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SEX EXPRESSION RESPONSE OF SPINACH AS AFFECTED BY BIOFERTILIZER AND GA_3 APPLICATION AND ITS RELATION TO YIELD, QUALITY AND SEED PRODUCTION

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ABSTRACT

The main objective of this study was to determine the response of sex expression and yield as well as its quality characteristics of spinach, in relation to planting date, biofertilizer and GA_3 treatments. Two field experiments were carried out during 2001/2002 and 2002/ 2003 winter seasons at the Agricultural Research Station, Faculty of Agriculture, Alexandria University, to investigate the effects of planting date (October and December) and the application of biofertilizer (Halex2) and/or GA_3 spray (25 ppm) as well as their interaction on vegetative growth, sex expression, chemical composition, yield of fresh leaves and its quality and seed production of spinach plant, cv. Saloniky.

The obtained results revealed that:

1- Sowing spinach seeds early in October significantly increased plant height, photosynthetic pigments and total sugar contents of leaves and seed yield and its quality, compared with the late planting date (in December). On the other hand, no significant differences were detected in number and area of spinach leaves, nitrogen content and C/N ratio, endogenous phytohormones and yield of fresh leaves and its quality, due to planting date.

2- Inoculation of spinach seeds with biofertilizer was more pronounced for almost studied characters, such as vegetative growth, chemical composition and yield of fresh leaves and its quality, than GA_3 and control treatments. In addition, biofertilizer significantly enhanced both cytokinin and ethylene contents and reduced GA_3 content of leaves.

3- Biofertilizer inoculation significantly increased also the number of vegetative male, female and monoecious spinach plants and reduced the number of extreme male plants which was significantly increased with GA_3 spraying. Sex expression response to biofertilizer and/or GA_3, in relation to endogenous hormone changes was also discussed.

4- The correlation coefficient studies confirmed the relationships between sex expression and total yield of spinach as well as its quality characteristics, in relation to the effect of biofertilizer and/or GA_3 application, at both planting dates.

5-On the other hand, the results revealed that GA_3 is needed beside biofertilizer inoculation in order to increase seed production of spinach and to improve its quality, under either early (October) or late (December) planting dates.

6- It can be recommended the use of biofertilizer (Halex2) for spinach production to reduce the mineral NPK-fertilizers and to increase the yield of fresh leaves and its quality, and the use of GA_3 beside biofertilizer to increase seed yield and its quality.

Keywords: Spinach, Sex expression, Planting date, Biofertilizer, GA_3.

INTRODUCTION

Spinach (*Spinacia oleraceae* L.) is an annual long day plant, grown in temperate regions exclusively for its leaves. It is considered as one of the most important vegetable crops in Egypt, used fresh, canned as well as frozen product. Its leaves are rich in several minerals and vitamins, such as ascorbic acid, carotene, riboflavin and small quantity of thiamine (Rubatzky and Yamaguchi, 1997).

Quality of spinach depends mainly on some factors among them are chlorophyll, calcium and iron leaf contents (Abd El-Bary *et al.*, 1997). On the other hand, environmental factors; i.e., temperature and light affect growth, yield and quality of spinach (Stino *et al.*, 1973 and Gonzalez and Marx, 1982) as well as spinach leaf contents of vitamins, calcium oxalate (El-Shiaty *et al.*, 1980 and Matsumoto and Yamagata, 1999) and nitrate (Cantliffe, 1972; Aworth *et al.*, 1978 and Breimer, 1982).

Spinach is classified as a dioecious plant which is not strictly true because varying sexual types occur. Plant types are either male, female or both. Thus, sex expression characteristics of spinach plants were classified by Rubatzky and Yamaguchi, 1997) as: i) extreme males produce only staminate flowers, minimal foliage, flower early and die after flowering, ii) vegetative males produce only staminate flowers, more foliage and flower slightly late than extreme males, iii) female plants produce only pistillate flowers, have well-developed foliage and are very late to flower, and iv) Monoecious plants produce both staminate and pistillate flowers, well-developed foliage and are slow to flower.

Moreover, the predominate sex expression of spinach plants is difficult to be determined prior flowering, although small plants usually are male. Therefore, female and vegetative males as well as monoecious plants are preferred because they are larger, slower bolting and higher yielding with best quality.

The most important environmental factors affecting the shifts in sex expression are day length, light intensity, temperature, mineral nutrition and composition of the ambient atmosphere (Culafic, 1999). The contents of endogenous phytohormones changed during plant ontogeny and depended on the photoperiod, in long day spinach plants, whereas, genetic and hormonal regulation of sex expression in spinach is discussed by Chailakhyan (1979) and Zeevaart and Cage (1993). It is shown that sex expression of this plant is regulated by gibberellins which are synthesized in leaves and cause male sex expression, and by cytokinins which are synthesized in the roots and cause female sex expression. These data indicated that sex expression in dioecious plants is the result of the interaction between the genetic apparatus and phytohormones.

Furthermore, Sorial (2000) reported that inoculation with biofertilizer (nitrogen fixing bacteria) with lower mineral nutrient levels changes endogenous hormones which may control sex expression of squash plants and leads to sex ratio modification.

This study, therefore, was undertaken over two years as an attempt to investigate the response of sex expression, yield and its quality to planting date, Halex2 and GA_3 treatments. The relationship between sex expression and yield along with its quality was also considered. Determination of the chemical composition and endogenous phytohormones of spinach leaves as affected by the studied treatments was also the second target of the present work.

MATERIALS AND METHODS

Two field experiments were conducted during the two successive winter seasons of 2001/2002 and 2002/2003, at the Agricultural Research Station, Faculty of Agriculture, Alexandria University, in order to study the effects of planting date, application of biofertilizer and gibberellic acid (GA_3) as well as their interaction on vegetative growth, sex expression, chemical composition and the productivity of spinach plants, cv. Saloniky (smooth leaved). In addition, the relationship between sex expression and total yield and its quality characteristics of spinach was also investigated. The soil of the experimental sites was silty clay loam in texture with pH 8.14 and 8.22, EC 3.11 and 3.31 mmhos/cm, available N 0.134 and 0.147%, available P 0.051 and 0.066% and available K 0.068 and 0.077%, in the first and second seasons, respectively.

The experimental treatments and design

The experiments included 8 treatments which were all combinations of two planting dates and four biofertilizer and GA_3 treatments, which were:

1- NPK fertilizers at the recommended levels (control treatment).

2- Biofertilizer inoculation + 50% of the recommended NPK levels (Bio treatment) (Niel, 2001).

3- GA_3 spraying + NPK fertilizers at the recommended levels (GA_3 treatment).

4- Biofertilizer + GA_3 + 50% of the recommended NPK levels (Bio + GA_3 treatment).

The experimental layout used was split-plot in randomized complete blocks design with three replications. Planting dates were considered as the main plots, whereas, the four biofertilizer and GA_3 treatments were considered as the sub-plots as they were randomly assigned within each main plot. Each sub-plot consisted of five rows, 4.5 m long and 0.7 m apart, occupying an area of 15.75 m^2.

Seeds of spinach were sown at both sides of the ridges on October 20^{th} and December 5^{th}, in the first season, however, the two planting dates were October 22^{nd} and December 6^{th}, in the second season.

NPK-fertilizers (control treatment) were used at the recommended levels, which were 100 kg ammonium nitrate (33.5% N), 150 kg calcium superphosphate (15.5% P_2O_5) and 50 kg potassium sulphate (48% K_2O) per feddan. P-fertilizer was applied one week before sowing, however, the levels of N and K-fertilizer were applied in two equal split applications; the first was

Halex2, a biofertilizer contains a mixture of growth promoting N-fixing bacteria of genera *Azospirillum*, *Azotobacter* and *Klebsiella*, which was kindly supplied by Prof. Dr. M.G. Hassouna, Biofertilization Unit, Plant Pathology Dept., Alexandria Univ., was used as seed inoculation treatment and was compared with mineral NPK-fertilizers. Spinach seeds were mixed with Halex2 before planting at the rate of 7.0 g/kg seeds.

A sodium salt of gibberellic acid (GA_3), commercially named Berelex, was used in this study. Berelex is produced in tablets form. Each tablet contained one gram of the active ingredient (GA_3) and easily soluble in water. Spinach plants were sprayed twice with GA_3 at the rate of 25 ppm (Chailakhyan, 1979). The first spray was applied three weeks after sowing (at the 2-3 leaf stage) and repeated at the same concentration at the flowering stage (for seed yield only).

Pest control and other cultural practices, such as cultivation and irrigation, were applied whenever it was necessary and as commonly recommended in the commercial spinach production. Harvest took place on January 5^{th} and February 15^{th}, in the first season, and on January 7^{th} and February 17^{th}, in the second season, when spinach plants had reached marketable size. Seed yield was obtained at the end of season, when spinach plants had reached the full maturity stage.

Data recorded

1. **Vegetative growth characters:** A random representative sample of 20 spinach plants was taken from the two outer rows of each sub-plot, three days after harvest, and the following growth measurements were recorded: plant height (cm), number of expanded leaves and leaf area (cm^2) per plant.
2. **Chemical analysis of leaves:** A random representative sample of 10 spinach plants was taken from the two outer rows of each sub-plot, five days after harvesting for determination of the following chemical analysis:

a) Leaf chlorophyll *a*, *b* and carotenoid contents, according to the method of Witham *et al.*(1971).

b) Total sugars and total carbohydrates were determined according to the method of Dubois *et al.*(1956), then total mass of carbon was calculated.

c) Nitrogen was estimated using microkjeldahl method (A.O.A.C., 1980), then C/N ratio was calculated.

d) Endogenous phytohormones, GA_3, IAA and cytokinin were extracted as described by Jones *et al.*(1980). Then it was determined using GLC in Arid Land Agricultural Research Unit, Faculty of Agriculture, Ain Shams Univ. Ethylene was measured using the modified method of Lizada and Yang (1979). Ten grams of fresh spinach leaves was taken from each sub-plot at the beginning of flowering as it was ground in liquid nitrogen and extracted overnight in 80% ethanol at 40°C. The crude was centrifuged and the supernatant was collected, evaporated to dryness and dissolved in 1 ml distilled water and the sample was injected into a varian 3700 gas chromatography filled with porapak CI column and flame ionization of ethylene was calculated and expressed as ng.100 g^{-1} fresh weight. Each hormone was presented as a percentage of the total hormones for each treatment.

3. **Sex expression:** All spinach plants of the medium row were taken, from each sub-plot, at the end of flowering stage and the beginning of the fruiting stage, and were used for determination of sex expression. Number of male (extreme and vegetative) plants, female plants and monoecious, was recorded. Then, the percentage of each group was calculated.

4. **Yield and its quality:** At harvest, weight of the harvested plants from the second row of each sub-plot was recorded and then converted to kg/m^2 and ton/fed. A random representative sample of 10 spinach plants was taken from each sub-plot and the following quality characteristics were determined:

a) Average plant weight (g).
b) Dry weight per plant (g) after drying at 70°C for 48 hours.
c) Dry matter content (%).
d) Ascorbic acid content (vitamin C) of leaves, using the titration method with 2,6-dichlorophenol indophenol dye, as described in A.O.A.C.(1980), and expressed as mg/100 ml of fresh juice.

5. **Seed yield and its quality:** All spinach plants in the remainder row (fourth row) from each sub-plot were left until the full plant maturity stage to determine the seed yield and its components. The following data were recorded:

a) Seed yield per m^2 (g).
b) Seed yield per feddan (kg).
c) Weight of 100 seeds in gram (seed index).
d) Seed germination percentage.
e) Dry matter content of seeds (%).

6. **Correlation coefficients:** All possible correlation coefficients (r) among the yield and its quality and sex expression of spinach plants as affected by the studied factors, in the second season, were calculated using Costat Software (1985).

All obtained data were statistically analyzed using Costat Software (1985), and treatment means were compared by using revised L.S.D test according to the procedure outlined by Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

1. Vegetative growth characters

As shown in Table (1), plant height was significantly affected by planting date, as this growth index tended to decrease in December planting, compared with that of October planting date, in both seasons. This may be due to the variation in temperature degrees during the growing seasons. This result was in agreement with those obtained by El-Sayed and Shehata (1990). Similar results were also found by Stino *et al.*(1973), who attributed this increase in plant height during the early planting date to the increase in the number and size of cells. In addition, Shinohare and Suzuki (1981) stated that the relatively warm weather and long photoperiod of October favours this phase of vegetative growth in leafy vegetable crops. However, the obtained results were not in agreement with those found by Abd El-Bary *et al.*(1997), who stated that vegetative growth of spinach plants was best when seeds were sown on December.

Table (1): Vegetative growth characters of spinach plants as affected by planting date, biofertilizer and GA_3 treatments and their interaction, in 2001/2002 and 2002/2003 winter seasons.

Parameters	Planting date	2001/2002 winter season					2002/2003 winter season				
		Treatments				Mean	Treatments				Mean
		Control	Bio.	GA_3	Bio+GA_3		Control	Bio.	GA_3	Bio+GA_3	
Planting height (cm)	October	35.50bc	37.27b	43.17a	44.43 a	40.09A	34.53bc	36.77 b	42.47a	43.87 a	39.41A
	December	33.13 c	35.37bc	41.53a	42.87 a	38.23B	31.80 c	34.53bc	40.93a	42.67 a	37.48B
	Mean	34.32 B	36.32 B	42.35A	43.65 A		33.17 C	35.65 B	41.70A	43.27 A	
Number of leaves	October	7.83 cd	9.83 a	8.07 c	8.50 b	8.56 A	7.37 cd	9.37 a	7.53 c	8.23 b	8.13 A
	December	7.27 e	9.70 a	7.60de	8.13 bc	8.18 A	7.07 d	9.27 a	7.37cd	8.10 b	7.95 A
	Mean	7.55 C	9.77 A	7.84 C	8.32 B		7.22 C	9.32 A	7.45 C	8.17 B	
Leaf area (cm^2)	October	305 cd	478 a	334bcd	386 b	376 A	291 c	458 a	319 bc	366 b	359 A
	December	293 d	463 a	323bcd	375 bc	364 A	282 c	456 a	314 bc	367 b	355 A
	Mean	299 C	471 A	328BC	381 B		286 C	457 A	317BC	367 B	

Values marked with the same alphabetical letters, within a comparable group of means, do not significantly differ, using revised L.S.D. test at 0.05 level.

On the other hand, values recorded in Table (1) also show that number of spinach leaves and leaf area were not significantly affected by planting date, in both seasons. These results, in general, were not in agreement with those obtained by Stino *et al.*(1973), El-Sayed and Shehata (1990) and El-Gizawy *et al.*(1992), who found that sowing seeds of Saloniky spinach cultivar on January significantly increased leaves number and leaf area as compared with seed sowing on November.

With regard to the effect of biofertilizer and/or GA_3 treatments on vegetative growth, data presented in Table (1) reveal that spinach plants were significantly taller when sprayed with GA_3 alone or with biofertilizer inoculation, than those of the control or biofertilizer alone treatments, in both seasons. Similar results were also obtained by Abd El-Fattah (1997) on broad beans and Liang *et al.*(1998) on spinach plants. On the other hand, data in Table (1) showed clearly significant increments in the number of leaves and leaf area of spinach plants when inoculated with biofertilizer alone, in both seasons, compared with the control and GA_3 treatments, followed by biofertilizer and GA_3 treatment. Similar finding was also obtained by Abd El-Fattah and Sorial (1998) on lettuce plant and Abd El-Fattah and Sorial (2000) on squash plant.

Beneficial effects of rhizosphere bacteria have most often been based on increased plant growth, faster seed germination and better seedling emergence. Plant growth-promoting rhizobacteria (biofertilizers) may induce growth promotion directly or indirectly (Lazarovits and Nowak, 1997), which together promote the vegetative growth to go forward (Jagnow *et al.*, 1991 and Carletti *et al.*, 1996).

The comparison among the means of various combined treatments of planting date and biofertilizer and/or GA_3, listed in Table (1), indicated that spinach plants which were inoculated with biofertilizer, at both planting dates, had the highest stimulating effects on number and area of leaves, in both seasons. However, GA_3 treatment, at both planting dates, gave the highest simulating effect on plant height.

2. Chemical analysis

a) Photosynthetic pigments

Data presented in Table (2) illustrated that chlorophyll concentrations (*a* and *b*) were significantly enhanced in October planting date as compared with that of December date, in both seasons. However, carotenoids content was significantly higher at the early planting date, only in the first season. The increase in plastid pigments in October planting might be attributed to length of photoperiod. Similar results were reported by El-Sayed and Shehata (1990) and Krause *et al.*(1999) on spinach plants, seemed to confirm this finding.

Table (2): Photosynthetic pigments, some biochemical compounds and C/N ratio of spinach leaves as affected by planting date, biofertilizer and GA_3 treatments and their interaction, in 2001/2002 and 2002/2003 winter seasons.

Parameters	Planting date	2001/2002 winter season					2002/2003 winter season				
		Treatments				Mean	Treatments				Mean
		Control	Bio.	GA_3	Bio+GA_3		Control	Bio.	GA_3	Bio+GA_3	
Chlorophyll (a) mg/g dry weight	Oct.	3.294 c	5.113a	2.842c	3.957 b	3.801A	3.231 c	4.920a	2.800cd	3.813 b	3.691A
	Dec.	3.121 c	4.913a	2.770c	3.846 b	3.663B	3.064cd	4.766a	2.678 d	3.770 b	3.570B
	Mean	3.207 C	5.013A	2.806D	3.901 B		3.148 C	4.843A	2.739 D	3.792 B	
Chlorophyll (b) mg/g dry weight	Oct.	1.618bc	2.349a	1.386c	1.879 b	1.808A	1.589bc	2.291a	1.366 c	1.821 b	1.767A
	Dec.	1.523 c	2.273a	1.334c	1.829 b	1.740B	1.446 c	2.225a	1.304 c	1.737 b	1.678B
	Mean	1.570 C	2.311A	1.360C	1.854 B		1.517 C	2.258A	1.335 C	1.779 B	
Carotenoids mg/g dry weight	Oct.	1.819cd	2.338a	1.572ef	2.003 bc	1.933A	1.763 c	2.278a	1.520de	1.955 b	1.879A
	Dec.	1.739de	2.207ab	1.467 f	1.916 cd	1.832B	1.711cd	2.258a	1.461 e	1.881 bc	1.828A
	Mean	1.779 C	2.272 A	1.520D	1.960 B		1.737 C	2.268A	1.491 D	1.918 B	
Total sugars mg/g dry weight	Oct.	12.5 d	17.1 a	15.3 c	16.6 ab	15.38A	11.5 e	17.0 a	15.1 c	16.5 ab	15.03A
	Dec.	10.8 e	16.2 b	13.1 d	15.1 c	13.80B	9.9 f	16.0 b	13.0 d	15.0 c	13.48B
	Mean	11.65 D	16.65 A	14.20C	15.85 B		10.70 D	16.50A	14.05 C	15.75 B	
Mass of carbon mg/g dry weight	Oct.	26.72 d	30.66 c	30.22 c	31.54 b	29.79A	27.16 d	31.09a	28.47 c	30.66 a	29.35A
	Dec.	25.40 e	32.40 a	31.68 b	31.10 c	30.15A	24.97 e	29.37b	26.72 d	29.78 b	27.71A
	Mean	26.06 B	31.53 A	30.95A	31.32 A		26.07 B	30.23A	27.60AB	30.22 A	
Nitrogen mg/g dry weight	Oct.	3.1 ef	4.1 b	3.2 de	3.7 c	3.53 A	3.0 e	4.1 ab	3.3 d	3.8 c	3.55 A
	Dec.	2.8 f	4.5 a	3.5 cd	3.8 bc	3.65 A	2.7 f	4.3 a	3.5 d	3.9 bc	3.60 A
	Mean	2.95 D	4.30 A	3.35 C	3.75 B		2.85 D	4.20 A	3.40 C	3.85 B	
C/N ratio	Oct.	8.62 c	7.48 e	9.44 a	8.52 cd	8.52 A	9.05 a	7.58 d	8.63 b	8.07 c	8.33 A
	Dec.	9.07 b	7.20 e	9.05 b	8.18 d	8.38 A	9.25 a	6.83 e	7.63 d	7.64 d	7.84 A
	Mean	8.85 AB	7.34 C	9.25 A	8.35 B		9.15 A	7.21 B	8.13 AB	7.86 B	

Values marked with the same alphabetical letters, within a comparable group of means, do not significantly differ, using revised L.S.D. test at 0.05 level.

Inoculation with biofertilizer recorded a significant increase in chlorophyll concentration compared to control and the other treatments. It was followed by Bio + GA_3 treatments, in both seasons. Meanwhile, GA_3 treatment decreased chlorophyll contents compared to the control plants. The previous results were in agreement with those reported by Nieto and Frankenberger (1990) with respect to the effect of biofertilizer and Abd El-Fattah *et al.*(1997) with respect to the effect of GA_3.

b) Total sugars and carbon mass

Comparing to control, all treatments led to significant increase in total sugars of spinach leaves, in both seasons, as this effect was more clear at the early planting date (Table 2). The same trend was observed for mass carbon. The highest total sugars and mass of carbon were recorded with biofertilizer treatment, in both seasons, compared to other treatments. Similar results were observed by Zdor and Anderson (1992), Haggag and Azzazy (1996) and Terry *et al.*(1996).

c) Nitrogen content and C/N ratio

The same trend as total sugars was observed for nitrogen concentration (Table 2). With respect to C/N ratio of spinach leaves, biofertilizer inoculation significantly reduced it, compared with the untreated plants and GA_3 treated plants. Cojhi *et al.*(1993) suggested that the inoculation with bacterial microorganisms would be capable of enhancement transfer of fixed nitrogen to the plant tissues in sugarcane. The lower C/N ratio under inoculation of rhizobacterial condition means high N content and this may be due to increasing uptake of ammonium (Rygiewiczp *et al.*, 1984) and nitrogen-containing solutes (Hamp *et al.*, 1995). It is believed that the proper balance of carbohydrates and nitrogen (C/N ratio) is needed for proper vegetative growth and flowering (Swiader *et al.*, 1994).

d) Endogenous phytohormones

It is clearly shown from the data presented in Table (3) that planting date did not significantly affect the endogenous hormones. GA_3 concentration was significantly increased with GA_3 spraying, meanwhile, the inoculation of spinach seeds with biofertilizer significantly reduced it. These results had the same trend at both planting dates.

Regarding IAA concentration, it can be noticed that there was a highly significant reduction in IAA content with GA_3 spraying as well as Bio + GA_3 treatments. Meanwhile, biofertilizer inoculation had no significant effect on IAA content, compared to control treatment at the early planting date, however, it significantly reduced it at the late one.

Endogenous cytokinin concentration recorded higher values with the application of biofertilizer, however, it recorded lower values with GA_3 spraying, compared to the control treatment. Moreover, inoculation with biofertilizer combined with GA_3 spraying significantly enhanced cytokinin content, at both planting dates. Similar results were obtained by Abd El-Fattah and Sorial (2000) on squash plants.

The more interesting data of endogenous ethylene content of spinach leaves (Table 3) which clearly showed that, at both planting dates, inoculation of spinach seeds with biofertilizer recorded highly significant increases in ethylene production, either when it was applied alone or with GA_3 spraying. Meanwhile, GA_3 treatment did not significantly affect ethylene contents, compared with the control plants. Similar results were found by Zhong *et al.*(1989) and Werf and Nagel (1996) on tomato and pepper plants, respectively. Moreover, inoculation with biofertilizer caused a highly significant increase in cytokinin/GA_3 ratio, compared to all other treatments. The results of Abd El-Fattah and Sorial (2000) seemed to confirm these findings.

Table (3): Endogenous phytohormones and Ck/GA ratio of spinach leaves as affected by planting date, biofertilizer and GA_3 treatments and their interaction, in 2001/2002 winter seasons.

Planting date	Treat-ments	GA_3		IAA		Cytokinin (Ck)		Ethylene		Ck/GA Ratio
		ng/100 g fresh wt.	% from total horm-ones	ng/100 g fresh wt.	% from total horm-ones	ng/100 g fresh wt.	% from total horm-ones	ng/100 g fresh wt.	% from total horm-ones	
October		115 A	29 A	99 A	25 A	62 A	16 A	126 A	31 A	0.775A
December		109 A	28 A	107 A	27 A	59 A	16 A	119 A	30 A	0.802A
	Control	145 B	37 B	115 A	29 A	54 C	15 B	81 C	20 C	0.369C
	Bio	43 D	11 D	108 B	28 A	79 A	20 A	170 A	43 A	1.828A
	GA_3	168 A	42 A	93 C	23 B	47 D	12 C	94 C	23 C	0.281C
	Bio+GA_3	93 C	24 C	95 C	24 B	63 B	16 B	143 B	37 B	0.678B
October	Control	150 b	38 b	110 b	28 b	55 c	14 c	82 f	20 f	0.367c
	Bio	45 e	11 e	105 bc	26 c	80 a	20 a	171 a	43 a	1.778a
	GA_3	170 a	42 a	90 e	22 e	48 d	12 d	98 d	24 d	0.282d
	Bio+GA_3	95 d	24 d	90 e	22 e	64 b	16 b	150 b	38 b	0.674b
December	Control	140 c	35 c	120 a	30 a	52 c	15 bc	80 f	20 f	0.371c
	Bio	41 e	10 e	111 b	29 ab	77 a	19 a	169 a	42 a	1.878a
	GA_3	165 a	42 a	96 de	24 d	46 d	12 d	90 e	22 e	0.279d
	Bio+GA_3	91 d	23 d	100 cd	26 c	62 b	16 b	135 c	35 c	0.681b

Values marked with the same alphabetical letters, within a comparable group of means, do not significantly differ, using revised L.S.D. test at 0.05 level.

Many investigators stated that bacterial inoculation changes plant growth and flowering by controlling growth regulators, especially those of gibberellins, cytokinins and IAA (Nieto and Frankenberger, 1990; Goicoechea *et al.*, 1995; Noel *et al.*, 1996 and Sorial, 2000), whereas, many *Azospirillum* strains produce several plant hormones such as IAA, IBA and cytokinin (Omay *et al.*, 1993). In addition, it was generally accepted that all plant hormones are interacting with other factors in the regulation of plant performance such as flowering (Metzer, 1995 and Taiz and Zeiger, 1998).

3. Sex expression

It was, generally, noticed from the data presented in Table (4) that planting date led to some significant effects on sex expression of spinach plants. Whereas, male plants were not significantly affected by planting date, in both seasons, however, female plants (%) significantly increased, in the second season at the latest planting date. On the other hand, monoecious plants significantly increased, in both seasons, when spinach seeds were sown early in October, compared to the late planting date (December). Similar effects of day length and temperature (planting date) on sex expression have been reported by Abdel-Samie (1958), Cantliffe (1981), Freeman *et al.*(1981), NeSmith and Hoogenboom (1994) and El-Keblawy and Lovett-Doust (1996) on Cucurbitaceae plants and by Culafic (1999) on spinach plants.

Table (4): Sex expression of spinach plants as affected by planting date, biofertilizer and GA_3 treatments and their interaction, in 2001/2002 and 2002/2003 winter seasons.

Planting date	Treat-ments	2001/2002 winter season				2002/2003 winter season			
		Male plants (%)		Female plants %	Mono-ecious (%)	Male plants (%)		Female plants %	Mono-ecious (%)
		Extreme	Vegetative			Extreme	Vegetative		
October		26.38 A	15.54 A	49.75 A	8.33 A	27.20 A	14.97 A	47.41 B	10.42 A
December		26.07 A	15.34 A	51.64 A	6.94 B	26.50 A	14.86 A	50.98 A	7.66 B
	Control	30.51 B	15.58 B	46.81 C	7.10 B	29.50 B	15.72 B	46.15 C	8.65 B
	Bio	7.99 D	17.77 A	65.14 A	9.11 A	8.72 D	16.59 A	64.69 A	9.99 A
	GA_3	44.89 A	12.32 C	36.06 D	6.73 B	46.31 A	11.06 C	33.17 D	9.46 A
	Bio+GA_3	21.53 C	16.09 B	54.78 B	7.61 B	22.89 C	16.31 A	52.77 B	8.04 B
October	Control	31.22 b	14.48 c	46.15 c	8.14 a	30.42 b	14.29 c	45.62 c	9.68 bc
	Bio	8.77 d	18.42 a	63.16 a	9.65 a	9.44 d	16.74 b	60.94 a	12.88 a
	GA_3	44.75 a	12.79 d	35.16 d	7.30 a	46.26 a	11.21 d	32.24 d	10.28 b
	Bio+GA_3	20.78 c	16.45 b	54.54 b	8.23 a	22.69 c	17.65 a	50.84 b	8.82 bc
December	Control	29.79 b	16.67 b	47.47 c	6.06 a	28.57 b	17.14 ab	46.67 c	7.62 cd
	Bio	7.21 d	17.12 a	67.12 a	8.56 a	8.00 d	16.44 b	68.44 a	7.11 d
	GA_3	45.02 a	11.85 d	36.96 d	6.16 a	46.36 a	10.91 d	34.09 d	8.64 c
	Bio+GA_3	22.27 c	15.72 b	55.02 b	6.99 a	23.08 c	14.96 c	54.70 b	7.26 cd

Values marked with the same alphabetical letters, within a comparable group of means, do not significantly differ, using revised L.S.D. test at 0.05 level.

Besides, biofertilizer inoculation significantly enhanced the induction of vegetative male and female plants (%) as well as monoecious plants (%) and significantly reduced the extreme male plants (%). This result being true in both seasons. The above mentioned treatment was followed by biofertilizer with GA_3 treatment. On the other hand, GA_3 treatment led to significant increase in extreme male plants (%), in both seasons, compared to all other treatments.

Moreover, the comparisons among the means of various combined treatments of planting date and biofertilizer and/or GA_3, listed in Table (4), showed also that spinach plants which were inoculated with biofertilizer, at both planting dates, had the highest number of female plants, in both seasons. However, it is important to show herein that spinach plants which were sprayed with GA_3, at both planting dates, had the highest number of extreme male plants, in both seasons.

The same trend was plotted between sex expression (Table 4) and endogenous hormones distribution (Table 3) due to the application of biofertilizer and/or GA_3, since Shiffress (1985) cited that the female expression of squash plants can be converted into monoecious by spraying with GA_3. In addition, endogenous gibberellin (GA) was higher in male plants, while endogenous cytokinin was higher in female plants of spinach (Khryznin and Chailakhyan, 1980). Moreover, Chailakhyan (1979) stated that the sex expression in spinach plants is regulated by gibberellins which are synthesized in leaves and cause male sex expression and by cytokinins which are synthesized in the roots and cause female sex expression. Furthermore, Ombrello and Garrison (1987) stated that there were higher levels of cytokinins in female than in male spears of asparagus and there were, also, higher Ck/GA ratio in female than in male spears and sex expression is controlled, in part, by Ck levels or by Ck/GA ratios (Table 3). In addition, biofertilizer inoculation seemed to reduce C/N ratio (Table 2) resulting in increasing female plants (Table 4) (Abd El-Fattah and Sorial (2000).

Yin and Quinn (1995) suggested that ethylene had overriding effect on GA_3 and acted more directly on sex expression in cucumber. Moreover, they demonstrated that only one growth regulator controlling sex expression and this is likely to be ethylene (Table 3). Similar conclusion, seemed to confirm the present results, was also obtained by Abd El-Fattah and Sorial (2000) on squash plants, using biofertilizer inoculation.

4. Total yield and its quality

Data presented in Table (5) show that planting date did not significantly affect the total yield of spinach, either per m^2 or per feddan and its all quality characteristics, in both seasons. Meanwhile, it can be noticed that the early planting date (October) tended to increase yield and its quality, compared with the late one, however, the detected differences were not significant. Stino *et al.*(1973) obtained heavier spinach plants at early planting date (October) compared with November planting date. Similar results were also found by Stino and Abel-Fattah (1973), El-Sayed and Shehata (1990) and more recently by Niel (2001). On the other hand, El-Gizawy *et al.*(1992) found that sowing seeds of Saloniky spinach cultivar on January 1st increased yield as compared with seed sowing on November. Contradictory results were also obtained by Abd El-Bary *et al.*(1997), who stated that the quality of spinach yield was best when seeds were sown on 10 December, compared with 10 October planting.

Data in Table (5), also, reveal that biofertilizer inoculation, in both seasons, exceeded the comparable control and GA_3 treatments, concerning the total yield of spinach plants and its quality characteristics, followed by biofertilizer with GA_3 treatment. The average increase was about 47.6 and 48.7%, with respect to total yield per feddan, in the first and second seasons, respectively, as compared to the control treatment.

Yield increases might be due to the fact that biofertilizer stimulates root growth, changes root morphology (Carletti *et al.*, 1996) and enhances uptake of minerals as reported by Ruiz-Lozano *et al.*(1995). It is also possible due to the involvement in phytohormones production (Table 3) (Noel *et al.*, 1996), which all together might cause promotion of vegetative growth characters (Table 1) and induction of some chemical and biochemical compounds (Table 2). Moreover, Pandy and Kumar (1989) related the yield increase in biofertilizer inoculated plants to the beneficial effects of the bacteria not only on N-fixation capacity but also to their ability to produce antibacterial and antifungal compounds, growth hormones and siderophores. Furthermore, yield increase and quality improvement of spinach due to biofertilizer inoculation might be attributed to the effect of biofertilizer on sex expression of spinach which was discussed above (Table 4), especially the increment of vegetative male, female and monoecious plants which have better yield and quality characteristics (Rubatzky and Yamaguchi, 1997), compared to the extreme male plants.

Table (5): Total yield of spinach plants and its quality as affected by planting date, biofertilizer and GA_3 treatments and their interaction, in 2001/2002 and 2002/2003 winter seasons.

Parameters	Plant -ing date	2001/2002 winter season					2002/2003 winter season				
		Treatments				Mean	Treatments				Mean
		Control	Bio.	GA_3	Bio+GA_3		Control	Bio.	GA_3	Bio+GA_3	
Yield per m^2 (kg)	Oct.	2.202cd	3.273a	2.005de	2.639 b	2.530 A	2.157cd	3.193a	1.903de	2.520 b	2.445A
	Dec.	2.084 de	3.058a	1.818 e	2.504bc	2.366 A	2.009de	3.003a	1.788 e	2.418bc	2.305A
	Mean	2.143C	3.166A	1.912 C	2.571 B		2.083 C	3.101A	1.846 C	2.469 B	
Total yield/fed. (ton)	Oct.	8.37 c	12.44a	7.61 cd	10.03 b	9.61 A	8.20 c	12.15a	7.23 cd	9.57 b	9.29 A
	Dec.	7.92 c	11.62a	6.91 d	9.51 b	8.99 A	7.63 c	11.41a	6.80 d	9.19 b	8.76 A
	Mean	8.15 C	12.03A	7.26 D	9.77 B		7.92 C	11.78A	7.02 D	9.38 B	
Average plant weight (g)	Oct.	28.13cd	40.97a	24.70de	32.60 b	31.60 A	27.70bd	39.97a	24.23de	31.77 b	30.92A
	Dec.	26.77d	38.83a	23.20 e	30.93bc	29.93 A	25.87ce	37.90a	22.73 e	30.13bc	29.16A
	Mean	27.45C	39.90A	23.95 D	31.77 B		26.78 C	38.93A	23.48 D	30.95 B	
Dry weight per plant (g)	Oct.	2.86 cd	4.29 a	2.44 ef	3.18 b	3.19 A	2.77 cd	4.20 a	2.33 e	3.25 b	3.14 A
	Dec.	2.69de	4.00 a	2.23 f	3.08 bc	3.00 A	2.42 de	3.99 a	2.18 e	3.14 bc	2.93 A
	Mean	2.77 C	4.15 A	2.34 D	3.13 B		2.59 C	4.10 A	2.26 D	3.19 B	
Dry matter content (%)	Oct.	8.76 c	11.68a	8.55 c	10.31 b	9.82 A	8.67 c	11.60a	8.48 c	10.21 b	9.74 A
	Dec.	8.59 c	11.46a	8.40 c	10.03 b	9.62 A	8.49 c	11.33a	8.33 c	9.85 b	9.50 A
	Mean	8.68 C	11.57A	8.47 C	10.17 B		8.58 C	11.46A	8.41 C	10.03 B	
Ascorbic acid content (mg/100g f.w.)	Oct.	90 de	102 a	95 c	100 ab	96.75 A	88 ef	100 a	93 d	98 ab	94.75A
	Dec.	88e	98 b	91 d	95 c	93.00 A	86 f	97 bc	90 e	95 cd	92.00A
	Mean	89.0 D	100.0A	93.0 C	97.5 B		87.0 D	98.5 A	91.5 C	96.5 B	

Values marked with the same alphabetical letters, within a comparable group of means, do not significantly differ, using revised L.S.D. test at 0.05 level.

Similar yield increases were also obtained as a result of different biofertilizers inoculation, such as those found by Abd El-Fattah and Sorial (1998) on lettuce, Abd El-Fattah and Sorial (2000) on squash and more recently by Niel (2001) on spinach plants. On the other hand, Liang *et al.*(1998) stated that GA_3 increased the photosynthetic rate, ascorbic acid content and shoot fresh weight of spinach plants, compared to the control plants.

The results in Table (5), also, show that there was a significant effect due to the interaction between planting date and biofertilizer and/or GA_3 treatments on total yield of spinach plants and its quality characteristics, in both seasons. Spinach plants which were inoculated with biofertilizer, at both planting dates, in general, achieved the highest increase in total yield and showed the best quality characteristics, in both seasons. Similar finding was also obtained by Niel (2001), who found that inoculum with *Azotobacter* or *Azospirillum* biofertilizer significantly increased the total yield of spinach, especially at the first sowing date of the two seasons (November planting), as compared to the second one (January planting).

5. Seed yield and its quality

Results in Table (6) pointed out that sowing spinach seeds early on October significantly increased seed yield, either per m^2 or per feddan and improved seed index (weight of 100 seeds), in both seasons. However, the early planting date led to significant improvement of seed germination (%) and dry matter content of seeds (%), only in the first season. Takao (1998) showed that growth and flower stalk development of spinach plants differed due to sowing dates. Similar results that confirm this finding were also obtained by Culafic (1999) on spinach.

Table (6): S eed y ield o f spinach plants and its quality as affected by planting date, biofertilizer and GA_3 treatments and their interaction, in 2001/2002 and 2002/2003 winter seasons.

Parameters	Planting date	2001/2002 winter season					2002/2003 winter season				
		Treatments				Mean	Treatments				Mean
		Control	Bio.	GA_3	Bio+GA_3		Control	Bio	GA_3	Bio+GA_3	
Seed yield per m^2 (g)	Oct.	49.67e	78.67bc	70.67d	86.67 a	71.42 A	47.67e	75.67bc	69.00cd	85.33 a	69.42A
	Dec.	477 e	74.33cd	68.33d	84.67ab	68.75 B	46.33e	73.00cd	66.67 d	82 67ab	67.17B
	Mean	48.67D	76.50 B	69.50C	85.67 A		47.00D	74.33 B	67.83 C	84.00 A	
Seed yield per feddan (kg)	Oct.	199 e	315 bc	283 d	347 a	286 A	191 e	303 bc	276 cd	341 a	278 A
	Dec.	191 e	297 cd	273 d	339 ab	275 B	185 e	292 cd	267 d	331 ab	269 B
	Mean	195 D	306 B	278 C	343 A		188 D	297 B	271 C	366 A	
Seed index (wt. of 100 seeds) (g)	Oct.	9.20 ef	11.00bc	9.80 d	11.67 a	10.42 A	9.17de	10.83bc	9.67 d	11.50 a	10.29A
	Dec.	9.03 f	10.87 c	9.70de	11.53ab	10.28 B	9.00 e	10.77 c	9.63 d	11.40ab	10.20B
	Mean	9.11 D	10.93 B	9.75 C	11.60 A		9.08 D	10.80 B	9.65 C	11.45 A	
Seed germination (%)	Oct.	75.33d	77.33 b	76.33c	79.00 a	77.00 A	74.33d	76.00bc	75.33 c	78.00 a	75.92A
	Dec.	74.33e	76.67bc	75.33d	78.33 a	76.17 B	73.00d	75.33 c	74.33 d	77.33ab	75.00A
	Mean	74.83C	77.00 B	75.83B	78.67 A		73.67C	75.67 B	74.83 B	77.67 A	
Dry matter content of seeds (%)	Oct.	91.67c	95.67 a	95.33ab	96.67 a	94.83 A	91.00c	95.00ab	94.67ab	96.33 a	94.25A
	Dec.	91.33c	95.33ab	94.67 b	96.33 a	94.42 B	90.67c	95.00ab	94.00 b	96.00 a	93.92A
	Mean	91.50B	95.50 A	95.00 A	96.50 A		90.83B	95.00 A	94.33 A	96.17 A	

Values marked with the same alphabetical letters, within a comparable group of means, do not significantly differ, using revised L.S.D. test at 0.05 level.

It is, also, important to state that the inoculation with biofertilizer and spraying with GA_3, together, significantly increased seed yield of spinach plants and improved almost its quality characteristics, compared to other treatments. This result was insistently observed in both seasons. In addition, this treatment, in general, might be the most effective treatment, at both planting dates, on increasing the seed yield and improvement of its quality. The increase in spinach seed yield per feddan was contributed to the increase in seed yield per plant and seed yield components. Similar results were also r eported b y A bd E l-Fattah (1997) o n s eed y ield o f b road b eans, with respect to the effect of GA_3 and by Saeed (2002) on seed yield of squash, with respect to the effect of biofertilizer inoculation.

6. Correlation coefficients

The correlation coefficients studied among vegetative growth, yield and its quality and sex expression (Table 7) showed positive and significant correlation coefficients between vegetative growth characters, expressed as leaf area, number of leaves and total yield and its components, in the second season. Similar results were also obtained by Abd El-Fattah *et al.*(1998) on spinach plants. In addition, there were positive and significant correlation coefficients between total yield as well as its quality and female plants (%). On the other hand, there were negative and significant correlation coefficients between yield and its quality and extreme male plants (%). However, the correlation coefficients between vegetative male plants or monoecious plants and yield as well as its quality were positive but not significant. Moreover, seed yield had no significant correlation coefficients with all parameters studied. These results confirmed the relationships between sex expression and total yield as well as its quality characteristics (Rubatzky and Yamaguchi, 1997).

Table (7): Correlation coefficients (r) among vegetative growth, yield and its quality and sex expression of spinach plants, in the second winter season of 2002/2003.

Character	Leaf area	Total yield	Average plant weight	Dry weight per plant	Dry matter content	Seed yield	Extreme male plants	Vegetative male plants	Female plants	Monoecious plants
Number of leaves	0.995**	0.959**	0.953**	0.961**	0.981**	0.62	-0.835*	0.474	0.832*	0.268
Leaf area		0.935**	0.926**	0.936**	0.968**	0.648	-0.806*	0.431	0.813*	0.227
Total yield			0.999**	0.998**	0.987**	0.483	-0.942**	0.665	0.916**	0.271
Average plant weight				0.997**	0.982**	0.453	-0.948**	0.678	0.920**	0.269
Dry weight per plant					0.986**	0.495	-0.940**	0.648	0.920**	0.253
Dry matter content						0.603	-0.911**	0.626	0.896*	0.216
Seed yield							-0.319	0.131	0.337	0.050
Extreme male plants								-0.830*	-0.985**	-0.035
Vegetative male plants									0.770*	-0.090
Female plants										-0.953**

* **Significant at 0.05 level of probability.**

** **Significant at 0.01 level of probability.**

In conclusion, the results of the present study support the fact that the inoculation with biofertilizer leads to increasing plant growth which causes significant increases in total yield of spinach plants and improves its quality characteristics, throughout the increase in vegetative male and female plants (%) and the reduction of extreme male plants (%). This obtained trend was observed, also, at both planting dates, either early on October or late on December. Moreover, the morphogenic influence of biofertilizer inoculation upon plant growth and total yield can be explained, in part, by the production of phytohormones which can interact with plants in the rhizosphere and cause dramatic effects on plant growth and development, including endogenous hormones and sex expression. The obtained results reveal, also, that the use of biofertilizers in spinach cultivation can substitute for 50% of mineral NPK. Hence, this treatment would save the high cost of chemical fertilizers as well as decrease the pollution of the environment. On the other hand, the results reveal that GA_3 is needed beside biofertilizer inoculation to increase seed yield of spinach plants and to improve its quality characteristics, under either early or late planting dates.

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إستجابة التعبير الجنسى لنباتات السبانخ لتأثير تطبيق كل من السماد الحيوى وحمض الجبرلليك وعلاقته بالمحصول وجودته وإنتاج التقاوى

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الهدف الرئيسى من هذه الدراسة هو تقدير العلاقة بين التعبير الجنسى لنباتات السبانخ وكمية محصول الأوراق وصفات جودته وعلاقة ذلك بميعاد الزراعة وتطبيق كل من السماد الحيوى والرش بحمض الجبرلليك، حيث أنه من المعروف أن هناك علاقة بين حالات الجنس فى السبانخ وكمية المحصول وجودته حيث تفضل النباتات المؤنثة والمذكرة الخضرية عن النباتات المذكرة التى تكون صغيرة وأقل جودة.
وقد أجريت تجربتان حقليتان أثناء الموسمين الشتويين ٢٠٠١/٢٠٠٢، ٢٠٠٢/٢٠٠٣، وذلك فى مزرعة التجارب الخاصة بكلية الزراعة – جامعة الأسكندرية لدراسة تأثير ميعاد الزراعة (خلال أكتوبر وديسمبر) تطبيق السماد الحيوى (هالكس٢) والرش بحمض الجبرلليك (٢٥ جزء فى المليون) سواء كل منهما منفردا أو مع بعضهما البعض، على صفات النمو الخضرى والتعبير الجنسى والتركيب الكيماوى ومحصول الأوراق الطازج وجودته وكذلك إنتاج التقاوى وجودتها لنباتات السبانخ من الصنف سالونيكى.
وقد أشارت النتائج إلى مايلى:

١- أدى زراعة بذور السبانخ مبكراً خلال شهر أكتوبر إلى الحصول على زيادة معنوية فى طول النبات وكل من محتوى الأوراق من الصبغات (كلورفيل أ،ب) والسكريات الكلية كما أدى لزيادة كمية محصول التقاوى وتحسين جودتها، وذلك بالمقارنة بميعاد الزراعة المتأخرة خلال شهر ديسمبر. ومن جهة أخرى لم يؤثر إختلاف ميعاد الزراعة معنوياً على عدد الأوراق ومساحتها ومحتواها من النتروجين ونسبة الكربوهيدرات إلى النتروجين ومحتواها من الهرمونات وكذلك محصول الأوراق الطازج وجودته.

٣- بينت النتائج أن إستخدام السماد الحيوى كان له تأثيرات إيجابية معنوية فقد أدى إلى تحسين صفات المجموع الخضرى وزيادة المحتوى الكيماوى للأوراق وزيادة المحصول الورقى وتحسين جودته وذلك بالمقارنة بالمعاملة رشاً بحمض الجبرلليك والنباتات الغير معاملة (الكنترول). بالإضافة إلى أن تطبيق السماد الحيوى قد أدى إلى الحصول على زيادة معنوية فى محتوى كل من السيتوكينين والإيثيلين فى الأوراق وتقليل محتواها من الجبرللين.

٣- كما أظهرت النتائج أن إستخدام السماد الحيوى قد أدى إلى زيادة فى نسبة عدد النباتات المذكرة الخضرية والمؤنثة والنباتات أحادية المسكن وهى التى تفضل من حيث كمية المحصول الورقى وجودته.
وقد تم مناقشة التغير فى التعبير الجنسى نتيجة المعاملة بالسماد الحيوى وحمض الجبرلليك على أساس التغيرات التى تحدثها هذه المعاملات فى محتوى الأوراق من الهرمونات النباتية (الجبرللين، السيتوكينين، الإيثيلين).

٤- أثبت حساب معامل التلازم تأييد وجود علاقة بين التعبير الجنسى ومحصول السبانخ وجودته وعلاقة ذلك بتطبيق معاملات السماد الحيوى وحمض الجبرلليك.

٥- من جهة أخرى بينت التجارب أهمية الرش بحمض الجبرلليك بجانب تطبيق السماد الحيوى للحصول على أعلى محصول للبذور وتحسين صفات جودتها وذلك فى حالة زراعة السبانخ فى كلا ميعادى الزراعة تحت الدراسة.

٦- يمكن التوصية من خلال نتائج هذه الدراسة بإستخدام تطبيق السماد الحيوى (هالكس٢) عند إنتاج السبانخ حيث يعمل ذلك على تقليل إستخدام الأسمدة الكيماوية المعدنية كما يعمل على زيادة المحصول الورقى للسبانخ وتحسين جودته، كما ينصح بإستخدام حمض الجبرلليك رشاً بجانب السماد الحيوى فى حالة إذا كان الغرض من الزراعة هو إنتاج التقاوى لزيادة محصول البذور وتحسين جودتها.