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Effect of Presowing Treatment with Growth Regulators on Different Growth and Yield Contributing Parameters in Soybean [*Glycine max* (L.) Merril]

Eun Oh Kwon* and Ja Ock Guh*

植物生長 調節劑의 種子處理가 콩의 生長과 收量構成要素에 미치는 영향

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ABSTRACT

To know the effect of presowing seed soaking with some growth substances (kinetin, IAA, ethrel and salicylic acid) on vegetative growth and yield contributing parameters in soybean [*Glycine max* (L.) Merrill] the investigation was undertaken. The salient features from the study are;

– All the treatments showed the enhancement effect on vegetative growth, viz. seediling emergence, germination percentage, plant height, number of leaves and branches per plant as compared with control, whereas salicylic acid delayed the seedling emergence process and lowered the germination percentage.

– Nodule initiation, number of nodules and nodule weight were hastened and increased respectively under all the treatments whereas IAA showed an opposite effect on all the parameters at the early stages of nodule development.

– Kinetin, IAA and ethrel showed the significant enhancement effect on the levels of biochemical parameters throughout the time of seed development whereas salicylic acid and water showed the tendency similar to that of control.

– Yield and its components, viz. number of flowers, pods and seeds per plant were significant increased under all the treatments but they didn't show any significant enhancement effect on number of seeds per pod and pod setting rate. One hundred seed weight was lower under all the treatments, indicating the dilution effect resulting from incapability of increased number and size of source to provide the increased size of sink with assimilates.

INTRODUCTION

The Soybean [*Glycine max* (L.) Merrill] produces a large number of flowers but most of them abscise and do not form optimal number of pod to their full potential. This large loss of potential yield in soybean is common even under optimal growing conditions. Van Schaik and Probst[29] reported that the abortion of flowers and pods was ranged from 43 to 81%. From one-third to one-half of total shedding was in the form of pods. Breveden *et al.*,[3] re-

* 全南大學校 農科大學 (Chonnam National University, Kwangju 500, Korea) 〈'87. 10. 5 接受〉

ported the influence of nutrition on flower and pod abortion in soybean. Potential sink does not seem to be a limiting factor in this crop, yet, it is the retention of flowers and their subsequent pod development which hampers the optimization of the yield.[9,11] Many researchers have modified soybean pod formation by physical removal of pods and/or leaves in different parts of soybean plant and observed the resulting adjustment in the yield.[1,7,9] Various kinds of growth regulators are known to affect pod yield[18] by making the plants photosynthetically more efficient thereby increasing the yield. The study on the yield potential of the plant is being done actively by the crop physiologists in terms of source (activity of chlorophylls, leaf area, and nitrogen content) and sink (grain, tuber etc.) and interrelationship between source and sink.[9] Nourallah[20] reported that foliar spray of NAA and BA to field grown soybean delayed monocrapic senescence (as measured on by leaf yellowing) and plant maturity but did not increase seed yield. The delay in leaf yellowing was greater with BA than with any other chemical tested. Ethrel caused siginificant reduction in seed yield and 100 seed weight through increased leaf abscission. This indicates that the function of leaves did not remain to the seed maturity time and sink was not changed.

Morandi *et al.*[18] observed that DPC reduced the stem and branch length of soybean without reducing the number of nodes. It modified the growth pattern of main stem. DPC also increased the harvest index due to the partition of assimilates favouring its accumulation in the seeds. Seshandrinath and Abba[24] also reported the improvement of reproductive efficiency by chemical manipulation in chickpea cv. L550. Two growth regulators, viz. IAA (2ppm) and BA (5ppm) singly and in combination were tried in chickpea. It was observed that application of IAA at pre-flowering and BA at post-flowering stage gave significant increase over untreated control in the production of total pods (119%), seed yield (137%) and harvest-index (111%). Singh and Kaur[25] observed increase in pod number of plant and yield of mungbean by the application of TIBA, 2,4,5-T, salicylic acid, β-naphthol and Atonik each for 10ppm. Maximum yield was obtained with 10ppm of β-naphthol. Gupta and Singh[10] observed increased number of pods per plant, shelling percentage, pod yield and 100 seed weight of groundnut treated by foliar application of 10ppm of 2,4-D and 40ppm of α-NAA. Similarly, the same results in groundnut treated with some phenolic compound increase in oil and protein content was also observed by Parmar *et al.*[21]

Any exogenous presowing soaking treatment of seeds with growth regulators for enhancing germination and better establishment of the crop canopy can be a milestone in yield improvement. Though the information on presowing soaking treatment of seeds is available, it is only at the germination stage in the laboratories but not at fields. Further the exogenous application of growth regulators was done preor post-flowering stage. Therefore, it is necessary to study the impact of presowing soaking treatment of seeds at the early stage of plant growth and how this impact can be further retained or can trigger the next step of development phase.

This experiment was focused on the fact whether this low concentration of growth regulators can trigger the enhancement effect at the early stage and this vigorous growth can be maintained throughout the growth period thus can result in higher yield. If yield is higher with the treatments, then this labor saving presowing soaking treatment can be practically applicable to soybean culture.

MATERIALS AND METHODS

Seeds: Healthy and uniform seeds of soybean seeds, cv. Bragg were selected and soaked in 10ppm of kinetin, IAA, ethrel and salicylic acid for 6 hours and dried in the shade for 48 hours. Seeds soaked in water were used as the control. Piror to sowing seeds were inoculated with good strain of *Rhizobium japonicum*.

Sowing: Sowing was done on July 5, 1984. The field was prepared after preirrigation and randomized block design with 3 replication was used. Plots consisted of with 50cm row width, 20cm between hills, and one plant per hill. The field was kept free of weeds without the use of chemicals.

Observations: Each of the three replications (blocks) of various treatments were sampled at definite intervals for recording the observations on physiological and biochemical parameters. While some of the samples were analysed fresh, the others were dried in an oven at 60°C and preserved for further analysis.

Seedling emergence: Seedling emergence time was noted when the first seedling emerged and expressed as seedling emergence days after sowing.

Germination percentage: Number of the seedlings established in each treatment was counted. This counted number has been used as the germination percentage.

Plant height: Three representive plants per treatment were selected and tagged for observation of periodical as well as final height. The height was measured with the help of scale in centimeter (cm) from the soil surface to the apex of the plant.

Studies on nodulation: Nodulation(nodule initiation) was noted when the first nodule appeared and expressed as nodule initiation days after sowing.

Determination of chlorophylls: Chlorophyll was determined by the method of Anderson and Boardman.[2] Fresh leaves were collected from the plants at random from each treatment. One hundred mili-gram (100mg) of leaves were ground throughly in 2ml of 80 percent acetone in a motar and pestle. The homogenized material was extracted with 80 percent acetone and centrifuged at 5000rpm. The supernatant containing chlorophyll was kept aside. The residue was again extracted with 80 percent acetone and final volume was made to 10ml with 80 percent acetone. Optical density was read at 645nm and 663nm wave length using spectronic-20.

The amount of chlorophyll was calculated by the following formula:

$$\text{mg Chl.a/g tissue} = (12.7OD_{663} - 2.69OD_{645}) \times V/(1000 \times W)$$

$$\text{mg Chl.b/g tissue} = (22.9OD_{645} - 4.68OD_{663}) \times V/(1000 \times W)$$

$$\text{mg total Chl./g tissue} = (20.2OD_{645} + 8.02OD_{663}) \times V/(1000 \times W)$$

Where, OD_{663} – Optical density at 663nm, OD_{645} – Optical density at 645mm, W – Weight of sample taken and V – Total volume of solution made, respectively.

Determination of nitrate reductase activity: Nitrate reductase activity was measured by the method of Jaworski.[12] Leaves and nodules were sliced with the help of razor blade and 100mg of these slices were seperately incubated at 25°C for 3 hours in 5ml of incubating medium consisting of 2.40ml of 0.2M potassium phosphate buffer (pH 7.5), 1.25ml of 0.02m KNO in phosphate buffer (pH 7.5), 1.25ml of 5% propanol, and 0.10ml of chloramphenicol (0.1mg/ml). The incubation was carried out in tightly sealed vials of 6ml capacity in dark. Nitrate is reduced to nitrite by the action of nitrate reductase which assured at the end of 3 hour incubation period. After incubation 0.4ml of aliquot of reaction mixture was removed from vials and it was mixed with 0.21m of 1% sulphanilamide in 3N HCL and 0.2ml of 0.2% N-naphthylene diamide hydrochloride (NEDH). After 20 minutes, 4ml of deionized water was added to each. tube. The pink color, so developed was measured at 540nm on a spectronic-20.

Flower initiation: Flower initiation was noted when the first flower appeared and expressed as flower initiation days after sowing.

Number of pods per plant: Already tagged plants were used for this observation and expressed as the mean value of pod per plant.

Number of flowers per plant: Number of flowers per plant were counted every other day starting from flowering to harvest by counting the number of flowers per plant that had emerged since the previous count.

Number of seeds per pod: Average number of seeds per pod was calculated by taking the mean value of total number as seeds obtained from 20 pods taken from tagged plants.

100 seed weight: 100 seed weight was taken from each of tagged plants and expressed as the mean value in grams (g).

Pod setting rate: The pod setting rate was calculated by dividing number of pods per plant by the number of flowers per plant and expressed in %.

Yield per plant: Yield per plant was calculated by taking the mean value of total seed weight obtained from 5 plants taken from each treatment.

RESULTS

Seedling emergence: Kinetin and IAA treatment showed hastening effect on seedling emergence but did not have any significance. Whereas SA showed significant negative effect on this (2.7) as against that of control (2.4).

Germination percentage: Kinetin, IAA and SA showed significant lower germination percentage than control. Ethrel also showed lower value than control but it did not show any significance.

Plant height: All the treatments showed enhancement effect on plant height at all the stages of growth as compared with control. But SA did not show significance. All the treatment indicates that enhancement effect in the early stage of growth which is retained throughout the growth thus make the plants have better canopy for photosynthesis.

Table 1. Effect of some growth substances on seedling emergence (days) and germination in soybean.

Treatment	Seedling emergence(days)	Germination (%)
Kinetin	2.3	63
IAA	2.3	65
Ethrel	2.4	67
SA	2.7	60
Control	2.4	69
C.D. at 5%	0.18	2.7

Table 2. Effect of some growth substances on plant height (cm) in soybean.

Treatment	Days after sowing					
	25	40	55	70	85	100
Kinetin	13.4	20.7	40.2	57.3	62.3	62.3
IAA	12.3	24.1	41.5	57.0	62.0	62.0
Ethrel	12.0	21.8	39.6	54.0	57.7	57.7
SA	10.0	19.7	35.2	42.2	48.0	48.0
Control	9.8	18.5	32.4	42.5	44.3	44.3
C.D. at 5%	1.9	2.3	9.2	6.7	3.3	3.3

Nodule initiation and number of nodules: Kinetin showed significant promoting effect but IAA showed significant negative effect on this parameter. This retardation effect by IAA was also coincided with those of Nandawal *et al.*,[19] who reported that in *Pisum sativum*, kinetin resulted in earlier nodule initiation, whereas IAA delayed this process and both the growth regulators brought about considerable increase in fresh and dry weight of nodules. In case of number of nodules, only kinetin showed more value than control.

Chlorophyll content: All the treatments showed an increase in the amount of chlorophyll a, b and total chlorophyll in leaves 25 days after flowering except SA treatment. This high amount of chlrophyll in later stage of seed development indicate higher photosynthetic function of leaves than that of control. Similar report has been obtained

Table 3. Effect of some growth substances on nodule initiation (days) and number of nodules in soybean.

Treatment	Nodule initiation (days)	Number of nodules (Days after flowering)					
		−15	0	15	25	35	45
Kinetin	20	35	36	55	57	45	31
IAA	25	50	31	80	42	40	32
Ethrel	21	27	35	52	60	37	31
SA	22	28	29	65	50	33	28
Control	22	333	25	63	45	35	25
C.D. at 5%	1.8	10.2	8.1	6.3	8.5	7.6	5.4

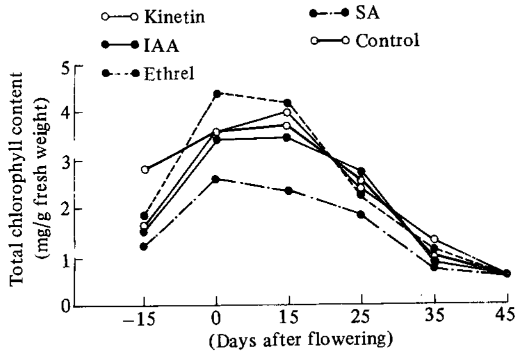

Fig. 1. Effect of some growth substances on chlorophyll content in leaves of soybean.

by El-Tahawi *et al.*,[8] and Yokoyama *et al.*,[31] who observed the increase in chlorophyll a and b in *Phaseolu vulgaris* by application of growth regulators and also the application of growth regulators has been reported to delay the onset and process of senescence in leaves, thus, keeping the leaves functional for longer time.[6]

Nitrate reductase activity: NRA increased upto 25 days after anthesis and sharply decreased upto seed maturity in leaves as well as in nodules. Kinetin, ethrel and IAA showed a significant higher NRA than control throughout the time course of seed development, whereas SA showed tendency similar to that of control.

Floral initiation: All the treatments caused earlier floral initiation than control and SA, IAA and kinetin showed significant effect. SA showed to be the best in hastening the floral initiation process indicating that phenolic substances were effective in changing the plant from vegetative growth to reproductive growth. These results are in line with those of Jensson and Svensson[13] who also reported the suppression of vegetative growth through the

Tble 4. Effect of some growth regulators on floral initiation in soybean.

Treatment	Floral initiation (days)
Kinetin	50.7
IAA	51.0
Ethrel	51.3
SA	50.4
Control	52.3
C.D. at 5%	1.2

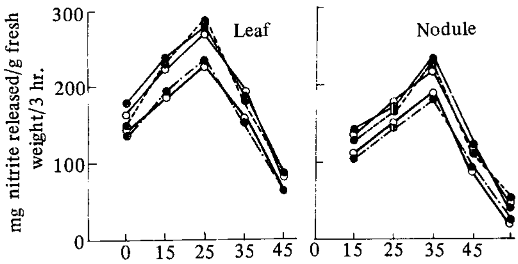

Fig. 2. Effect of some growth substanses on nitrate reductase activity in leaves and nodules of soybean (Abb.: refer to Fig. 1).

phenolics treatment. Phenols may cause enhancement of IAA oxidase activity, uncoupling of respiration and oxidative phospholylation or direct inhibitory effect on various enzymes leading to repression of vegetative growth.[14,27] It was further observed that phenolics promoted floral initiation in various crops.[5,28] This result was further confirmed by that of Zeiher *et al.*[32] who reported that hastening effect on floral initiation lengthened the duration of seed filling and high positive correlation between seed yield and duration of seed filling in soybean.

Variance: The treatment show the significant variance in all the yield components in soybean, whereas the replication does not show any significance.

Table 5. Anaysis of variance in yield components in soybean soaked in some growth regulators.

SV	df	F	P	PSR	SPD	SPL	SW	Yield
Replication	2	10.5	1.1	3.9	0.003	14.7	0.3	0.4
Treatment	5	8813.0*	1112.6*	151.4*	0.028*	9160.6*	4.9*	33.4*
Error	10	237.5	104.3	36.6	0.01	398.5	1.9	1.8

*: significant at 5% level.
Note: F: Number of flowers per plant, SPD: Number of seeds per pod,
P: Number of pods per plant, SPL: Number of seeds per plant,
PSR: Pod setting rate, SW: 100 seeds weight.

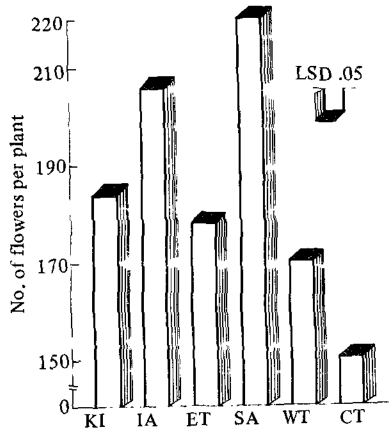

Fig. 3. Variations in number of flowers per plant as influence by various treatments (KI: Kinetin, IA: IAA, ET: Ethrel, SA: Salicylic acid, WT: Water, and CT: Control).

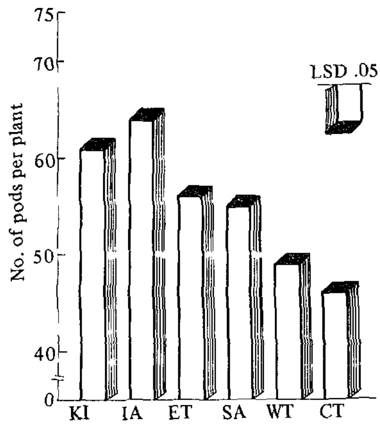

Fig. 4. Variations in number of pods per plant as influenced by various treatments (Abb.: refer to Fig. 1).

Number of flowers per plant: The flower number shows significant increase in all the treatments. Among the treatments, SA shows remarkably the highest value (220) followed by IAA (205) as against 169 in that of control.

Number of pods per plant: The pod number shows significant increase in all the treatments. Among the treatments, IAA showed the highest value (64) followed by kinetin (61) as compared with 49 in that of control.

Pod setting rate: Pod setting rate was not significantly increased in all the treatments except in SA which showed significant decreased effect (25.0) as against 29.2 in that of control.

Number of seeds per pod: Only ethrel showed significant enhancement effect on this parameter (2.80) as compared with that of control (2.68).

Number of seeds per plant: All the treatments showed significant enhancement effect on this parameter. IAA showed the highest value (174.7) as compared with 131.3. in that of control.

100 seed weight: All the treatments showed very significant 100 seed weight, lower than control. Control showed the highest value (11.27).

Seed yield: Seed yield was significantly increased by all the treatments as compared with control. Kinetin showed the highest value (17.8) and IAA (17.7) ranked next to it.

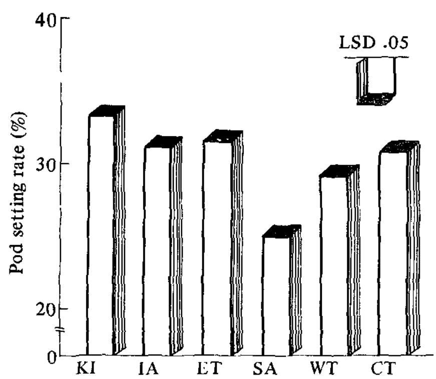

Fig. 5. Variations in pod setting rate (%) as influenced by various treatments (Abb.: refer to Fig. 3).

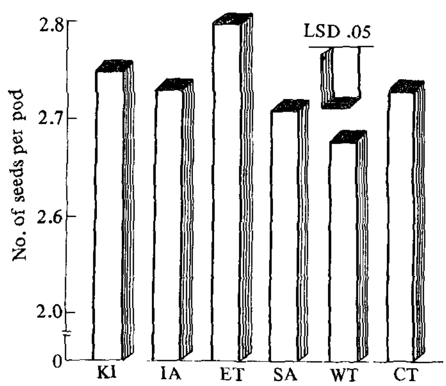

Fig. 6. Variations in number of seed per pod as influenced by various treatments (Abb.: refer to Fig. 3).

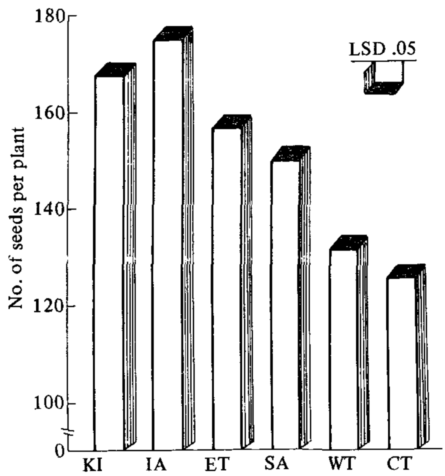

Fig. 7. Variations in number of seed per plant as influenced by various treatments (Abb.: refer to Fig. 3).

Correlation coeffecient: Yield showed highly positive correlation with number of pods per plant and number of seeds per plant. Whereas 100 seed weight showed the negative correlation with yield indicating that increased size and number of sink could not be filled up sufficiently even though size and number of source were increased by the treatment. This means the dilution effect of assimilates.

DISCUSSION

The data showed the effect of presowing soaking treatment with growth regulators on yield, its components and partial correlation coefficient between these components. All the treatments showed

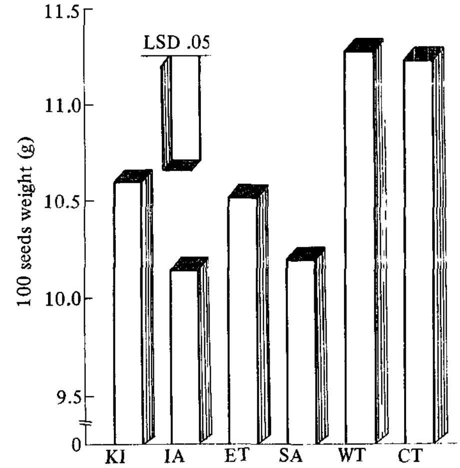

Fig. 8. Variations in 100 seeds weight (g) as influenced by various treatments (Abb.: refer to Fig. 3).

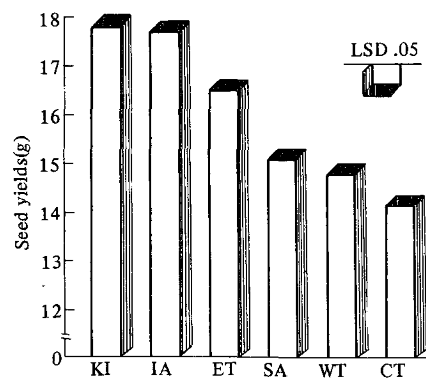

Fig. 9. Variations in seed yields(g) per plant as influenced by various treatments (Abb.: refer to Fig. 3).

Table 6. Partial correlation coefficient between different yield components in soybean soaked in some growth regulators.

	F	P	PSR	SPD	SPL	SW
P	0.67					
PSR	−0.50	0.31				
SPD	−0.03	0.43	0.27			
SPL	0.62*	0.99**	0.36	0.22		
SW	−0.88**	−0.78**	0.17	−0.20	−0.82**	
Yield	0.41	0.95**	0.57	0.23	0.96**	0.63*

**: Significant at 1% level. *: Significant at 5% level.

Note: F: Number of flowers per plant, P: Number of pods per plant, P: Pod, PSR: Pod setting rate, SPD: Number of seeds per pod, SPL: Number of seeds per plant, and SW: 100 seeds weight, respectively.

significant increase of yield by increasing yield components except 100 seeds weight in various ways. Kinetin showed best performance in increasing all the yield components and it was followed by IAA. These results are in agreement with that of Rehaman[23] who reported that in *Cajanus cajan* cytokinins increased the number of sink and size of individual sink which are mainly responsible for increasing the yield Kinetin possibly increases the endogenous level of free IAA and GA (gibberelic acid) which help in seed development, IAA treatment might have increased the endogenous level of GA and auxins, whereas the level of abscisic acid decreased thereby increasing the ratio between growth promoters and inhibitors.[26] Number of flowers per plant in kinetin, IAA and ethrel also increased tremendously over that of control and the number of pods per plant was although more than control but was less pronounced as compared with the increase of number of flowers per plant. Wiebold *et al.*[30] and Carlson *et al.*[4] reported that first formed flowers, and in the top nodes of the plant canopy had maximum pods and their seed weight were highest. Similar result was reported in pigeon pea.[22] Dybing and Lay[6] observed in soybean that the process of senescence was accelerated by the development of pods probably because of competition for assimilates and other nutrients between reproductive and vegetative sinks and growth reulators delayed the onset of senescence process in leaves thus increasing the reproductive efficiency. Whereas Kumas & Nanda,[17] on the other hand reported an increased mobilization of reserve material for vegetative meristems to change into reproductive stage associated with floral induction in *Impatiens balsamian* by SA. In contrast to this, SA showed the lowest pod setting rate resulting from the highest number of flowers per plant among the treatments in the present study.

Although all the treatments showed the enancement effect in yield components but they showed reverse effect in 100 seeds weight. These results are contrary to those of others who reported the increased 100 seeds weight in soybean with DPC[18] and in mungbean with NAA and TIBA.[26] It can be explained that lower 100 seeds weight of treatments than that of control is not direct effect of growth regulators but the capacity of source was not able to cope with the increased size of sink. This result is in line with that of Kwon[16] who reported that even though growth regulator increased the capacity and capability of source in soybean i.e. chlorophyll content, plant canopy, nitrate reductase activity in leaves but could not match the demand from sink. Kokubun and Watanabe[15] also observed this kind of dilution effect in soybean that as the number of pods decreased, 100 seeds weight increased and he concluded that yield was determined by source activity between flowering and early seed development and by sink capacity during seed development. In general, growth regulators have very short effective term ranging from 10 to 25 days. Therefore, it is a little difficult to link the effect of presowing soaking treatment of seeds with growth regulators to the yield which comes at least 100 days after application of these chemicals. But yield can not be determined by only one factor or at on time but by many factors which the plant face during vegetative and reproductive growth. Therefore it is very important to consider the effect of these chemicals at the early stage of growth and development of plants. We have seen that these growth regulators enhanced the growth of early stage thus taking the plants to next stage of growth and development. This vigorous growth established at the seedling stage was maintained throughout the life of plant because of high chlorophyll contents and nitrate reductase activity. These indicate that photosynthetic and nitrogen fixing apparatus are functional during the seed development.[8] Further SA belonging to phenolic compounds and known to be inhibitor to plant growth also showed the possibility to be applicated for soybean culture. Even though SA did not show any signifiant enhancement effect on the growth as compared with the other treatments. But it showed the highest enhancement effect on floral initiation, thereby reducing the vegetative growth period and lengthening the reproductive growth period. This was in line with that of Zeiher *et al.*[32] who also reported that hastening effect on floral initiation lengthened the duration of seed filling and high positive correlation between yield and duration of seed filling period in soybean.

From all these results, the following conclusion was drawn. Low concentration of growth regulators belonging to promoting line (IAA and kinetin) and inhibiting line (SA and ethrel) showed the possibility to be applicated in agriculture by presowing treatment, because of making plants lead to next receptive stage. It seems, therefore, that further study is needed to determine the optimal concentration and its mechanism in enhancement effect. Low 100 seed weight is still indicating that the potential capacity of sink it not a limiting factor of yield but source is a limiting factor. Therefore, it is needed to study in the direction of increasing source in soybean and increasing the reproductive efficiency.

摘 要

본실험 "식물생장조절제의 콩종자처리가 수량 구성요소간의 상관계수에 미치는 영향'은 식물생장조절제의 콩종자처리가 콩의 초기생육에 어떠한 영향을 주며 이러한 초기영향이 생육후기까지의 지속여부 및 수량 구성요소에 미치는 영향을 연구하기 위한 것이다. 사용한 종자는 Bragg로서 10 ppm의 kinetin, ethrel, IAA and salicylic acid에 침지한 후 근류균 인공접종후 파종하였다. 대조구로는 물에 침지한 종자를 사용하였다. 시료는 주기적으로 포장에서 채취하여 초기생장, 근류균형성 생화학적 변화(엽록소함량, Nitrate Reductase활성도)및 수량 구성요소 등을 보았으며 결과는 다음과 같다.

1. kinetin, IAA 및 Ethrel은 초기생육을 촉진시켰으나 페놀계통의 SA는 발아과정을 지연시켰으며 가장 낮은 발아율을 보였음.

2. kinetin, ethrel, SA는 근류형성을 촉진했으나 IAA는 초기근류형성을 다소 지연시켰으나 후기에는 촉진시켰음.

3. kinetin, ethrel, IAA는 엽록소함량 및 N-RA에 유의성 있는 증가효과를 보였으나 SA는 대조구와 비슷한 경향을 보였음.

4. SA는 개화촉진 및 개화수 증가에 가장 효과적이었는데 이는 페놀계통의 화학물질이 작물체의 영양생장에서 생식생장 단계로의 전환을 촉진하고 있음을 보였으며 이러한 생식생장기관의 증대가 수량증대에 기여한 것으로 나타남.

5. 전 처리구에서는 수량 및 수량구성요소(꽃수, 협수, 주당립수)가 유의성 있는 증가를 보였으나 협당립수 및 결협률에는 유의성이 없었음.

6. 수량은 협수($r = 0.962$)와 가장 높은 정의상관관계를 보였으나 100립중과는 부의 상관관계를 보임($r = -0.634$).

7. 100립중의 경우 전 처리구가 대조구보다 낮았는데 이는 식물생장조절제의 처리가 동화생산기관(source) 및 저장기관(sink)을 동시에 증가시켜 수량증가효과를 가져왔으나 증가된 생산기관(source)이 증가된 저장기관(sink)에 동화물질을 충분히 공급할 수 없음에 따른 동화물질 분배상의 희석효과(dilution effect)로 보여짐.

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