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PHYSIOLOGICAL STUDIES ON THE TUBERIZATION OF POTATO PLANTS

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Introduction

Although the potato is one of the most principal vegetable crops in Hokkaido and large amounts of potatoes are transported to the other parts of Japan every year, little attention has been paid to the physiological behavior of potato tubers. The understanding of the mechanism of tuber formation in *Solanum tuberosum* L. is not only a matter of scientific interest from the view point of plant physiology, but also it is practically important in the field of agriculture.

A series of reports of physiological, morphological and biochemical studies on potato plants have been made by TAGAWA et al. from 1948 to 1955 (98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114). The general developmental characteristics of potato plant during its entire life cycle were investigated in considerable detail by these workers.

In spite of these extensive basic research, numerous points remained to be clarified, especially on the specific mechanism of tuberization in potato plant.

It has been generally accepted that the potato tuber is—in the strict sense of the word—morphologically one of the peculiar shape of stem as storage organ which is a rich accumulation in starch.

Furthermore the fact that the tuber formation of this plant was essentially controlled with the variations of various environmental conditions such as the daylength and temperature, indicates that the tuber would be artificially formed at will on any part of the plant except on the root, if the environmental conditions is favorable for the onset of tuberization.

Indeed this concept is supported with a number of evidences established by many investigators. As one of the typical instance of irregular tuber formation, so-called the sprout tubers were readily formed on the sprout immedi-

ately emerged from the senile tubers. Likewise evidence has been shown that the aerial tuber formation being found on the stem nodes of potato plants above the ground was a real common appearance, if the plants were treated with girdling on the basal part of stems in order to retard the downward transport of nutrient substances. Thus it seems safe to conclude that there are virtually numerous cases of tuberization in potato plant.

It would be, therefore, intriguing to know the fundamental principles of tuber formation of potato plant from the physiological point of view.

The present investigation was undertaken in order to study on the most important internal factor concerning the initiation of tuberization under the various conditions, particularly on the substantial variation of growth pattern of the plant and the related organs as stolon to the tuberization.

Before going further, the author wishes to acknowledge his indebtedness to those who have directly or indirectly aided him in his work. His gratitude is first expressed to Prof. Dr. Takashi TAGAWA, Prof. Dr. Keisaku TAGUCHI of Hokkaido University who have guided the author while this project was being carried out and have kindly read the manuscript; and Prof. emeritus Dr. Eikichi SAWADA of Hokkaido University who has kindly arranged the experimental field of potato plant in the Farm belonged this university and encouraged the author during the work. Grateful acknowledgement is also made of the facilities for supplies of experimental materials given by the Directors of Hokkaido Agricultural Experimental Station and Hokkaido Central Foundation Seed Potato Farm.

I Review of literature

In the normal growing phase of potato plant, the stolons emerged exogenously from the nodes of stems under ground on fifth days after sprouting above ground, then simultaneously started to linearly grow until the onset of tuber formation on their tips. After the elongative growth of stolon for about two weeks or less, its tip began to swell followed to form new tuber (69, 130). YASUI postulated a opinion that the cease of elongative growth of stolon resulted in stimulative the commencement of secondary cell division for the sake of tuberization (131).

With regard to the starting time of tuberization of this plant, the similar results have been reported by several investigators (43, 45). NODA (68) and SUGI and ANDO (94) have ecologically studied on the relationship between the tuber and flower bud formations, indicating that there was no direct correlation with these two morphological variations in spite of coincide of starting time

as to the tuber and flower bud formations. According to the earlier investigations conducted by ARTSCHWAGER (4, 5), the morphological characteristics of potato tuber during its developmental stage were outlined.

Recently NODA and YAMAMOTO (69) have histologically studied on the progressive development of stolon and tuber, in turn a particular attention has been paid to an accumulation of starch grains started to occur in the vicinity of endodermis tissue of the stolon tips immediately after the cease of stolon elongation and subsequent ones of starch grains spreaded out all over the tissues of tuber including cortex and pith concomitant with the advance of development of tuber.

With reference to this problem, TAGAWA and OKAZAWA (114) also represented the evidence that the biosynthesis of starch in the tuber was wholly dependent upon the phosphorylase activity by means of not only histochemical detection but quantitative determination of this enzyme. Similar results were also reported by HORI (34). In all respects, the results of these biochemical studies were thoroughly consistent with the NODA's opinion as described above.

In the earlier articles (2, 88), general problems concerning the variations of nutrient substances in the potato tuber during its developmental stage were outlined. In further recent studies on these problems, TAGAWA and OKAZAWA (99, 101, 106, 110), have advanced the views with respect to the metabolisms of various forms of carbohydrates and nitrogenous compounds in the tubers during the developmental stage of them. According to the summarized results of these investigations, it was ascertained that maximal amount of reducing sugar was accumulated in the stolon tips briefly before the initiation of swelling of stolon tips accompanied with attaining to a high activity of their respiration, after which a gradual decrease of sugar content and an increasing accumulation of starch started concomitant with a decline of respiratory activity with the lapse of maturing process of tubers. In conclusion, these might be the most important significance at the initiative stage of tuber formation as regards not only the metabolic pattern of carbohydrates but also morphological variation of stolon tips.

This assumption was thoroughly supported with many further investigators (100, 103, 107, 112). On the other hand, it has been also found the facts that the tops of potato plants play a significant rôle for tuber formation from the view-point of the translocation of nutrient substances (41, 57, 104, 111, 112).

According to the survey conducted by TAGAWA and SAKAI, it may be at least assumed with certainty that potassium in the plant might play a significant rôle for the sake of nitrogen metabolism of potato tuber during its developmental stage.

This is also compatible with the respect of earlier investigations being postulated by MARTIN, BROWN and SPRAGUE (57), and JAMES (41).

While the numerous physiological studies on the development of potato tuber were carried out as described above, virtually no adequate explanation has been given on the direct mechanism of tuber formation.

BERNARD (9) in his classical work suggested that the tuber formation of potato plant is brought about by the symbiotic relationship between the plant and fungus. At present this fungus theory has not been entirely discarded.

On the other hand, an environmental condition such as the daylength was considered to be one of the most reasonable factor for causing to change the growth pattern of potato plant, especially for induction of its tuber formation. The earliest publications with reference to this problem were reported by WERNER (126, 127), who indicated that an excessive accumulation of synthesized carbohydrate in potato plant due to an insufficient supply of nitrogen source and unfavorable growing conditions as short daylength and the low temperature, resulted in spontaneously the initiation of tuber formation. The similar evidences were demonstrated by several investigators (19, 61, 91).

With respect to the varietal differences of potato plant in induction to the tuberization many investigators have already advanced the view that the late varieties of potato plant are generally more sensitive to the short daylength in order to form tuber than early varieties (77, 93, 94, 95). There also accumulated several reports as to the tuberization of wild species of potato (17, 25, 116). With regard to the influence of environmental condition on the tuber formation, it led us to general conclusion that tuber formation of potato plant is retarded by the long day condition contrary to the stimulation by the short daylength.

In recent years, GREGORY (23) made survey on the tuber forming stimulus as the internal factor for the tuberization, who reported that this stimulus was formed in the top of potato plant growing under the short day condition at the low night temperature (17°C).

CHAPMAN (13) has further advanced the view that this stimulus was formed only in the upper young leaves of potato plant being subjected to over fourteen cycles of short day treatment and transferred downwardly to stimulate the tuber formation on the stolon tip, irrespective of the insufficient supply of carbohydrate for the sake of starch synthesis in the formed tuber. In addition, he also pointed out that the stimulus moved down only vertical direction. In Japan, ITO and KATO (38) has also represented the evidence concerning the occurrence of similar stimulus in the etiolated potato shoots, they assumed that this seems to be some kind of auxin-like substance.

According to the late investigation by MADEC and PERENNEC (56), the

raw sap extracted from tuber-induced potato plant was injected into the basal internode of the non-induced cutting and it was successfully induced artificial tuberization on the cutting. It must not be overlooked that there seems to be substantially an existence of the tuber forming substance in the sap of induced plant, notwithstanding that the chemical natures of this substance still remain obscure. Unfortunately there is no further development of the study on the stimulus, especially the physiological characteristic of stimulus remains to be clarified.

On the other hand, there are considerably many investigations concerning the irregular tuber formation of potato plant, namely, the formations of aerial tuber, secondary growth tuber and sprout tuber. Usually the aerial tuber is formed on the node of stem of potato plant above the ground, being attributable to the block of downward movement of the nutrient substances through the stem by means of girdling and injury by some fungus infection or some other treatment (12, 16, 86, 121, 124, 128).

According to the most reasonable assumption as to the inductive mechanism of secondary growth tuber formation, when the developing tuber under the ground was subjected to the severe dry condition for a considerable long duration, the development of tuber temporarily ceased. Afterward the sufficient supply of water followed to be given to the soil with the considerable high temperature on account for development of tuber. The tubers resulted to grow again irregularly to form so-called secondary growth tuber (4, 35, 62, 95, 119). Therefore it seems to be considered that the moisture and temperature in the soil might be the most important factor for causing to form the secondary growth tuber (11, 117, 118). TAGAWA (97) and NAKA (65, 66) have studied on the carbohydrates variations in the secondary growth tuber and informed that the metabolism of reserved carbohydrates in the tuber was disturbed by the variation of soil condition and followed to change the developmental pattern of tuber.

It is also real common to find the potato tubers with very irregular shape of sprout such as the sprout tuber, due to the senility of tuber during storage being kept for a long duration over their rest period (20, 92, 123, 124, 125).

According to the opinion represented by TAGAWA and NAKA (101), it seems reasonable to assume that the formation of sprout tuber might be due to changes of the reserved substances in the mother tuber and of the respiration rate of the sprout and tuber according to their storage condition. Furthermore there were also accumulated several investigations on the appropriate environmental conditions for the sprout tuber formation (44, 81, 82, 83, 84, 85).

In spite of the extensive researches with reference to the tuberization of potato, numerous points remains to be clarified, especially on the physiological and biochemical mechanism of tuberization.

II Tuber formation in sterile culture

According to the experimental results of extensive studies by authors, the variations of carbohydrates and nitrogenous compounds as nutrient sources seem to play an important rôle for tuber formation of potato plants. In addition, it is generally recognized that tubers are ready to form in any part of potato stem tissues when they were subjected to a favorable condition for induction of tuberization (127, 128). Many investigators have studied on sterile culture of potato tissues, namely on callus formation of tuber parenchymatous tissue and tuberization on stem tissues (6, 13, 14, 28, 59).

Therefore the main purpose of the present experiment is concerned with the effects of environmental conditions on the tuber formation of potato stem segment in sterile culture.

Materials and methods:

Potato tubers, var. Irish Cobbler, were used as materials for sterile culture. These tubers were soaked in Usuplun (Methoxyethylmercury chloride) solution for twenty minutes in order to perform the surface sterilization, then followed to plant in the moist sand in dark room at 23–27°C. After about three weeks, the etiolated shoots grew up about 25 cm in length. Stem segments including a node taken from the basal part of etiolated shoots were used for culture throughout the experiments, except where otherwise noted.

Sterilization of stem segments..... The various kinds of chemicals were used as the germicides for sterilization of stem tissues. The cuttings of potato plants were commonly contaminated by bacterias and fungi, not only on the surface of stems but inside of ones.

As is shown in Table 1, one per cent mercuric chloride solution was generally the most effective germicide for the sterilization of seeds, however was highly toxic for living tissue. Accordingly it was not recommended for sterilization of potato stem tissue. Conversely "Antiformin" solution (sodium hypochlorite solution) was the most favorite ones for potato tissues because of less toxicity. Therefore the sterilization was held with 5% Antiformin solution supplemented with 0.001% Tween-20 throughout all experiments. After the sterilization, stem segments were washed five times by sterilized distilled water, followed by soaking in sterilized water in order to remove the germicide. All these procedures were carried out in the sterile chamber illuminated by the

TABLE 1. Effects of various germicides on the sterilization of potato stem segments

Germicide ¹⁾	Time of incubation (min)	Rate of contamination (%)		Toxity of germicide ²⁾
		10-day old culture	20-day old culture	
0,1 % Mercuric chloride	5	75	90	±
	15	58	71	++
	30	23	30	+++
10 % Calcium hypochlorite	5	72	81	—
	30	61	72	—
	60	53	60	—
	90	31	42	+
20 % Antiformin ³⁾	10	72	91	±
	30	65	85	+
	90	40	62	++
	120	23	38	++
10 % Antiformin	10	72	89	—
	30	61	74	—
	90	18	31	±
	120	15	29	+
5 % Antiformin	30	28	36	—
	60	9	12	—
	90	9	9	—
	120	6	7	±
75% Ethanol +5% Antiformin ⁴⁾	90	5	5	—
5% Antiformin with 0.02% Tween 20	90	7	8	—
0.2 % Uspulun ⁵⁾	30	78	100	—
	60	65	90	—

- 1) All germicides are water solution.
- 2) Toxity grades of germicides mean as follows; — none, ± little slightly + slightly, ++ severe, +++ highly severe.
- 3) Common name of sodium hypochlorite solution.
- 4) Stem segments were incubated in 75% ethanol for 2 sec., and subsequently in 5% Antiformin solution for 90 min.
- 5) Commercial name of methoxyethylmercuric chloride.

ultraviolet light.

Nutrient medium... The basic nutrient medium consisted of water, mineral salts, trace elements and sucrose. The water which was used throughout the experiments, had previously been redistilled in hard glass equipment to eliminate any toxic substances. The mineral salts and trace elements which were added to the nutrient medium according to NITSCH (67) are follows:

Mineral salts solution :

Ca (NO ₃) ₂ ·4H ₂ O	500 mg
KNO ₃	125
MgSO ₄ ·7H ₂ O	125
KH ₂ PO ₂	125
FeC ₆ O ₅ H ₇ ·5H ₂ O	10
Water up to	1000 ml

The trace element mixture had the following composition :

H ₂ SO ₄ (sp. gr. 1.83)	0.5 ml
MnSO ₄ ·4H ₂ O	3000 mg
ZnSO ₄ ·7H ₂ O	500
H ₃ BO ₃	500
CuSO ₄ ·5H ₂ O	25
Na ₂ MoO ₄ ·2H ₂ O	25
Water up to	1000 ml

In summary : one liter of the basic medium contained the mineral salt solution, 1 ml of the trace element mixture and sucrose. The basic medium was adjusted to an initial pH 6.0 by the addition of 0.2 mol NaOH or 0.2 mol HCl.

The agar which had been used to solidify the culture medium, washed carefully for about five days with the distilled water to wash out any trace impurity such as auxin, and then added to the nutrient medium to make 0.5% in final concentration. Twenty ml of medium was poured into each test tube (25 × 150 mm) used as culture container. They were autoclaved for 20 minutes at 15 lb. pressure.

Experimental Results ;

(1) Effect of sucrose concentration in the medium on tuber formation :

As pointed out previously in the earlier articles (104, 107), it has good reason to assume that the tuber formation of potato might be attributed to an excessive accumulation of carbohydrate in the tips of stolons or the senile sprouts. It seems of interest, therefore, to evaluate with respect to the effect

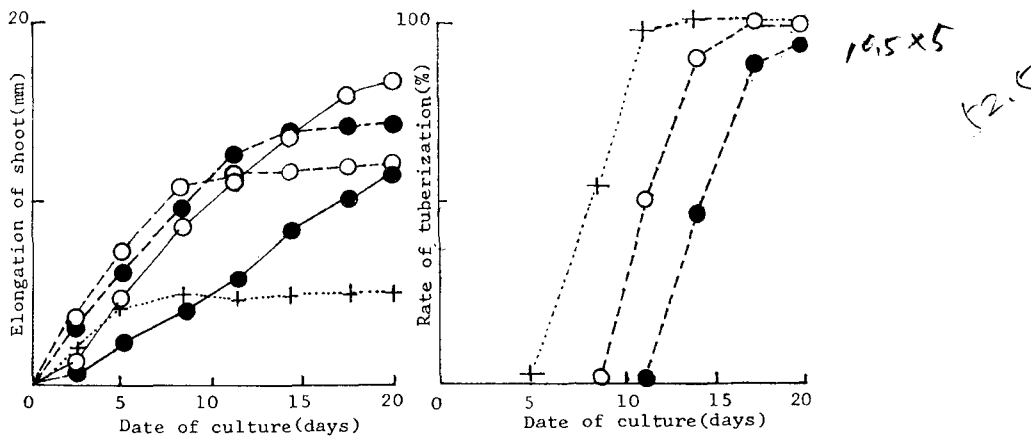


Figure 1. Effect of sucrose concentration in the nutrient medium on the shoot elongation and tuberization of stem segment.
 Sucrose concentration; ●—● 2%, ○—○ 4%, ●—● 6%, ○—○ 8%, +---+ 10%.

of sucrose concentration in the nutrient medium upon the tuber formation of stem segment.

From an inspection of the data showing in Figure 1 and Plate 1, it is readily seen that the frequency of tuberization on the stem segment was progressively enhanced with increasing the sucrose concentration of medium. This fact indicated with certainty that the tuber formation might be presumably

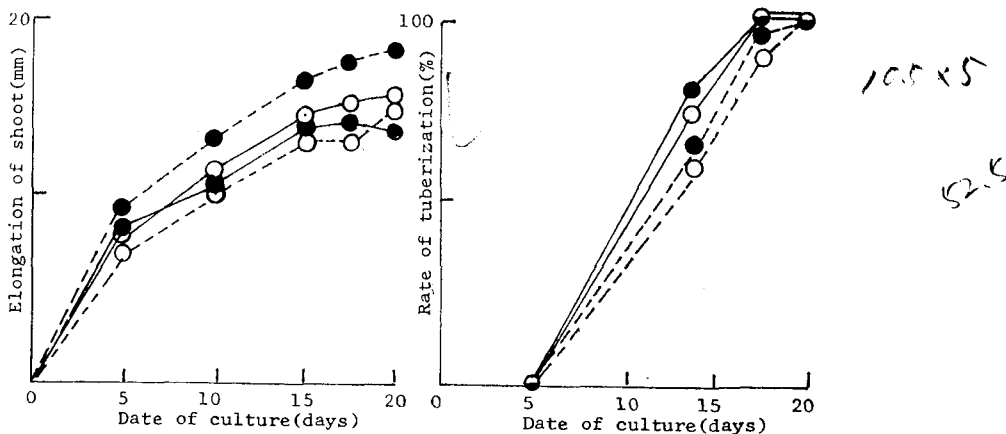


Figure 2. Effect of the various sugars as carbohydrate source in the nutrient medium on the shoot elongation and tuberization of stem segment.
 ●—● sucrose, ○—○ glucose, ●--● fructose, ○--○ maltose.

ascribed to excessive supply of sugar to the stem segment, so far as the present case of culture using the induced stem as materials.

(2) Effect of several kinds of sugars on tuberization :

It has come to be generally accepted as a fact that there are many kinds of soluble sugars in the potato plant as glucose, fructose and sucrose. It would be, therefore, intriguing to know the availability of these sugars for the initiation of tuberization of stem segment. As the experimental result was represented graphically in Figure 2, there was virtually no significant difference between effect of these sugars in the medium upon the onset of tuberization.

(3) Effect of environmental temperature on tuberization :

In the present culture, eight per cent of sucrose in final concentration was supplied to the basic medium, and the stem segments were cultured at three different temperatures of 12°, 23° and 27°C.

As is seen in Figure 3, the tuber formation on the stem segments cultured at 23°C started a little earlier than those cultured at other temperatures. In the culture at 12°C, the emergence of a lateral shoot on the stem segment was somewhat delayed by the swelling of shoot due to the raising of relative humidity in the culture container. At 27°C culture, the linear growth of lateral shoot emerged from the stem segment was enhanced slightly which in turn caused a retardation of tuberization.

It has come to be generally accepted as a fact that the most favorable

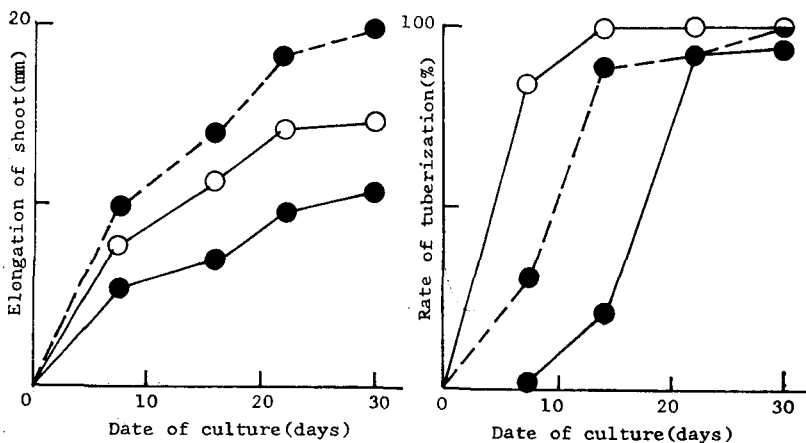


Figure 3. Effect of cultural temperature on the shoot elongation and tuberization of stem segment.

Temperature; ●—● 12±1°C, ○—○ 23±1°C, ●--● 27±1°C.

temperature for the normal development of potato plant, particularly for the onset of tuberization, might be at about 18°C. However culturing the stem segments at two different temperatures of 18°C and 26.5°C, Mes and MENGE (59) found no difference on the mode of tuber formation between the two.

So far as the present culture experiments show, the temperature of 27°C seems to be too high for the tuber formation on the stem segment. At the same time, there remains little reason to doubt the assumption that the culture temperature of 12°C is too low for the development of the new tuber. The observations made in the above experiments lead one to the conclusion that the optimum temperature for the tuber formation on the stem segment may be at around 20°C.

(4) Effect of the hydrogen ion concentration in the nutrient medium on the tuber formation:

As one of the most important environmental factors in sterile culture, the influence of pH value of the nutrient medium on the growth of stem segment was studied. The stem segments were cultured on four portions of the basic nutrient medium with supplement of 8% sucrose, of which pH value were adjusted at 4.0, 5.0, 6.0 and 7.0 respectively.

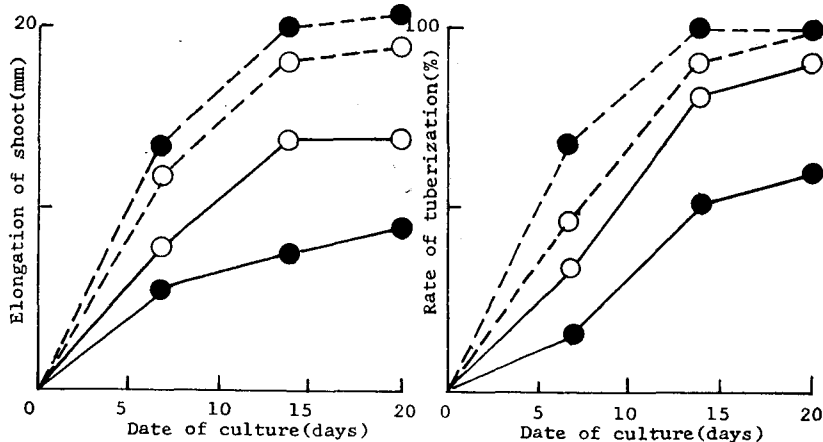


Figure 4. Effect of pH variation of the nutrient medium on the shoot elongation and tuberization of stem segment.
 pH value; ●—● 4.0, ○—○ 5.0, ●—● 6.0, ○—○ 7.0.

As is shown in Figure 4, the optimal pH value for the development of lateral shoot and tuber formation on the stem segments is in the neighborhood of pH 6.0. However, on the culture medium at pH 4.0, not only the distinct inhibition of growth of lateral shoot, but also a retardation of onset of

tuberization were observed.

In the previous paper (98, 114), the writers clearly established that the enzymes, such as amylase and phosphorylase, which are responsible for the carbohydrate metabolism in potato plants, would play an important rôle in the new tuber formation. Likewise evidences have accumulated to show that the optimal pH value of these enzymes ranges between pH 6.0 and 7.0 inclusively (3, 58, 114). Accordingly, it seems reasonable to assume that the variation of pH in the medium may itself control the tuberization of stem segments not directly but indirectly being correlated with the nutritional metabolism of stem segments.

- (5) Effect of various ratios of carbohydrate to nitrogen in the medium on the tuber formation :

According to the results stated previously, the onset of tuberization on the stem segments is correlated with the increase of sugar content in the medium. In the case of abnormal tuber formation, particularly on the sprout tuber, a marked accumulation of carbohydrate in the tip of sprout in a form of starch was recognized. At the same time, the soluble nitrogen content also decreased (102, 104). In the present experiment showing in Table 2, the apical tips of etiolated shoot were rich in carbohydrate, but the basal portion were poor.

TABLE 2. Carbohydrate and nitrogen contents in the various parts of the stem segment in the etiolated shoots.

	stem segment			
	1st node	2nd node	3rd node	4th node
Water content (%)	99.4	96.4	95.5	95.2
Total carbohydrate content (mg)	317.6	337.1	353.1	367.1
Total nitrogen content (mg)	43.9	36.4	31.7	27.9
Ratio of carbohydrate to nitrogen content	7.3	8.2	11.1	13.1

(mg/Dry wt. gm)

The nitrogen content in the etiolated shoots was inversely related to the carbohydrate contents. By comparing the ratios of carbohydrate to nitrogen in the tissues as shown in Table 2, it is apparent that the apical tips which do not form usually the tuber on the nutrient medium, showed a low value of this ratio and, on the contrary, the basal parts of stem segments which are

formed tuber easily, have a higher value of this ratio.

From an inspection of this data of Table 2, the ratio of carbohydrate to nitrogen in the stem segments seems to be highly correlated to the tuber formation. In the next experiment, the culture tests were carried out under varied combinations of two different sugar concentration and three nitrogen ones.

The tuber formation on the stem segments is at least partly, if not entirely, controlled by the ratios of carbohydrate to nitrogen contents in the medium (Figure 5). [Namely, the time of initiation of tuber formation became earlier

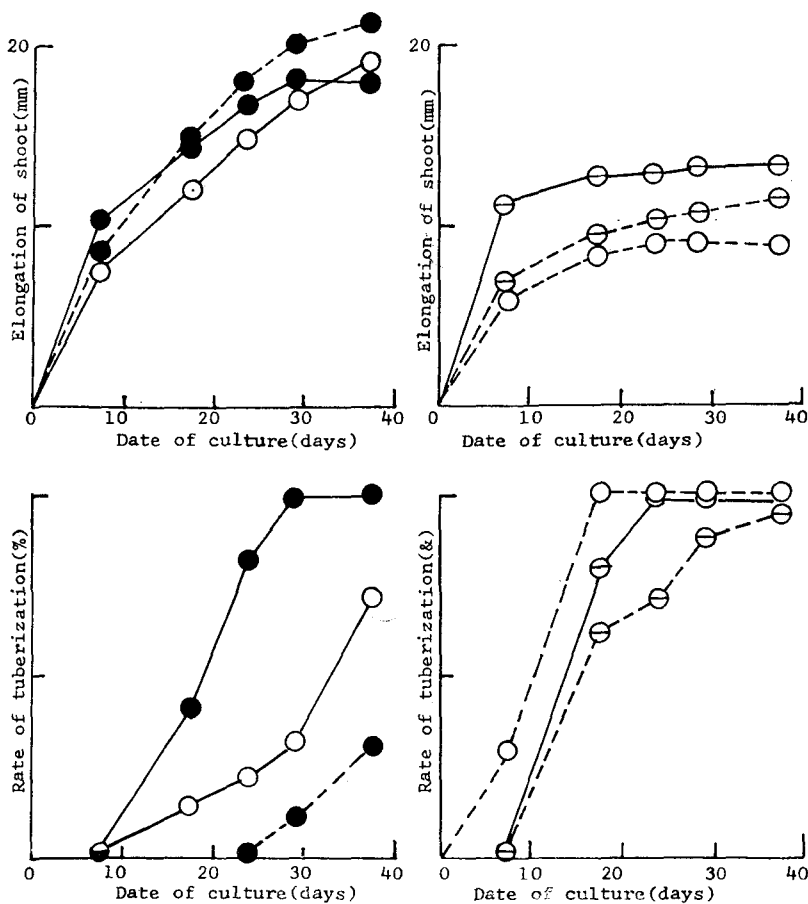


Figure 5. Effect of the variation of ratios between sugar and nitrogen contents in the nutrient medium on the shoot elongation and tuberization.

- sucrose 2%+nitrogen 0 ppm, ○--○ sucrose 8%+nitrogen 0 ppm.
- sucrose 2%+nitrogen 50 ppm, ⊖--⊖ sucrose 8%+nitrogen 50 ppm.
- sucrose 2%+nitrogen 250 ppm, ⊖--⊖ sucrose 8%+nitrogen 250 ppm.

with raising of this ratio. At the later stage of the culture, however, it is hard to find any close correlation between the tuberization and this ratio.

(6) Effect of the senescence of mother tubers on the tuber formation:

In order to obtain the further information on the effect of senescence of mother tuber upon the tuberization, the present cultures were devised. Apical tips of sprout cutting from the tuber immediately after sprouting and the basal stem segments which were obtained from the etiolated shoot of 25 cm in length were cultured on the nutrient medium individually. Although the basal stem segments of etiolated shoot became faster to form tuber, concomitant with an increase of sugar concentration in the medium, conversely apical pieces of sprouts resulted no tuber formation, regardless of any increment of sucrose concentration in the medium (Table 3).

TABLE 3. Difference of initiation of tuberization between apical pieces of sprout and basal stem segment of shoot

Materials	Sucrose conc. of nutrient medium (%)					
	2	4	6	8	10	12
Basal stem segments	-	±	+	+	+	+
Apical pieces of sprouts	-	-	-	-	-	-

(+) means tuber formation and (-) no tuber formation.

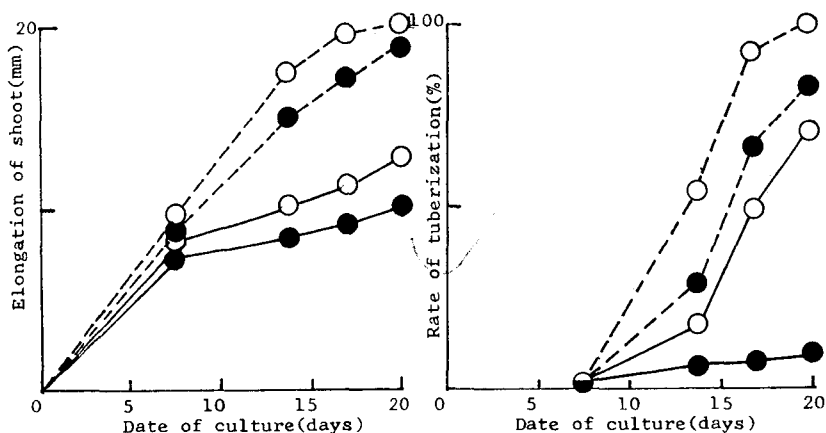


Figure 6. The shoot elongation and tuber formation of stem segments with the different nodes of same main shoot.

●—● first node, ○—○ second node, ●--● third node, ○--○ fourth node.

10.5 x 5
52.5

Regard to culture of stem segment obtained from various part of the etiolated shoots, the tuber forming activity was inversely related to increasing distance from the tips to the each node (Figure 6 and 7).

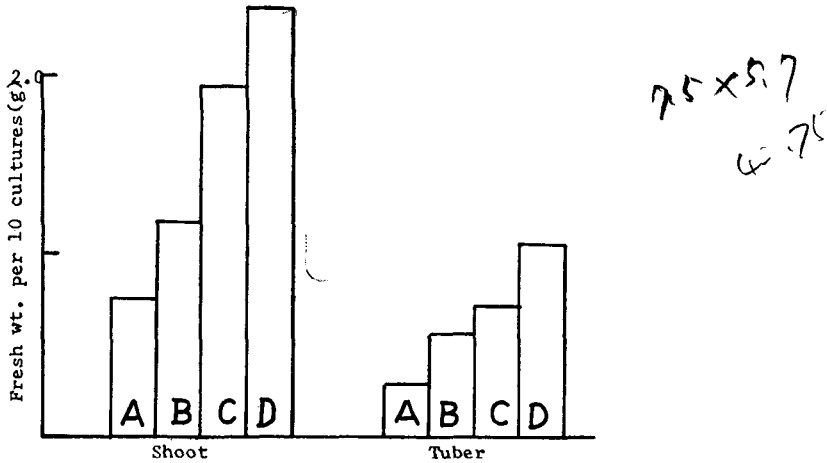


Figure 7. Fresh weights of shoots and tubers which were formed on each stem segment with different node of main shoot. (20-day old culture).
 A=first node, B=second node, C=third node, D=fourth node.

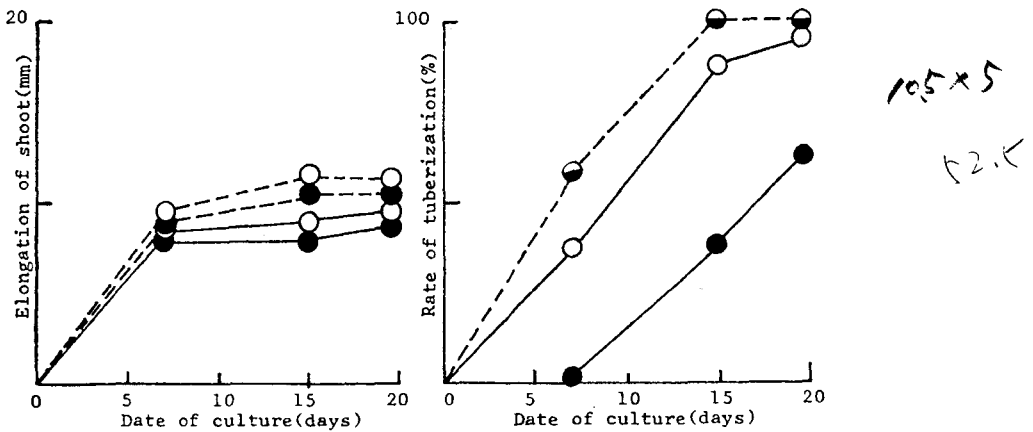


Figure 8. The shoot elongation and tuber formation of stem segments of main shoot which grew from senile tuber.
 ●—● first node, ○—○ second node, ●—● third node, ○—○ fourth node.

The tuber formation on the stem segments of the sprout of the senile tuber stored in a cellar for about six months, was considerably easier and more rapid than that on the stem segments obtained from young tuber just after the termination of rest period (Figure 7 and 8). In other words, the tuber formation on the apical piece of shoot is very difficult regardless of the raising of sugar concentration in the medium.

This fact may be attributable to the difference in their physiological ages between each stem segments of the same shoot. The senescence of tuber may also have an influence on tuber formation.

(7) Effect of the exogenous growth substances on tuberization :

According to an inspection of the previous data, it must not be overlooked that the physiological age of potato tuber might play rather more important rôle on the initiation of tuberization, than the carbon nutrient sources as sugar supplied to the medium exogenously. This assumption seems to be imply the possibility, therefore, that the changes of endogenous growth substances due to the advance of senescence of tuber during the storage period may be closely associated with the onset of tuberization.

Several investigators reported on the enhancement of potato tuberization by means of the treatments with α -naphthaleneacetic acid (NAA) or 2, 4-dichlorophenoxyacetic acid (2, 4-D) (38, 83). At the same time, it would be also expected some influence of gibberellin on its tuberization, based on the views of previous evidences (37, 50).

It is required to elucidate the effect of the growth substances on tuberization of stem pieces which are obtained from the shoot at the different physiological age.

a) Effect of NAA

Nutrient mediums containing 0.05, 0.1 and 0.5 mg/l of NAA respectively were used for culture of stem pieces. As showing in the result of culture with apical tips of sprouts, a retardation of shoot elongation and an excellent yield of tuber were observed in response to appropriately high NAA concentration in the medium (Figure 9). A tentative interpretation of this fact is proposed. It is based on the assumption that the auxin may serve as an artificial stimulant of tuber formation for even the non-induced potato plant, if it would be applied appreciably at the high concentration which is sufficient for the inhibition of straight growth of potato shoot. On the other hand, no significant difference in the appearance of tuberization on the induced stem segments was illustrated depend on the variation of NAA concentration in the medium (Figure 10).

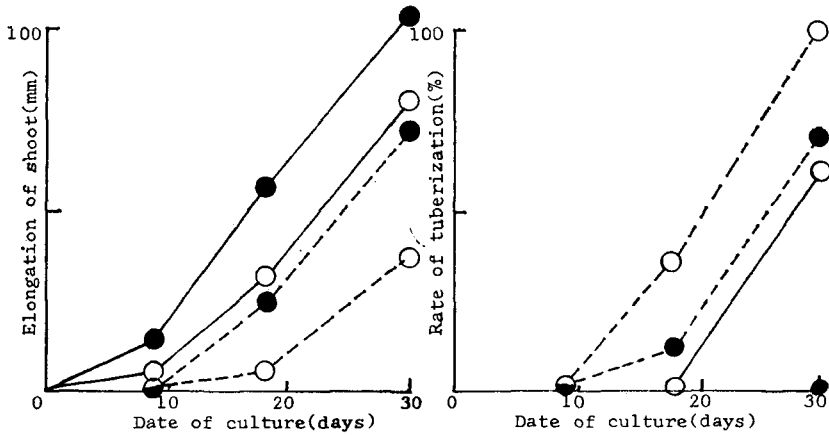


Figure 9. Effect of NAA application to the nutrient medium on the shoot elongation and tuberization of apical piece of sprout. Conc. of NAA; ●—● 0 ppm, ○—○ 0.05 ppm, ●—● 0.1 ppm, ○—○ 0.5 ppm.

10.5 x 5
52.5

10.5 x 5
52.5

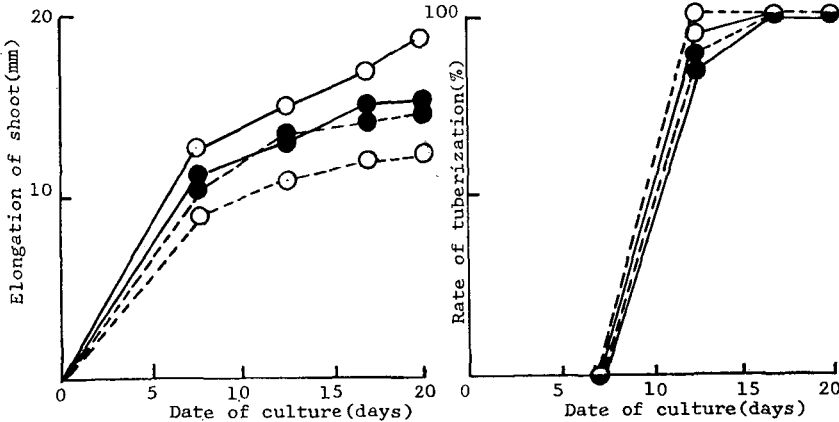


Figure 10. Effect of NAA application to the medium on the shoot elongation and tuberization of the stem segment cut from the basal part of main shoot. Conc. of NAA; ●—● 0 ppm, ○—○ 0.05 ppm, ●—● 0.1 ppm, ○—○ 0.5 ppm.

b) Effect of 2, 4-D

In this experiment, the effect of 2, 4-D on the tuberization of stem segments was investigated. The concentrations of 2, 4-D were 0.01, 0.1, 1.0 and 10.0 mg/l in the medium respectively. An optimal concentration of 2, 4-D for excellent development of the lateral shoot emerged from stem segment was at

0.1 mg/l. At above this concentration, namely at 1.0 and 10.0 mg/l, the linear growth of shoot was markedly retarded (Figure 11, 12 and 13 and Plate II). Especially at the concentration of 10 mg/l, it was always followed by substantial tuber formation (Plate II-B). Accordingly regard to the tuber formation of

1.05 x 10.5
5.7.7K

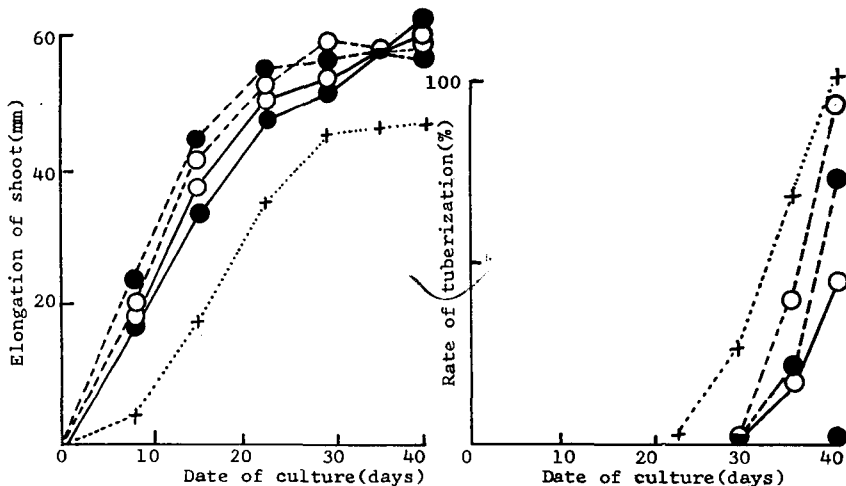


Figure 11. Effect of 2, 4-D application to the medium on the shoot elongation and tuberization of apical piece of sprout.
Conc. of 2, 4-D; ●—● 0 ppm, ○—○ 0.01 ppm, ●—● 0.1 ppm, ○—○ 1.0 ppm, +··+ 10.0 ppm.

5 x 10.5
5.2.7K

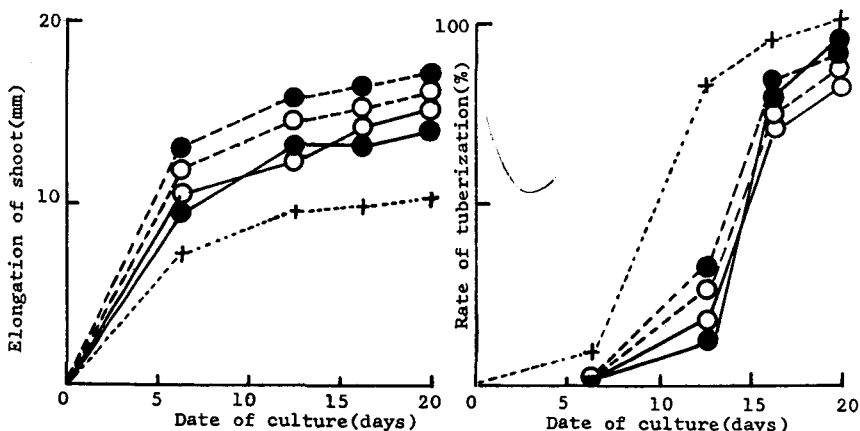


Figure 12. Effect of 2, 4-D application to the medium on the shoot elongation and tuberization of the stem segment cut from the basal part of main shoot.
Conc. of 2, 4-D; ●—● 0 ppm, ○—○ 0.01 ppm, ●—● 0.1 ppm, ○—○ 1.0 ppm, +··+ 10.0 ppm.

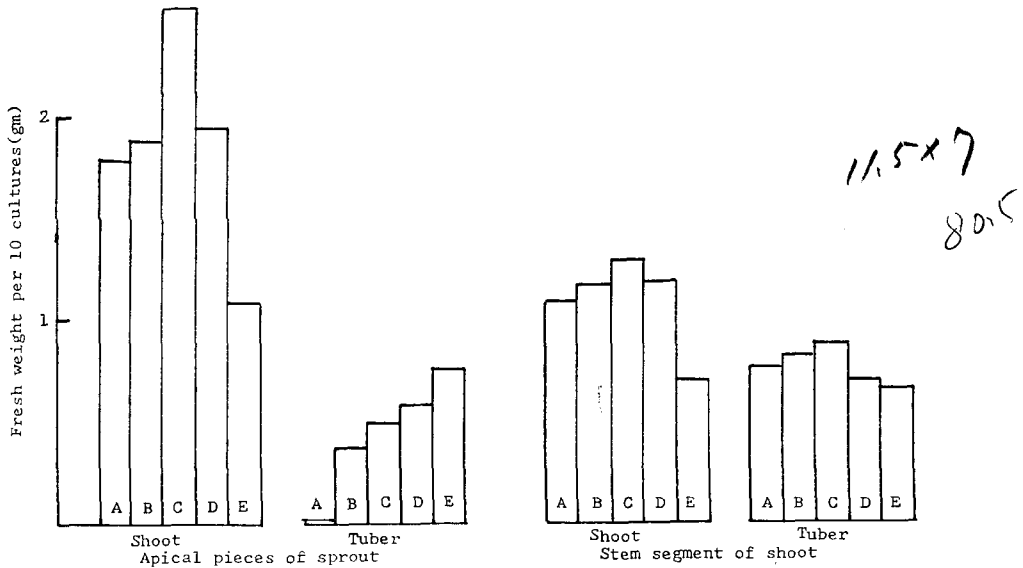


Figure 13. Variation of fresh weights of shoots and tubers with application of 2, 4-D (40-day old culture).
 Conc. of 2, 4-D; A=0 ppm, B=0.01 ppm, C=0.1 ppm, D=1.0 ppm, E=10.0 ppm.

stem segment, the sensitivity and the magnitude of the response to auxin were somewhat different due to the variation of auxins, but the effect of auxin on their tuberization might be substantially similar as expectation.

c) Effect of gibberellin

It should be pointed out that the addition of gibberellin significantly increased the linear growth rate of lateral shoot. On the contrary, an inhibition of tuberization on the stem segment was resulted in the medium with addition of gibberellin, and its inhibitory activity is intensified by raising the gibberellin concentration (Figure 14, 15 and 16, and Plate III). The evidence presented in this experiment indicates that the application of gibberellin to medium exerted a detrimental effect on the initiation of tuberization on the induced stem segment.

From the results of these experiments, it was confirmed that the induction to the tuber formation of potato stem segments was progressively shifted with an advance of physiological age of plant. In other words, the non-tuber induced tissues such as the young sprouts and apical tips of shoots were not successful to form tuber even under the most favorable nutritional condition. At the case of the external application of excessive high concentration of auxins,

5 x 7
75

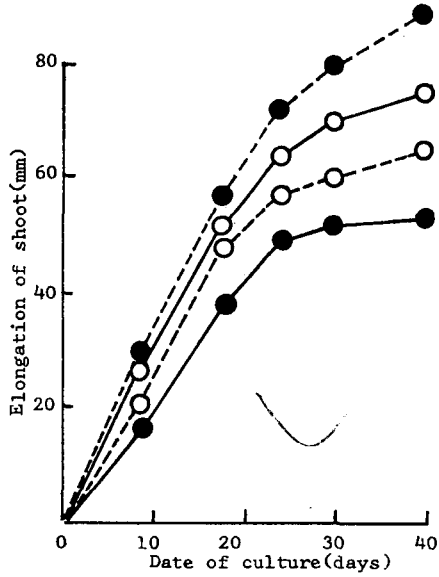


Figure 14. Effect of gibberellin application to the medium on the shoot elongation of apical pieces of sprout.
 Conc. of gibberellin; ●—● 0 ppm, ○—○ 0.1 ppm, ●--● 1.0 ppm, ○--○ 10.0 ppm.

10.5 x 5
62.5

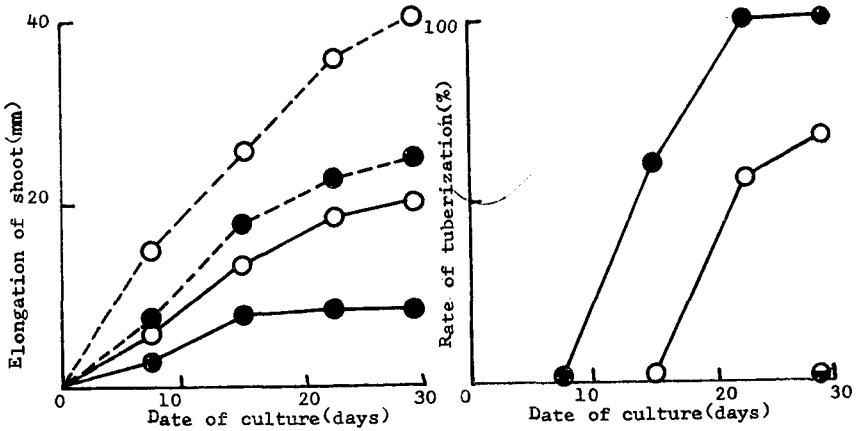


Figure 15. Effect of gibberellin application to the medium on the shoot elongation and tuber formation of the stem segment cut from the basal part of main shoot.
 Conc. of gibberellin; ●—● 0 ppm, ○—○ 0.01 ppm, ●--● 0.1 ppm, ○--○ 1.0 ppm.

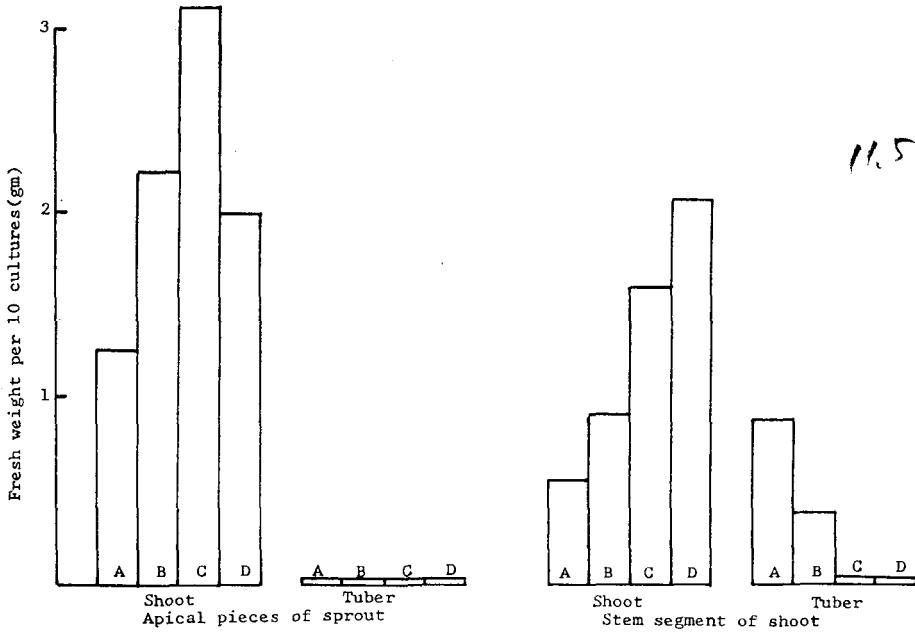


Figure 16. Variation of fresh weights of shoots and tubers with application of gibberellin (40-day old culture).
 Conc. of gibberellin for apical pieces of sprout; A=0 ppm, B=0.1 ppm, C=1.0 ppm, D=10.0 ppm.
 Conc. of gibberellin for stem segment of shoot; A=0 ppm, B=0.01 ppm, C=0.1 ppm, D=1.0 ppm.

it resulted in the marked retardation of linear development of the lateral shoots from the stem segments and succeeded by substantial enhancement of tuberization.

On the other hand, tuber-induced tissues as stem segments of etiolated shoots which were more advanced physiological age than that of young sprouts, were easy to form tuber without reference to any nutritional condition of medium.

Judging from these results, it seems very reasonable to assume, therefore, that the induction to the onset of tuberization in potato plant might conceivably be mediated due to not variation of major nutritional elements but the change of the balance between the levels of some tentative natural growth substances in the potato plant. Accordingly the carbohydrate nutrition is significantly beneficial for tuberization but is not essential.

This assumption was confirmatory supported by the evidence that the some synthetic auxins and gibberellin being applied to the stem segment exogenously were virtually change the mode of tuberization.

III Effects of gibberellin on the tuber formation of potato plant

In relatively recent years, it has come to be generally accepted as a fact that the gibberellin treatment is conspicuous effective for breaking the dormancy of potato tuber (50, 120). MACLOAD, et al. (54) and HUMPHRIES (37) have advanced a further views that an irregular tuber formation of potato plant during the growing stage also occurred by means of foliar treatment of gibberellin.

These facts seems to imply, therefore, that gibberellin might play an important rôle for the tuberization of potato.

Indeed, the potato plant is induced to initiate the tuber formation under the short day condition and its tuberization is thoroughly arrested with subjecting to the long day condition. However it resulted in opposite mode of growth as to tuberization, if top of plant was treated with adequate dosage of gibberellin even under the similar short day condition. It seems, therefore, safe to conclude that an application of gibberellin has a quite similar activity for the suppression of tuberization as that being subjected to long day condition (22, 52).

On the other hand, a great number of investigators have been studied extensively concerning an occurrence of endogenous natural gibberellins in the various kinds of higher plants (8, 26, 27, 51, 55, 64, 78, 79, 89).

In the present investigation, in extending the works on the occurrence and variations of endogenous gibberellin-like substances in the different tissues of potato plant, particular attention was given to a study on the physiological influences of these substances on the induction of tuber formation of the potato plant which being subjected the short day or long day conditions.

Materials and Methods:

The main materials employed were *Solanum tuberosum* L. var. Irish Cobbler, Norin No. 1 and Kamiya-imo. It has been known that Kamiya-imo is essential to be subjected rather longer duration of short day condition in order to cause tuberization than that of Norin No. 1, and tuber formation of Irish Cobbler is most insensitive for the variation of daylength.

Extraction of natural gibberellin... As the solvent, 80% ethanol was chiefly used throughout the experiments. Materials were extracted with 80% ethanol for 24-hr at 2°C. Then this extract was filtered and the residue was washed thoroughly with ethanol and filtered again. The two filtrates were mixed together, then ethanol was evaporated off at 50°C under reduced pressure. The aqueous residue was filtered and fractionated into neutral and acidic

fractions with ether by the procedure described by LARSEN (48). After condensation of acidic fraction at 50°C, the samples were chiefly developed paper chromatographically with iso-propanol / ammonium hydroxide / water (10 : 1 : 1) as solvent. The chromatograms were cut equally into ten or fifteen pieces and each piece was placed in the petri-dish of 3.3 cm in diameter. As the control, a piece obtained from below the starting line of chromatogram was used in the same way as stated above.

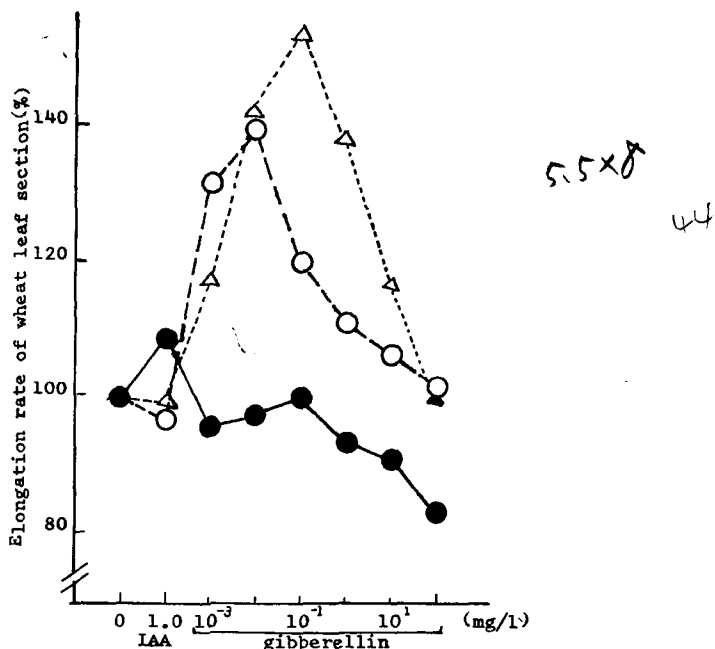


Figure 17. Sensitivities of leaf section for gibberellin at the various growing stage of etiolated wheat seedling; length of coleoptile, ●—● 18~25 mm, ○--○ 40~50 mm, △...△ 60~90 mm.

The activity of gibberellin was assayed by the modified wheat leaf section test developed originally by RADLEY (78). As is shown in Figure 17, it was evident that the elongation of leaf section obtained from about 60 mm of etiolated wheat seedling in length was promoted parallel with increase of the applied gibberellin concentration covering range from 10⁻⁴ to 10⁻¹ mg/l inclusively, while there is absolutely no effect of IAA application concerning the elongation of leaf section.

Accordingly, wheat seedlings about 60 mm in length for gibberellin bioassay were used exclusively throughout the present experiments.

From each selected shoot, 5 mm section was cut at 3 mm above the base of the shoot. Primary leaves were pushed out from the coleoptile cylinder with a fine glass rod, and ten sections were placed on each chromatogram piece in the test dish in order to incubate for 24-hr in dark at 25°C.

After incubation the elongation of sections was measured and results were expressed in term of percentage compared with the elongation of control section.

Experimental results:

(1) Characteristic behaviors of endogenous gibberellin in the potato plant.

a) Paper chromatographic partition of ethanol extract

The middle part of 50-day old potato plant, var. Irish Cobbler subjected to short day condition (8-hr. daylength) for 14 days were used as material for this experiment. The extracts of leaves were chromatographically partitioned with six different development solvents described as follows.

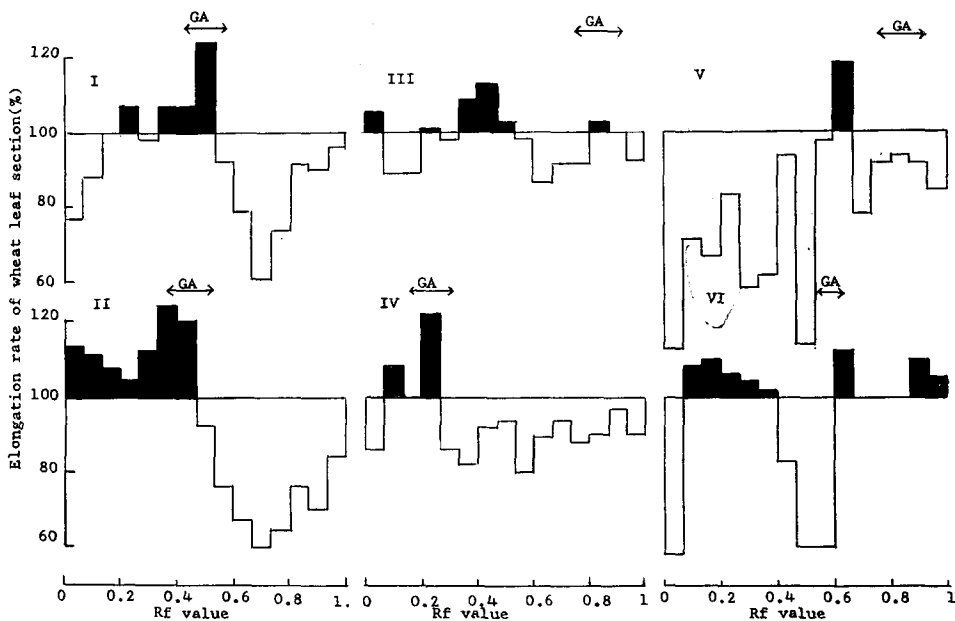


Figure 18. Histograms of wheat leaf test of ethanol extracts obtained from potato leaves.

Solvent for chromatography, I chloroform/ether/water/formic acid (20 : 4 : 2 : 1), II iso-propanol/ammonium hydroxide/water (10 : 1 : 1), III ethanol/3N-ammonium hydroxide (8 : 2), IV n-buthanol/1.5N-ammonium hydroxide (3 : 1), V n-buthanol/acetic acid/water (95 : 5 : 30), VI benzene/pyridin/3N-ammonium hydroxide (3 : 6 : 1). \longleftrightarrow shows Rf value of fungal gibberellic acid.

- (I) chloroform / ethanol / water / formic acid (20 : 4 : 2 : 1)
- (II) iso-propanol / 28% ammonium hydroxide / water (10 : 1 : 1)
- (III) ethanol / 3N-ammonium hydroxide (8 : 2)
- (IV) n-butanol / 1.5N-ammonium hydroxide (3 : 1)
- (V) n-butanol / acetic acid / water (95 : 5 : 30)
- (VI) benzene / pyridine / 3N-ammonium hydroxide (3 : 6 : 1)

According to the histograms of Figure 18, promoting areas (Rf 0.46–0.53 in solvent I, Rf 0.40–0.46 in solvent II, Rf 0.20–0.26 in solvent IV and Rf 0.60–0.66 in solvent VI) coincided approximately with the Rf values of control gibberellin on each solvent.

Determination of the Rf value of control gibberellin which is "Gibberellin Kyowa" containing over 90% of gibberellin A₃, was carried out by means of ultraviolet light method described by OVERBEEK (70).

As showing in the histograms of solvent III and V, however, the Rf value of control gibberellin corresponded with Rf of inhibiting area on each chromatogram against expectation.

Concerning the fact, it can be substantially deduced that the promoting action by natural gibberellin might be masked with some inhibiting substances such as the inhibitor- β .

b) Relationship between natural gibberellin and natural inhibitor in the potato leaves

As stated above, there seems to be within the bound of possibility that a function of gibberellin in the potato extract might be masked partially due to the coexistence with the inhibitor- β in it.

Recently CORCORAN and PHINNEY (15) have postulated that diffusates from the immature seed of *Ceratonia siliqua* L. and *Erioboteya japonica* LINDL. markedly inhibited gibberellin induced growth.

Present experiment was undertaken to know physiological relation between natural gibberellin and inhibitor- β in potato leaves.

In order to obtain the preparation of inhibitor- β , the extract of potato leaves was partitioned by means of the paper chromatography using solvent II as described above. The region of chromatogram ranging between Rf 0.75 and 0.85, was cut off and reeluted by ether. The ether elute was divided equal volume of four portions. Each elute was absorbed with a piece of filter paper (20 × 20 mm). After evaporation of ether from the paper at room temperature, each paper contained inhibitor- β extracted from 30 gm in fresh weight of potato leaves, then was placed in the petri-dish with 0.3 ml. gibberellin solution of 0, 0.001, 0.01, 0.1 mg/l in concentration, respectively.

As control test, same series of gibberellin solution without addition of inhibitor- β were prepared as the similar way for the leaf section test.

Figure 19 is shown the result of present experiment. Gibberellin induced growth of wheat leaf section was progressively promoted keeping pace with the increment of gibberellin concentration in the test solution without inhibitor- β .

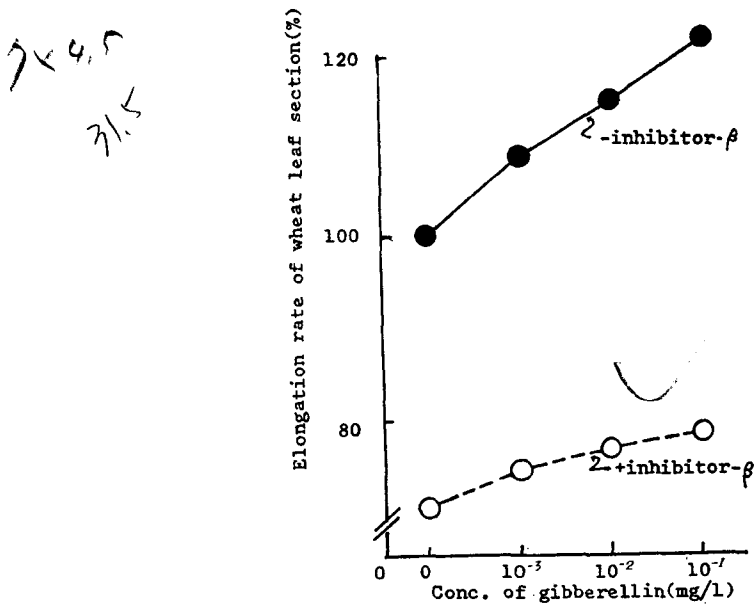


Figure 19. Effect of inhibitor of potato leaves on gibberellin activity.

In the solution in presence of inhibitor- β , contrarily, it resulted about thirty per cent reduction of the gibberellin induced growth on an average throughout all series of test solution.

This fact indicates, therefore, that a natural inhibitor such as inhibitor- β has a retarding function to the growth of wheat leaf section, not only its normal growth but gibberellin induced growth.

c) Isolation of natural gibberellin from natural inhibitor.

While the fungal gibberellin is considerably hard soluble in chloroform (96), HEMBERG (32) reported that natural inhibitor- β extracted from potato tubers was freely soluble in chloroform. In accordance with HEMBERG's opinion, aqueous residue of ethanol extract of potato leaves was reextracted twice with the same volume of purified chloroform prior to its ether extraction in order to discard the contaminated inhibitor- β from its aqueous residue.

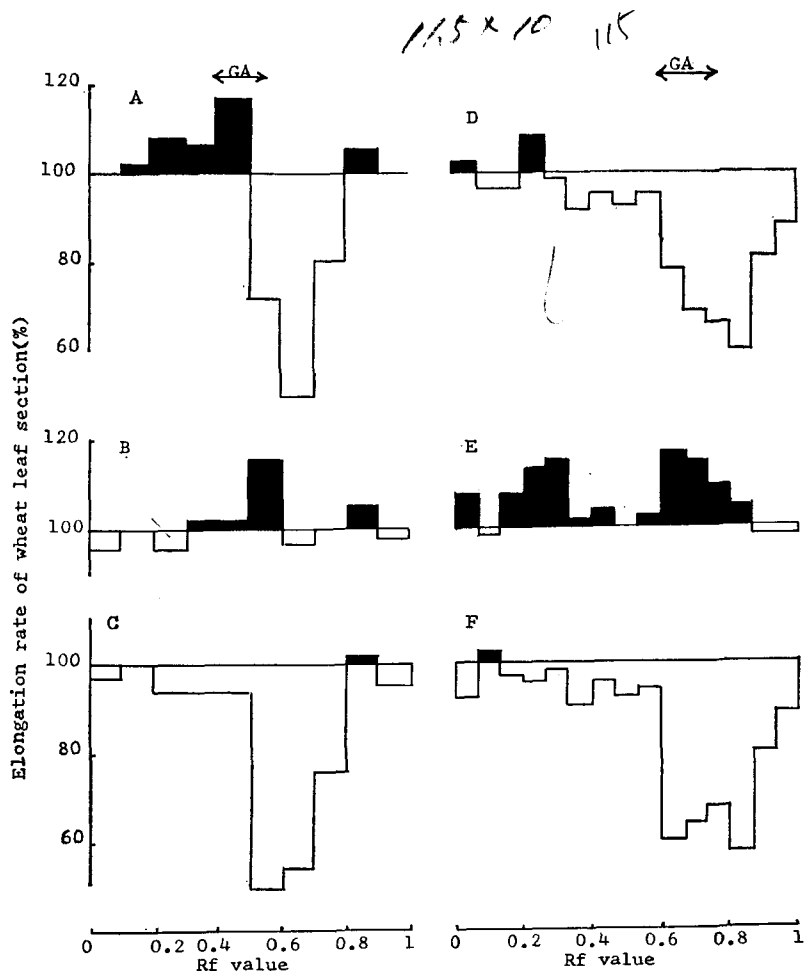


Figure 20. Isolation of natural gibberellin from natural inhibitor of potato leaves.

A and D—Histograms of wheat tests of ether extracts without pretreatment of chloroform, chromatogram A was developed by solvent-II and D by solvent-III.

B and E—Histograms of wheat tests of ether extracts with pretreatment of chloroform, chromatogram B was developed by solvent-II and E by solvent-III

C and F—Histograms of wheat test of chloroform fraction, chromatogram C was developed by solvent-II and F by solvent-III.

According to the histograms of Figure 20, there was no promoting area on the chromatogram of ether extract without the pretreatment of chloroform, at the same Rf value as that of gibberellin being detected on the control chromatograms.

However it resulted in a remarkable promotion of leaf section growth on the chromatogram of ether extraction with the pretreatment of chloroform, and the one of promoting area on the chromatogram (R_f 0.60–0.72) was approximately coincided with that of control gibberellin.

It appeared that removal of inhibitor- β from the extract might be a suitable procedure for the partial isolation of gibberellin.

On the other hand, it must not be overlooked the important evidence which was demonstrated by HAYASHI and RAPPAPORT (27), indicating that there was also virtually a chloroform-soluble gibberellin in the potato tubers. However it could not be detected the presence of the similar chloroform-soluble gibberellin in the potato leaves as that in the tuber, so far as concerning the present experiment.

- d) Comparison between the wheat leaf section test and ultraviolet light test concerning natural gibberellin on the paper chromatogram.

The present experiment was evaluated with respect to the detection of gibberellin on the sulfuric acid treated paper chromatogram by means of the irradiation of ultraviolet light as stated by OVERBEEK (96), comparing with the results obtained by bioassay such as wheat leaf section test.

As shown the results with solvent II and III for development of chromatography (Figure 21, I and II), a similar deep purple colored band of fluorescence on the same regions of chromatogram (R_f 0.42–0.60 and R_f 0.58–0.69) obtained from potato leaves and that from control gibberellin were recognized under the irradiation of ultraviolet light.

At the same time, promoting areas (R_f 0.40–0.60 in I and R_f 0.50–0.70 in II) were distinguished on the chromatograms by leaf section tests.

Furthermore, it was detected some other fluorescence bands on the chromatogram of potato leaves such as light purple, light blue and yellow, but these areas of chromatogram were not associated with results of bioassay, besides deep purple band described above.

However some of endogenous gibberellin existing in the potato leaves seems to be possible to identify by fluorescence caused by irradiation of ultraviolet light.

- e) Resistances of gibberellin obtained from potato plant to heating, acid and alkaline treatments.

Fungal gibberellin is known to be stable in acidic and neutral solution, while denatured in basic solution (46).

In order to see the change of stability with heating, acidic and basic solution treatments, the aqueous solution of potato extract was subjected to

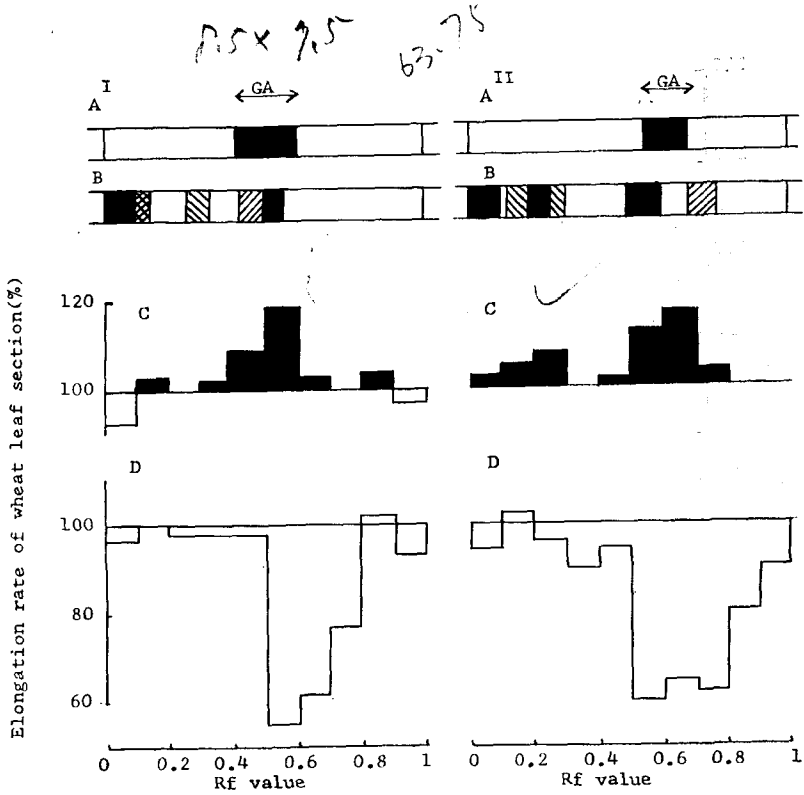


Figure 21. Comparison between the wheat test and UV test concerning natural gibberellin on the chromatograms of group I were developed by solvent-II and that of group II by solvent-III. A—Fluorescent band of fungal gibberellin used as control. B—Fluorescent bands of ethanol extract of potato leaves. C—Histograms of wheat test of ether extracts with pretreatment of chloroform. D—Histograms of wheat test of chloroform fraction.

■ deep purple ▨ yellow ▧ light blue ▩ light violet

heat with 0.5 mol HCl and 0.5 mol NaOH on the boiling water bath for 60 minutes.

According to the results showing in Figure 22, the gibberellin activities extracted from potato plants were quite stable in the plain water and 0.5 mol HCl solution by heating just as the fungal gibberellin is. On the other hand, a remarkable reduction of these gibberellin activities were resulted in the treatment of 0.5 mol NaOH solution.

So far as the results of leaf test, ultraviolet light test and acid and alkaline

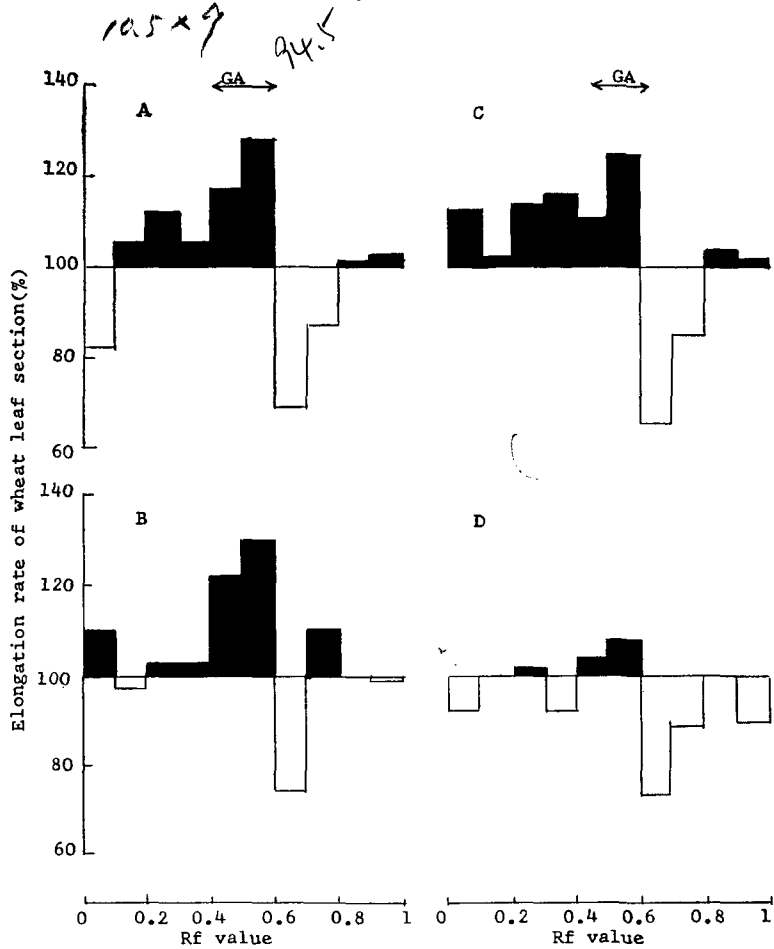


Figure 22. Resistance of gibberellin of potato leaves to heating, acid and alkaline treatments.
A—control, B—heating treatment, C—acidic treatment, D—alkaline treatment.

treatments which were made using the paper chromatogram obtained from the ethanol extract of potato tissues, it may be concluded that there are some gibberellin-like substances in the potato tissues. At least one of these substances may be regarded as the gibberellin itself, or seems to be highly similar to the gibberellin.

(2) Distribution of natural gibberellin in potato plant.

These experiments were made to see the distributions of gibberellin-like substances in the various organs.

a) Leaves

Ten gm, twenty gm and thirty gm in fresh weight of leaves obtained from growing potato plant immediately before the initiation of flower bud formation, were extracted as usual way, respectively.

Each extract was fractionated by the paper chromatography, followed by

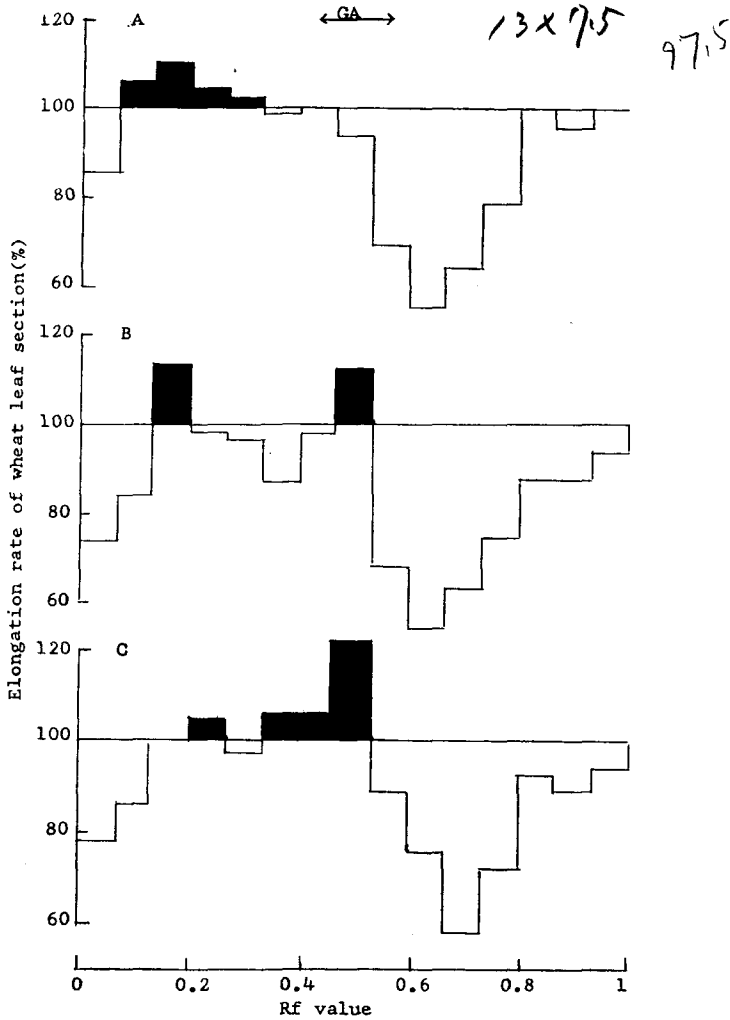


Figure 23. Variation of gibberellin activities in various fresh weights of potato leaves (var. Norin No. 1).
 A—Histogram of extract obtained from 10 g leaves.
 B—Histogram of extract obtained from 20 g leaves.
 C—Histogram of extract obtained from 30 g leaves.

the wheat leaf test to measure gibberellin activity.

As is shown in Figure 23, growth of leaf sections induced by extracts of leaves was enhanced with the increase of fresh weight of sample, which promoting areas on the chromatograms of samples wholly coincided with that of control gibberellin ranging from Rf 0.45 to Rf 0.56, however 10 gm extract caused no stimulation on this area.

b) Tubers

As to the gibberellin content in the cortex of tuber, it generally contained much more amount of gibberellin than that in the leaves (Figure 24, 25 and 26).

With regard to the distribution of gibberellin activities in the tuber of

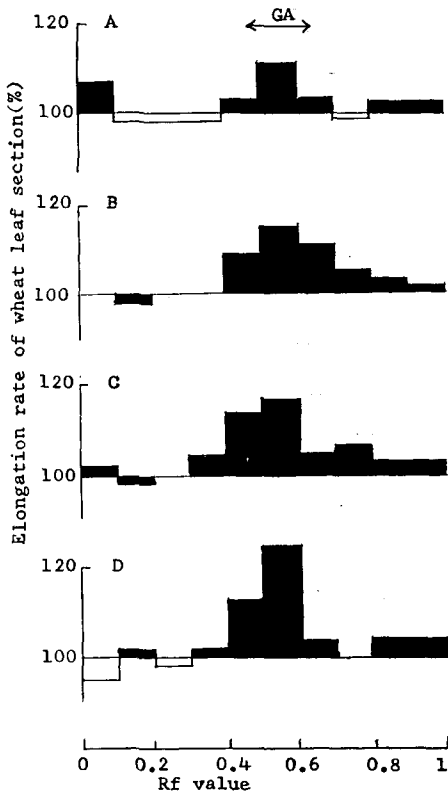


Figure 24. Variation of gibberellin activities in various fresh weights of potato tubers. (var. Irish Cobbler). Histograms of extracts obtained from potato tubers, 1.5 g (A), 3.0 g (B), 6.0 g (C) and 9.0 g (D).

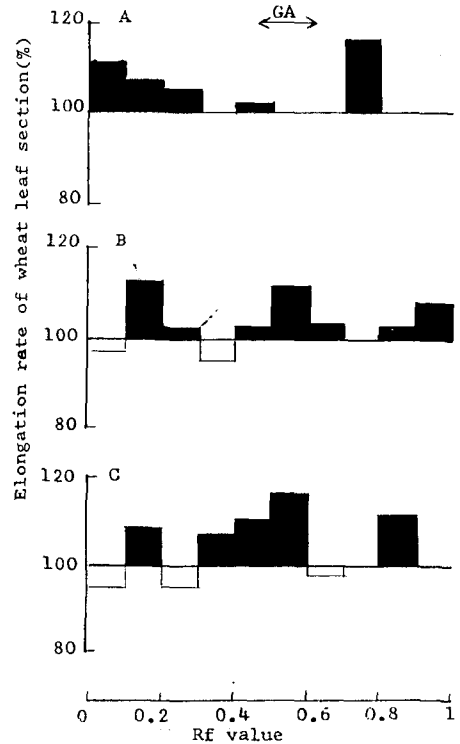


Figure 25. Variation of gibberellin activities in various fresh weights of potato tubers (var. Norin No. 1). Histograms show extracts of potato tubers, 1.5 g (A) 3.0 g (B), and 6.0 g (C).

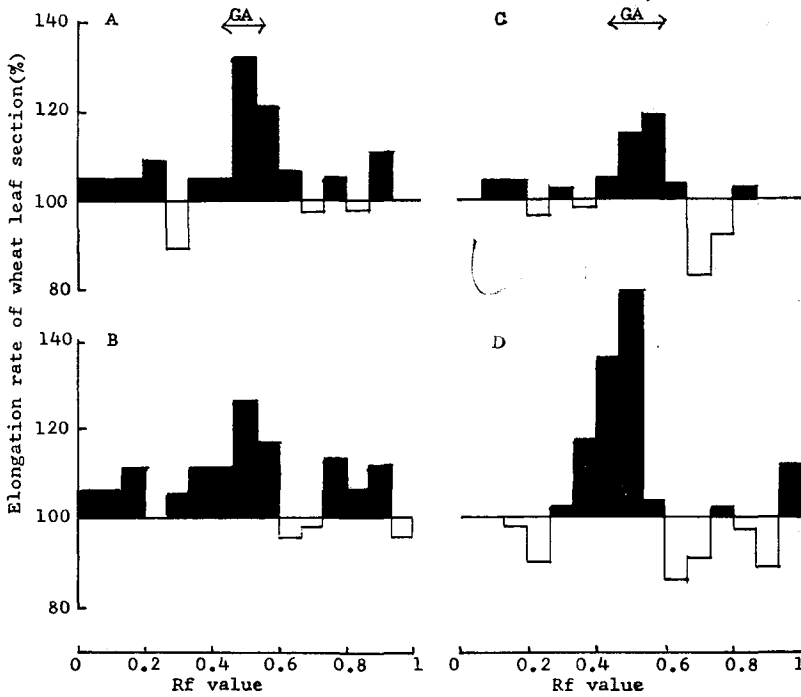


Figure 26. Variation of gibberellin activities in the various parts of tubers.
 A—apical part of tuber, B—cortex of tuber,
 C—pith of tuber, D—sprout.

Irish Cobbler potato at the beginning of its sprouting stage, a higher activity in the cortex tissue of tuber, particularly in the vicinity of apical buds was ascertained than that in the pith tissue. The highest activity was found in the young sprouts (Figure 26-D).

c) Stems

As mentioned in the previous chapter, it was recognized some specific characters due to differences of the physiological ages concerning the tuberization on the stem segments obtained from various parts of the etiolated shoots.

This experiment was made to measure the gibberellin content in each stem segment.

From the result being shown in Figure 27, amounts of gibberellin in the etiolated shoot of potato decreased in inverse proportion to the advancing of its physiological age, namely a maximum amount of gibberellin was found in the apical piece of shoot and a minimum amount was in the basal part of it.

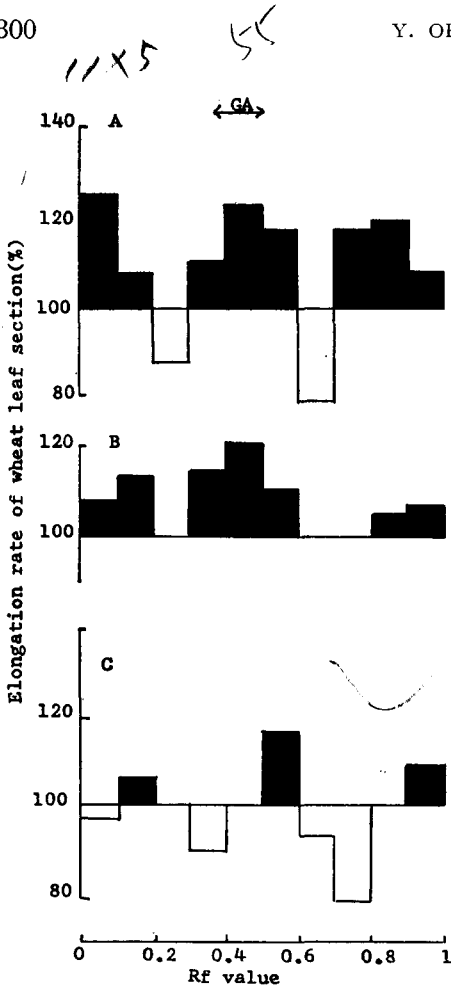


Figure 27. Gibberellin activities in the various parts of potato shoots. A—apical part of shoot, B—middle part of shoot near the third node, C—basal part of shoot near the fifth node.

It was suggested that the endogenous gibberellin may be of most significant for breaking of potato dormancy.

Meanwhile the present data also made to appear the remarkable increment of gibberellin activities in the young buds and cortex tissues in the neighborhood of buds just after sprouting, which seems to be a substantial evidence that natural gibberellin in the tuber might cause a promotion of sprouting involving to enhance the termination of dormacy.

On the relative amount of natural gibberellin in the various parts of potato plant, the following series was eventually recognized in order: sprout > stem tip > tuber > middle part of stem > leaflet.

According to the assumption stated by LOCKHART (51), it has been considered that natural gibberellin-like substance might probably be formed in the apical growing point of stem. Therefore his opinion was sufficiently supported by the result of present investigation as to the distribution of natural gibberellin in potato plant.

On the other hand, LIPPERT (50) has established an opinion concerning the breaking of potato dormancy by means of the gibberellin treatment and suggested that natural gibberellin occurred in the potato tuber may also play, at least partially, some significant rôle being connected with the termination of dormancy and promotion of sprouting.

In fact, SMITH and RAPPAPORT (89) had demonstrated that the concentration of endogenous gibberellin in the potato tuber remained low during its rest period but markedly increased immediately after the end of rest period.

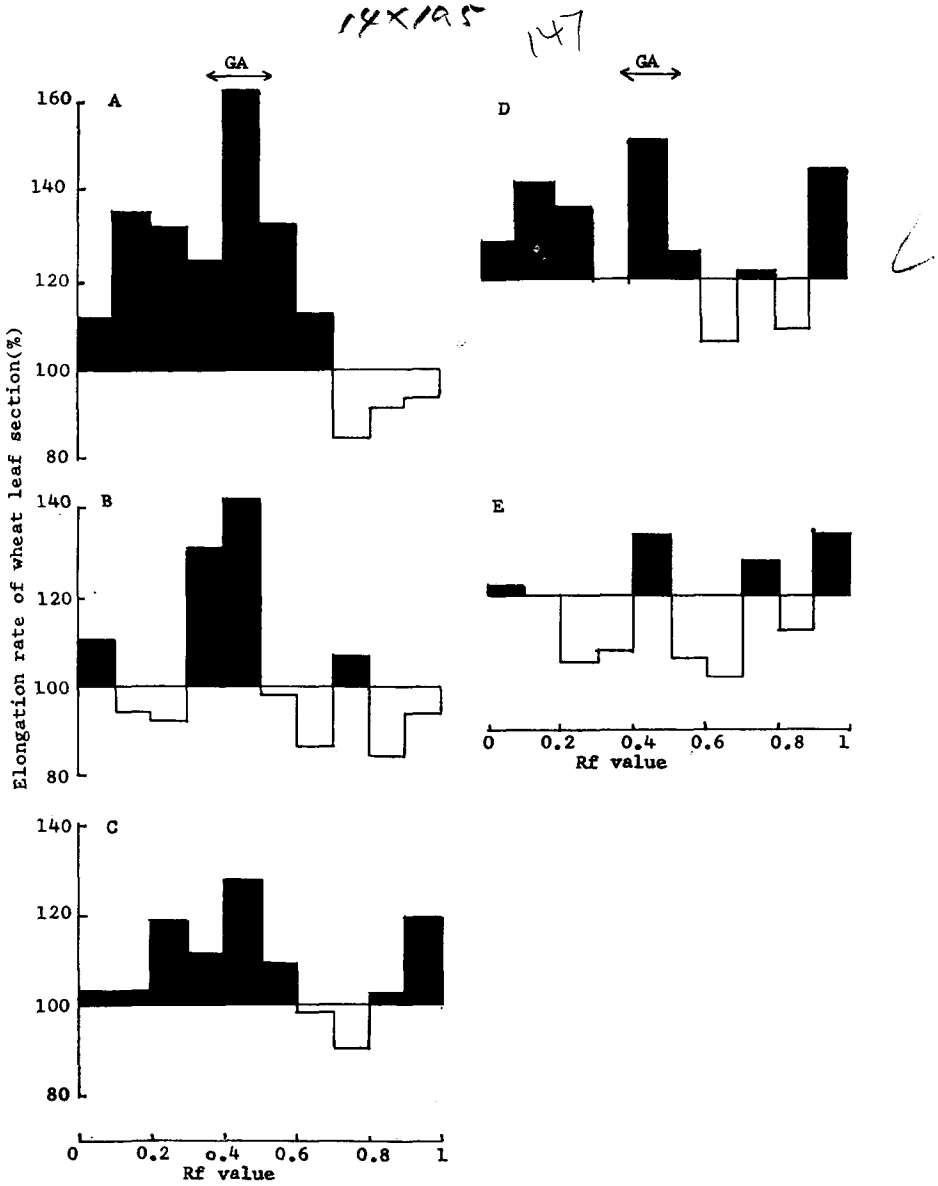


Figure 28. Progressive variation of natural gibberellin activities in the stolon tips and young tubers.
 A—Stolon tip at its linear growing period.
 B—Stolon tip just before the starting of tuberization.
 C—Stolon tip at the initiation of swelling.
 D—Young tuber just after tuberization.
 E—Young tuber at further developing stage.

(3) Physiological relationship between gibberellin and tuber formation of potato.

a) Progressive variation of natural gibberellin activities in the stolon tips and young tubers.

Samples for extraction of gibberellin in the stolon tips and young tubers during the growing season of potato plant, were obtained from many various stages as indicated below :

- (1) Stolon tips at their linear growing period
- (2) Stolon tips just before the starting of tuberization
- (3) Stolon tips at the initiation of swelling
- (4) Young tuber just after tuberization
- (5) Young tuber at further developing stage.

The experimental data are shown as histograms in Figure 28, in which "Solvent II" were used for development of chromatogram.

It was found two restrict promoting areas (Rf 0.1-0.3 and Rf 0.4-0.6) in the histograms which obtained from acid fraction of extracts of stolon tips at its developmental stage, a decreasing tendency of gibberellin activities was generally recognized with morphological transformation of stolons involving specific change of growth pattern from linear growth to tuberous development.

However, when the stolon tips developed to tuber, a new promoting region occurred on the chromatogram ranging from Rf 0.9 to 1.0. At the further developmental stage of young tuber, inhibiting activities was found on some regions of chromatogram (Rf 0.2-0.4 and Rf. 0.6-0.9), and their activities increased inversely with the lowering of gibberellin activities.

In conclusion, contents of natural gibberellins were rich in the stolon at its vigorous growing stage, then decreased gradually with an onset of tuberization.

b) Effect of foliar application of gibberellin upon tuber formation of potato.

It has long been accepted the tuber formation of potato plant was promoted by the treatment of short daylength and reversely resulted by long day condition (1, 14, 23, 61, 76,77, 91, 94, 126, 127).

On the other hand, some of long day plants were induced to initiate the flower bud formation by gibberellin treatment without subjecting to long day condition (22, 52).

In the present experiment, potato plants growing about 10 cm in height were treated with three contrasting photoperiods describing as follows: (1) a 16-hr daylength followed by an 8-hr dark period, (2) an 8-hr daylength followed by a 16-hr dark period, and (3) groups of short day plant as described in (2)., were foliar sprayed with 50 ppm gibberellin water solution immediately before and after the short day treatment.

TABLE 4. The effects of gibberellin treatment on the tuberization of potato plants which were subjected with long day or short day conditions.

A, Foliar application of gibberellin just before the starting of photoperiodic treatment.

	Top of plant		Stolon		Tuber	
	Height (cm)	No. of shoot	Number	Length (cm)	Number	Fresh Wt. (gm)
Long day condition	52.0	9.6	4.0	5.0	0.0	0.0
Short day condition	28.9	6.2	2.8	3.4	2.2	0.4
Short day condition with gibberellin treatment	73.2	11.5	4.5	14.3	0.0	0.0

B, Foliar application just after the end of photoperiodic treatment.

	Top of plant		Stolon		Tuber	
	Height (cm)	No. of shoot	Number	Length (cm)	Number	Fresh Wt. (gm)
Long day condition	49.0	14.0	13.2	13.0	0.0	0.0
Short day condition	32.5	7.2	2.3	4.8	2.3	5.1
Short day condition with gibberellin treatment	38.5	11.2	17.1	9.8	1.6	1.8

From the results summarized in Table 4 and Plate IV, it was apparently observed an active elongation of stolon with no yield of tuber on the plants which were subjected to long day condition. On the contrary, rapid formation of tubers on the tips of stolons was resulted on the plant by the treatment of short photoperiods.

Furthermore, the plants on which a foliar application of gibberellin was made immediately before starting of short day treatment, formed no tuber on the stolons, similarly as plants subjected to long day condition, even if the top of plant was thoroughly subjected to short day treatment for the induction of tuberization.

When the plants were subjected to 14 cycles of short photoperiod followed by the application of gibberellin, the tubers which once formed under the short day condition, started again to sprout and resulted in the new emergence of stolons from the main stems under the ground.

From the facts stated above, it was confirmed that a gibberellin treatment might certainly the tuberization of potato as similar as the plants subjected to

long day condition.

c) *Effect of photoperiod on the variation of natural gibberellin content in the potato plant.*

In order to know a physiological relationship between the tuber formation and variation of natural gibberellin content in the potato plant, the present experiments were carried out.

“Norin No. 1” and “Kamiya-imo” are considerably photosensitive varieties for the induction of tuberization.

These potato plants which were already raised up to about 20 cm in length, were subjected to two different daylengths using the same procedure stated in the foregoing experiment.

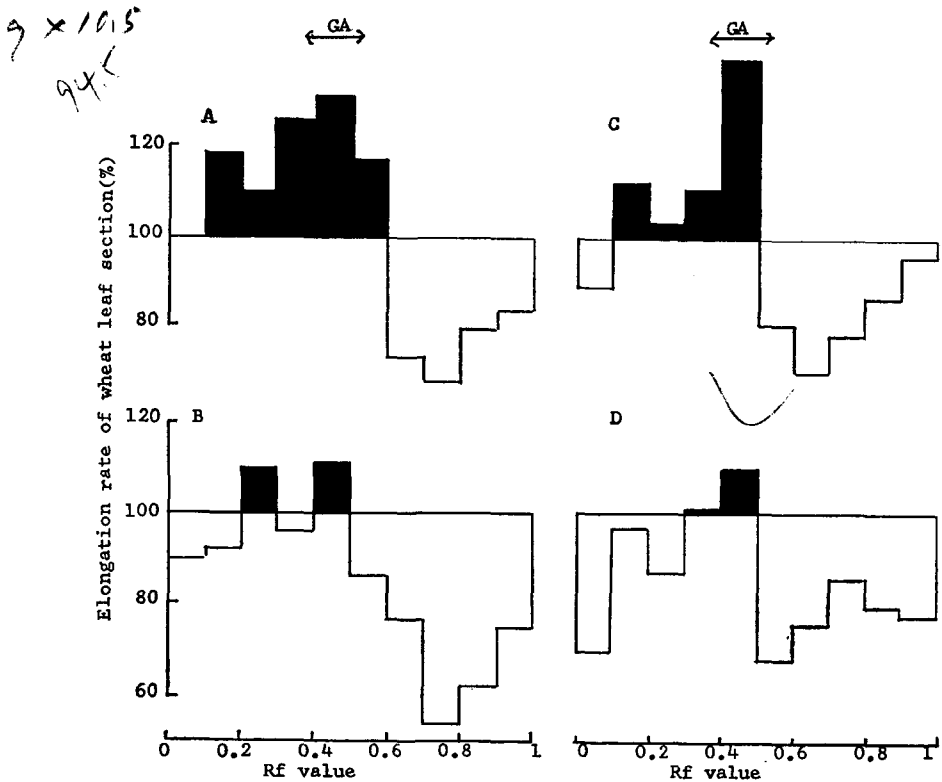


Figure 29. Effect of photoperiodic treatment on the gibberellin activities in potato leaves.

- A—Norin No. 1 (16-hr daylength).
- B—Norin No. 1 (8-hr daylength).
- C—Kamiya-imo (16-hr daylength).
- D—Kamiya-imo (8-hr daylength).

After treatment of 7-cycles photoperiods, the apical tips of plant including young unfolding leaves were harvested to use as materials for the assay of natural gibberellin.

According to the histograms showing in Figure 29, there was apparently a promoting area on the chromatogram obtained from leaves growing under long day condition, and which area coincided closely with the Rf value of pure gibberellin on the control chromatogram which was ranged from Rf 0.5 to 0.6. It was also recognized less activity of gibberellin on the chromatogram of leaves obtained the short day plant than that from long day plant.

Consequently it seems to be in all likelihood that the variation of natural gibberellin in the potato leaves due to the change of daylength might be closely related to the induction of tuberization.

d) Effect of environmental temperature on the variation of natural gibberellin content in the potato plant.

Potato variety, Norin No. 1 was used as materials to grow at two different temperature, 13°C and 18°C. When the plants grew about 20 cm in height, they were moved to the green house from June 19, to June 28, of which temperature was regulated automatically at 13°C and 18°C. At the end of treatment, young leaves were harvested for the determination of gibberellin content.

The effect of varied temperature on the gibberellin activities in the potato leaves was illustrated histographically in Figure 30.

In the both of histograms, it is shown a considerable gibberellin activity on the chromatogram in a wide range of Rf 0.0-0.5, particularly maximum

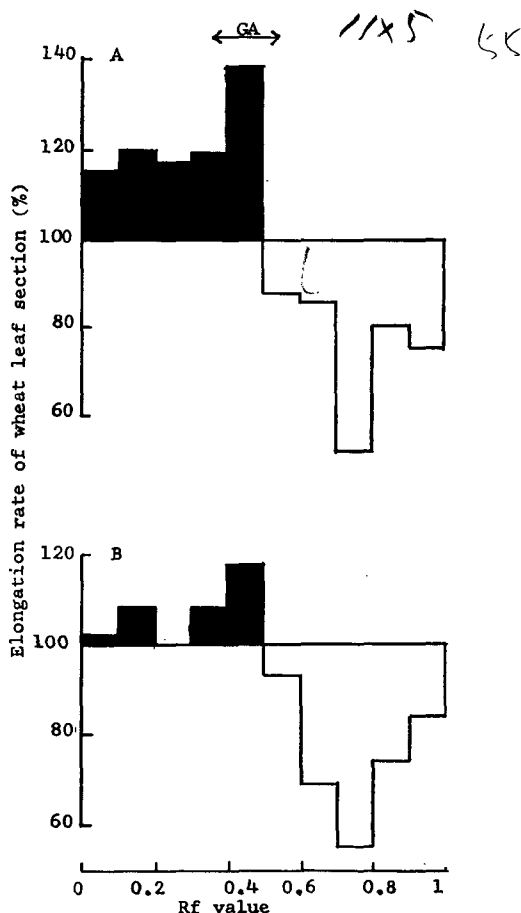


Figure 30. Effect of environmental temperature on the gibberellin activities in potato leaves. A—18°C, B—13°C.

activity was found in the region between Rf 0.4 and 0.5, which region corresponded with the Rf value of pure gibberellin on the chromatogram.

It was also recognized a much more stimulation of gibberellin activity due to the rise of environmental temperature. The plant grown at 18°C has considerably higher activity of gibberellin than that at 13°C. In contrast to the result as to gibberellin activity, a higher activity of inhibitor was found in the plant raised at 13°C than that at 18°C. This fact indicated that the gibberellin content in the potato plant increased with rising the environmental temperature.

e) Varietal differences of gibberellin activities in the potato plants.

Many investigators have reported that there are considerable differences of photoperiodic responses of potato plant for the induction of its tuberization (1, 93, 94). Variety of Irish Cobbler has, for instance, less responsibility for photoperiod than Norin No. 1 and Kamiya-imo, and D-805 variety need to subject the conspicuous longer duration of short daylength in order to form tuber.

Accordingly, the study on the variation of gibberellin content in the various varieties of potato was performed. Irish Cobbler, Norin No. 1, Kamiya-imo and D-805 were used as materials. These potato plants about 15-20 cm in height raising under long day condition (18-hr daylength) were harvested. Three gm of young leaves obtained from each variety of plant was extracted for bioassay.

From the histograms of Figure 31, it is generally seen that the major part of gibberellin activities was found in the region of relative low portion of chromatogram, contrary to the activities of inhibitors in that of high portion of ones. In all respect, the maximum activity of gibberellin was detected in the area of Rf value ranging Rf 0.4-0.5, where is corresponded with the Rf value of pure gibberellin on the control chromatogram.

On the relative amounts of gibberellin in these varieties, briefly, the following series was ascertained in order

Irish Cobbler > D-805 > Kamiya-imo > Norin No. 1

On the other hand, the following series concerning the content of inhibitors was recognized in order

Irish Cobbler > Norin No. 1 > D-805 > Kamiya-imo

According to the results stated above, it seems to be quite evident that the natural gibberellin content in the leaves of these varieties increased parallel with the incline of photo-sensitivity responding for tuberization, except the case of Irish Cobbler.

Notwithstanding much amount of gibberellin in the Irish Cobbler, the less

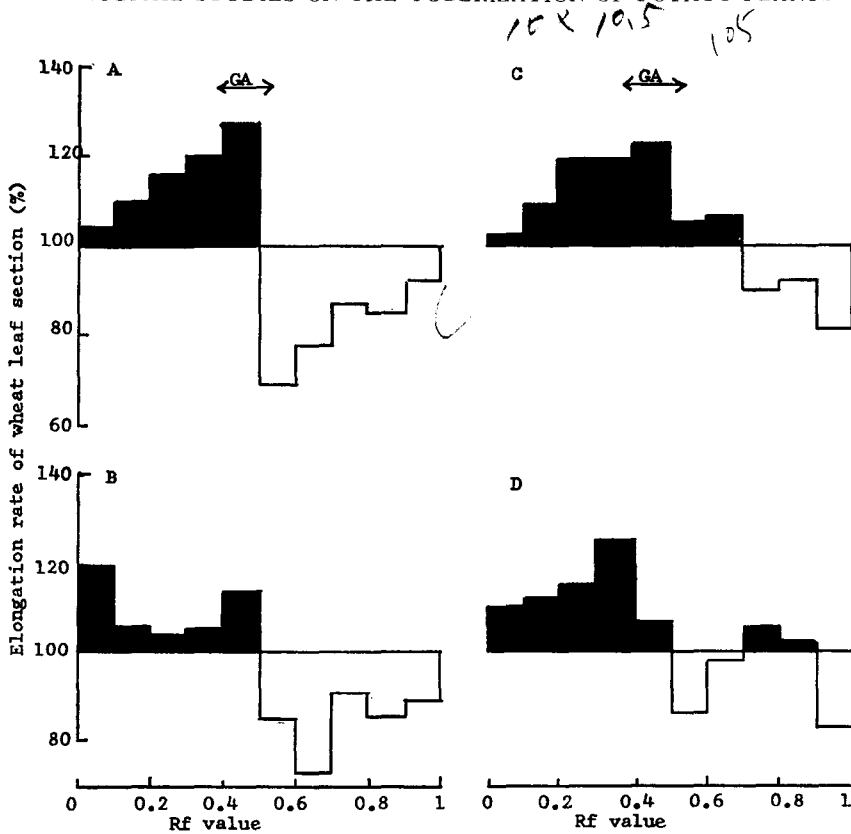


Figure 31. Varietal variation of gibberellin activities in the potato plant.
 A—Irish Cobbler, B—Norin No. 1, C—Kamiya-imo, D—D-805.

sensitivity of this variety as to photo-response for tuberization might be likely to explain that there is also predominant amount of inhibitor in this variety.

Because of its inhibition to the gibberellin activity, tuber formation of this variety would be much easier than other varieties.

It seems to be considerable possible that varietal differences of tuberization on the growing plant might be due to the occurrence of gibberellin activity in the leaves.

(4) Relation between the irregular tuber formation and natural gibberellin

- a) Progressive change of natural gibberellin in the tubers with the advance of their senescence.

When the potato tubers were continued to store at room temperature even after the termination of their dormancy, they lost their moisture remarkably and followed to result extreme dehydration due to an increasing activity of

respiration. With regard to this fact, TAGAWA and OKAZAWA (105) has postulated previously an opinion that the senescence of potato tuber attributed to its dehydration might be one of the cause, even if indirectly, forming irregularly the sprout tuber.

To investigate the relation between to irregular tuberization and changes of gibberellin content in the tuber, this experiments were carried out. Potato tubers, variety Irish Cobbler, were stored in a cellar at room temperature, which terminated the dormancy at the middle of December, then started to sprouts. Sprouts emerged from tuber were removed on 15, February and 20, March. Assays of gibberellin in the tuber were performed just after the end of dormancy (6, January) and at the advanced stage of senility of tuber (18, April). Each sample was obtained from the cortex tissue of tuber.

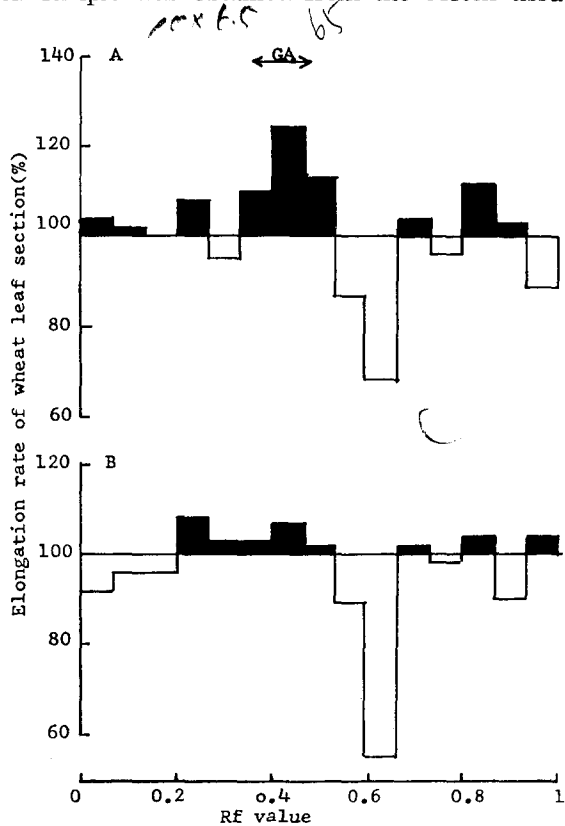


Figure 32. Progressive change of gibberellin activities in the tubers with advance of their senescence.

A—Sprouting stage of tuber (January, 6).

B—Senescent tuber (April, 18)

As is presented in Figure 32 and 33, the maximum activities of natural gibberellin on the chromatograms obtained from each sample were also located in the region ranging from Rf 0.4 to 0.5. Growth promoting rate by gibberellins in the potato being sampled on 6th of January and 18th of April were 124% and 106%, respectively. On the other hand, the growth inhibiting substances were found at Rf 0.5-0.7 of which activities increased with progress of senility.

Briefly, concomitant with the advance of senility, a decrease in gibberellin activity and an increase in inhibitor one were ascertained. From an inspection of the present data, therefore, it will be assumed that the natural gibberellin in the tuber might play an important rôle in the arrestment of irregular tuber formation.

b) Effect of gibberellin treatment on the irregular tuber formation of potato.

It would be important to study the effect of gibberellin treatment on the sprouting of senile tuber, in order to support the concept of its arresting function for irregular tuber formation.

On 23th of April, senile tubers which were the similar samples used in previous experiment, were soaked in the various concentrations of gibberellin solution supplemented with 0.05% Tween-20 for 10 minutes at 25°C. The concentrations of gibberellin arranged for this experiment were 0, 5, 25 and 50 mg/l and 12 tubers were used for each treatment. After incubation, treated tubers were planted in the wooden boxes containing rich soil, then these boxes were stood to allow in the dark room at 13°C, which temperature in favorable condition for the sprout tuber formation (80, 81, 122).

On 3-rd of June, the tubers were harvested for observation. The results

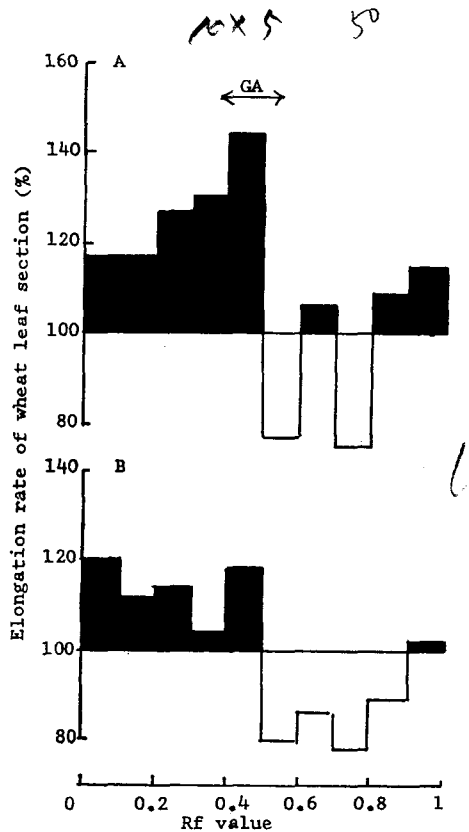


Figure 33. Variation of gibberellin activities in the potato sprouts occurred at 13°C. A—Sprout of non-senile tuber. B—Sprout of senile tuber.

TABLE 5. Effect of gibberellin treatment on sprout tuber formation of the senile tuber

	Concentration of gibberellin (mg/l)			
	0	5	25	50
Length of stem (cm)	17.9	18.1	23.0	25.0
Number of stem	5.0	4.5	9.0	9.5
Weight of stem (gm)	2.7	2.3	1.2	0.3
Number of tuber	4.0	3.0	0.0	0.0
Weight of tuber (gm)	3.0	0.2	0.0	0.0

are summarized in Table 5.

The length and number of etiolated stems emerged from the senile tubers increased directly proportional to the increment of gibberellin concentration used for these treatment, resulting a striking contrast to the decrease of weights of stems and tubers.

No sprout tuber formed on the senile tuber treated with 25 mg/l and 50 mg/l of gibberellin and a considerable prevention of tuberization with 5 mg/l in gibberellin were also ascertained (Plate V-C).

Accordingly it is clearly shown that an application of gibberellin to the senile tuber nevertheless prevents sprout tuber formation under conditions otherwise favorable to it, and this fact is undoubtedly compatible with the assumption of previous survey regard to the functional behaviors of endogenous gibberellin in the tuber.

c) Variation of natural gibberellin content in the light exposed tuber.

SHIMA and ITO (114) conducted a study on the light exposure treatment of potato tuber using for seed pieces, and reported that greened tubers with this treatment were enhanced to form the vigorous sprouts and promoted further development of young tubers even until the later growing stage.

In the present experiment, a recovery of vigorous sprouting activity of the senile tuber would be expected by means of the light exposure treatment of tuber.

Senile tubers used in the previous experiment were placed on the bench in the phytotron (18°C) for 30 days in order to expose the natural day light. Another group of similar senile tubers were stored in the dark room of the same phytotron. At the end of treatment both groups of tubers were immediately planted in the wooden box filled with rich soil and transferred to the dark room regulated temperature at 13°C. On 7th of August, all plants were harvested.

According to the results showing in Table 6, the poor growth of shoot and the formation of many sprout tubers were recognized on the senile tuber without being exposed to the diffuse light, showing contrast to the vigorous shoot development and mere slight formation of irregular tuber on the treated mother tubers (Plate V-A. B).

TABLE 6. Effect of light exposure treatment of tuber on the formation of sprout tuber

	Length of shoot (cm.)	Weight of shoot (gm.)	Number of shoot	Number of formed tuber	Weight of tuber (gm.)
Non-treatment	10.6	13.5	5.0	6.5	10.3
Light exposure treatment	34.0	70.0	10.4	2.0	1.6

The march of vigorous sprouts from senile tubers was also resulted by the treatment of exposing to diffuse light as similar as the gibberellin treatment.

Therefore a recovery of occurrence of the endogenous natural gibberellin in the senile tuber with this treatment will be expected quite within the bound of possibility.

To explore this possibility, the variation of gibberellin activity contained in the senile tuber were measured comparing with that in the young tuber.

According to the results represented in Figure 34, natural gibberellin content in the cortex tissues of senile tuber was conspicuously low as compared with that in the young tuber.

In the case of senile tuber exposed to diffuse light, on the contrary, a remarkable increase of gibberellin activities was recognized as expected.

Although the gibberellin content in the sprout of senile tuber was also considerably poor compared with that of the non-senile tuber, its amount resulted in remarkable rise steeply as same extent as that of young tuber with the treatment of exposing to light (Figure 35).

Consequently, as to the variation of natural inhibitor activity in the sprout of senile tuber, resulted a considerable decline with this treatment.

Accordingly a recovery of vigorous sprouting activity from the greened senile tuber seems to be, not perfectly, explicable on the basis of the fact, namely, a remarkable increase of gibberellin activity with the light exposure treatment of tuber.

Therefore the facts described above lead eventually to the conclusion that

11X10.5

115.5

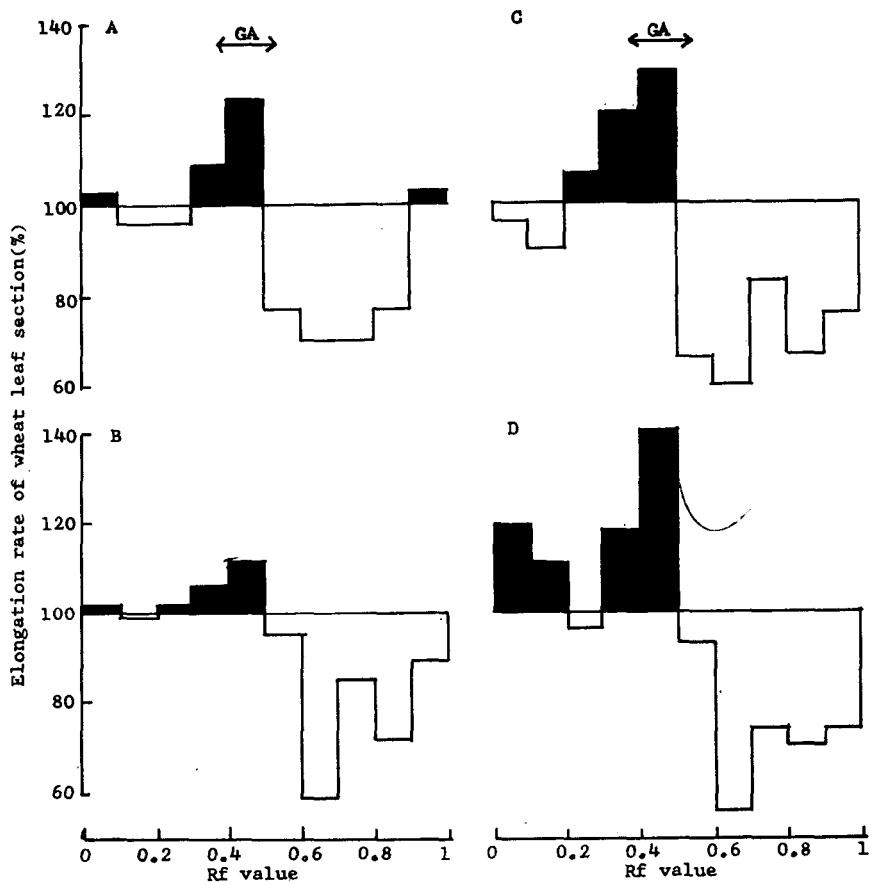


Figure 34. Variation of gibberellin activities in the potato tubers treated with light exposure.

- A—Non-treated young tuber.
- B—Non-treated senile tuber.
- C—Light exposed young tuber.
- D—Light exposed senile tuber.

the joint effect of gibberellin and inhibitor on tuber formation of potato plant may be considered as the algebraic sum of their effects, the former refers to negative effect and the latter to a positive one.

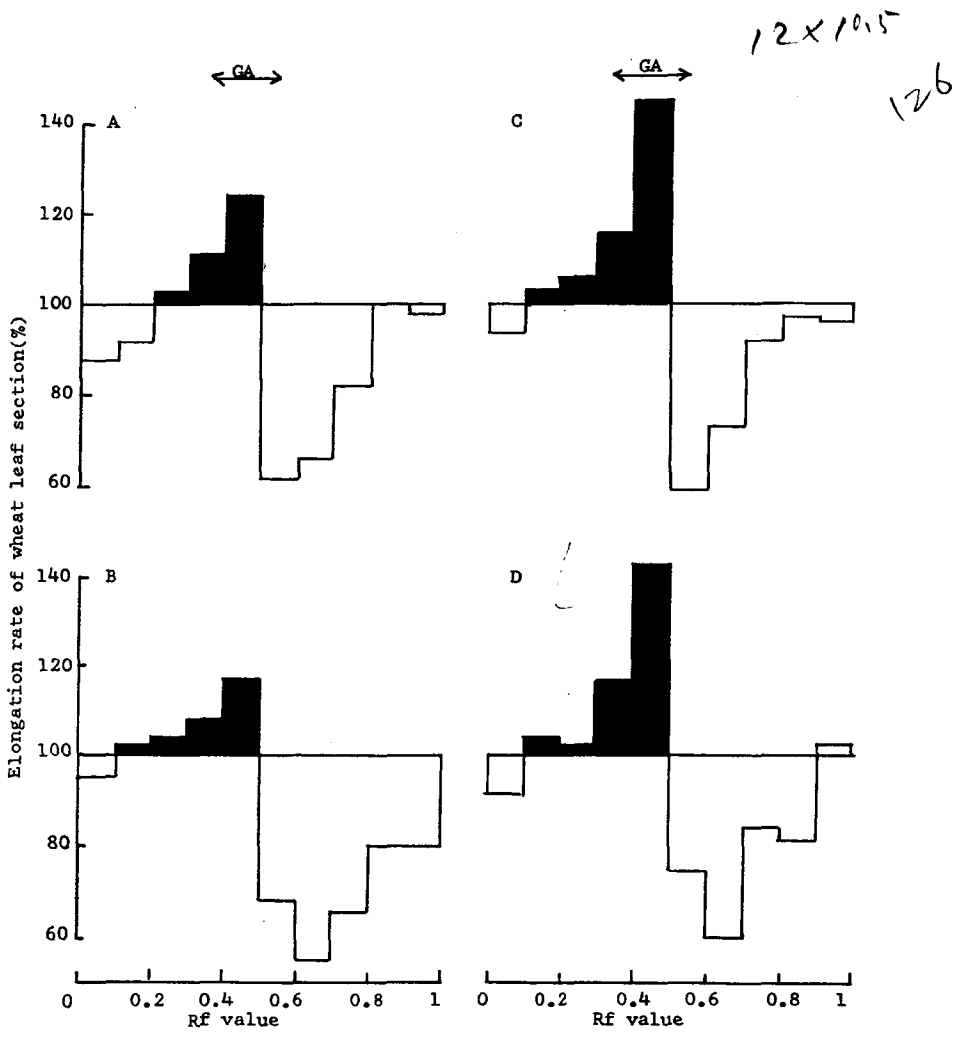


Figure 35. Variation of gibberellin activities in the potato sprouts emerged from the light exposed tubers.

- A—Sprouts obtained from the non-treated young tubers.
- B—Sprouts obtained from the non-treated senile tubers.
- C—Sprouts obtained from the light exposed young tubers.
- D—Sprouts obtained from the light exposed senile tubers.

IV Influence of auxin on tuber formation of potato plant

Informations of auxin occurred in the potato tuber have been accumulated by many workers, (10, 18, 24, 39, 40, 42, 53, 60, 63, 90), and demonstrated its physiological behaviors and distribution in the potato tuber. According to the detail investigations conducted by HEMBERG et al, the physiological functions of endogenous growth substance in the potato tuber as to the usefulness for the understanding of its dormancy were almost thoroughly outlined. With regard to the studies on the identification of auxins in the potato tuber, HEMBERG (30, 31) and PACHECO (73) reported that the major part of auxins in the potato tubers were identified as indoleacetic acid, accompanied by a slight amount of indoleacetaldehyde.

Although HAYWARD (29) has already represented the assumption that the cease of linear growth of potato stolon being followed by the initiation of tuberization seemed to be wholly associated with the change of apical dominance due to the auxin action, little attention has been virtually paid to the physiological relationships between the initiation of tuberization and variations of auxin content in the potato plant.

Therefore it is fundamental to a proper understanding of the functional pattern of auxin catabolism during the tuber forming stage of potato plant.

Materials and Methods:

The materials employed were potato varieties; Irish Cobbler, Norin No. 1, and Kamiya-imo.

Extraction of auxin from potato plant was carried out by means of the procedure somewhat similar to that of BOYSEN-JENSEN (10).

As the solvent, the peroxide free ether was chiefly used throughout the experiment. After the extraction for 48-hr with ether changing three times, the extracts supplemented with 20 ml water were combined and condensed at 50°C under the reduced pressure. Immediately after the condensation of extract in order to remove ether, aqueous residue was filtered to discard the insoluble chlorophyll in water phase, then adjusted pH value to pH 8.0 with the addition of appropriate volume of 8% sodium bicarbonate solution. Subsequently this neutrized aqueous solution was extracted with ether in order to bring the neutral fractions into ether phase and followed to again acidify to pH 2.8 with 15% tartaric acid. This acidified aqueous solution was extracted three times with ether to obtain ether solution of the acidic substances. Each ether extract was condensed to slight volume of ether solution.

After condensation of each fraction at 50°C, the samples were developed on paper chromatography with isopropanol / ammonium hydroxide / water (10 : 1 : 1).

The chromatograms obtained were cut transversely into ten equal pieces, and each piece was placed in a 4 cm petri dish. As control a piece of paper obtained from the plain chromatogram was used in the same way as stated above. Three ml of culture solution which was prepared M/50 potassium biphosphate supplemented with 2% sucrose was added to each dish. Thereafter ten pieces of 2 mm length of subapical section of *Avena* coleoptiles were soaked in the culture solution for 20-hr at 25°C in dark and their length were measured and results were expressed in terms of percentage compared with the control elongation.

Experimental results:

(1) Variation of auxin content during the normal development of potato.

a) Variations of auxin content in the stolon tips and young tubers at the tuber forming stage.

From an inspection of the result represented histographically in Figure 36, it was found two promoting areas on the chromatogram obtained from the extract of stolon tips at their linear growth stage of which Rf values are ranging from 0.6 to 0.9. Then immediately before the commencement of tuberization on the stolon tips, another promoting area was found on the chromatogram ranging from Rf 0.3 to 0.4, and it was confirmed that it is indoleacetic acid (IAA), because of essentially no difference in the Rf value of this auxin in comparison with that of IAA. In addition the result of EHRICH'S reaction of this auxin was virtually the same as that of pure IAA.

With the advance of tuber development, the rise of auxin activity identified as IAA in the young tuber were ascertained, contrary to the disappearance of auxin activity which was found on the chromatogram ranging between Rf 0.6-0.7.

As to the growth-inhibitor in the developing tuber, it started to occur soon after the disappearance of auxin as described above. The distribution of this inhibitor was found to be about Rf 0.6 on the chromatogram.

According to the investigations by BENNET-CLARK (7) and HEMBERG (33), the inhibitor extracted from the potato tuber was found at Rf 0.6-0.7 on the similar chromatogram developed with ammoniac isopropanol and determined as the inhibitor- β .

Consequently the inhibitor demonstrated in the present experiment may be the same to inhibitor- β comparing with Rf values reported by BENNET-CLARK.

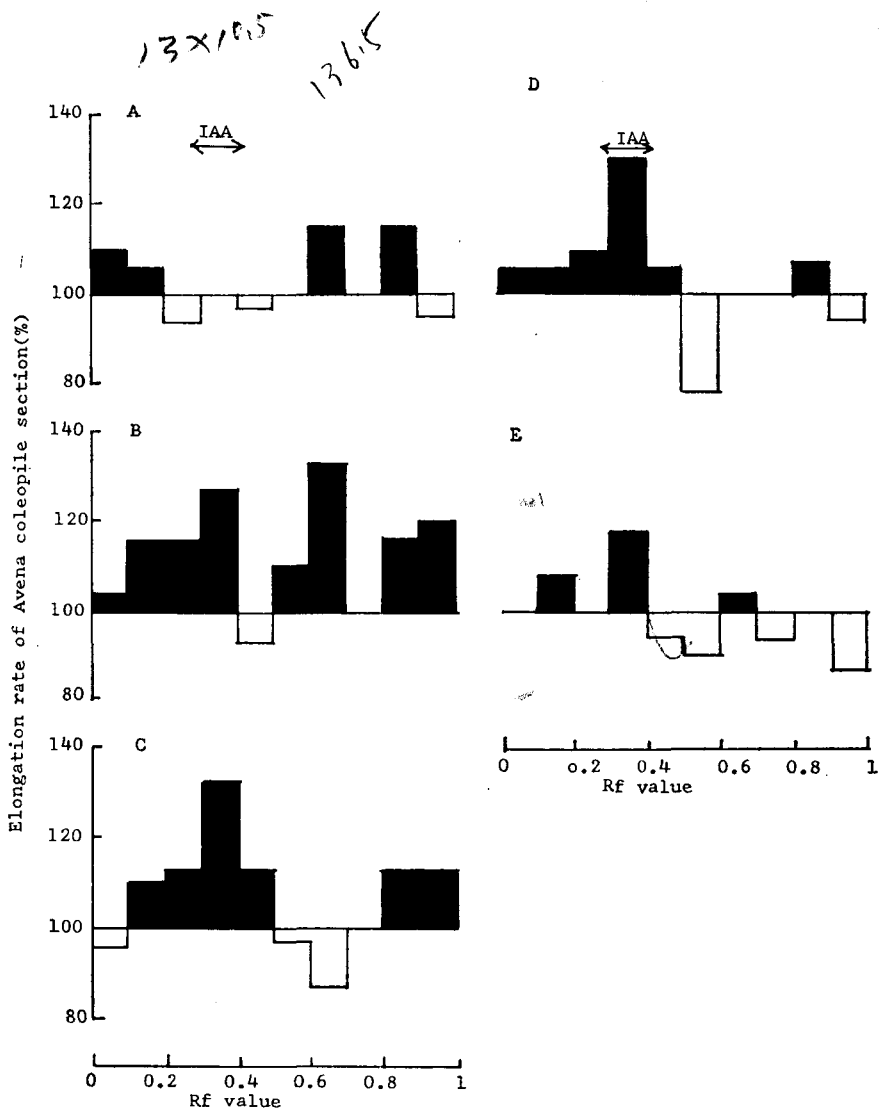


Figure 36. Progressive changes of endogenous auxin content in the stolon tips and young tubers.

A—Stolon tip at its linear growing period.

B—Stolon tip just before the starting of tuberization.

C—Stolon tip at the initiation of swelling.

D—Young tuber just after tuberization.

E—Young tuber at further developed stage.

b) Effect of different daylength on the variation of auxin content in potato leaves.

WITSH (129) and LEOPOLD (49) reported that auxin content and its formation in the leaves increased by a proportionate rise to the long day condition. A similar fact was also confirmed in the leave of potato plant.

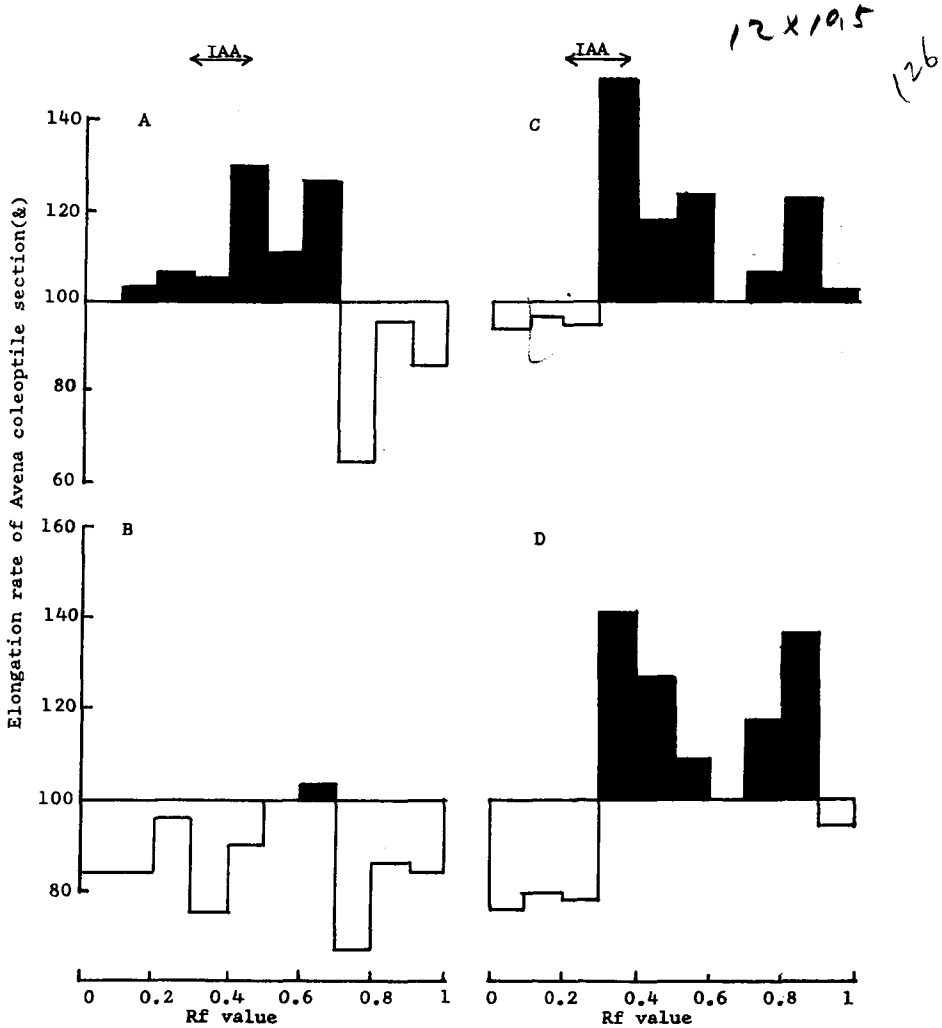


Figure 37. Effect of photoperiodic treatment on the endogenous auxin contents in the potato leaves.
 A—Norin No. 1 (16-hr daylength), B—Norin No. 1 (8-hr daylength), C—Kamiya-imo (16-hr daylength), D—Kamiya-imo (8-hr daylength).

In fact, a considerable decrease of auxin content and an increase of inhibitor one were most pronounced with the young leaves of potato plant, particularly of Kamiya-imo subjected to short daylength for ten days (Figure 37).

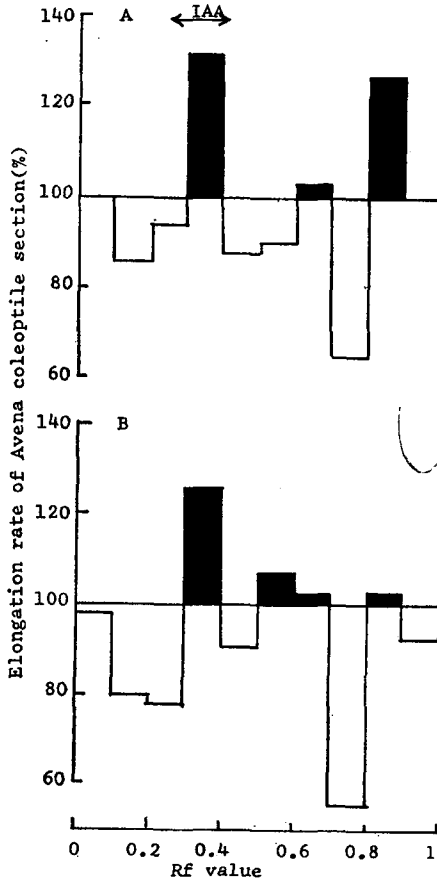
ABE and TAKAHASHI (1) are of the opinion that the response of potato plant for short day condition in order to be induced tuberization was actually more sensitive in Kamiya-imo and Norin No. 1 than that in Irish Cobbler. This fact indicated that a decline of auxin content being concomitant with an incline of inhibitor- β content in the short day plant might be likely one of the favorable condition to the onset of its tuber formation.

c) Effect of environmental temperature on the variation of auxin contents in potato leaves.

The potato plants used as materials were grown at two different environmental conditions, and all procedure for sampling was the same as experiments of natural gibberellin assay stated in the previous chapter.

Figure 38 shows that there are two promoting areas (Rf 0.3-0.4 and Rf 0.8-0.9)—former one may be considered as IAA—and an inhibiting zone (Rf 0.7-0.8) on the chromatogram (ammoniac isopropanol solvent) which were obtained from each extract of potato leaves grown at 18°C and 13°C. However a significant reduction of auxin content in the leaves was ascertained when

Figure 38. Effect of environmental temperature on the endogenous auxin content in the potato leaves.
A—18°C, B—13°C.



exposed at 13°C of environmental temperature.

As to the favorable conditions for the induction of tuberization of potato plant, WERNER has made it obvious (126, 127) that relative low temperature as an environmental condition was as much effective as short day condition. It was also recognized a marked decrease of auxin content in the potato leaves

treated with the short daylength as described in the results of foregoing experiment.

These facts proposed a tentative interpretation, therefore, that a reduction of auxin with an increase of inhibitor might be at least, though not entirely, correlated with the tuber formation.

d) Varietal difference of auxin content in the potato leaves.

Irish Cobbler, Norin No. 1 and Kamiya-imo were used as materials for the different varieties of potato plants.

It is generally recognized, there are three groups of promoting zone (Rf 0.0-0.1, Rf 0.2-0.7 and Rf 0.8-0.9) and a slight or no inhibiting zone (Rf 0.1-0.2 and Rf 0.7-0.8) on the chromatograms obtained from the potato plants of each variety (Figure 39).

In this case of experiment, a particular shape of histograms as compared with histograms obtained from other previous experiments seems to be due to different growing stage for sampling.

There can be no doubted, as a whole, that a reduction of total auxin in the leaves throughout all varieties of potato runs parallel to the increase of sensitivity for daylength in order to induce the tuberization of potato.

(2) Relation between the irregular tuber formation and auxin.

a) Progressive change of auxin content in the tubers with the advance of their senescence.

In order to see the changes of auxin activities in the cortex tissue of potato tuber which were stored for a long duration over its dormant period,

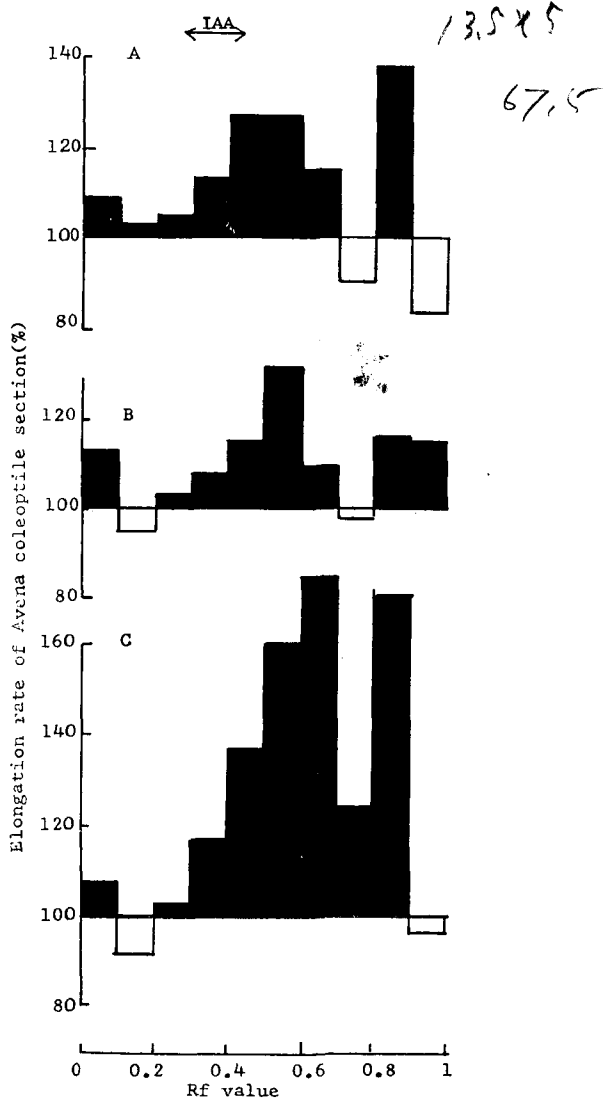


Figure 29. Varietal variation of endogenous auxin contents in the potato plants. A—Irish Cobbler, B—Norin No. 1. C—Kamiya-imo.

the present experiment was carried out.

A remarkable decline of auxin content, particularly of IAA-type auxin in the tuber, was resulted with the advance of its senility (Figure 40). This fact indicates that a irregular poor sprouting from the senile tuber may be partially depend upon the deficiency of auxin source in the tuber.

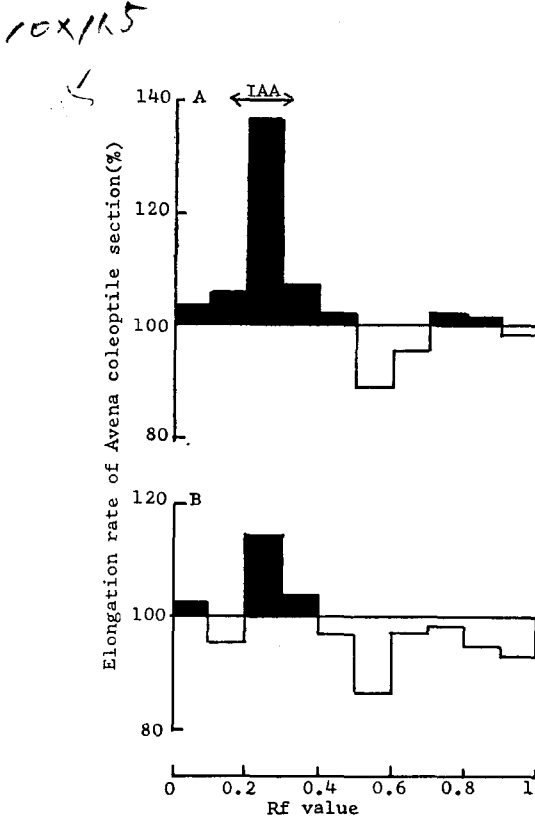


Figure 40. Progressive changes of endogenous auxin contents in the tubers with advance of their senescence.
A—Sprouting stage of tuber (January, 6).
B—Senile tuber (April, 18).

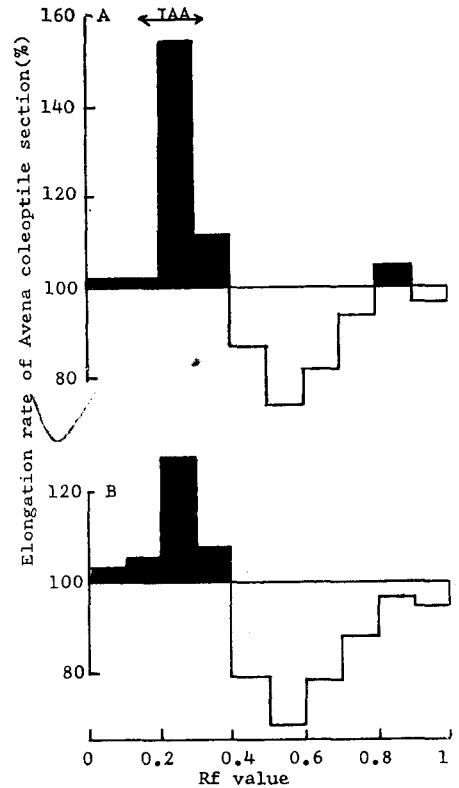


Figure 41. Variation of auxin contents in the potato sprout occurring at 13°C.
A—Sprout of non-senile tuber.
B—Sprout of senile tuber.

b) Variation of auxin content in the sprout emerged from the senile tuber at low temperature.

This experiment was carried out using the sprouts which emerged from the senile tuber planted at low temperature (13°C) as materials for auxin assay.

Comparing with the sprout grown from non-senile tuber, an auxin content in the senile sprout was considerably low and high in inhibitor content (Figure 41).

c) Variation of auxin content with exposure treatment to the diffuse light.

An increase of auxin content in the senile tuber due to exposure to diffuse light might be reasonably attributed to the rejuvenation of senile sprouting. The light exposure treatment performed in a similar way as the gibberellin experiment as stated in the previous chapter.

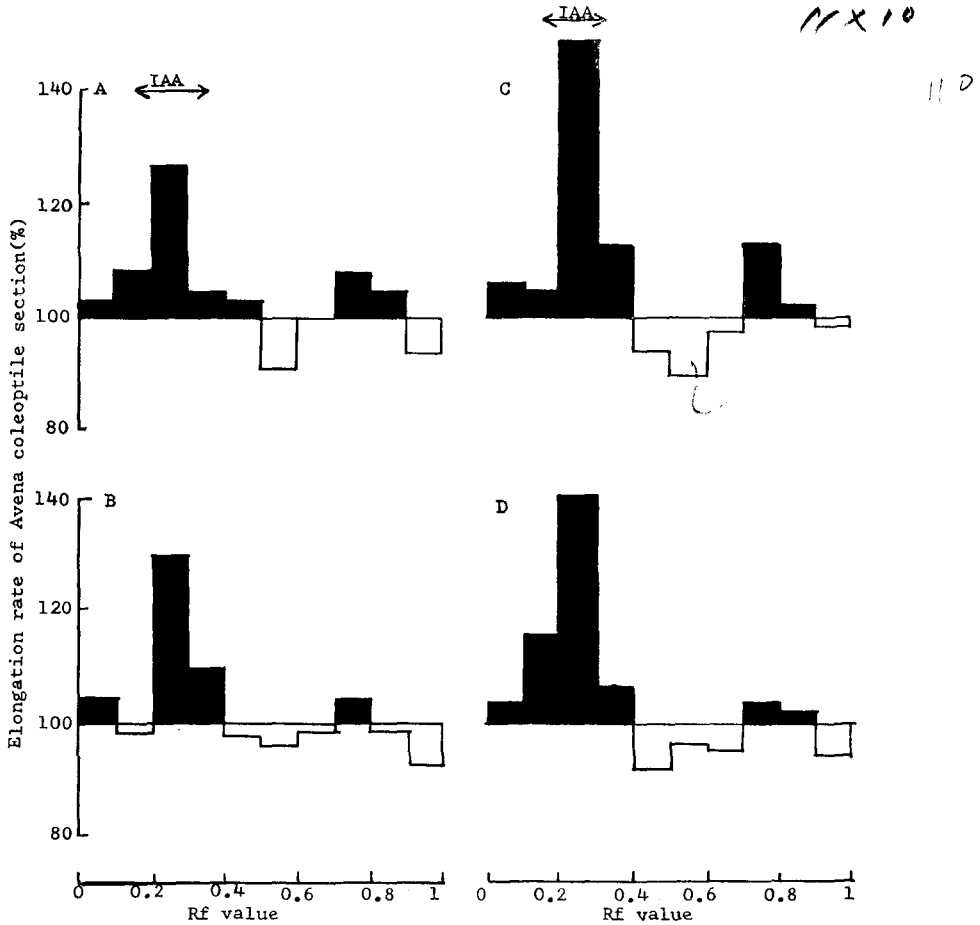


Figure 42. Variation of auxin contents in the potato tubers treated with light exposure.

- A—Non-treated young tubers.
- B—Non-treated senile tubers.
- C—Light exposed young tubers.
- D—Light exposed senile tubers.

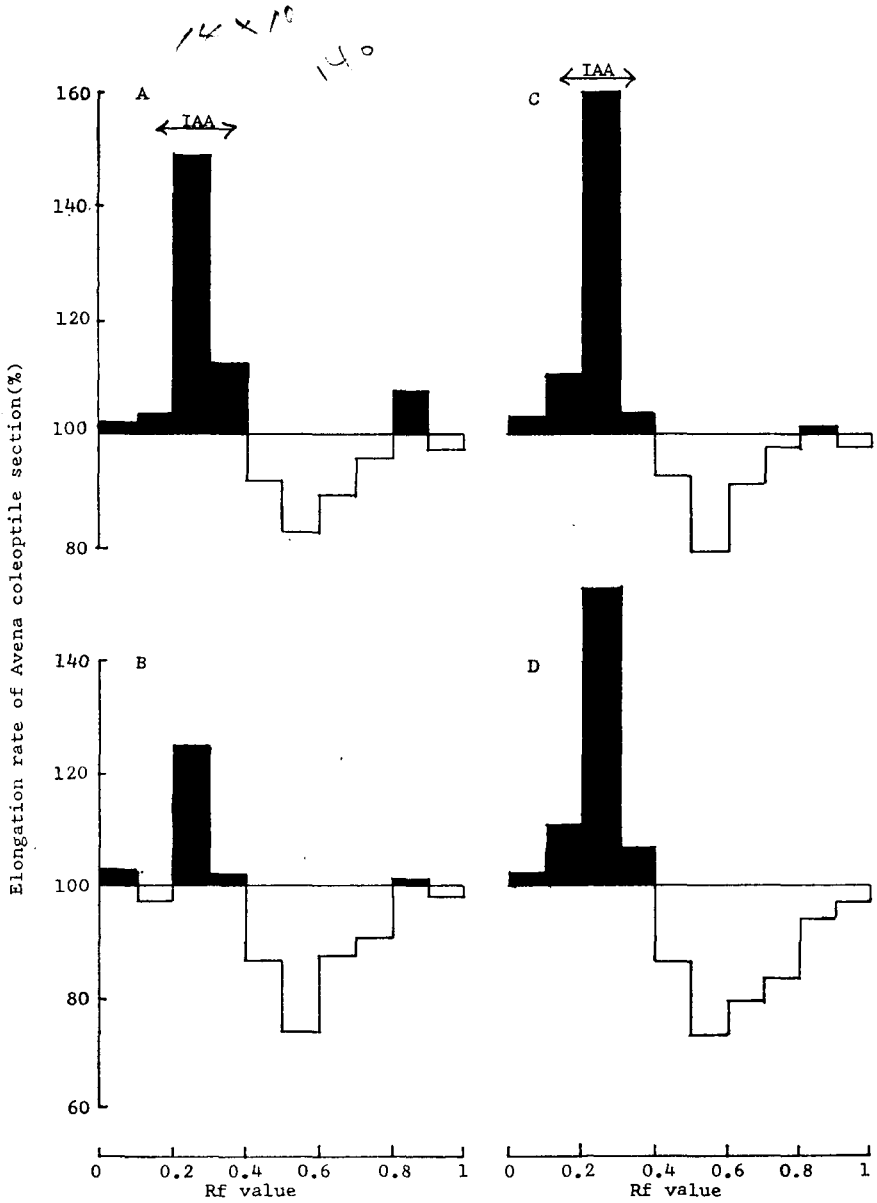


Figure 43. Variation of auxin contents in the potato sprouts emerged from the light exposed tubers.

- A—Sprouts obtained from non-treated young tubers.
- B—Sprouts obtained from non-treated senile tubers.
- C—Sprouts obtained from light exposed young tubers.
- D—Sprouts obtained from light exposed senile tubers.

According to the results shown in Figure 42, the increases of auxin contents resulted not only in the senile tuber but also non-senile tuber, which increase was wholly attributable to that of IAA in the tubers. While a slight increase of inhibitor content—presumably as inhibitor- β —was also recognized, it was rather less comparing with a noticeable increase of auxin activities.

In the case of sprouts emerged from the treated tubers, it was also recognized the similar tendency of auxin metabolism involving inhibitors in response to the light treatment as the case of tuber. Namely a remarkable accumulation of auxin, especially as IAA, and somewhat decrease of inhibitor- β were resulted (Figure 43).

V Discussion

Since the potato tuber is one of the storage organs of plants, it must be, as a matter of course, essentially provided a large amount of carbohydrates to the organ such as stolon tip where the tuber is formed. According to the results stated in the earlier papers (99, 105, 107), it was recognized a remarkable accumulation of soluble carbohydrates, particularly that of the reducing sugar in the stolon tips of potato plants prior to the initiation of tuber formation.

In the cases of irregular tuber formations such as the sprout tuber and secondary growth tuber, it was also confirmed similarly a temporary accumulation of sugar (65, 97, 108).

These facts at least may be concluded with certainty, therefore, that this accumulated sugar might evidently afford the raw materials which is necessary for the synthesis of reserved starch in the potato tuber. In addition, this tentative interpretation was thoroughly supported by the fact that a rapid decline of soluble sugar content in the stolon tips runs parallel to a gradual increase of starch accumulation immediately after the onset of tuberization (105).

On the contrary, the variations of nitrogen compounds at the earlier stage of tuber formation was not so distinguish in comparison with that of carbohydrates described in the previous papers (102, 105). So far as can be judged from the nutritional point of view, it may be very substantial that a excessive supplies of carbohydrates to the organs where tuber is formed, were the most principal factor responsible for the tuber formation.

On the other hand, it was also ascertained that the tuberization of potato plant depends greatly upon the environmental conditions such as short day-length, temperature and so on. These facts are inconsistent with the nutritional theory about tuber formation, because of unfavorable condition for the photosynthesis of carbohydrate in the potato leaves.

GREGORY (23) is of the opinion that the sugar level in the nutrient medium of stem segment culture *in vitro* is not the sole determining factor for tuber formation.

With reference to the evidence reported by CHAPMAN (14), the unfolding young leaves of potato plants in the vicinity of apical tips have rather less photosynthetic activity than the old matured leaves, but were apparently more sensitive to the short daylength responsible for tuberization than matured ones. Therefore an accumulation of sugar in the stolon tip before the onset of tuberization is not essentially the primary factor, but is one of the analytical result being consequent on the induction of tuberization.

A more detailed argument in support of this opinion can be found in the results of present investigation as to the sterile culture of potato stem segment. So far as stem segment culture of etiolated potato shoots, it might be divided into two groups with respect to the tuber forming activity on the stems, namely, the one is non-induced stem segments as to the tuberization, irrespective of the favorable condition of culture medium, and the other is induced stem segments being ready to form tuber by itself under any unfavorable circumstances. As showing in Figure 1, the rate of tuber formation on the stem segments obtained from the basal part of etiolated shoot apparently raised with proportional to the increase of sucrose concentration in the nutrient medium. Meanwhile the stem segment cut from any parts of etiolated shoots growing on the senile tubers was readily resulted in the initiation of tuberization even on the medium containing insufficient sucrose concentration. From the inspection of these facts, the tuber formation on the induced stem segment was promoted keeping pace with the increase of sucrose concentration in the nutrient medium. In other words, the sucrose concentration in the nutrient medium is a sole determining factor for tuberization, so far as the induced stem segment was used as materials. However, no tuber formation occurred irrespective of the increase of sugar content in the medium, if the apical tips obtained immediately after sprouting were employed as cultural materials. Accordingly the tuber formation of potato *in vitro* could not be decided wholly by the excessive supply of carbohydrate from the nutrient medium, but it seems to be much depend upon the physiological age of stem tissue used as cultural materials.

When the apical tips of sprouts which are physiologically younger than the basal stem segments were cultured with the application of appropriately high concentrations of auxins (NAA, or 2,4-D), the tuber formation was brought about on the apical tip of sprout, concomitant with the suppression of the linear growth of stem. Accordingly the significant availability of auxin

for the starting of tuberization on the stem pieces obtained from non-induced shoots was recognized, whereas no pronounced effect on tuberization, not only stimulative but detrimentally, in the case of induced stem segments. In other words, the linear growth of axillary shoots which emerged from the induced stem segments was slightly enhanced whenever the concentration of auxin was relatively low, and the tuber formation was not retarded. These facts might be undoubtedly interpreted that a recovery of linear growth and suppression of tuberization on the induced stem segment was not attained even after such old stem tissue was rejuvenated with auxin treatment. It seem very likely, therefore, that the action of auxin may be a primary factor in the tuber formation of potato plant.

When the gibberellin in place of auxin was applied to the culture medium, not only the linear growth of the shoots was promoted, but also the tuber formation of the potato was completely suppressed, regardless of the sugar amounts supplied in the medium.

From the experimental evidences stated above, it seems justifiable to conclude that while an excessive supply of carbohydrate to the various parts of potato stems is a favorable factor for the tuber formation, it does not seem to be the sole determining factor. Some other factor, for example, some kind of growth regulators as gibberellin may play a more significant and principal rôle in controlling the tuber formation in potatoes.

Recently many investigators have reported the evidences of occurrence of gibberellin-like substances from species of several different families of flowering plants, such as *Leguminosae*. Such occurrence holds true in the case of potato plant according to the present investigation. These facts obtained in the present experiments suggest the possibility, therefore, that the endogenous natural gibberellin in potatoes may also play an important rôle in their tuber formation.

From the results of wheat leaf test, ultraviolet light test and acid- and alkaline-treatments which were made using paper chromatogram obtained from ethanol extracts of potato tissues, it may be concluded that there are some gibberellin-like substances in the various tissues of potato plants.

On the relative amounts of natural gibberellin contained in the various parts of the tissues, the following series was recognized in order:

sprout > stem tip > tuber > middle part of stem > leaf

LOCKHART (51) has already advanced the view that apical regions of shoot in the higher plants might be considerably a locus of gibberellin production. Consequently the results of present experiments is not contradictory to LOCKHART'S assumption.

It is further important to know that the natural gibberellin shows certain variation in content in the leaf blades, according to the environmental conditions, e.g., a considerable decrease in amount was recognized as resulting from subjection to short day (8-hr. daylength) and low temperature (13°C), both of which are the most favorable conditions for tuberization of potatoes. Similarly, a decline of gibberellin activity in the tubers also resulted, keeping pace with the senility of tubers which are stored for a long duration of time over their rest period. As is seen from Table 6, the senile tubers recovered their sprouting activity, and also the rate of abnormal tuber formation of the sprout declined sharply, when such tubers were exposed to diffused light in the green house for about one month. At the end of this treatment, the amounts of natural gibberellin contained in the treated tubers and sprouts were found to have increased markedly.

Furthermore it was also clearly confirmed the fact that a retardation of irregular tuber formation on the senile tuber by the soaking treatment with gibberellin solution. In the light of above consideration, it would be consequently expected the possibility that the endogenous natural gibberellin in the potato may conceivably behave similar way as that of the exogenous gibberellin. In the majority of case, it was actually resulted indeed the distinguished decline of the endogenous gibberellin activity in the tissue of potato plant being placed under the unfavorable condition for the induction of tuberization. These facts suggested that natural gibberellin in the potato plants seems to be one of the inhibiting factors for the tuberization of potato.

Subsequently this assumption is also compatible with the evidences that the tuber formation on the induced stem segment was suppressed by the application of gibberellin in the medium.

In fact, in the light of consideration as to the stimulation of tuberization, the experimental results stated above demonstrated a clear-cut relationship between the variations of endogenous gibberellin contents in the plant and that of environmental conditions such as the day length and temperature.

On the other hand, it was apparently evident that the tuberization on the non-induced potato plant was stimulated by the treatment with 2-chloroethyl trimethylammonium chloride (CCC), which acts antagonistically to gibberellin (72).

In addition, an inhibiting effect of gibberellin treatment on tuberization was also recovered by the retreatment with CCC. Consequently CCC may serve to alter the behavior of tuber formation, so far as the initiation of tuber formation.

Although a conclusion as to the natural stimulus of tuberization in potato

plant must be drawn with caution, it would be assumed the presence of natural anti-gibberellic substances such as inhibitor- β .

It is unfortunate that sufficient data were not collected to explain with greater certainty the tuber forming stimulus except a recent MADEC's information (56).

With regard to the functional behaviors of gibberellin, it may at least be concluded with certainty that the gibberellin serve as an inhibitor for the tuberization, not only exogenously but also endogenously.

With reference to the variation of natural auxin level established in the potato plant at its tuber forming stage, they are real resemble to the variation of natural gibberellin at the same stage.

However there was no significant effect of synthetic auxin treatment on the growing plant, except the stimulation of tuberization with a relatively high concentration of auxin treatment *in vitro*.

It have been made evident by the several workers that the occurrence of gibberellin activity required the presence of auxin (47, 71, 115). Accordingly it was also assumed that a marked inhibiting effect of gibberellin on the tuberization may probably enhanced, at least partially, dependent upon the exogenous or endogenous supply of auxins at their suitable concentration.

Judging from the results stated above, it may be reasonable to assume that the growth regulating substances, such as natural gibberellin, auxin and inhibitor contained in the potato would be closely connected with its tuber formation.

Under the environmental conditions such as the short daylength and low temperature, the formations of small amounts of natural gibberellin and large amounts of natural inhibitor in the potato plants were resulted, which in turn caused the induction of tuber formation. On the other hand, due to the counteraction between gibberellin and inhibitor which are formed in the potato plant under the condition of long day or high temperature, the potato plant continued its vegetative development without any tuber formation. Therefore the fact described above lead eventually to the conclusion that the joint effect of gibberellin and inhibitor on the tuber formation of potato plant may be considered as the algebraic sum of their effects, the former refers to negative effect and the latter to a positive one. At the same time, of course auxin itself may also be responsible for the tuberization, but indirectly.

Summary

With the hope that some clue might be found as to the relationship between the formation of potato tubers and the effects of some growth substances on it, the present investigation was undertaken, with special reference to the tuberization mechanism from the physiological point of view. The materials used were "Irish Cobbler, Norin No. 1, Kamiya-imo and D-805 varieties of potato plant (*Solanum tuberosum* L.). The experimental results obtained may be summarized as follows:

In order to see strikingly the effects of various nutrient substances and growth regulators on the tuber formation, the stem pieces of potato plants were cultured aseptically on the synthetic media. The tuber formation of the tuber-induced stem segments obtained from the etiolated shoots of potato plant was enhanced with raising the concentrations of sucrose or ratio of sugar to nitrogen content in the medium, however no significant effect on the growth pattern of tuberization was recognized. On the other hand, only gibberellin completely inhibited the tuber formation of induced stem segments followed to stimulate the linear growth of lateral shoots of them, but no appreciable change in tuberization response to auxin was resulted.

Stem pieces cut from the non-tuber induced sprout yielded no tuber, irrespective of the most favorable condition of the nutrient medium.

In this case of culture, it resulted in the suppression of linear growth of stem and subsequently formed tuber, when the supra-optimal concentrations of NAA or 2, 4-D was externally applied to the medium.

It was also found that some gibberellin-like substances are contained in the various tissues of potato plants. On the relative amounts of these natural gibberellin contained in the various parts, the following series was recognized in order:

sprout > stem tip > tuber > middle part of stem > leaf

The activities of the natural gibberellins and auxins showed certain variation in the leaves, according to the environmental conditions, e.g. a considerable low activity was recognized when the plants were subjected to short-day (8 hours daylength) and low temperature (13°C) condition, both of which are the most favorable for tuberization of potato.

Similarly, decline of gibberellin and auxin activities in the tubers also resulted, keeping pace with the advance of senescence of tubers which were stored for a long duration of time over their rest period.

These senile tubers recovered their sprouting activity, and also the rate

of abnormal tuber formation on the sprout declined sharply, when such tubers were exposed to diffused light. The increases in activities of gibberellin and auxin in the treated tubers and sprouts were found. However the abnormal tuber formation on the senile tuber was completely suppressed with the gibberellin treatment, whereas the auxin treatment of senile tuber could not exert no appreciable effect on the abnormal tuberization of sprouts. Therefore it may be reasonably assumed that the increase of gibberellin level in the potato plant may be one of the principal factors to counteract with the tuber formation.

On the other hand, the amount of the inhibitor- β in the tuber displayed a tendency to increase by such treatment as short daylength and low temperature or by advance of senescence of tuber, which are favorable condition for the tuber formation. It was also ascertained an interest fact that the growth promoting activity of gibberellin would be masked by this inhibitor. Therefore the inhibitor- β may play an important rôle in the stimulation of tuberization. Eventually it would be concluded that the joint effect of gibberellin and inhibitor on tuber formation of potato plant may be considered as the algebraic sum of their effects, the former refers to negative effect and latter to a positive one. At the same time, auxin itself may also be responsible for the tuberization, but indirectly.

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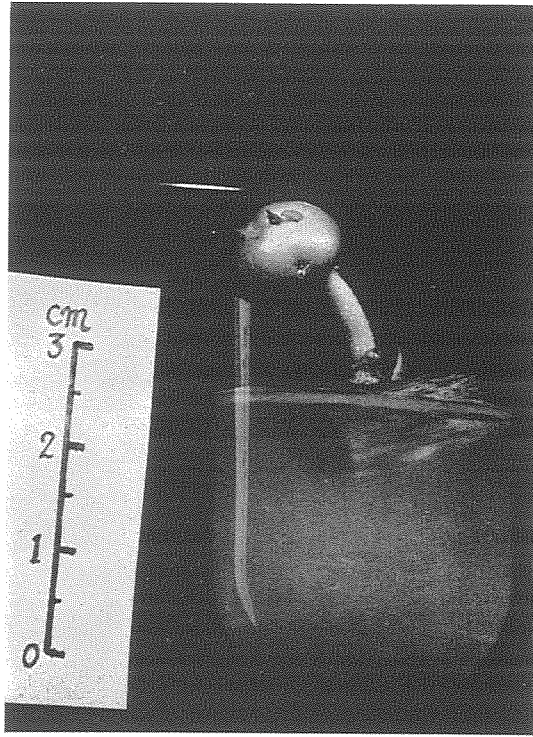
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Plate I Tuber formation of potato stem segment on the nutrient medium (20-day old culture).

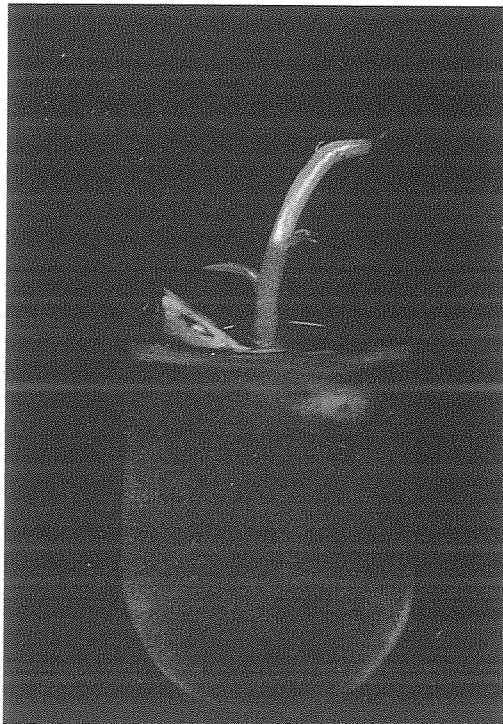
A—Tuber formation of stem segment on the medium supplemented with 4% of sucrose.

B—Lateral shoot formation of stem segment on the medium supplemented with 2% of sucrose.



9.7 x 7
67.9

[A]



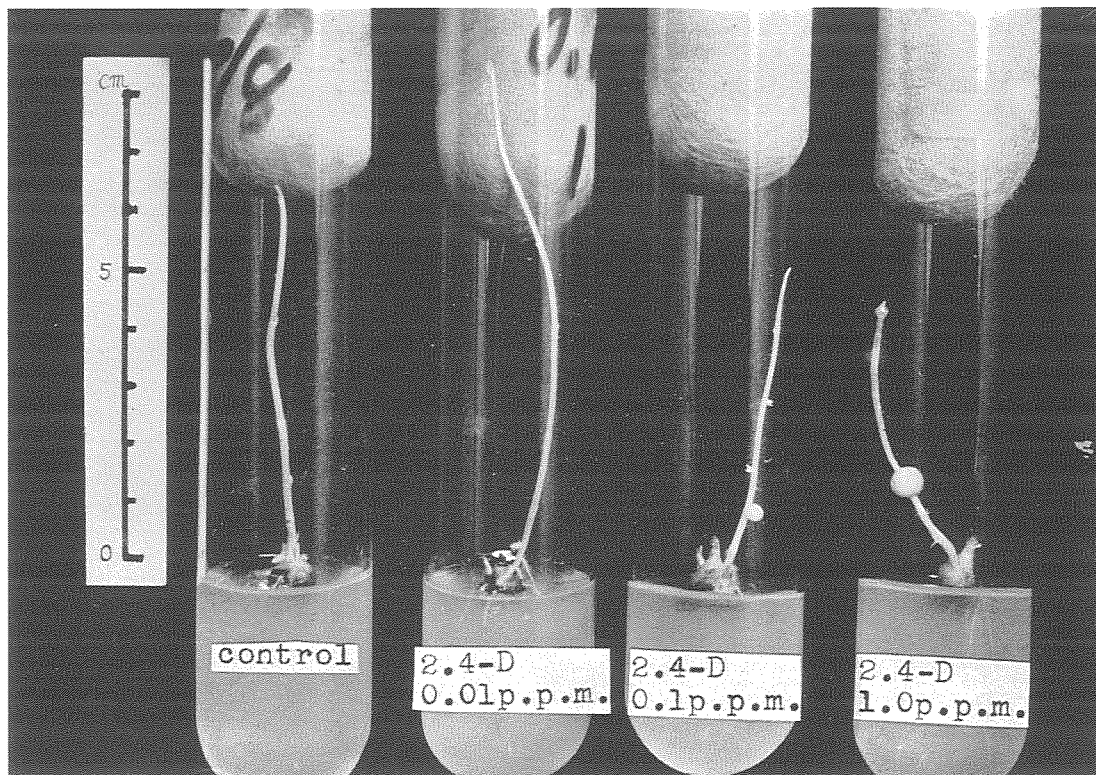
7.6 x 6.6
63.7

[B]

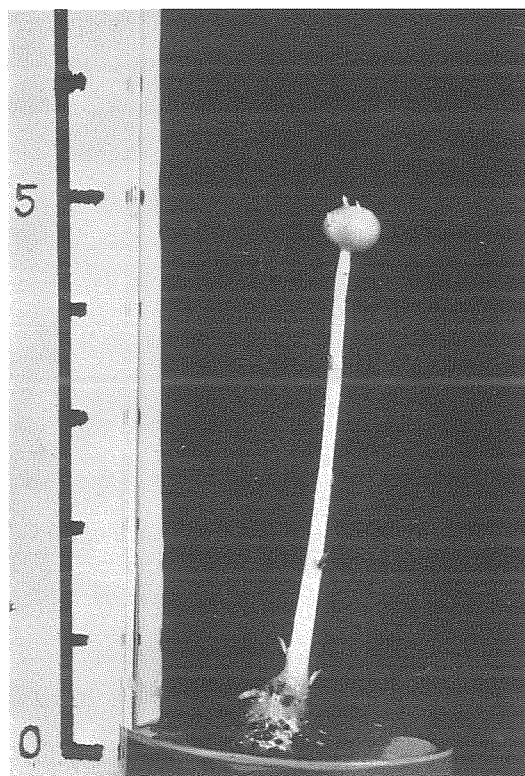
Plate II Effect of 2, 4-D on the tuberization of apical pieces of potato sprout (40-day old culture).

A—Promotion of tuberization and inhibition of shoot elongation with 2, 4-D treatment.

B—Tuber formation on the apex of shoot emerged from excised sprout, due to the significant effect of 2, 4-D.



[A]



[B]

10.2x6.9
70.38

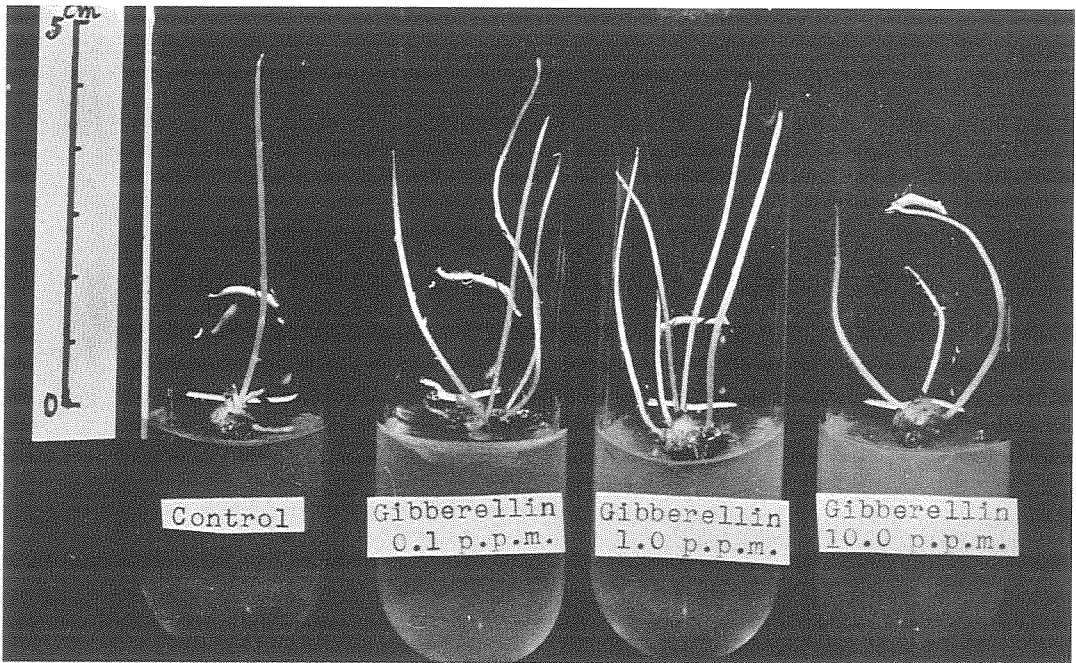
Plate III Tuber formation and shoot elongation of potato stem pieces on the nutrient medium.

A—Promotion of shoot elongation on the apical pieces of potato sprout due to the application of gibberellin in the medium.

B—Inhibition of tuberization on stem segment of potato shoot by the gibberellin application in the medium.

87X14

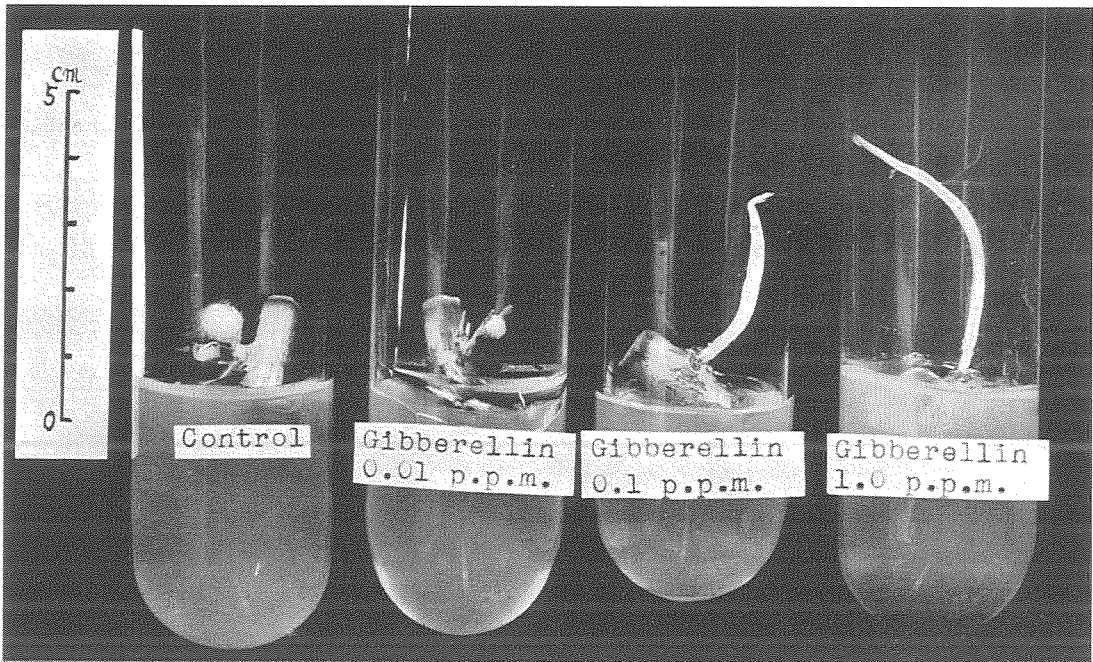
1212



[A]

87X143

124.41



[B]

Plate IV Stolons of potato plants on their growing stage.

A—Stolons were obtained from the plant growing under the long day condition (16-hr daylength).

B—Stolons were obtained from the plants growing under the short day condition (8-hr daylength).

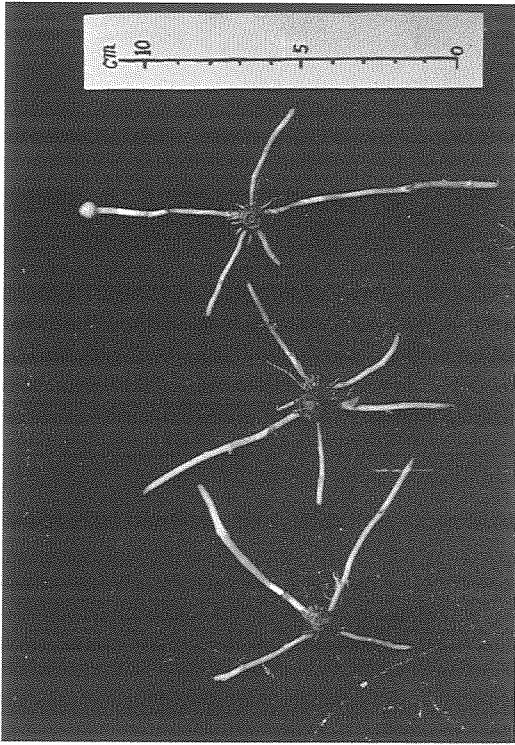
C—Stolons were obtained from the short day plants which were sprayed with 50 ppm gibberellin solution at the beginning of short day treatment.

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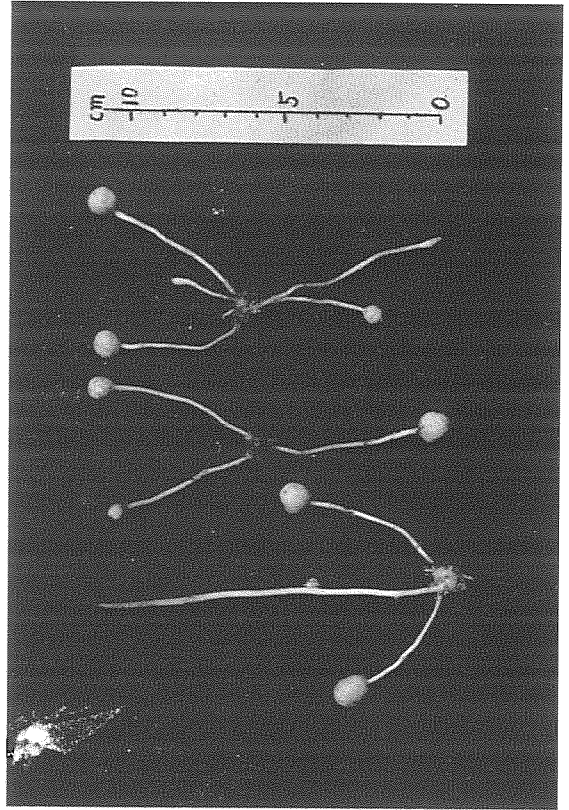
6.8 x 9.8
6664

7.3 x 10.6
199.38

Plate IV

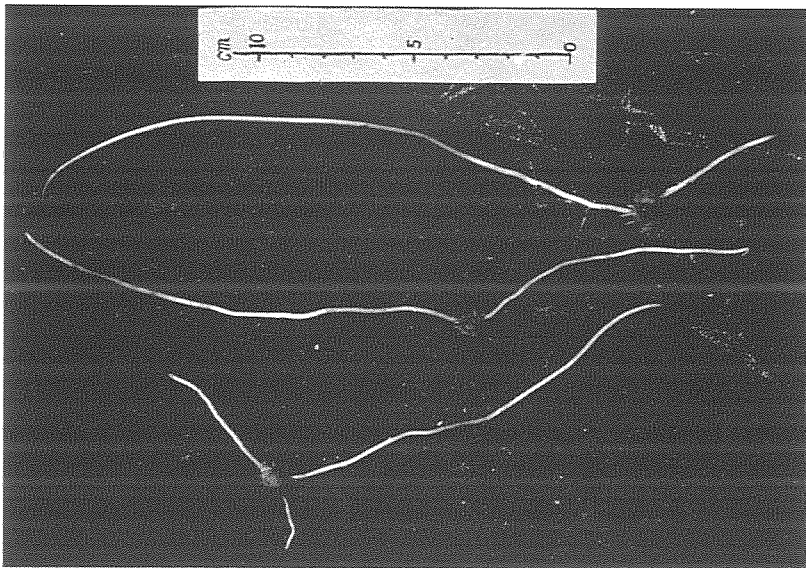


[A]



[B]

1.6 x 7.5
79.5



[C]

Plate V Irregular tuber formation of potato plant.

A—Picture shows sprout tuber formation on the senile tuber without treatment of light exposure.

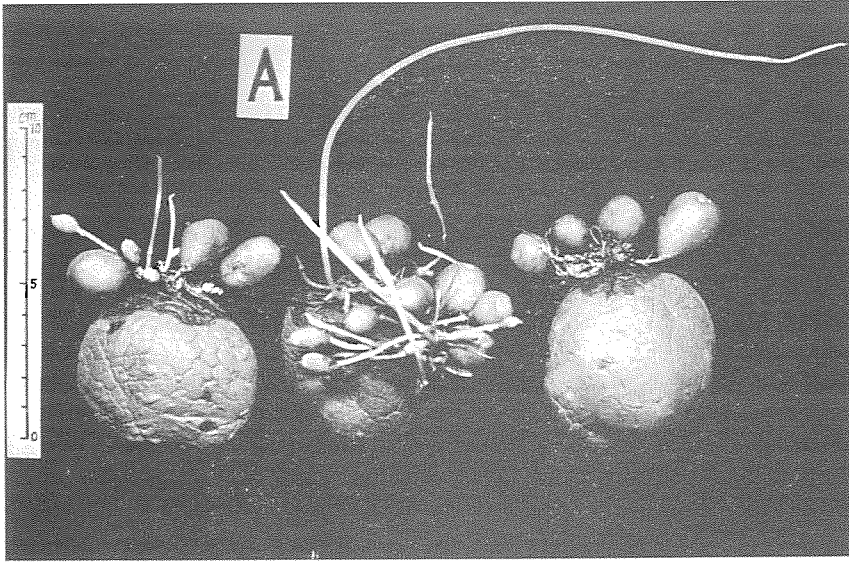
B—Etiolated shoot with slight formation of sprout tubers emerged from the semile tuber.

C—Effect of gibberellin treatment on the sprout tuber formation of potato. left—non-treatment with gibberellin, right—treatment with 25 mg/l of gibberellin.

7.3 x 11.2 81.76

Y. OKAZAWA

Plate V

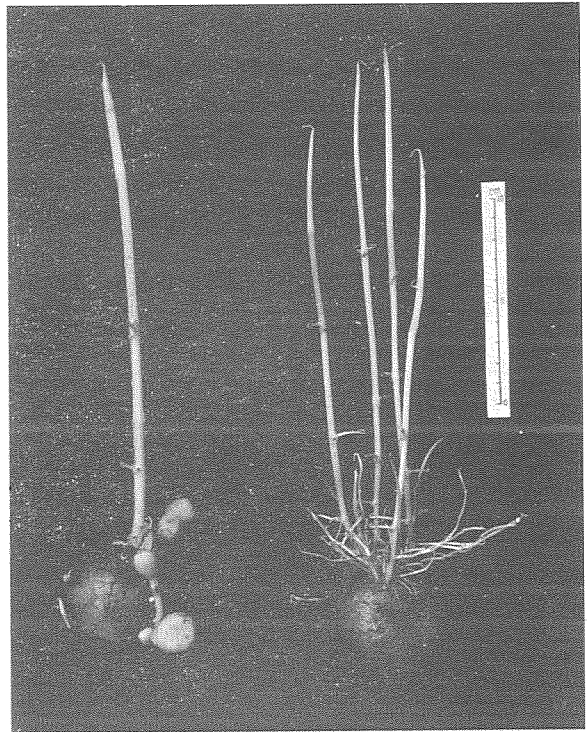


6.7 x 11.6 77.72

[A]



[B]



7.7 x 7.6 73.72

[C]