Flower Bud Formation of Sacred Lotus (*Nelumbo nucifera* Gaertn.): A Case Study of ‘Gyozankouren’ Grown in a Container

Fumiko Ishizuna and Nobuhiro Tsutsumi¹

Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Tokyo, Japan 113-8657

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Abstract. The genus *Nelumbo* consists of two species, *N. nucifera* and *N. lutea*. *N. nucifera* is an ornamental and edible plant that is widely cultivated. Earlier studies of sacred lotus (*N. nucifera*) flowers focused mainly on morphology, phyllotaxis, leaf arrangements, and flower development. During the growing season, sacred lotus produces one foliage leaf at each node. Flower buds emerge from the abaxial side of the basal part of the foliage leaf. However, the number of blooming flowers is much less than the number of foliage leaves. Little is known concerning flower bud formation during lotus plant development. This is the first experimental study to reveal that every node has one flower bud even in the dormant shoot apex and that most of the formed flower buds aborted in the course of floral development. Our results suggest that flower bud formation of sacred lotus is independent of daylength. On the other hand, whether a formed bud reaches blooming may depend on environmental factors.

The genus *Nelumbo* consists of two species, *N. nucifera* Gaertn. (sacred lotus) and *N. lutea* Willd (American lotus). Generally, *N. nucifera* has pink or white flowers and is mainly distributed in Asia, Australia, and Russia, whereas *N. lutea* has yellow flowers and is distributed mainly in the United States (Heywood, 1993).

Sacred lotus is a perennial aquatic plant that grows in shallow water in marshes and lakes. It has rhizomes that horizontally creep under the water in the soil (Esau and Kosakai, 1975) (Fig. 1A). The rhizome consists of nodes and the sections of stem between the nodes, called internodes (Fig. 1A). In the temperate zone, where winters are cold, the shoots sprout in the spring and produce shoot and root systems with peltate foliage leaves, some of which float on the water surface and some of which rise above the water at right angles to the rhizomes. Flowers bloom in midsummer. At the end of the growing season, the internodes enlarge and become fleshy tubers, which act as storage organs (Esau and Kosakai, 1975).

Sacred lotus has been cultivated for food (the rhizome and fruits are edible), medicines,

and ornaments. There is some evidence that it was used in China as early as 6000 to 7000 years ago (Wang and Zhang, 2004). Flowers of sacred lotus have been used as offerings or tributes by Hindus and Buddhists. Sacred lotus is generally cultivated by asexual propagation from the rhizome. In Thailand and south Asian counties, sacred lotus are commercially grown for cut flowers (Imsabai et al., 2010). In Japan, cut flowers of sacred lotus are available in the market for a limited period in midsummer. Techniques for regulating the flowering of sacred lotus have not been established. During the growing season, sacred lotus produces foliage leaves regularly at every node. The number of blooming flowers is generally much smaller than the number of foliage leaves. Earlier studies showed the position of flower bud emergence. Flower buds of sacred lotus emerge from the abaxial side of the basal part of the foliage leaves (Kumazawa, 1979; Miki, 1927; Takhatajan, 1969; Wigand and Dennert, 1888). Scanning electron microscope observations showed the development of floral elements (e.g., petals and sepals) in different floral developmental stages (Hayes et al., 2000). Minamikawa (1974) stated that latent flower buds formed at most nodes but did not give any experimental data. It has remained unclear whether flower buds form at every node and at what time of the year flower buds form. It is difficult to answer these questions because the plants can extend horizontally for over 5 m and most of the plant body grows in the soil under water.

In this study we sought to answer these questions by planting 20 sacred lotus and pulling up their rhizomes for examination at

different times over an 11-month period (mid-May to mid-April). Our results show that during the growing season from spring to fall, flower buds formed at every node. On the other hand, most of the formed flower buds aborted at various floral developmental stages.

Materials and Methods

Rhizomes of *N. nucifera* ‘Gyozankouren’ were provided by the Institute for Sustainable Agro-ecosystem Services, Graduate School of Agricultural and Life Sciences, The University of Tokyo. Cultivar Gyozankouren is typical of sacred lotus in size and number of petals (≈30 petals) with pink flowers (Watanabe, 1990). Based on a study of simple sequence repeat markers in *Nelumbo* cultivars, cv. Gyozankouren belongs to a group that includes cv. Jodairen, cv. Ittenshikai, cv. Ukesho, cv. Rinnohren, and others (Kubo et al., 2009). Each primary rhizome was prepared for observation in the form of a rhizome consisting of a shoot apex and the following two internodes. An axillary bud on the node after the shoot apex was removed to make sure that each rhizome contained only one growing point at the time of planting.

The rhizomes (n = 20) were planted on 10 May 2009, each in a 80-L plastic container (diameter, 58 cm; depth, 47.5 cm) with loam soil ≈15 cm in depth. The containers were kept outside under natural light conditions and irrigated regularly to keep the water level at 15 cm above the soil surface. Ten grams of fertilizer [Daiya-amino (5N–5P–5K); Mitsubishi Shoji Agri-Service Co., Tokyo, Japan] was applied to each container at ≈2-week intervals during the growing season. Flower buds formation was examined from 8 Aug. 2009 [90 d after planting (DAP)] to 18 Apr. 2010 (343 DAP) (Table 1). For observations of flower buds, the entire plant was taken from the container and washed with tap water to remove soil from the rhizomes. Roots on the nodes were removed. Some of the primary rhizomes were lost during removal of the soil. The plant was discarded after the observations. On 24 Sept. (137 DAP), 9 Oct. (150 DAP), 31 Oct. (172 DAP), and 25 Nov. (199 DAP), two samples were chosen, one with blooming flowers and one without blooming flowers (Table 1). All nodes including the insides of those shoot apex were dissected and some of them were subjected to microscopic observation.

In this article, “node set” is defined as the set of the attachments to the node such as scale leaves, flower bud, etc. The attachments are described in detail in the “Results” section. Flower buds were judged to be aborted when 1) they stopped growing and failed to flower; 2) other attachments to the nodes continued to grow; and 3) the shoot apex of the main shoot continued to produce younger nodes.

Results and Discussion

Flower buds were continuously present within the shoot apex. The shoot apex was

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¹To whom reprint requests should be addressed; e-mail atsutsu@mail.ecc.u-tokyo.ac.jp.

Fig. 1. Plant architecture of *N. nucifera*. (A) A schematic diagram of plant architecture of *N. nucifera*. (B) A rhizome collected during the growing season (4 Sept. 2009, 117 d after planting). (C) A rhizome collected during the dormant season (25 Nov. 2010, 199 d after planting). pr = primary rhizome; rh = rhizome; st = shoot tip; i-n = internode; n = node; l = foliage leaf; p = petiole; ab = axillary bud; ro = roots; dashed-square = enlarged rhizome; r = the mark with the white labels = flower petiole; numbering = the node number on the main axis from primary rhizome. Scale bar B–C: 50 cm.

Fig. 2. Positions of flower buds within the shoot apex in the growing and the dormant seasons. (A) A typical shoot apex. (B) Same shoot apex as in A after removal of two scale leaves (s1, s2). (C) Inside of a shoot apex collected in the growing season (4 Sept. 2009, 117 d after planting) after removal of scale leaves and ochrea. f' = nested sets of flower buds; l' = foliage leaf; oc' = ochrea. (D) A typical flower bud within a shoot apex collected in the growing season (4 Sept. 2009, 117 d after planting). (E–F) Typical flower buds within shoot apices in a sample collected in the dormant period (25 Nov. 2009, 199 d after planting). The form and size of flower buds did not differ between the growing season and dormant period. sa = shoot apex; s = scale leaf; l = foliage leaf; f = flower bud; ab = axillary bud; oc = ochrea; arrowhead = the position of the flower bud forms. Scale bar A–B: 1 cm, C–F: 500 µm.

Fig. 3. Anatomy of flower buds at *N. nucifera* nodes. (A) A typical node, consisting of two scale leaves (s1, s2), a foliage leaf (l), an ochrea (oc), an axillary bud (ab), and roots (r). (B) Dissection of the node shown in A. s1, s2 = scale leaves; l = foliage leaf; oc = ochrea; ab = axillary bud; r = roots. Arrow = growing direction; arrowhead = the position flower bud forms. (C–F) Flower buds on nodes of rhizomes collected on different dates. (C) Node 14, 9 Oct. 2009 (150 d after planting). (D) Node 15, 9 Oct. 2009 (150 d after planting). (E) Node 18, 9 Oct. 2009 (150 d after planting). (F) Node 18, 4 Sep. 2009 (90 d after planting). Insets in C and D show enlarged views of the flower bud. Scale bar A–B: 1 cm, C–F, enlarged view in the left bottom corner of C–D: 500 µm.

tightly ensheathed in two scale leaves (Fig. 2A). Removal of the two scale leaves (s1 and s2) from the shoot apex revealed a foliage leaf (l), an ochrea (oc), and a flower bud (arrowhead) (Fig. 2B). Removal of the ochrea (oc) (Fig. 2B) and smaller sets of scale leaves (not shown) inside the ochrea (oc) revealed an axillary bud (ab), a smaller flower bud (f'), a foliage leaf (l'), and an inner ochrea (oc') (Fig. 2C). Another smaller set consisting of an axillary bud and shoot tip wrapped with two scale leaves had already formed inside the inner ochrea (oc') of Figure 2C (not shown). Two to three nested node sets including flower buds in each set had formed inside the shoot apex wrapped with scale leaves.

The plant structures on a typical day in the growing season (4 Sept. 2009, 117 DAP, Fig. 1B) and on a typical cay in the dormant period (25 Nov. 2010, 199 DAP, Fig. 1C) were similar except that the latter had an enlarged rhizome at the distal end. Nodes on the main axis are numbered from the primary rhizome (Fig. 1B). At the end of the growing season (late September to October), several distal internodes began to enlarge. At the same time, the leaves began to turn yellow. In November, the plants shed their leaves and entered the dormant period until March (Fig. 1C). Figures 2C–D and 2E–F show flower buds within a shoot apex collected in the growing season

and the dormant period, respectively. No obvious difference was observed between these flower buds (Figs. 2D and 2F), and the flower buds were continuously produced within the shoot apex. Flower buds observed within the shoot apex, which formed at the end of the growing season, seemed to be alive during the dormant period. The flower buds of the dormant period showed no signs of decay or damage (Table 1^y).

A flower bud formed at every node. The components of an intact and dissected node are shown in Figures 3A and 3B, respectively. Each node set consists of two scale leaves (s1, s2), a foliage leaf (l), a flower bud (f), an axillary bud (ab), roots (r), and an ochrea (oc) that wrapped the next internode (Fig. 3A–B). One flower bud emerged from the abaxial side of the basal part of the foliage leaf (Fig. 3B–F, arrowheads). During the growing season (May to October), one flower bud formed at every node (Table 1). Since the time of planting, the shoot apices of primary rhizomes continuously produced nodes until the beginning of the dormant period. The highest node number in Table 1 for each sample indicates the total number of nodes in the sample. After the growing season, each plant had 24 or 25 nodes on the main axis. These results indicate that the rhizome elongation and node formation of all samples were almost synchronized and that the elongation growth stopped near the end of the growing season. Nodes 1 to 13 on the 8 Aug. sample (90 DAP, Table 1) were produced between the planting time (10 May) and the sampling time (8 Aug., 90 DAP) and flower buds formed at all nodes. Growth of the rhizome was essentially complete by early October. So it can be assumed that the 13th node formed just before 8 Aug. (90 DAP) and the 19th node formed just before 4 Sept. (117 DAP). In some samples, the first few nodes from the primary rhizome had started to decay on the observation date, so it could not be determined whether flower buds were present. These buds are labeled indeterminate (i) in Table 1.

Most flower buds aborted at various developmental stages. These results indicate that every node develops that every node has one flower bud even in the dormant shoot apex. Only some of the buds bloomed. However, of the 14 plants examined, three had three bloomed flowers, three had two bloomed flowers, one had one bloomed flower, and the others had no flowers. Except for one bloomed flower at Node 17, all the other bloomed flowers were at Nodes 9 to 14. Most of flower buds were aborted in the course of floral development. The floral developmental stages of the aborted flower buds were different (Fig. 3C–F). Sepals could not be seen clearly in flower buds that aborted in the early stage of floral development (Fig. 3D) but some of the flower buds developed further.

An almost completely developed floral organ with pinkish sepals is shown in Figure 3F. The flower bud on Node 14 of Plant 6 in Table 1 did not develop further (Fig. 3C),

Table 1. Occurrence of flower buds and flowers within the shoot apices and at nodes of *N. nucifera* during the growing and dormant seasons.^z

Node no.	Sample no.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Sampling date													
	8 Aug.	4 Sept.	24 Sept.	24 Sept.	9 Oct.	9 Oct.	31 Oct.	31 Oct.	25 Nov.	25 Nov.	7 Jan.	21 Feb.	18 Apr.	18 Apr.
	DAP													
	90	117	137	137	150	150	172	172	199	199	242	287	343	343
1	+	+	<i>i</i>	<i>i</i>	<i>i</i>	<i>I</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>i</i>	+	+	<i>i</i>	<i>I</i>
2	+	+	<i>i</i>	<i>i</i>	<i>i</i>	<i>I</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>I</i>
3	+	+	+	<i>i</i>	<i>i</i>	<i>I</i>	<i>i</i>	<i>i</i>	<i>i</i>	<i>i</i>	+	<i>i</i>	+	+
4	+	+	+	<i>i</i>	<i>i</i>	<i>I</i>	<i>i</i>	+	<i>i</i>	+	+	<i>i</i>	+	+
5	+	+	+	<i>i</i>	+	<i>i</i>	<i>i</i>	+	+	+	+	<i>i</i>	+	+
6	+	+	+	+	+	+	<i>i</i>	+	<i>i</i>	+	<i>i</i>	+	+	+
7	+	+	+	+	+	<i>i</i>	+	<i>i</i>	<i>i</i>	<i>i</i>	+	+	+	+
8	+	+	+	<i>i</i>	+	+	+	+	+	+	+	+	+	+
9	F	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	F	+	+	+	+	+	+	+	+	+	+	+	+
11	F	+	F	+	+	+	+	+	+	+	+	F	+	+
12	F	+	F	+	+	+	+	+	+	+	+	F	+	+
13	+	F	+	+	F	+	+	+	F	+	+	F	+	+
14	+ ^y	F	+	+	F	+	+	+	F	+	+	+	+	+
15	+ ^y	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+ ^y	+	+	+	+	+	+	+	+	+	+	+	+	+
17		+	+	+	+	+	F	+	+	+	+	+	+	+
18		+	+	+	+	+	+	+	+	+	+	+	+	+
19		+	+	+	+	+	+	+	+	+	+	+	+	+
20		+ ^y	+	+	+	+	+	+	+	+	+	+	+	+
21		+ ^y	+ ^y	+ ^y	+	+	+	+	+	+	+	+	+	+
22		+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y
23			+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y
24					+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y	+ ^y
25							+ ^y	+ ^y	+ ^y	+ ^y				

^zEach plant was taken from its container on the indicated day and examined for flower bud formation at each node. All examined samples were discarded after the observations.

^yThe shoot apex and within the shoot apex.

+ = flower bud was identified (aborted flower buds); F = flower bud reached to flowering; *i* = indeterminate; Node no. = the number of the node from the primary rhizome; DAP = days after planting.

whereas the flower bud on Node 14 of Plant 5 reached blooming (Table 1).

Flower bud formation of sacred lotus is independent of daylength. During the growing season from spring to fall, flower buds regularly formed at every node (Table 1). At the end of the growing season, the distal internodes of rhizome enlarge in girth and become fleshy tubers as storage organs. It was suggested that daylength and phytochrome play roles in rhizome enlargement of *N. nucifera* (Masuda et al., 2006, 2007). In potato, flowering and tuberization have a common photoperiod pathway mediated by phytochrome (González-Schain et al., 2012; Navarro et al., 2011; Rodríguez-Falcon et al., 2006). However, the present result indicated that flower bud formation of sacred lotus was not noticeably affected by daylength. Although one flower bud was formed at every node, most of the formed flower buds were aborted at various floral developmental stages. Only 16 (7%) of 232 identified flower buds bloomed. The remaining 216 flower buds were aborted at different sizes and at different stages of floral development. It is surprising that sacred lotus continuously form non-viable flower buds throughout the growing season. In this study, we were not able to clarify the conditions required to blooming. The nodes where the blooming flower emerged were limited to the nodes produced in the shoot apex during a certain period of time (Table 1).

The preceding results indicate that every node has one flower bud even in the dormant shoot apex. Our results also show that most of the formed flower buds aborted in the course of floral development. Our results suggest that flower bud formation of sacred lotus is independent of daylength. On the other hand, whether a formed bud reaches blooming may depend on environmental factors.

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