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Characteristics of reproductive effect and phenology of *Polygonatum stenophyllum* grown in riverside in Paju-si

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파주시 하천변에 서식하는 층층둥굴레의 계절학과 번식생태학적 특성

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Abstract

Polygonatum stenophyllum Maxim. is a native perennial herb to Korea belonging to family Liliaceae. Although it was removed from the endangered species list, since it inhabits the riverside, it has been threatened with damage to its populations due to frequent disturbances. In order to reveal the basic ecological characteristics of *P. stenophyllum,* This study was identified the life cycle of the aboveground part and was measured the number of flowers and fruits, which are reproductive organs of *P. stenophyllum* in riverside in Paju - si during the two years. As a result, aboveground part of *P. stenophyllum* appeared and grew rapidly until the end of May to about 1m. In early May, two buds per one peduncle were formed up to six pairs per floor. The flowers bloomed in turn from the base of the stem and to the top floor and the blooming occurred when the number of leaves was at least three. Fruits were formed in mid-June and matured by the end of September. The numbers of flowers and fruits were revealed that flowers bloomed in turn from the bottom of the stem to the top floor, the most intensively bloomed in the center part of the stem, and did not bloom from the floor closest to the ground and above the highest 5th node. This basic ecological characteristics of the *P. stenophllyum* grown in riverside identified in this study will be useful as basic data for their conservation.

Key words : Polygonatum genus, Phenology, Reproductive organs, Number of flower, Number of fruit, Ecological characteristic

요 약

층층둥굴레는 백합과에 속하고 한국에 자생하는 다년생 초본이다. 이는 멸종위기생물종 목록에서 해제되었지만 하천변에 서식하기 때문에 빈번한 교란으로 개체군은 훼손 등 위협에 처해있다. 따라서 층층둥굴레의 기초 생태학적 특성을 밝히기 위해, 본 연구는 2년 동안 파주시의 하천변에서 자생하는 층층둥굴레의 지상부의 식물계절학 조사와 번식기관인 꽃과 열매의 수를 측정하였다. 그 결과, 층층둥굴레의 지상부는 4월 말에 출현하고 5월 말까지 약 1m로 빠르게 생장하였다. 5월 초, 1개의 꽃자루 당 2개의 꽃봉오리가 줄기 마디에 최대 6쌍까지 형성되었고, 5월 중순부터 개화하였다. 열매는 6월 중순부터

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형성되어 9월 말까지 성숙하였다. 층층둥굴레의 꽃과 열매는 줄기의 기저부부터 정단부까지 차례로 달렸고, 꽃과 열매의 수는 줄기의 중앙 부분에서 가장 많이 형성하였고, 지면과 가장 가까운 마디부터 최대 5마디까지는 형성하지 않았다. 본 연구에서 밝힌 하천변에서 자생하는 층층둥굴레의 기초 생태학적 특성은 이들의 보전을 위한 기초 자료로 유용할 것이다.

핵심용어 : 둥굴레속, 계절학, 번식 기관, 꽃 수, 열매 수, 생태학적 특성

1. Introduction

Polygonatum stenophyllum Maxim. is a perennial herb belonging to genus Polygonatum of family Liliaceae and a native plant of Korea (Lee, 2006; Barbour et al., 2015). Unlike other species of Polygonatum genus, stem of *P. stenophyllum* is vertical and it's leaf arrangement is verticillation (Song et al., 2009). Globally, it is distributed in the northern hemisphere such as Russia and three northeast areas of China (Heilong Jiang, Jilin, and Liaoning Provinces) (Chung et al., 2014). In Korea, it is distributed from the border area near the Tumen River to Gangwon-do, Gyeonggi-do, Chungbuk, and Gurye in Jeonnam (Ann et al., 2003; Song et al., 2009; Hwang, 2015) so that Korea is considered as the southernmost distribution area of *P. stenophyllum* (Hyun, 2015; NIE, 2015).

Until recently, the distribution of new natural habitats and populations has been consistently identified so that *P. stenophyllum* was removed from the endangered species list in 2017 (NIE, 2017). However, since *P. stenophyllum* inhabits the riverside, it has been threatened with damage to its populations due to frequent disturbances such as the four-river project and river development(Lee et al., 2017). Although *P. stenophyllum* were restored in replacement habitat as an alternative, the replacement habitat formed were inappropriate due to lack of basic ecological information on *P. stenophyllum* population resulting in negative effects on the maintenance of the populations (Kim, 2017; Lee et al., 2017).

Domestic studies on the *P. stenophyllum* include studies on the soil environment and vegetation structure of natural habitats (Song et al., 2009), comparison of genetic diversity between *P. stenophyllum* and *Polygonatum involucratum* (Chung et al., 2014), individual distribution status (Hwang, 2015), assessment of replacement habitats using the habitat suitability index (Lee et al., 2017), effects of invasive plants on the growth response of *P. stenophyllum* Maxim. (Kim, 2017; Kim et al., 2018), and distribution characteristics, population threat factors, and sustainability assessments (Kim et al., 2019), and until recently, diverse studies have been continuously conducted to found measures for sustainability of *P. stenophyllum*.

In particular, in order to maintain individual and populations, it is important to identify the phenology of plant growth, flowering, and fruition of the relevant species corresponding to seasonal changes (Kim, 2012) because temperatures and precipitation that change along with seasons are closely related to the life cycles of plants. In addition, since the yields of flowers and fruits of plants are determined by changes in surrounding environmental factors (Kim, 2012; Lee et al., 2018), studies on reproductive ecological responses such as flower and fruit formation are indispensable (Lee et al., 2018). Although studies on the plant phenology and reproductive ecological responses or the characteristics of reproductive organs of other species of Polygonatum genus (Guitian et al., 2001; Hasegawa and Kudo, 2005; Min, 2017) have been actively conducted, studies on the plant phenology and reproductive ecology of *P. stenophyllum* are insufficient.

Therefore, focusing on identifying the basic ecological characteristics of species for the maintenance and protection of *P. stenophyllum* populations that inhabit the riverside, in this study, the life cycles of the aboveground part of individuals were identified through the plant phenology records of the habitats of *P. stenophyllum* and the numbers of flowers and fruits, which are reproductive organs, were measured to study the reproductive ecological characteristics.

2. Materials and Methods

2.1 Study materials and Survey area

P. stenophyllum's stems are straight, and its leaves hang on the nodes in a verticillate arrangement with 4-6 leaves per stem. The leaves are 5-10mm wide, have no petiole, and have clear and gloss main veins (Autor's observation) (Fig. 1-ⓐ). Flowers are bell-shaped and are in pale green. Two flowers bloom per peduncle (3mm) at the leaf axil (Fig. 1-ⓑ). Each flower has six stamens and one pistil (Fig. 1-ⓒ). The fruits are round, and the pericarp ripens in green to dark green and each fruit has 1 to 6 seeds, which are yellow in color (Fig. 1-ⓓ,ⓔ). The underground part is composed of rhizomes and roots, and the rhizomes undergo vegetative reproduction (Fig. 1-ⓕ). *P. sibiricum*, a similar species, has 2-3cm wide leaves and the leaf apices are curled. Up to eight flowers bloom per peduncle, which is 3 to 7.5cm long, and one fruit contains 1 to 2 seeds (Autor's observation).

The study area are located at the riverside (37° 81'66.33″ N, 126° 78'49.42E″) at the point where Galgokcheon originated from Galgok-ri, Beopwon-eup, Paju-si,

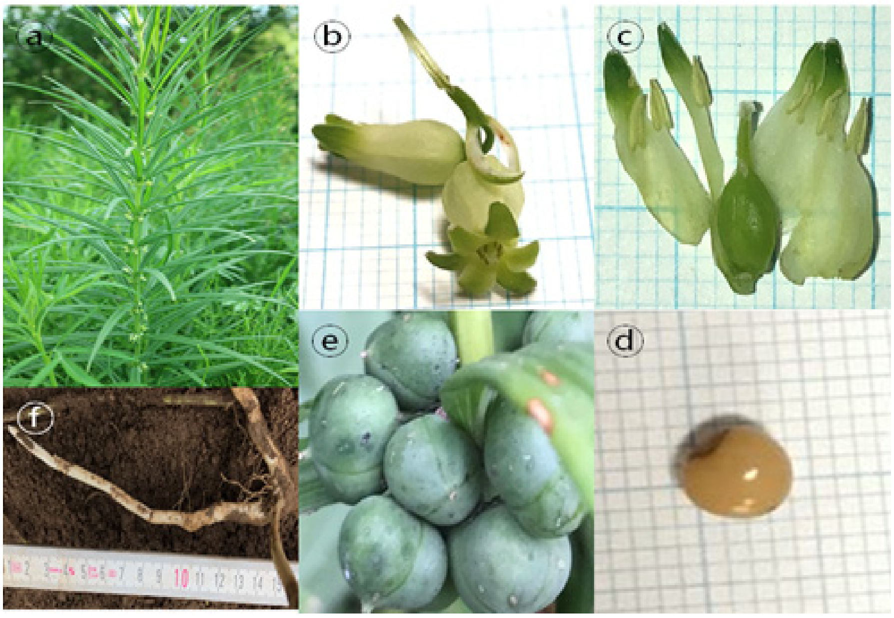

Fig. 1. Aboveground part ⓐ, flowers ⓑ, stamen and pistil ⓒ, seed ⓓ, fruits ⓔ, belowground (rhizomes and root) ⓕ of *Polygonatum stenophyllum*.

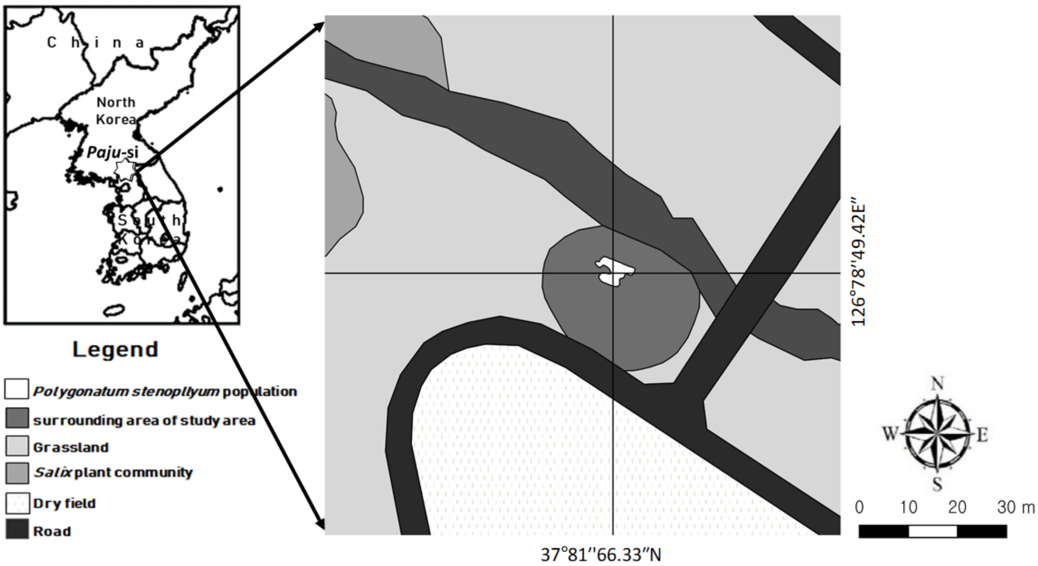

Fig. 2. Map of study area and landscape.

Gyeonggi-do, joins Munsancheon, the first tributary of the Imjin River(Fig. 2). The surrounding landscape comprises grasslands, *Salix* plant community, roads and fields. The altitude of the study area was 13m, and the distance from the river was about 10m. Since the study area was close to the river, *P. stenophyllum* population was flooded due to the flooding of the river during the rainy season. In addition, road construction, farming, and fishing activities were conducted in the surroundings leading to frequent human interference throughout the year.

As for the atmospheric environment of the study area, temperature and precipitation climate maps were prepared using the meteorological data (KMA, 2018). In 2017, the average air temperature was 14.3 ℃, the minimum temperature was −4.6 ℃, the maximum temperature was 25.3 ℃, and the average precipitation 101 mm. In 2018, the average air temperature was 12.8 ℃, the minimum temperature was −6.8 ℃, the maximum temperature was 26.5 ℃, and the average precipitation was 91.62 mm (Fig. 3). In order to understand the soil physicochemical characteristic of study area, We measured and analyzed in surface of soil the organic matter (%), water content (%) and pH. We gather the soil in study area and analyzed Organic matter and water content in laboratory. pH was measured the pH instrument (DM-5, TAKEMURA). As a result, soil organic matter was 6.28 %, water content was 10.43 % and pH was 6.8.

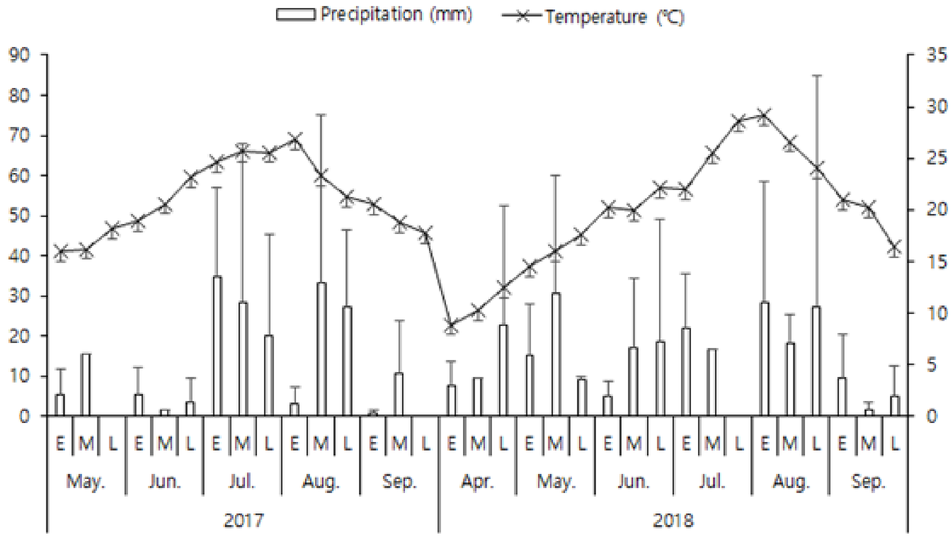

Fig. 3. Monthly total precipitation (mm) and monthly air temperature (℃) during study period in Paju-si. The bar means total precipitation (mm) and line means monthly temperature (℃). Alphabet of axis of abscissas mean E; early days, M; middle days, L; last period.

As for the plant species composition in *P. stenophyllum* populations, no tree layer or shrub layer appeared, and all emerged species were herbs. To determine the degrees of occupation of species of *P. stenophyllum*, the coverages and frequencies of herbaceous plants were obtained in June, and the relative coverages and relative frequencies were calculated and summed up to calculate the importance value (IV). As for IV of individual species, *P. stenophyllum* had the highest value at 40.0 followed by *Urtica angustifolia* (32.3), *Ambrosia trifida* (7.8), and *Chelidonium majus* var. *asiaticum* (2.8), *Phragmites japonica* (1.9), *Chenopodium album* var. *centrorubrum* (1.7), *Stellaria aquatica* (1.4), *Galium spurium* (1.3), *Rumex crispus* (1.1), *Equisetum arvense* (0.9), *Calystegia japonica* (0.5), *Agropyron ciliare* (0.6), *Calamagrostis epigeios* (0.6) and *Artemisia selengensis* (0.5). *P. stenophyllum* formed vegetation with species that appear in areas adapted to seasonal water level fluctuations and disturbed riverside such as *C. japonica* and *Humulus japonicus* hindered the growth of *P. stenophyllum* by winding or twisting the aboveground part and fruits. In particular, *A. trifida*, which is an at least 2m high invading plant, was widely distributed within *P. stenophyllum* populations thereby affecting the maintenance of individuals of *P. stenophyllum*.

2.2 Study method

2.2.1 Plant phenology

Surveys were conducted from May to September 2017 (1st year) and from April to September 2018 (2nd year), and 30 ea (1m x 1m) quadrats were installed in the region where the density of *P. stenophyllum* was the highest. In mid-May in the first year and in mid-April in the second year, 10 individuals were randomly selected per quadrat, labels were attached to the aboveground part of *P. stenophyllum*, and the plant phenology was investigated. The aboveground part was considered to have appeared when the epicotyl surrounded by the coleoptile came out of the ground. The growth of the aboveground part was considered as vertical growth, and leaf unfolding was considered to have occurred when all leaves expanded. Blooming was considered to have occurred when five petals began to unfold after buds were formed. Fruition was regarded to have occurred when the fruit with green rind came into bearing. Fall foliage was regarded to have occurred when the color of changed from green to yellow. The life cycle was considered to have ended when 1/2 of alive leaves fell. Observations were carried out every month, and a plant phenology table was prepared after designating 1st to 10th of a month as the early stage, 11th to 20th as the middle stage, and 21st to 31st as the late stage.

2.2.2 Reproductive ecological measurement

In mid-May 2017 and early May 2018, which were the flowering seasons of *P. stenophyllum*, 10 individuals that were flowering and had the aboveground parts with similar heights were selected per quadrat (a total of 300 individuals were selected). The numbers of flowers were measured by floor (the number of nodes was expressed as floor) from the flowers hanging on the node of the stem closest to the ground (Floor, F1) to those hanging on the top floor. Thereafter, in order to grasp changes in the number of flowers for two years, the number of flowers in the first year was subtracted from the number of flowers in the second year and the resultant value was divided by the number of flowers in the first year and multiplied by 100 to obtain the rate of change (%).

In late July 2017 and mid-June 2018, which were the fruiting seasons of *P. stenophyllum* 10, individuals that formed fruits and had the aboveground parts with similar heights were selected per quadrat (a total of 300 individuals were selected). Thereafter, the numbers of fruits were measured by floor (the number of nodes was expressed as floor) from the fruits hanging on the node of the stem closest to the ground (Floor, F1) to those hanging on the top floor. Thereafter, in order to grasp changes in the number of fruits for two years, the number of fruits in the first year was subtracted from the number of fruits in the second year and the resultant value was divided by the number of fruits in the first year and multiplied by 100 to obtain the rate of change (%).

3. Results and Discussion

3.1 Plant phenology

The plant phenology of the aboveground part of *P. stenophyllum* was observed for two years (Fig. 4) and according to the results, *P. stenophyllum* is a reproduced and grew in a year. The appearance of the aboveground part of *P. stenophyllum* was not accurately grasped in the first year but was identified on April 28 in the second year. In the second year, leaf unfolding occurred simultaneously with the appearance of the aboveground part and the plant grew until flowers bloomed. Buds were formed on May 16 in the first year and May 13 in the second year, and flowers bloomed on May 22 in the first year and May 22 in the second year. The flowers of *P. stenophyllum* bloomed in turn from the bottom of the stem to the top and the blooming occurred when the number of leaves was at least three. Two flowers bloomed per peduncle hanging on the leaf axil and up to six pairs of flowers bloomed per floor. The fruition occurred on July 26 in the first year and June 18 in the second year. Simultaneously with fruition, fall foliage gradually progressed from F1 to the top floor. All leaves of the individuals began to fall on September 27 in the first year and September 8 in the second year.

In the case of the *P. stenophyllum* in the study area, the aboveground part appeared in the late spring (mid-April) when water canals were opened from the surrounding vegetation and the life cycle ended in early autumn, September. To compare the foregoing with the findings of Hasegawa and Kudo (2015), who studied the plant phenology of three species of Polygonatum genus in Japan (42° 39'N), *P. odoratum* var. *maximowiczii* inhabited the lower layer of deciduous forests and the aboveground part appeared in late April and completed the growth, leaf unfolding, and the formation of buds by the end of May. On the other hand, the aboveground part of *P. involucratum* inhabiting the edge of forests appeared in early May, while that of *P. humile* inhabiting sand dunes without water canals appeared in mid-May. Given the foregoing, as the light environment stabilizes, the appearance of the aboveground part is delayed, and the rapid growth of the aboveground part and the formation of buds before the water canals are closed are judged to be necessary for efficient photosynthesis under seasonally changing light conditions (Houle, 2002; Hasegawa and Kudo, 2015). The life cycle of

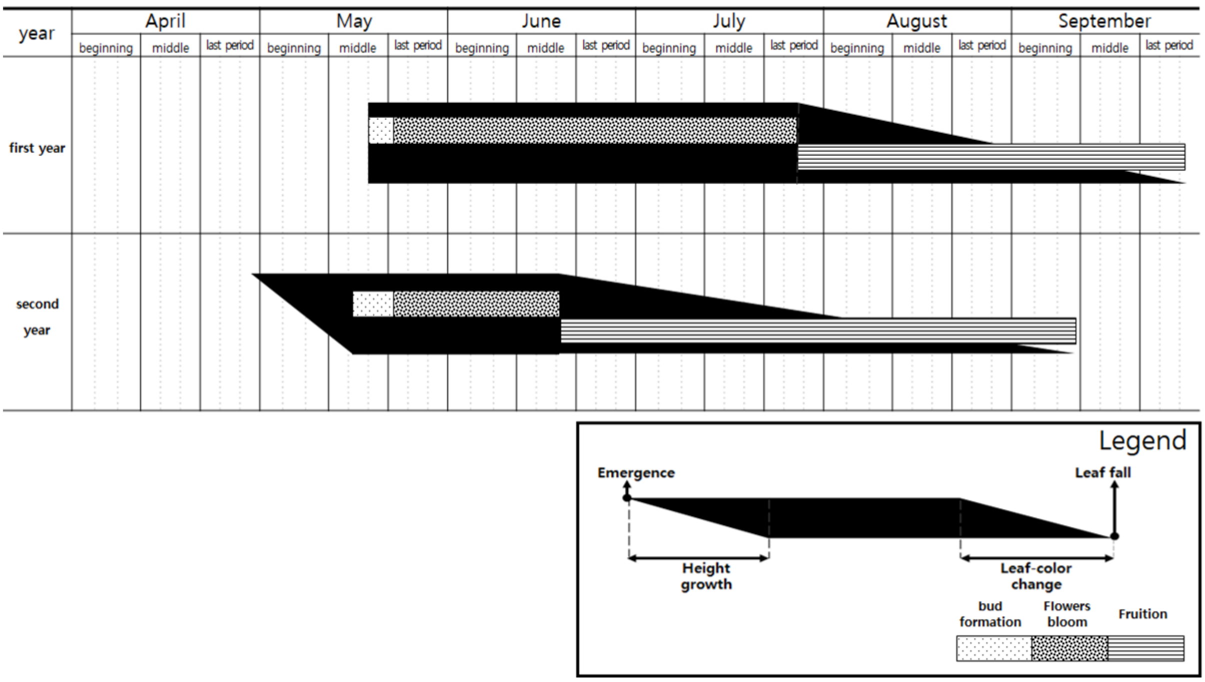

Fig. 4. Schema of phenology of aboveground of *Polygonatum stenophyllum* during study period in the permanent quadrat.

P. stenophyllum finished about two weeks earlier in the second year than in the first year. When the average meteorological temperatures in August of the relevant years were compared, the average temperature in the second year was found to be about 2.8 ℃ higher than that in the first year. Lee et al. (2017) analyzed the plant phenology and its correlation with air temperature. As a results, the correlation between the plant phenology in spring with the air temperature was relatively high, and temperature rise accelerated the average flowering date. Therefore, in this study the plant phenology of the second year are considered to be faster than the first year due to the air temperature rise.

3.2 Reproductive ecological response

As for the flowers of *P. stenophyllum* in the first year, 0 to 8 flowers bloomed in a floor from the 6th floor (F6) at the lowest to the 16th floor (F16) at the highest, and the average number of flowers bloomed per floor (ea ± standard deviation) was 1.56 ± 0.73. The number of flowers was the largest in the 8th floor (F8) and decreased in floors closer to the highest floor (F16) (Fig. 5-ⓐ). In the second year, 0 to 14 flowers bloomed in a floor from the 6th floor (F6) at the lowest to the 20th floor (F20) at the highest, and the average number of flowers bloomed per floor (ea ± standard deviation) was 1.80±1.93. The number of flowers was the largest in the 8th floor (F8) and decreased in floors closer to the 20th floor (F20) (Fig. 5-ⓐ). The average number of flowers per floor ranged from 0 to 14, ranging from 6th floor (F6) to 20th floor (F20), with 1.80 ± 1.93. Flowers were the most on the 8th floor (F8), and the number of flowers tended to decrease as you climb up to the 20th floor (F20) (Fig. 5-ⓐ). *P. stenophyllum* bloomed in turn from the base of the stem to the top floor, similarly to other species of Polygonatum genus (Yoon et al., 2002). In the case of *P. involucratum*, the number of flowers per stem was the smallest at 1.5 ± 0.7 (Yoon et al., 2002). In the case of *P. humile*, the flowers per stem were formed 2.7 ± 1.1 from the third to the eighth floor at the highest (Hasegawa and Kudo, 2005). In the case of *P. odoratum*, the average number of flowers per aboveground part 6.8 ± 2.3 (Guitian et al., 2001). In the case of *P. sibiricum*, which is a species similar to *P. stenophyllum*, the number of flowers per stem was 25.2 ± 12.0, which was the largest. Similarly, in the case of *P. stenophyllum,* the average number of flowers was 26.96 ± 16.40 (standard of the second year) (Fig. 5-ⓐ). The flowers of *P. stenophyllum* bloomed in turn from the bottom of the stem to the top floor. The number of flowers was the largest in the center of the stem and no flower was formed from the floor closest to the ground (F1) to the fifth floor (F5) and above the highest floor (Fig. 5-ⓐ). Polygonatum genus including *P. sibiricum*, *P. odoratum*, *P. odoratum* var. *pluriflorum*, *P. humile* had few or no flower at the lower part of the stem similarly to *P. stenophyllum* (Yoon et al., 2002). The reason why Polygonatum genus forms flowers in the center of the stem instead of the base of the stem was found to be the fact that the leaves that grow in the nodes close to the ground are products produced from the resources from by the underground part and these leaves invest more resources in growth rather than reproductive organ formation (Min, 2017). *P. stenophyllum* is considered to show similar results.

The fruits of *P. stenophyllum* came into bearing from the 6th floor (F6) at the lowest to the 15th floor (F15) at the highest in the first and second years and were not formed from the floor closest to the ground in which leaves grow(F1) to the 5th floor (F5). The number of fruits formed in one floor ranged from 0 to 13 in the first year and from 0 to 10 in the second year. The average number of fruits per floor (ea ± standard deviation) was 1.57 ± 0.70 in the first year and 0.92 ± 0.78 in the second year. In the first year, the number of fruits was the largest in the 9th floor (F9) and tended to decrease in floors closer to the 15th floor (F15) (Fig. 5-ⓑ). In the second year, the number of fruits was the

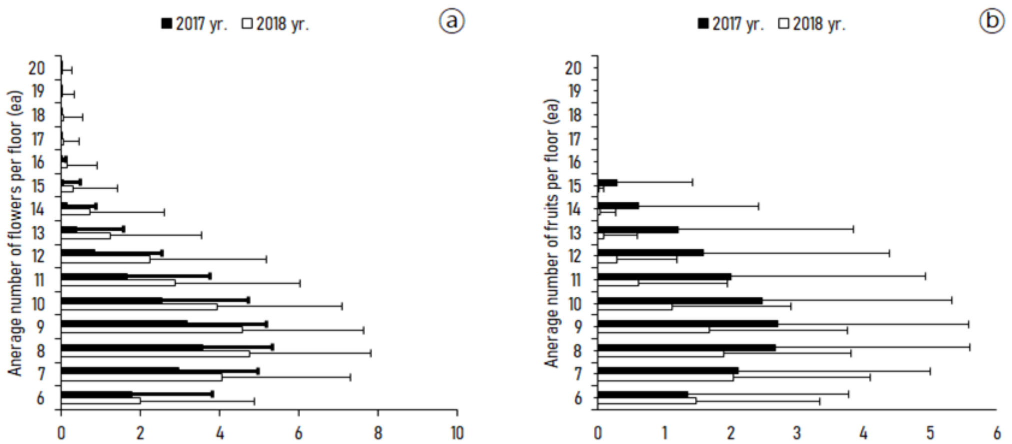

Fig. 5. Average number (ea) of flowers (ⓐ) and fruits (ⓑ) per floor of *Polygonatum stenophyllum* during two years.

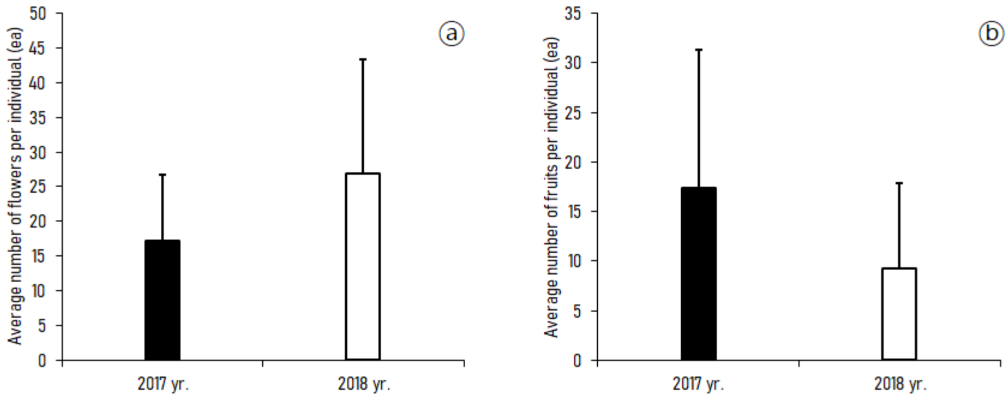

Fig. 6. Average number (ea) of flowers (ⓐ) and fruits (ⓑ) per individual of *Polygonatum stenophyllum* during two years.

largest in the 7th floor (F7) and tended to decrease in floors closer to the 15th floor (F15). The fruits of *P. stenophyllum* were not formed from the first floor (1F) to the fifth floor (5F) but were formed from the 6th floor (6F) on average. The number of fruits was large in the center of the stem (7F ~ 9F) and tended to decrease in floors closer to the top floor (Fig. 5-ⓑ). In the case of *P. odoratum* that showed results contrary to this study, the number of fruits increased in floors closer to the base of the stem (Guitian et al., 2001).

In the case of *P. stenophyllum*, as for the numbers of reproductive organs (flowers and fruits) formed per individual (ea ± standard deviation), the number of flowers and the number of fruits were 17.19 ± 9.54 and 17.34 ± 14.04, respectively in the first year and 26.96 ± 16.40 and 9.23 ± 8.66, respectively in the second year(Fig. 6-ⓐ, ⓑ). When the rates of change of the reproductive organs were compared for two years, it could be seen that whereas the number of flowers increased by 56%, the number of fruits decreased by about 47%. In particular, in the second year, the average number of fruits decreased by about 35% compared to the average number of flowers formed in one individual. The loss and drops of fruits are caused by incomplete pollination and fertilization, food intake by herbivores, physical damage due to plants, and rapid changes in environmental factors (precipitation, temperature, etc.) (Kang and Min, 1994). The largest cause of the decrease in the number of fruits in the second in the study area is assumed to be the precipitation because precipitation increased by 298 mm in May-June, which is the flowering period of *P. stenophyllum*. In addition, *P. stenophyllum* which is pollinated by bumblebees (Chung et al., 2014) is considered to have been incompletely pollinated and fertilized due to the heavy rainfall.

4. Conclusions

The aboveground part of *P. stenophyllum* in riverside appeared in early spring and the life cycle ended as the leaves were falling in early September. In early May, two buds were formed per one peduncle up to six pairs per floor. The flowers of *P. stenophyllum* began to bloom from the bottom of the stem in turn to the top and bloomed when the number of leaves was at least three. The fruits were formed in mid-June and matured until the end of September. The flowers and fruits of *P. stenophyllum* were not formed in the bottom and top floors but were formed from the 6th to 20th floors on average and were the most intensively formed in the center of the stem. The number of flowers and fruits decreased from the base of the stem to the tip. Overall, the rapid flowering and the fruit maturation for a long period of time after the appearance of the aboveground part are considered to be a strategy of *P. stenophyllum* to be prepared for seasonal variation in the unstable habitats.

사 사

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