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Journal

Scientia Horticulturae　volume 159, Pages 113-121

Published

30 July 2013

URL

https://doi.org/10.1016/j.scienta.2013.04.038

Growing carrots hydroponically using perlite substrates

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Abstract

Perlite substrate and nutrient solution were studied for growing carrot [*Daucus carota* L. cv. Dr. Carotene 5] hydroponically. Three independent studies were conducted to determine the size of perlite particle and concentrations of nutrient solution for growing carrot hydroponically by evaluating their effects on growth, root yield and qualities of carrot. In the first study, carrots were grown in 1.2 or 5.0 mm with 12.5, 25, 50 and 75% □Enshi□ nutrient solution. Plants grown in 1.2 mm perlites with 50 and 75% nutrient solution produced about 15 and 28% higher root yield, respectively than those plants grown in 5.0 mm with same concentration, which was attributed by its longer roots. In the second study, carrots were grown in 0.6, 1.2, 2.5, and 5.0 mm perlites and with 25, 50, 100, 200, and 300% of nutrient solution. It was found that, carrot plants grown in 0.6 mm perlite supplied with 100% nutrient solution produced significantly higher root yield compared to larger perlite particles and higher concentrations of nutrient solution. In the third study, once used perlites of 0.3, 0.6, and 1.2 mm particle and 50, 75, 100, and 150% nutrient solution were investigated and greater root yield was obtained from carrots grown in 0.6 mm than in 0.3 or 1.2 mm perlite with 75% nutrient solution. Carrots grown in 0.3 mm perlites produced shorter roots, wider near the proximal end and whitish in the distal end due to excessive water content causing oxygen deficiency. Carrot root length was greatly hampered leading to decreased root yield in plants grown in 0.3 mm perlites compared to other perlite sizes at all concentrations except 100%. This ultra fine perlite hold excessive water causing oxygen deficiency in the substrate air zone and as a result roots become whitish with reduced amount of carotenoids content. Reused perlite culture in the third study lead to maximum root yield at 75% nutrient solution whereas it was with 100% in the second study, which was possibly due to residual nutrient available in the reused perlite which in turn lowers the demand of nutrients in the second culture. Year and growing season along with reused perlite has results in lower root yield and quality in the last two cultures in autumn 2009 and 2010. Therefore,

we recommended 0.6 mm perlite and 100% (for first culture) or 75% (for second culture) □Enshi□ nutrient solution for growing carrots hydroponically with maximum yield and higher quality.

Key words: *Daucus carota* L.; Soilless culture; Nutrient solution concentration; residual nutrient

1. Introduction

The carrots [*Daucus carota* L.], rich in carotenoids, is among the top-ten most economically important vegetable crops in the world, in terms of both area of production and market value (FAOSTAT, 2012; Fontes and Vilela, 2003). It has been widely used as a material for juice production both alone and mixed with other vegetables and fruits with high quality of taste, sweetness and flavor. Regular intake of carrot juice can help us to stay away from different degenerative diseases like cataracts, glaucoma, cardiovascular complications, and even cancer as it has high antioxidant properties. In this regard, hydroponically grown carrots provide outstanding quality, flavor and nutrition (Gichuhi et al., 2009). Therefore, hydroponics system has been evaluated for growing potatoes, sweet potatoes, sugar beets and peanuts for controlled ecological life support system (Hill et al., 1992). It allows clean cultivation of roots as the most of pests are soil born which is no longer a concern in hydroponics. Compared to traditional systems, hydroponic carrots are grown with a bigger assurance of nutritional content. Since a regular nutritional testing is conducted in the hydroponic growing system, so it can be more easy defined whether the desired amount of nutritional content is present in the carrots or not. In addition, undesired nutrient contents, for instance nitrites, heavy metal contamination can be easily kept away from the system.

Root vegetables are often discouraged to grow by hydroponics possibly because of the poor root development. Carrot is a root vegetable which forms numerous hairy roots with a reduced tap root in the nutrient solution. Moreover, inside nutrient solution this storage organ form hypertrophy due to the ample supply of water and nutrient resulting decrease in length and weight compared to non-submerged condition (Terabayashi et al., 2008). Therefore, appropriate moisture content of growth medium is crucial for optimum growth and development of the storage root (Eguchi et al., 2008).

Growth media can provide proper moisture content for producing cleaned, bright color, and uniform shaped carrot roots in hydroponics culture system. It can also provides mechanical support to the plant and encourage tap root enlargement. Therefore, use of suitable soilless media can increase both marketable yield and quality of root crops by many folds (Hanna, 2009). Rockwool has been found to be the most prevalent media for growing horticultural crops hydroponically (Smith, 1998). It has been investigated to evaluate growth characteristics and yield of three cultivars and suggested as a suitable method for growing carrots hydroponically (Islam et al., 2008). However, recently the perlite has emerged as an excellent medium with versatile use. It has been widely used to grow many horticultural crops including tomatoes, cucumber, melon, peppers, lettuce and rose (Szmidt et al., 1988; Hochmuth and Hochmuth, 2003; Rodriguez et al., 2006; Cantliffe et al., 2003; Frezza et al., 2005; Fascella and Zizzo, 2005). Perlite is widely preferred as it encourages faster root development, reduces risk of damping off, avoids water logging and provides an optimum balance of air and water. Its strong attraction for water automatically draws up solution from the reservoir at the same rate that the plants remove water leaving excess solution in the reservoir. Therefore, optimum moisture level can be maintained around root, and this is a significant advantage over rockwool, which has less capillary action.

Dr. David A. Hall from United Kingdom mentioned that tomato crops hydroponically grown in perlite have produced average yields 7% higher than crops grown in rockwool (A research report issued by the Perlite Institute Inc.). Currently many vegetables and ornamentals are being grown in hydroponic grow bags throughout the world. Melons are being grown in Florida, Holland and US, commercial cut flowers, strawberries, and orchids are being grown in 100% hydroponic perlite containers. In addition, used perlite can be cleaned and disinfected as needed and recycled for many years can reduce time and expense, as it is not organic in nature and physically and chemically stable (Hanna, 2005, 2010). Therefore, the use of perlite has many advantages over rockwool as soilless culture media. So far, many researchers around the world have compared the perlite with other soilless media for hydroponic culture of cucumber (Abul-Soud et al., 2003; Schon and Peggy Compton, 1997), water melon (Yetisir et al., 2006; Raja Harun et al., 1991; Guler et al., 1995), tomato (Inden and Torres, 2004; Lee et at., 1999; Ghehsareh et al., 2011), strawberry (Hochmuth et al., 1998), lettuce (Siomos et al., 2001; Tapia and Caro, 2009) and many other corps (Gul et al., 2005; Samartzidis et al., 2005). However, until now there are little researches on growing hydroponic root vegetables using perlite as substrate. Soilless culture of carrot using the perlite substrate has not been reported yet. Thus the effort of our present research is to grow the carrot hydroponically in perlite substrate. Growth and yield of different crops depends significantly of the sizes of perlite. For example, water holding capacity is greatly depends on this characteristic. Ultrafine perlite has smaller pores spaces that hold water strongly, in turns larger particle size perlites has less moisture retention capacity. Therefore, selection of the suitable particle size of perlite for carrot is important for maximizing root yield. Concentration of culture solution is another yield determining factor to be investigated for growing carrots hydroponically. Therefore, finding the suitable perlite size along with optimal concentration of culture solution is a great advantage for maximizing carrot yield in hydroponics.

Thus, the objectives of this study were to select the most suitable perlite particle size and the optimum concentration of the nutrient solution by evaluating their effects on growth, yield and quality of carrots grown hydroponically and to assess the feasibility of reusing perlite substrate in the successive culture.

2. Materials and methods

2.1. Carrot cultivar

Carrot cultivar 'Dr. Carotene 5', an orange type with ruby to inside, was used as the plant material in this study. The cultivar has excellent sweet taste, strong vigor even in reduced fertilizer recommendation. The seeds were collected from Takii & Co. Ltd. Kyoto, Japan.

2.2. Perlite substrate

Perlites of different particle size (0.3, 0.6, 1.2, 2.5, and 5.0 mm) were used as substrate for growing carrot in soilless hydroponics. The perlites were collected from UBE Industries, Ltd. Tokyo, Japan, is mainly composed of porous igneous rock which is heated, foamed and milled to an appropriate particle size. This light weight medium is sterile, inert, non-toxic, non-decomposable and easy to handle with enhanced water retention and aeration capacity.

2.3. Enshi nutrient solution

A series of □Enshi□ nutrient solution (12.5, 25, 50, 75, 100, 200 and 300% of full strength solution) were applied for growing carrot in soilless hydroponics. A full-strength □Enshi□ nutrient solution contains the following amounts of salts 1000 L^{-1} of tap water: 950 g of $Ca(NO_3)_2 \cdot 4H_2O$; 810 g of KNO_3; 500 g of $MgSO_4 \cdot 7H_2O$; 155 g of $NH_4H_2PO_4$; 3 g of H_3BO_3; 2 g of $ZnSO_4 \cdot 7H_2O$; 0.05 g of $CuSO_4 \cdot 5H_2O$; 0.02 g of $NaMoO_4$; and 25 g of NaFe-EDTA (Hori, 1966).

2.4. Climatic conditions of the experimental site

Three independent studies were conducted to investigate the perlite particle size and nutrient solution concentrations for growing carrot using soilless hydroponic system at the greenhouse (20 m × 5 m) of Experimental Research Center for Biological Resources Science, Shimane University. During study period in autumn 2009, the average temperature was about 4 °C greater than spring 2009 but average solar irradiation and rainfall was lower. About 44 W m^{-2} greater average solar radiations were recorded in autumn 2009 than that of autumn 2010.

2.5. Hydroponic culture of carrot using 1.2 and 5.0 mm perlite and 12.5, 25, 50 and 75% of nutrient solution

A 2 × 4 factorial experiment was conducted in a randomized complete block design with three replications to determine the effects of 1.2 mm and 5.0 mm perlites and 12.5, 25, 50 and 75% nutrient solution on the growth, root yield and quality of 'Dr. Carotene 5'. Seeds were sown in the plastic container (54 × 34 × 20 cm) filled with 30 liters of perlite on 4 March 2009. In three rows 11 hills per container were made, and three seeds were placed in each place at about one cm depth.

From 14 March 2009, one liter of each concentration of nutrient solution was sprinkled to the carrot plants everyday to provide the sufficient amount of water and nutrients. After one month, when the leaves were starting to spread, two seedlings were removed leaving one healthy plant per place. At the end of study on 2 June 2009 growth of carrot plants were measured as leaf numbers, length, fresh, and dry weight whereas, yield was measured as the length, diameter, and fresh weight of roots. Leaves of each carrot plant were collected in brown paper bag (230 × 318 cm, K Roll Special 1gou, Fukusukekogyo Co. Ltd. Tokyo, Japan) kept in constant temperature oven (DKN 812, Yamato Scientific Co. Ltd. Japan) for measuring the dry weight at 80 °C for 72 hours. Fresh weight of carrot roots were measured with an electrical balance (Tuning-Fork Balance, CJ-620, Shinko Denshi Co. Ltd. Tokyo, Japan).

2.6. Hydroponic culture of carrot using 0.6, 1.2, 2.5 and 5.0 mm perlite and 25, 50, 100, 200 and 300% of nutrient solution

Based on the results of previous culture, a 4 × 5 factorial experiment was conducted in a randomized complete block design with three replications to determine the effects of 0.6, 1.2, 2.5, and 5.0 mm perlites and 25, 50, 100, 200, and 300% of □Enshi□ nutrient solution on the growth, root yield and quality of 'Dr. Carotene 5'. Seeds were sown in plastic container (42 × 32 × 30 cm) filled with 40 liters of test perlites on 26 Aug. 2009 (Fig. 1). In three rows 7 hills per container were made and three seeds were placed in each place at about one cm depth. From 10 September 2009 one liter of each concentration of nutrient solution was sprinkled to the carrot plants everyday to provide enough water and nutrients. On 29 September 2009 when the leaves were starting to spread, two seedlings were thinned out leaving one plant per hill. Other cultural practices were done as described in first study.

2.7. Hydroponic culture of carrot using 0.3, 0.6, and 1.2 mm perlite and 50, 75, 100 and 150% nutrient solution

A 3 × 4 factorial experiment was conducted in a randomized complete block design with three replications to determine the effects of once used perlites of 0.3 (a special grade obtained from UBE Industries, Ltd. Tokyo, Japan), 0.6, and 1.2 mm and 50, 75, 100, and 150% of □Enshi□ nutrient solution on the growth, yield and qualities of roots of ‘Dr. Carotene 5’. Seeds were sown in plastic container (44 × 32 × 30 cm) filled with 40 liters of test perlites on 22 September 2010. In three rows 7 hills per container were made and three seeds were placed in each place at about one cm depth covering with vermiculite. After germination each container was supplied with 500 ml of 50% nutrient solution until start of nutrient solution concentration treatments. Two plants were thinned out leaving one plant per pit on 18 October 2010 after the leaves were spread. Carrot plants were sprinkled with 500 ml of each concentration of nutrient solution everyday to provide sufficient water and nutrients. Other cultural practices were done according to the previous studies. At the end of the study, growth characters like leaf numbers, length, and dry weights and yield in terms of length, diameter, and fresh weights of root were measured.

2.8. Carrot root quality analysis

Carrot roots were sliced and frozen at –30 ºC for subsequent analysis of soluble solids, carotenoids and ascorbic acid content. On the day of analysis root were kept out of freezer to obtain juice. The extracted juices were collected in 50 ml volumetric flask. Further root samples were mashed in mortar and pestle for juice extraction and then the collected juice were mixed, and analyzed for soluble solid content. About 0.4 ml of the mixed juice was placed onto the prism surface of pocket digital refractometer (PAL-1, Atago Ltd., Tokyo, Japan) and soluble solid contents were recorded. Repeated measures were conducted by washing the prism surface by distilled water and also rinsed with the test juice.

Carotenoids content were measured from carrot juice. The frozen root samples (1.5 g) were placed in a mortar with small amount of acetone (99%) and then grounded using the pestle. This has been done at least three times until the fiber become white i.e. there is no carotene remains in the root samples. The extracts were filtered using qualitative filter paper (Advantec Grade no. 131, 185 mm thickness) in funnel on a 50 ml volumetric flask. Acetone was added several times on the samples in filter paper and finally the volume was measured up to the mark of 50 ml volume. Carotenoids content were measured at wavelengths of 443, 475, 492 and 505 nm by spectrophotometer (U-2900, Hitachi High Technologies Corporation, Tokyo, Japan) following the method developed by Nagata et al., 2007. Concentration of α-carotene, β-carotene and lycopene were determined by measuring the absorbance at 443, 475, 492 and 505 nm, i.e., A_{443}, A_{575}, A_{492} and A_{505}, respectively and performing calculations. The equations, with correlation coefficients between prediction and measurement (r), and the standard errors of prediction (SEP), are as follows: α-carotene (mg/L) = $0.847A_{443} + 3.218A_{475} - 1.499A_{492} - 3.519A_{505} - 0.119$ ($r = 0.965$, SEP = 0.231); β-carotene (mg/L) = $-1.488A_{443} + 4.844A_{492} - 2.352A_{505} + 0.098$ ($r = 0.946$, SEP = 0.228); lycopene (mg/L) = $0.256A_{443} - 1.984A_{492} + 5.088A_{505} - 0.237$ ($r = 0.996$, SEP = 0.139).

Ascorbic acid contents were measured following 2,4-dinitrophenylhydrazine (DNP) colorimetry. Carrot juice (0.5 ml) were taken in 50 ml glass test tube then 0.5 ml of 10% meta-phosphoric acid solution, 1 ml of distilled water, 1 ml of 0.03% 2,6-dichlorophenol-indophenol (DCP), 2 ml of thiourea, and 1 ml of DNP was added to the samples sequentially following three hours incubation at 37 ºC in water bath (BW400, Yamato Scientific Co. Ltd. Japan). After incubation 5 ml of 85% H_2SO_4 were added keeping the samples in iced water. After 30 minutes cooling ascorbic acid content were measured at 520 nm by spectrophotometer.

2.9. Residual mineral nutrients analysis

The twice used perlites from the third study were collected to determine the residual mineral nutrients compared with once cultured perlites and also with new perlite. The collected perlite samples were dried in constant temperature oven at 80 °C for 72 hrs. 100 g of oven dried perlites were soaked with 100 ml distilled water and shacked at 150 rpm (Bio-Shaker BR-43FL, Japan) overnight. The supernatant were filtered with Whatman No. 131 filter paper and the filtrate were analyzed for major mineral nutrients by with compact Twin NO_3^- meter (B-343, Horiba Ltd. Kyoto, Japan) for NO_3^-, spectrophotometer (UVmini 1240, Shimadzu Corporation, Kyoto, Japan) for PO_4^{3-} and atomic absorption spectrophotometer (Z-5010, Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}.

2.10. Statistical analysis

Analysis of variance was performed to test for significant interactions between sizes of perlite and concentrations of nutrient solution and their effects on the plant growth, yield and carrot root quality in all three studies. Mean separations were performed by Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$ level of significance.

3. Results

3.1. Effects of perlite sizes (1.2 and 5.0 mm) and concentrations (12.5, 25, 50 and 75%) of nutrient solution

3.1.1. Growth and root yield

In the first study, interaction of particle sizes of perlite and concentrations of nutrient solution had no significant effects on growth and yield variables of carrot except root diameter and fresh weight (Table 1). Size of perlite substrate had a significant effect on growth and yield variables of 'Dr. Carotene 5' except length of leaves. Number, and dry weight of leaves per plant, length, diameter and fresh weight of carrot roots were greater in plants grown in smaller perlite particles (1.2 mm) than grown in the bigger particles (5.0 mm). Carrots grown in 1.2 mm perlite yielded about 55% higher fresh roots per plant at 75% than 12.5% whereas, it was not differ significantly than 50% nutrient solution. Overall results showed an increasing trend in growth and yield variables of carrot with the increase in concentrations of nutrient solution ranged from 12.5 to 7.5%. At the highest concentration of 75% nutrient solution significantly greater leaf dry weights, root lengths and fresh weights were measured in plants grown in smaller perlite particles (1.2 mm) than plants grown in the bigger particles (5.0 mm).

3.1.2. Carrot root qualities

Root qualities of carrot in terms of soluble solids, α- and β-carotene, and ascorbic acid content were not affected by the interaction of perlite sizes and concentrations of nutrient solutions used in this experiment (Table 2). Two perlite particles (1.2 and 5.0 mm) investigated had no influence on the above qualities of carrot root. Soluble solids content was differed in carrot root grown in different concentrations of nutrient solutions but carotenoids and ascorbic acid content were not affected significantly. It was significantly increased in root grown with 50 or 75% of nutrient solution than grown with other concentrations.

3.2. Effects of perlite sizes (0.6, 1.2, 2.5 and 5.0 mm) and concentrations (25, 50, 100, 200 and 300%) of nutrient solution

3.2.1. Growth and root yield

There were no significant interaction between particle sizes of perlite and concentrations of nutrient solution on the growth and yield variables except root length (Table 3). All the growth parameters were greater in carrot plants grown in 0.6 mm than other perlite sizes supplied with five concentrations of nutrient solution. Highest dry weight of leaves was recorded from plants grown with 100% of nutrient solution in 0.6 mm perlite which was attributed by maximum leaf length and higher number of leaves. Significantly higher root yield (146.3 g fresh weight per plant) attributed by longer and wider carrots were harvested from plants grown in the 0.6 mm perlite with 100% nutrient solution. It is evident that carrots grown in 0.6 mm with 100% nutrient solution produced about 30, 58 and 43% greater root yield than those grown in 1.2, 2.5, and 5.0 mm perlite, respectively with the same concentration. Although carrot plants grown with 200% nutrient solution in 1.2 and 2.5 mm perlite produced significantly similar dry matter as with 100% nutrient solution in 0.6 mm, but root yield was not improve greatly in perlite particle size higher than 0.6 mm. Therefore, 100% nutrient solution was found to be optimal for maximize carrot root yield in hydroponics.

3.2.2. Carrot root qualities

All the qualities of carrot root except β-carotene were significantly influenced by the particle sizes of perlite, the concentration of nutrient solution and/or their interaction (Table 4). Plants grown in perlite smaller than 1.2 mm produced roots with higher soluble solids, ascorbic acid and lycopene content whereas those qualities were found to be decreased with the increase of particle size. α-carotene in roots was not differ in particle sizes greater than 1.2 mm but it was decreased in the smallest perlite particles (0.6 mm). In 2.5 and 5.0 mm perlite α-carotene content was higher at higher concentrations (100 to 300%) but lycopene was found higher at 50 or 100% concentration. Lycopene content in root were higher in roots grown with lower concentrations (25 and 50%) of nutrient solution and it was decreased with the increase of concentrations. These results indicated that quality roots were produced by the carrot plants grown in smaller perlite particles than those grown in larger particles.

3.3. Effects of reused perlite sizes (0.3 0.6, and 1.2 mm) and concentrations (50, 75, 100 and 150%) of nutrient solution

3.3.1. Growth and root yield

There were no significant interaction between particle sizes of perlite and concentrations of nutrient solution on the growth and yield variables except dry weight of leaves and root diameter (Table 5). Size of perlite size had a significant effect on the growth and yield variables of carrot except number of leaves whilst concentrations of nutrient solution showed no influence on these characters. Length of leaves was higher in plants grown in 0.3 or 0.6 mm perlite in all the concentrations compared to 1.2 mm perlite except 75 and 150% nutrient solution. Dry weight of leaves was increased significantly in 0.3 mm perlite with 50 to 100% nutrient solution compared to other perlites sizes having any concentration of nutrient solution. Therefore, vigorous growth of carrot plants was evidenced in the smaller particles of perlite than larger particle sizes. Carrot root length was greatly hampered in plants grown in 0.3 mm perlites compared to other perlite sizes at all concentrations except 100%. Decrease in root length in the smallest perlite is possibly due to compact muddy substrate. Fresh weight of root was higher in plants grown in 0.6 mm perlites with 75% nutrient solution than in 0.3 mm. However, compared to second study, root yield was lower in 0.6 and 1.2 mm perlite at 50-150% nutrient solution. The reason might be the variation in climatic factors between the growing seasons in two different years.

3.3.2. Carrot root qualities

Carrot root qualities such as soluble solids, β-carotene, and ascorbic acids were affected significantly due to the interaction of perlite sizes and concentrations of nutrient solution except α-carotene and lycopene (Table 6). Soluble solids content were significantly higher in roots obtained from 0.3 mm perlite at all the nutrient concentrations compared with other perlites except 1.2 mm with 100% nutrient. Although α-carotene was not significantly influenced by the perlite particles and nutrient concentrations, β-carotene, lycopene and ascorbic acid were affected significantly without any definite pattern. β-carotene was significantly higher in carrot roots grown in 0.6 mm with 100 or 150% and also in 1.2 mm with 75-150% nutrient solution. In case of carrot grown in 0.6 and 1.2 mm perlite with 75-150% can produce root with higher lycopene compared to carrot grown in 0.3 mm with all concentration except 150%. In general whitish color carrot roots were harvested from 0.3 mm perlite at all concentrations of nutrient solution. It also indicated that lower amount of β-carotene and lycopene in the carrot roots grown with 50% nutrient solution.

4. Discussion

In the first study, plants grown in 1.2 mm perlites with 50 and 75% nutrient solution produced about 15 and 28% higher root yield, respectively than those of plants grown in 5.0 mm with the same concentrations (Table 1). This higher root yield was attributed by its longer root. Although the media volume per container was equal between two perlite sizes (~30 L), there may have shortage of water and mineral nutrients for the plants grown in bigger particles of perlites compared with the smaller particles. The bigger particles of perlite with large pore spaces allow the nutrient solution pass through quickly with minimum nutrient solution to retain. Thus results indicated that smaller particles had better performance than bigger particles. Considering the above results we have investigated perlite particle sizes ranging from 0.6 to 5.0 mm in the following study. At the same time, to find out the upper limit of nutrient solution concentration we have also studied a series concentration (25, 50, 100, 200 and 300%) in the following cultures. Results clearly indicated that carrot grown in 0.6 mm with 100% of nutrient solution significantly improved the plant growth and root yield compared to lower and/or higher concentrations. These results indicated that carrot roots qualities were improved when grown in smaller perlite particles than those grown in larger particles. In order to find out the lowest suitable perlite particle size, another experiment was conducted to grow carrot plants in 0.3 mm perlites.

Recent research results revealed that perlite can be recycled for many years to reduce production cost without a negative impact on tomato yield (Hanna, 2005, 2006). Therefore, in this study we reused the perlites of previous culture to evaluate its feasibility of growing carrot hydroponically. Fresh weight of carrot roots was higher in plants grown in 0.6 mm perlite than 0.3 mm irrespective of concentration of nutrient solution. The reason can be realized from root parameters in Table 5. Carrot roots obtained from plants grown in 0.3 mm perlites were found as short, wider near the proximal end and whitish in color. This ultra fine perlite hold excessive water causing oxygen deficiency in the substrate air zone and as a result roots become whitish with reduced amount of carotenoids (Table 6). Root cell layer when observed under Olympus fluorescent microscope (BX51N-34-FL2) this phenomenon is clearly evidenced (Fig. 2). Increase in root diameter toward the proximal end of carrot storage root was found remarkable with Deep Flow Technique (DFT), resulting in a spindle shape of the root and was often followed by radial cracking along the axis (Terabayshi et al., 1997). From the above results it is clear that 0.6 mm is the suitable perlite size for growing carrot hydroponically.

In the third study, maximum root yield was obtained from carrots grown with 75% nutrient solution whereas it was with 100% in the second study. This difference in root yield was possibly due to residual nutrient available in the reused perlite which in turn lowers the demand of nutrients in the second culture. The NO_3^--N content of first cultured perlite was two times higher than second cultured perlite, therefore, in the second culture carrot plants uptake about 50% residual nitrogen from the reused perlites (data not shown). As a result, the second culture of perlites requires lower doses of fertilizers than the first culture. In the first and second study, carrots were grown in new perlite substrates and we supplied one liter of nutrient solution to each container every day, but in third study it was 500 ml per container every day. This suggests lower requirement of water and nutrient in the reused perlite substrates in the following year's culture. It was reported that 50% nutrient solution with it half level of NO_3-N appeared to be the optimum concentration for production of quality turnip in hydroponics (Asao et al., 2005). Therefore, we found that 50-75% concentration of nutrient solution is the optimal for growing carrots hydroponically in a recycled perlite (Fig. 3).

However, in the third study the values of all the yield parameters of carrot plants grown in 0.6 and 1.2 mm were lower than those in the second study for nutrient solution concentration of 50-150%. The similar phenomenon was found for the root quality characteristics. Season and year of planting have significant effects on the yield of crops. Similarly, the variation in values of growth and yield parameters of carrot between these two studies might be due to the variation in environmental controls in two different years. Another other possible reasons possibly the reused perlite substrate in the third study. We investigated the feasibility of reutilizing perlite substrate in the economic point of view as it can save money, time and labor. Reuse of perlite may cause the media compaction, salt buildup, left over root residues, and pest contamination for the successive crop. These risks were found as potential for tomato crops grown in reused perlite without processing by Hanna and Smith (2002). However, Hanna (2005) suggested that cleaned and disinfected used perlite can be recycled to save the growers money without reducing tomato yield. In our case, the carrot was grown in reused perlite as it has less chance of the above risk of recycling substrate. It has not extensive root system, broad leaves and profuse branching as in tomato. Thus we evaluate the once used perlite in the successive culture of carrot. Our results also suggest that properly cleaned and disinfected perlite can be reused in the succeeding crops.

5. Conclusion

Carrot cultivar ‘Dr. Carotene 5’, an orange type with ruby to inside, was grown in soilless hydroponics using different perlite particle sizes and several concentrations of □Enshi□ nutrient solution. Three independent studies/cultures were conducted during spring 2009, autumn 2009 and autumn 2010 under greenhouse condition. First study results showed greater growth and yield variables of carrots grown in smaller (1.2) mm perlite than the bigger (5.0 mm). It also evident an increasing trend in growth and yield variables of carrot with the increase in concentrations of nutrient solution ranged from 12.5 to 75%. Therefore, perlite sizes smaller than 0.6 and between 0.6 and 5.0 mm were investigated in the following culture (second study) with several fold increased concentration of nutrient solution (100, 200 and 300%). All the growth parameters were greater in carrot plants grown in 0.6 mm than other perlite sizes supplied with five concentrations of nutrient solution and significantly higher root yield attributed by longer and wider carrots were harvested from plants grown in the 0.6 mm perlite with 100% nutrient solution. In order to find out the lowest suitable perlite particle size 0.3 mm perlite was included in the third study. Recent research results revealed that perlite can be recycled for many years to reduce production cost without a negative impact on tomato yield (Hanna, 2005, 2006). In this connection, our third study was designed to evaluate the feasibility of reusing perlites of previous culture. Fresh weight of root was higher in plants grown in 0.6 mm perlites with 75% nutrient solution than in 0.3 mm. Carrot root length was greatly hampered leading to decreased root yield in plants grown in 0.3 mm perlites compared to other perlite sizes at all concentrations except 100%. This ultra fine perlite hold excessive water causing oxygen deficiency in the substrate air zone and as a result roots become whitish with reduced amount of carotenoids content. Reused perlite culture in the third study lead to maximum root yield at 75% nutrient solution whereas it was with 100% in the second study, which was possibly due to residual nutrient available in the reused perlite which in turn lowers the demand of nutrients in the second culture. The seasonal variation and impact of reused perlite together played role in lowering the root yield in autumn 2010 culture (third culture) than autumn 2009 culture (second culture).

Finally, it is clear that the growth, yield and quality of carrot were influenced greatly by the size of the perlite particle. It also was verified that the nutrient solution concentration can be decreased with the reused perlite. From the above studies we concluded that the suitable particle size of perlite is 0.6 mm and the concentration of □Enshi□ nutrient solution is 100% (for first culture) or 75% (for second culture) for growing carrot hydroponically. Reuse of perlite for growing carrot may cause the media compaction, salt buildup, and other associated risks; therefore our results also suggest that properly cleaned and disinfected perlite can be reused in the succeeding crops.

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Table 1

Interaction effects of particle sizes of perlite and concentrations of Enshi nutrient solution on the growth characters and root yield of ‘Dr. Carotene 5’ grown in soilless hydroponics during spring 2009.

Size of perlite (mm)	Conc. of NS[a] (%)	Number of leaves[b]	Leaf length (mm)	DW of leaves (g)	Root length (mm)	Root diameter (mm)	FW of root (g)
1.2	12.5	10.2 ab[c]	481.7 e	3.2 de	149.3 a	37.1 cd	73.7 d
	25	11.1 ab	550.7 bcd	4.2 d	148.0 a	38.5 bc	80.1 cd
	50	12.1 a	628.9 a	6.9 b	160.1 a	44.0 a	113.0 a
	75	11.7 ab	621.9 a	8.1 a	158.1 a	44.5 a	113.9 a
5.0	12.5	9.3 b	499.5 de	2.4 e	88.3 c	31.7 e	42.7 f
	25	10.1 ab	530.2 cde	3.2 e	104.3 bc	34.2 de	58.6 e
	50	10.9 ab	608.3 ab	5.6 c	117.8 b	41.6 ab	88.2 bc
	75	10.7 ab	578.6 abc	6.7 b	115.7 b	43.6 a	98.7 b
Significance							
Size of Perlite		*	ns	**	**	**	**
Conc. of NS		*	**	**	**	**	**
Interaction		ns	ns	ns	ns	*	*

[a] Concentration of half strength Enshi nutrient solution.

[b] Parameters were measured on per plant basis; Fresh weight (FW), Dry weight (DW).

[c] Means within column followed by different letters are significant according to the Tukey's test at $P < 0.05$.

[ns, *, **] Non-significant or significant at $P < 0.05$, 0.01, respectively.

Table 2

Interaction effects of particle sizes of perlite and concentrations of Enshi nutrient solution on the root quality parameters of ‘Dr. Carotene 5’ grown in soilless hydroponics during spring 2009.

Size of perlite (mm)	Conc. of NS[a] (%)	Soluble solids (%)	α-Carotene (mg / L)	β-Carotene (mg / L)	Ascorbic acid (mg / 100 ml)
1.2	12.5	6.5 b[b]	0.80 a	0.54 a	8.3 a
	25	6.8 b	0.53 a	0.57 a	6.1 a
	50	7.4 ab	0.60 a	0.63 a	7.3 a
	75	8.8 a	0.53 a	0.51 a	7.9 a
5.0	12.5	6.8 b	0.70 a	0.54 a	6.0 a
	25	6.9 b	0.74 a	0.37 a	8.4 a
	50	7.6 ab	0.58 a	0.72 a	5.9 a
	75	8.1 ab	0.72 a	0.60 a	5.9 a
Significance					
Size of perlite		ns	ns	ns	ns
Conc. of NS		**	ns	ns	ns
Interaction		ns	ns	ns	ns

[a] Concentration of half strength Enshi nutrient solution.

[b] Means within column followed by different letters are significant at $P < 0.05$ according to the Tukey's test (n = 33).

[ns, **] Non-significant or significant at $P < 0.01$, respectively.

Table 3

Interaction effects of particle sizes of perlite and concentration of Enshi nutrient solution on the growth characters and root yield of 'Dr. Carotene 5' grown in soilless hydroponics during autumn 2009.

Size of perlite (mm)	Conc. of NS[a] (%)	Number of leaves[b]	Leaf length (mm)	DW of leaves (g)	Root length (mm)	Root diameter (mm)	FW of root (g)
	25	12.8 ab[c]	613.2 a-e	7.6 ab	125.4 ab	49.5 ab	124.8 ab
	50	13.2 a	651.6 abc	8.0 ab	128.6 a	47.9 bc	121.8 ab
0.6	100	13.1 a	713.1 a	10.5 a	130.8 a	54.9 a	146.3 a
	200	12.6 ab	637.6 a-d	8.4 ab	113.1 a-d	49.5 ab	109.3 bcd
	300	12.3 ab	606.5 a-e	8.3 ab	106.0 bcd	48.0 bc	98.1 b-f
	25	11.4 ab	608.3 a-e	5.3 b	112.6 a-d	42.7 cde	82.1 d-h
	50	11.1 ab	638.0 a-d	6.2 ab	111.6 a-d	40.9 def	81.3 d-h
1.2	100	11.4 ab	651.9 abc	7.4 ab	121.9 abc	46.7 bcd	112.5 bc
	200	10.8 ab	579.1 b-e	6.3 ab	104.6 cde	39.4 ef	81.7 d-h
	300	9.9 b	501.6 e	4.7 b	108.4 bcd	37.7 ef	68.7 gh
	25	12.8 ab	527.3 de	4.3 b	100.4 de	38.7 ef	81.0 e-h
	50	11.9 ab	577.5 b-e	4.3 b	97.5 de	38.5 ef	76.6 e-h
2.5	100	12.8 ab	652.1 abc	7.1 ab	102.8 cde	42.3 cde	92.7 c-g
	200	11.2 ab	606.5 a-e	6.0 ab	98.8 de	38.8 ef	77.3 e-h
	300	10.4 ab	552.9 cde	4.5 b	85.4 efg	36.0 f	53.9 h
	25	11.4 ab	579.1 b-e	4.7 b	94.2 def	42.8 cde	79.8 e-h
	50	10.9 ab	599.8 b-e	5.2 b	97.6 de	41.7 def	81.3 d-h
5.0	100	12.1 ab	666.7 ab	7.7 ab	104.5 cde	46.3 bcd	102.4 bcd
	200	10.8 ab	585.4 b-e	5.8 b	73.3 g	41.6 def	73.4 fgh
	300	9.9 b	534.6 de	5.1 b	76.8 fg	38.9 ef	58.0 h
Significance							
Size of perlite		**	**	*	**	**	**
Conc. of NS		**	**	ns	**	**	**
Interaction		ns	ns	ns	*	ns	ns

[a] Concentration of half strength Enshi nutrient solution.

[b] Parameters were measured on per plant basis; Fresh weight (FW), Dry weight (DW).

[c] Means within column followed by different letters are significant according to the Tukey's test at $P < 0.05$.

[ns, *, **] Non-significant or significant at $P < 0.05$, 0.01, respectively.

Table 4

Interaction effects of particle sizes of perlite and concentrations of Enshi nutrient solution on the root quality parameters of ‘Dr. Carotene 5’ grown in soilless hydroponics during autumn 2009.

Size of perlite (mm)	Conc. of NS[a] (%)	Soluble solids (%)	α-Carotene (mg/L)	β-Carotene (mg/L)	Lycopene (mg/L)	Ascorbic acid (mg/100 ml)
0.6	25	6.8 b-g[b]	0.34 e	0.99 a	1.80 a	16.2 de
	50	8.0 a-d	0.68 a-e	0.79 a	1.20 abc	37.3 ab
	100	6.6 a-e	0.34 de	0.81 a	1.49 ab	40.4 a
	200	8.6 abc	0.65 b-e	0.59 a	0.48 cde	44.9 a
	300	9.0 ab	0.59 b-e	0.51 a	0.12 e	43.2 a
1.2	25	7.9 a-d	0.40 cde	1.07 a	1.87 a	32.7 abc
	50	9.2 a	0.94 ab	0.78 a	0.78 b-e	40.8 a
	100	6.8 b-g	0.92 ab	0.69 a	0.34 cde	26.4 bcd
	200	7.6 a-e	0.83 abc	0.59 a	0.10 e	43.4 a
	300	7.1 a-f	0.80 a-d	0.63 a	0.00 e	18.5 de
2.5	25	6.5 c-h	0.57 b-e	0.53 a	0.51 cde	21.8 cde
	50	6.3 d-h	0.55 b-e	0.78 a	1.55 ab	19.6 de
	100	5.6 e-h	0.72 a-e	0.48 a	0.47 cde	17.4 de
	200	6.5 d-h	1.13 a	0.52 a	0.07 e	20.2 cde
	300	5.2 fgh	0.43 cde	0.89 a	0.84 b-e	15.3 de
5.0	25	4.3 h	0.58 b-e	0.78 a	1.06 a-d	11.4 e
	50	4.8 gh	0.59 b-e	0.76 a	1.29 abc	15.4 de
	100	4.3 h	0.82 abc	0.70 a	0.56 cde	13.9 de
	200	6.5 c-h	0.80 abc	0.59 a	0.24 de	26.4 bcd
	300	6.7 c-g	0.91 ab	0.69 a	0.16 de	14.6 de
Significance						
Size of perlite		**	**	ns	**	**
Conc. of NS		**	**	ns	**	**
Interaction		**	**	ns	**	**

[a] Concentration of half strength Enshi nutrient solution.

[b] Means within column followed by different letters are significant at $P < 0.05$ according to the Tukey's test (n = 21).

[ns, **] Non-significant or significant at $P < 0.01$, respectively.

Table 5.

Interaction effects of particle sizes of perlite and concentrations of □ Enshi□ nutrient solution on the growth characters and root yield of 'Dr. Carotene 5' grown in soilless hydroponics during autumn 2010.

Size of perlite (mm)	Conc. of NS[a] (%)	Number of leaves[b]	Leaf length (mm)	DW of leaves (g)	Root length (mm)	Root diameter (mm)	FW of root (g)
0.3	50	9.7 a	453.8 ab[c]	4.3 abc	99.1 bc	40.8 a	66.0 abc
	75	9.5 a	460.4 ab	4.6 ab	99.9 bc	39.4 abc	59.4 bc
	100	11.2 a	466.2 a	5.7 a	107.4 abc	40.5 ab	64.6 abc
	150	9.6 a	409.9 ab	3.0 cde	89.2 c	38.7 a-d	57.0 bc
0.6	50	9.3 a	438.3 ab	3.1 b-e	120.0 ab	34.3 de	77.3 ab
	75	9.5 a	434.2 ab	3.6 bcd	126.0 a	36.4 a-e	86.4 a
	100	10.2 a	424.5 ab	2.7 de	116.1 ab	36.8 a-e	68.3 abc
	150	10.0 a	435.1 ab	3.6 bcd	117.2 ab	37.1 a-e	70.5 abc
1.2	50	9.5 a	397.5 b	3.8 bcd	117.6 ab	35.0 cde	65.8 abc
	75	10.0 a	423.2 ab	2.8 cde	122.8 a	34.9 de	63.8 abc
	100	9.2 a	394.2 b	1.9 e	108.2 abc	33.1 e	50.9 c
	150	9.7 a	413.6 ab	3.5 bcd	119.5 ab	36.1 b-e	67.6 abc
Significance							
Size of perlite		ns	**	**	**	**	**
Conc. of NS		ns	ns	ns	ns	ns	ns
Interaction		ns	ns	**	ns	*	ns

[a] Concentration of half strength □ Enshi□ nutrient solution.

[b] Parameters were measured on per plant basis; Fresh weight (FW), Dry weight (DW).

[c] Means within column followed by different letters are significant according to the Tukey's test at $P < 0.05$.

[ns, *, **] Non-significant or significant at $P < 0.05$, 0.01, respectively.

Table 6

Interaction effects of particle sizes of perlite and concentration of Enshi nutrient solution on the root quality parameters of 'Dr. Carotene 5' grown in soilless hydroponics during autumn 2010.

Size of perlite (mm)	Conc. of NS[a] (%)	Soluble solids (%)	α-Carotene (mg/L)	β-Carotene (mg/L)	Lycopene (mg/L)	Ascorbic acid (mg/100 ml)
0.3	50	7.8 ab[b]	0.29 a	0.46 bcd	0.29 bc	9.5 b
	75	8.2 a	0.28 a	0.46 bcd	0.18 c	11.6 ab
	100	7.9 ab	0.30 a	0.43 cd	0.22 bc	11.0 ab
	150	7.7 ab	0.30 a	0.45 bcd	0.34 abc	12.0 ab
0.6	50	6.0 de	0.26 a	0.43 cd	0.23 bc	16.9 ab
	75	5.5 e	0.29 a	0.45 bcd	0.35 abc	5.6 b
	100	6.4 de	0.28 a	0.49 a-d	0.48 abc	16.9 ab
	150	6.3 de	0.32 a	0.48 a-d	0.37 abc	6.8 b
1.2	50	6.2 de	0.23 a	0.30 d	0.20 c	15.3 ab
	75	6.9 bcd	0.31 a	0.67 a	0.95 ab	8.2 b
	100	7.6 abc	0.25 a	0.70 a	1.03 a	10.5 ab
	150	6.6 cde	0.31 a	0.60 abc	0.79 abc	22.3 a
Significance						
Size of perlite		**	ns	**	**	ns
Conc. of NS		**	ns	**	*	*
Interaction		**	ns	**	ns	**

[a] Concentration of half strength Enshi nutrient solution.

[b] Means within column followed by different letters are significant at $P < 0.05$ according to the Tukey's test (n = 21).

[ns, *, **] Non-significant or significant at $P < 0.05$, 0.01, respectively.

Figure captions:

Fig. 1. Hydroponic culture system of carrot using perlite substrates in 100 m^2 greenhouse of Shimane University, Matsue, Japan.

Fig. 2. Epidermal cells carrot roots observed under Olympus fluorescent microscope (× 100) showed carotene pigmentation responsible for orange color of carrots (Left figure represents root cells of carrot grown in 0.6 mm perlite supplied with 50% nutrient solution, and the right figure is the root cells of carrot grown in 0.3 mm perlite with 50% nutrient solution).

Fig. 3. Carrot roots grown in 0.6 mm of recycled perlite with 50, 75, 100, and 150% (from left to right) of □ Enshi□ nutrient solution. Results indicated that 50 and 75% of nutrient solution produced longer and wider carrot root leading to maximum yield.

Fig. 1. Hydroponic culture system of carrot using perlite substrates in 100 m^2 greenhouse of Shimane University, Matsue, Japan.

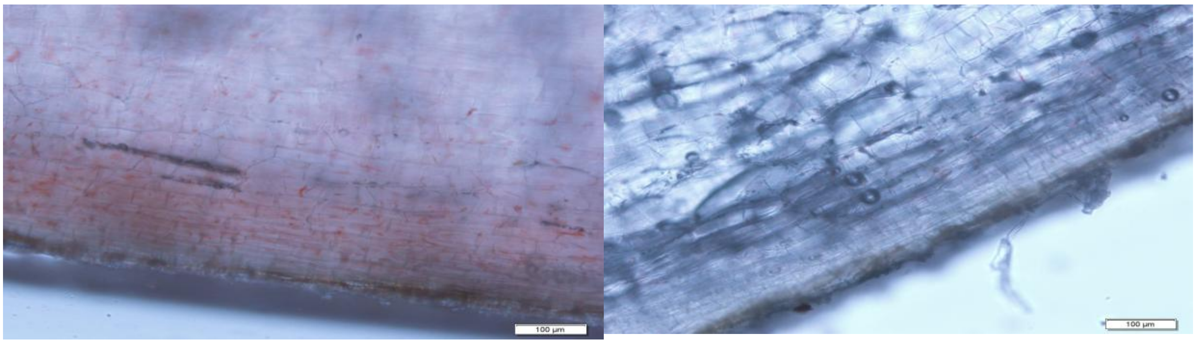

Fig. 2. Epidermal cells of carrot roots observed under Olympus fluorescent microscope (× 100) showing carotene pigmentation responsible for orange color of carrots (Left figure represents root cells of carrot grown in 0.6 mm perlite supplied with 50% nutrient solution, and the right figure is the root cells of carrot grown in 0.3 mm perlite with 50% nutrient solution).

Fig. 3. Carrot roots grown in 0.6 mm of recycled perlite with 50, 75, 100, and 150% (from left to right) of □ Enshi□ nutrient solution. Results showed that 50 and 75% of nutrient solution produced longer and wider carrot root leading to maximum yield.