PHYSIOLOGICAL STUDIES ON THE MECHANISM OF TUBERIZATION IN POTATO PLANTS

Part 2. On the physiological relationship between the habit of the sprout and the tuber formation of potato plant *in vitro*

By

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Although the effects of environmental factors on the tuber formation of potatoes have been extensively studied, virtually no adequate explanation has been given on the mechanism of the tuber formation.

The understanding of the mechanism of tuber formation in *Solanum tuberosum* L., is not only a matter of scientific interest from the viewpoint of plant physiology, but also is practically important in the field of agriculture. BERNARD (1902) in his classical work, suggested that the tuberization of potato plant is brought about by the symbiotic relationship between the plant and a fungus. At present this fungus theory has not been entirely discarded. DOSTEL (1945), DEUSE (1947) and ITO and KATO (1951) are of the opinion that the tuber formation of potato plant may be correlated to certain stimulus like a plant growth hormone.

On the other hand, the view expressed by DRIVER and HOWKES (1943) is that the tuber formation of potato plant may be controlled easily by raising the plants under the adequate combination of light and temperature conditions. According to their opinion, namely, the combination of short photoperiod and low night temperature, gives a good yield of tuber, while the condition of long photoperiod and high night temperature results in no tuber formation.

Recently MES and MENGE (1954) have reported that the tuber formation on the stem segments of potatoes is promoted when the plant is cultured in darkness at a high concentration of sugar in the nutrient medium, while the conditions of relatively low sugar concentration of the nutrient medium and in the light encourages the leaf development.

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In the previous paper, the writer has reported that the small tubers could be enabled to form spontaneously on the stem segments under the sterile condition, and in that case the higher the sucrose content in the nutrient medium, the easier the tuber formation.

The main purposes of the present investigation are: (1) to see the effects of the physiological ages of the sprouts on the tuber formation on the short stem pieces of potatoes cultured under sterile condition, and (2) to find out if the environmental factors, such as cultural temperature and pH values of nutrient medium, share in the tuberization of stem segments.

Materials and Methods

In the present experiments, the early variety "Irish Cobbler" obtained from the Central Foundation Seed Potato Farm, Hiroshima, Hokkaido was used as the material. To keep the stem pieces sterile on the nutrient medium during each experient, the following treatments were made. The potato tubers which had been stored in the cellar for the period from the end of October 1955 to the beginning of February 1956, were dipped into a Uspulun solution diluted with 800 parts of water for 30 minutes, and then planted in the wooden box filled with moist soil. They were allowed to stand in a dark room at 25–27°C for two or three weeks. When the etiolated sprouts were about 25 cm. in height, they were harvested and the apical tips and stem segments of the sprouts were cut off to be used for the experiments.

These tissues were then tranferred into sterile jars, and all subsequent treatments were carried out in a sterile chamber under the illumination of an ultraviolet lamp.

The sterilization procedures of the living plant tissues were made as follows. At first the experimental materials were dipped in a diluted Antiformin solution about an hour for the sterilization, then this solution was drained off and the materials were washed carefully five times with sterile, distilled water, then the materials were transferred to sterile Petri dishes in which two sheets of filter paper were applied to remove excess moisture.

The materials were then planted with one tissue segment each on the nutrient media filled in the test tubers.

The agar which had been used to solidify the culture medium,

was washed carefully for about five days with distilled water to wash out any trace of impurity such as auxin, and then added to the nutrient medium to make 0.5% in the final concentration. The cultures were generally incubated on shelves in a thermostat (20°C).

The basal 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 (1951) are as follows:

Mineral salts solution:

$Ca(NO_3)_2 \cdot 4H_2O$	500 mg
KNO_3	125 mg
$MgSO_4 \cdot 7H_2O$	125 mg
KH_2PO_4	125 mg
$FeC_6O_5H_7 \cdot 5H_2O$	10 mg
Water up to	1000 ml

The trace element mixture had the following composition.

H_2SO_4 (sp. gr. 1.83)	0.5 ml
$MnSO_4 \cdot 4H_2O$	3000 mg
$ZnSO_4 \cdot 7H_2O$	500 mg
H_3BO_3	500 mg
$CuSo_4 \cdot 5H_2O$	25 mg
$Na_2MoO_4 \cdot 2H_2O$	25 mg
Water up to	1000 ml

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

Experimental results and discussion

(1) Influence of 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 *in vitro*. The stem segments obtained from the etiolated sprouts were cultured on four portions of the basic nutrient medium in the presence of 8% sucrose in final

concentration, of which pH values were adjusted at 4.0, 5.0, 6.0 and 7.0 respectively.

TABLE 1. Influence of pH of the nutrient medium on the tuber formation of stem segment.

pH of nutrient medium	Number of cultures	Rate of tuber formation		
		7 days	14 days	21 days
4.0	19	15.7 %	47.3 %	56.1 %
5.0	20	30.0	80.0	90.0
6.0	20	65.0	100.0	100.0
7.0	20	45.0	90.0	100.0

(cultured at 23°C)

As is seen from table 1, the pH optimum for the development of stem segment and the tuber formation of potato tissues cultured on the nutrient medium 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 the axillary shoot on the stem segment, but also the retardation of the onset of tuber formation were observed.

In previous papers (TAGAWA and OKAZAWA 1948, 1955), 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 role in the new tuber formation. Likewise evidences have accumulated to show that the optimum pH of these enzymes ranges between pH 6.0 and 7.0 inclusively (ARREGUIM-DOZANO and BONNER 1949, MARUO 1950).

Accordingly, it seems reasonable to assume that the variation of pH of the cultural medium may itself control the tuberization of stem segments not directly but indirectly being correlated with the nutritional metabolism of the stem segments.

(2) Influence of temperature of culture on the tuber formation of the stem segment.

In the present culture experiment, 8% of sucrose in final concentration was added to the basic nutrient medium, and the stem segments were cultured at the three different temperatures of 12 ± 1°C, 23 ± 1°C and 27 ± 1°C.

As is seen from table 2, the tuber formation on the stem segments

cultured at 23°C started a little eariler than those cultured at other temperatures. In the culture at 12°C, the emergence of an axillary bud on the stem segment was delayed somewhat by the swelling of the sprout due to the increase of the relative humidity in the culture vessel. When cultured at 27°C, on the contrary, the linear growth of the axillary shoot which emerged on the stem segment was promoted a little, which in turn caused the retardation of the new tuber formation on that segment.

TABLE 2. Influence of cultural temperature on the tuber formation of the stem segment.

Culture temperature	Number of cultures	Rate of tuber formation			
		7 days	14 days	21 days	28 days
12±1°C	15	0 %	20 %	93.3 %	93.3 %
23±1°C	15	80	100	100	100
27±1°C	15	33.3	86.6	93.3	100

(cultured at pH 6.0)

It has come to be generally accepted as a fact that the most favorable temperature for the development of potato plants, particularly for their tuberization, might be at about 18°C. However, on culturing the stem segments at two different temperatures of 18°C and 26.5°C, MES and MENGE (1954) found on difference on the mode of tuber formation between the two.

So far as the present culture experiments *in vitro* 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.

(3) Influence of the various ratios of carbohydrate to nitrogen in nutrient medium on the tuber formation *in vitro*.

According to TAGAWA and OKAZAWA (1952 b.), a marked accumulation of reducing sugar in the apical part of the stolon was ascertained with the growth of potato plants, followed by the swelling of the stolon

tips. At this time the onset of accumulation of storage starch grains was recognized in the tuber cells, particularly in the vicinity of the vascular ring. In the case of an abnormal tuber formation, such as the sprout tuber, the sugar which was formed by the action of amylase in the mother tuber, translocates to the new sprout, and this sugar accumulates there in the form of starch. At the same time the soluble nitrogen content in the new sprout decreased (TAGAWA and OKAZAWA 1952 b).

In the present experiment, as is shown in table 3, the apical tips of etioloted sprouts were rich in carbohydrate, but those in the basal parts of the sprouts were poor.

The nitrogen content in the etiolated sprouts was inversely related to the carbohydrate contents.

TABLE 3. Carbohydrate and nitrogen contents in the various parts of the stem segment in the etiolated sprouts.

	Stem segments			
	1st node	2nd node	3rd node	4th node
Water content	94.4 %	96.4 %	95.5 %	95.2 %
Total carbohydrate content	317.6 mg	337.1 mg	253.1 mg	267.1 mg
Total nitrogen content	43.9 mg	36.4 mg	31.7 mg	27.9 mg
Ratio of carbohydrate content to nitrogen content	7.3	8.2	11.1	13.1

(mg/dry weight g)

By comparing the ratios of carbohydrate to nitrogen in the tissues as shown in table 3, 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 easily, have a higher value of this ratio. So far as can be judged from the data stated above, the ratio of carbohydrate to nitrogen in the stem segments seems to be highly correlated to the tuber formation. In the next experiments, the culture tests were carried out under varied combinations of two different sugar concentrations and three nitrogen concentrations.

As seen from the experimental results presented in table 4, the tuber formation on the stem segments is at least partly, if not entirely,

controlled by the ratio of carbohydrate to nitrogen in the nutrient medium. Namely, the time of initiation of the tuber formation became earlier as this ratio increased. At the later stage of the culture, however, it is hard to find any close correlation between the tuber formation and this ratio.

TABLE 4. Influence on the tuber formation of the stem segment of varied ratios of carbohydrate to nitrogen content in the cultural medium.*

Sugar conc. in medium	Nitrogen conc. in medium	Number of cultures	Rate of tuber formation
2 %	0 p.p.m.	20	50 %
2	50	20	10
2	250	20	0
8	0	17	100
8	50	18	100
8	250	20	65

* The test plants were cultured at 23°C and pH 6.6. The rates of tuber formation were checked at 18 days after plants were set in the culture.

(4) Influences of the physiological age of tubers and of the sprouts of potatoes on the tuber formation.

OKAZAWA (1955) has recently reported the fact that the tuber formation on potato stem segments *in vitro* is closely related to the sugar concentration in the nutrient medium. This will furnish some substantiation for the results obtained recently by MES and MENGE (1954). While GREGORY (1956) is of the opinion that the sugar level in the nutrient medium is not the sole determining factor for tuberization, he concluded that the tuber formation might be promoted by some certain stimulus which arises in the above ground parts of potato plants under specific condition of temperature and photoperiod and has a nature able to be translocated across the grafting point.

On the other hand, it has long been known that irregular tuber formation, such as sprout tubers are formed if or when the mother tubers are stored for a long period or under the condition of unfavorable high temperature [DE VRIES (1878), WELLENSICH (1924)].

According to TAGAWA and NAKA (1949) and TAGAWA and OKAZAWA (1952, a) the formation of an abnormal tuber such as a sprout tuber,

might be attributed to the changes of reserve substances in the mother tuber and of the respiration rate of the sprout and tuber due to the senility of mother tuber developing the storage. However, much remains to be explained concerning the mechanism of tuber formation, particularly the relationship between the physiological ages of the sprouts and the tuber formation should be made clear.

In order to obtain further information on the different mode of tuber formation on the apical tips which were obtained just after the germination of the tuber and on the basal stem segments which were obtained from the etiolated sprouts of about 25 cm. in length, the present experiment was carried out. The results are shown in table 5.

TABLE 5. Difference of the tuber formation on the apical tips and on the basal stem segments of sprouts.*

Materials cultured	Sucrose concentration in nutrient medium (%)					
	2	4	6	8	10	12
Basal stem segments	−	±	+	+	+	−
Apical tips	−	−	−	−	−	−

* The test plants were cultured at 23°C and pH 6.0; (+) in the table means tuber formation and (−) no formation, respectively.

In the case of the culture with the basal stem segments, the tuber was formed easily concomitant with the increase of sugar concentration in the nutrient medium. In the case of the apical tip segments, on the contrary, no tuber formation resulted regardless of the increase of sucrose concentration in the nutrient medium (Plate 2).

TABLE 6. Tuber formation on the stem segment obtained from the different node of the same sprout.*

Number of node	Number of culture	Rate of tuber formation			
		7 days	14 days	16 days	21 days
1st node	20	0 %	6.6 %	6.6 %	10.2 %
2nd node	19	0	8.3	50.0	65.0
3rd node	18	0	7.6	65.0	75.0
4th node	19	0	53.3	80.0	100.0

* Each culture medium contained 8% sucrose; cultures made at 23°C and pH 6.0.

When the segments obtained from the various parts of the same etiolated shoot were cultured, the tuber formation itself as well as the size of new tuber formed is inversely related to the distance from the tips at which the stem segment was obtained (Table 6).

The tuber formation on the stem segments of the sprout of the senile tuber stored in a cellar for about 6 months, was considerably easier and more rapid than that on the stem segments obtained from young tuber just after the termination of rest period (Table 7).

TABLE 7. Tuber formation on the stem segment obtained from the different nodes of sprout on the senile tuber.*

Number of node	Rate of tuber formation	
	7 days	14 days
1st node	0 %	30 %
2nd node	35	80
3rd node	65	100
4th node	65	100

* Each culture medium contained 8% sucrose; cultures made at 23°C and pH 6.0.

In other words, tuber formation on the apical stem segment is very difficult regardless of the sugar concentration in the culture medium. This fact may be due to the difference in their physiological ages between each stem segment respectively, obtained from the same sprout. At the same time, the senility of the tuber from which the stem segments were obtained for culture test, may have an influence in the tuber formation.

The view expressed by SHIBUYA (1950, 1951) is that the aerial tuber formation of potato plant in darkness may depend upon the apical dominance of the sprout, which in turn is related to the auxin content in the potato plant.

Judging from the results obtained in the present investigation, it seems quite reasonable to assume that the physiological age of the stem segment may be of importance in the production of the potato tuber as an internal factor.

On the other hand, some cultural conditions, such as sugar concentration, pH value, the ratio of carbohydrate to nitrogen in nutrient

medium and culture temperature, also play parts in tuber formation of potato plants as the environmental factors.

Summary

The present investigation was carried out to study the influences of the environmental and internal factors on the tuber formation on the stem stegments cultured under sterile condition. The experimental results obtained may be summarized as follows.

(1) When the stem segments obtained from the etiolated sprouts were cultured at 23°C and pH 6.0, small new tubers were formed most easily. It does not seem very likely that the environmental conditions described above are the sole essential factors for the tuber formation.

(2) The apical segments of the sprouts which were obtained immediately after the emergence of potato tubers, produced no formation of any new tubers *in vitro*, regardless of the sugar concentration in the nutrient medium. The tuber formation on the stem segments of the etiolated sprouts was inversely related to the distance from the apex at which the stem segments were obtained.

(3) It seems quite reasonable to assume from the facts stated above that the physiological ages of the sprouts and the tubers may be of important significance in respect to the tuber formation on the stem segments *in vitro*.

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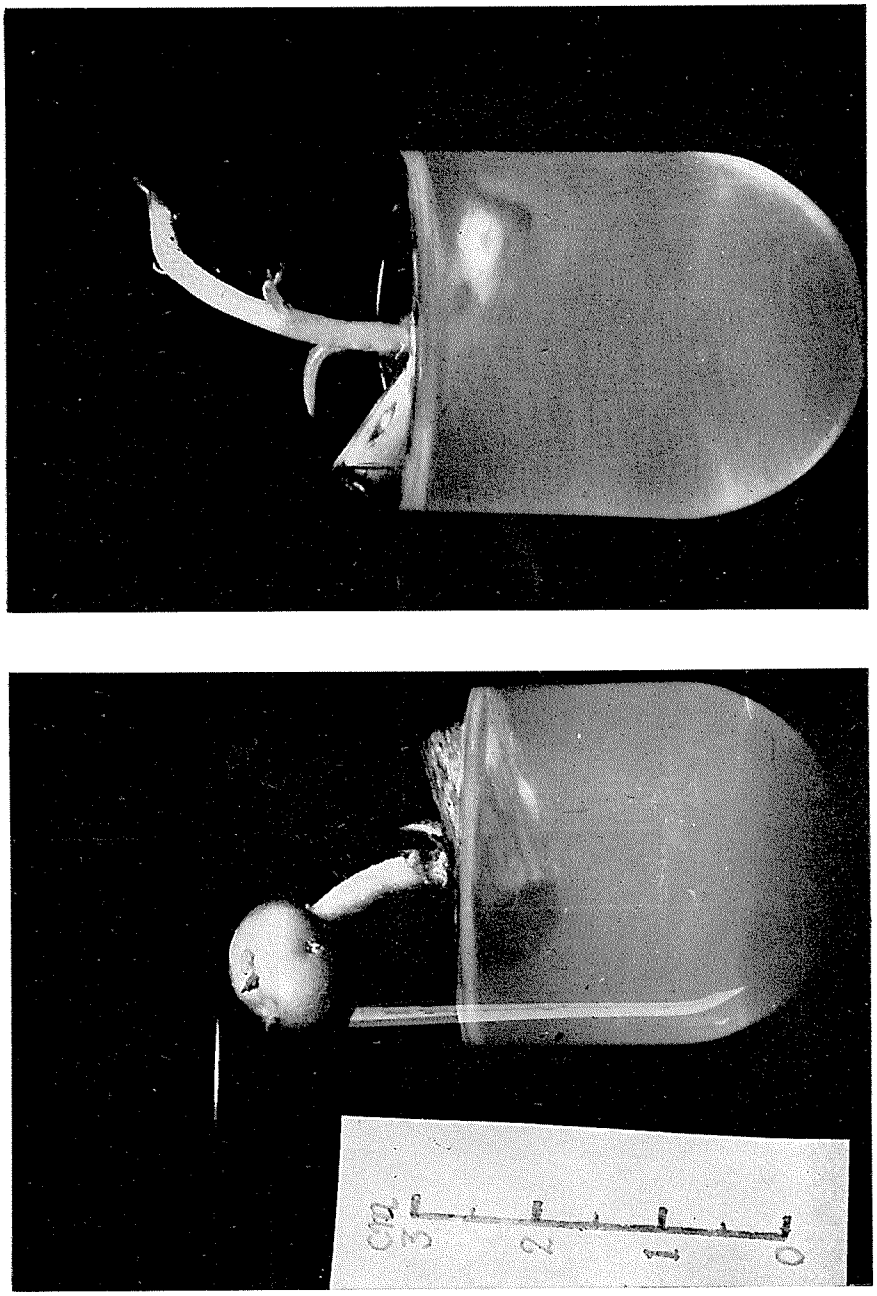
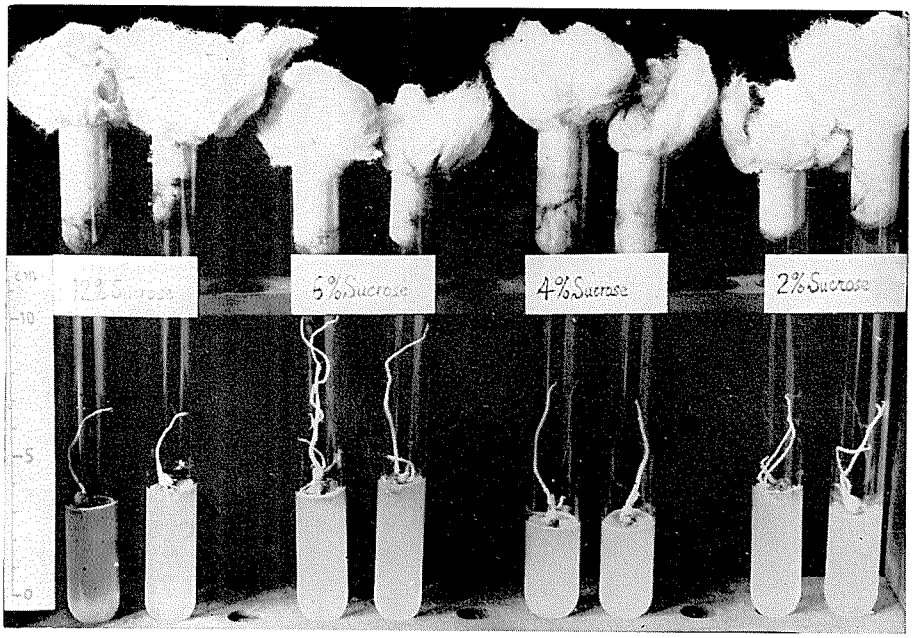


The cultures of stem segments on the basic nutrient medium containing 8% sucrose (left) and 2% sucrose (right) at the culture age of 30 days.



The cultures of the apical tips of the sprouts obtained just after the germination, on the nutrient mediums containing various concentration of sugar. This picture was taken culture age of 30 days.

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Errata

Page	Line	Delete	Insert
185	Tab. 3	253.1 mg 267.1 mg	353.1 mg 367.1 mg
186	Tab. 4	pH 6.6	pH 5.6
187	Tab. 5	12 —	12 +