PHYSIOLOGICAL RESPONSE OF CANOLA PLANTS (*BRASSICA NAPUS* L.) TO TRYPTOPHAN OR BENZYLADENINE

Mona G. DAWOOD, Mervat S. SADAK

Botany Department, National Research Centre, Egypt

e-mail: monagergis@yahoo.com

Two pot experiments were conducted at the National Research Centre during two successive seasons to investigate the influence of two foliar treatments -to10 and12 weeks old plants- with tryptophan or benzyladenine at 25,50,75 mg/L on the growth of canola plants (Brassica napus L.var. Pactol) as well as their influence on the yield; photosynthetic pigments; endogenous hormones and the chemical composition of the yielded seeds. Exogenous application of tryptophan or benzyladenine considerably increased plant growth as shoot height; shoot circumference; number of leaves; fresh and dry weight of shoot; leaf area and number of inflorescences/plant. The effect was more pronounced with 75mg/L tryptophan or benzyladenine. Significant increases in the yield parameters were recorded from treatment with 75mg/L tryptophan or benzyladenine. These increases were 8.9% and 15.6% respectively in the number of pods/plant; 5.6% and 17.4% in the number of seeds /pod; 33.2% and 24.5% in the weight of pods/plant; 31.7% and 21.6% in the weight of seeds/ plant. Photosynthetic pigments showed the same tendency with the highest concentration of tryptophan or benzyladenine. The increase percentage reached 52.5% and 43.2% respectively in total chlorophylls; 38.3% and 37.9% in carotenoids. The quantitative amounts of endogenous GA3 and IAA increased concomitant with decrease in ABA in response to all treatments. The contents of oil; protein; total carbohydrates and phenolic compounds of the yielded canola seeds increased gradually with concentrations of tryptophan or benzyladenine. It is worthily to mention that treatment with tryptophan at 75mg/L showed the highest increases in the chemical constituents of the yielded seeds than the other treatments. Total saturated fatty acids decreased while total unsaturated fatty acids and essential fatty acids increased due to all treatments .Special attention was paid to the influence of tryptophan or benzyladenine on the amount of erucic acid in the yielded seeds which was noticeably decreased with the two compounds. The decrease percentage due to tryptophan was 18.8% and 25.9% using 25 or 50mg/L, whereas using benzyladenine with 25 or 50 mg/L decreased the amount of erucic acid to 30.6% and 23.5% respectively. Higher concentration from the two compounds approximately had no effect.

Keywords: *Tryptophan ; Benzyladenine; Canola; Brassica napus; Growth; Oil; Protein; Carbohydrates; Phenolics; Erucic acid.*

There is no doubt that the need of traditional edible oils will increase due to the growth of population all over the world. In Egypt, over 80% of the oil consumed is imported from abroad due to limited local production. Therefore, renewable resources in oils are required. To overcome this problem, one of suggestion is introducing new oil crops (*Brassica napus* L.). Canola, an oilseed crop, is a genetically altered and improved version of rapeseed belonging to cruciferae family, brassica genus. Canola seeds have an economic value associated with high quality oil and protein. Canola oil is edible oil having 2% or less erucic acid and used as salad and cooking oil. The defatted meal contains 34 - 40% protein having less than 30 micromoles/g glucosinolates and used as an animal feeds or organic fertilizer.

The use of plant growth regulators is directed in general, to improve yield quality and /or quantity of many crops through regulating and adjusting the balance of endogenous hormones level in favour of normal physiological process and in turn yield. Tryptophan treatments could increase growth rate and yield of wheat [23].

Benzyladenine is used to regulate growth, chemical composition and yield parameters of plants, this may be due to its effect on the cell division and cell enlargement via the increase in cell wall extensibility [20, 17] delay of senescence; promotion of lateral buds development; grain growth and fruit set [40].

The aim of the present work was to investigate the physiological changes induced by foliar application of tryptophan or benzyladenine on canola plants.

MATERIAL AND METHOD

Two pot experiments were conducted during two successive seasons (2005/2006 and 2006/2007) at the National Research Centre, Giza, Egypt. Canola seeds (*Brassica napus* L.var. Pactol) were obtained from Oilseed Department, Agricultural Research Centre, Giza, Egypt.

Each experiment was carried out in pots (30 cm in diameter) filled with clay soil. The seeds were sown at a depth of about 3cm in the middle of November in the two seasons. Ca-superphosphate was applied at a rate of 10g/ pot before sowing. Nitrogen fertilizer (as urea) was applied at the rate of 2g/ pot twice to 3 and 5 weeks old plants. Tryptophan or benzyladenine were applied foliarly twice at 25, 50 and 75mg/L to 10 and 12 weeks old plants. Each experiment comprised 7 treatments with 15 replicates in complete randomized design.

Plant samples were collected 1 week after the second spraying treatment (13 weeks old) to study their influence on the growth; photosynthetic pigments and endogenous hormones. Moreover, other sample were collected (15 weeks old) to study the changes in growth parameters.

At harvest (27th April) in two seasons, the following items were studied, shoot height; number of pods/ plant; weight of pods/ plant; weight of seeds/ plant; number of seeds/ pod and seed index. Air dried seeds were ground into a fine powder for analysis.

photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined according to [26]. Acidic hormones (IAA, GA3 and ABA) were determined by Gas Liquid Chromatography. Extraction was carried according to the method adopted by [31] and methylation according to [38]. Seed oil content was determined using soxhlet apparatus and petroleum ether (40-60 °c) according to [4]. The protein content was determined by microkjeldahl method according to [4]. Total carbohydrates were determined colorimetrically according to the method of [32]. Total phenolic compounds were determined according to [34]. Methyl esters of fatty acids were prepared from an aliquot of total lipid according to [15]. Identification and quantitative determination of fatty acid were performed using Gas Liquid Chromatography.

Data were statistically analyzed using the least significant difference at 5% level of probability according to [33].

RESULTS AND DISCUSSIONS

The data presented in (*tab.1*) show that exogenous application of tryptophan or benzyladenine at 25,50,75mg/L significantly improved the growth parameters of canola plants in most cases during the different stages of growth such as shoot height; shoot circumference; number of leaves; fresh and dry weight of shoots; leaf area and number of inflorescence /plant. This effect was more pronounced with 75mg/L tryptophan or benzyladenine. These results are in agreement with those reported by [22] who stated that foliar application of benzyladenine caused significant increase of soybean plant height; fresh and dry weight/plant; leaf area and leaf area index. Moreover, benzyladenine application to pepper plants induced significant increase in plant height; number of branches and number of leaves/plant [2]. Such increase in growth parameters in response to benzyladenine application can be attributed to the increase of cell elongation and /or cell division [20 ,17].The stimulatory effect of foliar application of tryptophan treatments on the growth parameters of canola plants is similar to those reported by [23,5, 36] in different plant species. In addition, foliar application of tryptophan caused significant increase in wheat growth even under saline conditions [14].

Significant increases in yield parameters (*tab.2*) were recorded with either tryptophan or benzyladenine at 75mg/L (except in the case of plant height). These increases reached 8.9% and 15.6% respectively in the number of pods/plant; 5.6 %and 17.4% in the number of seeds /pod; 33.2% and 24.5% in the weight of pods/plant; 31.7% and 21.6% in the weight of seeds/ plant. The improvement in the yielded canola seeds as well as its components resulted from tryptophan application are in agreement with those obtained by [41]who stated that spraying tryptophan to strawberry enhanced growth; yield and fruit quality. Whereas, the improvement due to benzyladenine application is similar to those reported by [22] who found that benzyladenine application to soybean increased the number of pods/plant and seed weight/plant. El Abagy [11] stated that foliar application of benzyladenine (25 and 50mg/L) to some faba bean cultivars caused significant increase in most growth parameters at different stages of growth as well as yield and its components. The data in the present investigation revealed that tryptophan treatments at 50 and 75 mg/L were more effective in inducing pronounced increments in seed weight/plant and pod weight/plant followed by benzyladenine at 75mg/L than other treatments. However, the significant increments in the number of seeds/pod and number of pods/plant were obtained by applying benzyladenine at 50 and 75 mg/L. The increase in yield and yield parameters could be attributed to the increase in the number of pods which in turn caused greater number of seeds. The increase in seed weight could be attributed to the promotive effect of tryptophan or benzyladenine in increasing the amount of assimilates as well as increasing their translocations from the leaves to the fruits and hence increase seed weight [20]. Ramaih[28] reported that the increase in growth as a result of tryptophan application may be due to its conversion to IAA.

Table 1

Effect of foliar application of tryptophan or benzyladenine on the growth parameters of canola plants(13 and 15 weeks old) (combined analysis of two seasons)

	13 weeks old					
Treatments	Shoot height (cm)	Number of leaves /plant	Shoot circumfere nce(cm)	Fresh weight of shoot(g) /plant	Dry weight of shoot (g)/plant	Leaf area(cm²/ plant)
Untreated plants	15.36	7.33	1.87	26.35	2.29	445.07
Try.(mg/L)						
25	17.33	8.16	2.40	27.33	2.41	517.63
50	18.00	8.83	2.50	27.95	2.44	505.13
75	18.33	8.67	2.58	29.93	2.52	566.98
B.A.(mg/L)						
25	17.83	7.58	2.22	28.63	2.38	462.42
50	19.83	8.00	2.32	29.93	2.80	492.84
75	19.33	8.50	2.37	30.67	2.87	586.25
LSD 5%	1.16	0.63	0.15	1.15	0.17	17.67

	15 weeks old				
Treatments	Shoot height (cm)	Number of leaves/plant	Number of Inflorescence/ plant	Fresh weight of shoot(g)/ plant	Dry weight of shoot (g)/plant
Untreated plants	74.56	15.58	4.17	90.0	8.50
Try.(mg/L)					
25	77.00	16.57	4.50	96.56	9.35
50	78.67	16.80	4.67	98.19	9.50
75	82.50	17.17	5.00	100.77	9.73
B.A.(mg/L)					
25	77.67	16.66	4.67	98.85	9.42
50	86.83	16.70	5.17	107.00	9.70
75	86.39	16.83	5.00	111.89	9.83
LSD 5%	1.55	0.37	0.20	4.49	0.63

Try.(tryptophan), B.A.(benzyladenine)

Table 2

Effect of foliar application of tryptophan or benzyladenine on the yield of canola plants and its components (combined analysis of two seasons)

Treatments	Shoot height(cm)	Number of pods/plant	Weight of pods(g)/ plant	Number of seeds/pod	Weight of seeds(g)/pl ant	Weight of1000 seeds(g)
Untreated plants	105.50	131.25	20.00	23.68	8.30	2.59
Try.(mg/L)						
25	113.53	136.50	21.75	23.99	9.60	2.49
50	119.83	139.13	25.06	24.21	10.52	2.57
75	109.83	142.92	26.63	25.00	10.93	2.64

Treatments	Shoot height(cm)	Number of pods/plant	Weight of pods(g)/ plant	Number of seeds/pod	Weight of seeds(g)/plant	Weight of1000 seeds(g)
B.A.(mg/L)						
25	111.27	136.42	20.59	27.13	9.87	2.63
50	113.22	145.34	23.74	27.50	9.93	2.62
75	109.63	151.66	24.90	27.80	10.09	2.63
LSD 5%	5.52	8.02	1.76	0.57	0.40	N.S.

N.S. (non significant).

Total chlorophyll and carotenoids (*tab.3*) increased significantly in response to either tryptophan or benzyladenine application at 75mg/L. The increase percentage reached 52.5% and 43.2% respectively in total chlorophyll; also, the increase percentage in carotenoids reached 38.3% and 37.9% respectively. Similar results were reported earlier in response to the application of tryptophan in other plant species [6, 14, 36].The influence of tryptophan on chlorophyll biosynthesis may be due to its role in IAA biosynthesis[5,7].The increase in the amounts of photosynthetic pigments of canola leaves in response to benzyladenine application is similar to those reported by [10]. Generally, the increase in pigment contents could be due to the promotion of pigment biosynthesis and /or retardation of pigment degradation. The increase in carotenoids content could protect chlorophylls against photoxidation process as reported by [27].Moreover, benzyladenine could retard chlorophyll breakdown via the inhibition of chlorophyllase [9].

Table 3

Effect of foliar application of tryptophan or benzyladenine on the photosynthetic pigments* of canola plants (combined analysis of two seasons)

Treatments	Chl. a	Chl. b	Chl. a+b	Carotenoids
Untreated plants	9.31	4.61	13.92	2.48
Try.(mg/L)				
25	10.31	4.38	14.69	2.76
50	11.12	4.32	15.44	3.06
75	14.89	6.34	21.23	3.43
B.A.(mg/L)				
25	11.13	4.16	15.32	3.07
50	11.59	4.37	15.96	3.23
75	13.98	5.95	19.93	3.42
LSD 5%	1.02	0.41	2.46	0.31

* mg/g fresh weight.

Data presented in (*tab.4*) show a marked increase in endogenous growth promoters (IAA and GA3) concomitant with a decrease in (ABA) due the different concentrations of tryptophan or benzyladenine. The increase was more pronounced at 50mg/L tryptophan. Such increases in the levels of the endogenous growth promoters could be attributed to the increase in their biosynthesis and/or decrease in their degradation and conjugation. The decrease in ABA content could be attributed to the shift of the common precursor isopentenyl pyrophosphate to the biosynthesis of cytokinins and /or gibberellins instead of ABA [17]. The increase in IAA and GA3 contents in shoot tissues treated with tryptophan or benzyl adenine concur with the increase in growth rate of canola plants. This effect is mainly due to the role of endogenous hormones in stimulating cell division and /or cell enlargement and subsequently the growth. The obtained data are in agreement with the findings of various studies. Harridy [16] demonstrated that tryptophan applied to *catharanthus roseus* plants increased IAA concentration. The increase of indoles may be attributed to the conversion of tryptophan to IAA as stated by [28]. Benzyladenine treatments increased the endogenous auxin content by retarding the destruction of endogenous auxin and preventing the transformation of active free auxin into inactive forms [19]. Endogenous active gibberellins increased by benzyladenine treatments as reported by [13]. Hala [14] showed that IAA and GA3 increased significantly and ABA decreased as a result of tryptophan application to wheat plants even under saline conditions.

Table 4

Effect of foliar application of tryptophan or benzyladenine on acidic endogenous hormones* of canola plants

Treatments	IAA	GA3	ABA
Untreated plants	20.21	23.02	26.99
Try.(mg/L)			
25	35.00	59.82	23.32
50	59.70	60.34	20.70
75	34.94	38.99	21.94
B.A.(mg/L)			
25	29.75	31.05	22.79
50	31.67	37.21	22.21
75	27.27	29.64	21.70

*ug/g fresh weight, mean of duplicate determinations.

The contents of oil; protein; total carbohydrates and phenolic compounds in the yielded canola seeds (*tab.5*) increased gradually with increasing the concentrations of tryptophan or benzyladenine. It is worthily to mention that treatment with tryptophan at 75 mg/L showed the highest increases in the chemical constituents of yielded seeds than the other treatments. The increase percentage was 5.0% in oil content; 8.2% in protein content; 23.6% in total carbohydrates and 23.6 % in phenolic compounds.

Data presented in (*tab.5*) show significant increase in the oil content of the yielded canola seeds. These results are in agreement with those obtained by [25,3] who pointed that foliar application of benzyladenine to soybean and datura induced significant increase in oil percentage. The increase in oil percentage as a result of benzyladenine treatments may be due to its promotive effect on nutrient transport to the developing seeds [12, 37]. The effect of tryptophan application on protein and carbohydrates was previously reported in other plant species [6, 14, 36]. Benzyladenine foliar application (*tab.5*) caused a marked increase in the protein percentage and total carbohydrates. This result is similar to those reported by [11] on faba bean. El- Meleigy [12] and Sun [35] reported that benzyladenine enhanced the accumulation of total carbohydrates and total N- contents in roselle. Cytokinins are known to activate enzymes which regulate carbohydrate metabolism. The increase in carbohydrate accumulation may be due to the decline in the carbohydrate degradation [8]. Hopkins and Huner [17] reported that benzyladenine promoted RNA and protein synthesis, while inhibited certain proteolytic enzymes [30].

Data presented in (*tab 5*) show that foliar application of tryptophan or benzyladenine at 50,75mg/L caused significant increase in phenolic compounds percentage in the yielded canola seeds. The effect of benzyladenine on the phenolic content was reported by [1] who showed that cytokinins increased phenolic content in cotton plants.The increase in phenolic content may be attributed to the increase in carbohydrate synthesis. This result is confirmed by [21] in *Helianthus annuus* and [29] in maize. The increase in total phenolic content was concur with the increases in IAA contents in shoots and led to the suggestion that most of phenolic compounds are diphenols and polyphenols which may inhibit IAA- oxidase activity resulting in auxin accumulation, which reflected in stimulating the growth and yield of plant as reported by [24].

Table 5

Effect of foliar application of tryptophan or benzyladenine on the chemical composition* of the yielded canola seeds (Combined analysis of two seasons)

Treatments	Oil	Protein	Total carbohydrates	Total phenolic compounds
Untreated plants	38.67	21.32	10.80	1.65
Try.(mg/L)				
25	39.30	21.55	12.01	1.68
50	39.95	22.48	12.52	1.96
75	40.62	23.06	13.35	2.04
B.A.(mg/L)				
25	39.52	21.54	11.10	1.65
50	39.70	22.16	11.61	1.75
75	40.31	22.63	12.07	1.78
LSD 5 %	0.48	0.26	0.10	0.09

*percent of whole seeds , on dry weight basis.

The results of the gas chromatographic analysis of the methyl esters of fatty acids of the yielded canola oil are shown in (*tab.6*). The obtained data revealed that all treatments caused a decrease in total saturated fatty acids (Ts) accompanied by an increase in total unsaturated fatty acids (Tu). Thus, ratio Tu/Ts also increased. Palmitic acid was the most predominant saturated fatty acid while oleic acid was the major unsaturated fatty acid. The distinct advantage of canola oil is its low saturated fatty acid content. Cytokinins may play a role in fatty acid synthesis, as well as desaturation and chain elongation reaction as mentioned by [18] who stated that the kinetin treatment increased unsaturated fatty acid at the expense of saturated fatty acids in *Helianthus annuus*.

Table 6

Effect of foliar application of tryptophan or benzyladenine on fatty acid composition* of the yielded canola oils.

Fatty acids	Treatments						
	Untreated plant	Tryptophan (mg/L)			Benzyladenine(mg/L)		
		25	50	75	25	50	75
Palmitic(C16:0)	3.96	4.39	4.06	4.61	4.04	4.60	3.92
Stearic(C18:0)	2.73	1.92	1.83	2.00	1.86	1.76	2.05
Oleic(C18:1)	55.73	56.25	57.50	56.34	57.00	56.37	57.23
Linoleic(C18:2)	14.68	15.20	15.14	15.64	15.90	15.82	14.92
Linolenic(C18:3)	6.97	6.69	6.99	7.52	7.55	7.42	6.94
Arachidic(C20:0)	0.97	0.92	0.99	1.01	0.94	0.95	0.99
Gadoleic(C20:1)	10.81	12.90	12.39	11.41	11.49	11.90	11.58
Behenic(C22:0)	0.43	0.39	0.41	0.36	0.38	0.47	0.40
Erucic(C22:1)	0.85	0.69	0.63	0.80	0.59	0.65	0.85
Total saturated fatty acids(Ts)	8.08	7.20	7.29	7.98	7.22	7.78	7.36
Total unsaturated fatty acids(Tu)	89.04	91.73	92.65	91.71	92.53	92.16	91.52
Tu/Ts	11.02	12.04	12.71	11.49	12.82	11.85	12.43
Total essential fatty acids(C18:2+C18:3)	21.65	21.89	22.13	23.16	23.45	23.24	21.86

* Percent of total fatty acids, mean of duplicate determinations.

The fat quality is usually valued according to the essential fatty acids since human nutrition required some of these fatty acids in the diet to prevent fatty acid deficiency diseases. All treatments used in this investigation caused an increase in essential fatty acids (C18:2+C18:3). The absence of low molecular weight of fatty acids(less than C12) from the yielded oilseeds of untreated and differently treated plants was known to enhance oil stability [39. Erucic acid in the yielded seeds was noticeably decreased with the two compounds. The decrease percentage due to tryptophan was 18.8% and 25.9% using 25 or 50mg/L, whereas using benzyladenine with 25 or 50 mg/L decreased the amount of erucic acid to 30.6% and 23.5% respectively. Higher concentration from the two compounds approximately had no effect. Since the quality of canola oil for human consumption is generally evaluated by its low erucic acid content as well as its low saturated fatty acid coupled with about 8-10% alpha linolenic acid, the present results in this work showed clearly that all treatments did not change this criteria.

CONCLUSIONS

The results of this study showed the possibility of using tryptophan or benzyladenine as a tool to increase the yield of canola seeds/plant as well as its oil, protein and carbohydrate content. Since the nutritional value of canola oil is closely related with the amount of erucic acid, special attention was paid to its content in the yielded seeds which was noticeably decreased with 25 or 50mg/L with either tryptophan or benzyladenine.

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