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Exploring the Role of Calcium in the Physiology of *Tulipa*: A Comparative Study across Different Cultivars

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Abstract: Cut tulip flower production, typically cultivated using hydroponic systems, often faces the challenge of stem toppling due to calcium (Ca) deficiency in the nutrient solution. Growers generally grow various tulip cultivars to meet market demands. In hydroponic production, plants require precise nutrient concentrations to promote growth and development, as the food reserves in the mother bulb are insufficient and vary depending on the plant cultivar. This study aimed to assess the impact of different tulip cultivars and Ca deficiency on tulip growth, flowering, and physiological changes. Two cultivars of tulip, namely, 'Strong Gold' and 'Orange Juice', were subjected to three distinct nutrient solution treatments, namely (1) deionized water (DI) only (without any plant nutrition added), (2) complete solution (Hoagland's complete nutrient solution), and (3) Ca deficiency solution. The results indicate that the 'Orange Juice' tulip was particularly susceptible to Ca deficiency, exhibiting stem toppling and flower abortion similar to those grown in DI. Ca deficiency led to a reduction in root length and leaf area but did not affect the plant height. Additionally, it had a negative impact on bulb qualities, resulting in a decreased bulb fresh weight and bulb circumference in both tulip cultivars. Furthermore, Ca deficiency and DI reduced the photosynthetic rates, and Ca deficiency increased the transpiration rate in the tulip. The leaf concentrations of nitrogen (N), phosphorus (P), and Ca were decreased under Ca deficiency and DI, while magnesium (Mg) levels increased under Ca deficiency treatment compared to the complete nutrient solution treatment.

Keywords: tulip; hydroponics; plant nutrition; calcium; flowering; quality; crop management

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1. Introduction

Tulip (*Tulipa gesneriana* L.) stands out as a prominent cut flower globally. With over 100 species and numerous commercially significant cultivars, it holds a central position in the floral market [1]. The Netherlands takes the lead in tulip production, contributing 88% (14,400 ha) to the global output [2]. The Dutch production amounts to an impressive 4.32 billion tulips, valued at 2.3 billion euro, serving as source material for cultivation in the subsequent season. Approximately 57% of these tulips (around 1.3 billion) are cultivated within the Netherlands for the production of cut flowers. The remaining tulips, totaling 1 billion (0.63 billion within the European Union and 0.37 billion outside the European Union), are exported to regions such as South and Southeast Asia and other countries [2].

The size of the mother bulbs is the primary determinant influencing growth and flowering in tulip production. Typically, a flowering-sized mother bulb has an approximately 12 cm or larger circumference. If the mother bulb is undersized, it either fails to flower or produces flowers of subpar quality [3]. Alongside the size of the mother bulbs, effective nutrient management during the planting period appears as another key factor influencing

the growth and flowering of tulips. For optimal bulb production, the recommended estimates for total kilograms per hectare of nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca) are 140 to 150, 40 to 50, 140 to 150, and 110 to 120, respectively [4].

The hydroponic cultivation of tulips gained traction as a horticultural practice in the Netherlands during the 1990s. Over 80% of Dutch cut tulips undergo hydroponic forcing, and this method is gaining popularity in various other countries. However, the latest technique, known as tulip deep flow hydroponics, which involves a specialized hydroponic tray [5], has drawbacks. These include the risk of root rot due to restricted water circulation or insufficient oxygen supply, an unstable method of securing bulbs on the tray, and the manifestation of Ca deficiency symptoms stemming from high root zone temperatures and inadequate nutrient combinations [6,7].

Ca stands as a necessary element in the growth and development of plants, with its mobility within plant tissues being limited. Primarily functioning outside the cytoplasm in the apoplasm, Ca is a mineral nutrient, and a significant portion of the total Ca^{2+} in plant tissue resides in the cell wall (apoplasm) [8]. Its pivotal role includes regulating plant growth regulator (PGR) actions, such as those of indolyl acetic acid (IAA), which promotes cell elongation and differentiation. A Ca deficiency can result in enlarged and undifferentiated cells due to the absence of structural stability. Moreover, the Ca^{2+} ion is indispensable for cell division, cell wall construction, and cell elongation in the meristematic tissue of both shoot and root vegetative points [9]. When forcing tulip bulbs without a Ca fertilizer, deficiency symptoms may emerge, including stem toppling, light green foliage, stunted leaves and scape, and flower bud abortion [6].

Using N, P, and K fertilizers to force tulip production may lead to plant Ca deficiency. Stem toppling, a prominent concern in the Dutch tulip industry, is linked to plant transpiration and contributes to Ca deficiency. Consequently, it becomes important to supplement the nutrient solution with both N and Ca for effective tulip forcing [7]. Despite this, there is a lack of research on the impact of Ca deficiency on the physiological aspects of tulips. Therefore, this study aims to explore the effects of Ca deficiency on the physiological characteristics of tulips by comparing two distinct cultivars.

2. Materials and Methods

2.1. Plant Materials, Growth Conditions, and Treatment

Tulip (*Tulipa hybrida*) 'Strong Gold' and 'Orange Juice' bulbs with a circumference of 12 cm were subjected to a 2-week rooting period at 9 °C until the roots and shoots reached approximately 5 cm in length. Subsequently, these plants were transplanted into a hydroponic system using a double-pot system with air added, provided by an air pump (Figure 1). The greenhouse environment was maintained at 20 ± 2 °C, with an average photosynthetic photon flux density (PPFD) of 442 μmole m^{-2} s^{-1} and relative humidity (RH) of 80%.

The experimental design followed a factorial method in a completely randomized design (CRD) involving two factors. The first factor related to the two tulip cultivars, 'Strong Gold' and 'Orange Juice', while the second was related to the three nutrient solution formulas. These formulas included the following: (1) plants were grown with deionized water (DI) only, without the addition of any fertilizers; (2) plants were grown with a complete nutrient solution containing all essential elements, with electrical conductivity (EC) maintained at 1.2 mS cm^{-1}, which served as the control treatment; and (3) plants were grown with a Ca deficiency nutrient solution, with EC maintained at 1.2 mS cm^{-1}.

The concentration of nutrient supply for both tulip cultivars, 'Strong Gold' and 'Orange Juice', in each treatment is detailed in Table 1.

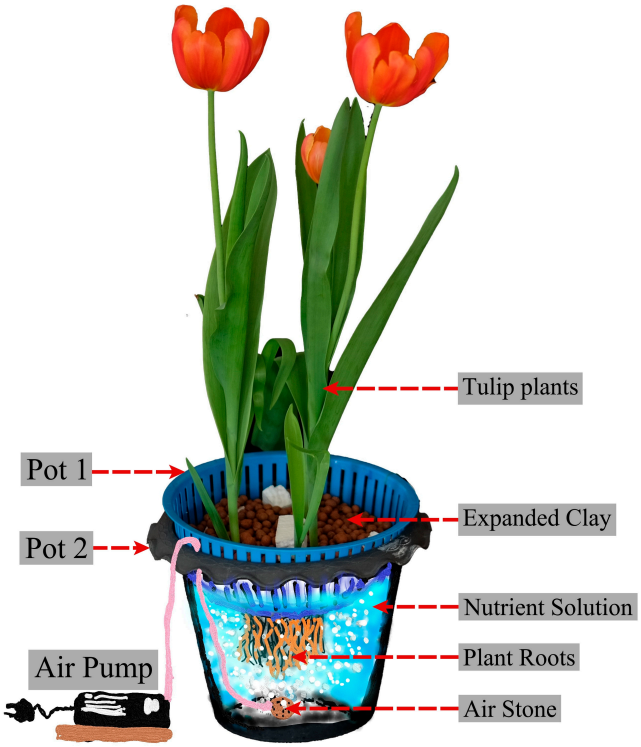


Figure 1. The hydroponic 'double-pot system' used in this experiment.

Table 1. The concentrations of nutrient supply for both 'Strong Gold' and 'Orange Juice' tulip cultivars in each treatment.

Nutrient Solution	Concentration (mg L^{-1})										
	N	P	K	Ca	Mg	S	Mo	Cu	Zn	B	Mn
DI	0	0	0	0	0	0	0	0	0	0	0
Complete	224	62	235	160	24	32	0.05	0.03	0.13	0.27	0.11
Ca deficiency	224	62	235	0	24	32	0.05	0.03	0.13	0.27	0.11

2.2. Measurement of Plant Morphology and Growth Characteristics

At the flowering stage (4 weeks after planting), 12 plant samples per treatment were chosen for data collection. Plant morphological characteristics were measured, including plant height, root length, and leaf area (LI-3100C, LI-COR Biosciences, NE, USA). Flowering characteristics were also observed in terms of flower stalk length, the percentage of flower abortion, and the percentage of stem toppling. The photosynthetic rate (μmole m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}), and stomatal conductance (mol m^{-2} s^{-1}) were measured using a portable photosynthesis measurement system incorporating an infrared gas analyzer (Lcpro+, ADC Bioscience, Hertfordshire, UK) at 11 a.m.

At eight weeks after planting, the bulb quality in terms of bulb fresh weight (FW) and bulb circumference was measured.

2.3. Chemical Analysis

Plants were randomly sampled from each treatment for chemical analysis during the flowering stage (4 weeks after planting). The plants were prepared using two rinses with deionized water and then dried in a hot-air oven at 60 °C for seven days. Nitrogen (N) and phosphorus (P) samples were digested through the Kjeldahl method. Subsequently, the N concentration was analyzed using the indophenol method, while the P concentration was determined using the ammonium molybdate method [10]; an atomic absorption spectrophotometer was used to determine potassium (K) and calcium (Ca) levels.

2.4. Determination of Element Mapping

During the flowering stage, the stems of tulip flowers in each treatment were cut to 10 cm, with the cutting position located 20 cm below the flower. To examine the distribution of elements (P, Ca, and K) in the area of stem toppling, a micro-X-ray fluorescence spectrometer (Bruker M4 Tornado) was used for mapping.

2.5. Statistical Analysis

The experiment followed a factorial method within a completely randomized design, with each treatment having four replications. Each replication comprised three bulbs. Statistical analyses were conducted using the Statistic 8 analytical software package (SXW Tallahassee, FL, USA). In instances of significant treatment effects, mean comparisons were executed using LSD at a significance level of 0.05.

3. Results

The impact of calcium (Ca) on the physiological responses of *Tulipa* 'Strong Gold' and 'Orange Juice' was assessed through various parameters, including plant growth, flowering, photosynthetic rates, stomatal conductance, transpiration rate, and nutritional content.

3.1. Vegetative Growth

The 'Strong Gold' cultivar exhibited a greater plant height and root length, measuring 43.9 and 17.1 cm, respectively, compared to 'Orange Juice', which measured 39.8 and 15.5 cm, respectively (Table 2). Conversely, the leaf area of 'Orange Juice' was larger than that of 'Strong Gold', as shown in Table 2.

Table 2. *Tulipa* 'Strong Gold' and 'Orange Juice' growth was affected by Ca deficiency at the flowering stage (4 weeks after planting).

Factor		Plant Height (cm)	Root Length (cm)	Leaf Area (cm^2)
Cultivar	Strong Gold	43.9 a	17.1 a	209.6 b
	Orange Juice	39.8 b	15.5 b	266.9 a
	$LSD_{0.05}$	*	*	*
Nutrient solution	DI	40.5 b	15.0 b	233.9 ab
	Complete	43.3 a	20.9 a	260.5 a
	Ca deficiency	41.7 ab	13.9 b	220.3 b
	$LSD_{0.05}$	*	*	*
Cultivar × Nutrient solution		*	*	*

* Means within the same column followed by different letters show significant differences between treatments with LSD test at $p \leq 0.05$.

When exposed to various nutrient solutions, the findings indicated that the cultivation of tulips in deionized water led to a reduction in plant height. Furthermore, both DI and calcium deficiency were associated with a decrease in root length (Table 2 and Figure 2). Additionally, the lack of Ca decreased the leaf area of the tulips in both cultivars. These results suggest that the food reserves in the mother bulb were inadequate to support average tulip growth in terms of plant height, root length, and leaf area.

3.2. Flowering

The flower stalk length of the 'Strong Gold' tulip typically reached 33.6 cm, shorter than that of the 'Orange Juice' variety. 'Strong Gold' did not experience any flower abortion or stem toppling, while 'Orange Juice' exhibited approximately 8% flower abortion and 33% stem toppling, as shown in Table 3.

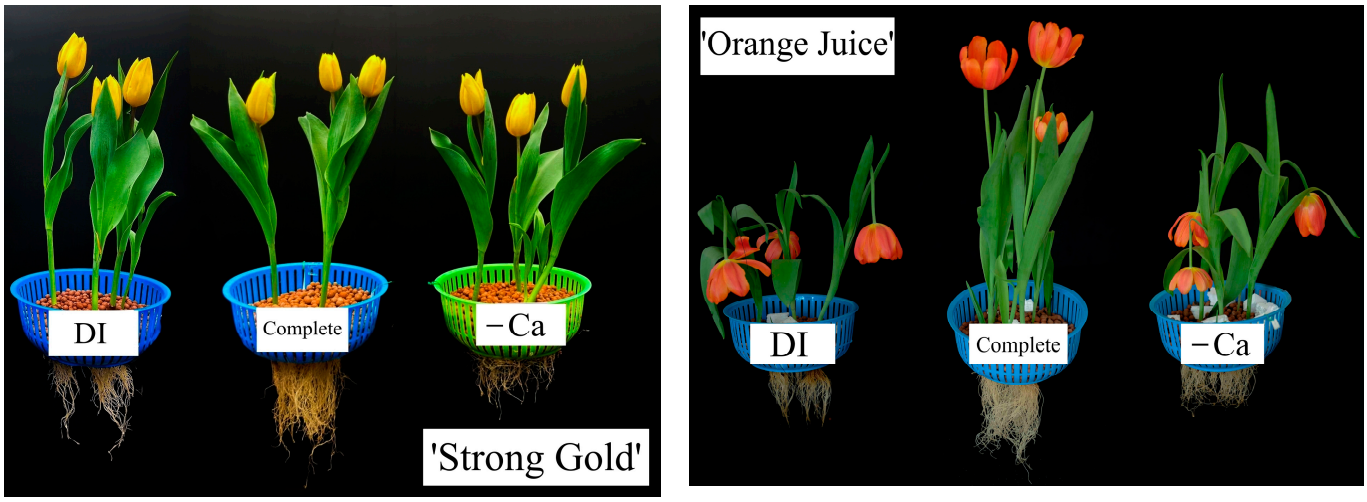


Figure 2. Growth and development of 'Strong Gold' and 'Orange Juice' tulips under different nutrient solution treatments at flowering stage (4 weeks after planting).

Table 3. Flower stalk length, flower abortion, and stem toppling of Tulipa 'Strong Gold' and 'Orange Juice' affected by Ca deficiency at 4 weeks after planting.

Factor		Flower Stalk Length (cm)	Flower Abortion (%)	Stem Toppling (%)
Cultivar	Strong Gold	33.6 b	0	0
	Orange Juice	37.5 a	8	33
	LSD $_{0.05}$	*		
Nutrient solution	DI	35.0 a	9	29
	Complete	35.6 a	0	0
	Ca deficiency	36.1 a	4	21
	LSD $_{0.05}$	*	-	-
Cultivar × Nutrient solution		*	-	-

* Means within the same column followed by different letters show significant differences between treatments with LSD test at $p \leq 0.05$.

Interestingly, the nutrient solution had no significant impact on the flower stalk length. However, when tulips were grown in DI and Ca deficiency solution, it led to a higher incidence of flower abortion, with rates of 9% and 4%, respectively. These conditions also triggered stem toppling, with rates of 29% for DI and 21% for the Ca-deficient solution, respectively (Table 3).

3.3. New Bulb Qualities

The circumference of the new bulb was not significantly different between the 'Strong Gold' and 'Orange Juice' cultivars. However, the new bulb FW of 'Orange Juice' was larger than that of 'Strong Gold', with 8.0 and 6.9 g, respectively. Growing plants with DI and Ca deficiency decreased the bulb FW and circumference compared with the control treatment (Table 4).

3.4. Photosynthetic Rate, Stomatal Conductance, and Transpiration Rate

The results indicated variations in the efficiency of photosynthesis between the two cultivars of tulip. Specifically, for the 'Orange Juice' cultivar, the photosynthetic rate, stomatal conductance, and transpiration rate were approximately 5.04 μmol m^{-2} s^{-1}, 0.15 mol m^{-2} s^{-1}, and 1.61 mmol m^{-2} s^{-1}, respectively, all of which were higher than the corresponding values in the 'Strong Gold' cultivar (Table 5).

Table 4. The Ca deficiency affected the bulb qualities of tulips 'Strong Gold' and 'Orange Juice' at 8 weeks after planting.

Factor		Bulb Fresh Weight (g)	Bulb Circumference (cm)
Cultivar	Strong Gold	6.9 b	7.15 a
	Orange Juice	8.0 a	7.18 a
	LSD $_{0.05}$	*	NS
Nutrient solution	DI	7.21 b	6.58 b
	Complete	8.63 a	8.43 a
	Ca deficiency	6.44 b	6.48 b
	LSD $_{0.05}$	*	*
Cultivar × Nutrient solution		*	*

* Means within the same column followed by different letters show significant differences between treatments with LSD test at $p \leq 0.05$.

Table 5. Physiological changes in leaves of *Tulipa* 'Strong Gold' and 'Orange Juice' at flowering stage (4 weeks after planting).

Factor		Photosynthetic Rate (μmol m^{-2} s^{-1})	Stomatal Conductance (mol m^{-2} s^{-1})	Transpiration Rate (mmol m^{-2} s^{-1})
Cultivar	Strong Gold	3.36 b	0.97 b	0.11 b
	Orange Juice	5.04 a	1.61 a	0.15 a
	LSD $_{0.05}$	*	*	*
Nutrient solution	DI	3.62 c	1.16 b	0.12 b
	Complete	4.73 a	1.32 ab	0.12 b
	Ca deficiency	4.23 b	1.39 a	0.15 a
	LSD $_{0.05}$	*	*	*
Cultivar × Nutrient solution		*	*	*

* Means within the same column followed by different letters show significant differences between treatments with LSD test at $p \leq 0.05$.

Interestingly, Ca deficiency reduced the photosynthetic rate but did not affect the stomatal conductance. Conversely, it increased the transpiration rate compared to the control and DI conditions, as shown in Table 5.

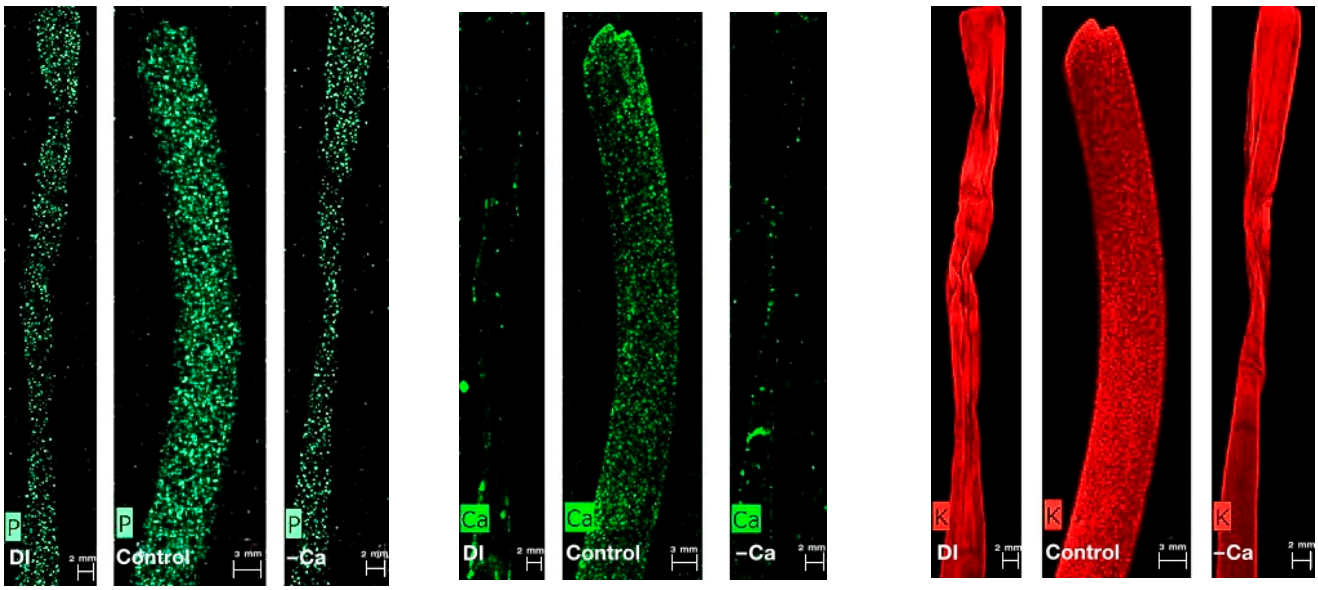
3.5. Nutritional Concentrations

The lack of Ca and DI decreased the total N (0.83 and 0.77, respectively), P (1.63 and 2.28, respectively), and Ca concentrations (6.25 and 5.60, respectively) in leaves, compared with the control treatment, with 1.97% N, 3.97% P, and 9.86% Ca. The nutrient concentration in leaves under the DI treatment was the lowest at 0.77% N, 2.28% P, and 5.60% Ca (Table 6). The total K under the DI treatment was lower than in the control and Ca deficiency treatments. On the other hand, Ca deficiency promoted the uptake of Mg; therefore, the total Mg in the leaves under Ca deficiency was the highest, at 5.65%, while that of control leaves was lower, at 2.47% Mg, as well as that of DI leaves, with 1.50% Mg. Figure 3 shows the P, Ca, and K mapping using a MicroXRF spectrometer (Bruker M4 Tornado). The result confirmed that the concentration of these elements was reduced under DI and Ca deficiency treatments compared to the control.

Table 6. Ca deficiency affected *Tulipa* 'Orange Juice' mineral nutrient concentrations at 4 weeks after planting.

Treatment	Total N (%)	Total P (%)	Total K (%)	Total Ca (%)	Total Mg (%)
DI	0.77 b	2.28 b	28.58 b	5.60 b	1.50 b
Complete	1.97 a	3.97 a	38.87 a	9.86 a	2.47 b
Ca deficiency	0.83 b	1.63 b	39.58 a	6.25 b	5.65 a
CV (%)	18.03	15.77	9.89	15.79	38.95
$LSD_{0.05}$	*	*	*	*	*

* Means within the same column followed by different letters show significant differences between treatments with LSD test at $p \leq 0.05$.



(**a**) (**b**) (**c**)

Figure 3. The mapping of P (**a**), Ca (**b**), and K (**c**) shown in the stems of 'Orange Juice' tulip grown under DI, complete, and Ca deficiency nutrient solution treatments.

4. Discussion

A lack of Ca^{2+} supply in the nutrient solution leads to a decrease in root extension within a few hours. This effect is more distinct in a Ca^{2+}-free nutrient solution than in distilled water [8]. These symptoms were also found in the present experiment. The root length of the tulip grown in the Ca deficiency solution was lower than that of the control and DI plants. However, it did not affect the plant height. The inhibition of root growth under Ca^{2+} deficiency has also been found in many plant species, such as begonia [11], grapevine [12], and narcissus [13]. The cessation of root growth under Ca deficiency is due to the function of Ca in counterbalancing the effects of high concentrations of other ions at the plasma membrane. Moreover, Ca is involved in cell division; therefore, the halting of root growth found in the 'Strong Gold' and 'Orange Juice' tulips was primarily a result of inhibited cell extension. Ca^{2+} regulates primary root growth by affecting auxin and cytokinin (CK) signaling and plays a role in abscisic acid (ABA)-inhibited primary root growth by transducing reactive oxygen species (ROS) signals or affecting ethylene biosynthesis [14]. Both the 'Strong Gold' and 'Orange Juice' leaf areas decreased when Ca^{2+} was not supplied. This result was also found in grapevine [12]. Some growers believe that the tulip bulb has enough food for growth and development. However, this research found that growing tulips in DI decreased the plant height, root length, new bulb fresh weight, and new bulb circumference and increased the percentage of flower abortion and stem

toppling. These results indicate that the food reserves in the mother bulb were insufficient for the normal growth and flowering of tulips.

The photosynthetic rate, stomatal conductance, and transpiration rate of tulips grown in the Ca deficiency solution differed depending on the cultivar, and the photosynthetic rate of 'Strong Gold' was significantly lower than that of 'Orange Juice. A deficiency in Ca^{2+} in plants can have a profound impact on their photosynthetic rate, a critical process for both energy production and overall growth. Additionally, Ca is involved in the regulation of stomatal opening and closure. Stomata are small openings on the plant's leaves that control gas exchange, including CO_2 uptake. In this research, Ca^{2+} deficiency increased the stomatal opening and transpiration rate compared with the DI and control treatments. On the other hand, Ca^{2+} deficiency decreased the photosynthetic rate of the tulips. These results may be due to the Ca^{2+} pool in chloroplasts decreasing under deficiency conditions; therefore, it affected the Ca^{2+} binding protein in the chloroplast and chloroplast membrane, such as s-adenosylmethionine transporter-like (SAMTL), and directly interacted with the cytoplasm signal molecules [15]. The Ca in chloroplasts can regulate the photosynthetic pathway, which is the primary source of energy supply in plant cells [16]. Furthermore, several enzymes involved in photosynthesis require Ca as a cofactor. When the Ca levels are insufficient, these enzymes may not function optimally. This disruption can impair the overall photosynthetic process [17,18].

In this study, the decrease in photosynthetic rate observed in the Ca deficiency treatment resulted in reduced root growth and an overall decrease in the plant's vitality. This might be due to Ca also playing a role in the energy transfer process within plant cells. In its absence, energy transfer mechanisms may not work efficiently, impacting the conversion of light energy into the chemical energy required for photosynthesis. Photosystem II (PSII) comprises redox enzymes embedded within the cellular membrane, and Ca ions (Ca^{2+}) play a pivotal role as cofactors in the assembly of activation sites [19,20]. One crucial component within PSII is the oxygen-evolving complex (OEC), which is responsible for breaking down water molecules [21]. The extrinsic proteins PsbQ, PsbP, and PsbO are integral to OEC [22,23] and possess close associations with CP47, the α subunit of cytochrome b559, and a small subunit within PSII [24]. PsbQ and PsbP indispensably rely on chloride ions (Cl^-) and Ca ions (Ca^{2+}) as essential cofactors [22,25]. Ca^{2+} ions linked to PsbO are sourced from the Mn_4CaO_5 cluster [19,26], and the stability of this Mn_4CaO_5 cluster is intricately connected to PsbO [27]. Ca ions are also actively involved in the s-state cycle, which plays a critical role in the breakdown of water molecules [28].

Ca^{2+} deficiency in plants can have a complex relationship with the content of other essential nutrients. This study observed decreased N, P, and Ca concentrations in tulip leaves but increased K and Mg concentrations, as shown in Table 6. Millikan et al. [29] reported that Ca was essential for nitrate reduction in protein synthesis, and Ca-deficient plants were incapable of absorbing and assimilating nitrates.

The interaction between Ca and P was reported by Jakobsen [30]. The Ca^{2+} absorption was increased by a soluble phosphorus supply. Ca and P often compete for uptake by plant roots. In Ca-deficient soils, plants might prioritize Ca absorption over P. This process can reduce P uptake and potentially lead to P deficiency symptoms. In a study conducted by Robson et al. [31], it was observed that when exposed to typical soil solution conditions with varying Ca and phosphate concentrations, several annual legume species exhibited a significant enhancement in phosphate absorption as the concentration of Ca in the flowing culture solutions increased.

Ca and K can interact in complex ways. Adequate Ca levels are necessary for the maintenance of cell membrane integrity, while K regulates ion movement across cell membranes. As observed in this study, Ca^{2+} deficiency can affect the plant's ability to maintain proper K levels in cells, as tulip leaves exhibited increased K concentrations compared to DI-treated plants.

When considering the Mg concentration, it is essential to note that Ca and Mg are divalent cations. Consequently, Ca^{2+} deficiency can disrupt the balance of cations and lead to competition between Ca and Mg for uptake by plants.

5. Conclusions

The response to Ca deficiency in tulips depends on the cultivar. This research revealed that the 'Orange Juice' tulip was particularly susceptible to low Ca concentrations compared to 'Strong Gold'. The Ca-deficient and DI tulips exhibited a reduced root length, a smaller leaf area, and lower photosynthetic rates, while the transpiration rates of the Ca-deficient plants increased compared to those in the control treatment, leading to stem toppling and flower abortion. Moreover, it affects the nutrient concentrations in the leaves, bringing about a mineral imbalance in cells. Therefore, as a result, it is advisable to supplement tulip cultivation in hydroponic systems with additional Ca at appropriate levels to mitigate these adverse effects.

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Conflicts of Interest: Author Weerasak Wichapeng was employed by the company Charoen Pokphand Agriculture Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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