Studies on Cool Injury in Bean Plants

VI. Direct and indirect effects of low temperature and levels of nitrogen and phosphorus in the nutrients on the yield components of soybeans*

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In this series of studies, the authors have shown that the damage to yield and yield components, caused by low temperature, was greatly affected by the conditions of N supply in the nutrient solutions in which the soybeans were grown^{1,2)}. Highly significant interactions between N supply and temperatures were obtained in the pod setting rate and fertile seed percentage as described in the fertility percent of ovules. The pod setting rate of the plants subjected to low temperature was closely related to the time of N application. N application before or during the first five days in a 15-day treatment of low temperature before anthesis, caused a significant decrease in the pod setting rate compared with the N free plots, and later application showed less damage. On the other hand, some of the flowers escaped from the adverse effects of low temperature, and flowers on the plants grown under favorable temperature showed high pod setting rate, with high N application. Furthermore, the plants grown under the high N conditions had a prolongation of the flowering period and an increase in the number of flowers.

These facts indicate that the high N conditions would result in two different responses to low temperature: Firstly, direct effects in increasing the damage described above, and secondly, indirect effects leading to better recovery after the end of low temperature stress.

On phosphate nutrition, an experiment has been done with a volcanic ash soil, which was high in the coefficient of phosphate absorbance⁴⁾. The plants grown in the highest rate of phosphate fertilizers used, showed best growth during the low temperature condition and smoothly recovered from the low tem-

perature stress. Highly positive relationships were obtained between the seed yield and stem weight, and between the number of pods and number of nodes on branches. It was apparent that the excellent vegetative growth insures better yield even in the low temperature treated plants. High phosphate application to volcanic ash soils would be effective in reducing the poor-growth type of injury stressed by unfavorable conditions including cool summer weather. To date, however, few studies have been done on the effects of P nutrition under low temperature conditions on bean plants.

Experiments were undertaken to elucidate the effects of P in relation to N nutrition with solution culture, on the pod setting rate and related characters of the plants which were subjected to low temperature. The first experiment was carried out to determine the direct effects of low temperature on the flowers, developed in the most sensitive stage to low temperature, as influenced by the levels of N and P applied. In the second experiment, the potentiality of compensation was compared with the plants which were preconditioned under different levels of N and P supplied, after the complete removal of flowers in a given period.

MATERIALS AND METHODS

In both experiment-I and II, the soybean cultivar 'Kitami-shiro' was planted in seed beds prepared with sand and soil about half to half in volume, in that soil nodulated soybeans had been grown. Three plants were transferred to a 1/5000-a sized pot containing 4 l of nutrient solution, when the unifoliate leaves fully opened. The roots in the nutrient solutions were aerated for 8 hours, in total, under a programmed schedule everyday. After the end of

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treatments on N and P nutrition, all of the plants were kept growing in the same nutrient containing the standard level of N (40 ppm), and P (20 ppm), until harvest time at the green bean stage. Solutions were changed at weekly intervals. All plants, except for the period subjected to low temperature, were grown in a natural light glass house, conditioned at 24°C for 14 hours by day and 10°C for 10 hours at night, of the phytotron. The experiments were carried out in 1972, from May to July, for experiment-I, and from August to October, for experiment-II.

In the first experiment, plants were grown in different levels of N and P for 3 weeks from the 23rd day after planting, as shown in Table 1. Plants were subjected to low temperature, at 15°C day and night, for 15 days in a natural light glass house of the phytotron, from the 23rd day after planting (L-1), or from the 29th day after planting (L-2). Control plants were grown under the 24°C/19°C condition throughout the experiment (fig. 1). Every plot consisted of 3 pots. In order to elucidate the direct effects of low temperature, flowers used for this experiment were allowed to set in a limited period of flowering, for 6 days from the average onset, besides a restriction in number at most 3 on each node of the main stems and 1-node branches, and 2 on each node of 2-node branches. The flowers other than described above were pinched off.

In second experiment, L-3 plants were simultaneously subjected to low temperature and various levels

Table 1 Treatment designations on nutrient and levels of nitrogen and phosphorus supplied, (ppm)—Experiment-I and -II—

Designation	N	P	Designation	N	P
N ₀ P ₀	0	0	N ₁ P ₂	5	20
N ₀ P ₁	0	4	N ₂ P ₀	40	0
N ₀ P ₂	0	20	N ₂ P ₁	40	4
N ₁ P ₀	5	0	N ₂ P ₂	40	20
N ₁ P ₁	5	4			

Note:

Salt used: N; NH₄NO₃
P; NaH₂PO₄

Period of treatments:

Exp.-I; three weeks from 23rd day after planting

Exp.-II; two or three weeks from 23rd day after planting

Fig. 1 Schedule of treatments on temperature and nutrient

of N and P combinations in the nutrient from the 23rd day after planting, and kept in a growing condition under different nutrients for 3 weeks, until 6 days after the end of the low temperature treatment. The corresponding controls were grown in various levels of N and P for 2 weeks, from 23rd day after planting. In order to simulate a heavy shedding of flowers caused by low temperature, flowers on both control and L-3 plants, which opened by 7th day from the average onset, were removed. After the complete removal of the first 7-day-flowers, the potentiality of compensation was compared among the plants preconditioned under different levels of N and P supplied. The plots consisted of 4 pots for L-3 and 3 pots for the control condition.

RESULTS

1) Direct effects of low temperature on the yield components as affected by the levels of N and P supplied

As the flowers on the plants were restricted in number and time of flowering, direct effects of low temperature treatments on the yield components were measured with the characters listed in Table 2. All of the 3 characters listed, were adversely affected by the low temperature treatments. The effects of N levels, temperatures (T) and N×T interaction on the pod setting rate exceeded the 0.1% level of significance. The mean pod setting rate of L-1 and L-2 reduced by 54% and 68% of control respectively. Pod setting rate was decreased with increased levels of N irrespective of P levels in the low temperature treated plots, especially in L-1, whereas no significant

difference was obtained in the control plots (table 3). N_0 plants showed the least damage caused by low temperature. Although the N application tended to increase damage caused by low temperature treatments, the magnitude of damage was closely related to the stage of flower development under low temperature, as seen in N_1 plots of L-1 and L-2. No significant effect was obtained from P application, $P \times N$, and $P \times T$ interaction.

The F values from variance analysis of fertile seed percentage were similar but smaller in comparison to those of the pod setting rate. Low temperature treatments reduced the fertile seed percentage by 77% in average. Fertilizer N significantly reduced fertile seed percentage only in the L-1 condition where the pod setting rate was also greatly decreased (table 4). None of these, the P levels, $P \times N$, and $P \times T$ interaction, were significant.

Although the number of ovules per pod was significantly decreased in the L-1 condition, the magnitude of reduction was smallest in the yield components measured. N supply tended to increase the number of ovules per pod while P did not. No significant interaction was obtained from $N \times T$ and $P \times T$.

The number of fertile seeds produced by a flower can be used for measuring the gross effects of treatments on yield components in the case of flowers restricted in number on each node. Using the number of fertile seeds per flower, the adverse

effects of N associated with time of low temperature treatment and levels of N supplied became more clear than that observed in each character concerned with the low temperature injury (table 5). A high level of N supply, N_2 , caused damage more extensively and severely than the N_1 , a low level of N supply, did. No significant difference was obtained among the levels of P supplied, but higher P application slightly lowered the average in low temperature treated plots.

Table 3 Pod setting rate as influenced by temperatures and levels of nitrogen and phosphorus supplied

Designation	L-1	L-2	Mean	Cont.
N_0P_0	54.7 a	48.4 ab	51.5 ab	66.6
N_0P_1	57.7 a	58.2 a	58.0 a	65.8
N_0P_2	49.2 a	40.9 bc	45.1 bc	65.0
N_1P_0	28.3 b	47.6 ab	38.0 cd	64.3
N_1P_1	28.6 b	43.6 abc	36.1 cde	59.9 N.S.
N_1P_2	29.3 b	49.4 ab	39.4 ab	68.0
N_2P_0	30.1 b	37.1 bc	33.6 def	66.7
N_2P_1	13.5 c	30.0 c	21.8 ef	63.7
N_2P_2	17.5 c	38.1 bc	27.8 ef	59.9
Grand mean	34.3	43.7	39.0	64.4
Mean	N_0 53.9 A	49.2 A	51.6 A	65.8
	N_1 28.7 B	46.9 A	38.0 B	64.1 N.S.
	N_2 20.4 C	35.1 B	27.8 C	63.4
	P_0 37.7	44.4	41.1	65.9
	P_1 33.3 N.S.	43.9 N.S.	38.8 N.S.	63.1 N.S.
	P_2 32.0	42.8	37.4	64.3

Note: Values in the same column, followed by the same letter are not significantly different at the 5% level based on Duncan's new multiple range test.

Table 2 Summarized results of variance analyses for pod setting rate, fertile seed percentage and number of ovules per pod
—Experiment-I—

Source	D. f.	F value Pod setting rate	Fertile seed %	No. ovules /pod
N level	2	28.09***	7.39**	21.16***
P level	2	1.22	—	1.01
$N \times P$	4	2.56	—	7.18***
Temp.	2	91.69***	79.21***	5.75**
$N \times T$	4	10.36***	2.93*	2.27
$P \times T$	4	—	1.96	1.05
Error	62			

Note: *, **, *** Significant at the 5%, 1% and 0.1% respectively

Table 4 Summarized results of fertile seed percentage as influenced by temperatures and levels of nitrogen and phosphorus supplied

Designation	L-1	L-2	Mean	Cont.
N_0	76.4 A	69.2	72.8 A	89.9
N_1	61.3 B	68.7 N.S.	65.0 B	86.5 N.S.
N_2	60.3 B	67.9	64.1 B	86.6
Mean	P_0 68.4	67.4	67.9	85.3
	P_1 64.3 N.S.	73.2 N.S.	68.7 N.S.	88.4 N.S.
	P_2 65.4	65.2	65.3	89.4

Note: Refer to note in Table 3.

Table 5 Summarized results of number of fertile seeds per flower as influenced by temperatures and levels of nitrogen and phosphorus supplied

Designation	L-1	L-2	Mean	Cont.
N ₀	1.046 A	0.853 A	0.950 A	1.563
N ₁	0.477 B	0.889 A	0.683 B	1.544 N.S.
N ₂	0.324 C	0.663 B	0.493 C	1.509
Mean				
P ₀	0.695	0.824	0.759	1.543
P ₁	0.579 N.S.	0.827 N.S.	0.703 N.S.	1.535 N.S.
P ₂	0.573	0.755	0.664	1.539
Grand mean	0.616	0.802	0.709	1.539

Note: 1. Refer to note in Table 3.

2. Cont. > L-2 > L-1, significant at the 5% level.

2) Productivity, after the complete removal of flowers in the early period of flowering, with the plants preconditioned under various levels of N and P combinations

After the complete removal of flowers that opened in the first 7 days, comparison of the productivity or potentiality of compensation was made with subsequent flowers on the plants, which were preconditioned under various levels of N and P combinations for 2 weeks for control plants or for 3 weeks for low temperature treated plants (L-3).

As the plants were harvested at the green bean stage, the number of seeds per pot was used for a character closely related to yield (table 6). F values from variance analysis exceeded the 0.1% level of significance for N and P levels supplied, treatment, and P×T interaction, and the 5% level of significance for N×T interaction (table 7). With higher N and/or P application prior to the onset of flowers tested, the number of seeds considerably increased in both the L-3 and control condition. The N₂P₂ and N₂P₁ plots produced the greatest number of seeds among 9 combinations of N and P levels. However, the rates of increase in seed number with N and/or P application were different between L-3 and corresponding controls. Generally, control plants produced more seeds than L-3 plants did, and the difference between two conditions grew larger with increased levels of N and/or P supply.

Summarized results of yield components are

Table 6 Number of seeds per pot, produced by the flowers opened after the complete removal of flowers in the early period of flowering, when preconditioned under different levels of nitrogen and phosphorus in the nutrients

Designation	L-3	Cont.	Mean
N ₀ P ₀	35.3 d	37.0 d	36.0 f
N ₀ P ₁	41.8 cd	60.8 c	49.4 de
N ₀ P ₂	48.8 bc	59.7 c	53.4 d
N ₁ P ₀	45.3 bc	38.3 d	42.3 ef
N ₁ P ₁	56.5 b	73.0 c	63.6 bc
N ₁ P ₂	50.3 bc	89.0 b	66.9 b
N ₂ P ₀	53.3 bc	59.9 c	56.0 cd
N ₂ P ₁	72.8 a	109.3 a	88.4 a
N ₂ P ₂	80.3 a	105.3 a	91.0 a
Grand mean	53.8	70.2***	
Mean			
N ₀	41.9 C	52.5 C	46.5 C
N ₁	50.7 B	66.8 B	57.6 B
N ₂	68.8 A	91.4 A	78.5 A
P ₀	44.6 B	45.0 B	44.8 B
P ₁	57.0 A	81.1 A	67.3 A
P ₂	59.8 A	84.7 A	70.4 A

Note: 1. Refer to note in Table 3.

2. *** Significant at the 0.1% level.

presented in Table 8. With the exception of ovule number per pod, yield components measured were improved with increased levels of N and/or P. In general, the means of the plots supplied with the highest level of N were significantly higher than the others. On the other hand, the means of the plots supplied with the highest level of P tended to show the highest values, but significant difference from the means of P₁ was obtained only in the pod setting rate of L-3. The interaction of P×T, or N×T was significant in many cases such as, number of seeds, pods and the pod setting rate. As shown in Table 6 and 8, yield components were improved by the application of N and/or P, but the magnitudes of response to the application were different between the two conditions. The plants which continued growing under the favorable condition showed better response to N and/or P application.

DISCUSSION

The previous papers have shown that the pod setting rate and fertile seed percentage were severely

Table 7 Summarized results of variance analyses for yield components —Experiment-II—

Source	D. f.	F value					
		No. seeds	No. pods	No. flowers	Pod setting %	Fertile seed %	No. ovules/pod
N level	2	89.46***	70.30***	9.36**	46.27***	9.83**	1.15
P level	2	66.38***	54.29***	20.86***	21.97***	5.69**	—
N×P	4	3.10*	1.91	6.46**	1.38	1.52	—
Treatment	1	67.38***	11.41**	—	13.59**	41.81***	16.59***
N×T	2	3.05*	1.11	3.86*	6.01**	—	—
P×T	2	16.02***	6.99**	—	4.42*	1.61	2.48
Error	49						

Note: *, **, *** Significant at the 5%, 1% and 0.1% levels respectively

Table 8 Yield components of the plants, after the complete shedding of flowers in the early period of flowering, when preconditioned under various levels of nitrogen and phosphorus in the nutrients

Designation	No. pods/pot		No. flowers/pot		Pod setting rate		Fertile seed percentage		No. voules/pod	
	L-3	Cont.	L-3	Cont.	L-3	Cont.	L-3	Cont.	L-3	Cont.
Mean	N ₀	27.0 C 28.5 C	112.3 B 120.4		23.9 C 23.6 C		72.5 B 79.5 B		2.18 2.30	
	N ₁	31.5 B 35.2 B	117.3 B 117.7 N.S.		26.7 B 29.4 B		72.5 B 81.1 B		2.23 N.S. 2.30 N.S.	
	N ₂	40.1 A 45.3 A	132.7 A 122.9		30.1 A 36.8 A		77.6 A 87.8 A		2.20 2.27	
	P ₀	27.8 B 25.8 B	111.0 B 106.0 B		25.0 B 24.8 B		72.3 78.0 B		2.22 2.24	
	P ₁	34.4 A 40.7 A	127.3 A 129.2 A		26.5 B 31.3 A		74.8 N.S. 85.5 A		2.21 N.S. 2.31 N.S.	
	P ₂	36.4 A 42.6 A	124.1 A 125.8 A		29.2 A 33.7 A		75.5 84.8 A		2.19 2.32	
Grand mean	32.9	36.4**	120.8	120.3	26.9	29.9***	74.2	82.8***	2.21	2.29***

Note: 1. Refer to note in Table 3

2. **, *** Significant at the 1% and 0.1% levels respectively

3. N.S. Not significant

influenced by low temperature during flower development before anthesis when the plants were supplied with fertilizer N in the early period of low temperature treatment^{1,2)}.

These results have been confirmed in experiment-I, in which a significant interaction existed between N×T in both characters. In L-1, only the plots supplied with N significantly lowered the pod setting rate, whereas N₀ plots showed no significant change, in comparison to correspondences in L-2. Among N-supply conditions, the N₁ plots which received only small amounts of N of 20 mg/3 plants/week during low temperature treatment, markedly differentiated the magnitudes of damage observed in L-1 and L-2, and that the former was severely damaged next to N₂ plots and the latter was less damaged to the same extent as the N₀ plots. At the

beginning of the L-1 treatment, the age of flowers used was 11 to 17 days before flowering under control condition, that was six days earlier than L-2. These differences of flowers caused significant change in pod setting rate and fertile seed percentage of L-1 and L-2. These results lead that the most sensitive stage to low temperature might be somewhat earlier as was indicated in the previous paper⁷⁾, and that the sensitivity of floral bud to low temperature might be enhanced with increase in level of N in the plant.

On the P nutrition, no direct effect of levels supplied and P×T interaction, was observed on the yield components measured. It indicates that the P application may not cause any adverse effects in relation to low temperature treatment.

It has been reported that the high potentiality of compensation in soybeans reduced the damage

caused by artificial shedding of flowers or pods^{5,6)}, or caused by cool summer weather in fields note 1) or low temperature treatments in the phytotron^{1,3)}, when the plants could grow under favorable conditions after the stresses. In experiment-II, the increase in levels of N and/or P supply, prior to the onset of the test flowers, promoted the recovery from the damage and produced more seeds per pot through the increase in number of pods, pod setting rate, number of flowers and fertile seed percentage. Differential responses to the N or P application were obtained in the yield components of L-3 and its control. The plants grown under control condition, responded favorably to higher levels of N and P, and produced more seeds. This difference might be due to the residual effects of low temperature treatment on the plants, such as depression of growth and some direct or indirect damage to the flowers used for the experiment. Although the highest level of P application tended to produce the largest number of seeds in average, the difference between P_1 and P_2 was not significant at the 5% level. These small responses at the highest level might be due to the comparatively small requirement of P by plants in comparison to N.

It was confirmed by the different way of experiment conducted in the first paper in this series that N application has two aspects: first, the positive effect promoted the recovery from the stress and secondly, the negative effect enhanced the sensitivity to low temperature during the early stage of flower development.

On the contrary, P application showed no considerable change in yield components in experiment-I where N application increased damage, besides it promoted better recovery from the stress as shown in experiment-II. In general, no particular change in yield components occurred, in the combinations of N and P levels supplied, in both low temperature

treated and control plots.

SUMMARY

1) Experiments were undertaken to elucidate the direct or indirect effect of P and N nutrition with solution culture, on the yield components of soybeans which were subjected to low temperature at 15°C for 15 days from the early-to mid-reproductive stage.

2) In order to determine the direct effects of low temperature on the yield components, flowers were allowed to set only in a limited period of flowering, besides a restriction in number on each node, in the first experiment. Pod setting rate and fertile seed percentage were decreased with increased levels of N irrespective of P levels in the low temperature treated plots. The magnitude of damage was closely related to the stage of flower development under low temperature. No significant effect was obtained from P application, $P \times N$ and $P \times T$ interaction.

3) After the complete removal of the flowers that opened in the first 7 days, comparison of the productivity, or potentiality of compensation was made with subsequent flowers on the plants, which were preconditioned under various levels of N and P combinations for 2 weeks for control plants or for 3 weeks for low temperature treated plots, in the second experiment. The increase in levels of N and/or P which were supplied, prior to the onset of the test flowers, promoted the recovery from the damage and produced more seeds per plant through the increase in number of pods, pod setting rate, number of flowers and fertile seed percentage.

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〔和 文 摘 要〕

豆類の冷害に関する研究

第6報 大豆の収量構成形質におよぼす低温ならびに窒素と磷供給濃度の直接および間接的影響

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大豆の収量構成形質におよぼす低温の直接、間接的影響が窒素と磷供給濃度によつてどのように変わるかを明らかにするため水耕法を用いて2つの実験を行なつた。実験Ⅰは直接的影響を検討するため冷害危険期の花を、一節当り2ないし3個に開花数を制限して供試した。窒素の供給水準を高めると磷供給水準のいかんにかかわらず、低温処理による結莢率ならびに稔実歩合の被害が大きくなつた。被害の程度は、低温下における花の発育時期と密接な関係が認められた。低温処理による直接的な障害の程度は、磷供給濃度によつて差がなく、また磷と窒素供給濃度ならびに磷供給濃度と温度条件といった交互作用も有意でなかつた。

実験Ⅱは、対照区および低温処理区のいずれも開花初期7日間の花を摘花し、その後に開花した花の子実生産が、摘花時までの2ないし3週間にわたる窒素ならびに磷供給濃度によつて影響を受けるかどうかを検討した。窒素および磷のいずれも供給濃度の高い場合に花数の増加や結莢率、稔実歩合の向上で、個体当りの稔実粒数が増加した。このように供給濃度を高めると障害を受けた後の補償能力は大きくなり被害を軽減する可能性が高まることが認められた。