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Excessive Calcium Accumulation in the Roots Is a Key Factor in Tipburn Incidence under High Ca Supply in Lisianthus (*Eustoma grandiflorum*) Cultivars

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Received: 23 July 2020; Accepted: 31 July 2020; Published: 2 August 2020

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Abstract: In lisianthus cultivars, the occurrence of tipburn is known to adversely affect quality and yield. However, information concerning the responses of tipburn incidence to different levels of Ca is limited. In addition, only a few studies have investigated the effect of potassium (K) concentration on Ca acquisition and distribution in lisianthus. To address this knowledge gap, we investigated tipburn incidence in three lisianthus cultivars (Umi honoka (UH), Reina white (RW), and Voyage peach (VP)) and the Ca and K concentrations in them under different concentrations of Ca supply (40, 80, and 120 ppm). These cultivars exhibited different responses to different concentrations of Ca supply. Tipburn was not observed in UH. In RW, tipburn incidence and severity significantly decreased with an increase in nutritional Ca concentration, because the Ca concentration in the tips of the top leaves significantly increased with Ca concentration. By contrast, in VP, tipburn incidence under all treatments was 100%, and there was no significant difference in the Ca concentration in the tips of the top leaves among the treatments, but the total Ca concentration significantly increased. VP was the only cultivar that significantly acquired and accumulated more Ca in the roots with an increase in nutritional Ca concentration. Overall, excessive Ca accumulation in the roots under high-Ca conditions inhibits the distribution of Ca to the tips of the top leaves and eventually manifests as tipburn in the cultivar. In addition, our results suggested that the content ratio of K in the nutrient solutions did not prevent Ca acquisition and distribution in lisianthus cultivars and that the K concentration has a negligible effect on the occurrence of tipburn.

Keywords: *Eustoma grandiflorum*; Ca acquisition; Ca distribution; K concentration; antagonism

1. Introduction

Calcium (Ca) is an essential plant macronutrient. It has structural roles in the cell wall and membrane and cellular signaling responses; it also acts as a counter-cation in storage organelles [1]. In horticultural crops, Ca deficiency disorder is a major problem affecting plant quality and yield, as it causes tipburn [2–6]. The occurrence of Ca deficiency disorder is known to be influenced by various environmental factors (e.g., light [7], humidity [8], and airflow [9]). However, there are no environmental control techniques to eliminate the occurrence of Ca deficiency disorder; farmers are often forced to select and produce cultivars with a high resistance to Ca deficiency disorder. Therefore, it is important to elucidate varietal differences among horticultural crops regarding Ca deficiency disorder.

Lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) is one of the most popular cut flowers in Japan. However, tipburn (Ca deficiency disorder) has a negative effect on its production. In extreme cases, tipburn causes the death of the growing point and thus reduces product value. Even small damage can delay the harvest time and increase maintenance efforts, such as bud picking. A previous study on 14 lisianthus cultivars indicated that tipburn-sensitive cultivars have a low ability to distribute Ca to the tips of upper leaves [10]. In addition, it has been suggested that the differences in the humidity and plant growth rate have negligible effects on tipburn incidence in the lisianthus cultivars [11,12]. A study on the responses of three cultivars to different concentrations of Ca supply has been reported [13], but there is no information on how Ca uptake and its distribution change before and after the onset of tipburn under high-Ca conditions. To determine the causes of the physiological disorder, it is necessary to investigate the changes in plants before and after the occurrence of tipburn. Thus, further research is needed to elucidate the varietal difference among lisianthus cultivars in responses to different Ca supply.

The occurrence of Ca deficiency disorder is reportedly influenced by the presence of other cations in the substrate. In particular, the concentration of potassium (K) in the substrate could have an effect on Ca acquisition and distribution. In a previous study on strawberries, antagonism was observed between K and Ca [14]; furthermore, the tipburn incidence in the cultivars was related to leaf Ca and K concentrations [15]. In rice [16] and barley [17], it has been reported that a high K concentration inhibits Ca uptake. Therefore, a high content ratio of K in the nutrient solution in our previous experiments [10–13] may have prevented Ca acquisition and distribution in some lisianthus cultivars. It is necessary to investigate the relevance of Ca and K concentrations in lisianthus cultivars.

In this study, to enhance our understanding of the varietal differences among lisianthus cultivars in the responses to different concentrations of Ca supply, we investigated the incidence of tipburn in three lisianthus cultivars, and Ca acquisition and distribution in them before and after the onset of tipburn. In addition, to discuss the effects of K concentration on Ca acquisition and distribution in lisianthus cultivars, we determined the K concentration in them at the end of the experiment.

2. Materials and Methods

2.1. Plant Material

Three lisianthus cultivars, Umi honoka (UH) (Sumika Agrotech Co., Ltd., Osaka City, Japan), Reina white (RW) (Sakata Seed Corporation), and Voyage peach (VP) (Sakata Seed Corporation, Yokohama, Japan), were selected for this study. These cultivars were selected based on the results for tipburn severity (as high, medium, or low damage) reported previously [10]. Seedlings were developed and raised as previously reported [10–13].

2.2. Treatments

Three treatments with different Ca concentrations (40, 80, and 120 ppm) were established. The nutrient solution for each treatment was prepared by dissolving nutrient salts in distilled water. The nutrient salts used in the 40 ppm Ca treatment were as follows. KNO_3 (Fujifilm Wako Chemicals U.S.A. Corporation, Richmond, VA, USA), 0.202 g/L; $Ca(NO_3)_2{\cdot}4H_2O$ (Fujifilm Wako Chemicals U.S.A. Corporation), 0.236 g/L; $NH_4H_2PO_4$ (Fujifilm Wako Chemicals U.S.A. Corporation), 0.038 g/L; $MgSO_4{\cdot}7H_2O$ (Fujifilm Wako Chemicals U.S.A. Corporation), 0.123 g/L; and Otsuka-house No. 5 L (OAT Agrio Co., Ltd., Tokyo, Japan), 0.4 mL/L. For the 80 and 120 ppm Ca treatments, in addition to these salts, $CaCl_2{\cdot}4H_2O$ (Fujifilm Wako Chemicals U.S.A. Corporation,) was added at concentrations of 0.1497 and 0.294 g/L, respectively. The theoretical values of the nutrient concentrations are shown in Table 1.

The plants were maintained in a phytotron (25/20 °C (14 h light period (250 µmol m^{-2} s^{-1} maintained using a fluorescent light)/10 h dark period), 60 ± 5% humidity, and 400 ppm CO_2) and supplied with the nutrient solution for 30 min every morning. We replaced the nutrient solution with fresh nutrient solution every 2 weeks. In the phytotron, the plants in each treatment were randomly divided into three plots for cultivation.

Table 1. Theoretical values of nutrient concentrations in each Ca treatment.

Nutrients (ppm)		NO_3-N	NH_4-N	PO_4-P	K	SO_4-S	Ca	Mg	Fe	B	Mn	Zn	Cu	Mo	Cl
Ca treatments	40 ppm	113	4.6	10.2	107	16	40	12	3.1	1.8	0.9	0.09	0.04	0.04	-
	80 ppm	113	4.6	10.2	107	16	80	12	3.1	1.8	0.9	0.09	0.04	0.04	71
	120 ppm	113	4.6	10.2	107	16	120	12	3.1	1.8	0.9	0.09	0.04	0.04	142

2.3. Sampling

Fifteen plants of each cultivar were randomly sampled at the start of the experiment (0 weeks). Subsequently, nine plants (3 plants × 3 plots) were sampled in each treatment at 4 weeks to determine the Ca acquisition and distribution within plants before the onset of tipburn. At the end of the experiment, 15 plants (5 plants × 3 plots) were harvested in each treatment at 8 weeks. The harvested plants were washed with distilled water and were divided into roots and shoots immediately. In addition, the first (top) and fourth (lower) leaves on the main stem were distinguished at 4 weeks to investigate the vertical Ca distribution in each cultivar. The leaf position was determined from the top to the base. The first (top), fourth (middle), and seventh (lower) leaves on the main stem were distinguished at 8 weeks.

2.4. Tipburn Severity and Incidence

Whole leaves were scored from 0 to 1 using an arbitrary tipburn severity index (0, asymptomatic; 0.2, deformed leaf margins; 0.5, leaf-tip chlorosis; 1, leaf-tip necrosis) [10–13]. Tipburn severity was determined using the following formula:

$$\text{tipburn severity} = \sum \{(\text{severity index} \times \text{leaf number})/\text{whole leaves number per pot}\} \times 100 \quad (1)$$

Tipburn incidence was defined as the percentage of plants exhibiting tipburn symptoms, {(number of plants exhibiting tipburn symptoms)/15 × 100}.

2.5. Determination of Ca and K Concentrations

The harvested samples were dried at 70 °C for 72 h, separated by organ, and weighed. The Ca concentration was measured using an atomic absorption spectrophotometer (Z-5300; Hitachi, Ltd., Tokyo, Japan). For each leaf position (top, middle, and lower), the top one-fifth (leaf tip) and the remainder (leaf base) of each leaf were distinguished and analyzed separately to investigate the horizontal Ca distribution within the leaves. The total Ca concentration (Ca concentration of the whole plant) was determined by dividing the whole-plant Ca concentration by the dry weight. The Ca concentration of the whole leaves was measured. The K concentration was measured using the same method to evaluate the effects of the K concentration on Ca acquisition and distribution.

2.6. Statistical Analysis

Data analyses were conducted using SPSS v.22.0 (IBM Corp. Japan, Tokyo, Japan). A one-way ANOVA was used to assess the effects of the treatments ($p < 0.05$). Then, homoscedasticity was assessed using Levene's test. If homoscedasticity was assumed ($p > 0.05$), the differences in the mean values were assessed using Tukey's b test. If it was not assumed ($p < 0.05$), Dunnet's T3 test was conducted with the 40 ppm Ca treatment as the control group. To evaluate the effects of K concentration on Ca acquisition and distribution, a correlation analysis was conducted.

3. Results

3.1. Tipburn Severity and Incidence

Tipburn was not observed in UH. In RW, the severity and incidence of tipburn significantly decreased with an increase in nutritional Ca concentration, with no tipburn under the 120 ppm treatment (Figure 1). By contrast, in VP, the incidence of tipburn under all treatments was 100%, although the severity showed a tendency to decrease with an increase in Ca concentration in the nutrient solution (Figure 1). In RW and VP, there was a significant correlation between the Ca concentration in the nutrient solution and tipburn severity (RW: r = −0.637, $p < 0.01$; VP: r = −0.664, $p < 0.01$).

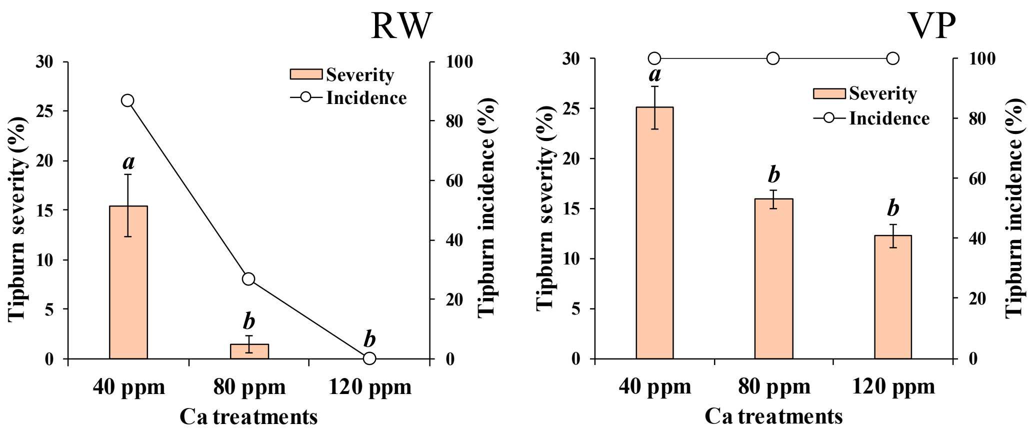

Figure 1. Tipburn severity and incidence in RW and VP under each treatment at the end of the experiment (8 weeks). Data are presented as mean ± SE. Significant differences among the means are indicated with different letters (regular font: Tukey's b test, $p < 0.05$; *italic font*: Dunnet's T3 test, $p < 0.05$). Cultivars: Reina white (RW) and Voyage peach (VP).

3.2. Plant Growth and Total Ca Concentrations

There were no significant differences in the plant dry weight among the three lisianthus cultivars at 4 and 8 weeks (Table 2). In addition, there was a slight difference in plant dry weight among the cultivars (Table 2). At 4 weeks (before the onset of tipburn), there was no significant difference in the total Ca concentration among the treatments (Table 3). By contrast, at 8 weeks, the total Ca concentration in all cultivars under the 80 ppm treatment was significantly higher than that under the 40 ppm treatment (Table 3). However, in RW, there was no significant difference between the 40 ppm and 120 ppm treatments.

Table 2. Total dry weight of each cultivar and treatment at weeks 0, 4, and 8.

Cultivar	Ca Treatment	Total Dry Weight (g)										
		0 Week			4 Weeks				8 Weeks			
UH	40 ppm	0.055	±	0.003	0.44	±	0.02	n.s.	3.44	±	0.15	n.s.
	80 ppm				0.43	±	0.02		3.75	±	0.16	
	120 ppm				0.49	±	0.04		3.23	±	0.22	
RW	40 ppm	0.037	±	0.002	0.43	±	0.04	n.s.	3.51	±	0.16	n.s.
	80 ppm				0.40	±	0.05		3.57	±	0.17	
	120 ppm				0.40	±	0.03		3.14	±	0.18	
VP	40 ppm	0.059	±	0.004	0.44	±	0.03	n.s.	3.59	±	0.19	n.s.
	80 ppm				0.46	±	0.03		3.58	±	0.20	
	120 ppm				0.48	±	0.03		3.20	±	0.20	

Data are presented as mean ± SE. n.s. represents no significant differences among the treatments (ANOVA, $p > 0.05$). Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

Table 3. Total Ca concentration in each cultivar and treatment at weeks 0, 4, and 8.

Cultivar	Ca Treatment	Total Ca Concentration (mg-Ca/kg-DW)				
		0 Week	4 Weeks		8 Weeks	
UH	40 ppm		2.5 ± 0.1		3.2 ± 0.0	*a*
	80 ppm	1.9 ± 0.1	2.5 ± 0.1	n.s.	4.1 ± 0.1	*b*
	120 ppm		2.6 ± 0.1		4.4 ± 0.1	*b*
RW	40 ppm		2.2 ± 0.1		3.9 ± 0.1	*a*
	80 ppm	2.2 ± 0.1	2.4 ± 0.1	n.s.	5.1 ± 0.5	*b*
	120 ppm		2.3 ± 0.1		4.7 ± 0.1	*ab*
VP	40 ppm		3.8 ± 0.2		4.3 ± 0.1	*a*
	80 ppm	1.8 ± 0.1	4.0 ± 0.2	n.s.	5.5 ± 0.4	*b*
	120 ppm		4.1 ± 0.1		7.1 ± 0.2	*c*

Data are presented as mean ± SE. Significant differences among the means are indicated with different letters (regular font: Tukey's b test, $p < 0.05$; *italic font*: Dunnet's T3 test, $p < 0.05$). DW represents dry weight. n.s. represents no significant differences among the treatments (ANOVA, $p > 0.05$). Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

3.3. Ca Distribution

3.3.1. Ca Concentration in Each Plant Organ before the Onset of Tipburn (at 0 and 4 Weeks)

In each organ, there was a slight difference in Ca concentration at the start of the experiment (0 weeks) among the cultivars (Table 4). The Ca concentration in the tips of the top (first) leaves was higher than that in the base in each cultivar (Table 4). At 4 weeks (before the onset of tipburn), there was no significant difference in the Ca concentration in the whole leaves, stems, and roots among the treatments in each cultivar (Figure 2). In addition, there was no significant difference in the Ca concentration at each leaf position among the treatments, except for that at the base of the lower (4th) leaves in UH (Table 5).

Table 4. Ca concentration in the shoots, roots, and tips and the base of the top (first) leaves of each cultivar and in each treatment at the start of the experiment (0 weeks).

Cultivar	Ca Concentration (mg-Ca/kg-DW)			
	Shoot	Root	Top (First) Leaves	
			Tip	Base
UH	1.4 ± 0.1	4.9 ± 0.2	1.7 ± 0.1	0.7 ± 0.0
RW	1.7 ± 0.1	6.0 ± 0.3	2.6 ± 0.1	1.2 ± 0.1
VP	1.2 ± 0.1	5.5 ± 0.4	1.8 ± 0.1	0.9 ± 0.0

Data are presented as mean ± SE. DW represents dry weight. Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

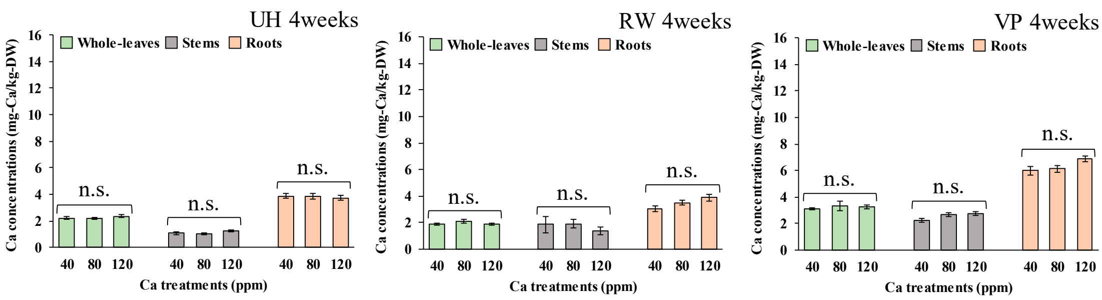

Figure 2. Ca concentration in whole leaves, stems, and roots of each cultivar and in each treatment at 4 weeks after the start of the experiment. n.s. represents no significant differences among the treatments (ANOVA, $p > 0.05$). Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

Table 5. Ca concentrations in the tips and bases of the top (first) and lower (fourth) leaves of each cultivar and in each treatment at 4 weeks after the start of the experiment.

Cultivar	Ca Treatment	Ca Concentration (mg-Ca/kg-DW)							
		Top (First) Leaves				Lower (Fourth) Leaves			
		Tips		Bases		Tips		Bases	
UH	40 ppm	2.35 ± 0.14	n.s.	1.61 ± 0.08	n.s.	3.51 ± 0.16	n.s.	2.16 ± 0.09	ab
	80 ppm	2.88 ± 0.23		1.65 ± 0.07		3.69 ± 0.17		2.07 ± 0.15	a
	120 ppm	2.56 ± 0.13		1.76 ± 0.12		3.44 ± 0.14		2.53 ± 0.12	b
RW	40 ppm	1.32 ± 0.11	n.s.	1.05 ± 0.16	n.s.	4.51 ± 0.68	n.s.	2.65 ± 0.30	n.s.
	80 ppm	1.34 ± 0.22		0.82 ± 0.09		3.34 ± 0.60		2.59 ± 0.24	
	120 ppm	1.42 ± 0.14		0.91 ± 0.10		3.85 ± 0.55		2.80 ± 0.32	
VP	40 ppm	2.02 ± 0.16	n.s.	0.98 ± 0.07	n.s.	4.70 ± 0.39	n.s.	4.04 ± 0.23	n.s.
	80 ppm	2.01 ± 0.25		1.10 ± 0.11		4.46 ± 0.33		4.00 ± 0.29	
	120 ppm	2.12 ± 0.18		1.27 ± 0.11		4.68 ± 0.34		3.87 ± 0.45	

Data are presented as mean ± SE. Significant differences among the means are indicated with different letters (regular font: Tukey's b test, $p < 0.05$; *italic font*: Dunnet's T3 test, $p < 0.05$). n.s. represents no significant differences among the treatments (ANOVA, $p > 0.05$). Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

3.3.2. Ca Concentration in Each Plant Organ after the Onset of Tipburn (at 8 weeks)

The Ca concentration at the end of the experiment (8 weeks) in each cultivar significantly increased with an increase in Ca concentration in the nutrient solution, except in the roots of UH and RW (Figure 3). In the top (first) leaves, the Ca concentration in the tips of all cultivars, except VP, increased with an increase in Ca concentration in the nutrient solution (Table 6). In the middle (fourth) leaves, all the cultivars showed an increasing tendency for the Ca concentration in the tips and bases with an increase in Ca concentration in the nutrient solution (Table 6). In the lower (seventh) leaves, there was no significant difference in the Ca concentration in the tips among the treatments, whereas that in the bases increased with the increase in Ca concentration in the nutrient solution (Table 6). In RW and VP, there was no significant correlation between the Ca concentration in the tips of the top leaves and tipburn severity (RW: r = −0.197, $p = 0.204$; VP: r = −0.117, $p = 0.456$).

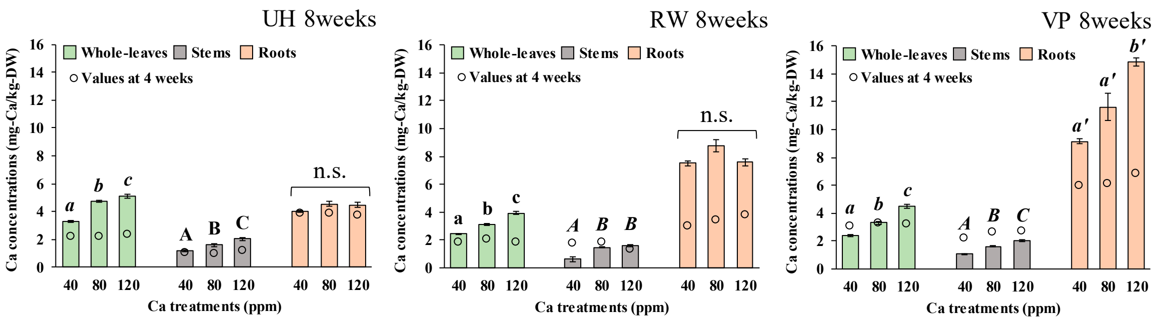

Figure 3. Ca concentration in whole leaves, stems, and roots of each cultivar and in each treatment at 8 weeks. Significant differences among means are indicated with different letters (regular font: Tukey's b test, $p < 0.05$; *italic font*: Dunnet's T3 test, $p < 0.05$). n.s. represents no significant differences among the treatments (ANOVA, $p > 0.05$). Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

Table 6. Ca concentrations in the tips and bases of the top (first), middle (fourth), and lower (seventh) leaves of each cultivar and in each treatment at the end of the experiment (Week 8).

Cultivar	Ca Treatment	Ca Concentration (mg-Ca/kg-DW)											
		Top (First) Leaves				Middle (Fourth) Leaves				Lower (Seventh) Leaves			
		Tips		Bases		Tips		Bases		Tips		Bases	
UH	40 ppm	5.0 ± 0.2	a	1.9 ± 0.1	*A*	4.6 ± 0.1	a′	3.0 ± 0.2	A′	5.1 ± 0.1		2.9 ± 0.1	A″
	80 ppm	5.5 ± 0.3	ab	3.6 ± 0.2	*B*	5.0 ± 0.2	a′b′	5.3 ± 0.1	B′	5.6 ± 0.5	n.s.	4.3 ± 0.1	B″
	120 ppm	5.9 ± 0.2	b	4.1 ± 0.4	*B*	5.4 ± 0.2	b′	5.9 ± 0.3	B′	6.0 ± 0.2		5.4 ± 0.1	C″
RW	40 ppm	2.0 ± 0.2	*a*	1.9 ± 0.2	A	3.0 ± 0.2	a′	2.0 ± 0.1	A′	5.2 ± 0.3		2.4 ± 0.1	*A″*
	80 ppm	4.2 ± 0.3	*b*	2.6 ± 0.1	B	3.7 ± 0.1	b′	3.0 ± 0.1	B′	5.3 ± 0.2	n.s.	3.1 ± 0.1	*B″*
	120 ppm	5.2 ± 0.3	*b*	3.2 ± 0.2	C	4.3 ± 0.2	b′	4.1 ± 0.1	C′	5.9 ± 0.4		3.9 ± 0.1	*C″*
VP	40 ppm	2.2 ± 0.2		2.9 ± 0.3	A	5.1 ± 0.5	a′	2.2 ± 0.5	*A′*	3.8 ± 0.4		2.9 ± 0.2	*A″*
	80 ppm	2.3 ± 0.5	n.s.	3.8 ± 0.5	AB	4.4 ± 0.4	a′	2.6 ± 0.1	*A′*	4.0 ± 0.8	n.s.	3.6 ± 0.2	*B″*
	120 ppm	2.6 ± 0.4		5.1 ± 0.6	B	7.0 ± 0.4	b′	3.8 ± 0.3	*B′*	3.9 ± 0.2		4.4 ± 0.4	*B″*

Data are presented as mean ± SE. Significant differences among means are indicated with different letters (regular font: Tukey's b test, $p < 0.05$; *italic font*: Dunnet's T3 test, $p < 0.05$). n.s. represents no significant differences among the treatments (ANOVA, $p > 0.05$). Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

3.4. K Concentration

In all cultivars, the K concentration increased with an increase in Ca concentration in the nutrient solution (Table 7), but VP exhibited no significant difference in K concentration in the tips of the top (first) leaves among the treatments ($p = 0.183$).

Table 7. Total K and K concentrations in the tips of top (first) leaves of each cultivar for each treatment at the end of the experiment (Week 8).

Cultivar	Ca Treatment	K Concentration (mg-K/kg-DW)			
		Total		Tips of Top (1st) Leaves	
UH	40 ppm	38.5 ± 0.5	a	25.3 ± 0.8	a
	80 ppm	41.4 ± 0.6	b	27.8 ± 1.0	ab
	120 ppm	44.5 ± 0.7	c	30.0 ± 1.1	b
RW	40 ppm	32.5 ± 0.8	a	26.7 ± 1.5	a
	80 ppm	36.4 ± 0.6	b	30.0 ± 1.5	a
	120 ppm	39.1 ± 0.7	c	37.1 ± 1.1	b
VP	40 ppm	27.8 ± 0.4	*a*	45.0 ± 4.0	
	80 ppm	45.6 ± 1.2	*c*	46.7 ± 5.1	n.s.
	120 ppm	39.0 ± 0.8	*b*	55.5 ± 3.3	

Data are presented as mean ± SE. Significant differences among the means are indicated with different letters (regular font: Tukey's b test, $p < 0.05$; *italic font*: Dunnet's T3 test, $p < 0.05$). n.s. represents no significant differences among the treatments (ANOVA, $p > 0.05$). Cultivars: Umi honoka (UH), Reina white (RW), and Voyage peach (VP).

There was no significant correlation between the total K concentration and total Ca concentration among the three cultivars (UH: r = 0.06, $p = 0.709$; RW: r = 0.014, $p = 0.93$; VP: r = 0.094, $p = 0.56$). In UH and RW, there was no significant correlation between the K concentration and Ca concentration in the tips of the top (first) leaves (UH: r = 0.08, $p = 0.621$; RW: r = 0.163, $p = 0.307$). In VP, there was a significant positive correlation between the K concentration and Ca concentration in the tips of the top (first) leaves (r = 0.408, $p = 0.008$). In addition, there was no significant correlation between K/Ca in the tips of the top (first) leaves and tipburn severity in RW and VP (RW: r = −0.061, $p = 0.689$; VP: r = −0.164, $p = 0.283$).

4. Discussion

4.1. Severity and Incidence of Tipburn under Different Concentrations of Ca Supply

Each of the three lisianthus cultivars exhibited different responses in terms of tipburn occurrence to the Ca concentration in the nutrient solutions (Figure 1). UH was considered a tipburn-resistant cultivar because tipburn was not observed in this cultivar. By contrast, RW was considered a tipburn-semi-sensitive cultivar, as the occurrence of tipburn could be prevented by supplying high concentrations of Ca in the nutrient solutions. This result was consistent with that reported in Azuma-no-kanori (AK) [13]. VP, in which the tipburn incidence was 100%, was considered a tipburn-sensitive cultivar. This is similar to the result reported in Celeb wine (CW) [13], in which Ca application was not an effective approach for alleviating tipburn in these cultivars.

4.2. Relevance of Ca Acquisition and Tipburn Incidence

In all cultivars, there was no significant difference in the total dry weight and Ca concentration at 4 weeks among the treatments (Tables 2 and 3). These results indicated that these cultivars did not take up a considerable amount of Ca under each treatment before the onset of tipburn. By contrast, at 8 weeks, all cultivars showed a tendency for increased total Ca concentration with an increase in Ca concentration in the nutrient solution (Table 3), but there was no significant difference in plant dry weight among the treatments (Table 2). In addition, RW and VP (tipburn-semi-sensitive and tipburn-sensitive cultivars) presented higher total Ca concentrations than UH (tipburn-resistant cultivar) under each treatment (Table 3). This is contrary to our hypothesis that tipburn-sensitive cultivars cannot acquire enough Ca even under high-Ca conditions. These findings demonstrated that Ca acquisition has a negligible effect on the occurrence of tipburn in lisianthus cultivars.

4.3. Relevance of Ca Distribution and Tipburn Incidence

In our previous study, the tipburn-sensitive cultivars, VP and CW [13], showed no significant difference in the Ca concentration in the tips of the top leaves among the treatments (Table 6). By contrast, the Ca concentrations in the roots of these species significantly increased with an increase in the nutritional Ca concentration, and the values under each treatment were higher than those in the tipburn semi-sensitive cultivars (RW and AK) and tipburn-resistant cultivars (UH) (Figure 3). Several studies have shown that excessive Ca accumulation in the vacuole by sCAX1 expression reduces the apoplastic Ca concentration, and this eventually leads to Ca deficiency disorder [18,19]. In addition, a recent study on lisianthus cultivars demonstrated that the increase in the distribution of Ca in the roots occurred before and after the onset of tipburn, and this accounted for the decrease in the distribution of Ca in the leaves and the occurrence of tipburn [12]. Therefore, it was suggested that excessive Ca accumulation in the roots under high-Ca conditions inhibits Ca accumulation in the tips of the top leaves, and this eventually manifests as tipburn in the sensitive cultivars. To elucidate the relevance of Ca distribution and gene expression, the findings will be implemented in the future.

4.4. Effects of K Concentration on Ca Acquisition and Tipburn Incidence

In all cultivars, there was no significant correlation between the total K concentration and total Ca concentration. This indicates that the K concentration in lisianthus cultivars had a negligible effect on Ca acquisition. In addition, there was no significant "negative" correlation between the K concentration and Ca concentration in the tips of top leaves. Thus, our findings suggest that the content ratio of K in the nutrient solutions does not prevent Ca acquisition and distribution in lisianthus cultivars and that the K concentration has a negligible effect on the occurrence of tipburn. In the future, the effects of the other cations (e.g., magnesium) in the nutrient solution on Ca acquisition, Ca distribution, and tipburn severity in lisianthus will be studied.

5. Conclusions

Under high-Ca conditions, the incidence of tipburn in VP was 100%, because there was no significant difference in the Ca concentration in the tips of the top leaves among the treatments. Our results suggest that excessive Ca accumulation in the roots under high-Ca conditions inhibits Ca distribution in the tips of the top leaves, and this eventually leads to tipburn in sensitive cultivars. In addition, the content ratio of K in the nutrient solutions did not influence Ca acquisition and distribution in lisianthus cultivars, suggesting that K concentration has a negligible effect on the occurrence of tipburn.

Author Contributions: All authors contributed to the study. T.K. and H.W. designed the study. T.K. wrote the manuscript. T.K. and M.S. collected the data. H.W. and M.A. provided suggestions. All authors reviewed and approved the manuscript.

Funding: This research was funded by JSPS KAKENHI, Grant Number JP19K15830.

Conflicts of Interest: The authors declare no conflict of interest.

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