International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.10, pp 163-172, 2016

Proline, antioxidant enzymes activity and productivity of snap bean as affected by bio-regulators application under two sowing dates

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Abstract : Two consecutive field experiments were conducted in the experimental farm, Faculty of Agriculture, Ain Shams University, Qalubia governorate during the two winter seasons of 2012/2013 and 2013/2014 to study the response of snap bean cv Bronco to two sowing dates (1th and 16th of October) and two bio-regulators i.e., salicylic acid (SA) at 0, 0.5 and 1.0 mM and putrescine(PUT) at 0 , 1.0 and 2.0 mM as well as the combination between both of them . The experiment was laid out in a split plot design with three replicates .Proline, antioxidant enzyme activities (POX ,CAT and SOD) and pod weight as well as total yield were determined. The results revealed that the higher significant value of proline content in snap bean leaves was recorded through the first sowing date. The combined application of SA at 0.5 mM and PUT at 2 mM mitigated and significantly enhanced the proline content . The first sowing date significantly increased the activity of POX and SOD compared with the second one .The maximum and significant response of POX ,CAT and SOD generated in plants when sprayed with SA in combination with PUT at higher rates. Sowing snap bean seeds on the 1st October induced significant maximum total yield. Application of SA or PUT at any concentration and their combination significantly increased total yield compared to the check untreated plants.
Key words : Snap bean, salicylic acid, putrescine, sowing dates, proline , a*ntioxidant enzymes activity* , yield.

1. Introduction

Snap bean, (*Phaseolus vulgaris* L.) belong to the family Fabaceae, It is used for it's fresh pods or dry seeds which are a good source of vitamin A, minerals, fibers and protein. Snap bean grown in a large scale for local and export in Egypt and its growth could be improved by its sowing on the proper date .Low temperature is one of the most important stress factors limiting the growth and productivity of snap bean. One of the reasons of these fluctuations in average yield is climate change. Consequently, sowing date is one of the most important factors which have a paramount effect on snap bean development, growth and biological yield (Fagnano *et al*., [1] and Compant *et al* [2]).So its growth could be improved by its sowing on the proper date. Therefore, cultivation snap bean in winter season (under chilling condition) was protected by covering plants with plastic sheet (under tunnels) whereas, in open field cultivation faced damage due to chilling stress. In order to tackle with chilling problem, growth bio-regulators are substances used to elevate the tolerance of plants to environmental stress and in the same time to improve yield and quality. Also sowing dates differ to the effect of all environmental condition on large scale on growth and yield of snap bean crop which differ widely from region to another as reported by many researchers. Moreover, sowing date is an important factor which significantly affects the timing and duration of vegetative and reproductive stages, consequently yield and it's components

The exposure of plants to unfavourable environmental conditions is usually accompanied by the production of reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Suzuki and Mittler,[3]). The excessive accumulation of ROS may induce oxidative damage to proteins, DNA and membrane lipids, or even cell death (Apel and Hirt, [4]). The ability to adjust the antioxidant system to changing ROS concentrations is vital in all species especially under stress conditions. In order to keep the amount of ROS in equilibrium, plants have evolved several enzymatic and non-enzymatic antioxidant systems. However, ROS not only cause oxidative damage to cells, but may also play an important role as signals in plants exposed to various stress conditions (Mittler *et al*.,[5]).

A promising approach to diminish environmental stress which induces crop losses is the foliar application with chemical desiccant on plants and recently, there is an increasing interest in the use of natural compounds approach to diminish environmental stress which induces crop losses and maintenance of fruit quality. (Khandaker *et al*. [6]).Salicylic acid(SA), a widely distributed compound in plants, belongs to a group of phenolic compounds. Salicylic acid could be considered as an endogenous plant growth regulator involved in the regulation of physiological processes and disease resistance mechanisms (Luo *et al*. [7]) that exogenous application of SA can aid plant tolerance with many abiotic stresses, such as: induced the increase in resistance to low temperature (Janda *et al*.[8]),

Polyamines have been found to play a key role in many physiological processes such as cell growth and development and response to environmental stresses(Kosson and Prange [9]) causing increases in growth which might be due to the enhancement of cell division activity (Galston [10]).Also enhanced biosynthesis enzymes, modulated several growth development processes , cell division, differentiation, flowering (Kakkar *et al*. [11]).

Salicylic acid and polyamine treatments have the potential for commercial control of environmental stresses Therefore, this work is an attempt to evaluate whether exogenous application with salicylic acid and putrescine could mitigate the adverse effect of low temperature on productivity of snap bean under two sowing dates and impact of SA and PUT application on proline and antioxidant enzymes activity .

2. Materials and Methods

Two consecutive field experiments were conducted at the experimental farm, faculty of Agriculture, Ain Shams University, Qalubia Governorate, under open field conditions to study the effect of two bio-regulators and their combination with the two sowing dates on the growth, productivity and quality of snap bean. The soil of the experimental sites was clay loam. The average minimum and maximum temperature during the growing seasons are represented in Table (1).

Table (1) Average minimum and maximum (C°) in Qalubia Governorate during winter seasons of 2012/2013 and 2013/2014 (Central laboratory for agri-climate, Dokki, Giza, Egypt.

Month	2012/2013			2013/2014		
	Maximum temperature	Minimum temperature.	Mean temperature	Maximum temperature	Minimum temperature	Mean temperature
October	30.51	20.99	25.75	28.27	18.25	23.26
November	26.85	19.19	23.02	26.96	15.25	21.28
December	24.7	17.05	20.87	20.8	9.1	14.95
January	20.77	9	14.88	21.34	8.62	14.98

2.1 Agricultural practices:

Seeds of snap bean (*Phaseoulus vulgaris*. L) cv Bronco were sown in appropriate soil content in two dates, i.e. on the1st and 16th of October during the two winter seasons of 2012/2013 and 2013/2014. The selection of these sowing dates was based on the results of Abou El Yazied [12]. During soil preparation calcium super-phosphate (15% P_2O_5) at 300Kg/fed, was added on rows at one time during the soil preparation.

Ammonium sulphate (20.5% N) and potassium sulphate (48% K_2 O) were first applied to the soil two weeks after sowing at the rate of 125 and 50 kg/ fed and an equal quantity was applied a month later. The surface irrigation was used and agricultural management, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture and land reclamation. Harvesting was carried out for each sowing date when pods reached the marketable harvest index (when the pods is bright green and the pod is fleshly).

2.2. Treatments and experimental design:

The experiments contained 18 treatments which were all combination of two sowing dates i.e., 1 and 16 of October and two bio-regulators treatments namely, salicylic acid (SA) at the rate of 0.5 and 1.0 mM and putrescine (PUT) at the rate of 0, 1.0 and 2.0 mM in various combinations by foliar spray method as follow :

1. Control treatment (spraying with water)
2. SA was applied at the rate of 1.0mM (designated as $SA_{1.0}$)
3. SA was applied at the rate of 0.5 mM (designated as $SA_{0.5}$)
4. PUT was applied at the rate of 2.0 mM (designated as $PUT_{2.0}$)
5. PUT was applied at the rate of 1.0mM (designated as $PUT_{1.0}$)
6. SA at the rate of 1.0mM + PUT at the rate of 1.0mM (designated as SA_1+PUT_1)
7. SA at the rate of 1.0mM + PUT at the rate of 2.0mM (designated as SA_1+PUT_2)
8. SA at the rate of 0.5mM + PUT at the rate of 2.0mM (designated as $SA_{0.5}$+PUT_2)
9. SA at the rate of 0.5 mM+ PUT at the rate of 1.0mM (designated as $SA_{0.5}$+PUT_1)

Twice foliar applications of Salicylic acid and Putrescine (Sigma Chemical Co. UK) were carried out with 10 d intervals using a hand-held sprayer. Salicylic acid was applied after 30 and 40 days from sowing while PUT was applied after 35 and 45 days from sowing. The experiment was laid out in a split–plot design with four replicates. The sowing date treatments were distributed in the main plots, whereas the bio-regulators treatments were randomly arranged in sub plots. The area of experimental plot was 14 m^2 consisted of five rows, each row was 4m length and 0.7m width in four replicates. The plant distance was 7 cm apart and on the top of the rows .

2.3. Studied characteristics:

2.3.1. Proline:

It was assayed in the fresh of the fourth expanded upper leaf during flowering stage according to the method described by Bates et al. [13].

2.3.2 Antioxidant enzymes activity*:*

Peroxidase (POX; EC 1.11.1.7) activity assayed using the method of Bergmeyer [14]. superoxide dismutase (SOD; EC 1.12.1.1) activity measured according to the method of Dhindsa *et al*. [15]. Catalase (CAT; EC 1.11.1.6) activity assayed according to the method of Chen *et al*. [16]. The enzyme activities were measured by using the Spekol Spectrocolorimeter (Carl Zeiss AG; Jena, Germany).

2.3.3 Pod fresh weigh and total yield :

Ten pods were taken randomly from the each harvest and subjected to determine pod fresh Weight. Snap bean were harvested at commercial maturity (i.e.,bright green , tender fleshy and with small green imature seeds). All green pods per plot were harvesting and weighed and total yield per he^{-1}were calculated.

2.4 Statistical analysis:

All data were subjected to an analysis of variance (ANOVA) for a factorial design, after testing for the homogeneity of error variances according to the procedure outlined by Gomez and Gomez[17]. Statistically significant differences between means were compared at $P \leq 0.05$ using Duncan's multiple range test.

3. Results and Discussion

3.1 Proline content

Proline is a stress indicators and believed to protect plant tissue against stress by acting as a nitrogen storage compound, osmosolute and hydrophobic protectant for enzymes and cellular structures, stabilize membrane structures during hostile conditions, and detoxify free radicals (Larher *et al*., [18]). Also, the accumulation of proline serves as a means of osmotic adjustment which improves plant's tolerance to adverse effects of abiotic stresses (Ma and Turner, [19]).

The effect of sowing dates and bio-regulators and their interaction on proline presented in Table (1). Comparison between sowing dates, the results showed that the higher significant value of proline content in snap bean leaves was recorded through the first sowing date i.e., 1th of October. This finding is unexpected and suggested that the plants in the second sowing dates which grown under more low temperature stress- induced lower proline content rather than first one. The possible explanation it may be argued due to the close relation between proline content and prevailing radiation, both intensity and fluctuation (Claussen, [20]). Since total radiation fluctuated strongly and often changed quickly during the course of the experiment and proline content dependent on the time (day) of radiation was recorded prior to each sampling date according to shift of sowing date .

Table (2) Mean comparison of the interaction effect of bio-regulators and sowing dates on proline content (µmol /100 g FW) of snap bean leaves during 2012/2013 and 2013/2014 seasons.

Treatments	proline content (µmol /100 gFW)					
	1 October	16 October	Mean	1 October	16 October	Mean
	First season			Second season		
Control	63.25^{a}	25.95^{c}	53.10B	74.09 $^{a-c}$	47.45^{i}	60.77 ^{B}
SA1.0	78.71^{a}	58.62^{ab}	63.66^{AB}	73.2^{a-d}	54.24^{hi}	63.72AB
SA 0.5	76.57^{a}	57.11^{ab}	61.84^{AB}	72.73^{a-e}	63.26^{c-h}	68.00^{AB}
PUT2	69.16^{a}	60.43^{ab}	64.79^{AB}	71.50a-e	60.44^{f-h}	65.97^{AB}
PUT1	71.10^{a}	59.96^{ab}	65.52 ^{AB}	71.19^{a-f}	57.11^{g-i}	64.15^{B}
SA1+PUT2	75.05^{a}	63.76^{ab}	69.40^{A}	80.31^{a}	61.87^{e-h}	71.09^{A}
SA1+PUT1	68.81^{a}	66.18^{ab}	67.49^{AB}	76.14^{ab}	62.96^{d-h}	69.55^{A}
SA0.5+PUT2	71.45^{a}	54.77^{ab}	63.10^{AB}	72.24^{a-e}	54.77^{hi}	63.50^{AB}
SA0.5+PUT1	73.54^{bc}	62.70^{ab}	68.12^{AB}	67.52^{b-g}	60.48^{f-h}	64.00^{AB}
Mean	71.40^{A}	65.12 ^{B}		73.21^{A}	58.06^{B}	

Values with common letter in the same column are not significantly different at 5% level

SA_1= Salicylic acid at 1 mM $SA_{0.5}$= Salicylic acid at 0.5 mM
PUT_1= Putrescine at 1 mM PUT_2= Putrescine at 2 mM

Respecting to the effect of bio-regulator treatments, the results show that, foliar application of SA or PUT at the two tested rates either individually or in combination increased proline as compared to respective control . The combined application of SA_1 and PUT_2 mitigated and significantly enhanced the proline content in both growing seasons. Also, the combined application SA_1 and PUT_1 increased proline content compared with control (the results were significant only in the second season). In recent investigation, Keshavarz *et al.* [21] on canola , suggest that proline was greatly affected by salicylic acid treatment under cold stress and its

Content was the highest in third day after treatment with 200 µM SA. In addition, Faizan *et al*. [22], on canola, Eraslan *et al*,.[23] on carrot and Gharib [24] on basil and marjoram found that exogenous SA application significantly increased the accumulation of proline.

Similar was the case with putrescine, our results are in agreement with previous findings of Öztürk and Demir [25] found that, putrescine increased proline content in spinach leaves. In recent study, Tamoor and Bango [26] who cleared that, putrescine-induced stress tolerance was mediated by the enhanced production of proline in wheat leaves.

Concerning the interaction between sowing dates and bio-regulators, results showed that, sown snap bean seeds on the 1th of October with either sole application of SA_1 or the combination of SA_1+PUT_2 were more effective and gave the highest proline content in the first and second season respectively.

3.2 Antioxidant enzymes activity

One of the most promising influences of various abiotic stresses including low temperature stress is the generation of oxidative stress that results from an increased level of reactive oxygen species (ROS) in cells exposed to stress (Apel and Hirt [4]). In addition, the antioxidant enzymes in apoplastic spaces of plants under environmental stresses, such as cold, and ozone were shown to play an important role in the regulation of stress response (Hernandez *et al*., [27]; Patykowski and Urbanek [28]; Atıcı and Nalbantoglu [29] and Tasgin *et al*., [30]). In order to repair the damage generated by ROS, plants evolve complex antioxidant metabolism and this includes enzymes like peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD).These antioxidant systems can be divided into two categories: one that reacts with ROS and keeps them at low levels, peroxidase, superoxide dismutase (SOD) and catalase, and one that regenerates the oxidised antioxidants, ascorbate peroxidase (APX) and glutathione reductase (Smirnoff [31]).

Also, Catalase and peroxidase activity is associated with scavenging of H_2O_2. An increase in its activities is related with increase in stress tolerance (Foyer *et al*., [32]). Involvement of SOD in temperature stress tolerance has been also advocated by Alscher et al. [33] and Hassanein et al. [34].

The effect of sowing dates, SA and PUT treatments and their interaction on antioxidant enzymes activity are presented in Table (3 &4). Regarding the effect of sowing dates, the results showed that, the first sowing date significantly increased the activity of POX in snap bean leaves compared with the second one .The same trend was also true for SOD while, the effect was insignificant in respect to CAT activity . A perusal of the data showed that , sown seeds in the first of October gave plants have better scavenging capacity and higher tolerance to low temperature stress whereas, plants of the second sowing date showed lower expression of the above enzyme activities and have little or in adequate H2 O2 scavenging mechanism and hence, poor low temperature tolerance . In this concern, Oloyede *et al*. [35] mentioned that as sowing date was delayed antioxidant activity of pumpkin fruits was reduced.

As for Bio-regulator spraying treatments, when considered as a single factor, the results indicated that, in both growing seasons, foliar application of SA or PUT alone increased POX, SOD and CAT activities. These increments were in parallel with increasing the concentration used, but did not reach the significant level with check treatment(in most cases). Moreover, the interaction treatments between SA and PUT followed almost exactly the trend of single factor behavior. The maximum and significant response generated in plants when sprayed with SA_1+$PUT_{2,}$ as compared to check as well as other spraying treatments . This stimulatory effect of SA + PUT on the investigated antioxidant activity may be attributed to the integrated of each other and such this integration could be suggested to increase the effect of the individual application .In other words , application of SA in combination with PUT have induced better scavenging capacity and induced higher tolerance to low temperature stress than control as well as other treatments .

There are previous publications concerning exogenous treatments of putrescine or salicylic acid are found to be effective in inducing antioxidant activity . Farooq *et al*. [36] reported that the maximum SOD activity recorded from spermine followed by SA.While, the maximum CAT and APX activities were observed from spermine followed by brassinosteroids under drought. stress. Also, Ghasemzadeh *and* Jaafar [37], found that exogenous application of SA observed significant increases in CAT, POX , SOD and proline in ginger .Similarly, SA induced transient increases in SOD and CAT enzyme activities in young tomato leaves

Similar was the case with Putrescine, in recent study, the putrescine-induced salt tolerance in wheat was mediated by stimulation in the activities of antioxidant enzymes, SOD and POX (Tamoor and Bano [26]). In addition , our results are in agreement with previous findings of Çakmak and Atıcı [38] reported that, under cold conditions, the putrescine treatments surprisingly increased enzyme activities in spring wheat .

Table (3) Mean comparison of the interaction effect of bio-regulators and sowing dates on POX content of snap bean leaves during 2012/2013 and 2013/2014 seasons.

Treatments	peroxidase activity(unit/mg protein)					
	1 October	16 October	Mean	1 October	16 October	Mean
	First Season			Second Season		
Control	1174.0^{c-e}	1202.9c-e	1188.4^{C}	1165.93^{e}	1003.50^{f}	1084.71^{E}
SA$_{1.0}$	1696.8^{ab}	1055.9d-f	1376.3^{A-C}	1658.57^{a}	1090.79^{ef}	1374.68^{B}
SA$_{0.5}$	1665.3^{ab}	773.3f	1219.3^{BC}	1681.36^{a}	1162.98^{e}	1268.88^{C}
PUT$_2$	1727.3^{a}	839.9ef	1283.6^{BC}	1510.95^{bc}	872.15^{g}	1191.55^{CD}
PUT$_1$	1297.2^{b-d}	1017.8d-f	1157.5^{C}	1157.72^{e}	856.39^{g}	1160.35^{DE}
SA$_1$+PUT$_2$	1620.7^{ab}	1666.9ab	1643.8^{A}	1609.21^{ab}	1596.67^{ab}	1602.94^{A}
SA$_1$+PUT$_1$	1487.0^{a-c}	1486.6a-c	1486.8A^{B}	1308.39^{d}	1503.35^{bc}	1450.87^{B}
SA$_{0.5}$+PUT$_2$	1483.9^{a-c}	1405.4^{a-d}	1444.6^{A-C}	1459.80^{c}	1466.^{42c}	1463.11^{B}
SA$_{0.5}$+PUT$_1$	1327.2^{a-d}	1400.3^{a-d}	1363.8^{A-C}	1459.93	1462.96^{c}	1461.44^{B}
Mean	1497.71^{A}	1205.45^{B}		1445.76^{A}	1223.91^{B}	

Values with common letter in the same column are not significantly different at 5% level
SA$_1$= Salicylic acid at 1 mM SA$_{0.5}$= Salicylic acid at 0.5 mM
PUT$_1$= Putrescine at 1 mM PUT$_2$= Putrescine at 2 mM

Table (4) Mean comparison of the interaction effect of bio-regulators and sowing dates on CAT and SOD content of snap bean leaves during 2012/2013 and 2013/2014 seasons.

Treatments	Catalase dismutase activity(unit/mg protein)			Superoxide dismutase activity (unit/mg protein)		
	1 October	16 October	Mean	1 October	16 October	Mean
	Second season			Second season		
Control	0.19^{e}	0.23^{de}	0.21^{C}	10.27^{bc}	7.25^{c}	8.76^{B}
SA 1.00	0.33^{bc}	0.22^{de}	0.28^{B}	12.78^{ab}	9.69^{bc}	11.24^{AB}
SA 0.5	0.26^{c-e}	0.21e	0.24^{BC}	10.86^{bc}	7.60^{c}	9.23^{B}
PUT2	0.27^{c-e}	0.21e	0.25^{BC}	9.88^{bc}	10.41^{bc}	10.15^{AB}
PUT1	0.22^{de}	0.26c-e	0.24^{BC}	8.79^{bc}	10.18^{bc}	9.48^{B}
SA1+PUT2	0.42^{a}	0.38^{ab}	0.40^{A}	15.44^{a}	12.83^{ab}	14.24^{A}
SA1+PUT1	0.24^{de}	0.31^{b-d}	0.27^{B}	10.02^{bc}	9.02^{bc}	9.52^{B}
SA0.5+PUT2	0.24^{de}	0.25^{c-e}	0.24^{BC}	8.46^{bc}	10.47^{bc}	9.47^{B}
SA0.5+PUT1	0.27^{c-e}	0.25^{c-e}	0.26^{BC}	9.98^{bc}	8.93bc	9.46^{B}
Mean	0.27^{A}	0.26^{A}		10.72^{A}	9.59^{B}	

Values with common letter in the same column are not significantly different at 5% level

SA$_1$= Salicylic acid at 1 mM SA$_{0.5}$= Salicylic acid at 0.5 mM
PUT$_1$= Putrescine at 1 mM PUT$_2$= Putrescine at 2 mM

Respecting the interaction between sowing dates and bio-regulator treatments, it was found that seeds sown on the 1th of October and applications of SA$_1$ (at the higher rate) or Put$_1$ (at the lower rate) singly showed the higher contents of POX in first and second season, respectively. Meanwhile, the highest significant increase in CAT and SOD were obtained by exogenous supplement with of SA$_1$+PUT$_2$ at the first sowing date in both seasons.

3.3 Pod number and total yield

The effect of sowing dates and bio-regulators and their interaction on pod weight and Pant al yield, (4). Sown snap bean seeds on the 1st October was the better proper sowing date giving the heavier pod weight. However, in the two growing seasons, sown snap bean seeds on the 1st October significantly produced higher plant yield than sowing on the 16th October. However, sowing snap bean seeds on the 1st October induced significant maximum total yield (10.31and 9.29 ton /he^{-1} with a downward trend for the late sowing 8.24 and 7.07 ton /he^{-1} in the first and second season, respectively. Sown snap bean seeds on the 1th of October was the better proper sowing date and the optimum time makes the best use of the available growth factor such as temperature and solar radiation at different stages of growth for high productivity. Akhtar *et al* , [39] reported that to get better crop yield, sowing time in terms of changed temperatures has been critical factor in various crops. In addition, The superiority of the 1st October sowing date in yield components may be resulted from their increase in vegetative growth characters of plant (data not shown) and led to the formation of higher photosynthesis which ultimately resulted in higher fresh pod yield . In contrast, the reduction in yield under late sown condition (16, October) could be attributed to poor development of vegetative growth due to unfavorable temperature prevailed during reproductive phase of the crop. Our results in a good agreement with the result obtained by Abou El- Yazied [12] reported that, the early sowing date (1st of October) gave the highest pod weight and total yield of snap bean.

Table (4) Mean comparison of the interaction effect of bio-regulators and sowing dates on pod number , total yield of snap bean plant during 2012/2013 and 2013/2014

Treatments	Pod weight						Total yield (ton/he^{-1})					
	1 October	16 October	Mean	1 October	16 October	Mean	1 October	16 October	Mean	1 October	16 October	Mean
	First season			Second season			First season			Second season		
Control	4.35^{a}	3.94^{b-e}	4.15^{A}	4.48^{ab}	4.17^{bc}	4.33^{A}	9.12^{k}	7.31^{q}	8.21^{I}	7.81^{j}	6.19^{o}	7.01^{i}
SA 1.0	3.98^{b}	3.45^{e}	3.72^{B}	4.36^{b}	3.62^{c-e}	3.99^{AB}	10.40^{e}	7.67^{p}	9.02^{F}	9.50^{e}	7.12^{l}	8.31^{E}
SA 0.5	4.20^{ab}	3.54^{e}	3.87^{B}	4.80^{a}	3.77^{cd}	4.29^{A}	9.64^{h}	7.26^{r}	8.45^{H}	9.10^{g}	7.00^{l}	8.07^{F}
PUT_2	4.11^{ab}	3.61^{de}	3.30^{C}	4.29^{bc}	3.54^{c-f}	3.92^{AB}	10.10^{f}	8.17^{n}	9.12^{E}	9.55^{d}	7.52^{k}	8.36^{D}
PUT_1	4.17^{ab}	3.72^{be}	3.95^{B}	4.99^{a}	3.76^{cd}	4.38^{A}	9.71^{g}	7.79^{o}	8.74^{G}	9.29^{f}	6.81^{m}	7.88^{G}
SA_1+PUT_2	3.40^{e}	3.23^{f}	3.32^{C}	3.91^{c}	3.47^{d-f}	3.69^{B}	11.40^{a}	9.48^{i}	10.43^{A}	10.40^{a}	8.05^{i}	9.19^{A}
SA_1+PUT_1	3.53^{e}	3.25^{f}	3.35^{C}	3.89^{c}	3.50^{de}	3.70^{B}	11.10^{b}	9.29^{j}	10.19^{B}	10.05^{b}	8.02^{i}	8.88^{B}
$SA_{0.5}+PUT_2$	3.64^{de}	3.42^{e}	3.53^{C}	3.90^{c}	3.42^{d}	3.66^{B}	10.86^{c}	8.93^{l}	9.88^{C}	9.74^{c}	7.29^{l}	8.55^{C}
$SA_{0.5}+PUT_1$	3.87^{b-e}	3.34^{ef}	3.61^{BC}	3.67^{c-e}	3.76^{cd}	3.72^{B}	10.45^{d}	8.45^{m}	9.45^{D}	8.29^{h}	6.55^{n}	7.40^{H}
Mean	3.92^{A}	3.50^{A}	4.15^{A}	4.25^{A}	3.67^{B}		10.31^{A}	8.24^{B}		9.29^{A}	7.07^{B}	

Values with common letter in the same column are not significantly different at 5% level

SA_1= Salicylic acid at 1 mM $SA_{0.5}$= Salicylic acid at 0.5 mM
PUT_1= Putrescine at 1 mM PUT_2= Putrescine at 2 mM

Regarding the bio-regulators spraying effect, the data showed that, in both seasons , control plants yielded the highest pod fresh weight compared to singly or combined applications. The results were significant in the first season while, the effect was significant with the combined application treatments in the second season. The pod weight data reflect the conflicting reports in the literature that SA and PUT application increased pod weight . The possible explanation of the reduction in pod weight with bio-regulators application maybe attributed to dry matter partitioned to pods is affected by pods load, so increasing the number of pods per plant with bio-regulators treatments (data not shown) diminishes the fraction of total biomass allocated to the pods which in turn decreased pod weight

However, application of SA or PUT at any concentration and their combination significantly increased total yield compared to the check untreated plants. When considering SA or PUT as a sole application, in most cases, PUT has shown a much better ability to promote yield and its components at the two tested rates. Moreover, when considering SA and PUT as a mixture , data revealed that, all combination treatments significantly surpassed yield and its components in comparison to control as well as single application (except of $SA_{0.5}$+PUT_1 with total yield in second season).

The most promising results obtained in total yield were the combination treatment of SA_1+PUT_2, followed in decreasing order by $SA_{1+}PUT_1$ then SA $_{0.5}$+$Put_{2.}$. The significant increments of total pod yield over control induced by these treatments were 27.0 %, 24.1% and 20.3% in the first season and 31.3 % , 26.9% and 22 % in the second season, respectively. These results may be attributed to the integrated effect of SA and PUT and such integration could be increase the individual application effect on vegetative growth, chlorophyll content and nutrient content which lead to increase photosynthetic assimilates production and consequently increased the yielding ability of plants. SA have been shown to be induced resistance of plants to low temperature (Hayat and Ahmed, [40]) and the increase happened by SA treatments may be also due to that SA increased flower longevity via inhibition of ethylene production (Lesilie and Romani [41]). In this concern, using SA as bio-regulator improved yield as well as quality of the seeds of vegetables legumes (Moustafa, [42]). On mung bean (Singh and Kaur [43]), on broad bean and dry bean (Awasthi *et al*. [44]; Zaghlool, *et al*. [45]) on sweet pepper (Abou El-Yazied [12]), on some legume plants (Wael *et al*., [46]) and on quinoa (Abd Allah et al [47]). Similar was the case with putrescine, the promotive effects of PUT on yield components in agreement with previous findings of Gahrib and Hanafy Ahmed [48] on pea and Hanafy Ahmed et al. [49] on snap bean.

Concerning the interaction between sowing date and bio-regulators treatments, obtained results, indicated that plants growing under the first sowing date and combined with the mixture of SA_1 + PUT_2 gave the greatest pod number/plant, plant yield and total and marketable yield, in both growing seasons.

In conclusion, it can be concluded that appropriate sowing date and bio-regulators spraying treatments can substantially increase the productivity of snap bean .Putrescine and salicylic acid can be easily applied to snap bean plants in the field. SA and Put singly or in combination, resulted in a significant increase in the proline and antioxidant activity enzymes .The maximum total yield of snap bean can be harnessed from the mixture of SA + PUT at higher rates thus opens up a new avenue for increasing yield and improving the quality of snap bean by carrying out early sowing on 1st October.

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