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Marcel Dieter Moll^{1*}, Nick Paul¹, Julian Elfers¹, David Stoddart¹, Ralf Pude^{1,2} **Impact of different Ca-Applications on Physiology, Yield and Fruit Quality of Tomato in Deep Water Culture**

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Impact of different Ca-Applications on Physiology, Yield and Fruit Quality of Tomato in Deep Water Culture

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Abstract

The tomato (*Solanum lycopersicum*) is a vital crop in German horticulture, with fruit quality being crucial for marketability. The 'Cypry RZ' variety was examined in Deep Water Culture systems to assess the impact of different calcium (Ca) applications. Experiments included adding CaCl or Ca-chelate to the nutrient solution, applying Ca directly to leaves, and comparing these with a control group without extra Ca. Adding CaCl significantly increased the fresh weight of class I fruits (fully colored; without blossom-end rot) compared to Cachelate and no calcium addition. However, Ca-chelate resulted in a significant increase in orange-coloured fruits and notably reduced blossom-end rot compared to the control. There was also a trend of CaCl reducing blossom-end rot. Additionally, Ca supplements positively influenced the overall plant physiology, as evidenced by vegetation indices Photochemical Reflectance Index (PRI) and Normalized Difference Vegetation Idex (NDVI). NDVI indicated higher vitality due to additional Ca (Ca-chelate and CaCl), while PRI indicated higher symptoms of stress and a lower Light Use Efficiency after added Ca to the nutrient solution. Overall, Ca-applications, particularly Ca-chelate, significantly improved the quality of 'Cypry RZ' tomatoes.

1. Introduction

Tomato (*Solanum lycopersicum*) is the economically most valuable vegetable in global horticultural production and belongs to the *Solanaceae* family (Razifard et al. 2020; Zhang et al. 2023). One of the most important effects on marketability is the occurrence of blossomend rot (BER). Yet, it remains unclear if BER is a symptom of calcium (Ca) deficiency or if other factors (e.g. stress) are causing the disintegration of cell membranes, thereby causing BER (Saure 2001). There are four main factors influencing BER: physiological, genetic, and agronomic factors, as well as abiotic stress. Not all are related to Ca-uptake (e.g. grafting, growth retardents), but most are somewhat related to Ca (Hagassou et al. 2019). Generally, BER is regarded as caused by stress (e.g. salinity, heat, light intensity) leading to higher quantities of reactive oxygen species (ROS), oxidative stress and lateron cell death (Saure 2014). Besides the effects of stress, different Ca treatments showed a significant reduction in BER symptoms (Coulibaly et al. 2023). Defoliating is a common practice in tomato cultivation. Pruning plants to 12-15 leaves allows for higher daily Ca transports and therefore reduces incidents of BER (Indeche et al. 2020).

Due to its unique elemental attributes, Ca can govern a variable spectrum of tasks in a crop's metabolism, which underlines the difficulties of the early categorization of Ca disorders (Hepler 2005). During soilless tomato cultivation (substrate culture), the uptake of Ca is suppressed under high K:Ca ratios and promotes physiological disorders of fruits (e.g. BER) connected to Ca deficiency symptoms (Sonneveld and Voogt 2009).

Modern production techniques allow for higher quality while simultaneously increasing yield as well as reducing environmental impact (D'Amico et al. 2023). In soilless cultivation, the nutritional value of fruits is considered higher than in soil based techniques (Goh et al. 2023). Today, deep water culture (DWC) is the most suitable option to reduce or increase an element for plant growth to a concentration where a deficiency or toxicity symptom is displayed on the tissue level (Nemali 2022).

As the exact dynamic of BER occurrence in tomato and Ca remains somewhat unclear, we investigated the effects of different Ca treatments as mitigation strategies in hydroponic (DWC) tomato cultivation. The key objectives were to identify the most suitable way of Ca administration. We hydpothesized that different ways of Ca-application (Ca-chelate or CaCl) differ in Ca-uptake efficiency and thus lead to different levels of reduction in BER. Additionally, the effects of different application techniques were hypothesized to have different effects on fruit count and fruit quality. Therefore, we used destructive as well as non-destructive measurements to determine fruit parameters as well as plants physiologic responses to the treatments.

2. Data, Methods and Approach

Plant material for tomato cultivar 'Cypry RZ' (Rijk Zwaan Welver GmbH, Germany) was cultivated from seeds sown into Grodan rockwool plugs (Brinkman Deutschland GmbH, Germany) which was performed on July $3rd$ 2023. At the stage of three leaves, plantlets were transferred onto the Deep Water Culture (DWC) system on July 21st 2023. Cultivation techniques (pinching, removal of older leaves, etc.) were performed based on standard methods (e.g. Peet and Welles 2005). Harvesting was performed on October 5th, 12th and 13th 2023, respectively. Single fruit weight as well as weight per truss (Kern PNJ 3000-2M, Kern & Sohn GmbH, Germany), colouring (Figure 1), and blossom-end rot were measured. To determine fruit quality, fruits were determined as class I if they were fully coloured and had no symptoms of BER.

Figure 1. Six stages of tomato colouring standard used for the determination of fruit colour. Stage 5 represents a fully coloured fruit, while stage 6 was considered "overripe". Besides biomass accumulation, Vegetation Indices (VIs) were calculated via Polypen RP 400 UV-VIS (PSI, Drásov, Czech Republic) measurements to assess plant physiology and stress response. The Normalized Difference Vegetation Index, NDVI in short, $((R_{NIR}-R_{Red})/(R_{NIR}+R_{Red}))$ gives insight into plant vitality and nutrient supply (Gamon et al. 1995) while the Photochemical Reflectance Index, in short PRI $((R_{531}+R_{570})/(R_{531}+R_{570}))$ indicates light use efficiency (LUE) and photosynthetic efficiency (Garbulsky et al. 2011). Data for VIs is shown for October $10th$, as impact of treatment is considered largest on the latest possible date of measurements.

The DWC system consisted of eight boxes (one for each plant) as well as a main reservoir, with a total water volume of 560 l. Canna Aqua Vega (Canna Deutschland GmbH) was used as fertilizer for the first 37 days after transplanting. After 38 days of cultivation, Canna Aqua Flores was used (Canna Deutschland GmbH). Calcium treatments were performed using 1 mmol of Ca-EDTA-chelate as well as CaCl (GPR RECTAPUR® 2-5 mm, purified, VWR International GmbH, Germany) in the nutrient solution. Ratios of K/N and K/Ca were 1.0 and 2.5, respectively. After Ca-application, EC values during the generative phase (using Canna Aqua Flores) were measured at 1.57 mS/cm, 1.53 mS/cm, and 1.37 mS/cm for CaCl, Ca-chelate and the control, respectively. Oxygen saturation was achieved using immersed air stones that were placed under each of the netpots. Additionally, a chiller was used to achieve constant water temperatures of 20 °C.To re-adjust pH levels, KOH (Advanced Hydroponics pH Up) and H_3PO_4 (Advanced Hydroponics pH Down Bloom) were used to keep pH between 5.8 and 6.2.

Data are given as boxplots, illustrating median, upper and lower quartile, and standard deviation. Statistical analysis was carried out within JMP Pro 17 (SAS Institute GmbH, Heidelberg, Germany) as well as Excel 2019. Under the given normal distribution (Kolmogorov-Smirnov test) as well as homoscedasticity (Levene test), one-way ANOVA and Tukey-HSD as post-hoc procedure were used to determine significant differences $(p \le 0.05)$.

3. Results and Discussion

The Ca-treatment showed to have significant impact on total truss weight of 'Cypry RZ' (Figure 2), with Ca-chelate increasing truss weight in comparison to the control significantly. CaCl (432.76 ± 117.14 g) yielded significantly higher fruit weight than Ca-chelate $(333.56 \pm 105.04 \text{ g})$. None of the treatments was significantly different to the fruit biomass/ freshmatter of the control group (357.57 \pm 102.57 g). Besides total fresh truss weight, fresh fruit weight for class I as measured. The CaCl-treatment yielded in significantly higher fruit weight (21.66 \pm 2.42 g) compared to Ca-chelate (19.39 \pm 3.74 g) and the control plants $(20.42 \pm 2.84$ g).

Figure 2. Single fruit weight of fruit classified as class I (A) and total truss weight (B) in g of 'Tomary RZ' for three Ca treatments (purple: Ca-chelate; orange: CaCl; green: control). Significant differences are indicated by letters after ANOVA and Tukey-HSD (*p* ≤ 0.05); ns: not significant

Fruit quality was significantly influenced by Ca-application (Figure 3). The amount of fruits with BER was significantly reduced by Ca-chelate (12.5 \pm 5.09) while CaCl (18.75 \pm 6.08) showed tendencies of a similar pattern of BER reduction in comparison to the untreated plants (30.5 \pm 8.58). Additionally, fruit count of class I was significantly increased via Cachelate (36.17 ± 4.96) and CaCl (31.75 ± 2.75) in comparison to the control group (23.5 ± 4.80) . Calculating the ratio of BER and class I fruits, Ca-chelate scored 34.6, CaCl 59.1, and the control 129.8.

Figure 3. Fruit count (number) with BER (A) and of class I classified fruits (B) of 'Tomary RZ' for three Ca treatments (purple: Ca-chelate; orange: CaCl; green: control). Significant differences are indicated by letters after ANOVA and Tukey-HSD (*p* ≤ 0.05; *n* = 4)

The results clearly underline that additional Ca-application significantly reduce the occurrence of BER and is therefore a suitable strategy to mitigate symptoms of BER. Nevertheless, BER was not completely prevented. Still, from all fruits that were categorized either in class I or BER (excluding all fruits that were not fully orange-coloured), 23% of Cachelate, 37% of CaCl and 56% of control plants had symptoms of BER. Therefore, additional Ca showed significant potential to reduce BER and thereby increase the amount of marketable fruits.

Hyperspectral Vegetation Indices revealed significant differences between treatments (Figure 4). Ca-chelate (0.733 \pm 0.020) and CaCl (0.714 \pm 0.017) vielded significantly higher NDVI scores than the control (0.702 ± 0.022) . Still, Ca-chelate scored higher NDVI than CaCl. These results indicate higher plant vitality due to the addition of Ca into the nutrient solution. As our experiment did not cover a whole season, this effect might have more pronounced impacts when covering a longer vegetation span.

PRI scored significantly lower in both Ca-treated groups. CaCl-treated plants scored 0.003 ± 0.021 in comparison to Ca-chelate (0.078 \pm 0.020) and control (0.097 \pm 0.032). This indicates the stress-induction of CaCl, possibly due to higher salt concentrations in the nutrient solution. Based on the PRI, LUE was highest in control plants with lowest scores for CaCl-treated plants (Figure 4). Other factors might also play a role here, e.g. water stress is often correlated with the PRI, especially in tomato. But low coefficients of determination $(R² = 0.453)$ indicate that PRI is not sufficient to determine water stress in tomato (Kittas et al. 2014).

In conclusion, VIs show a two-fold picture of tomato under different Ca-treatments. While NDVI indicated higher vitality due to additional Ca (Ca-chelate and CaCl), PRI indicated higher symptoms of stress and a lower LUE after added Ca to the nutrient solution. Therefore, a repetition of this experiment will bring clearity on the physiological response of tomato, related to VIs.

Figure 4. Vegetation Indices (A: NDVI; B: PRI) on 10.10.2023 for three Ca treatments (purple: Ca-chelate; orange: CaCl; green: control). Significant differences are indicated by letters after ANOVA and Tukey-HSD (*p* ≤ 0.05; *n* = 36)

4. Conclusions

The application of Ca-chelate significantly enhances overall fruit quality, notably increasing the proportion of class I fruits while mitigating the incidence of BER. For our experiment, no statistically significant influence on single-fruit weight was observed following the application of Ca-based treatments. Using Ca-chelate and CaCl as added Ca-source, CaCl-treated plants exhibited higher truss weights relative to those treated with Ca-chelate. Physiological indicators of stress (Vegetation Indices: NDVI and PRI) were detected for CaCl-treated plants. Our findings clearly show that Ca-chelate holds great potential to increase marketable class I fruits of tomato in a DWC system.

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