

Effect of different nutrient levels on anthocyanin and nitrate-N contents in turnip grown in hydroponics

T. Asao, H. Kitazawa, K. Washizu, T. Ban and M.H.R. Pramanik

Faculty of Life and Environmental Science, Shimane University, 2059 Kamihonjo, Matsue, Shimane, 690-1102, Japan. E-mail: asao@life.shimane-u.ac.jp

Abstract

Effect of different concentrations of nutrient solutions on the growth of a Japanese turnip (*Brassica rapa* L. rapifera group 'Tsudakabu') was evaluated through hydroponics culture. All nutrients levels were maintained at 25, 50 and 75 % of a full strength nutrient solution with their full or half (50 %) $\text{NO}_3\text{-N}$ content in each level. The nutrient solution at 50 % concentration with 50 % $\text{NO}_3\text{-N}$ was effective enough to grow quality turnip. Anthocyanin content in roots significantly increased with the decreasing concentration of nutrient solution irrespective of $\text{NO}_3\text{-N}$ level in them. $\text{NO}_3\text{-N}$ level in the nutrient solutions had no marked effect on $\text{NO}_3\text{-N}$ content in turnip. Fifty percent nutrient solution with its half level (50 %) of $\text{NO}_3\text{-N}$ (4 m mol l^{-1}) appeared to be optimum for production of quality turnip in hydroponics.

Key words: Anthocyanin, hydroponics, $\text{NO}_3\text{-N}$, turnip

Introduction

'Tsuda-kabu' (*Brassica rapa* L. rapifera group) is a local turnip cultivar popularly grown at Shimane prefecture in Japan. The cultivar is red in color with comma-shaped root. The crop is normally sown in September and harvested in December. Harvesting of turnip in early December is profitable mainly for sending it as a year-end gift in Japan. However, in September sowing of turnip in field condition is often delayed for incessant rainfall. Besides, turnip in soil culture needs extra labor for harvesting and washing. In hydroponics, turnip cultivation is less influenced by environmental change, and harvesting and processing of turnip is also convenient. Root vegetables are often discouraged to grow by hydroponics possibly for poor root development. However, Grant *et al.* (1993) had grown sweet potato in a greenhouse and found that fresh and dry weights of storage roots and foliage were maximum in a modified half-strength Hoagland solution (Hoagland and Arnon, 1950) both in NFT (nutrient film technology) and also split-root in deep-water culture (Sherif *et al.*, 1995). Removal of foliage significantly reduced the storage-root yield and shoot biomass of sweet potato in hydroponic culture (David *et al.*, 1995). Minitubers of potato were sufficiently produced using NFT (Rolot and Seutin, 1999) and the aeroponic cultivation system (Ritter *et al.*, 2001). However, report on cultivation of turnip by hydroponics is quite meager. In soil cultivation, the most part of the 'Tsuda-kabu' root is commonly found above ground and only a few centimeter of the root is inserted into the soil. So the cultivation of 'Tsuda-kabu' might be profitable by hydroponics.

The shoots and roots of turnip are often pickled / used in processing foods. Anthocyanin, responsible for red pigment, is an added quality of turnip to serve delicious foods. Thus, large amount of anthocyanin in roots is connected with quality of turnip. In grapes, it is well known that cultural practices such as fertilizers, degree of crop load, and degree of pruning as well as

temperature greatly affect the pigmentation (Dokoozlian *et al.*, 1995). Faust (1965) found that excess nitrogen fertilizer decreased anthocyanin accumulation in the pericarp of grapes. *In vitro* accumulation of anthocyanin in the leaves of grape was decreased by the substrate including $\text{NO}_3\text{-N}$ (Pirie and Mullins, 1976). Plants metabolize $\text{NO}_3\text{-N}$ for production of amino acid and protein when huge amount of sugars is consumed. Anthocyanin is a secondary metabolite of flavonoid group. Sugar in one of the constituents and thus seem to be necessary for the formation of anthocyanin. A competition for sugars between anthocyanin and protein productions in plants may exist (Faust, 1965).

Optimum nutrient level in nutrient solution may be a precondition for harvesting quality product and maximum yield through hydroponic culture. In this study, effects of different concentrations of nutrient solution on the growth of turnip in hydroponics were investigated.

Materials and methods

Plant cultivation: Seeds of a Japanese turnip Tsuda-kabu were sown in vermiculite moistened with tap water in a plastic cell-tray on 11 September 2002. On 19 September, eighteen seedlings at the first leaf-stage were transplanted to each plastic container ($63 \times 48 \times 23 \text{ cm}$) placed in a greenhouse at Shimane University, Japan (Fig. 1). The container was filled with 50 l of continuously aerated (3.8 l min^{-1}) Enshi nutrient solution (Hori, 1966). Full strength nutrient solution contain the following amounts of salts per 1000 l tap water: 950 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 810 g KNO_3 ; 500 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 155 g $\text{NH}_4\text{H}_2\text{PO}_4$; 3 g H_3BO_3 ; 0.02g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 2g $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$; 0.05g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; and 0.02g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

The test nutrient solution were treated at three levels (25, 50, 75 % of full strength solution) adding their full or half-strength (50 %) $\text{NO}_3\text{-N}$ at each level. The desired $\text{NO}_3\text{-N}$ and K^+ levels were adjusted with KCl as well as with KNO_3 . The nutrient solutions were renewed at two-week intervals until harvesting. After

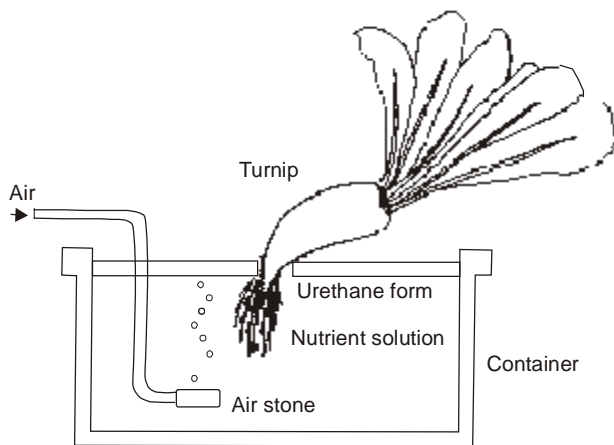


Fig. 1. Hydroponic system used for turnip cultivation

Table 1. Enshi nutrient solutions^a

Chemicals	Amounts ^b (m mol liter ⁻¹)
Ca(NO ₃) ₂ ·4H ₂ O	4.03
KNO ₃	8.02
MgSO ₄ ·7H ₂ O	2.03
NH ₄ H ₂ PO ₄	1.35
H ₃ BO ₃	0.05
ZnSO ₄ ·7H ₂ O	7.64×10 ⁻⁴
MnSO ₄ ·5H ₂ O	8.30×10 ⁻³
CuSO ₄ ·5H ₂ O	2.00×10 ⁻⁴
Na ₂ MoO ₄ ·2H ₂ O	9.71×10 ⁻⁵
NaFe-EDTA	0.06

^a Full-strength; ^b Amounts of salts per liter of tap water. (Hori, 1966)

thinning out the seedlings at three times (Oct. 10, Oct. 25 and Nov. 8), six seedlings were placed in each container and three containers were used for each treatment. Electrical conductivity (EC) and pH of the nutrient solutions were checked regularly. EC ranged from 0.5 to 0.8 (for 25 %), 1.0 to 1.3 (for 50 %) and 1.6 to 1.8 dS m⁻¹ (for 75 %) and pH ranged from 7.5 to 8.0, 7.0 to 7.8 and 6.4 to 7.4, respectively. The EC and pH of the 25, 50 and 75 % nutrient solutions with their full- and half-strength NO₃-N content were almost similar. The mean air and water temperatures during the experiment ranged from 7.4 to 27.1 and from 9.4 to 27.4 °C, respectively. The crop was harvested on 21 November 2002. After harvesting the crop, data were recorded on the leaf number per plant, maximum leaf length and width, leaf color (MINOLTA SPAD-502), root diameter, fresh and dry weights of leaves and roots.

Table 2. Effects of concentrations of nutrient solutions on the growth of turnip cv. Tsuda-kabu

Nutrient solution	Treatment NO ₃ -N (m mol liter ⁻¹)	Number of leaves	Maximum leaf length (cm)	Maximum leaf width (cm)	Leaf color (SPAD)	Maximum root diameter (cm)	Fresh weight (g)		Dry weight (g)	
							Leaf	Root	Leaf	Root
0.75u ^x	12	14.7a	52.7a	16.7a	32.1ab	17.4	201.6a	143.3	14.4a	7.6
	6	14.0ab	52.0a	15.0abc	34.0a	17.6	189.6ab	137.2	13.7ab	7.3
0.50u	8	15.0a	53.0a	16.1ab	31.0ab	18.3	207.9a	161.6	14.7a	7.5
	4	14.7a	51.0a	14.5bc	31.6ab	17.9	183.6ab	149.9	13.2ab	8.1
0.25u	4	13.8ab	47.0ab	13.7cd	28.8b	17.7	153.7b	155.2	10.7b	6.9
	2	11.7b	42.8b	12.4d	29.8ab	16.9	96.9c	129.0	7.2c	7.0
						NS		NS		NS

^x The concentration of nutrient solution; one unit (1.0u) is standard concentration.

Different letters within a column indicate significance at 5% level by the Tukey's test.

Determination of anthocyanin concentration: Harvested roots (upper parts) were bored with a cork borer to have three skin discs (10 mm in diameter). Anthocyanin pigments were extracted from those discs with 1% hydrochloric acid-methanol (20 ml) for 24 hours at room temperature (Hiratsuka *et al.*, 2001). The extracts were collected for each treatment and their absorbance was measured at 530 nm by spectrophotometer (UV-1240mini, Shimadzu Co.). The absorbance was treated as anthocyanin level in root. Each measurement was replicated three times.

Determination of nitrate nitrogen content: The dried leaves and roots were ground by an automatic grinder. The powdered roots or leaves (25 mg) were extracted with distilled water (25 ml) for one hour at 30°C by gentle shaking in an electric shaker. The extracts were filtered and the filtrates were analyzed by Cataldo method (Cataldo *et al.*, 1975). Five ml of 5 % salicylic acid-H₂SO₄ was added to the mixture (50 ml) and kept at room temperature for twenty minutes. Then the mixture was shaken with 2 M NaOH (5 ml) and cooled down at room temperature. Absorbance of the extracts were measured at 410 nm by a spectrophotometer (UV-1240mini, Shimadzu Co.). The data were expressed as g kg⁻¹ DW using KNO₃ as a standard.

Results and discussion

Plant cultivation: Turnip plants were grown hydroponically using different concentrations of nutrient solution. The crop was harvested about two weeks earlier than in soil culture. The size and color of roots from hydroponic culture were similar to that of turnip grown in soil culture, and the size of leaves in the former was larger than that in latter (data not shown).

Leaf number, leaf length and width, fresh and dry weights of leaves all decreased significantly at the lowest concentration of nutrient solution (25 %) compared to those at high concentrations (75 or 50 %) (Table 2). Presence of full and half-strength NO₃-N in 75 and 50 % nutrient solution did not show significant difference in growth. The fresh and dry weights of root were significantly decreased at the lowest concentration (half-strength NO₃-N at 25%). Leaf color and root diameter did not vary for changes in concentration of nutrient solutions. The growth of turnip was similar for the nutrient solution at 50 % concentration or more, even with half-strength NO₃-N. The results revealed that the Enshi nutrient solution at 50 % concentration with 4 m mol l⁻¹ NO₃-N was effective enough to grow quality turnip in hydroponics.

Anthocyanin concentration and nitrate nitrogen content:

Anthocyanin content in the turnip roots increased with decreasing concentration of nutrient solution (Table 3). Responses of turnip to half-strength $\text{NO}_3\text{-N}$ at all concentrations were similar to that of full-strength $\text{NO}_3\text{-N}$ level. $\text{NO}_3\text{-N}$ contents in the shoots and roots of turnip were decreased with the decreasing $\text{NO}_3\text{-N}$ level in the nutrient solution. $\text{NO}_3\text{-N}$ contents in shoot and root at 25 % using half-strength $\text{NO}_3\text{-N}$ was significantly decreased. The $\text{NO}_3\text{-N}$ content of root was lower than that of shoot.

Table 3. Effects of concentration of nutrient solution on anthocyanin in root and nitrate nitrogen in shoot and root of turnip cv. Tsuda-kabu

Nutrient solution	Treatment $\text{NO}_3\text{-N}$ (m mol liter ⁻¹)	Anthocyanin in root ^x	Nitrate nitrogen(g kg ⁻¹ DW)	
			Shoot	Root
0.75u ^y	12	0.46c	15.6a	3.8a
	6	0.49c	17.2a	4.1a
0.50u	8	0.53b	15.6a	2.5b
	4	0.55b	13.0b	2.8b
0.25u	4	0.66a	14.2b	2.4b
	2	0.74a	4.8c	1.5c

^x The absorbances of the extracts at 530 nm.

^y The concentration of nutrient solution; one unit (1.0u) is standard concentration.

Different letters within a column indicate significance at 5% level by the Tukey's test.

Pirie and Mullins (1976) reported that anthocyanin accumulation in grape leaves decreased with nutrient contents including $\text{NO}_3\text{-N}$ in an *in vitro* culture. Anthocyanin concentration in root was inversely proportional to the content of nitrate nitrogen in root. However, when $\text{NO}_3\text{-N}$ content at 50 % nutrient solution concentration was changed from full (8 m mol l⁻¹) to half (4 m mol l⁻¹), $\text{NO}_3\text{-N}$ content and anthocyanin contents in the turnip root were not affected. And using the half strength of 50 % nutrient solution and the full strength of 25 % nutrient solution at the same $\text{NO}_3\text{-N}$ concentration, the $\text{NO}_3\text{-N}$ content of root was unchanged and anthocyanin content in root was increased. When the $\text{NO}_3\text{-N}$ content in the nutrient solution was changed from full to half at 25 % concentration, the $\text{NO}_3\text{-N}$ content of root was decreased by about 63 % of that and anthocyanin content in root was unchanged. Thus, there was no strong relation between $\text{NO}_3\text{-N}$ concentration and anthocyanin contents in the roots of turnip. The results revealed that the anthocyanin content in the roots of turnip is strongly influenced by nutrients concentration as a whole rather than the concentration of $\text{NO}_3\text{-N}$ in the nutrient solutions. Even excess nitrogen fertilizers decreased the accumulation of anthocyanin in the pericarp of grapes (Faust, 1965). Thus, it appears that at medium concentration of nutrient solution (50 %), variation of $\text{NO}_3\text{-N}$ contents may not interact

with the formation of proteins and anthocyanin contents in turnip. Horiguchi (1989) reported that in corn (*Zea mays* L.), phosphorus deficiency caused a significant accumulation of anthocyanin in the leaves, while nitrogen deficiency did not. In red lettuce (*Lactuca sativus* L.) and red cabbage (*Brassica oleracea* L.) manganese deficiency caused decreases in anthocyanin contents in their leaves (Horiguchi, 1989). The results indicate that the effects of other nutrients except $\text{NO}_3\text{-N}$ might be associated with the formation of anthocyanin in turnip.

Finally, it may be concluded that medium range (50 %) nutrient solution with 4 m mol l⁻¹ $\text{NO}_3\text{-N}$ may be treated as optimum concentration for production of quality turnip by hydroponic culture.

References

- Cataldo, D.A., M. Haroon, L.E. Schrader and V.L. Youngs, 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Common. Soil Sci. Pl. Anal.*, 6: 71-80.
- David, P.P., A.A. Trotman, D.G. Mortley, C.K. Bonsi, P.A. Loretan and W.A. Hill, 1995. Foliage removal influences sweetpotato biomass yields in hydroponic culture. *HortScience*, 30: 1000-1002.
- Dokoozlian, N., D. Luvisi, M. Moriyama and P. Schrader, 1995. Culture practices improve color, size of Crimson Seedless. *California Agric.*, 49: 36-40.
- Faust, M. 1965. Physiology of anthocyanin development in McIntosh apple - Relationship between protein synthesis and anthocyanin development. *J. Amer. Sor. Hort. Sci.*, 87: 10-19.
- Grant, P.J., J.Y. Lu, D.G. Mortley, P.A. Loretan, C.K. Bonsi and W.A. Hill, 1993. Nutrient composition of sweetpotato storage roots altered by frequency of nutrient solution change. *HortScience*, 28: 802-804.
- Hiratsuka, S., H. Onodera, Y. Kawai, T. Kubo, H. Itoh and R. Wada, 2001. Enzyme activity changes during anthocyanin synthesis in Olympiagrape berries. *Sci. Hortic.*, 90: 255-264.
- Hoagland, D.R. and D.L. Arnon, 1950. The waterculture method for growing plants without soil. *Calif. Agr. Expt. Sta. Circ.*, 347.
- Hori, H. 1966. *Gravel Culture of Vegetables and Ornamentals. 3. Nutrient Solution*, Yokendo. Tokyo, Japan, pp. 60-79.
- Horiguchi, T. 1989. Effects of nitrogen, phosphorus, and manganese deficiencies on the formation of anthocyanin and other phenolic compounds in plants. *Jpn. J. Soil Sci. Plant Nutr.*, 60: 226-232.
- Pirie, A. and M.G. Mullins, 1976. Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate, and abscisic acid. *Plant Physiol.*, 58: 468-472.
- Ritter, E., B. Angulo, P. Riga, C. Herran, J. Reloso and M. Sanjose, 2001. Comparison of hydroponic and aeroponic cultivation systems for the production of potato minitubers. *Potato Res.*, 44: 127-135.
- Rolot, J. L. and H. Seutin, 1999. Soilless production of potato minitubers using a hydroponic technique. *Potato Res.*, 42: 457-469.
- Sherif, M.A., P.A. Loretan, A.A. Trotman, D.G. Mortley, J.Y. Lu and L.C. Garner, 1995. Split-root nutrition of sweetpotato in hydroponic systems. *Acta Hortic.*, 401: 121-130.