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PHYSIOLOGICAL FACTORS IN THE TUBEROUS ROOT FORMATION OF THE SWEET POTATO PLANT

By

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In the previous reports (1—3), the senior author showed that the young tissues of roots, favored with the supplement of growth hormone, carbohydrates and sufficient nitrogen, are stimulated into the tuberous form even after considerable growth.

The purpose of this paper is to describe the relationships of those factors with tuberous root formation, estimating the amount of those substances in the plant tissues quantitatively.

Experiments

1. The general tendency of the tuberous root formation in the sweet potato stem cuttings.

It is indicated in Table 1 that the general tendency of tuberous root formation in the successive nodes of sweet potato stem cutting is basipetally in descending order (average percentage of tuberous roots of the apical nodes being 40 per cent and that of the basal nodes less than 20 per cent).

Table 1. General tendency of the tuberous root formation. Five nodes stem cuttings of Norin-No. 5 sweet potato plant, pinched above the first mature leafed node, were set in the field on Aug. 1 in a horizontal position and examined on Nov. 5.

Average length * of lateral shoots						Average ** number of roots					Average *** number of tuberous roots					Average weight of tuberous roots					
Node number						Node number					Node number					Node number					
1	2	3	4	5	Total	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	Total
106.0	88.9	58.0	34.7	—	287.6	3.6	4.0	3.8	3.7	6.5	1.7	1.7	1.5	1.2	0.5	105.8	73.0	46.5	25.0	21.2	271.5

* Lateral shoots over 10 cm long.

** Roots over 5 cm long.

*** Tuberous roots over 1.0 cm in diameter.

The weight of the tuberous roots of the successive nodes is in the same order, shoot growth being also in the same order.

2. Distribution of the native growth hormone, carbohydrates and nitrogenous constituents and the stem cuttings of sweet potato plant.

a). *Distribution in the nine nodes stem cuttings. Experimental procedure:* The amount of the growth hormone, carbohydrates and nitrogen in the successive nodes of the stem cuttings were determined. Also, those contained in the leaf-blades of the successive nodes were determined.

Estimation of growth hormone: Thirty samples from the respective nodes (6g) and thirty leaf-blades or 50g of the leaf-blades from the respective nodes were collected and extracted with ether in the cold (0°C) and estimated by the *Avena* curvature test.

Determination of carbohydrates and nitrogen: Carbohydrates were determined by the Somogyi method and nitrogen by the semi-micro Kjeldahl method.

Results: The amount in the stem. It is shown in Fig. 1 that the amount of the growth hormone contained in the successive nodes (per 30 samples) is in the following descending order (III, apical portion, I and V, VII, IX), the amount of carbohydrates being basipetally in the ascending and nitrogen in the descending order.

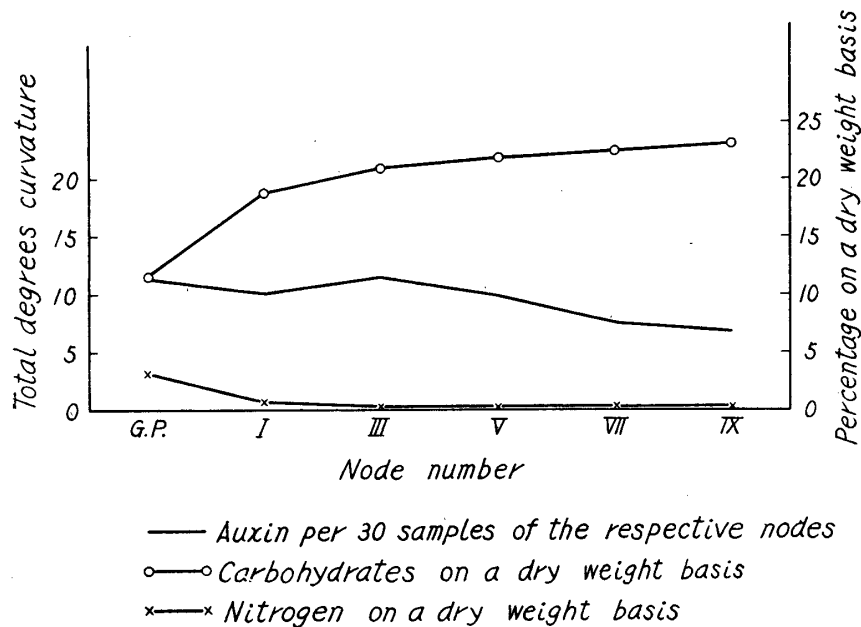


Fig. 1. Amount of auxin, carbohydrates and nitrogen in the successive nodes of the stem cutting.

The amount in the leaf-blade. The amount of the growth hormone in the leaf-blades of the successive nodes expressed on a fresh weight basis (50 g samples) is basipetally in the descending order, the amount of carbohydrates

expressed on a dry weight basis being basipetally in the ascending and the amount of nitrogen parallel with the border line (Fig. 2).

The amount of growth hormone expressed on the basis of an individual whole leaf-blade (per 30 leaf-blades) is the resultant of both factors, the leaf area and the hormone content of the unit volume of tissues. The abrupt ascent of the content on a fresh weight basis is shown at the younger nodes. Except for the last basal node, the area of an individual leaf enlarges parallel with the node number (Fig. 3). It is apparent that as leaf matures, the area of leaf-blade increases and the hormone synthesis per unit volume of tissues

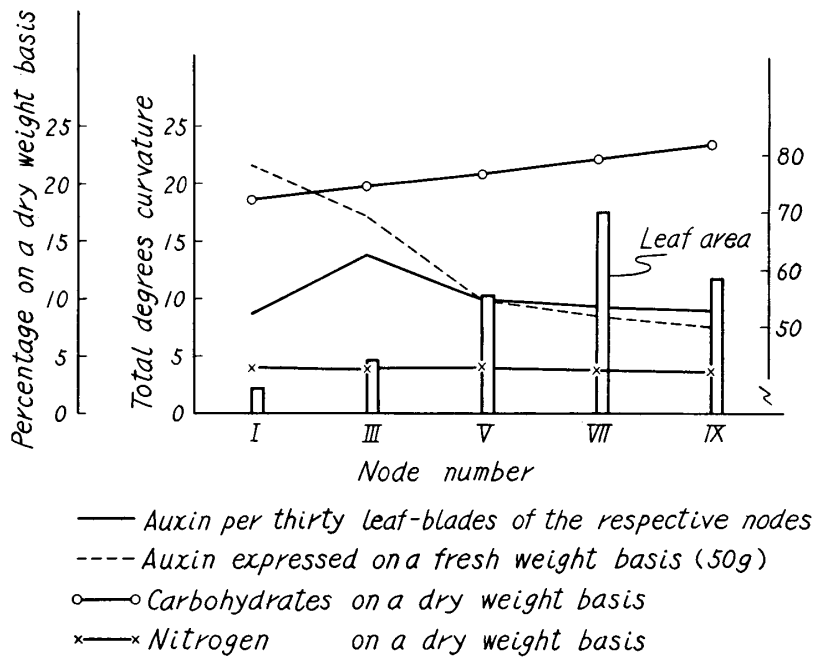


Fig 2. Amount of auxin, carbohydrates and nitrogen in the leaf-blade of the successive nodes of the stem cuttings.

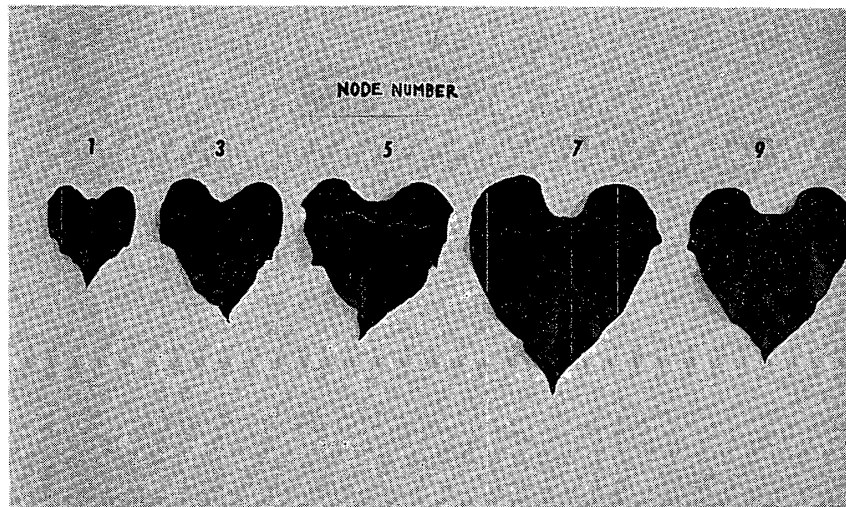


Fig 3. Leaf size of the respective nodes of nine nodes stem cuttings.

decreases.

It is indicated in Fig. 2 that the amount of growth hormone in the successive leaf-blades expressed on an individual whole weight basis is in the following order (III, V, I, VII, IX).

b). *Effect of the synthetic growth substances upon the distribution of the growth hormone in the stem cuttings.*

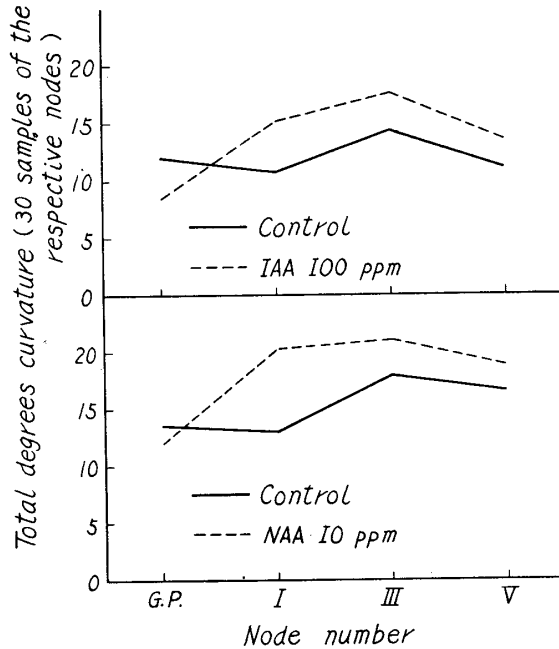


Fig 4. Effect of the synthetic growth substances (NAA and IAA) upon the distribution of the growth hormone in the stem cuttings.

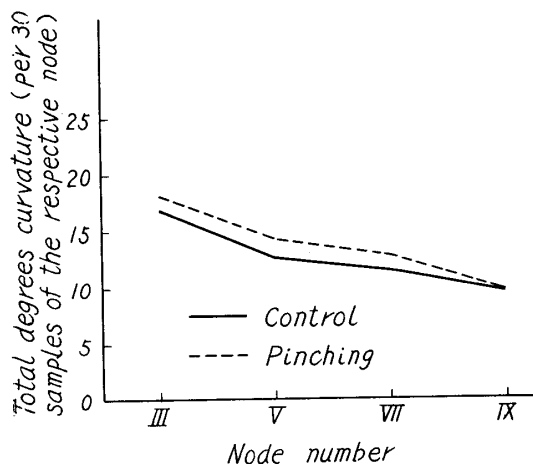


Fig 5. Effect of pinching upon the distribution of the growth hormone in the stem cuttings.

Experimental procedure: Two basal nodes of five nodes stem cuttings were immersed for 24 hr in Indoleacetic acid (IAA 100 ppm) or Naphthaleneacetic acid (NAA 10 ppm) solution.

Results: It is apparent from Fig. 4 that the amount of growth hormone expressed on a fresh weight basis in the cuttings much increased by the external supplement of the synthetic growth substances. The basal immersion of the cuttings, however, induced no significant change in the profile of the nodal hormone content. Localized increase caused in response to the basal absorption was scarcely found in the profile.

c). *Effect of pinching upon the distribution of growth hormone in the stem cuttings.*

Experimental procedure: Nine nodes stem cuttings were pinched above the third mature leafed node. After 20 hr, the inserted cuttings were pulled out and examined.

Results: The amount of growth hormone in the cuttings expressed on a fresh weight basis increased acropetally in the ascending order. It is apparent that this order is concerned with the responsiveness to pinching as shown

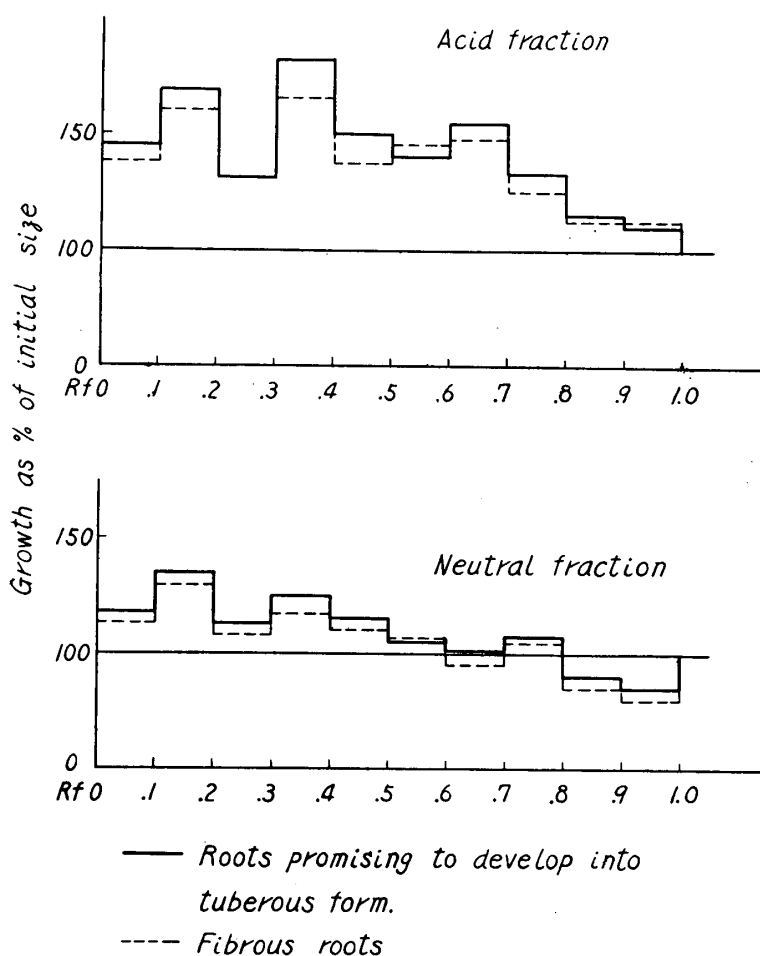
by the sprouting tendency of the secondary shoot growth (Fig. 5).

3. The root development and the native growth hormone.

a). The amount of growth hormone in the young roots.

Experimental procedure: The amount of growth hormone in the young roots about 5 cm long was determined by the Avena straight growth test. Roots showing signs of developing into fleshy form were collected from the single node stem cuttings of the sweet potato plant in contrast with the fibrous roots.

One gram sample (40 roots collectively) was quantitatively extracted with peroxide free ether in the cold for 20 hr. After three extractions, the total extracts were concentrated to 50 ml and were fractionated into acid and neutral fractions according to Hemberg (4). Each fraction was chromatographed in the solvent, (isopropanol, ammonia and water in a ratio of 8:1:1). After 16 hr of solvent migration the paper was dried and cut into ten equal sections. The sections were eluted with 2 cc of 3 per cent sucrose solution. Ten



The solid line gives the growth of the controls.
Fig. 6. Chromatograms of ether extracts of young roots per 1 gram sample (40 roots collectively).

coleoptile sections were placed in each dish in the dark room at 25°C and their growth was measured after 24 hr.

Result: It is apparent from Fig. 6 that the hormone content in the roots promising to develop into tuberous form is higher than that in the fibrous roots.

The data presented in Fig. 6 indicate that there are two major peaks representing two different hormones in the chromatograms of the extracts of sweet potato roots. One corresponds to the Rf of IAA and the other to the Rf of Accelerator α . In neutral fraction the inhibitor is found between Rf 0.8 and Rf 1.0.

b). The amount of growth hormone in the developing roots.

Experimental procedure: The amount of growth hormone in the young:

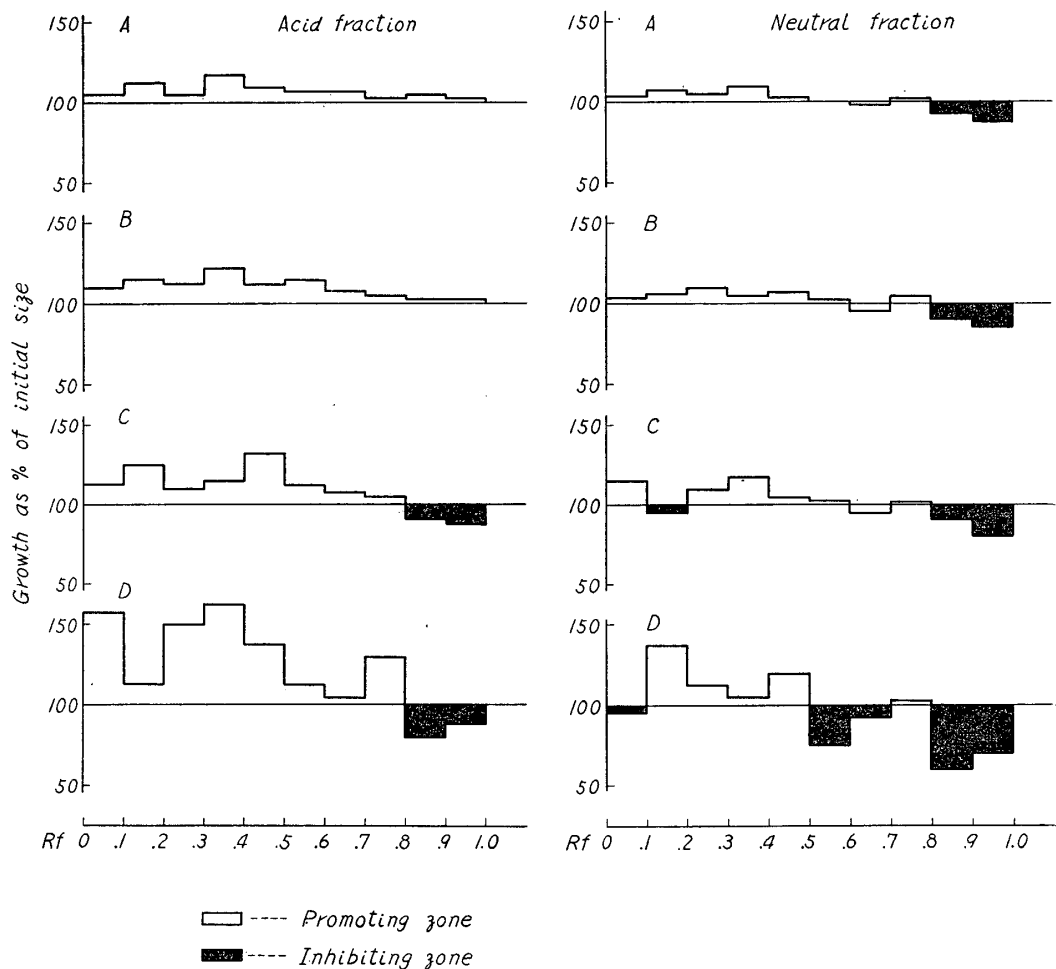


Fig 7. Diagrams showing the distribution of growth promoting substances on paper chromatograms from roots (5 roots) of sweet potato.

- a...Young fibrous roots
- b...Matured fibrous roots
- c...Whip-shaped transitory roots
- d...Developing tuberous roots

fibrous roots, matured fibrous roots, whip-shaped transitory roots, which failed to develop into tuberous form, and in the developing tuberous roots from a single node stem cuttings was respectively determined. Five tuberous roots inclusive of thickened middle portion and exclusive of the proximal slender portion were collected with the identical portions of the roots of the other types.

Result: It is shown in Fig. 7 that the amount of the thickening growth activity is correlated with the amount of the growth hormone in the developing root. In Fig. 7, two major peaks are found in the chromatogram and it is shown that the growth hormone which increase in amount in the developing root of the sweet potato plant is IAA.

4. Role of leaf-blade and the synthetic growth substance in the tuberous root formation.

Experimental procedure: Single node (the fourth node) stem cuttings with or without leaf-blade were tested for tuberous root formation. Cuttings were immersed for 20 hr in water or Indoleacetic acid (IAA 100 ppm) solution.

Result: Water immersed cuttings without leaf-blade were all dead and IAA solution immersed cuttings without leaf-blade were all alive, retaining leaf petiole and induced roots, but no new shoots and no tuberous roots developed (Fig. 8).

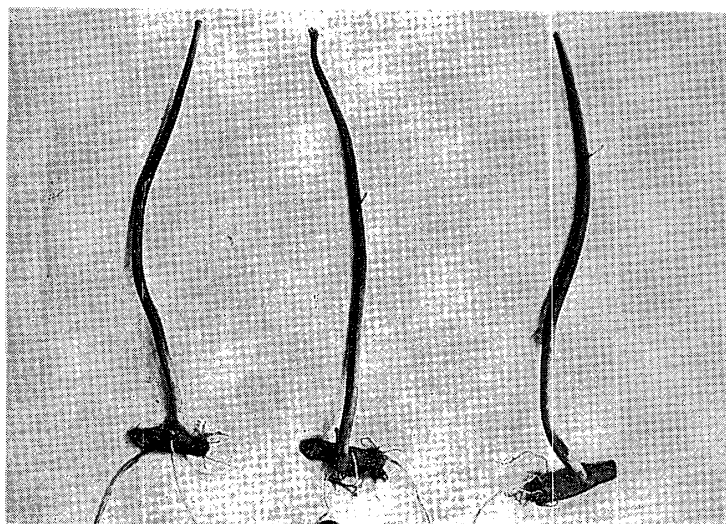


Fig 8. Rooting and retained leaf-petiole on the single node stem cuttings, removed leaf-petiole and immersed in IAA (100 ppm) solution before insertion.

Roots, tuberous roots and new shoots developed from the cuttings with leaf-blade, and the addition of IAA so remarkably invigorated those development that the roots thickened immediately at or near the proximal end point (Table 2, Fig. 9). Relative responses of the shoot growth and tuberous root development are parallel (correlation coefficient between the weight of shoot

Table 2. Effect of the growth substance (IAA) on the tuberous root formation of single node stem cuttings. Single node stem cuttings of Norin-No. 5 sweet potato plant were set in the field on Aug. 5 and examined on Nov. 5.

Treatment	Length of lateral shoot cm	Weight of lateral shoot g	Number of roots	Number of tuberous roots	Weight of tuberous roots g
None	81.5	160.0	5.7	3.0	75.0
IAA 100 ppm	98.0	180.0	7.5	4.8	107.5

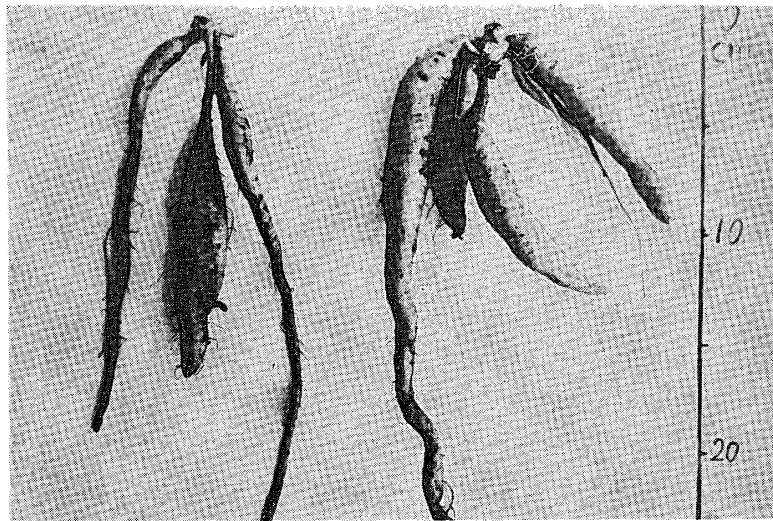


Fig 9. Development of tuberous roots with or without presence of IAA

Left: No presence of IAA

Right: In the presence of IAA; roots beginning to enlarge just at the proximal end point.

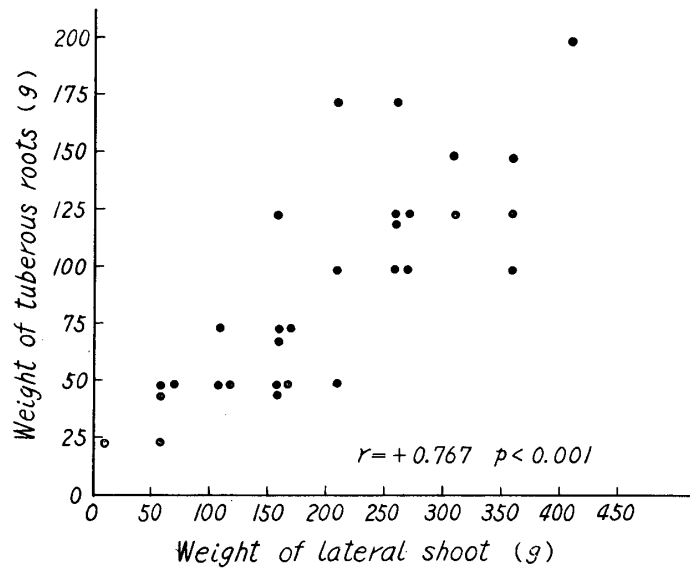


Fig 10. Relation of the tuberous roots to the shoots (represented by fresh weight)

and of tuberous root being $r=0.767$, $p<0.001$) (Fig. 10).

5. Influence of the external supplement of Naphthaleneacetic acid, sucrose and urea upon the root development.

a). *Single node stem cuttings.*

(1) *Effect of the external supply before planting.*

Experimental procedure: The second node with 0.5 cm stem pieces on both sides were taken from eighty uniform vines. After 20 hr immersion in 10 ppm Naphthaleneacetic acid solution in the presence of 5 per cent sucrose or 0.1 per cent urea, single node stem cuttings were set in the moist sand in the dark room, the temperature being kept at 25° C. After ten days the plants were dug out and examined.

Result: It is shown in Table 3 that the rooting was stimulated by NAA. NAA with sucrose favored root elongation and thickening. Notwithstanding the increased protoxylem bands in the root tissues, NAA with urea checked root elongation and thickening.

Table 3. Effect of NAA, urea and sucrose on the rooting and root development of single node stem cuttings.

Treatment	Number of roots	Length of the longest root cm	Total length of roots cm	Number of protoxylem	Root diameter mm
None	2.8	7.7	15.4	5.9	0.91
NAA 10 ppm	3.7	8.8	24.6	6.0	0.94
NAA 10 ppm + 5% sucrose	3.8	9.1	28.1	6.0	0.98
NAA 10 ppm + 0.1% urea	3.5	6.2	11.9	6.3	0.87

(2) *Effect of the external supply after rooting.*

Experimental procedure: Rooted cutting were supplied with Naphthaleneacetic acid (NAA 1 ppm), sucrose (5 per cent) and urea (0.1 per cent) and NAA in the presence of sucrose or urea by the method of brushing over both sides of the leaf-blade twice daily. Ten days after the treatment, the cuttings were dug out and examined.

Result: Foliar application of NAA, sucrose, or urea after rooting induced the same results as with the application by immersion method (Table 4).

Microscopic observation revealed that the wider ranges of the young root tissues of the cuttings treated with NAA were occupied by the undifferentiated tissues as compared with the nontreated root tissues (Fig. 11).

Table 4. Effect of foliar application of NAA, urea and sucrose after rooting on the thickening growth of roots developed from the single node stem cuttings.

Treatment	Number of roots	Length of the longest root cm	Total length of roots cm	Root diameter mm
None	2.2	3.8	6.1	1.08
NAA 1 ppm	3.0	4.0	14.6	1.12
NAA 1 ppm + 5% sucrose	3.2	12.6	27.3	1.20
NAA 1 ppm + 0.15 urea	3.0	3.6	5.8	0.99
5% Sucrose	3.2	10.8	26.2	1.18
0.1% Urea	3.0	3.0	5.1	0.97

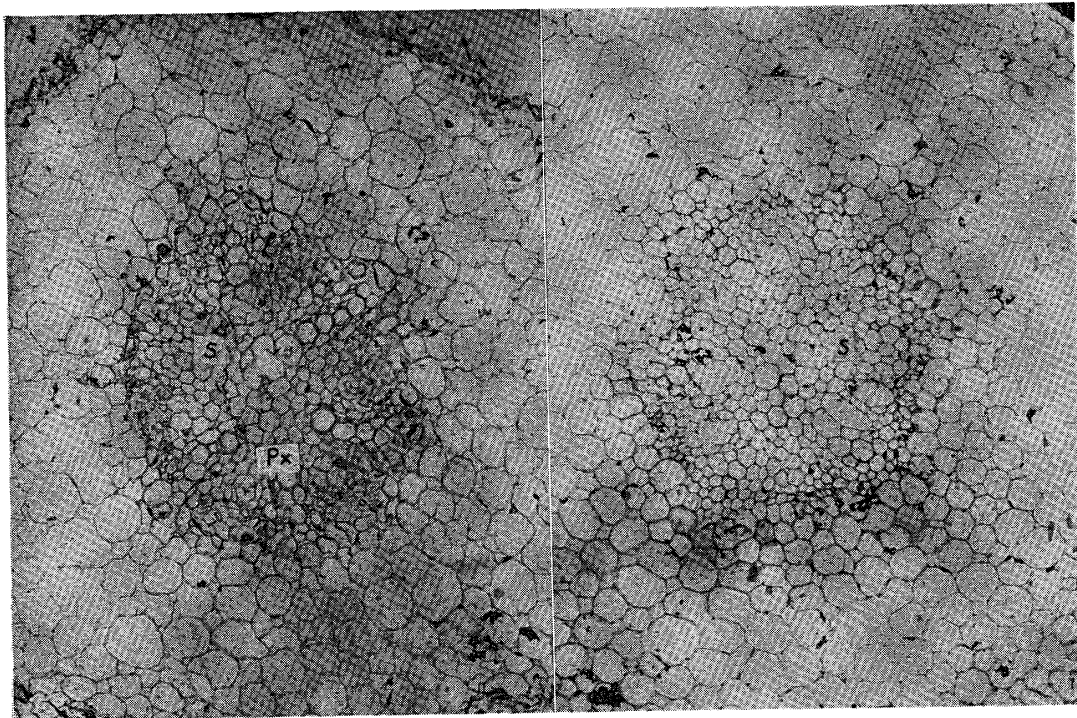


Fig. 11 Microscope photographs of sections of root tissues from NAA treated and nontreated sweet potato plants.

Left: Tissues from nontreated plants

Right: Tissues from NAA treated plants

($\times 150$)

C: Cortex S: Stele Px: Protoxylem

b). *Five nodes stem cuttings.*

Experimental procedure: Five nodes stem cuttings with or without leaf-blades were used for tuberous root formation. Cuttings were immersed for 20 hr in water or NAA (10 ppm) solution before pinching of the apical portion above the first matured leafed node. Cuttings were set in the field on Aug. 5 in a horizontal position and dug out on Nov. 5.

Result: The supplement of the growth substance by the basal immersion method favored both the shoot and the root development of the cuttings with or without leaf-blades, especially in the basal portion. It is shown in Table 5 that the total length growth and the total weight development of the fleshy roots are favored with the initial leaf area and the growth substance.

Discussion

The productiveness of the sweet potato plant is highly concerned with the growth performance during the nursery bed stage. The wide, broad leaves and succulent, thickened stem growth is the sign of high productivity. Such an appearance is, in turn, reflecting the inner nutritional conditions responsible for the tuberous root formation.

The data presented in this report indicate clearly that the external supplement of carbohydrate and the synthetic growth substances stimulate rooting and the root enlargement. The tuberous root formation is highly concerned with the amount of carbohydrate and the native growth hormone contained in the plant tissues.

Koshimizu and Nishida (5) have suggested, by the diffusion method, that a certain growth hormone in the sweet potato stem is concerned with the root enlargement. But to be certain that those substances are concerned with the enlargement of the sweet potato root, it is necessary to determine the amount of those substances in the tissues of the related organs. The identification of the hormone concerned with the enlargement of the roots has been accomplished by the use of paper chromatography using *Avena* straight growth test as indicators of the presence and amount of hormones as they separate on the paper. With this technique, it has been shown that the growth hormone controlling the root enlargement is Indoleacetic acid. Carbohydrates supplement is needed for the roots to continue further development.

Summary

The root enlargement of the sweet potato plant is favored with the external supplement of carbohydrates and the synthetic growth substances.

Changes in the amount of carbohydrates, nitrogen and the growth hormones preceding or accompanying the root enlargement were examined with

Table 5. Effect of external supplement of NAA upon the stem cuttings with or without leaves. Five nodes stem cuttings of Norin-No. 5 sweet potato plant, pinched above the first mature leafed node, were set in the field on Aug. 5 in horizontal position and examined on Nov. 5. (20 samples per treatment)

Treatment	Number of lateral shoots					Average length of lateral shoots					Average number ** of roots					Average number *** of tuberous roots					Average weight of tuberous roots									
	Node number					Node number					Node number					Node number					Node number									
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
No removal of leaves	None	17	12	10	1	96.0	78.9	46.0	27.7	—	248.6	3.5	4.2	3.8	3.8	6.0	1.6	1.8	1.5	1.0	0.3	95.5	62.0	36.5	25.0	20.8	239.8			
	NAA	19	18	16	13	4	77.5	66.5	35.0	20.0	—	199.0	3.6	3.8	4.8	5.0	7.1	1.8	2.0	1.8	1.8	1.4	72.0	50.5	28.0	23.5	28.0	202.0		
Leaves removed except 1st node	None	19	12	8	4	2	88.6	65.9	36.4	21.8	—	212.7	3.5	4.0	3.3	3.7	5.7	1.6	1.0	0.8	0.8	0.4	85.0	48.3	24.7	18.6	15.5	192.5		
	NAA	20	16	15	8	3	75.2	55.0	24.0	18.0	—	172.2	3.8	3.8	3.5	4.5	7.5	2.0	1.6	1.4	1.5	2.1	65.5	38.2	20.5	16.5	19.7	160.0		
Leaves removed except 3rd node	None	17	11	12	3	1	77.4	48.5	60.5	20.0	—	206.4	3.3	3.8	3.8	3.5	5.9	1.8	1.4	1.2	1.0	0.5	68.3	32.8	51.1	15.0	18.4	185.6		
	NAA	19	16	17	8	4	60.0	42.0	46.8	17.0	—	165.8	4.0	3.8	4.0	4.0	6.7	2.0	1.9	1.8	1.5	1.5	48.0	35.7	38.5	15.0	16.0	153.2		
Leaves removed except 5th node	None	18	12	9	6	2	72.0	54.0	33.0	19.0	—	178.0	3.2	3.3	3.0	3.4	5.8	1.2	1.2	1.0	0.9	0.8	53.0	39.0	16.5	29.0	22.0	159.5		
	NAA	19	17	15	11	10	62.0	42.0	28.5	15.4	12.0	169.0	3.2	3.5	3.5	4.3	6.8	1.6	1.4	1.3	2.0	2.2	39.0	35.7	23.0	20.0	29.0	146.7		

* Lateral shoots over 10 cm long.

** Roots over 5 cm long.

*** Tuberous roots over 1.0 cm in diameter.

the single node stem cuttings or with the long stem cuttings of the sweet potato plant.

The growth hormone concerned with the root enlargement was identified as Indoleacetic acid. This compound, initially, comes from the stem and the leaf-blade.

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