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Physiological Effect of Putrescine and Heat Hardening on *Nigella sativa* L. Plants

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ABSTRACT

Two seasons pot experiment was carried out to study the effect of presowing heat stress in presence and absence of foliar application of putrescine on vegetative growth and some chemical constituents of *Nigella sativa* L. plants. Foliar application of putrescine to Nigella plants significantly increased growth and yield parameters, more so with 5 mM putrescine. Foliar application of putrescine (5 mM) combined with heat stress at 50ºC for 15 min caused the greatest increase in growth and yield parameters above the control. Foliar application of putrescine at 5 mM significantly increased total sugars, total nitrogen and total protein percent in the seeds. Total fixed oil percent and yield/plant were also positively affected by putrescine treatments. Presowing seed treatment with 50˚C for 15 min significantly increased total sugar, total nitrogen, total protein percent, total fixed oil percent and yield. Foliar application of putrescine (5 mM) combined with presowing seed treatment with 50°C for 15 min caused the greatest increases in total sugars, total nitrogen, total protein percent, total fixed oil percent and yield of *Nigella sativa* L. plants. Presowing seed treatment at 50ºC for 30 min combined with foliar application of putrescine at 1 mM caused the greatest values of total unsaturated fatty acids compared with other treatments, while application of putrescine alone at 1 mM produced the lowest value. The greatest value of total saturated fatty acids was observed in plants treated with 5 mM putrescine, while presowing seed treatment with 50ºC for 30 min resulted in the lowest value of saturated fatty acids. Presowing heat stress at 50ºC for 15 min combined with foliar spray with 5 mM putrescine caused the greatest increase in total unsaturated/total saturated fatty acids ratio compared to other treatments. The results were discussed in relation to the effect of heat hardening and putrescine application.

Key Words: *Nigella sativa* L.; Putrescine; Heat hardening

INTRODUCTION

Nigella sativa L. (Black Cumin) is an annual herb with an erect, branched stem and alternate, finely divided, feathery, greyish-green leaves. The bluish-white, star-shaped flowers are terminal and solitary. Petals are absent. The fruit is a globose capsule with small black, rough seeds. The flowers produce abundant pollen and are attractive to bees (Stodola & Volák, 1992). The ripe seeds have a camphor-like scent and a bitter, later aromatic taste. Their constituents include saponin, an essential oil, a bitter compound (nigelline) and tannins. These substances give black cumin diuretic, cholagogic, carminative, anthelmintic, antispasmodic, galactagogic and emmenagogic properties. The seeds are mainly used for digestive and menstrual disorders, and for bronchitis. They are, however, slightly poisonous (Stodola & Volák, 1992).

Exposure of seeds or plants to sublethal environmental stress often increases their adaptation to subsequent extreme environmental factors. For example, plants become adapted to sublethal temperature for long periods, or to high temperature for short period (Yarwood, 1967; Ageeva & Lutova, 1971). This pre-treatment increased the thermostability of the plants. This phenomenon of acquired adaptation has been interpreted in different ways (Alexandrov, 1964; Levitt, 1972). Henckel (1964) attributed the effect of heat stress to the increased activity in the cell. He also suggested that the hardened plant had relatively high energy level, increased hydration capacity of the protoplasmic colloids and decreased viscosity. Khalil *et al*. (1983) reported that hardening of wheat grains by exposing them to 50˚C for 15 and 30 minutes increased the height of the treated plants. Khalil and Moursy (1983) reported that presowing heat hardening of tomato seeds at 50 and 60˚C increased stem length, number of leaves and leaf area. Gamal El-Din (1992) also found that the number of leaves, fresh and dry weights of leaves and branches of *Hyoscyamus muticus* L. were significantly increased as a result of heat hardening and the most effective treatments were the exposure to 40˚C for 40 minutes and 50˚C for 10 minutes. Similar results were also obtained by Tarraf (1999) on fenogreek and Gamal El-Din and Reda (2003) on wheat plants.

Polyamines are small ubiquitous molecules that have been involved in nearly all developmental processes, including the stress response (Perez-Adamor *et al*., 2002). They are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and

differentiation, related to aging and senescence, and usually involved in plant responses to stress (Flores & Galston, 1982; Friedman *et al*., 1989). Polyamines are low-molecular weight polycations, which are involved in the regulation of growth and stress, probably by binding to negatively charged macromolecules (Smith, 1985; Altman & Levin, 1993; Messiaen *et al*., 1997).

The aim of this investigation was to study the effect of heat hardening and putrescine on the growth and some metabolic changes of *Nigella sativa* L.

MATERIALS AND METHODS

Plant material and growth conditions. *Nigella sativa* L. seeds were obtained from the Horticulture Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. A pot experiment was carried out in the experimental farm of the botany department, National Research Centre, Dokki, Giza, Egypt, during two successive seasons (2000/2001 and 2001/2002), respectively. Batches of seeds were soaked in water until their humidity reached 55 %. Then, the seeds were either exposed to room temperature (ca. 25 °C) or 50˚C for 15 minutes or 50˚C for 30 minutes. On 15th October 2000 and on 19th October 2001, the seeds were sown in pots 30 cm diameter, each pot contained 8 kg clay loam soil. Treatments were distributed in complete randomized block design with five replications, five pots each. Fifteen days after sowing, the seedlings were thinned to the most three uniform plants in each pot. Each pot received equal and adequate amounts of water and fertilizers. Phosphorous as calcium superphosphate was mixed with the soil before sowing at the rate of 4.0 g/pot. Three g of nitrogen as ammonium sulphate in three applications (one g each) with intervals of two weeks started 30 days after sowing. Also, two g of potassium sulphate were added as soil application. Other agricultural processes were performed according to normal practice.

Nigella sativa L. plants received the two different heat hardening treatments as well as untreated plants were sprayed with the freshly prepared solution of putrescine (0, 1 or 5 mM), after 60 days of planting. Tepole was added (1g/l of spraying solution) as wetting agent. The volume of the spraying solution was maintained just to cover completely the plant foliage till drip. Distilled water was sprayed in the same previous manner on untreated plants (control plants).

Measurement of growth parameters. Plant height (cm), number of branches per plant, fresh and dry weights of leaves and stems (g/plant), number of capsules/plant, weight of capsules (g/plant), weight of seeds (g/plant) and weight of straw (g/plant) were determined.

Chemical analysis. Represented samples of the seeds of each treatment were subjected to the following different chemical analyses. Determination of total sugars were carried out according to Dubois *et al.* (1956). Total nitrogen (modified micro-Kjeldahl) was determined as described by Jackson (1973) and from which protein was calculated.

Fixed oil content of each sample was individually extracted from dried seeds and fatty acids percentage was also estimated according to A.O.A.C. (1970). The methyl esters prepared from oil samples and standard materials were analysed by Hewlett Packard gas chromatograph (HP 6890, USA) equipped with a dual flame ionization detector. The separation of fatty acid methyl esters was carried out with a capillary column: Agilent 1909M-413 (length 30 m; diameter 320 µm; thickness 0.25 µm). Column was used with temperatures program of 70º to 190ºC at 8ºC/min. The injector and detector temperatures were maintained at 250º and 300ºC, respectively. The pressure of carrier gas (nitrogen) was 18 kg/cm^2, chart speed 0.5 mL/min. The relative percent of each compound was determined according to the peak area by Vraian 4370 integrator. Different fatty acids were identified by matching their retention times with those of the authentic samples under the same conditions.

Statistical analysis. Data obtained (means of the two growing seasons) were subjected to standard analysis of variance procedure. The values of LSD were calculated, whenever F values were significant at 5 % level as reported by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Growth measurements. Data presented in Table I indicate that foliar application of putrescine to *Nigella sativa* plants significantly increased plant height, number of branches, number of leaves, fresh and dry weights of leaves and stems, in particular plants treated with 5 mM Put. Weight of straw, number of fruits/plant, weight of seeds/fruit, weight of seeds/plant followed the same trend as previous growth parameters. These results are in agreement with those obtained by Youssef *et al.* (2004b) who reported that putrescine increased growth of some Datura species. Egea-Cortines and Mizrahi (1991) indicated that high levels of free polyamines influence growth by affecting cell division and low levels through increasing cell expansion. Smith (1982) and Slocum & Galston (1985) reported that polyamines stimulate growth of several higher plants. In addition, polyamines can act as a sourse of nitrogen which stimulates growth, and also can bind to negatively charged molecules and stabilises them (Smith, 1982; Martin-Tanguy, 1997).

Heat hardening, in particular 50˚C for 15 min, increased the growth parameters measured in this study (Table I). Similar effect of the heat hardening was obtained for number of fruits/plant, weight of fruits, weight of seeds/plant, weight of fruits/plant as well as weight of straw/plant (Table I). Supporting our results is that, Khalil *et al*. (1983), Khalil and Moursy (1983) and Moursy *et al*. (1983) reported that heat hardening of wheat grains and tomato seeds increased their growth. Gamal El-Din (1992)

also found that *Hyoscyamus muticus* L. was significantly increased as a result of heat hardening. Henckel (1964) attributed the growth stimulatory effect of heat hardening to the increased activity in the cell, relatively high energy level, increased hydration capacity of the protoplasmic colloids and decreased viscosity. In addition, Alexandrov *et al*. (1970) also suggested that heat hardening activated some metabolizing substances in the cell and interacted with protein as stabilizing material.

A combination of putrescine and heat hardening significantly promoted growth, yield criteria and chemical constituents of *Nigella sativa* L. plants. Heat hardening of 50ºC for 15 min followed by foliar application of 5 mM putrescine caused the greatest increase in seed yield relative to the control (Table I). Talaat and Shalaby (1998) repored that presowing heat hardening of *Calendula officinalis* L. plants followed by foliar spraying of brassinosteroid increased growth, carbohydrate, nitrogen and oleanolic acid levels.

Supporting these results, Moursy *et al*. (1983) reported that hardening of wheat grains by exposing them to 50˚C for 15 and 30 minutes increased the plant height of the treated ones.

Chemical constituents. Data presented in Table II show that foliar application of Put to *Nigella sativa* L. plants significantly increased total sugars %, total nitrogen and total protein % of the seeds, the effect was more pronounced with 5 mM Put. Total fixed oil % and oil yield / plant were also positively affected by putrescine treatments (Table II). In this connection, Youssef *et al*. (2004a) reported that foliar application of Put to *Matthiola incana* plants significantly increased the contents of photosynthetic pigments, total nitrogen, total oil % and yield. Polyamines have been indicated to activate protein synthesis (Pegg, 1986; Serafini-Fracassini, 1991).

Presowing seed treatment of 50˚C for 15 min significantly increased total sugar %, total nitrogen %, total protein % total fixed oil % and oil yield/plant (Table II). Presowing seed treatment (50˚C for 15 min) followed by 5mM Put showed the greatest increases in total sugars, total nitrogen %, total protein %, total fixed oil % and yield in the seeds of *Nigella sativa* L. plants. Reda and Hegazy (1973), Khalil *et al.* (1983) and Gamal El-Din (1992) obtained significant increase in total carbohydrate and total nitrogen contents in the grains of maize, wheat and *Hyoscyamus muticus*, respectively, after presowing heat hardening of the seeds. Talaat (1993) also reported that subjecting seeds of *Calendula officinalis* L. to high temperature before sowing significantly increased total carbohydrate, total nitrogen % and oleanolic acid (mg/g), especially in plants treated at 45˚C.

Exposure to high temperature may show its effect on plants through membrane changes (Santarius, 1973), increased protein synthesis (Henckel, 1964) and increased content of DNA and RNA (Baker & Jung, 1970).

Fixed oil composition. The relative percentages of fatty acids extracted from *Nigella sativa* seeds are presented in Table III. Twelve fatty acids were identified as major fatty acids (more than 10%), minor fatty acids (1-10%) and traces (less than 1%). The unsaturated fatty acid Linoleic acid (18:2) was found to be the major one in all treatments and ranged from 32.54% to 66.51% followed by oleic acid (18:1) ranged from 1.45% to 19.47% (Table III).

Presowing seed treatment at 50ºC for 30 min followed

Table I. Effect of putrescine and heat hardening on the growth of *Nigella sativa* plants (mean of two seasons).

Treatments	Plant height (cm)	No. of branches	No. of leaves	Fresh weight of leaves (g)	Fresh weight of stems (g)	Dry weight of leaves (g)	Dry weight of stems (g)	No. of fruits /plant	weight of fruits /plant (g)	Wt. of seeds /plant (g)	weight of straw (g)
Effect of Interaction											
Control	29.00	3.67	25.67	1.47	1.30	0.42	0.35	12.67	1.60	0.51	1.64
1mM Put	31.67	4.67	28.33	2.43	1.41	0.54	0.43	17.33	1.83	0.58	2.05
5 mM Put	35.00	4.67	32.67	2.53	2.33	0.67	0.55	20.33	2.53	0.83	2.42
50ºC for 15 min	34.00	4.33	33.00	1.64	2.28	0.53	0.37	16.33	3.03	1.00	1.94
50ºC (15) →1mM Put	35.00	4.67	37.00	3.03	2.62	0.76	0.60	19.00	3.72	1.26	2.12
50ºC (15)→ 5 mM Put	37.33	5.00	42.67	3.77	3.84	0.90	0.81	22.67	4.30	1.46	3.86
50ºC (30 min)	29.00	4.33	28.00	1.87	1.48	0.46	0.36	11.00	1.70	0.51	1.71
50ºC (30 min)→1 mM Put	34.33	4.67	31.67	2.05	2.02	0.66	0.43	13.00	2.53	0.78	2.41
50ºC (30 min)→ 5 mM Put	35.67	4.67	41.00	2.52	2.19	0.72	0.57	16.67	3.70	1.15	3.10
LSD (5%)	1.92	N.S.	2.71	0.34	0.31	0.06	0.03	1.04	0.34	0.11	0.33
Effect of Temp.(mean)											
Control	31.89	4.33	28.89	2.14	1.68	0.54	0.44	16.78	1.98	0.64	2.04
50ºC for 15 min	35.44	4.67	37.56	2.81	2.91	0.73	0.59	19.33	3.68	1.24	2.64
50ºC (30 min)	33.00	4.56	33.56	2.15	1.90	0.61	0.45	13.56	2.64	0.81	2.40
LSD (5%)	1.11	N.S.	1.56	0.20	0.18	0.04	0.02	0.60	0.20	0.06	0.19
Effect of Putrescine (mean)											
Control	30.67	4.11	28.89	1.66	1.69	0.47	0.36	13.33	2.11	0.67	1.76
1mM Put	33.67	4.67	32.33	2.50	2.02	0.65	0.48	16.44	2.69	0.88	2.19
5 mM Put	36.00	4.78	38.78	2.94	2.79	0.76	0.64	19.89	3.51	1.15	3.13
LSD (5%)	1.11	N.S.	1.56	0.20	0.18	0.04	0.02	0.60	0.20	0.06	0.19

Put = Putrescine, → means first treatment followed by the second one.

Table II. Effect of putrescine and heat hardening on chemical constituents of *Nigella sativa* plants.

Treatment	Oil %	Oil yield (ml/plant)	Total sugars%	Total nitrogen %	Protein %
		Effect of Interaction			
Control	15.42	7.90	7.15	2.30	14.38
1mM Put	17.99	10.52	10.50	2.40	15.00
5 mM Put	18.45	15.41	17.00	3.25	20.31
50ºC for 15 min	17.70	17.70	15.83	2.75	17.19
50ºC (15) →1mM Put	21.10	26.66	18.35	2.80	17.50
50ºC (15)→ 5 mM Put	23.40	34.20	22.20	3.00	18.75
50ºC (30 min)	15.63	8.00	9.59	2.50	15.63
50ºC (30 min)→1 mM Put	18.05	14.17	16.50	2.60	16.25
50ºC (30 min)→ 5 mM Put	21.00	24.09	18.50	2.75	17.19
LSD (5%)	1.06	2.61	1.85	0.14	0.90
		Effect of Temperature			
Control	17.29	11.28	11.55	2.65	16.56
50ºC (15 min)	20.73	26.19	18.79	2.85	17.81
50ºC (30 min)	18.23	15.42	14.86	2.62	16.35
LSD (5%)	0.61	1.51	1.07	0.08	0.52
		Effect of Putrescine			
Control	16.25	11.20	10.86	2.52	15.73
1mM Put	19.05	17.12	15.12	2.60	16.25
5 mM Put	19.05	24.57	19.23	3.00	18.75
LSD (5%)	0.61	1.51	1.07	0.08	0.52

Put = Putrescine, → means first treatment followed by the second one.

Table III. Effect of putrescine and heat hardening on fatty acids constituents of the fixed oil of *Nigella sativa* plants

Treatment/ Fatty acids %	Control	1mM Put	5 mM Put	50ºC (15 min)	50ºC (15 min) →1mM Put	50ºC (15 min) → 5 mM Put	50ºC (30 min)	50ºC for (30 min)→ 1 mM Put	50ºC (30 min) →5 mM Put
Caprylic 8:0	1.39	2.57	0.00	5.63	1.74	1.00	0.16	0.91	1.50
Capric 10:0	0.44	0.32	0.00	0.15	0.00	0.00	0.17	0.00	0.76
Lauric12:0	0.44	0.43	0.00	0.06	0.00	0.00	0.40	0.00	0.00
Myrestic 14:0	0.64	1.18	2.20	0.09	1.16	0.00	0.16	0.70	0.75
Palmetic 16:0	0.43	0.30	14.88	0.09	1.34	2.08	2.32	1.70	2.22
Stearic 18:0	0.50	0.62	2.10	4.72	3.01	0.86	0.47	0.88	0.71
Total saturated	3.82	5.42	19.18	10.75	7.25	3.95	3.67	4.18	5.94
Palmetoleic 16:1	0.41	3.95	0.00	5.48	1.42	1.15	0.40	0.65	0.97
Oleic 18:1	10.02	21.27	3.24	1.45	18.38	18.49	12.75	19.47	18.76
Linoleic 18:2	49.50	32.54	60.22	66.51	49.94	54.56	56.59	58.52	53.77
Linolenic18:3	2.10	4.66	3.5	8.06	1.52	6.66	1.09	4.8	4.16
Eicosenoic 20:1	0.77	0.63	4.55	3.88	3.51	3.16	2.68	2.91	2.82
Eicosadienoic 20:2	3.24	2.96	2.13	1.52	1.38	1.35	0.63	0.65	0.88
Total unsaturated	66.04	66.01	73.64	86.89	76.15	85.36	74.14	87.00	81.36
Total identified	69.86	71.43	92.82	97.64	83.40	89.31	77.81	91.19	87.30
Unsat./Sat.	17.30	12.18	3.84	8.08	10.50	21.63	20.19	20.79	13.70

Put = Putrescine, → means first treatment followed by the second one

by foliar application of putrescine at 1 mM Put resulted in the greatest value of total unsaturated fatty acids (87.00%) compared with other treatments (Table III). Application of Put at 1 mM produced the lowest value (66.01%). On the other hand, the greatest value of total saturated fatty acids (19.18%) was observed in plants treated with 5 mM Put, while the lowest value of saturated fatty acids (3.67%) resulted from presowing seed treatment at 50ºC for 30 min. Total unsaturated/total saturated fatty acids ratio ranged from 3.84 to 21.63 (Table III). Foliar application of Put at 5 mM resulted in the lowest total unsaturated/total saturated ratio of fatty acids (3.84), while presowing heat hardening at 50ºC for 15 min combined with foliar spray with 5 mM Put caused the greatest increases in this ratio, compared to other treatments (Table III). It might be suggested that changes in fatty acids composition as a result of presowing heat hardening might be through increased activity in the cell (Henckel (1964) and increased content of DNA and RNA (Baker & Jung, 1970). Meanwhile, polyamines might be affecting fixed oil composition through their effect on activating the synthesis of some enzymes related to fatty acids metabolism. Supporting this suggestion, polyamines have been indicated to activate protein synthesis (Pegg, 1986; Serafini-Fracassini, 1991) and affect enzyme activities of fatty acid synthesis (Brown *et al*., 1991).

In conclusion, more work needs to be done to begin to understand the mechanism by which presowing heat hardening and putrescine treatments are affecting the metabolism of fixed oil constituents and whether these effects can be reproduced in other plant species.

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