, Root length; SL, Shoot length; RFW, Root fresh weight; SFW, Shoot fresh weight; RDW, Root dry weight; SDW, Shoot dry weight; ns, non-significant; *, **, ***, significant at 0.05, 0.01 and 0.001 levels, respectively

Table 2. Mean squares from analysis of variance (ANOVA) of data for morphological attributes of linseed plants under foliage applications of IAA and GA3 and their interaction at the vegetative stage

Source	df	RL	SL	RFW	SFW	RDW	SDW
IAA	2	8.800**	217.040**	0.027**	0.670***	2.227*	0.173*
GA3	2	3.827*	0.108**	0.013**	0.003*	0.009**	0.001*
Interaction IAA×GA_3	2	3.827*	0.133ns	0.013*	0.003**	0.009*	0.001*
Error	13	13.508	30.984	0.003	0.182	0.008	0.037
Total	19						

RL, Root length; SL, Shoot length; RFW, Root fresh weight; SFW, Shoot fresh weight; RDW, Root dry weight; SDW, Shoot dry weight; ns, non-significant; *, **, ***, significant at 0.05, 0.01 and 0.001 levels, respectively

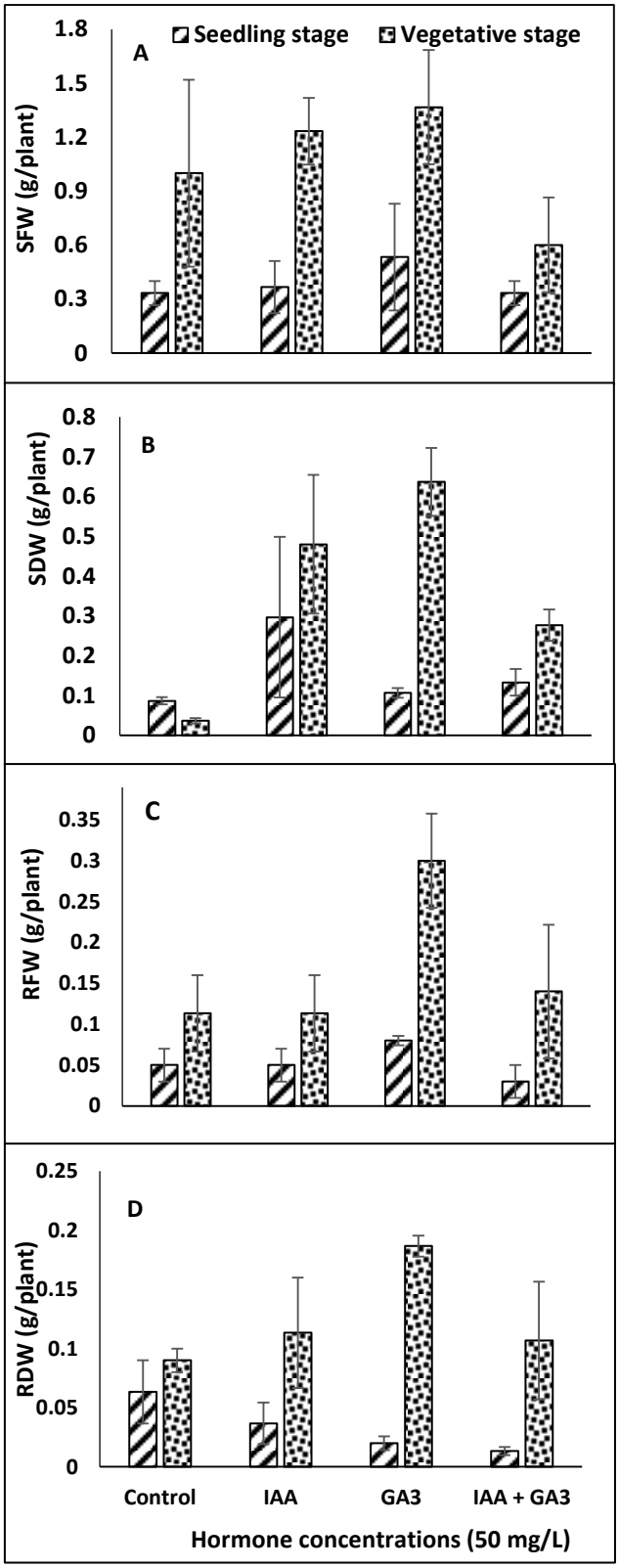

Figure 1. Growth attributes of linseed plants when different concentrations of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) were applied at the seedling and vegetative stages. (A) Shoot fresh weight, (B) Shoot dry weight, (C) Root fresh weight, and (D) Root dry weight.

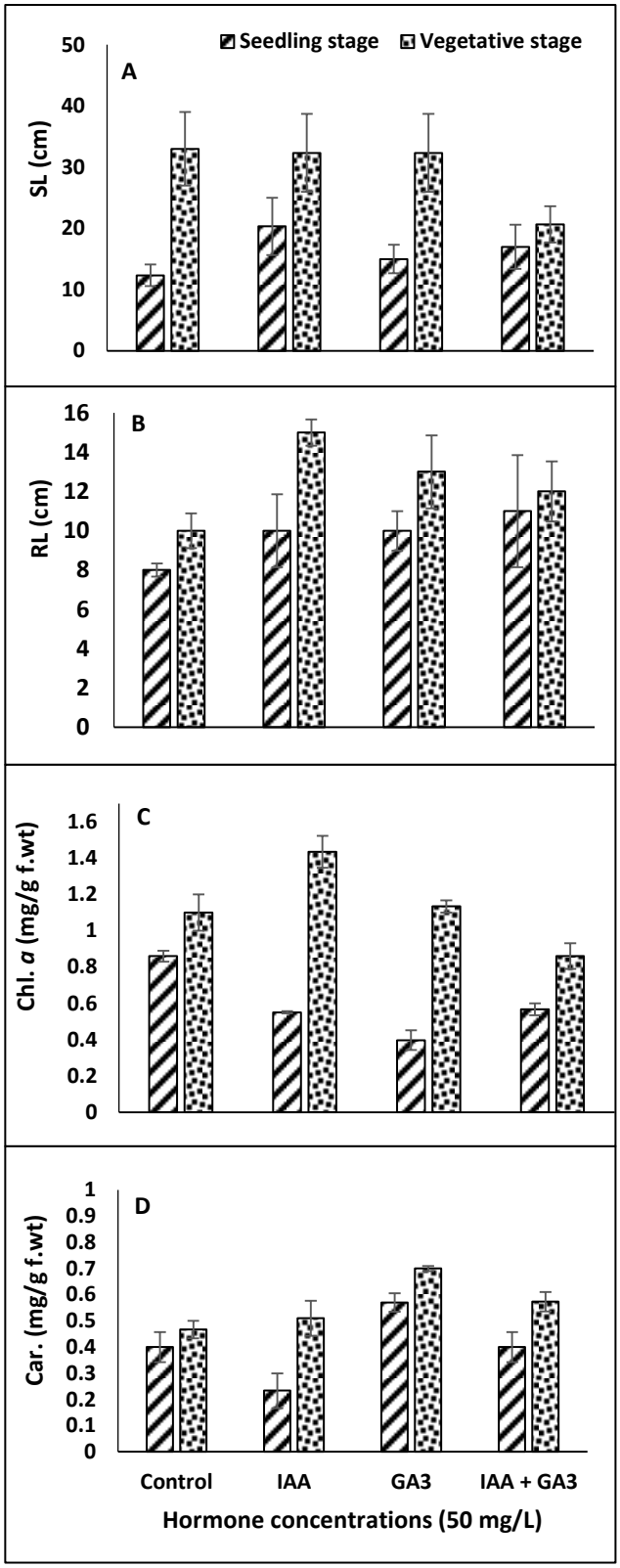

Figure 2. Shoot and root length, and chlorophyll a and carotenoid contents of linseed plants when different concentrations of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) were applied at the seedling and vegetative stages. (A) Shoot length (cm), (B) Root length (cm), (C) Chlorophyll a (mg/g f.wt.), and (D) carotenoids (mg/g f.wt.)

IAA has been reported to stimulate cell enlargement that results in higher growth as observed in mung bean (Singh and Rathore, 1998). Malik et al. (1992) noted that foliar application of IAA caused an increase in the weight of shoot in three important leguminous crops, pea, chick pea and lentil, due to the development of numerous branches. GA_3 was reported to expand stem length and number of blooms per plant (Lee et al., 1999). The antagonistic effect of GA_3 and IAA has also been reported. For example, an increased concentration of GA_3 promoted plant growth as observed in pea (Bora and Sarma, 2006), whereas that of IAA restricted plant growth in *Cassia absus* (Hussain et al., 2011). Similarly, Ayala-Silva et al. (2005) demonstrated that application of GA_3 expanded plant height, while IAA diminished plant tallness most likely because of the increment in stem diameter, which dropped down the shoot development. Aloni et al. (2006) suggested that increased number of roots promotes root fresh and dry weights. Long roots also play an important role in growth promotion. For example, longest roots were observed in potatoes with the joint application IAA and GA_3 (Haque et al., 2009). It is believed that low doses of plant hormones are more effective in promoting growth than their high doses (Rastogi et al., 2013). For example, Vwioko and Longe (2009) showed that only low doses of IAA are effective for plant growth promotion. Naeem et al. (2004) advocated that hormones in low concentrations can regulate growth, differentiation and development, either by promotion or reduction of these processes. and allow physiological processes to happen at their usual rate (Gulluoglu, 2004).

Both IAA and GA_3 had a significant effect on chlorophyll 'a' of linseed. Combined applications of IAA + GA_3 enhanced 20% chlorophyll a contents at the seedling stage (**Figure 2; Table 3**). At the vegetative stage, foliar spray of IAA showed 68% increase in Chl. a contents (**Figure 2**). Carotenoid content was significantly affected by exogenously-applied both hormones. Carotenoids were observed to be increased by 72% at the seedling stage. For example, IAA showed 33% increase in carotenoids at the seedling stage. At the vegetative stage, 85% increase was noted by GA_3 in Chl a contents (**Figure 2**). Overall, photosynthetic pigments (chlorophyll a and carotenoids) were increased with the applications of IAA and GA_3. Plants having a deficiency of hormonal influence cause a reduction in the chlorophyll contents, which results in leaf damage. Our findings were supported by those of Bora et al. (2007).

Effect of IAA, GA_3 and IAA+GA_3 on physiological attributes

Physiological attributes were improved significantly with the foliar applications of both PGRs applied singly or jointly (**Tables 3 & 4**). Photosynthetic rate increased 38% with GA_3 applied at the seedling stage. At the vegetative stage, GA_3 and IAA showed 55% and 10% enhancement in photosynthetic rate, respectively (**Figure 3**). Moreover, higher rate of transpiration was also noted with PGRs. At the seedling stage GA_3 improved transpiration of linseed plants (**Figure 3**). Higher sub-stomatal conductance CO_2 concentration was noted at the seedling and vegetative stages under foliar spray of IAA and GA_3 (**Figure 3**). Stomatal conductance was higher at the seedling stage than at the vegetative stage (**Figure 3**).

Table 3. Mean squares from analysis of variance (ANOVA) of data for physiological attributes of linseed plants under foliage applications of IAA and GA3, and their interaction at the seedling stage

Source	df	*A*	*E*	C_i	g_s	*Chl.a*	*Car*
IAA	2	1.175**	9.102***	11851.5**	0.060**	5.272**	6.436*
GA_3	2	0.002*	0.093**	107.5**	0.001ns	7.124**	2.035**
Interaction IAA×GA_3	2	0.002**	0.091***	107.5*	0.001*	2.569*	7.326*
Error	13	1.146	0.252	986.01	0.016	5.052	1.598
Total	19						

A, Photosynthetic rate; *E*, Transpiration rate; *Ci*, Sub-stomatal CO_2 concentration; g_s, Stomatal conductance; *Car*, Carotenoids; ns-non-significant; *, **, ***, significant at 0.05, 0.01 and 0.001 levels, respectively

Table 4. Mean squares from analysis of variance (ANOVA) of data for physiological attributes of linseed plants under foliage applications of IAA and GA3, and their interaction at the vegetative stage

Source	df	*A*	*E*	C_i	g_s	*Chl.a*	*Car*
IAA	2	10.215***	0.480***	4140.5***	2.004**	8.726**	1.088*
GA_3	2	0.554**	0.630**	244.23*	0.064*	5.688**	6.722**
Interaction IAA×GA_3	2	0.554***	0.630***	244.2*	0.064ns	7.38ns	1.088*
Error	13	7.666	5.666	5.66	0.058	4.055	1.088
Total	19						

A, Photosynthetic rate; *E*, Transpiration rate; *Ci*, Sub-stomatal CO_2 concentration; g_s, Stomatal conductance; *Car*, Carotenoids; ns-non-significant; *, **, ***, significant at 0.05, 0.01 and 0.001 levels, respectively

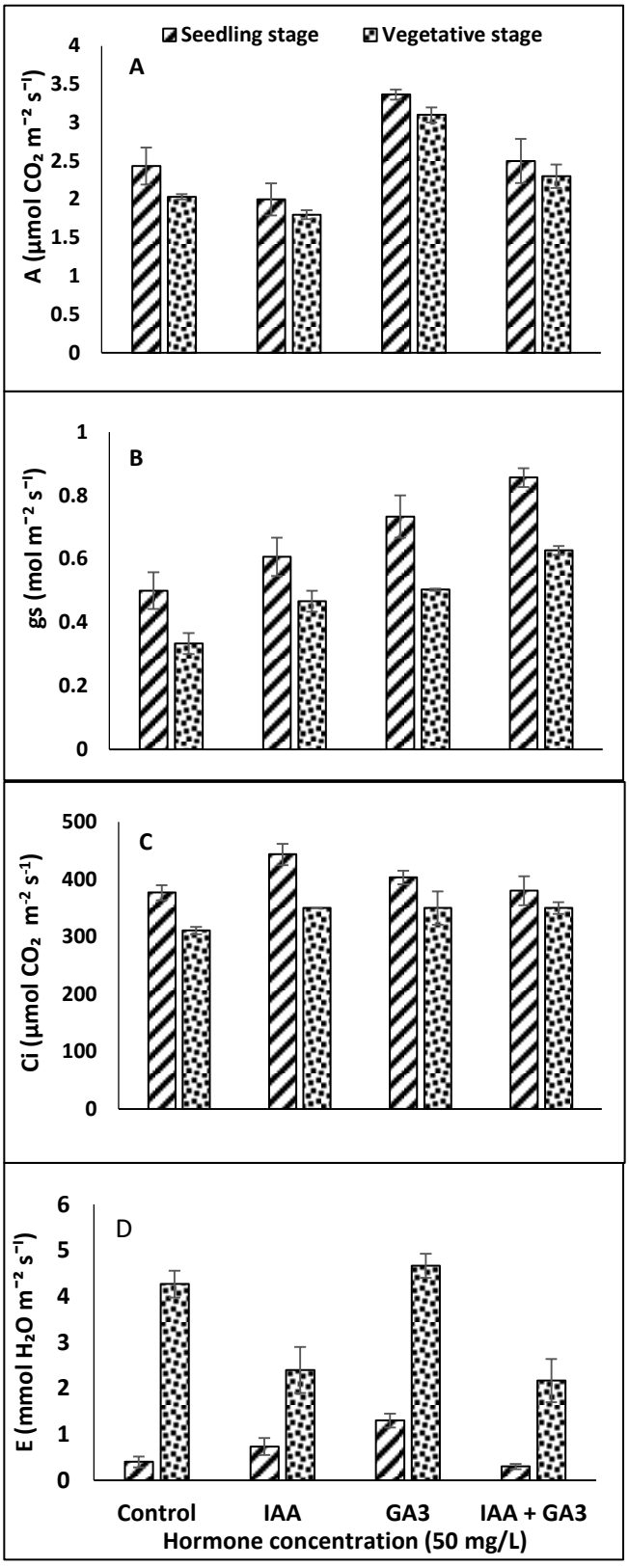

Figure 3. Gas exchnage attributes of linseed plants when different concentrations of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) were applied at the seedling and vegetative stages. (A) Photosynthetic rate (*A*; µmol CO_2 m^{-2} s^{-1}), (B) Stomatal conductance (g_s; mol m^{-2} s^{-1}) , (C) Internal CO_2 Concentration (C_i; µmol CO_2 m^{-2} s^{-1}), and (D) Transpiration rate (*E*; mmol H_2O m^{-2} s^{-1}).

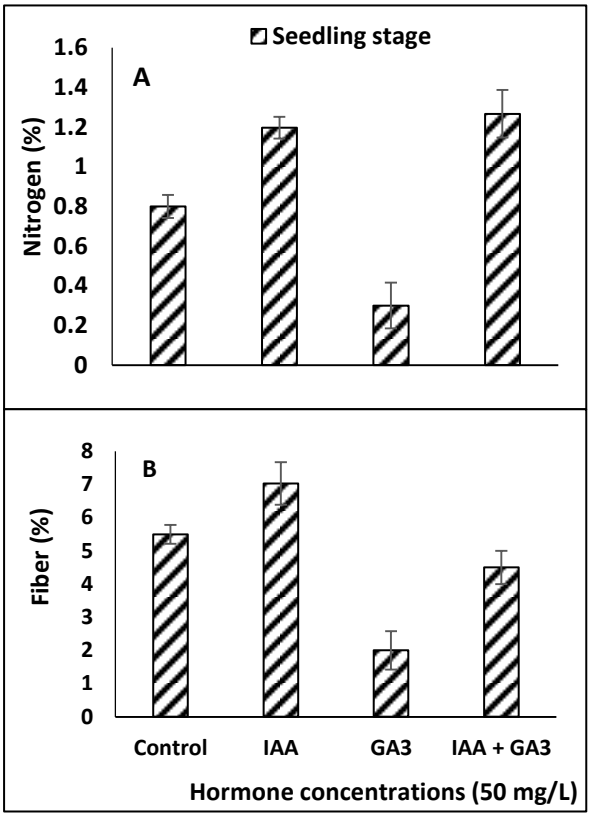

Figure 4. Nitrogen and fiber contents of linseed plants when different concentrations of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) were applied at the seedling stage. (A) Nitrogen (%), (B) Fiber (%).

In many studies it has been noted that IAA and GA_3 increased the physiological attributes as Yuan and Xu (2002) observed that GA_3 improved the value of g_s in broad beans, but in *Triticum aestivum,* GA_3 had no consistent effect on both g_s and *E* (Ashraf et al., 2002), whereas in *Mentha spicata,* GA_3 reduced the values of g_s and *E* (Singh et al., 1999). Thus, the effects of IAA or GA_3 seem to vary with the type of plant species.

Asada (1999) demonstrated that reduction in carbon dioxide assimilation results in low rate of photosynthesis in plants as improper balance could cause side reactions by forming active oxygen species (AOS) in some physiological events. Plants lacking essential hormones have less chlorophyll concentration, resulting in damaged leaves. Plants with darker habitats have an adequate supply of growth regulators like cytokinins and auxins (Bora et al., 2007).

Biochemical attributes

Nitrogen percentage in linseed was increased significantly with the applications of IAA and GA_3. Applications of IAA increased N by 1.19% and fibre by 7% (**Figure 4**). Application of GA_3 and combined application of both PGRs also increased the N and fibre contents (**Figure 4**). Effect of PGRs was also significant on total antioxidant activities (**Table 5**).

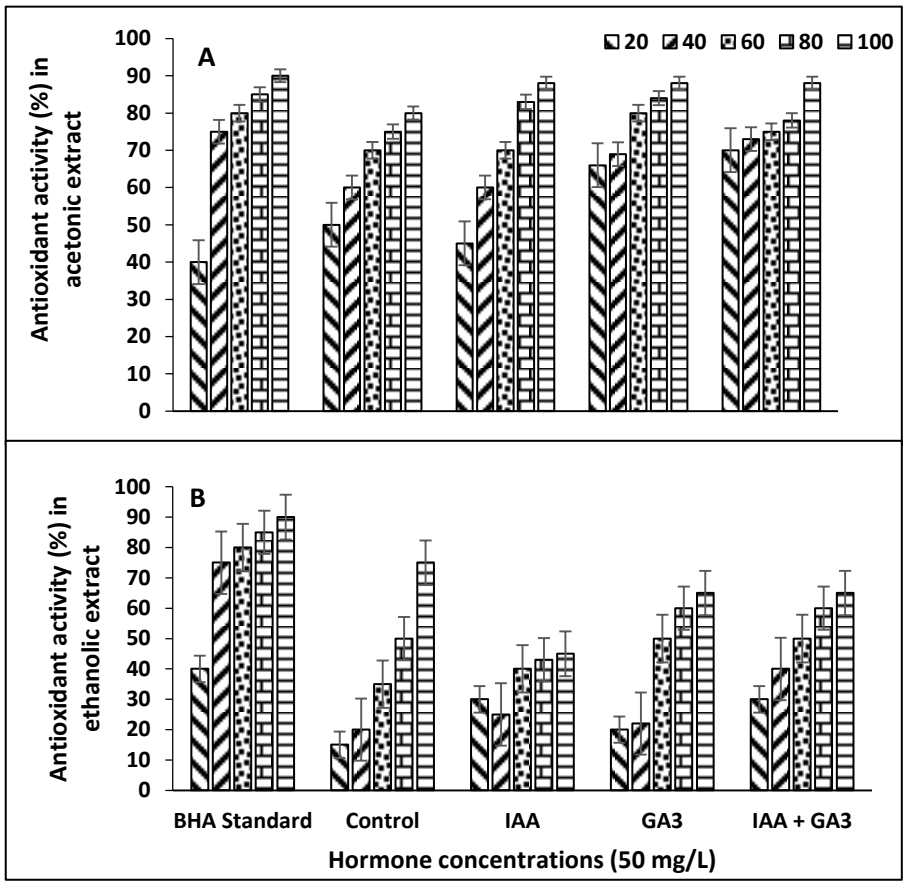

Figure 5. Antioxidant activity (%) of linseed plants when different concentrations of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) were applied at the seedling stage. (A) Antioxidant activity (%) in acetonic extract, (B) Antioxidant activity (%) in ethanolic extract

Table 5. Mean squares from analysis of variance (ANOVA) of data for N, total protein and fiber of linseed plants under foliage applications of IAA and GA3, and their interaction at the seedling and vegetative stages

Source	df	NSS	NSV	TPS	TPV	FSS	FSV
IAA	2	0.425**	0.628***	13.217***	24.514**	0.018**	0.029n*
GA3	2	0.123*	0.365*	8.2143**	14.288**	1.214*	2.449*
Interaction IAA×GA_3	2	0.246**	0.365**	9.251**	14.288**	6.442ns	12.470*
Error	13	0.112	0.140	3.212	5.502	0.008	0.016
Total	19						

NSS, Nitrogen at seedling stage; NSV, Nitrogen at vegetative stage; TPS, Total protein at seedling stage; TPV, Total protein at vegetative stage; FSS, Fiber at seedling stage; FSV, Fiber at vegetative stage; ns, non-significant; *, **, ***, significant at 0.05, 0.01 and 0.001 levels, respectively

Total antioxidant activity (%) in ethanolic extract of linseed showed 80% anti-oxidant activities (**Figure 5**), whereas those determined in acetonic extract had 100% activity with combined treatments of IAA and GA_3 (**Figure 5**).

There are several studies where PGRs are reported to enhance N, fibre and antioxidant activities. For example, Lima et al. (1987) have shown that foliar application of IAA caused a considerable increase in N content from 60% to 80%. An increase in the nitrogen content was also noted in the plants of *Vicia faba, Cladophora dalmatica, Ulva lactuca, Jania rubens* and *Pterocladia pinnata* under the application of growth regulators (El-Sheekh and El-Saied, 1999). In falx, yield, fibre strength and quality were also enhanced by the foliar spray of IAA and GA_3 (Ayala-Silva et al., 2005). Mckenzie and Deyholos (2011) reported that treatment of GA_3 caused stem enlargement, extension, multiplication, cell division and thickening in bast fibre of linseed. Gokani and Thaker (2002) also achieved similar results by exhibiting a significant influence of the hormones on both quality and extension of fibre in cotton (*Gossypium hirsutum* L.). Similarly, in the same crop, hormones promoted fibre production and elongation (Gokani et al., 1997; Gialvalis and Seagull, 2001). Earlier, Mckenzie and Deyholos (2011) reported similar findings as of the current study that gibberellic acid caused enlargement and extension bast fibre of linseed.

Table 6. Pearson correlation between morphological, photosynthetic, chlorophyll content, and biochemical attributes of linseed

	RL	SL	RFW	RDW	SFW	SDW	Ci	gs	A	E	Chl. a	Car.
RL	1											
SL	0.61	1										
RFW	0.46	0.64	1									
RDW	0.49	0.73	0.91	1								
SFW	0.63	0.91	0.80	0.84	1							
SDW	0.73	0.56	0.75	0.75	0.68	1						
Ci	-0.31	-0.63	-0.46	-0.60	-0.64	-0.06	1					
gs	-0.83	-0.48	-0.38	-0.33	-0.38	-0.57	0.16	1				
A	-0.15	-0.31	0.34	0.00	-0.02	0.03	0.16	0.16	1			
E	0.38	0.86	0.86	0.83	0.88	0.48	-0.70	-0.32	0.08	1		
Chl a	0.79	0.74	0.61	0.79	0.79	0.65	-0.72	-0.55	-0.30	0.61	1	
Carot.	0.37	0.33	0.82	0.62	0.62	0.50	-0.37	-0.25	0.71	0.64	0.39	1

-9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 0.1 0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9

RL, Root length; SL, Shoot length; RFW, Root fresh weight; RDW, Root dry weight; SFW, Shoot fresh weight; SDW, Shoot dry weight; C_i, Sub stomatal CO_2 conductance; g_s, Sub-stomatal conductance; *A*, photosynthetic rate; *E*, transpiration rate; Chl.a, Chlorophyll a; Car, Carotenoids

Pearson correlation

Pearson correlation showed differences based on regression (*r*) values (**Table 6**). There was a strong correlation between shoot fresh weight and shoot length (*r*= 0.91). The most negative weak correlation was observed between stomatal conductance and root length (*r*= -0.83). A weak positive correlation was noted between stomatal conductance CO_2 and photosynthetic rate (**Table 6**).

Principal component analysis

Principal component analysis was conducted between morphological, physiological, chlorophyll and carotenoid attributes at the seedling and vegetative stages. The Eigen value of the first 3 components were 7.15, 2.01, and 1.45, respectively. Root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, shoot length, root length and chlorophyll 'a' had a positive correlation shown in components first and second, which contained Eigen value of more than one (**Figure 6**).

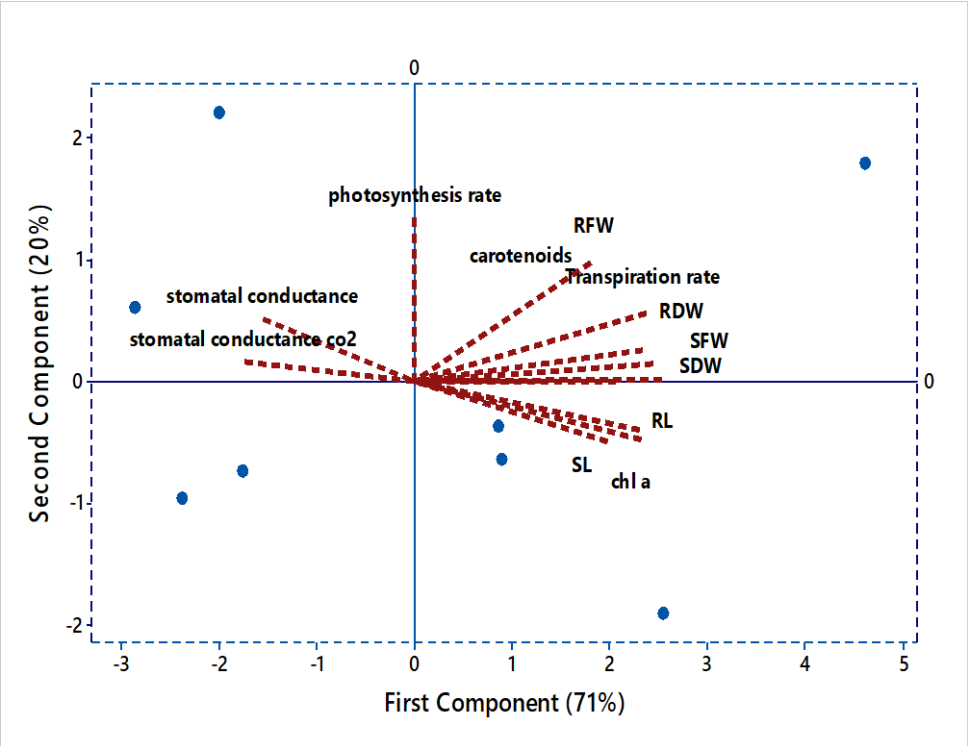

Figure 6. Principal components analysis (based on correlation matrix) of morphological, photosynthetic, chlorophyll content, and biochemical attributes of linseed. Biplot vectors are trait factor loadings for PC1 and PC2 of 12 measured traits

RL, Root length; SL, Shoot length; RFW, Root fresh weight; RDW, Root dry weight; SFW, Shoot fresh weight; SDW, Shoot dry weight; Chl.a, Chlorophyll a

Conclusions

Both PGRs, IAA and GA_3 can be successfully employed to enhance growth, and regulate physio-biochemical attributes to increase the linseed productivity. IAA and GA_3 can be applied individually or jointly to attain maximal growth and yield of linseed.

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