Tuberization in *Apios* (*Apios americana* Medikus)

I. Developmental morphology of tuber*

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Abstract: The formation process of rhizome to tuber in *Apios americana* Medikus (apios, groundnut) was investigated. The rhizome is a stem, which is shown by its morphological and anatomical structures. It consists of several nodes and internodes, stipule-like structures, scale-like leaves with axillary buds, and a hook-shaped tip. Several roots were developed on the rhizome just at the basal side of each node. Tubers of apios formed with the swelling of each internode of the rhizome, and they developed at basal internodes through internodes near the rhizome tip. In general, the first tuber developed at the 1st through the 4th internodes of rhizome. Three developmental processes could be recognized during tuberization in each internode: (I) tuber initiation, (II) extension of swelling areas, and (III) dominant enlargement of tuber. Tuberization took place at 8 weeks after planting. The swelling of internodes initiated at the basal side of the axillary bud of the node, extended basipetally and enlarged laterally. The swelling areas of the internodes coincided with the location of root development which occupied 20 to 50% of the length of the internode. Thus, a whole internode did not always swell. In a field, however, a whole internode or several successive short internodes were sometimes tuberized.

Key words: Apios, *Apios americana* Medikus, Axillary bud, Groundnut, Internode, Rhizome, Tuber, Tuberization.

アピオス (*Apios americana* Medikus) における塊茎の形成 第1報 肥大過程の外部形態観察: Juliarni・後藤雄佐・中村貞二・高橋 清・星川清親 (東北大学農学部)

要 旨: アピオス (アメリカホドイモ) における、「いも」の形成過程を調べた。まず、肥大する部分の組織形態から「いも」となる器官が根ではなく、地下茎であることを確認した。地下茎には多くの節があり、それぞれの節には鱗片葉と腋芽、および托葉に相当する器官がみられた。地下茎の先端部は釣り針のような形態を呈していた。地下茎の肥大が最初に観察されたのは植え付け後8週目であった。肥大は地下茎の基部付近の節間から始まり茎頂に向かって各節間で起こった。地下茎の最も基部側で肥大していた節間は第1節間から第4節間のいずれかであった。個々の「いも」は原則として、1つの節とその基部側の1つの節間が肥大したものであった。地下茎の伸長および肥大の過程で地下茎の腋芽が伸長し、地下茎が枝分かれすることもあった。節間の肥大過程の観察を基に、塊茎の形成過程を「塊茎の形成開始」、「肥大範囲の拡大」、および「塊茎の肥大」の3つの生育段階に分けた。塊茎の形成開始は腋芽着生節の基部側の節間が膨らみ始めることで確認できる。続いて膨らみの範囲が基部側に拡大する。塊茎が形成される範囲は、節間全体ではなく、根の出現している範囲とほぼ一致し、節間の20~50%を占めた。膨らむ範囲がほぼ決まる頃から、その部分で急速に肥大が進む。ポット栽培で肥大過程の観察に用いた地下茎の節間の長さは6~11 cmであった。圃場栽培のものには希に短い2~3 cmの節間が存在し、その節間の全域が肥大し、隣接する節間が融合した塊茎となることもあった。

キーワード: アピオス, アメリカホドイモ, いも, 腋芽, 塊茎形成, グランドナッツ, 節間, 地下茎。

Legume is one of the most important crops in the world. Among about 18,000 species of Leguminosae, only limited species produce edible tuber. These tuberous legumes are found in humid tropical and subtropical region. Most of them are wild plants which have never been cultivated²⁾.

Vietmeyer¹⁹⁾ noted that 25 or more species of tuberous legumes are needed to be explored. One of them, *Apios americana* Medikus

(apios or groundnut), was once an important Indian food over the entire eastern half of North America. In its natural habitat, apios was found growing under light intensities ranging from full to partial sunlight^{5,15)} and could be found near stream or lowland that are subject to flooding¹⁷⁾.

Apios has several advantages as a crop: its tuber contains about 16% to 17.5% of crude protein (dry weight basis)^{21,22)}, it has benefits in nitrogen fixation due to the formation of a root nodule²⁰⁾, and has shown a capacity to

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grow in water-logged and acidic soils⁶⁾.

We have observed the tuber development of apios for many years. Blackmon and Reynolds⁶⁾ and Reynolds et al.¹⁷⁾ described that tubers of apios were produced along rhizome, as we observed. However, no mention of tuberization in apios has been found in the literature. This study was undertaken to describe the developmental morphology of tuberization in apios.

Materials and Methods

The experiment was conducted at the Tohoku University experimental field. Seed tubers, 6–8g fresh weight of red vine clone¹⁰⁾, were planted on April 24, 1993 in 1/2000a Wagner's pots at a rate of 3 tubers/pot. Planting depth was 5 cm. As basal fertilizers, 0.2 g of N, 0.6 g of P₂O₅, and 0.6 g of K₂O were applied. All pots were placed outdoors.

The three plants were sampled each week after 6 weeks since planting to study tuber development. At each sampling, the underground parts were carefully obtained, washed out, fixed and preserved in formalin-acetic acid-alcohol (FAA) solution.

The growth habits and morphology of the rhizome in plants grown in the field were also examined. The practices for culture were similar, as described in our previous papers^{9,10)}. In the field experiment, which was different from pot experiment, plants were sampled as was necessary.

Because it was not easy to identify the nodes which were initiated at the base of the rhizome, we named the node located about ≥ 1 mm apart from the seed tuber as the 1st node, and the internode between the 1st and the 2nd node as the 1st internode, respectively (Fig. 2). Development of the first tubers in rhizomes took place at different internodal positions. However, the 2nd internode and 3rd internode tended to have a higher incidence of swelling of the first tubers in rhizome. In this experiment, tuberization at the 2nd internode and the 3rd internode of the longest rhizomes was mainly observed since development of the first tubers at those nodes was more uniform during observation.

Samples of aerial stem, tuberized organ, and seed tuber root prior to tuberization (by 5 weeks after planting) were fixed in FAA solution, dehydrated in water-ethanol-butanol

series and embedded in paraffin wax. Sections were cut at 10 μ m using a rotary microtome, and stained with hematoxylin for anatomical observation.

Results and Discussion

1. Anatomy and morphology of rhizome

It has not been obvious in apios which organ is the tuber in botany. Some researchers have termed the tubers of apios as swelled root^{1,2)}, while others described them as swelled underground stem or rhizome^{6,17)}.

We observed the anatomy of the tuberized organ prior to tuber formation (Fig. 1A) and compared it with those of aerial stem (Fig. 1B) and the root (Fig. 1C), to identify which organ is the tuber. The structure of tissue to tuberized organ was similar to that of aerial stem, and different from that of root. Tissue arrangement from the outside towards the center, i.e. epidermis, cortex, vascular bundles and pith, in the tuberized organ was the same as in the aerial stem. However, the cortex of tuberized organ was thicker than that of aerial stem. The tuberized organ has a typical anatomical structure of dicotyledonous plant stem where vascular bundles are arranged encircling the pith^{8,14)}. Each vascular bundle composed of xylem on the inside facing the pith and phloem on the outside. The vascular cambium exists between xylem and phloem. Anatomical observations described above strongly suggest that the tuberized organ was not a root, but an underground stem.

In this study we have observed the tetrarch young root. The metaxylem vessels were located in the central region of the root and a whole xylem was surrounded by vascular cambium which is responsible for the later secondary thickening. The cortex was limited by endodermis and epidermis (Fig. 1C).

In our previous report we have reported that seed tuber of apios had 2 or 3 buds at its distal end. One of the buds rises to form a shoot, while others grow and become rhizomes⁹⁾ (Fig. 2). The rhizome of apios was similar to that of potato stolon in having some elongated internodes, small scale-leaves, and a hook-shaped tip^{13,18)}. Thus, morphologically, the rhizome was a stem, similar to the stolon of potato. The picture of a node with a stipule-like structure, scale-like leaf with the axillary bud, and roots primordia is shown in Fig. 3.

2. Tuberization

Tubers of apios arose as the swelling of internodes. Generally, development of the first tuber in a rhizome occurred at the 1st through the 4th internodes. Tubers developed along rhizome at basal internodes through internodes near the rhizome tip. Non-swelling internodes or stunted tubers sometimes existed in the rhizome.

Fig. 4 shows tuber development at the 2nd internode of rhizome. There was no indication of tuberization by 6 weeks after planting. Diameter of the 2nd internode at this time was about 2 to 3 mm. The outer skin (epidermis and some cortical cells) of internode just at the basal side of the 3rd node was torn when the tuber developed at 8 weeks after planting. The swelling continued to extend basipetally as seen in tuber growth at 12 weeks after planting, and enlarged laterally. Diameter at the middle part of the swelling at 19 weeks after planting was about 9 mm. The axillary bud of this tuber has already elongated to produce a branch which will give rise to a number of tubers with time.

Fig. 5 shows tuber development in a branch of rhizome of plant grown in field. Similar to the pot experiment, development of the first tuber in the branch of rhizome of plant grown in field occurred at the 1st through 4th internodes. In rhizome of plant grown in field, the axillary buds near the rhizome tip did not always elongate. Even through they elongated, they produced only a few or no tuber (untuberized branches).

3. Developmental stages in tuberization

Fig. 6 illustrates tuberization at the 3rd internode of the longest rhizome. This schematic drawing was made based on sequential observations of tuberization at the 3rd internode of the pot experiment, and was also confirmed by field and other experiments^{11,12}. The following three developmental stages were identified in tuber development at any inter-

Fig. 1. Transverse sections of young tuberized organ (A), aerial stem (B) and root (C). Bars are 0.1 mm. co : cortex, en : endodermis, ep : epidermis, mx : metaxylem, ph : phloem, pi : pith, px : protoxylem, vc : vascular cambium, xy : xylem.

Fig. 2. Schematic drawing of developing rhizomes on seed tuber.

Fig. 3. A node of rhizome with axillary bud (ax), roots primordia (rp), scale-like leaf (sc) and stipule-like structure (sp). Bar is 1 mm.

Fig. 4. Tuber development at the 2nd internode of rhizome. Bar is 1 mm. Magnifications for all photographs are the same. Arrow indicates the axillary bud which elongated forming a branch of rhizome. The roots of tuber at 19 weeks after planting (WAP) has been cut.

Fig. 5. Rhizome of plant grown under field conditions developing a branch which produced several tubers. Bar is 5 cm. BR : branch of rhizomes, MR : main rhizome, N : node, RT : rhizome tip.

node of rhizome : (I) Tuber initiation : This stage could be identified by swelling of internode just at the basal part of the axillary bud of the 4th node. In the observed rhizome, this phenomenon could be seen at 8 weeks after planting. (II) Extension of swelling areas : the stage at which the swelling extended basipetally. We noticed this stage occurred from 8 through 15 weeks after planting. Most of the swelling areas coincided with the regions where some roots emerged ("rooting zone"). However, we do not know the relationship of root to tuberization at present and further investigation will be needed. In the observed rhizomes, the length of internode between the 3rd and 4th nodes varied from 6 to 11 cm, and the "rooting zone" occupied 20% to 50% of those lengths. Thus, swelling did not occur at a whole length of internode. In plants grown under field conditions, however, tubers which arose from the swelling of a whole length of

Fig. 6. Schematic drawing of tuberization at the 3rd internode of rhizome.

Fig. 7. Successive tubers of plant grown under field conditions. One of the axillary buds of successive tuber elongated forming a branch which produced another successive tuber. Details are the same with those in Fig. 5.

internode, or developed from several successive short internodes (fused tuber), were seen (Fig. 7). (III) The lateral enlargement of swelling: This stage overlapped with stage II and it seemed to commence 15 weeks after planting. The lenticels, the dark brown areas which surround the roots, were obvious at this stage.

Tuberization at other internodal positions of rhizomes may occur in a similar manner as for the 2nd and 3rd internodes, regardless of earlier or later tuber initiation and faster or slower in rate of bulking.

The observations described above suggest that tuber development in apios was different from that in potato. In the case of potato,

tuber arises as terminal swelling of a stolon which has many internodes^{3,4,7,16,18}, whereas in *apios*, tuber arose as swelling of each internode of a rhizome.

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