Cold-Induced Interveinal Chlorosis and Defective Root Formation Observed in *Lilium* × *formolongi*

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Abstract

We tried to identify the frequency of chlorosis occurrence and defective root formation under the low temperature conditions observed in *Lilium* × *formolongi* using a cultivar 'Green Lily Alp' ('Alp') as a model. First, we confirmed that 'Alp' plants exhibited more severe interveinal chlorosis than did *L.* × *formolongi* plants. The highest index for interveinal chlorosis in 'Alp' plants occurred in a study from November 2016 to April 2017 and was 4.1, compared with an index for *L.* × *formolongi*. A significant difference was observed in root dry weights, with stem roots weighing 60 mg and 260 mg and basal roots weighing 240 mg and 780 mg per plant in symptomatic and asymptomatic 'Alp' plants, respectively. The index for interveinal chlorosis occurrence was 0 under the control 25/10℃ (day/night) temperature treatment but 0.7 under the cooler 15–19/10℃ treatment. Total chlorophyll content and basal root dry weight were significantly lower ($P<0.05$) under 15–19/10℃ treatment than under the control. These results suggest that the extreme frequency and occurrence of low temperature-induced interveinal chlorosis and defective root formation in 'Alp' plants is induced by the low root zone temperature.

Key Words: Chlorophyll content, Environmental factor, 'Green Lily Alp', Root zone Temperature, Winter.

1. Introduction

Lilium spp. are common ornamental plants and are grown all over the world, and its year-round production is one of important themes in the floricultural industry. *Lilium* × *formolngi* produces pure-white colored flowers and are frequently used as a cut flower. 'Green Lily Alp' ('Alp') is a cultivar of *L.* × *formolngi* with green flower and frequently exhibits chlorosis on the leaf (Fig. 1). Chlorosis occurrence in lily (*Lilium* spp.) is referred to as interveinal chlorosis[1, 2]. Our hearing from a grower suggested that 'Alp' plants exhibited interveinal chlorosis and defective root formation more severely and more frequently than did the original *L.* × *formolongi* in early March.

Chlorosis is a physiological disorder characterized by a fading in leaf color as a result of a decrease in chlorophyll content. Chlorosis occurrence causes serious problems in agricultural and horticultural production, such as low yield and low product quality. In particular, studies on chlorosis in peach (*Prunus persica*), pear (*Pyrus communis*), soybean (*Glycine max*), and peanuts (*Arachis hypogaea*) reported significant yield decreases[3–6], and studies on geranium (*Pelargonium* × *hortorum*) and rose (*Rosa* spp.) reported damage to the ornamental value of chlorotic plants[7, 8]. Reports on grapefruit (*Citrus paradise*), orange (*C. reticulata*), peach (*P. persica*), corn (*Zea mays*), mung bean (*Vigna radiata*), and rice (*Oryza sativa*) indicated that the low temperature is a causative factor for the occurrence of chlorosis[9–14]. Further, reports on

Received December 22, 2019
Accepted February 7, 2020

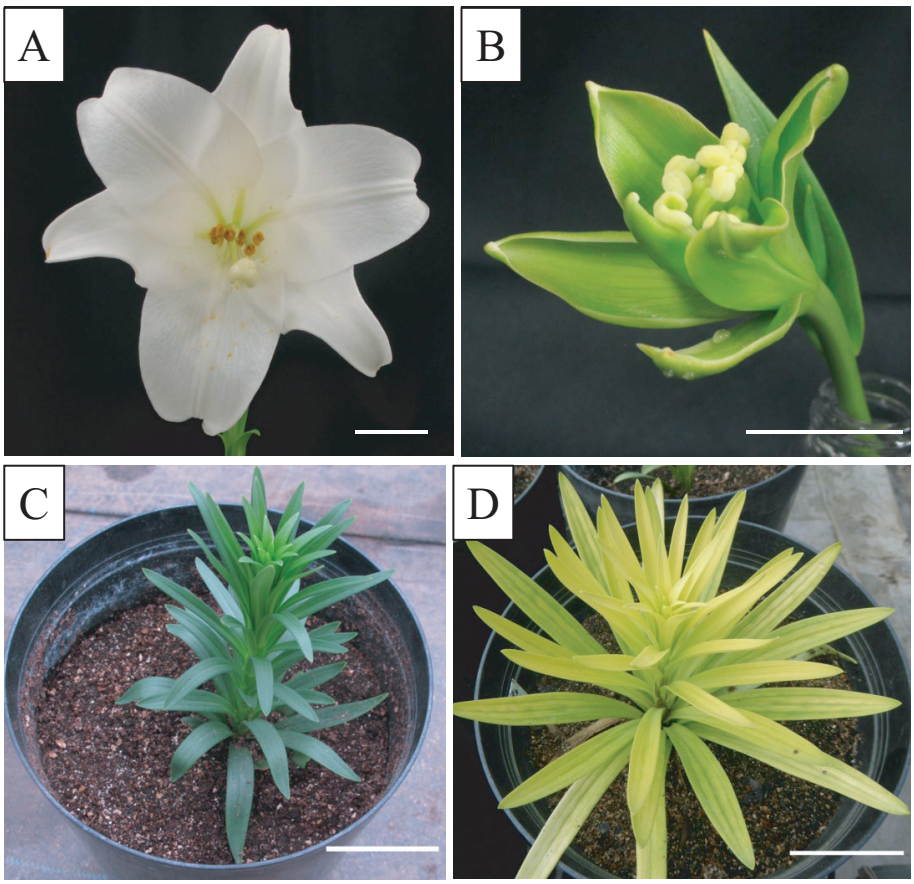

Fig. 1. The appearance of flowers and shoots of *L.* ×*formolongiand* 'Alp'.
A: Flower of *L.* ×*formolongi*; B: Flower of 'Alp' plant; C: Shoot of an asymptomatic 'Alp' plant; D: Shoot of a interveinal chlorosis exhibiting 'Alp' plant. Scale bars in (A) and (B) = 20mm. Scale bars in (C) and (D) = 50mm.

soybean (*G. max*), corn (*Z. mays*), beech (*Fagus sylvatica*), and Tilia (*Tilia cordata*) have indicated that low root zone temperature decreased root weight[15–17]. Therefore, the low temperature conditions of Nagano prefecture in spring might cause chlorosis occurrence and defective root formation.

In the present study, we tried to elucidate a contribution of the low temperature conditions on the occurrence of chlorosis and the defect in root formation in *L.* × *formolongi* using 'Alp' plants as a model. Firstly, we confirmed that 'Alp' plants exhibited cold-induced interveinal chlorosis more frequently and its expression was more severe than in the original *L.* × *formolongi*. The frequencies and degrees of interveinal chlorosis occurrence were compared between *L.* × *formolongi* and 'Alp' plants, and root formation was compared between asymptomatic and interveinal chlorosis exhibiting 'Alp' plants. The impact of low temperature on the frequency and severity of interveinal chlorosis in 'Alp' plants was evaluated under artificial climate-controlled conditions. Finally, the contribution of low temperature conditions on chlorosis occurrence and defective root formation was discussed.

2. Materials and Methods

2.1 Plant materials

'Alp' plants and *L.* × *formolongi* plants that had reverted from 'Alp' were obtained as bulbs from a lily grower in Komagane City, Nagano Prefecture, Japan. Plants of both genotypes were derived *in vitro* from the plants obtained. Plants of *L.* × *formolongi* were obtained as adventitious shoots using *in vitro* scaling, whereas shoot apical meristem cultures of 'Alp' plants were set up according to a conventional method, and regenerated plants were propagated by *in vitro* scaling. Plants were cultured on modified Murashige and Skoog (MS medium), containing MS macroelements, namely 1,650 mg·L^{-1} NH_4NO_3, 1,900 mg·L^{-1} KNO_3, 440

mg·L^{-1} $CaCl_2 \cdot 2H_2O$, 370 mg·L^{-1} $MgSO_4 \cdot 7H_2O$, and 170 mg·L^{-1} KH_2PO_4; MS microelements, containing 31 mg·L^{-1} H_3BO_3, 120.5 mg·L^{-1} $MnSO_4 \cdot 4H_2O$, 53 mg·L^{-1} $ZnSO_4 \cdot 7H_2O$, 4,150 μg·L^{-1} KI, 1,250 μg·L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 125 μg·L^{-1} $CuSO_4 \cdot 5H_2O$, 125 μg·L^{-1} $CoCl_2 \cdot 6H_2O$, and 38 mg·L^{-1} Fe-Na_2-EDTA; and MS organic elements, containing 100 mg·L^{-1} myo-inositol, 5 mg·L^{-1} glycine, 1,250 μg·L^{-1} nicotinic acid, 1,250 μg·L^{-1} pyridoxic acid, and 100 μg·L^{-1} thiamine hydrochloride acid[18]. The modified MS medium containing 3% (w/v) sucrose, was solidified with 0.3% gellan gum. The pH was adjusted to 5.8 prior to autoclaving at 121℃ for 15 min. After autoclaving, 100 mL of the modified MS medium was solidified in a 75 mm × 75 mm × 100 mm polycarbonate box. Cultures were incubated in the culture room under a 12 h day-length provided by fluorescent lights at photon flux density of 240 μmol·m^{-2}·s^{-1} at 20–30℃. Then, plants were transplanted to 193 mL cell trays filled with 155 mL of medium consisting of Metro Mix 250 (SunGro Horticulture Distribution, USA): vermiculite (Fukushimabami, Japan) = 2: 1 (v/v) and acclimated in the culture room for one month. During acclimation, plants were fertilized every two weeks using a 1:1000 diluted solution of Hanakoujyo (N: P: K = 50: 100: 50 mg·mL^{-1}; Sumitomo Chemical Garden Products Inc., Japan) and watered appropriately. After acclimation, plants were transplanted to 19.5 cm-diameter pots filled with 3 L above-mentioned medium and grown in a greenhouse at the Shinshu University experimental farm (Minamiminowamura, Japan). Considering the average temperature of Komagane City from mid-April to early May, the greenhouse was heated with an oil stove set at a minimum of 10 ℃, and side window was open automatically when the inside temperature came up to 20℃. Plants were fertilized as described above every two weeks and were watered appropriately.

2.2 Evaluation of leaf color

Five fully expanded leaves on the first to fifth nodes were evaluated for occurrence of interveinal chlorosis, using a leaf color scale (LEAF COLOR SCALE for PADDY RICE; Fujihira Industry Co., Ltd., Japan). In the leaf color scale, the leaf color scale values were sectioned into seven by the brightness of green, from light (leaf color scale value = 1) to dark (leaf color scale value = 7). Interveinal chlorosis occurrence on a leaf level was scored as an interveinal chlorosis index (ICI). Plants with leaves that exhibited color equivalent to the leaf color scale values 1, 2, 3, 4, or 5 were classified as ICI 5, 4, 3, 2, or 1, respectively. Plants with leaves that exhibited color equivalent to the leaf color scale value 6 or 7 were classified as ICI 0.

2.3 Total chlorophyll content

One leaf segment 1 cm × 1 cm was sampled from each of the five leaves evaluated for ICI. Each leaf segment was soaked in 1 mL N, N-dimethylformamide in a 2 mL plastic tube in the dark at 4℃ for 24 h, under which conditions the total chlorophylls were extracted. Absorbances at wavelengths of 663.8 nm and 646.8 nm were measured using a spectrophotometer (UV-1200; Shimadzu Corporation, Japan). Total chlorophyll content per fresh weight was determined according to the method of Porra et al.[19].

2.4 Root formation

Stem roots and basal roots were collected and dried at 60℃ for 72 h (Fig. 2), before obtaining and recording the dry weights of stem roots and basal roots per plant.

2.5 Root zone temperature

Root zone temperature was recorded using a data logger (Ondotori Jr. TR42; T&D, Japan). Root zone temperature under day/night air temperature variations of 15/10℃ (day/ night), 19/10℃ and 25/10℃ in incubators in Exp. 3 was monitored for 24 h.

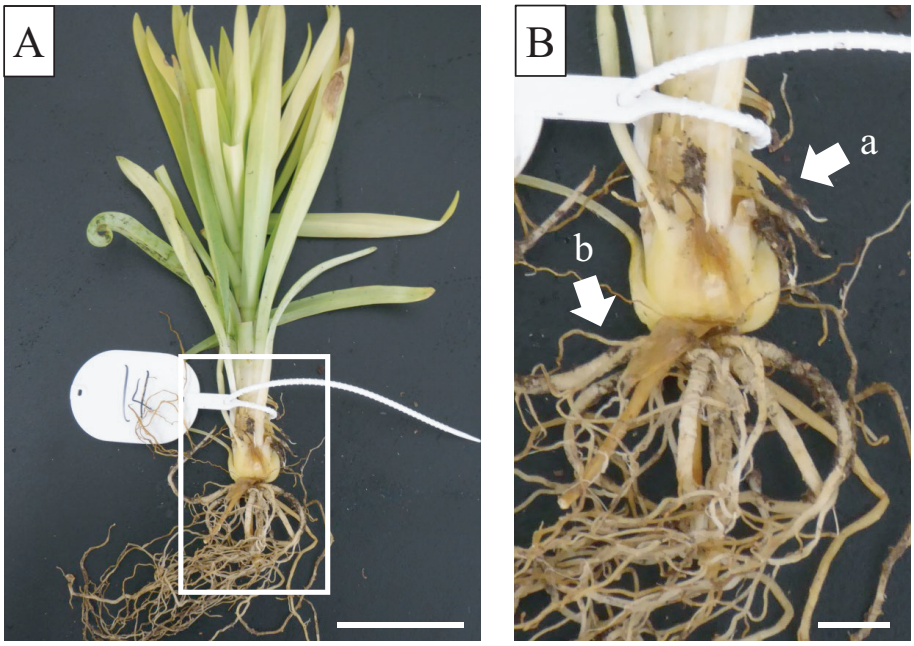

Fig. 2. Root formation of an 'Alp' plant.

A: Entire plant of an 'Alp' plant; B: Root system of an 'Alp' plant, corresponding to the area indicated by the square in (A). a: Stem root; b: Basal root. Scale bars in (A) and (B) = 50 mm and 10 mm, respectively.

2.6 Comparison of interveinal chlorosis occurrence between *L.* × *formolongi* and 'Alp' (Exp. 1)

Occurrence of interveinal chlorosis was compared between *L.* × *formolongi* and 'Alp' plants. Plant materials planted in the 19.5 cm-diameter pots were prepared as described. The experiment was replicated three times as follows; replication 1: acclimation was started on October 26, 2016 and planted on November 30, 2016, replication 2: acclimation was started on December 1, 2016 and planted on December 31, 2016, replication 3: acclimation was started on January 6, 2017 and planted on February 6, 2017. A total of sixteen or fifteen plants of each of *L.* × *formolongi* and 'Alp' were used for each replication. ICIs were determined three months after planting according to the condition of 'Alp' plants exhibiting interveinal chlorosis in a farmer's greenhouse (Fig. 1D). Our hearing result from a famer suggest that interveinal chlorosis in 'Alp' plants frequently occurs in late April, whereas asymptomatic leaves are produced after May in unheated greenhouse in Komagane City. In order to monitor the seasonal change in ICIs, the ICI values of *L.* × *formolongi* and 'Alp' plants in replication 1 were recorded every two weeks from February 15, 2017 to April 29, 2017.

2.7 Interveinal chlorosis occurrence and root formation (Exp. 2)

Fifteen *in vitro*-grown 'Alp' plants were acclimated from September 30, 2016 and planted on November 30, 2016. On March 31, 2017 when the plants had grown to 15 cm shoot length, three interveinal chlorosis exhibiting (symptomatic) 'Alp' plants (ICI: 5) and three asymptomatic 'Alp' plants (ICI: 0) were randomly selected. The stem roots and basal roots from each plant were harvested, dried and weighted and comparisons were made between asymptomatic and symptomatic 'Alp' plants.

2.8 Effect of temperature on occurrence and severity on interveinal chlorosis (Exp. 3)

After acclimation, 'Alp' plants were transplanted to 15 cm-diameter pots filled with 1.4 L above-mentioned medium and grown under two different environmental conditions in incubators (BioTRON LH-241S; Nippon Medical & Chemical Instruments Co., ltd., Japan) for three months. All plants were grown under a 12 h day-length and either 25/10℃ (day/night) condition as a control and 15–19/10℃ conditions as a low temperature treatment (Fig. 3). In the low temperature treatment, the day temperature was changed

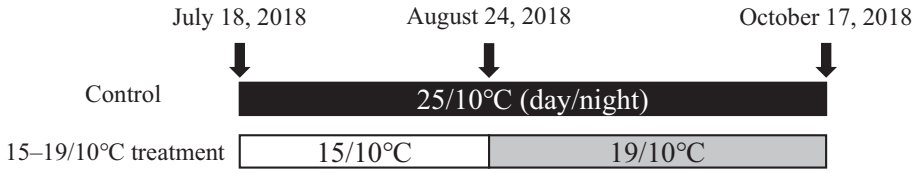

Fig. 3. Temperature conditions in Exp. 3.

'Alp' plants were grown under the 12 h day-length and 25/10℃ (day/night) conditions as a control and grown under the 12 h day-length and 15–19/10℃ conditions as a low temperature treatment. Day temperature in the low temperature treatment was changed from 15℃ to 19℃ after day 39 from the start of the treatment to promote plant growth.

from 15℃ to 19℃ after day 39 from the start of the treatment to achieve plant growth promotion. The ICI, total chlorophyll content, and the root dry weights were determined three months after the start of the treatment.

2.9 Statistical analysis

In Exps. 1, 2, and 3, Student's *t*-test was used for analyzing the difference of the ICIs, the root dry weights, and the total chlorophyll contents. The significance of the ICI value difference among three different replications in Exp. 1 was analyzed by analysis of variance followed by Tukey's multiple range test to compare means.

3. Results

3.1 Comparison of interveinal chlorosis occurrence between *L.* × *formolongi* and 'Alp' (Exp. 1)

The mean ICIs of 'Alp' plants were significantly higher than those of *L.* × *formolongi* in all three replications. The mean ICIs of 'Alp' plants ranged from 1.8 to 4.1 in three replications and those of *L.* × *formolongi* ranged from 0.7 to 2.3 (Table 1). With regard to the 'Alp' plants, the mean ICI value in the replication 2 conducted from late December 2016 to late March 2017 was 4.1 and was the highest of the three replications (Table 1). The mean ICI value in the replication 3 conducted from early February 2017 to late April 2017 was 1.8 and was the lowest (Table 1). The mean ICI values of *L.* × *formolongi* in replication 1 and 2 were similar and higher than the ICI value in replication 3. The mean ICI values of *L.* × *formolongi* and 'Alp' plants in replication 1 decreased steadily from mid-February 2017 to late April 2017 (Fig. 4). In late March, the mean ICI value of 'Alp' plants in replication 1 was 2.0 and was equivalent to the mean ICI values of *L.* × *formolongi* in replications 1 and 2 (Table 1; Fig. 4).

Table 1. Comparison of interveinal chlorosis indexes between *L.* × *formolongi* and 'Alp'.

Replication	Period of treatment	Genotype	ICI[z]	
1	From November 30, 2016 to March 3, 2017	*L.* × *formolongi*	2.3±0.3[y]	a[x]
		Alp	3.4±0.2	b
		t-test[w]	**	
2	From December 31, 2016 to March 31, 2017	*L.* × *formolongi*	2.3±0.3	a
		Alp	4.1±0.2	a
		t-test	**	
3	From February 6 to April 29, 2017	*L.* × *formolongi*	0.7±0.1	b
		Alp	1.8±0.2	c
		t-test	**	

[z] Occurrences of interveinal chlorosis were evaluated as the interveinal chlorosis index (ICI). Five fully expanded leaves on the first to fifth nodes were evaluated for the occurrence of interveinal chlorosis using a leaf color scale. Plants with leaves that exhibited the color scale values 1, 2, 3, 4, or 5 were classified as ICI 5, 4, 3, 2, or 1, respectively. Plants with leaves that exhibited the color equivalent to the leaf color scale values 6 or 7 were classified as ICI 0.

[y] Mean ± SE (n = 16).

[x] Means followed by different letters are significantly different at $P<0.05$ by Tukey's multiple range test (n = 16) in each genotype.

[w]** indicates significant difference within each replication at $P<0.01$ by *t*-test (n = 16).

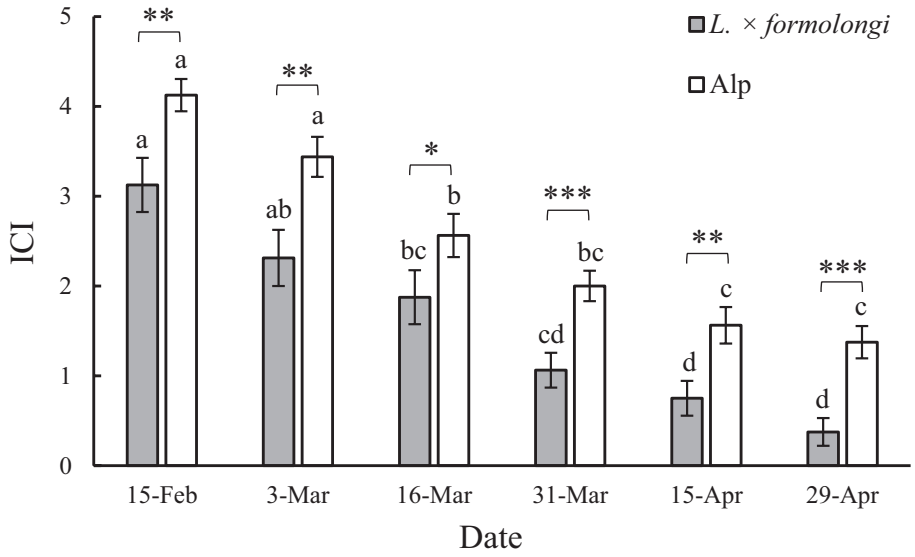

Fig. 4. Change of ICIs in *L.* ×*formolongi* and 'Alp' plants from mid-February to late April (replication 1).
Bars indicate ±standard error (SE) (n = 15, 16). *,**, and *** indicate significant
difference between the genotypes within each replication at $P<0.05$, 0.01, and 0.001, respectively, by *t*-test (n = 15, 16). Means followed by different letters are significantly different among the dates in each genotype at $P<0.05$ by Tukey's multiple range test.

3.2 Interveinal chlorosis occurrence and root formation (Exp. 2)

The differences of shoot growth statuses other than leaves color between symptomatic 'Alp' plants and asymptomatic 'Alp' plants were not observed (Fig. 5A, B). The mean dry weights of stem root and basal root were significantly lower in symptomatic as opposed to asymptomatic 'Alp' plants. The mean dry weights of stem root for symptomatic and asymptomatic 'Alp' plants were 60 mg and 260 mg, respectively, and those of basal root were 240 mg and 780 mg, respectively (Fig. 5C).

3.3 Temperature impact on interveinal chlorosis occurrence (Exp. 3)

The mean ICI of 'Alp' plants of the 25/10℃ control was 0.0, while that of the 15–19/10℃ treatment was 0.7, although the difference was not significant ($P > 0.05$) (Table 2). The mean total chlorophyll content and the dry weights of the basal roots were significantly lower in the 15–19/10℃ treatment than in the control (Table 2). The mean total chlorophyll contents were 540 μg·g FW^{-1} and 646 μg·g FW^{-1} in 15–19/10℃ treatment and the control, respectively (Table 2). The mean dry weights of the basal roots were 1,080 mg and 2,040 mg in the 15–19/10℃ treatment and the control, respectively: the mean dry weights of the basal roots of the 15–19/10℃ treatment and the control were greater than those of asymptomatic 'Alp' plants maintained in the greenhouse conditions in Exp. 2 (Table 2; Fig. 5C). Stem root formation was not observed in 'Alp' plants under either treatment in Exp. 3. The mean root zone temperatures in the control (25/10℃), 19/10℃, and 15/10℃ conditions of Exp. 3 were 16.7℃, 15.6℃, and 14.2℃, respectively.

Table 2. Interveinal chlorosis index, total chlorophyll content, and the dry weight of the basal root of 'Alp' plants maintained in the control and the low temperature treatment.

Treatment	ICI[z]	Total chlorophyll content (μg·g FW^{-1})	Dry weight of basal root (mg)	Number of expanded leaf
Control	0.0±0.0	645.8±13.5	2040±271	17.3±2.2
15–19/10℃	0.7±0.4[y]	539.8±34.3	1080±151	13.0±2.5
t-test[x]	N.S.	**	**	N.S.

[z] Occurrences of interveinal chlorosis were evaluated as the interveinal chlorosis index (ICI). Five fully expanded leaves on the first to fifth nodes were evaluated for the occurrence of interveinal chlorosis using a leaf color scale. Plants with leaves that exhibited the color scale values 1, 2, 3, 4, or 5 were classified as ICI 5, 4, 3, 2, or 1, respectively. Plants with leaves that exhibited the color equivalent to the leaf color scale values 6 or 7 were classified as ICI 0.
[y] Mean ± SE (n = 3).
[x] ** indicates significant difference at $P<0.01$ by *t*-test (n = 3). N.S. indicates no significant difference.

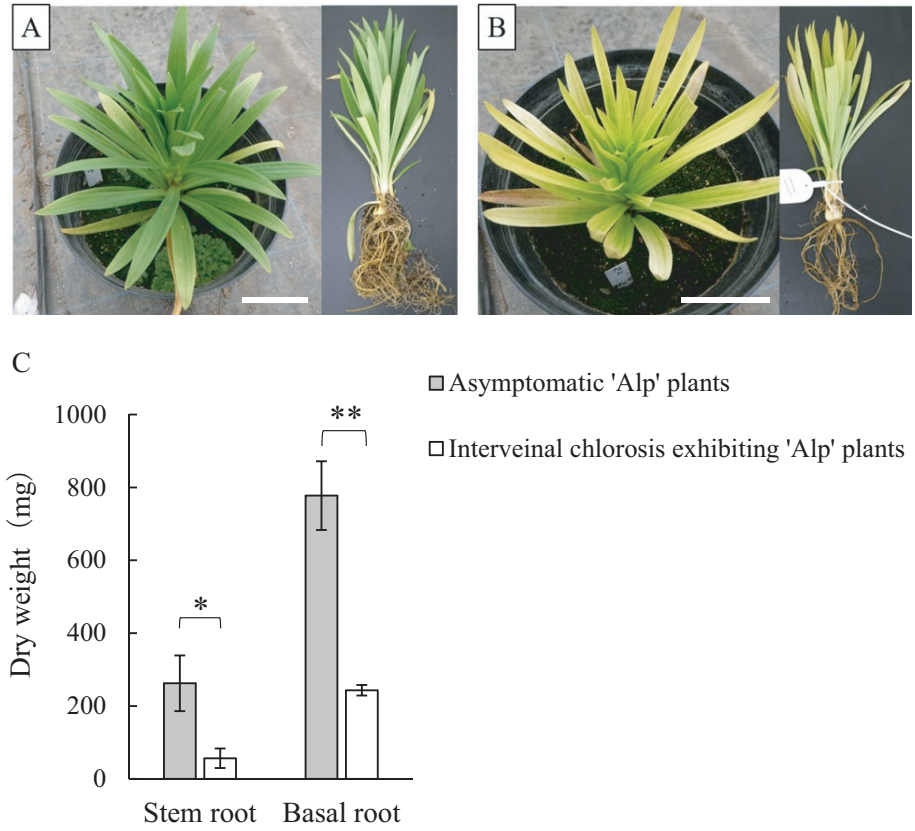

Fig. 5. Comparison of roots formation between asymptomatic 'Alp' plants and interveinal chlorosis exhibiting 'Alp' plants.

A: Asymptomatic 'Alp' plant; B: Interveinal chlorosis exhibiting 'Alp' plant; C: Comparison of dry weights of stem root and basal root between asymptomatic 'Alp' plants and interveinal chlorosis exhibiting 'Alp' plants. Scale bars in (A) and (B) = 50 mm. Bars in (C) indicate ±standard error (SE) (n = 3). * and ** indicate significant difference at $P<0.05$ and 0.01, respectively, by *t*-test (n = 3).

4. Discussion

'Alp' plants exhibited interveinal chlorosis more severely and more frequently than did the original *L.* × *formolongi*, and the symptomatic 'Alp' plants showed smaller root formation (Table 1; Fig. 5). The lower chlorophyll content is a typical symptom of chlorosis and has been reported in many chlorosis-exhibiting plants such as rhododendron (*Kalmia latifolia*), sanyeqing (*Tetrastigma hemsleyanum*), wheat (*Triticum durum*), pothos (*Epipremnum aureum*), and rice (*O. sativa*)[20–24]. Mineral deficiency is one of the factors most commonly responsible for the occurrence of chlorosis in many plant species[25–31]. For example, magnesium deficiency or molybdenum deficiency decreased chlorophyll content in arabidopsis (*Arabidopsis thaliana*), kiwi fruit (*Actinidia deliciosa*), and wheat (*T. durum*)[32–34]. Therefore, contribution of a mineral deficiency due to the defect in root formation might be a candidate factor for the occurrence of interveinal chlorosis in 'Alp' plants.

The ICI of 'Alp' plants showed seasonal change and was highest in the replication conducted from late December to late March (Table 1; Fig. 4). This finding suggested that a winter-specific environmental factor caused the interveinal chlorosis in 'Alp' plants in our experimental condition, with the temperature and day length being considered as candidate factors. Several studies have reported that low temperature condition can induce the occurrence of chlorosis[9–14]. Moreover, Tewari and Tripathy (1998) reported that cold stress suppressed chlorophyll synthesis in cucumber (*Cucumis sativus*) and wheat (*T. aestivum*)[35]. Further, in the cut flower production field of 'Alp', the occurrence of interveinal chlorosis was observed from April to May under unheated greenhouse conditions (data not shown). This period had a longer day length than all

replications of Exp. 1. Hence, the low temperature was left as the sole candidate factor for the seasonal change in occurrence of interveinal chlorosis.

In Exp. 3, 'Alp' plants maintained under the 15–19/10℃ condition exhibited higher mean ICI and lower chlorophyll contents than those in the control plants (Table 2). However, the ICIs were lower than the 'Alp' plants evaluated in Exp. 1 (Table 1; Table 2). To understand the difference in the ICIs between the artificial climate condition and the experimental condition of Exp. 1, we attempted to compare the mean root zone temperature between the two conditions. Unfortunately, the root zone temperature was not recorded in Exp. 1. Thus, using the greenhouse set at the same conditions in Exp. 1, the mean root zone temperatures were recorded using a pot plant of 'Alp'. The mean root zone temperatures from mid-January to mid-February and from late March to mid-April 2018 were 12.7℃ and 17.9℃, respectively, under that greenhouse condition. According to the results of Exp. 1, root zone temperatures of 12.7℃ and 17.9℃ would approximate to the temperatures that induced or did not induce interveinal chlorosis, respectively. The mean root zone temperature of the 15–19/10℃ treatment was 1.5℃ higher than that in the greenhouse from mid-January to mid-February. Hence, moderate interveinal chlorosis occurrence in the 15–19/10℃ treatment, as opposed to under the greenhouse conditions, would be due to the higher root zone temperature. Differences in amounts of basal and stem roots could also contribute to the ICI difference between Exps. 1 and 3. In 15–19/10℃ treatment in Exp. 3, the higher dry weight of basal root than symptomatic 'Alp' plants in Exp. 2 and the loss of stem root formation were observed (Table 2; Fig. 5C). The basal roots support the plants physically, and the stem roots have a crucial role to play in absorbing nutrients in lily[1]. The basal root formation would not particularly contribute to the suppression of interveinal chlorosis occurrence, by contrast, the greater stem root formation would suppress interveinal chlorosis occurrence as observed in the asymptomatic 'Alp' plants in Exp. 2. Control 'Alp' plants in Exp. 3 never produced stem roots, and no interveinal chlorosis occurrence was observed (Table 2). Those 'Alp' plants produced far more basal roots than did asymptomatic 'Alp' plants in Exp. 2 (Table 2; Fig. 5C). Favorable basal root formation might compensate for the lack of stem root formation in *L.* × *formolongi*.

In conclusion, it was revealed that 'Alp' plants exhibited interveinal chlorosis more severely and more frequently than did *L.* × *formolongi* and that the symptomatic 'Alp' plants showed a defect in root formation. This defect in root formation could be induced by the low root zone temperature in spring, which might decrease mineral absorption and induce the interveinal chlorosis occurrence. Defective root formation might be a factor linking low temperature condition and chlorosis occurrence, and further study on the phenomena will provide valuable information for understanding the occurrence of chlorosis under low temperature conditions in many plant species. We are now analyzing the relationships among the occurrence of chlorosis, the defect in root formation, and the low temperature conditions.

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低温条件下におかれたユリで頻発する葉脈間クロロシスへの根の形成不良の関与

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本研究では，シンテッポウユリの変異体である‘グリーンリリアルプ’（‘アルプ’）をモデルとして，多くの植物種で認められる低温条件下で起こるクロロシスと根の形成不良との関係を調査した．長野県の生産農家への聞き取りによると，‘アルプ’は変異前のシンテッポウユリと比較して，春に葉脈間クロロシスが多発し，葉脈間クロロシスを発症した株では健常株と比較して，根の形成量が少ないという．本研究では，まずこの現象を確認した．2016年11月から2017年４月にかけて，暖房温度を10 ℃に設定した加温ハウスで管理したシンテッポウユリと‘アルプ’との間で葉脈間クロロシスの発生程度を比較した．その結果，調査した３反復のすべてでシンテッポウユリと比較して‘アルプ’で葉脈間クロロシスの発生程度が有意に高かった．特に，12月下旬に定植し，３月下旬にクロロシスの発生程度を評価した‘アルプ’で葉脈間クロロシスの発生程度が最も高く，発生程度の値は約4.1であった．‘アルプ’の健常株と葉脈間クロロシスの発生株との間で根の形成量を比較したところ，前者と比較して後者で茎出根と底出根共に形成量が有意に少なく，健常株ではそれぞれ乾物重が約260 mg，約780 mg であり，葉脈間クロロシスの発生株ではそれぞれ約60 mg，約240 mg であった．以上より，春に‘アルプ’で起こる葉脈間クロロシスの多発には，低温による根の形成不良が関与すると考えた．この仮説を検証するために，25／10 ℃（昼／夜）および15–19 ℃／10 ℃（昼／夜）で‘アルプ’を管理し，葉脈間クロロシスの発生程度，葉での総クロロフィル含量および底出根の形成量を比較した．15–19 ℃／10 ℃区では25／10 ℃区と比較して，葉脈間クロロシスの発生程度が高く，25／10 ℃区では0，15–19 ℃／10 ℃区では約0.7であった．しかし，両者の間で有意差は認められなかった．15–19 ℃／10 ℃区では25／10 ℃区と比較して，葉での総クロロフィル含量および底出根の形成量が有意に少なかった．25／10 ℃区と15–19／10 ℃区のそれぞれで，葉での総クロロフィル含量はそれぞれ約646 μg･g FW^{-1}，約540 μg･g FW^{-1}であり，底出根の形成量はそれぞれ約2040 mg，約1080 mg であった．以上より，‘アルプ’での葉脈間クロロシスの発生には，低温に起因する根の形成不良が関与すると考えられた．また，多くの植物種で認められる低温条件下でのクロロシスの発生は，根の形成不良に起因することが強く示唆された．

キーワード：環境要因，‘グリーンリリアルプ’，クロロフィル含量，根圏温度，冬季