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Using Some Growth Regulators for Improving Growth and Productivity of Pea under Late Sowing Conditions

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Abstract: Two field experiments were conducted at private farm, El Gammaliah, Dakahlia Governorate, Egypt during the two successive winter seasons of 2018 and 2019 to study the effect of some growth regulators applied as (soaking, spray and combined) on pea plants grown under late sowing conditions. Fourteen treatments were arranged in randomized complete block with three replicates. The treatments were control; soaking in water; soaking in IAA (indole-3-acetic acid) at 25 ppm, NAA (1- naphthalene acetic acid) at 25 ppm, GA₃ (gibberellic acid) at 200 ppm and BR (brassinolide) at 5 ppm; spray with the same growth regulators at the same concentrations; and the combined between them (soaking with spray). The studied characteristics were plant height; number of branches plant⁻¹; total green pods yield fed⁻¹ and leaves content of nitrogen, phosphorus and potassium (%) and DPPH (free radical scavenging activities). The combined method (soaking with spray) recorded the highest results in all studied characteristics followed by spray and soaking methods, respectively. Moreover, NAA recorded the best result compared with other growth regulators under the experimental conditions. From the results of this study, it can be suggested that using NAA at 25 ppm as seed soaking for 2 hours before sowing and spraying three times was an effective practice to overcome the adverse effect on pea plants grown under late conditions.

Key words: Pea · Soaking · Spray · Growth regulators · Growth · Yield

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important leguminous vegetable crops in Egypt for local consumption and exportation. It is the second most important food legume in the world after common bean [1]. Pods of pea contain high amount of protein (21-25 %), carbohydrates, amino acids, vitamins, phosphorus, iron, magnesium and calcium and low contents of fiber. Pea is a cool climate vegetable crop whereas the growing period is extended under cool conditions. Its cultivation requires cool weather and partly moist climatic condition and the increase in temperature above 20°C decreases the yield and quality of immature seeds [2]. At the late sowing date, relatively high temperature and dry climate are unsuitable for flowering and pod development of pea [3].

Therefore, it is necessary to improve the growth and productivity of pea growing under stress conditions at late seasons. Plant growth regulators are one of the promising approaches to improve the productivity of crops under biotic and abiotic stress conditions. Plant growth regulators are organic substances synthesized chemically and when they are produced endogenously by plants, they called phytohormones or plant hormones [4, 5]. They help the plants adapt to changing environments, by mediating plant growth and development, nutrient allocation and source/sink transitions [6]. Also, they play an essential role in the regulation of signal transduction pathway involved in the induction of plant stress response and may be useful to return metabolic activities to their normal levels [7, 8].

Auxin is the first class growth regulator that was discovered. IAA (indole-3-acetic acid) is the natural auxin while the synthetic produce auxins are NAA (1- naphthalene acetic acid) and IBA (indole-3-butyric acid) [9]. Auxin is involved in mediating a number of essential plant growth and developmental processes, such as cell elongation, division and differentiation, induction of root growth and flower and fruit development, nutrient allocation and source-sink relationship [6, 10]. Additionally, auxin plays a vital role in plant responses to different biotic and abiotic stresses

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where it integrates stress signals from different plant hormones and modulate redox pathway to regulate root development as a stress-induced morphological response [11].

Another plant hormone is gibberellic acid (GA_3) belongs to gibberellins (GA_3) which are a large family of tetracyclic di-terpenoid plant growth substances. Gibberellic acid (GA_3) regulating several processes of plant development like cell elongation and cell division, cell wall extensibility, seed germination, stem and hypocotyl elongation, leaf expansion, induction of floral initiation, sex determination and flower and seed development [12-14]. Exogenous application of GA₃ can helps the plants to adapt the abiotic stresses. GA₃ has been shown to be beneficial for providing a mechanism to regulate the metabolic process as a function of sugar signalling and antioxidative enzymes and has a crosstalk with SA in the regulation of source-sink relation under abiotic stress [15].

Brassinolide (BR) belonging to brassinosteroids (BRs) is a new class of plant hormones that play an essential role in plant development. It is a natural plant hormone first isolated from the pollens of rapeseed plant (Brassica napus L.) [16]. Brassinosteroids (BRs) regulate multiple physiological and development processes including seed germination, cell division and elongation, differentiation, rhizogenesis, vascular flowering, senescence, abscission, maturation, regulation of carbohydrate assimilation and allocation and activation of photosynthesis [17, 18]. In addition, BRs induced the synthesis of IAA and GA in plant [19], stimulated the synthesis of heat-shock proteins and antioxidant enzymes and can be used as an antistress agent in a wide range of biotical and abiotic stresses [20].

The effect of growth regulators varies under different concentrations, methods of application, time of application, weather conditions, stress and genotypic differences [21, 22]. Therefore, this study aimed to evaluate the effect of some growth regulators with different methods on growth, yield and quality of pea under stress conditions at late season.

MATERIALS AND METHODS

The present study was carried out on pea plants (*Pisum sativum* L.) cv. Master-B in a private farm, El-Gammaliah, Dakahlia Governorate, Egypt during the two successive seasons of 2018 and 2019 to study the effect of some growth regulators (IAA, NAA, GA₃ and

BR) applied as soaking, foliar and combination between soaking and foliar application, in addition to soaking in water and control (spraying with tap water) treatments. The experimental soil was clay loam with pH 8.20, EC 1.13 ds.m^{-1} , organic matter 2.42 %, available nitrogen 44.20 mg kg⁻¹, available phosphorous 4.64 mg kg⁻¹ and available potassium 352.00 mg kg⁻¹. The mean monthly temperature and relative humidity during 2018 and 2019 seasons prevailing in El-Gammaliah region are presented in Table (1).

Seeds were inoculated before sowing with root nodules bacteria (*Rhizobium leguminosarum*) and sown on 20th January in the first and second season. Seeds were planted on rows with 20 cm spacing among rows and 10 cm among plants. The experimental design was complete randomized block design (CRBD), with three replicates. The plot area was 12 m² and included 3 ridges; each ridge was 5 m length and 0.80 m width. All recommended cultural practices for pea production were followed according to the Egyptian Ministry of Agriculture. The experiment included 14 treatments as shown in Table (2).

The seeds were soaked for two hours before sowing and the foliar treatments were applied three times (at 15, 30 and 45 days after sowing). The above growth regulators were obtained from Al-Gommhoria Company for chemicals. The seeds of pea were obtained from Horticulture Research Institute (HRI), Agricultural Research Center (ARC), Egypt.

The Following Data Was Recorded

Vegetative Growth Traits: Ten plants from each plot were randomly taken at 50 days after sowing from each plot to determine plant height (cm), number of branches plant⁻¹, stem diameter (cm), fresh weight plant⁻¹ and dry weight plant⁻¹ in each season.

Pod Yield and its Components: At harvest time, number of seeds pod⁻¹, average pod weight (g), number of pods plant⁻¹ and total green pods yield fed⁻¹ were determined.

Chemical Contents: Samples of leaves from each plot were randomly taken to measure and determine the following parameters: N (%) according to [23], P (%) according to the method of [24], K (%) according to [25] and antioxidants activity (free radical scavenging activities) towards the stable free radical DPPH(1, 1-diphenyl-2-picrylhydrazyl) was determined by the method of [26] and expressed as μ gmL⁻¹.

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|---------------|------------|------------|------|-------------|
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| Air temperature (°C) | | | | | | |
|----------------------|-------|-------|-----------------------|--|--|--|
| Month | Min | Max | Relative humidity (%) | | | |
| | | 2018 | | | | |
| January | 11.78 | 18.64 | 69.22 | | | |
| February | 12.89 | 20.94 | 67.19 | | | |
| March | 14.45 | 25.21 | 55.34 | | | |
| April | 16.35 | 26.63 | 57.59 | | | |
| | | 2019 | | | | |
| January | 9.65 | 18.11 | 59.89 | | | |
| February | 10.70 | 19.36 | 63.70 | | | |
| March | 12.17 | 21.03 | 63.85 | | | |
| April | 14.38 | 23.92 | 59.19 | | | |

| Table 1: Monthly temperature and relative humidit | v of the experimental site (| El-Gammaliah. Dakahlia Go | vernorate) during 2018 and 2019 |
|---|------------------------------|---------------------------|---------------------------------|
| | | | |

Table 2: Details of the studied treatments

| Treatments | Details |
|-----------------|---|
| T ₁ | Control. |
| T ₂ | Soaking in water. |
| T ₃ | Soaking in IAA (indole-3-acetic acid) at 25 ppm. |
| Γ_4 | Soaking in NAA (1- naphthalene acetic acid) at 25 ppm. |
| Γ ₅ | Soaking in GA ₃ (gibberellic acid) at 200 ppm. |
| Γ_6 | Soaking in BR (brassinolide) at 5 ppm. |
| Γ ₇ | Spray with IAA (indole-3-acetic acid) at 25 ppm. |
| Γ_8 | Spray with NAA (1- naphthalene acetic acid) at 25 ppm. |
| Г9 | Spray with GA ₃ (gibberellic acid) at 200 ppm. |
| Γ_{10} | Spray with BR (brassinolide) at 5 ppm. |
| Γ ₁₁ | Soaking and Spray with IAA (indole-3-acetic acid) at 25 ppm. |
| Γ ₁₂ | Soaking and Spray with NAA (1- naphthalene acetic acid) at 25 ppm. |
| Γ_{13} | Soaking and Spray with GA ₃ (gibberellic acid) at 200 ppm. |
| T ₁₄ | Soaking and Spray with BR (brassinolide) at 5 ppm. |

Statistical Analysis: The data were analyzed using SAS software Version 9.1 according to [27] and the differences among means were compared by using Duncan's multiple range tests (DMRT) at 0.05 levels according to [28].

RESULTS AND DISCUSSION

Vegetative Growth: The response of pea plants to soaking, spraying and the combined treatments of growth regulators are presented in Table (3). Concerning soaking treatments, all soaking treatments increased vegetative growth attributes compared with control plant. The seeds soaked in brassinolide (BR) exhibited the tallest plants while the values of other studied characters (number of branches plant⁻¹, stem diameter and fresh and dry weight) were highest in naphthalene acetic acid (NAA) followed by gibberellic acid (GA₃), indole acetic acid (IAA), brassinolide (BR) and water soaking treatments, respectively.

As to spray treatments, the differences between them and control were significant in all parameters. Brassinolide spray treatment recorded the highest values of plant height only, whereas naphthalene acetic acid treatment gave the highest values in other parameters, i.e., number of branches plant⁻¹, stem diameter, plant fresh weight and plant dry weight in the two seasons.

The combined treatments between spray and soaking (Table 3) showed significant effect on vegetative growth characters of pea plants. Soaking pea seeds and spraying with brassinolide exhibited the tallest plants while soaking and spraying with naphthalene acetic acid gave the highest values of other parameters compared with all studied treatments in both seasons of the study followed by GA_{33} IAA and BR.

Brassinolide application either as soaking or spraying or combined gave the highest plants of pea compared with other treatments. That may be due to the role of brassinolide in cell elongation and divisional activities by activating cell wall loosening enzymes [29] and synthesis of IAA and GA₃ in plant and the increase in plant height may probably due to their cumulative action [19]. Also, BRs promote lateral root development through increasing acropetal auxin transport [30]. Under normal growth or stress conditions, brassinolide can regulate heat shock proteins (HSPs) and can induce heat tolerance of stressed plants [31].

| | Plant height (cm) | | No of branches plant ⁻¹ | | Stem diameter (cm) | | Plant fresh weight (g) | | Plant dry weight (g) | |
|-----------------|-------------------|-----------------|------------------------------------|-----------------|--------------------|-----------------|------------------------|-----------------|----------------------|-----------------|
| Treatments | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd |
| Control | 51.00h | 51.00d | 1.07h | 1.00i | 0.290f | 0.295f | 13.967j | 15.02m | 5.007g | 5.120i |
| Soaking | | | | | | | | | | |
| Water | 52.00h | 52.00d | 1.33gh | 1.50h | 0.303ef | 0.305ef | 17.883i | 19.5501 | 6.723ef | 6.033h |
| IAA | 57.00de | 57.00bc | 1.53fg | 1.80fg | 0.310de | 0.315de | 19.580g | 21.000i | 6.007f | 6.300h |
| NAA | 54.00g | 54.00cd | 2.00de | 2.40d | 0.315cd | 0.320de | 21.670e | 23.417g | 8.103c | 8.303d |
| GA ₃ | 55.00fg | 56.33c | 1.93de | 2.00ef | 0.312de | 0.317de | 19.767fg | 21.900h | 6.193f | 6.900g |
| BR | 58.00d | 58.00bc | 1.53fg | 1.67gh | 0.308de | 0.319de | 18.763h | 19.967j | 6.007f | 6.127h |
| Spray | | | | | | | | | | |
| IAA | 56.00ef | 58.00bc | 2.13cd | 2.37d | 0.320bc | 0.325d | 21.507e | 23.267g | 7.083e | 7.497ef |
| NAA | 55.00fg | 55.00cd | 2.63ab | 2.80 b | 0.325bc | 0.330cd | 25.143c | 27.667c | 8.703bc | 8.967b |
| GA ₃ | 57.00de | 57.33bc | 2.33bc | 2.53cd | 0.323bc | 0.327cd | 23.200d | 25.417e | 7.973cd | 8.500cc |
| BR | 61.00c | 60.66b | 1.80ef | 1.93ef | 0.315de | 0.320de | 19.910f | 21.933h | 6.980e | 7.200fg |
| Spray+Soaking | | | | | | | | | | |
| IAA | 66.00b | 66.33a | 2.50ab | 2.73bc | 0.335bc | 0.340bc | 26.207b | 26.233d | 8.200bc | 8.693b |
| NAA | 56.00ef | 56.33c | 2.80a | 3.10a | 0.357a | 0.364a | 28.867a | 30.147a | 10.283a | 10.717 |
| GA ₃ | 62.00c | 61.00b | 2.60ab | 2.83b | 0.340ab | 0.345b | 26.393b | 28.667b | 8.917b | 9.007b |
| BR | 68.00 a | 68.00a | 1.80ef | 2.03e | 0.325bc | 0.330d | 23.117d | 24.900f | 7.283de | 7.590e |

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Mean pairs followed by different letters are significantly different ($P \le 0.05$) according to the Duncan's multiple range test.

The improvement effect of auxins (IAA and NAA) on pea vegetative growth may be related to the role of auxins in cell division and elongation and induction of root growth [10], leading to an increase in plant fresh weight. Likewise, IAA increases photosynthetic activities [32] and serves as a signaling molecule necessary for the coordination of growth and development of plant organs [33] improves nitrogen metabolism in pea plant grown under stress conditions and can alter the expression of stress responsive genes [34, 35].

Regarding GA₃, it stimulates cell division and elongation, increases internode length and improves nodulation via the positive effect on seedling roots [36, 37]. Furthermore, GA₃ improves the photosynthesis through its influence on photosynthetic enzymes, leaf area index, light interception and enhanced use efficiency of nutrients [38] and plays a crucial role in early plant responses to adverse environmental conditions by increasing SA biosynthesis [39].

Similar results were recorded by Shahid *et al.* [40] and Priya [16] on brassinolide, Husen *et al.* [41] and Aldesuquy *et al.* [42] on IAA, Vadeo [43] on NAA and Hussain *et al.* [44] on GA₃.

Pod Yield and its Component: Table (4) shows that soaking treatments significantly increased number of seeds pod⁻¹, average pod weight, number of pods plant⁻¹ and total green pod yield fed.⁻¹ compared with control. NAA soaking treatment was the best followed by GA₃, IAA and water, respectively in both seasons. Also, NAA applied as spray recorded the highest values compared with other spraying treatments and control. Regarding the

combined applications, soaking and spraying with NAA, GA_3 , IAA and BR, respectively gave the highest values of all studied characters, i.e., number of seeds pod^{-1} , average pod weight, number of pods $plant^{-1}$ and total green pod yield fed.⁻¹ compared with soaking or spraying treatments and control (Table 4) under stress condition (Table 1).

The improvement of pea pod yield in response to auxin and gibberellin treatments may be due to their role in uptake more nutrients, increasing photosynthetic rate and enhancing sink-source relationship, which led to increasing plant growth (Table 3), higher dry matter produced and transferred topods [45] and consequently increasing yield (Table 4).

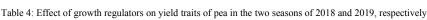
Concerning brassinolide, it has an effective role in photosynthesis through increasing the activity of Rubisco enzyme and the capacity of CO_2 assimilation in the Calvin cycle [46]. Also, brassinolide induces more translocation of assimilates to the developing seeds during seed filling phase, thus improving the seed weight [40].

These results were agreed with those obtained by El-Sayed *et al.* [47] on brassinolide, Khalid *et al.* [48] on IAA, Thomson *et al.* [37] on NAA and Singh *et al.* [49] on GA₃.

Chemical Contents: Table (5) shows the impact of growth regulators (IAA, NAA, GA₃ and BR) as soaking or sprays or combined on leaves content of N, P, K and DPPH (free radical scavenging, an accepted mechanism for screening the antioxidant activity of plant extracts).

| | No of seeds | No of seeds pod ⁻¹ | | Average pod weight (g) | | No of pods $plant^{-1}$ | | Total green yield fed -1(ton/fed | |
|-----------------|-----------------|-------------------------------|-----------------|------------------------|--------|-------------------------|---------|----------------------------------|--|
| Treatments | 1 st | 2 nd | 1 st | 2 nd | 1 st | 2 nd | | 2 nd | |
| Control | 7.50h | 7.70g | 3.00f | 3.10g | 6.99e | 7.06e | 4.197i | 4.399i | |
| Soaking | | | | | | | | | |
| Water | 8.067g | 8.30f | 3.20e | 3.30f | 7.20d | 7.29d | 4.615h | 4.816h | |
| IAA | 8.50ef | 8.70de | 3.37de | 3.44de | 7.37cd | 7.46c | 4.931fg | 5.129fg | |
| NAA | 8.70cd | 8.90bc | 3.46cd | 3.57cd | 7.41cd | 7.50c | 5.131de | 5.351de | |
| GA ₃ | 8.60de | 8.80cd | 3.45cd | 3.54cd | 7.39cd | 7.49c | 5.101ef | 5.307de | |
| BR | 8.30fg | 8.60ef | 3.30e | 3.40ef | 7.35cd | 7.44c | 4.847gh | 5.061gh | |
| Spray | | | | | | | | | |
| IAA | 8.80cd | 9.00bc | 3.51c | 3.59bc | 7.45bc | 7.55c | 5.361cd | 5.425cd | |
| NAA | 9.00cd | 9.20bc | 3.60ab | 3.67ab | 7.51bc | 7.59c | 5.411bc | 5.565bc | |
| GA ₃ | 8.90cd | 9.10bc | 3.55bc | 3.64ab | 7.49bc | 7.59c | 5.315cd | 5.529cd | |
| BR | 8.70cd | 8.90bc | 3.47cd | 3.56cd | 7.40cd | 7.48c | 5.131de | 5.256ef | |
| Spray+Soaking | 5 | | | | | | | | |
| IAA | 9.10bc | 9.20bc | 3.67ab | 3.76ab | 7.75a | 7.75b | 5.697ab | 5.808ab | |
| NAA | 9.80 a | 10.00a | 3.75 a | 3.83 a | 7.80a | 7.90a | 5.844 a | 6.048 a | |
| GA ₃ | 9.50ab | 9.70a | 3.71a | 3.80a | 7.78a | 7.89ab | 5.773ab | 5.996a | |
| BR | 9.00cd | 9.30b | 3.60ab | 3.68ab | 7.64ab | 7.74bc | 5.506ab | 5.693bc | |

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Mean pairs followed by different letters are significantly different (P < 0.05) according to the Duncan's multiple range test.

Table 5: Effect of growth regulators on chemical composition of leaves in the two seasons of 2018 and 2019, respectively

| Treatments | N% | | Р% | | K% | | DPPH (µg mL | ') |
|-----------------|-----------------|-----------------|---------------------|-----------------|---------|-----------------|-----------------|-----------------|
| | 1 st | 2 nd | 1 st | 2 nd | | 2 nd | 1 st | 2 nd |
| Control | 2.521 | 2.40h | 0.413b | 0.418b | 1.577h | 1.650h | 20.520i | 19.813h |
| Soaking | | | | | | | | |
| Water | 2.52j | 2.50gh | 0.415b | 0.420b | 1.600h | 1.700gh | 27.866h | 26.000g |
| IAA | 2.73i | 2.80ef | 0.417b | 0.422b | 1.703fg | 1.850f | 51.514c | 50.295c |
| NAA | 2.95fg | 3.00de | 0.419b | 0.424b | 1.800de | 1.950de | 49.620d | 49.187cd |
| GA ₃ | 2.83h | 2.93de | 0.417b | 0.423b | 1.750ef | 1.850f | 35.666g | 34.666f |
| BR | 2.64j | 2.70fg | 0.416b | 0.421b | 1.653gh | 1.750g | 36.958g | 35.333f |
| Spray | | | | | | | | |
| IAA | 3.00f | 3.10cd | 0.511a | 0.515a | 1.850de | 1.950de | 55.590b | 53.333b |
| NAA | 3.23d | 3.300ab | 0.511a | 0.517a | 2.000c | 2.133bc | 54.736b | 53.467b |
| GA ₃ | 3.10 e | 3.20bc | 0.511a | 0.516a | 1.898d | 2.000d | 45.366f | 44.934e |
| BR | 2.92g | 3.00de | 0.510a | 0.515a | 1.803de | 1.900ef | 47.608e | 47.739d |
| Spray+Soaking | 3 | | | | | | | |
| IAA | 3.31c | 3.40ab | 0.516a | 0.518a | 2.103b | 2.200b | 57.702a | 54.333b |
| NAA | 3.53a | 3.60a | 0.515a | 0.520a | 2.253a | 2.350a | 58.743a | 56.968a |
| GA ₃ | 3.41b | 3.50ab | 0.514a | 0.519a | 2.1990a | 2.300a | 45.000f | 44.500e |
| BR | 3.32c | 3.30ab | 0.512a | 0.517a | 2.000c | 2.083c | 51.000cd | 47.500d |

Mean pairs followed by different letters are significantly different (P < 0.05) according to the Duncan's multiple range test

It is clear that among soaking treatments, NAA recorded the highest content of N, P, K and DPPH in leaves compared with other treatments. The same trend was obtained in spray and combined treatments, which NAA came first and control was the latest in both seasons of the study. It is notable that the differences among all studied growth regulators applied in soaking or spray or combined were insignificant in both seasons of the study concerning phosphorous content. Among all investigated growth regulators, NAA applied as combined (soaking + spray) recorded the highest content

of chemical constitutes followed by GA₃, IAA and BR (soaking + spray).

The enhancement in leaves content of nutrients and DPPH in response to auxin, maybe due to its important roles in lateral root formation and activates the plasma membrane H+-ATPase, provide the driving force for the uptake of numerous nutrients [50-52]. Additionally, auxin enhancing the activities of antioxidant enzymes such as POD, CAT and SOD in plants [53]. In pea, Ochoa [22] found that IAA increased CAT activity by protecting plants against oxidative stress.

In case of GA_3 , it enhances nitrogen uptake due to the increase in shoot growth, which requires more N from the soil [38]. Ca^{2+} and other nutrients uptake increased by GA_3 application may be involved in stress tolerance by regulating antioxidant metabolism and reduced the lipid peroxidation of the cell membrane [54]. Also, GA_3 can regulate the metabolic process as a function of sugar signalling and antioxidative enzymes under abiotic stress [15].

Likewise, brassinolide changes root permeability and cation exchange capacity of the root and that enhanced water and nutrient uptake resulting in increased protein synthesis [55]. Besides, brassinolide enhanced the activities of antioxidant enzymes seemed to play an important role in scavenging the reactive oxygen species (ROS) [29] through regulating the expression of genes involved in the biosynthesis of antioxidant enzymes in plants [56].

Similar observations were found by Garget *al.* [57] on brassinolide, Hussain *et al.* [44] on IAA, Singh *et al.* [58] on NAA and Husain *et al.* [59] on GA₃.

The results of this study suggested that using NAA at 25 ppm as seed soaking for 2 hours before sowing and spray 3 times (15, 30 and 45 days after sowing) was an effective practice to overcome the adverse effect on pea plants grown under late conditions.

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