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# Effect of Partial Excision of Early Taproots on Growth and Components of Hydroponic Carrots

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**Abstract:** Hydroponics provides a stable root environment that can be easily controlled. In this paper, we investigated the effect of partial excision of early taproots of hydroponic carrots on their growth and components. Carrot taproots were excised after 30 days from sowing at 5 cm, 10 cm, and 15 cm from the stem base (C5, C10, and C15) and compared with nonexcised control plants. Time-course measurements revealed the taproot lengths of C10 and C15 plants gradually decreased. After 28 days of treatment, C5 taproot tips showed the most rounded shape among root-excised plants. Control plants possessed long taproots that were not enlarged at the site more than 15 cm from the stem base. Taproot fresh weight was lower in C5 plants and higher in C15 plants compared with controls. Although taproot sugar concentrations did not differ between treatments, total phenol concentration was higher in C5 taproots. These data suggest that partial removal of early taproots can regulate the shape and ingredients of hydroponic carrots.

Keywords: hydroponic carrots; taproot excision; taproot shape; total phenol; root contraction

## 1. Introduction

Hydroponics is an efficient method of growing plants without the use of soils. In Japan, hydroponic cultivation of leafy and fruit vegetables has been introduced to commercial crop cultivation in the form of plant factories [1]. However, commercial cultivation of root vegetables using hydroponics has yet to be established.

In recent years, research on hydroponics for root vegetables, including potatoes and sweet potatoes, has been conducted using various systems. Using sandy medium-based hydroponics, sweet potato storage roots have shown to thicken at sites higher than the groundwater level [2,3]. A newly designed rockwool slab-based hydroponic system has also been demonstrated to produce thickened storage roots of sweet potatoes [4]. In this system, storage roots developed between the surface of the hydroponic solution and nutrient-absorbed rockwool slabs, but not in the hydroponic solution. When sweet potatoes were cultivated in a two-layer system consisting of an upper vermiculite-containing pot and a lower nutrient solution, the roots thickened in the pots, but not in the nutrient solution [5]. In potatoes, aeroponics can continuously produce small tuberous roots only in the upper root zone where they are in direct contact with air [6]. These findings suggest that the water status around roots is an important determinant of root thickening in root vegetables.

Carrots are root vegetables that are available and consumed around the world. Edible roots, known as taproots, contain a variety of secondary metabolites such as carotenoids and phenolic compounds, which are believed to promote human health [7]. Several studies have demonstrated that carrot taproots can be grown using hydroponics [8–15]. Using a perlite substrate as a hydroponic growing medium, researchers have found that the size of taproots can be determined in part by the size of perlite particles [12]. In a rockwool-based hydroponic system, taproot growth was influenced

by the space of the sink enlargement [10]. Carrots have also been grown experimentally using nutrient film technique (NFT) and deep flow technique (DFT) hydroponic systems, which do not need substrate media [9,11,13–15]. In two distinct DFT experiments, oxygen supplementation to water-immersed roots by aeration has been shown to be required for carrot taproot enlargement [9,15]. Using a DFT system, it has also been demonstrated that increasing the root zone temperature can alter the growth and components of carrot taproots [13].

For consumers, the shape of carrot taproots is an important factor in both the appearance and processing efficiency of taproots and can directly affect market prices. In hydroponically grown carrots, the shape of the taproots tends to be irregular. In rockwool-based hydroponics, taproot development depends on the surrounding airspace [10]. In this study, taproot shape was not uniform, even with identical treatment. Using NFT hydroponics, carrots formed irregular taproots containing many branch roots [11]. Similar taproot branching has been observed in DFT hydroponics [15]. In another DFT study, hydroponic carrot taproots formed without branching and elongated more than those grown in soil [9]. Hydroponic cultivation methods that produce taproots that resemble those of soil-grown carrots are required. To improve the quality of hydroponic carrots, we examined the effects of partial carrot taproot excision at the early cultivation stage on growth and components.

#### 2. Materials and Methods

#### 2.1. Plant Growth and Experimental Conditions

An experimental timeline is shown in Figure 1. Carrot seeds (*Daucus carota* L. cv Tokinashigosun, Takii, Co. Ltd., Kyoto, Japan) were sown in  $2 \times 2 \times 2$  cm sponge cubes and grown at 20 °C under 200 µmol m<sup>-2</sup> s<sup>-1</sup> of photosynthetic photon flux (PPF) for 16 h under fluorescent lamps (FLs; FL40SBR-A; NEC Co., Tokyo, Japan). At 10 days after sowing, seedlings with a single taproot extending from the bottom of the cubes were transferred to a DFT hydroponic system with continuous aeration. The nutrient solution was based on a half-strength culture solution of A-type Otsuka House Solution (Otsuka Agri Techno Co. Ltd., Tokyo, Japan). The nutrient solution was changed every two weeks. To avoid entangling of seedling roots, roots were untangled every three or four days.

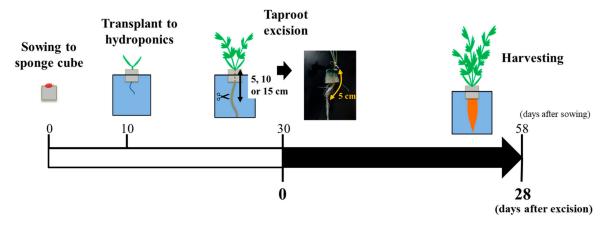


Figure 1. Experimental timeline in this study.

Early taproot excision treatments were conducted 30 days after sowing. Only plants with active root tips were selected for root excision. Taproot tips were excised 5 cm, 10 cm, and 15 cm from the base of the stem. Fibrous roots that branched off from the remaining taproots were left even if they extended longer than the cut size. The taproots of control plants were not excised. After root excision, the plants were immediately transferred to a new DFT system with a 25 cm deep box. Plants were cultivated at 20 °C under 250  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> PPF for 16 h under FLs. Growth parameters of leaf number, shoot length, and taproot diameter were measured at 0, 1, 2, 3, 5, 6, 7, 8, 10, 12, 14, 16, 19, 21, 23, and 28 days after taproot excision. At 28 days after taproot excision, the plants were harvested, and growth parameters

and components were then analyzed. Photographs of the taproots were taken after the removal of fibrous roots.

### 2.2. Measurement of Total Phenol Concentration

Total phenol concentration was measured using the Folin–Ciocalteu method with modification [16]. The middle part of each taproot was sliced and homogenized with a mortar and pestle. A 50 mg homogenized sample was transferred to a microtube (1.5 mL), to which 500  $\mu$ L of 90% methanol was added. The sample was stirred vigorously and was centrifuged at  $10,000\times g$  for 5 min. The supernatant (50  $\mu$ L) was diluted to 650  $\mu$ L with distilled water and mixed with 50  $\mu$ L of phenol reagent. After adding 300  $\mu$ L of 5% sodium carbonate, the mixture was incubated at 25 °C for 30 min. Absorbance of the supernatant was measured at 765 nm, and a standard curve was prepared using gallic acid. Absorbance was converted to total phenolic concentration in milligrams of gallic acid per gram of fresh sample weight.

#### 2.3. Measurement of Soluble Sugar Concentration

Sliced taproot segments were homogenized with a mortar and pestle, and the homogenates were filtered with filter paper (No. 1, Whatman plc, Maidstone, UK) to remove tissue debris. The concentration of soluble solids was measured using an Atago PAL-1 Handheld Digital Brix Refractometer (Atago, Japan).

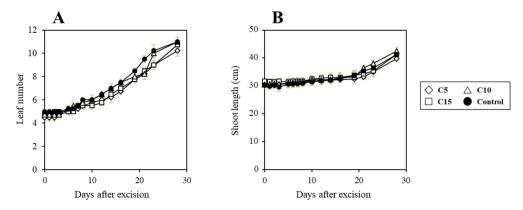
#### 2.4. Data Analysis

Data obtained for each parameter were analyzed with the statistical package JMP (SAS Institute, Cary, NC, USA). Differences among treatments were determined by one-way analysis of variance. Mean comparisons were made using the Tukey–Kramer honest significant difference multiple range test at p < 0.05.

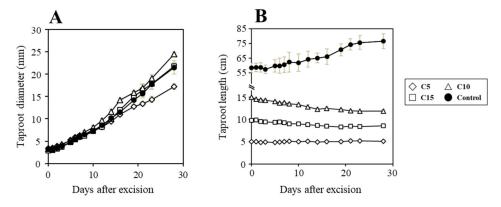
#### 3. Results and Discussion

Thirty days after sowing, hydroponic carrots had a single taproot approximately 60 cm long with a maximum diameter of 3 mm. In our experiment, taproot enlargement started at this point. To examine the effects of taproot excision, the tips of the taproots were removed at this time 5 cm (C5), 10 cm (C10), and 15 cm (C15) from stem base. The growth of the shoot and taproot of these plants was compared with that of nonexcised control plants. A time course observation of leaf number and shoot length revealed no significant differences between plants throughout the experimental periods (Figure 2). By contrast, a time course measurement of maximum taproot diameter showed a suppression of taproot enlargement in C5 plants compared with C10, C15, and control plants from 20 days after excision (Figure 3A). Taproot length at 0 day after excision was the cut length after root tip removal, except for nonexcised control plants (Figure 3B). In control plants, taproot length gradually increased from 5 days after excision treatments, reaching approximately 80 cm at 28 days after treatments (Figure 3B). The suppression of taproot elongation observed in the days after treatment in control plants was probably due to transplantation stress. In C15 and C10 plants, taproot lengths gradually contracted after 28 days of treatment to approximate lengths of 12 cm and 9 cm, respectively (Figure 3B). In C5 plants, no clear change in taproot length was observed. Root contraction phenomena have been observed in several plants as a developmental process [17-24]. The lower parts of radish taproots have been shown to contract during taproot thickening [19]. In our study, taproot contractions were induced when taproots were cut to a 10 or 15 cm length from the base of stem, but not when taproots were cut to a 5 cm length. This suggests that carrots are equipped with a taproot contraction mechanism at a site more than 5 cm from the stem base. In *Trifolium pratense*, contraction of roots has been shown to cause the pull of aboveground parts into the soil [24]. As roots do not experience soil resistance in hydroponics, hydroponic carrots may be able to contract their taproots more easily than soil grown carrots.

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**Figure 2.** Time course changes in leaf number (**A**) and shoot length (**B**) of carrots after partial taproot excision. Vertical bars represent  $\pm$  SE (n = 4).



**Figure 3.** Time course changes in taproot diameter (**A**) and taproot length (**B**) of carrots after partial taproot excision. Vertical bars represent  $\pm$  SE (n = 4).

Plants were harvested 28 days after excision, and growth and components were then investigated. Enlargement of taproots was observed in all experimental plots (Figure 4). Control plants had longer taproots than other plants with excision treatments. The enlarged parts of control taproots resembled those of C15 taproots, whereas control taproots over 15 cm from the root base exhibited little enlargement (Figure 4). Control plant roots resembled taproots in another hydroponic carrot study [9]. Carrot taproots are known to experience increases in cell numbers and weight during taproot enlargement [25], suggesting that these cell growth activities may exist only within 15 cm of the stem base in hydroponic carrots. In plants with excised taproots, the tips of the taproot were more rounded when taproots were cut to a shorter length (Figure 4). Early taproot excision treatments may therefore be a useful method of improving the root tip shape in hydroponic carrots.

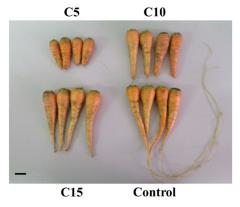
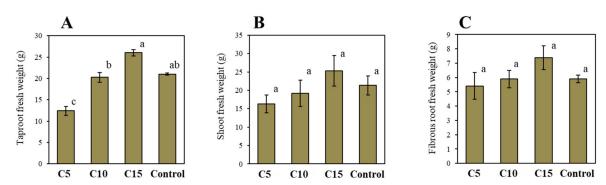


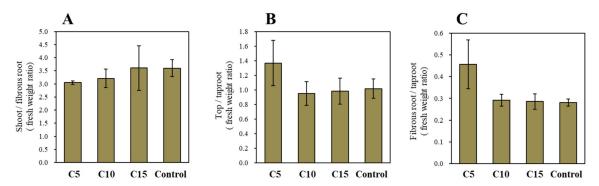
Figure 4. Taproot of carrots 28 days after partial taproot excision. Bar = 2.0 cm.

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Taproot fresh weight was highest in C15 plants followed by C10 and C5 plants among taproot-excised plants (Figure 5A). Control plants showed weights similar to those of C10 plants (Figure 5A). This indicates that early excision of taproots to shorter lengths may reduce the ability to accumulate photosynthetic products to taproots. The fresh weight of the shoot and fibrous root showed the same tendency as taproot fresh weight (Figure 5B,C). There was no difference in the ratio of shoot and fibrous root, but the ratios of shoot/tuberous roots and fibrous/tuberous roots tended to increase in C5 plants (Figure 6). These findings also suggest a decrease in the accumulation of photosynthetic products to C5 taproots. Sink size and activity have been proposed to be important determinants of partitioning of photosynthetic products to the whole plant [26]. In cucumbers, when sink size was limited by reducing fruit numbers, sink portioning of photosynthetic product was also reduced, especially when the number of fruits was severely restricted [27]. In C5 plants, not only taproots but also fibrous roots were more removed compared with other root-excised plants, and a temporary reduction of root function was induced which limited the growth in the whole plant.



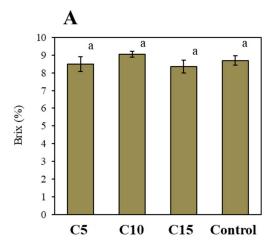
**Figure 5.** Effect of partial taproot excision on fresh weights of taproot (**A**), shoot (**B**), and fibrous root (**C**) of carrots 28 days after partial taproot excision. Vertical bars represent the means  $\pm$  SE (n = 4). Different letters indicate significant differences as determined by Tukey's multiple comparison test (p < 0.05).

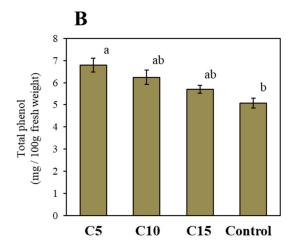


**Figure 6.** Effect of partial taproot excision on the ratios of plant organs of carrots 28 days after partial taproot excision. (**A**) (Shoot fresh weight)/(fibrous root fresh weight). (**B**) (Shoot fresh weight)/(taproot fresh weight). (**C**) (Fibrous root fresh weight)/(taproot fresh weight).

Sugar concentrations in the taproots measured by Brix did not differ between treatments (Figure 7A). However, the total phenol concentration in taproots was highest in C5 plants, followed by C10, C15, and control plants (Figure 7B). Phenolic compounds are produced in response to various environmental stresses, such as light, temperature, drought, and wounding [28,29]. Given that C5 taproots contained more wounded parts compared with C10 and C15 plants, continuous wound stress at a root cut site may trigger higher production of phenolic compounds in C5 plants.

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**Figure 7.** Effect of partial taproot excision on the concentration of sugar (**A**) and total phenols (**B**) of carrot taproots 28 days after partial taproot excision. Vertical bars represent the means  $\pm$  SE (n = 4). Different letters indicate significant differences as determined by Tukey's multiple comparison test (p < 0.05).

In root vegetables, sink development is regulated by a variety of plant hormones such as auxin, cytokinin, and gibberellic acid [30–33]. In carrots, gibberellic acids function as negative regulators of taproot enlargement [34,35]. Wang et al. [35] have shown that treatment of gibberellic acid to the soil triggers a reduction in taproot development, accompanied by the accumulation of lignin in the secondary xylem of taproots. In radishes, gene expression that is involved in lignin synthesis was reportedly reduced during taproot thickening [36]. Lignification of storage roots has also been reported in the restriction of root thickening in sweet potatoes [5,37]. These findings suggest that root treatment of hormones controlling the lignification could be a valuable strategy to increase sink biomass in root vegetables.

#### 4. Conclusions

Hydroponics in environmentally controlled systems such as plant factories are thought to be required for high-value crops to address high cultivation costs [38]. Enhancement of constituent compounds that foster human health will likely increase the value of crops. Regulation of light, temperature, and hormone treatments has been shown to increase phenol content in carrot taproots [13,39,40]. Therefore, by combining these environmental regulatory efforts, our early root excision method can be a useful tool for efficiently growing high-value carrots.

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