

14. PHYSIOLOGICAL STUDIES OF SOYBEANS IN JAPAN

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Preface

The physiological study of soybeans has a rather short history in Japan and it may be said that regular studies in this field started in or after 1946. Most of these studies have been developed in order to solve the problems which arose in the courses of breeding and cultivation experiments. For instance, researches in germination, flowering, and seed-setting began in relation to experiments on the planting and harvesting time. The photoperiodic response of soybeans and the development of their flower organs also have been studied in this connection. Experiments on the growth and yield of soybeans in diluvial soil grew into researches in soybean physiology relating to soil moisture and then have developed into such studies as the relation between nutrient uptake and inner chemical components, the ripening of seed, and the mechanism of the biosynthesis of chemical components in the seed.

On the other hand, soybeans were grown in the past as an intercrop with others, as wheat, barley, corn or mulberry, and damage caused by shading was a problem to be solved in the cultivation of them. Under the circumstances, experiments on reducing such damage grew into studies on branching and leaf functions and, unifying with experiments on high-yielding and the method of growth analysis, have developed into studies on dry matter production and photosynthesis.

In Japan, soybeans are produced mainly in Hokkaido and Tohoku district, the cold areas where they are often damaged by low temperature and insufficient sunshine. Therefore, physiological studies are also being carried on to prevent the damage.

In this paper an outline of the physiological studies of soybeans in Japan is briefly described together with their courses in the future.

Germination

The temperature suitable for germination of soybean seed was found to be 2–4°C at minimum, 44°C at maximum, and 34–36°C at optimum (Inoue 1953)¹⁴⁾. Many studies have been carried out about the changes of chemical components of soybean seeds with germination, making clear the behaviors of organic acids (Watanabe *et al.* 1967)⁶⁶⁾, amino acids (Kasai *et al.* 1966 a, b, 1967 a, b)^{15, 19–21)}, lipids (Toyosawa *et al.* 1970 a, b, c)^{63–65)} and the 11s and 7s components of protein (Shibasaki *et al.* 1971)⁵⁹⁾ as well as the changes of sugars. Sugars contained in soybean seed before germination are mostly sucrose and stachyose, but sucrose becomes the main sugar with germination, and glucose also increases with it (Matsushita 1967)⁴³⁾. Thus, the process through which reserve substances are used for making new organs in plant body are gradually coming in to light.

When the germinating ability of seed was examined in a soybean variety which reached the maturity of seed about 85 days after flowering, it was observed that immature seeds of soybean plants reaped as early as 35–40 days after flowering could germinate well if they were allowed to ripe as they were in the pods on the harvested plants, and the seeds which were harvested at the time when they had reached the maximum in length and width about 50 days after flowering showed a good germinat-

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ing ability without any such after-ripening. These data suggest that the developmental stage when harvested and dried seeds become to show a fixed ratio of length to width characteristic of the variety may be taken as a criterion for judging their germinating ability (Ozaki *et al.* 1956⁵⁵). It is known that summer soybeans which are harvested under high temperatures in warm districts are low in the viability of seed, but the ability is improved when the harvesting time is delayed by late seeding (Kumamoto *et al.* 1963)³⁵). This appears to be the same phenomenon as the rapid loss of the viability of soybean seeds in the tropics, through there are few reports about this problem after that. In recent years it has been reported in other crops that ethylene has relation to the growth of roots and cotyledons, and the germination of old seeds which generate little ethylene can be promoted by supplying them with ethylene from outside (Takayanagi *et al.* 1971)⁶⁰). This result is considered to show that the low germinating ability of old seed, a symptom of senility, is due to a reduced function of a system relating to the generation of ethylene which acts to stimulate the synthesis of an enzyme system or hormones necessary for germination. As to the germination of soybean seed, it is expected, in the same way, that the problem of low viability will be solved in future not only by the amounts of reserve protein and oil but also as a phenomenon which has relation to enzyme activity or other.

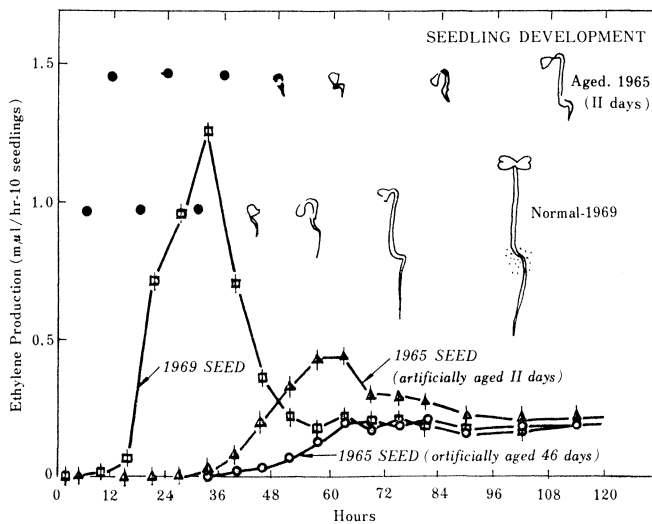


Fig. 1.

Development and shedding of floral organs

In the course of experiments on the cultivation of soybeans, anatomical studies of the development of floral organs and researches in sterility and flower shedding from the developmental viewpoint were carried out to make the causes of these failures clear. In these studies complicated developmental process from the flower-bud differentiation stage to the first trifoliate leaf stage in the seed was examined, and it was shown that the flower shedding was mostly due to the stop of embryonic development after fertilization, the occurrence of which was the highest in frequency at the early stage of proembryo (3–4 days after flowering) followed by the latter stage of proembryo (10–15 days after flowering) and the stage of cotyledon elongation (about 20 days after flowering). This means that there are three important stages when the development

of soybean seed is liable to stop. The stop of the development of seed comes from a competition of the development of floral organs with the growth of others for nutrients and water in the flowering and seed setting stages, and the importance of manuring and field management in these periods has been pointed out (Kato *et al.* 1954 a, b)^{22,23}.

Table 1. Percentage of abortives of seeds in matured pods

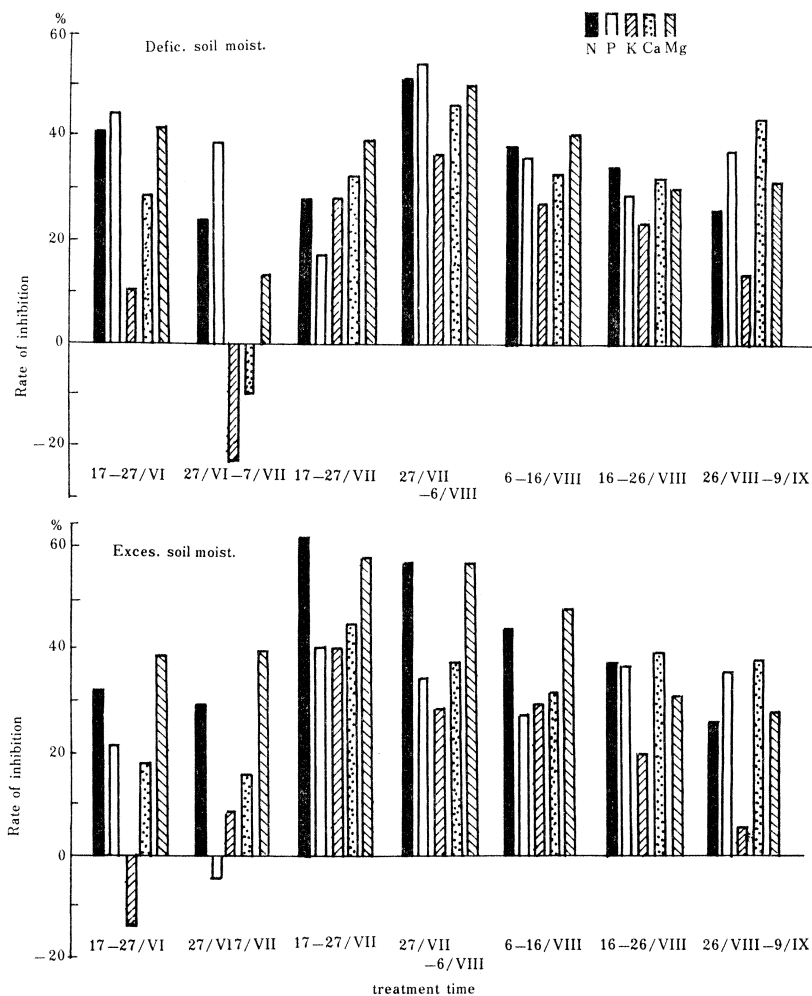
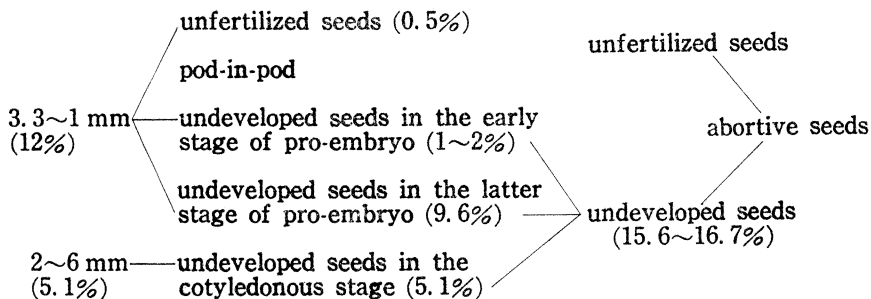


Fig. 2.

Soil Moisture

In Japan there is a large area of fields of volcanogenous diluvial soil where the soil moisture, until a depth of 15 cm, is mainly influenced by rainfall and shows wide fluctuations. Under such conditions soil moisture is considered to be a factor causing unstable production of soybeans, and many studies have been carried out on the growth and yield of soybeans with special reference to soil moisture in order to take measures to meet the situation. According to Fukui (1965)¹⁾, soybeans respond characteristically to soil moisture for each growing stage. Both excess and insufficient soil moistures at early growing stage act to inhibit the vegetable growth of treated soybeans. These treatments at the flowering stage increase shedding of flowers and pods and those at the first half of the ripening stage increase screenings, while the treatments at the latter half of ripening stage suppress the increase of average single seed weight. Such influence of soil moisture soybean is regarded as a result of a reduced absorbing function of roots, because morphological changes of root tissues, a reduced respiratory rate and low exudation activity are observed in these plants. Furthermore, the analysis of inorganic components proved that the absorption of N, Ca, Mg and P was remarkably inhibited in them. And the analysis of organic matter showed that an insufficient soil moisture reduced the photosynthetic activity so as to decrease the amount and percentage of carbohydrate contained in the plants, while an excess of soil moisture inhibited the functions of the systems relating to the use of sugar and starch, inducing an abnormal accumulation of those material in soybean plants.

Based on such experimental results, the so-called deep ditching cultivation method has been devised to stabilize high production of soybeans in diluvial fields. This method is characterized by making deep seed-bed positions, in which a sufficient amount of fertilizer is mixed with soil to grow soybean plants in the positions under less variable conditions of soil moisture and hilling is increased to extend the root zones of them.

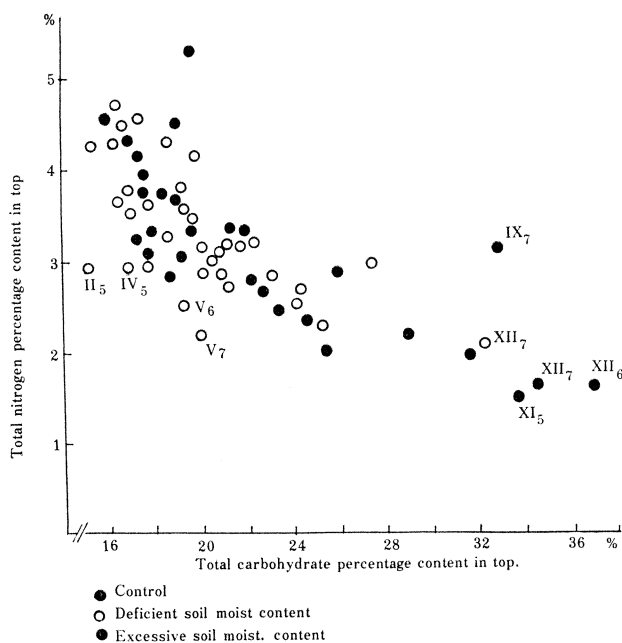


Fig. 3.

Nutriophysiology

Nutriophysiological studies have been carried out in soybeans with relation to manuring technique, nutritional diagnosis, growth, yield, and chemical components of

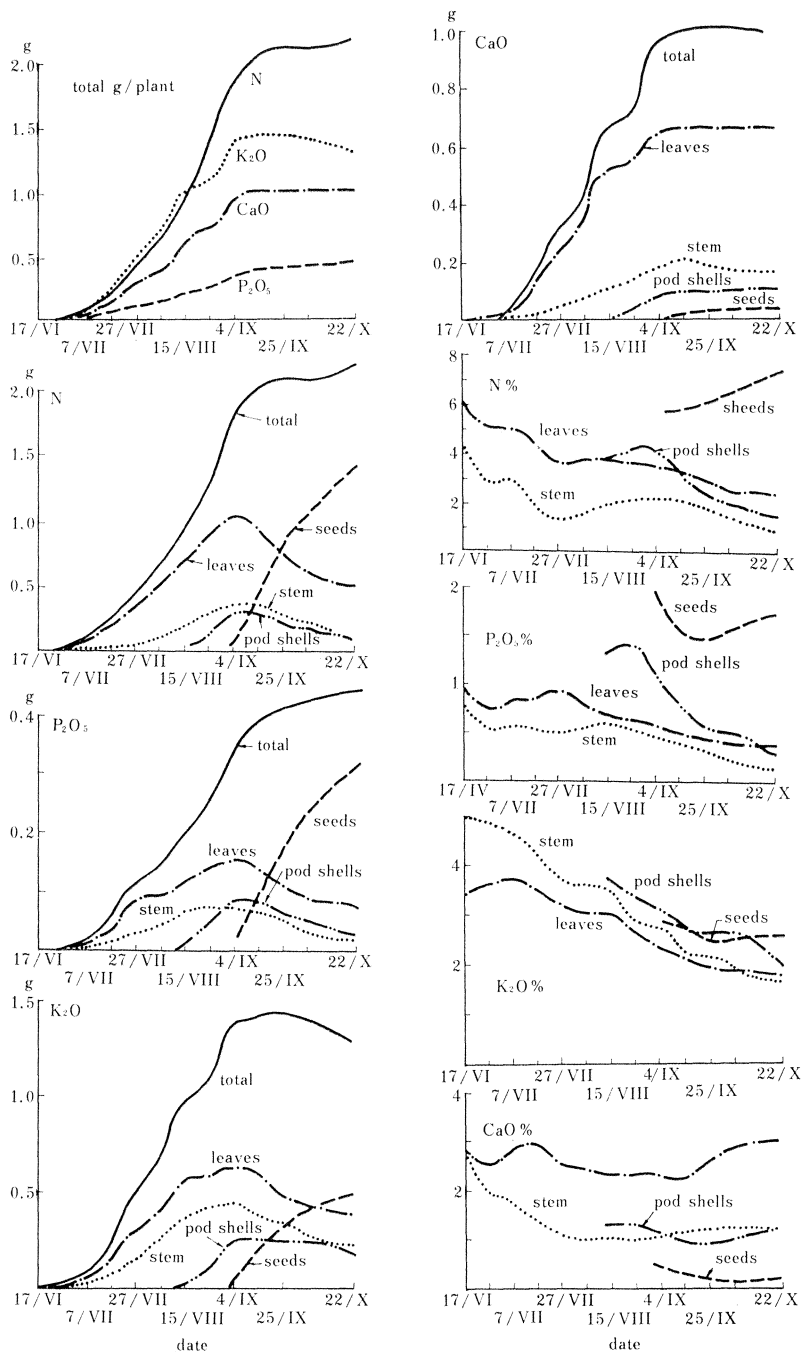


Fig. 4.

seed. According to Hirai (1961, 1962)^{12,13)} and Togari *et al.* (1955)⁶¹⁾, the absorption by soybeans of nutrient elements, as N, P, K and Ca, is rather small in amount until the flowering time, but it increases rapidly after that in accordance with the increase of dry matter in the plants, reaching the maximum at the pod-filling stage, though the absorption of P and N, which will be incorporated mainly into the components of seed, still continues to increase. They also examined the changes of each organ and each component with growth.

Nutritional effects of phosphorus and magnesium have been studied specially by Murayama *et al.* (1957 a, b)^{44,45)} and Hashimoto *et al.* (1953 a, b, 1954, 1955)⁸⁻¹⁰⁾, respectively. According to them, each character of a soybean plant has a time-limit to respond well to the supply of phosphorus as follows: formation of vegetable organ, until an earlier time; weight of pods, until about 2 weeks after the flowering time; size of bean, until a much later time. The oil content of soybean seed increased with the amount of phosphorus supplied. Magnesium was found to be an important element for forming pod shells. But pectin-Ca could be substituted for pectin-Mg, and *vice versa*, and Ca was transferred to pod shells when Mg supply was insufficient. They indicated from these results that the measurement or the acetic acidsoluble or the total Mg and Ca contents of pod shell was useful for nutritional diagnosis for Mg and Ca.

To make clear the part played for seed production by each nutrient element of fertilizer and the relation between the physiological state and seed production in soybeans, Konno (1967, 1969, 1971)^{28,30,31)} made studies by using an automatic irrigation type gravel culture and indicated that the especially important nutrient elements to be supplied for the growth and seed production of soybeans were N, P and K at the pod-filling stage. He considered as follows: these elements influence the growth and seed production in various means, that is; N being materials for protein synthesis, P relating to protein metabolism and Ca relating to translocation of chemical components into pods and seeds or its metabolism. According to him, increase an increased amount of fertilizer is applied to soybeans, a well-balanced increase of nutrient elements is more effective than an increase of a single element.

Table 2. Total amount of absorbed mineral elements during the treatment period (July 13-27)

		Absorbed elements per plant				
		N	P	K	Ca	Mg
C		1,186	204	701	398	187
-N	a	42	77	75	32	36
	b	44	70	58	20	21
-P	a	102	47	78	87	75
	b	102	58	86	99	93
-K	a	94	98	32	114	115
	b	79	80	33	86	91
-Ca	a	105	118	98	76	126
	b	98	118	93	96	119
-Mg	a	80	80	63	111	83
	b	106	115	61	124	103

C: mg; -N~-Mg: relative values of the control (100);
a: treatment with minus element solution;
b: foliar application of the omitted element

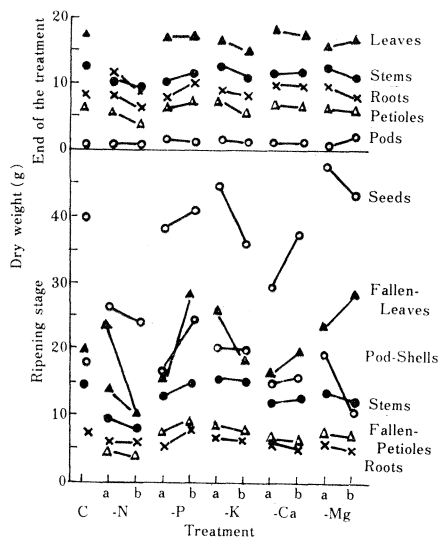


Fig. 5.

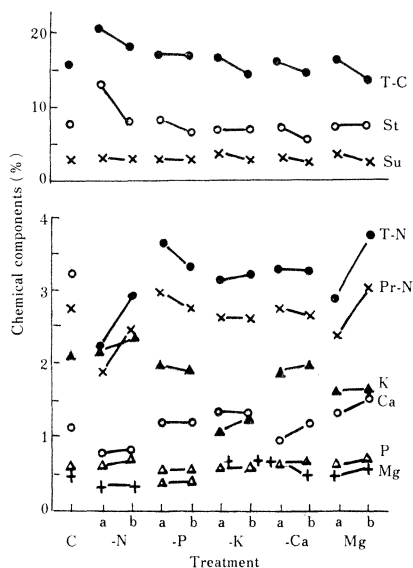


Fig. 6.

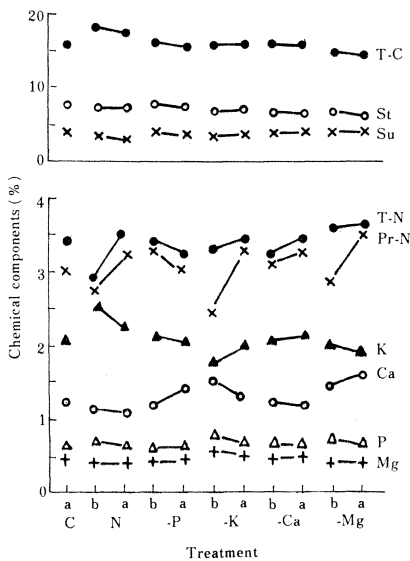


Fig. 7.

As to the effect of the nitrogen fixed by root nodule bacteria and that of combined nitrogen on the growth of soybeans, studies have been carried out in the Hokkaido National Agricultural Experiment Station by using a soybean line free from root nodules and an isolate with the nodules as materials. The results show that there is a positive correlation between the amino-N content of stems and the growth of stems and leaves, that is, when soybeans are supplied with an increased amount of combined N, the stems and leaves increase in amino-N content and grow thickly. On the other hand, considering the distribution of enzymes, allantoin-N appears to be hardly decomposed in stems and leaves and have no relation to vegetative growth. But it is utilized for filling

out seed, and its accumulation increases in stems with the development of root nodules. Therefore, it is estimated that the nitrogen fixed by root nodule bacteria is effectively used for filling out seed through allantoin-N (Kushizaki *et al.* 1964 a, b; Ishizuka *et al.* 1970)^{16,40,41}. According to Hashimoto (1971)⁷, the presence of root nodules has great meaning of the second half of the growing stage when nitrogen requirement of individual soybean plants is the greatest, but at the first half of stage combined N can be substituted for fixed N, and even at the second half of growing stage, the contribution of fixed-N to the development of pods can be large according to the season.

Kato (1966)²⁴ has reported that both import and re-export of P, K, Ca and N take place at the same time in the same leaf in the daytime and the movement of these elements has intimate relation to the presence of growing parts on the top of soybean plant, because the elements cease to move almost completely when growing parts are removed.

In addition to the above-mentioned, studies were carried out in the Kyushu Agricultural Experiment Station on the ideal type of growing process by using gravel culture supplied with different concentrations of nutrient elements, and it was indicated that the increase of the concentrations was effective at the second half of growing stage (Matsumoto *et al.* 1967)⁴². In the Tohoku National Agricultural Experiment Station an artificial culture medium of ion-exchange resin mixed with gravel has been developed as an adsorbent for nutrient elements. This is in use for studying the characteristics of nutrient uptake (Ohba *et al.* 1969)⁵⁰.

Dry matter production and photosynthesis

In the past, soybeans were grown as an intercrop with others, as wheat, barley and mulberry, under shaded condition during the early growing stage. In the Tohoku National Agricultural Experiment Station, Oizumi *et al.* (1955, 1959)^{51,52} studied the mechanism of yield increase in soybeans of intercrop and observed that after the spindly growth caused by shading, the plants showed a growth-stopping stage, regrowing stage and growth-retarding stage in turn. They also noticed that carbohydrate and nitrogen showed characteristic changes in the plants prior to each of these stages. This work developed into studies of the mechanism of branching and the function of leaves, becoming the starting point to studies on dry matter production and photosynthesis.

Studies on branching (Oizumi 1962)⁵³ show that it has regularity and is influenced by sunshine and the amount of available carbohydrate supplied for the axillary buds.

The leaf emergence on the main stem was found to have one or two turning points and regularity, being influenced by the temperature. On a stem the leaves above the 8th or 9th one are different from the lower leaves in regard to their elongation, life, weight and chemical components. The lower leaves are comparatively shorter in life and have intimate relation to the growth of the plant at the early growing stage as well as the development and elongation of branches, while the upper leaves have relation to the filling out of seeds (Oizumi *et al.* 1962)⁵⁴.

Ojima *et al.* (1965)⁴⁸ studied the photosynthetic rate of single leaves. According to them, the photosynthetic rate is low in young single leaves before unfolding, becoming higher with elongation of them. It reaches the maximum after the completion of unfolding and remains on the level for a short period before the beginning of decrease. Changes of crop growth rate and net assimilation rate with growth were also examined in a soybean community of a field, and it was observed that the optimum leaf area index was not fixed, varying with the intensity of light and the nitrogen content of the leaf (Ojima *et al.* 1966)⁴⁹.

Kumura *et al.* (1965, 1968 a, b, 1969)³⁶⁻³⁹ observed that the photosynthetic rate of single leaves increased sharply with the intensity of light up to 15 Klx, then gently

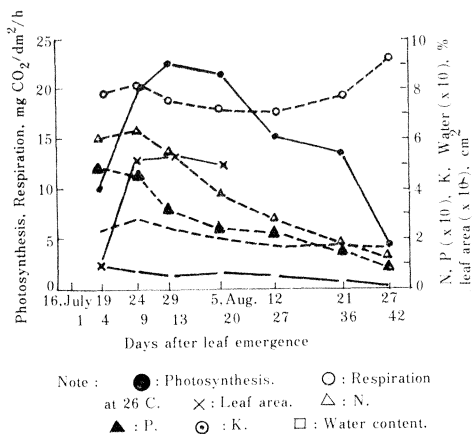
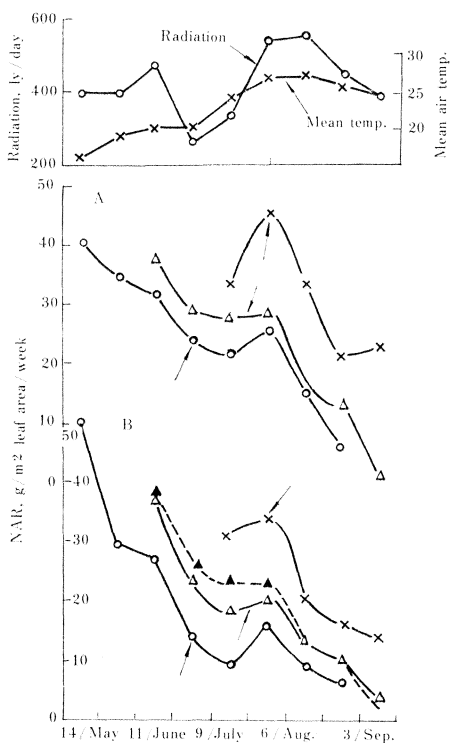
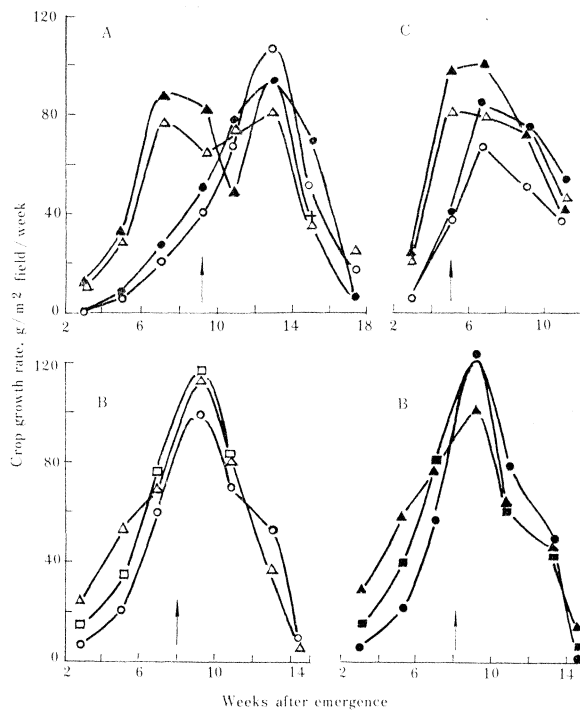


Fig. 8.



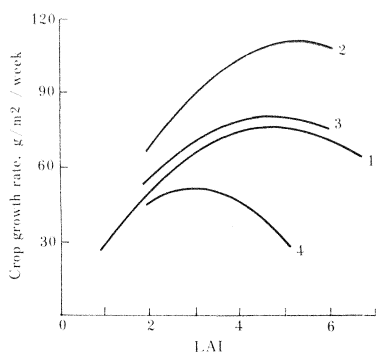
A : 10 plants/m². B : 40/m², but
 ▲ : 20/m². ○ : Early sowing.
 △ : Standard sowing. × : Late sowing.
 Average of light and heavy manuring
 plots. Arrow shows the flowering time.

Fig. 9.



A : Early sowing. B : Standard sowing. C : Late sowing.
 Open symbols : Light manuring. Solid symbols : Heavy manuring.
 ○ : 10 plants/m². □ : 20 plants/m². △ : 40 plants/m².
 Arrow shows the flowering time.

Fig. 10.



- 1 : Vegetative growth stage.
 2 : The period of 3 weeks after the flowering time.
 3 : The period from the 3rd week to the 5th after the flowering time.
 4 : The period from the 5th week to the 7th after the flowering time.

Fig. 11.

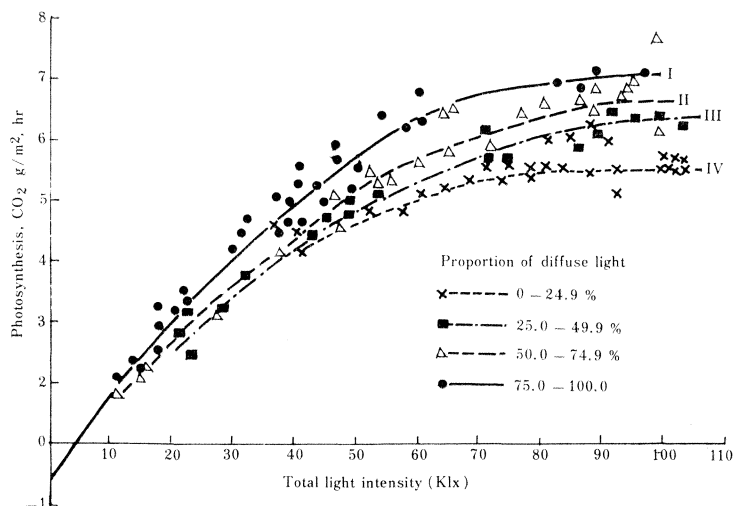


Fig. 12.

up to about 25 Klx, showing a slight or little increase thereafter. They indicated that: the photosynthetic rate in a soybean community increases with the quantity of diffused light, even if the horizontal light intensity is constant; when stems grow under shaded conditions, the leaves become shade leaves which show a very reduced photosynthetic rate under strong light, and their apparent photosynthetic rate under weak light increases; infrared rays penetrate most easily into the leaf canopy, being followed by green light; the photosynthetic rate is the highest under red light followed by green and blue. They also examined the structure and properties of the photosynthetic system of a community.

Kawashima (1969, a, b)^{25,26)} studied the orientation-adjusting movement of leaves in soybeans and found that all the leaves in a community moved so as to receive uniform light intensity on their surface, that is, the movement was useful for dissolving the light saturation phenomenon in the photosynthesis of a community.

Ripening and chemical components of seed

To increase the production of soybean, it is necessary to secure the production of as many beans as possible and to increase the weight of each bean by reducing the shedding of flowers and pods as well as sterility. Therefore, the studies of the ripening process from flowering to maturity were started on the basis of such improvement. In addition, from the viewpoint that soybean seed contain very high concentrations of protein (about 40%) and oil (about 20%) as compared with the seeds of other crops, the studies of ripening have developed into researches in the process of the production of the chemical components in seed to obtain much of these nutritionally important substances efficiently.

Table 3. Seed composition of several crops (dry weight %)

Crop	Crude Protein	Crude oil	Soluble non nitrate	Fiber	Ash
Soybean	42.59	20.46	28.10	4.65	4.20
Rice	10.14	2.54	84.66	1.15	1.50
Wheat	14.45	1.97	78.61	2.89	2.08
Maize	10.6	5.1	80.0	2.7	1.7
Adzuki bean	21.36	1.22	65.18	12.24	
Peanut	20.74	48.09	16.92	2.56	2.69
Rape	21.57	48.34	16.59	9.07	4.42

(Konno, 1968 cited from several literatures)

As to the ripening of soybean seed, the following processes were observed in the first place: a soybean pod elongates rapidly for about 10 days after flowering and reaches its final size in both length and width about 20 days after flowering; the length and width of a seed start to increase a little earlier than the pod reaches the maximum length, and the thickness of both pod and seed begins to increase at about the time when the pod ceases to elongate; these increases in size continue until about the leaf yellowing time, and the seed decreases in size as it dries until full ripening is reached in company with the change in shape from a ellipsoid to a sphere; the increase in dry weight of a seed begins about 3 weeks after flowering and continues to about the leaf yellowing time (Furutani *et al.* 1950³⁾.

Histochemical observations of the changes of reducing sugar, starch, oil and pro-

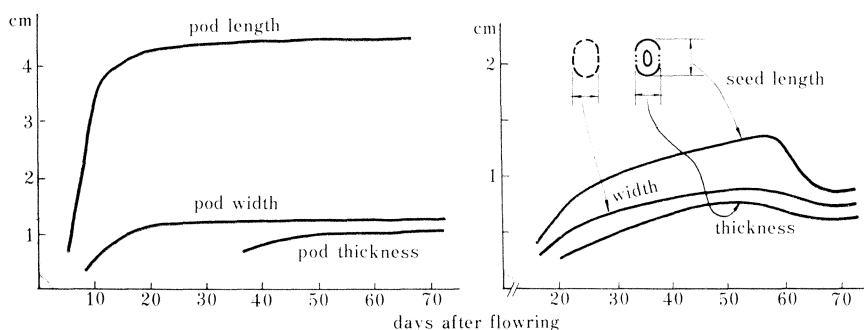


Fig. 13.

tein in the seed coat and cotyledon also have been reported (Kamata 1952 a, b)^{17,18}).

The changes of chemical components in the seed with ripening were studied by Konno (1966, 1969)^{27,29} and others.

The sugars contained in flowers, very young pods and seeds are mostly fructose, glucose and stachyose. Among these sugars almost nothing but sucrose begins to increase at about the beginning of the seed filling stage. Raffinose and stachyose appear at about the leaf yellowing time and increase. The sugar content of the dry matter at the ripening stage amounts to about 8.5 percent, made up as follows: ca. 60% sucrose, ca. 10% raffinose, and ca. 30% stachyose.

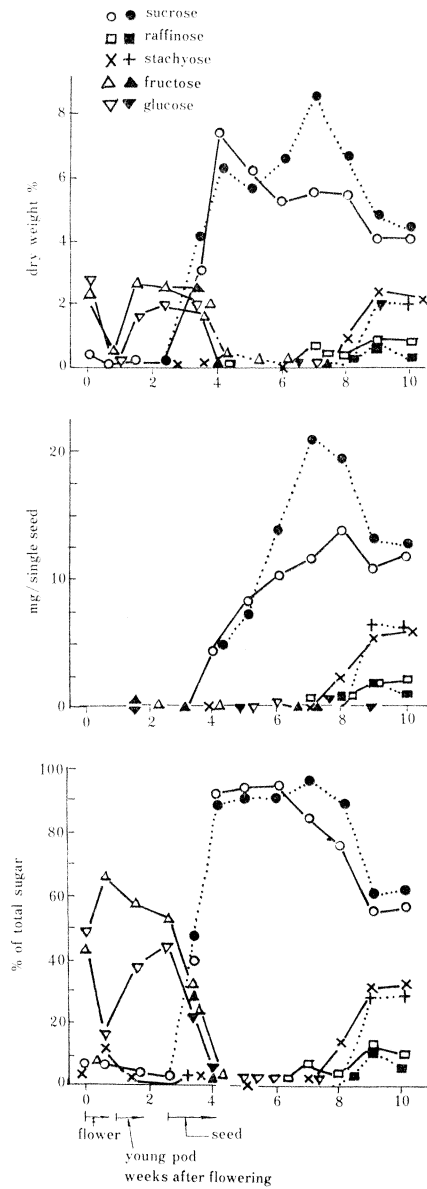


Fig. 14.

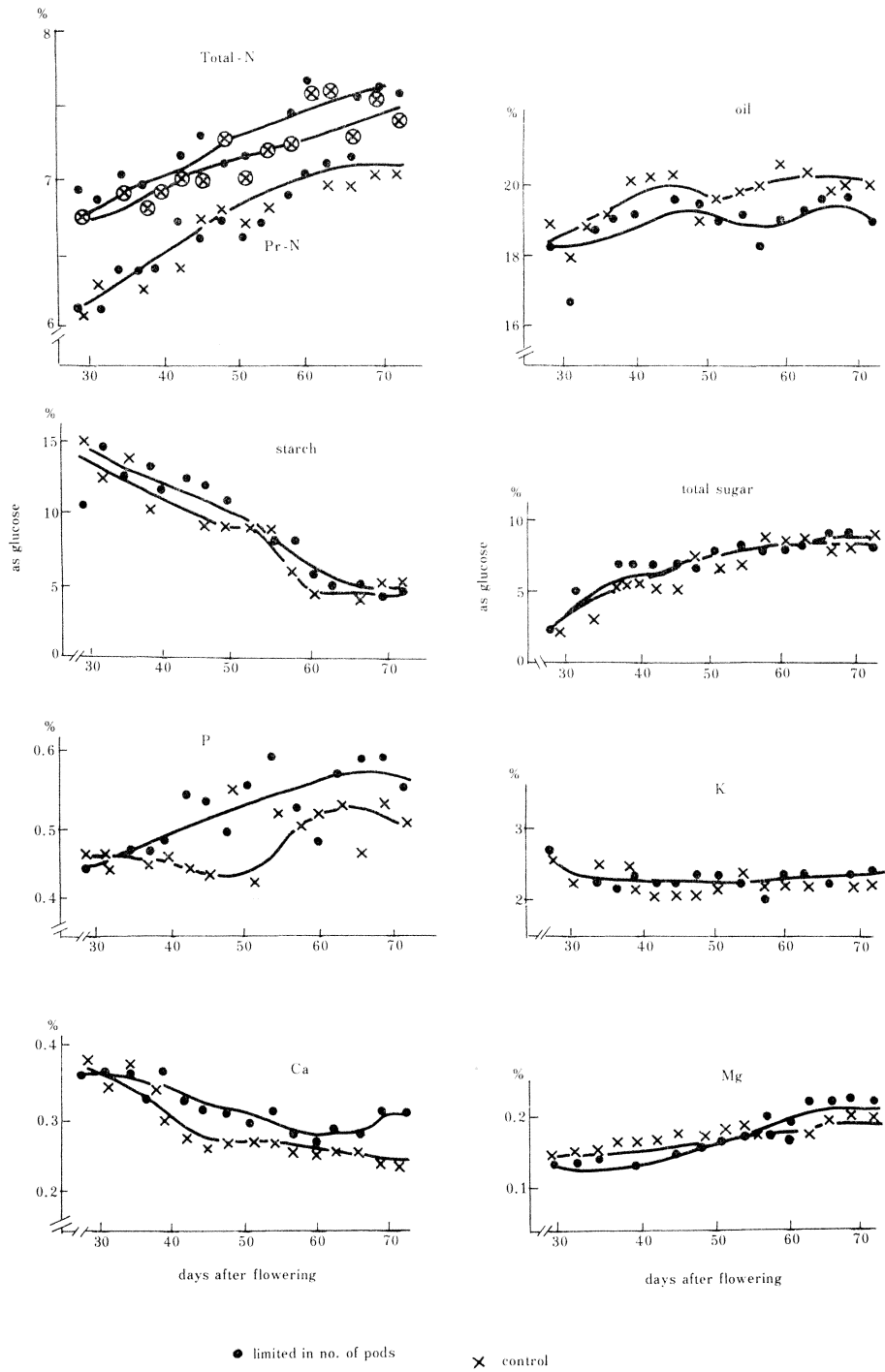


Fig. 15.

Starch is temporarily accumulated in the cotyledon until leaf yellowing time and then decreases, suggesting that it is probably changed into sugar and used for synthesizing other substances. Protein increases in the seed with ripening until after the leaf yellowing time, while oil content is rapidly increases in about two weeks corresponding the first half of the period when seed weight increases rapidly and remains on nearly the same level thereafter, that is, oil increases at nearly the same rate as the increase in seed weight. The water content of seed decreases as the organic matters mentioned above accumulate with ripening: about 90% at the early growing stage, about 60% at the leaf yellowing time, then decreasing rapidly to 20% at the ripening stage. These changes indicate that there is an important turning point in water physiology before ripening. Changes of other inorganic components, organic acid, amino acid and fatty acid were also studied, and, as a result, the processes of the production of chemical components in seed have been made clear to some extent.

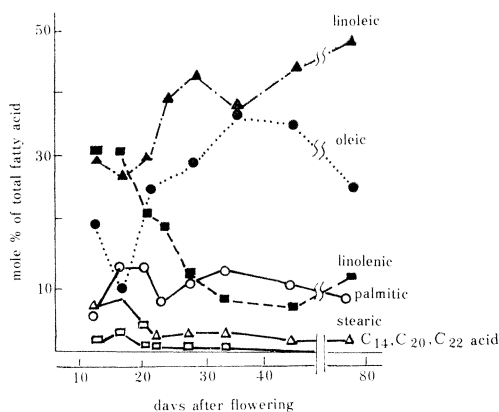


Fig. 16.

The relation between the environment and the chemical components, for instance, an increase in oil content by high temperatures during the ripening stage or by much application of phosphorus has been studied (Nitta 1952, 1953)^{46,47}.

Studies have been made on the structures and properties of the 7s and 11s components of globulin obtained from soybean protein (Kosiyama 1965, 1966, 1967, 1970; Fukushima 1967)^{2,32-34}.

Cool weather damage

In Japan, soybeans are mainly produced in Hokkaido and Tohoku district, the cold areas where the production is often reduced remarkably by the influence of low temperature and insufficient sunshine in the growing period as actually experienced in 1971. Studies of countermeasures for such damage have been done mainly in the Hokkaido National Agricultural Experiment Station.

The number of pods, ovule fertilization percentage and seed weight per plant, which have intimate relation to the yield, are most liable to be influenced by cool weather (15°C and below) in a period from about 15 days before flowering to the flowering stage (Toriyama *et al.* 1957; Saito *et al.* 1961, 1970)^{56,57,62}. This is due to increased failures of fertilization caused by cool weather through poor pollen development, reduced dehiscence ability of anthers, bad pollen dispersion and low pollen germination (Goto *et al.* 1942)⁴. And the damage is greater when soybeans are grown under nitro-

Table 4. Effect of nitrogen application on the soybeans

(Hashimoto, et al. 1970)

Effect of N supply condition on pod setting rate (%)

Effect of N supply condition on fertility per cent of ovules (%)

N supply condition	24°C/19°C condition	Low temperature treated condition	N supply condition	24°C/19°C condition	Low temperature treated condition
5-1	48.9	13.4*	5-1	79.8	50.5*
5-2	53.1	22.4	5-2	85.0	46.8*
5-3	47.4	26.4	5-3	78.9	53.1*
5-4	43.8	17.6*	5-4	74.7	52.3*
5-5	44.2	21.2	5-5	75.1	60.1
5-6	53.8	25.7	5-6	75.9	49.7*
10-1	45.5	14.8*	10-1	69.5	42.9*
10-2	52.2	12.3*	10-2	81.4	50.2*
10-3	41.6	18.6*	10-3	78.2	51.3*
10-4	56.1	32.4	10-4	80.7	55.7
10-5	55.9	25.9	10-5	76.2	49.9*
10-6	53.2	28.1	10-6	79.0	60.7
10-7	43.0	34.7	10-7	74.3	58.6
15	64.7	12.5*	15	75.8	54.9*
0	41.4	32.5	0	69.5	67.0
Average	49.7	22.6	Average	76.9	53.8

* Exceed least significant difference ($P < 0.05$) from the "O" plot in the low temperature treated condition

* Exceed least significant difference ($P < 0.05$) from the "O" plot in the low temperature treated condition

gen-rich conditions in this period (Hashimoto *et al.* 1970 a, b)^{5,6}. But, when soybean plants are subjected to low temperature at earlier time than the above, their recovery from the damage is better under nitrogen-rich conditions. In addition, it was shown that the absorption of water and phosphorus was greatly inhibited by low temperature and cool-weather damage to soybeans could be mitigated by much application of nitrogen, phosphorus and organic matter. The effect is estimated as follows (Satake *et al.* 1969)⁵⁸: the nitrogen-rich conditions due to much application of organic matter is different from similar conditions caused by application of quick-acting manure in a point that the nitrogen is changed into inorganic forms at a rate comparable to the growth of soybean plants and makes the growth of them vigorous; the nitrogen not only increases the number of nodes and flowers but also extends the flowering time so as to be helpful for diversification of risks; when the temperature is low, the organic nitrogen is inhibited to change into inorganic forms so as to reduce the damage caused by too much supply of nitrogen, and when a high temperature is restored, it supply soybeans again with much available nitrogen to promote their recovery from the damage.

It was proved that damage by cool weather to pollen development could be avoided when 1,000 ppm solution of growth regulator B-9 in water was sprayed two times at a distance of 5 days in the period when the damage was liable to occur. The mechanism of the action of the chemical is now under study.

Conclusion

As stated above, physiological studies of soybeans in Japan was started with an intention of increasing the production of bean and have developed into various fields

of plant physiology. But the researchers working now in these fields are not so large in number, about 20 persons in total, though their studies cover many subjects including photosynthesis, dry matter production, nutrient uptake, chemical components of seed, and cool weather damage. We expect that studies on photosynthesis and community structure with special reference to the efficient use of the solar energy will make progress in future together with researches in the nutrient uptake and metabolism, the mechanism of the production of chemical components in the seed and the properties of certain useful substances, for instance, the structure and utility of protein. These research results may be serviceable for the breeding and cultivation of soybeans and for the utilization and processing of the seed.

Discussion

T. Sanbuichi, Japan: It is said that usual wild types of soybean have higher protein contents than the cultivated ones. Dr. Kaizuma and Dr. Fukui reported the same results yesterday in this symposium. We observed many times that these wild types showed very good germination in our experimental fields. Usual native varieties which have high protein contents, in Thailand also showed good germination. Have you any experimental results about the genetic correlation between the protein contents and germinating ability? Or will you give me any information about it?

Answer: Unfortunately, I have not got any further information, now.

(A complementary explanation upon this problem was made at the General Discussion held later. See page 240).

P. P. Kurien, India: During the development of soybean seeds, did you get any evidence of the presence of galactose, mannose or uronic acids as the grain matures? Is this particularly influential in determining the gums and mucilages in legume seeds?

Answer: Compounds combined with galactose (rafinose, stachyose) were found, but neither free galactose nor manose was detected. The others were not investigated.

T. Narikawa, Japan: You have explained that the shedding of flowers or pods is caused by a competition for nutrients and water. I think, however, that the shedding is only a result of compensation of flowers. How do you think about it?

Answer: I have intended to say that the shedding of flowers is caused by the want of nutrients and water which are necessary for the development of flowers that have put forth in abundance simultaneously. It may be said as a "competition" from the outside view-point, but it also may be said as a "compensation" for plant itself. After all, if I might give a guess, I should say that it is rather a regulating or controlling function of the plant.

C. P. Cheng, Republic of China: Please give us the temperature ranges of the cool weather you mentioned in your paper.

Answer: It is below 15°C.

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