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Enhancement quality and quantity of faba bean plants grown under sandy soil conditions by nicotinamide and/or humic acid application

Mona Gergis Dawood^{1*}, Yasser Refaai Abdel-Baky¹, Mohamed El-Sayed El-Awadi¹ and Gehan Shaker Bakhoum²

Abstract

Background: Two field experiments were conducted at the Research and Production Station, National Research Centre, El-Nubaria Province, El-Behira Governorate, Egypt, during the two successive winter seasons of 2014/2015 and 2015/2016. This work aimed to study the enhancement effect of foliar application of nicotinamide at 5, 10, and 20 mg/L and/or humic acid at 5% on quality and quantity of faba bean plants (cultivar Sakha 4) grown under sandy soil conditions.

Results: Data show that nicotinamide at 5, 10, and 20 mg/L and/or humic acid at 5% had a positive effect on growth parameters, photosynthetic pigments, seed yield, and yield components as well as some biochemical constituents of the yielded faba bean seeds (total carbohydrate, total phenolic content, proline, and free amino acids). Individually, nicotinamide treatments had a more positive effect than humic acid treatment and their effect was increased by increasing nicotinamide concentrations. The most promising treatment appeared due to the interaction between nicotinamide at 10 mg/L and humic acid. Hence, this treatment increased plant dry weight/plant by 32.67%, total photosynthetic pigments by 113.2%, seed yield/feddan by 110.16%, total carbohydrate by 9.4%, total phenolic content by 0.33%, proline by 24.83%, and free amino acids by 21.33%.

Conclusions: We can conclude that nicotinamide and/or humic acid had a positive effect on growth parameters, photosynthetic pigments, seed yield, and yield components as well as some biochemical constituents of the yielded faba bean seeds. The most promising treatment appeared due to the interaction between nicotinamide at 10 mg/L and humic acid.

Keywords: Vicia faba, Humic substance, Nicotinamide, Sandy soil

Introduction

Faba bean (*Vicia faba* L.) is one of the most important winter legume crops and a major source of protein for both human and animal nutrition (Crepon et al., 2010). Mature seeds of faba bean are good sources of protein (about 25% in dried seeds), starch, cellulose, vitamin C, and minerals (Hamilton, 2005). They are used for animal feed where, broken seeds are mixed into animal diet and the vegetative parts of the plants are used as the animal

fodder (Akcin, 1988). At the same time, its cultivation leads to an increase of soil N compounds (Hungria and Vargas, 2000).

The newly reclaimed sandy soil is mostly exposed to a combination of environmental stress conditions including low water availability, saline water, saline soil, nutrient deprivation, temperature fluctuations, and high irradiances. In winter, when subjected to low temperature during the different stages of growth and development, plants show several biochemical and physiological dysfunctions (Berger et al., 1993). Such disturbances led to a reduction of growth and poor quantity and quality of the yield (Wang, 1982). In this concern, great efforts must be

Full list of author information is available at the end of the article



^{*} Correspondence: monagergis@yahoo.com

¹Botany Department, National Research Centre, 33 El Bohooth St. (former El Tahrir St.), Dokki, Cairo 12622, Egypt

paid to increase plant tolerance to such conditions, via selecting tolerant genotypes and applying the optimum cultural practices and/or treating the seeds (before sowing) or plants (at different growth stages) with some growth-regulating substances, that play an important role in helping plants to overcome partially the unfavorable conditions and avoid its negative effects on yield quantity and quality.

Vitamins are required in a trace amount to maintain normal growth and proper development of plant (Bassouny et al., 2008). These compounds act as coenzyme systems and play an essential role in the regulation of metabolism. Therefore, these vitamin supplements are known to enhance plant activities and did not have toxic or mutagenic action (Bronzetti et al., 2001).

Nicotinamide (vitamin B3/niacin) is a water-soluble vitamin and a well-characterized constituent of the pyridine dinucleotide coenzymes NADH and NADPH, which are involved in many enzymatic oxidations—reduction reactions in living cells (Sadak et al., 2010). In addition, nicotinamide is a stress-associated compound that induces and regulates secondary metabolic accumulation and/or the manifestation of defense metabolism in plants (Berglund, 1994; Berglund and Ohlsson, 1995). Nicotinamide is considered as one of the growth-regulating substances which in minute quantities can alter some physiological aspects of plants (Bearder, 1980). Robinson (1973) reported that nicotinamide acts as a coenzyme in the enzymatic reactions by which carbohydrates, fats, and proteins are metabolized and involved in photosynthesis and respiration. Soaking of Pisum sativum L. seeds with nicotinamide at the two dates of sowing significantly increased growth and yield components concomitantly with increases in the amounts of IAA, photosynthetic pigments TSS, free amino acids, proline, and phenolic contents as reported by Sadak (2016). Nicotinamide resulted in a significant improvement of physiological and biochemical parameters as well as the concentrations of soluble sugars, proline, amino acids, and total N and other mineral contents of faba bean plants (Abdelhamid et al., 2013).

The humic substance is formed through the chemical and biological humification of plant and animal matter by the biological activities of microorganisms. These substances influence plant growth directly and indirectly. The indirect effects on soil fertility include (i) the increase in the soil microbial population including beneficial microorganisms, (ii) improved the soil structure, and (iii) the increase in the cation exchange capacity and the pH buffering capacity of the soil. Regarding direct effects, humic compounds may have various biochemical effects either at cell wall or in the cytoplasm, including increased photosynthesis, respiration rates, enzymatic activities, protein synthesis (Biondi et al., 1994; Nardi et al., 2002), and plant hormone-like activity (Chen and Aviad, 1990). Humic acid is an important component of humic substances that

contains many functional groups situated at the carbon chain. They could be acidic (e.g., carboxylic acid and phenol), alkaline (e.g., amine, imines), or neutral groups (e.g., alcohol, aldehyde, ketone, ether, ester, and amide) which improve plant growth (Kadam and Wadje, 2011). Humic acid improves physical (Varanini et al., 1995), chemical, and biological properties of soils (Keeling et al., 2003; Mikkelsen, 2005), controlling soil-borne diseases and improving soil health and nutrient uptake by plants, mineral availability, fruit quality, etc., (Atiyeh et al., 2002; Rahmat et al., 2010; Mauromicale et al., 2011). Moreover, humic acid stimulates plant enzymes/hormones and improve soil fertility in an ecologically and environmentally benign manner (Sarir et al., 2005; Mart, 2007). The hormone-like activities of humic acid in particular auxin, cytokinin, and gibberellins are well documented in various papers (Varanini and Pinton, 2001; Pizzeghello et al., 2002; Chen et al., 2004). When humic acid was added to clay compacted soils, it helps break up clay compacted soils; assists in transferring micronutrients from the soil to the plant; enhances water retention; increases seed germination rates; improves water, air, and root penetration; and stimulates development of microflora population in soils (Mackowiak et al., 2001). When humic acid was applied to sandy soils, it adds essential organic material necessary for water retention, thus improving the root growth and enhancing the sandy soil ability to retain and not leach out vital plant nutrients (Khaled and Fawy, 2011).

This work aimed to study the enhancement effect of the application of nicotinamide and/or humic acid on quality and quantity of faba bean plants grown under sandy soil.

Materials and methods

Plant materials and experimental conditions

Two field experiments were conducted at the Research and Production Station, National Research Centre, El-Nubaria Province, El-Behira Governorate, Egypt, during two successive winter seasons of 2014/2015 and 2015/2016. The seeds of faba bean cultivar (Sakha 4) were obtained from the Legumes Crops Research Department, Ministry of Agriculture, Egypt, and selected for uniformity in size and color.

The soil of the experimental site was sandy soil. Mechanical, chemical, and nutritional properties of the experimental soil site (30 depths) were reported in Table 1 and were analyzed according to Chapman and Pratt (1978).

Table 1 Mechanical and chemical analyses of experimental soil

Sand (%)		,	Soil texture		ECds/ m				
93.7	3.9	4.78	Sandy	7.8	0.5	0.24	9.2	3.6	23.3

Regarding fertilization, P2O5 as calcium superphosphate (15.5%) and K_2O as potassium sulfate (48–52%) were added during seed bed preparation at the level of 31 and 24 kg/fed, respectively, while nitrogen fertilizer as ammonium nitrate (33.5%) was added at the rate of 75 kg N/fed. Seeds of faba bean were sown in hill spaced 20 cm apart at both sides of the ridge on the middle of November during the two growing seasons. Thinning was carried out at 15 days after sowing to leave two plants per hill. The experiments were laid out in a randomized complete block design with three replicates/treatments. The experimental unit was 10.5 m² (1/400 fed.) 3-m long and 3.5-m wide, and 60 cm apart between ridges. The plants were sprayed twice at 45 and 60 days after sowing with freshly prepared solutions of nicotinamide at 5, 10, and 20 mg/l and humic acid at 5% individually or in combination. Meanwhile, untreated plants were sprayed by distilled water to serve as a control. Irrigation was carried out using the sprinkler irrigation system where plants were watered every 5 days for 2 h.

Data recorded

Plant samples were collected after 75 days from sowing for measurement of some growth parameters (plant height, leave and branch number/plant, fresh and dry weights/plant) and photosynthetic pigments in fresh leaf.

At harvest, the following characters, number of pods/plant, number of seeds/pod, seed yield/plant (g), and 100 seeds weight (g) were recorded at a random of ten guarded plants from each plot. The whole plot was harvested to determine seed yield/feddan (kg). The yielded seeds were cleaned and crushed to determine total carbohydrate, total free amino acid, proline, and total phenolic contents.

Chemical analysis

Chlorophyll a, chlorophyll b, and carotenoids were determined using a method described by Moran (1982). The phenol-sulfuric acid method was used for the determination of total carbohydrates (Dubois et al., 1956). Total phenolic compounds were determined according to the method described by Zhang and Wang (2001). Proline was estimated according to Bates et al. (1973). Free amino acid content was determined with the ninhydrin reagent method (Yemm and Cocking, 1955).

Statistical analysis

The average of two seasons was statistically analyzed as a randomized complete block design according to Snedecor and Cochran (1980), and the least significant difference (L.S.D.) for each was calculated at 0.05 level of probability by using the MSTATC (1989) program in this connection.

Results

Vegetative growth parameters

Table 2 showed that nicotinamide and/or humic acid application caused marked increases in plant height, number of branches and leaves/plant, and fresh and dry weight/plant. These parameters were increased gradually by increasing nicotinamide concentration. Moreover, data indicated that nicotinamide treatments individually were more effective than humic acid. On the other hand, it was noted that interaction between nicotinamide at 10 mg/L and humic acid caused the highest significant increases of all vegetative growth parameters relative to control. Hence, this treatment increased plant dry weight/plant by 32.67% over control.

Table 2 Effect of nicotinamide and/or humic acid on vegetative growth parameters of faba bean plants at 75 days after sowing (data are combined of two seasons)

Treatments	Plant height (cm)	Number of branches/ plant	Number of leaves/ plant	Plant fresh weight (g)	Plant dry weight (g)
Control	34.00	2.33	19.83	35.39	9.91
Humic acid 5%	36.50	2.33	21.50	40.63	10.82
Nicotinamide 5 mg/L	37.50	2.66	22.50	43.30	11.10
Nicotinamide 10 mg/L	38.00	2.83	23.00	44.35	12.60
Nicotinamide 20 mg/L	38.50	2.83	24.67	46.85	13.38
Nicotinamide 5 mg/L + humic acid 5%	37.00	2.33	23.17	37.74	11.20
Nicotinamide 10 mg/L + humic acid 5%	38.00	3.00	29.17	51.46	14.05
Nicotinamide 20 mg/L + humic acid 5%	37.50	2.33	22.83	37.85	10.59
L.S.D. at 5%	3.28	N.S	6.19	3.13	1.51

Table 3 Effect of nicotinamide and/or humic acid on photosynthetic pigments (mg/g) of fresh faba bean leaf at 75 days after sowing

Treatments	Chlorophyll a	Chlorophyll b	Carotenoid	Chlorophyll a + chlorophyll b	Total photosynthetic pigments
Control	1.464	0.399	0. 062	1.863	1.925
Humic acid 5%	1.574	0.541	0.281	2.115	2.396
Nicotinamide 5 mg/L	1.702	0.878	0.207	2.580	2.787
Nicotinamide 10 mg/L	1.925	1.104	0.207	3.029	3.236
Nicotinamide 20 mg/L	1.838	1.339	0.168	3.177	3.345
Nicotinamide 5 mg/L + humic acid 5%	1.567	0.734	0.155	2.301	2.456
Nicotinamide 10 mg/L + humic acid 5%	2.167	1.668	0.270	3.835	4.105
Nicotinamide 20 mg/L + humic acid 5%	2.043	1.296	0.143	3.339	3.482
L.S.D. at 5%	0.42	0.71	0.10	1.03	0.94

Photosynthetic pigments

All applied treatments caused marked increases in all components of photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids) (Table 3). Regarding nicotinamide treatments, it was notated that plant photosynthetic pigments were increased by increasing nicotinamide concentration, whereas the interaction between nicotinamide at $10\,\mathrm{mg/L}$ and humic acid caused the highest significant increases in total photosynthetic pigments relative to other treatments. Hence, it increased the total photosynthetic pigments by 113.2% over control.

Seed yield and yield components

Nicotinamide and/or humic acid treatments caused obvious increases of pod number/plant, seed number/pod, weight of 100 seeds, seed yield/plant, and seed yield/feddan relative to control (Table 4). Nicotinamide application individually at different concentration increased seed yield and its components relative to control by increasing nicotinamide concentrations. The interaction between nicotinamide at 10 mg/L and humic acid showed the maximum

increases in seed components and seed yield. Hence, this treatment increased seed yield/feddan by 110.16% relative to control

Some biochemical constituents of the yielded seeds

All concentration of nicotinamide and humic acid individually or in combination caused significant increases in carbohydrate content, total phenolic content, proline, and free amino acids relative to control (Table 5). The interaction between nicotinamide at 10 mg/L and humic acid increased carbohydrate, phenolic, proline, and free amino acid contents by 9.40%, 0.33%, 24.33%, and 21.33%, respectively, relative to control.

Discussion

Vegetative growth parameters

Table 2 shows that nicotinamide and/or humic acid application caused marked increases in shoot height, number of branches and leaves/plant, and fresh and dry weight/plant. On the other hand, it was noted that interaction between nicotinamide at 10 mg/L and humic acid

Table 4 Effect of nicotinamide and/or humic acid on seed yield and yield components of faba bean plants

Treatments	Number of pods/ plant	Number of seeds/ pod	Weight of seeds/plant (g)	Weight of 100 seeds (g)	Seed yield (kg/ feddan)
Control	10.00	3.00	25.77	70.30	346.30
Humic acid 5%	10.17	3.33	26.80	70.50	360.50
Nicotinamide 5 mg/L	13.33	3.33	37.77	74.43	507.70
Nicotinamide 10 mg/L	14.00	3.50	39.70	78.20	528.60
Nicotinamide 20 mg/L	16.00	3.50	46.00	83.10	618.30
Nicotinamide 5 mg/L + humic acid 5%	14.00	3.33	39.57	78.63	532.10
Nicotinamide 10 mg/L + humic acid 5%	17.17	3.50	54.13	88.73	727.80
Nicotinamide 20 mg/L + humic acid 5%	10.33	3.17	27.03	78.47	362.90
L.S.D. at 5%	1.131	N.S.	2.93	5.44	103.70

Table 5 Effect of nicotinamide and/or humic acid on some biochemical constituents of the yielded faba bean seeds

Treatments	Total carbohydrate (%)	Total phenolic content (mg/g)	Proline (mg/g)	Free amino acids (mg/g)
Control	39.98	18.51	27.86	118.00
Humic acid 5%	42.15	24.20	32.26	129.60
Nicotinamide 5 mg/L	41.54	21.92	26.90	125.10
Nicotinamide 10 mg/L	41.67	22.93	31.38	131.80
Nicotinamide 20 mg/L	42.44	24.08	33.11	136.30
Nicotinamide 5 mg/L + humic acid 5%	42.60	23.08	31.95	130.60
Nicotinamide 10 mg/L + humic acid 5%	43.74	24.66	34.78	158.10
Nicotinamide 20 mg/L + humic acid 5%	41.40	23.19	31.50	143.60
L.S.D. at 5%	1.34	0.85	0.69	6.17

caused the highest significant increases of all vegetative growth parameters relative to control. The stimulatory effects of nicotinamide on plant growth were found to be correlated with the increase in content and activity levels of endogenous promoters (Kumari et al., 1987; Wilkins, 1989). Nicotinamide is known as a growth-regulating factor that influences many physiological processes such as biosynthesis of enzymes, nucleic acids, and proteins and acts as a coenzyme (Hathout, 1995). These results are in agreement with those obtained by others Zhang et al. (2000), El-Bassiouny et al. (2005), Hassanein et al. (2009), Sadak et al. (2010), and Erdal et al. (2011).

On the other hand, Karakurt et al. (2009), El-Nemr et al. (2012), and Said-Al Ahl et al. (2016) showed that foliar spray of humic acid enhanced plant growth, yield, and quality in a number of plant species due to its action on different physiological and metabolic processes. Humic acid increased nutrient absorption, cell division, photosynthesis (Atiyeh et al., 2002), respiration, biosynthesis of nucleic acid and enzyme, and overall, dry weight of the plant (Ulukan, 2008; Said-Al Ahl et al., 2016). Moreover, application of humic acid to foliage and to soil increased auxin, cytokinin, and gibberellin levels in plants (Abdel--Mawgoud et al., 2007). Likewise, humic acid is a hormone-like substance, its auxin-like activity stimulated the cell division and cell elongation (Mato et al., 1971; Schnitzer and Weightman, 1974), and this in turn improved plant growth (Abdel-Mawgoud et al., 2007). Furthermore, El-Ghozoli (2003) and Meganid et al. (2015) confirmed that humic acid was able to produce positive effects in improving fresh and dry biomass of faba bean and common bean plants.

Photosynthetic pigments

All applied treatments caused marked increases in all components of photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids) (Table 3). The enhancement effect of nicotinamide on photosynthetic pigments is confirmed by Sadak et al. (2010) and Abdelhamid et al.

(2013) on sunflower and faba bean. Nicotinamide might be protecting chloroplast and their membrane and maintaining their integrity and protect chloroplast from oxidative damage (Taylor et al., 1982; Munne-Bosch et al., 2001). In addition, the effect of nicotinamide on the biosynthesis of chlorophyll might be attributed to its activation of enzymes that regulate photosynthetic carbon reduction (Taylor et al., 1982). On the other hand, the positive effect of humic acid on photosynthetic pigments could be attributed to an increased in CO₂ assimilation and photosynthetic rate (Ameri and Tehranifar, 2012). These increases might be due to the role of humic acid in increasing rubisco enzyme activity and then increased the photosynthetic activity of plants and its yield (Delfine et al., 2005). Humic acid might be caused an enhancement in the synthesis of the chlorophyll and/or delayed chlorophyll degradation even under different stress as reported by Nardi et al. (2002) and Meganid et al. (2015). The increases in photosynthetic pigment content were due to the humic acid application in agreement with those obtained by Ameri and Tehranifar (2012), Bakry et al. (2013), and El-Bassiouny et al. (2014) on different plants.

Seed yield and yield components

Nicotinamide and/or humic acid treatments caused obvious increases of the pod number/plant, seeds number/pod, weight of 100 seeds, seed yield/plant, and seed yield/feddan relative to control (Table 4). The increase in seed yield and yield components due to nicotinamide treatments might be attributed to the effect of vitamins on enhancing protein synthesis and delaying senescence (Sahu et al., 1993). On the other hand, Srivastava (1995) stated that humic acid application increased photosynthetic rate, nutrient uptake from the soil to leaves, and translocation of these nutrients from the leaves to seeds, thereby enhancing seed yield without spending any energy as well as without any loss in transit. Humate treatment enhanced overall metabolism of crop plants and overall photosynthetic rate and hence the yield in general (Zeng, 2002). Moreover, Moraditochaee (2012)

recommended that humic acid significantly increased seed yield, straw yield, and biological yield of peanut. Many researchers concluded the enhancing effect of humic acid on growth, yield, and nutrient uptake by many crops (Neri et al., 2002; El-Desuki, 2004; Saruhan et al., 2011).

Some biochemical constituents of the yielded seeds

All concentration of nicotinamide and humic acid individually or in combination caused significant increases in carbohydrate content, total phenolic content, proline, and free amino acids relative to control (Table 5). These results could be explained by several authors, i.e., Robinson (1973) who revealed that nicotinamide acts as a coenzyme in the enzymatic reactions of carbohydrates, fats, and protein metabolism and involved in photosynthesis and respiration. Tarraf et al. (1999) reported that foliar application of nicotinamide to lemon grass plants significantly increased total carbohydrate and crude protein. Moreover, Sana and Ota (1977), Bearder (1980), and Hathout et al. (1993) reported that nicotinamide plays an important role in increasing pigments, carbohydrates, nitrogen, RNA, and DNA contents in plants. Mohamed et al. (1989) and Sadak et al. (2010) found that nicotinamide increased the level of carbohydrates of wheat and sunflower plant. These results are in agreement with those obtained by Hassanein et al. (2009) in maize and Sadak et al. (2010) in sunflower plant.

On the other hand, Ferrara and Brunetti (2010) observed the positive effect of HA on the photosynthetic metabolism in berry leaves, accompanied by an increase of soluble sugars and this change appeared to be mediated by variations of the activity of the main enzymes involved in carbohydrate metabolism (Merlo et al., 1991).

Regarding phenolic contents, total phenolic contents play a significant mechanism in the regulation of plant metabolic processes and consequently of overall plant growth (Lewis and Yamamoto, 1990). Moreover, phenolic contents act as a substrate for many antioxidant enzymes, so, it mitigates stress injuries (Khattab, 2007). Unlu et al. (2010) showed that humic acid application significantly improved antioxidant compounds in pepper fruit and this effect was mainly on carotenoids and phenolic compounds.

In respect to proline, total amino acids, and the treatment with humic acid and/or nicotinamide increased significantly free amino acid contents and proline of faba bean sees when compared with control. These obtained results are in agreement with those obtained by Peymaninia et al. (2012) on wheat, Bakry et al. (2013) on flax, and El-Bassiouny et al. (2014) on the wheat plant.

Many functions have been postulated for proline accumulation in plant tissues, and proline and free amino acids could be involved in the osmotic adjustment of plants (Delavari et al., 2010) and could also be a

protective agent of enzymes and membranes (Gzik, 1996). When a plant is subjected to unfavorable conditions as the newly reclaimed sandy soil, plants maintain their water content by the accumulation of compatible organic solutes acting as osmoprotectants, as proline, in their cytoplasm (Bandurska, 1993). This means that the inhibitory effect of unfavorable conditions on faba bean plant was alleviated by nicotinamide and/or humic acid treatments through increasing proline synthesis and/or enhancing the biosynthesis of other amino acids and their incorporation into protein. Barakat (2003) stated that vitamins probably improve growth of plants and attributed to inhibiting proline synthesis and/or enhancing the biosynthesis of other free amino acids and their incorporation into protein. In addition, vitamins might act as activators of protein synthesis via significant alteration in the enzymes related to protein metabolism (Kodandaramaiah, 1983). Humic acid influences the respiration process, the amount of sugars, amino acids, and nitrate accumulated (Boehme et al., 2005).

Conclusion

It could be concluded that nicotinamide at 5, 10, and 20 mg/l and/or humic acid at 5% showed a positive effect on growth parameters, photosynthetic pigments, seed yield, and yield components as well as some biochemical constituents of the yielded faba bean seeds (total carbohydrate, total phenolic content, proline, and free amino acids). The most promising treatment was appeared due to the interaction between nicotinamide at 10 mg/L and humic acid.

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Author details

¹Botany Department, National Research Centre, 33 El Bohooth St. (former El Tahrir St.), Dokki, Cairo 12622, Egypt. ²Field Crops Department, National

Research Centre, 33 El Bohooth St. (former El Tahrir St.), Dokki, Cairo 12622, Egypt.

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