

Changes of Ascorbic Acid and Nitrate Content in Lettuce by Unbalanced Nutrient Solution

Yang Ho Park[†], So Hyeon Park, Jae Hong Park, Ju Young Lee, Byoung Choon Jang, and Ki Sang Lee

National Institute of Agricultural Science and Technology, Seodun-Dong 249, Gwonseon-Gu, Suwon-Si 441-707, Korea

ABSTRACT: This study was to verify that the uptake inhibition and accumulation of nitrogen in different potassium levels. Lettuce was used as model plant in this study and grown in pot of 10cm's in diameter and depth with mixture media of vermiculite and perlite under supply of different culture solution for three weeks. Nitrogen absorption at root was inhibited by increased potassium concentration in nutrient solution, and nitrate accumulation of plant was depended on absorption of nitrogen because nitrate content of 0 K level was 4-5 times higher than that of 2 K level. Concentration of ascorbic acid was decreased by increasing the nitrogen absorption, since ascorbic acid (AsA) content of 2K level was higher than those of 0K level in both of old leaf and flesh leaf.

Keywords: n uptake inhibition, nitrate accumulation, ascorbic acid content, potassium levels

Much amount of the ascorbic acid synthesized at the cytoplasm and mitochondria (Wheeler *et al.*, 1998), and it exists at the cytoplasm and chloroplast. The concentrations of ascorbic acid are 2-4 mM (Foyer *et al.* 1983) in leaf cells usually, but much higher at the cytoplasm (5-10 mM) and chloroplast (25-50 mM) Horemans *et al.*, 2000). There are three forms of ascorbic acid in the plant mainly L-ascorbic acid (AsA, reduced form), monodehydroascorbic acid (MDHA, partly-oxidized form) and dehydroascorbic acid (DHA, oxidized form). AsA in plant cell rolls antioxidants by changing those three forms Foyer & Halliwell, 1976; Noctor & Foyer, 1998.

Increased application of nitrogen fertilizers has been shown to decrease the content of ascorbic acid in many plants. There are also reports indicating that nitrogen fertilizer may have no effect or may even increase the content of ascorbic acid in some plants (Table 1). Part of the controversy may be due to 1) amount of nitrogen fertilizer used relative to that considered optimum for plants under a given set of experimental conditions, and 2) the fact that the response of ascorbic acid to increase fertilizer rates may to through a maxima and then decrease when the application

rate is increased beyond a given value (Mozafar, 1993).

Purpose of this study is to confirm the changes of ascorbic acid and nitrate content in lettuce by unbalanced nutrient solution.

MATERIALS AND METHODS

Plant

Lettuce seeds (*Lactuca sativa* L.) were purchased from Danong Co. and germinated in bed soil which was Baroker. Seoul Agricultural materials Co. Ltd at green house. Lettuce was transferred after 20 days and was grown in pots (10cm (D) × 10cm(H)) which were filled with vermiculites and perlite (1 : 1). Nutrient solution was given 300 mL every 2 to 3 days. The general nutrient solution contained macro-nutrients as mM, Ca(NO₃)₂ · 4H₂O, 1.5; NH₄H₂PO₄, 0.5; NH₄Cl, 1.5; KCl, 4; MgSO₄ · 7H₂O, 0.5; and micro-nutrients as μM, H₃BO₃, 20.6; CuSO₄ · 5H₂O, 0.16; MnSO₄ · 2H₂O, 4.5; ZnSO₄ · 7H₂O, 0.34; Fe-chelate, 0.34 (Yamazaki, 1978) and shifted to unbalanced nutrient solution after 10days. Unbalanced nutrient solutions were 6N-0K-1P, 6N-0.5K-1P and 6N-2K-1P. 6N-0K-1P contained macro-nutrients as mM, Ca(NO₃)₂ · 4H₂O, 11; NH₄H₂PO₄, 0.5; NH₄Cl, 10.5; KCl, 0; MgSO₄ · 7H₂O, 0.5; and micro-nutrient was same as above. 6N-2K-1P contained macro-nutrients as mM, Ca(NO₃)₂ · 4H₂O, 11; NH₄H₂PO₄, 0.5; NH₄Cl, 10.5; KCl, 2; MgSO₄ · 7H₂O, 0.5; and micro-nutrients was same as above. 6N-0.5K-1P contained macro-nutrients as mM, Ca(NO₃)₂ · 4H₂O, 11; NH₄H₂PO₄, 0.5; NH₄Cl, 10.5; KCl, 8; MgSO₄ · 7H₂O, 0.5; and micro-nutrients was same as above. Plants were measured growth rates which were leaf length, leaf width and number of leaf every one week. Plant samples were analyzed by divided to new leaf(1-4th leaf from top), old leaf (5-8th from top), and root. And used only leaf blade.

Measurement of nitrate content

Plant samples were homogenized with liquid nitrogen. And they were preserved until to analyze at -80 °C. Homogenized samples(0.5 g) were weighted and extracted in 5 mL of deionized H₂O for 1h at shaker(140 rpm). Then they were

[†]Corresponding author: (Phone) +82-31-290-0320 (E-mail) pl23pyh@rda.go.kr

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Table 1. Effect of nitrogen fertilization on the concentration of ascorbic acid in plants (Mozafar A. 1993).

Effect	Plant
Increase	Apple, Cabbage, Cauliflower, Cherry, Chili Cucumber, Eggplant, Guava, Horse-radish, Lettuce, Mango, Oat(leaf), Peach, Pineapple, Potato. Spinach, Sweet potato. Tomato. Kohlrabi(leaf)
No effect	Cabbage, Carrot, Cauliflower, Cherry, Currant, Grapefruit, Orange, Pineapple, Potato. Radish, Satsuma, Sweet potato. Tomato, Turnip greens
Decrease	Apples, Brussels sprouts, Cabbage, Chinese Cabbage, Cantaloupe, Carrot, Cauliflower, Chard Chili Cucumber, Endive, Kale, Grape fruit, Leek, Lemon, Lime, Mandarin, Onion, Orange, Peach, Pepper, Pomegranate, Potato, Radish, Raspberry, Spinach, Stock beet, Strawberry, Tomato, Turnip greens

filtered with NO.2 filter paper. Nitrate was measured by CFA (Auto Analyzer 3: BRAN+LUEBBE) method (Yang Ho Park *et al.*, 2006).

Measurement of Ascorbic acid content: A hydrazine method for AsA and DHA measurements was employed based on determination of their food content (Ko, 1982). 1 gram of leaf powder (homogenized with liquid nitrogen) was added 10 mL of 5% (w/v) metaposphoric acid, shaken at 140 rpm for 1 hour, and passed through a NO.6 filter paper. To determine DHA and DHA plus AsA (the later was oxidized with 2,6-dichlorophenolindophenol solution) in the 1-mL extract, 2% (w/v) thiourea was added, placed in a water bath at 37 °C for 3 hrs, and moved to ice water. After addition of 2.5 ml of 85% (w/v) H₂SO₄ and 0.5 mL of 2% (w/v) 2,4-dinitrophenylhydrazine (in 9 N H₂SO₄), the extract was placed at room temperature for 30 mins, and the absorption at 520 nm was measured by a HITACHI U-2000 spectrophotometer. The difference of DHA plus AsA and DHA was AsA amount.

Measurement of Mineral content: Determination of inorganic factors was based on Walinga method (Walinga I. *et al.*, 1989). Method of plant digestion was that 0.3 g of leaf powder (homogenized dried plant) was added 3.3mL of 368 mM in 84.7% H₂SO₄ and let ride overnight. Then digested at 100 °C for 1hr and digested at 300 °C for 2-3 hrs until solutions changed to colorless (added 2 mL H₂O₂ every 20 mins) [Solution should be let cooling when added H₂O₂ then re-digested]. Those digested solutions were analyzed nitrogen, phosphorus by colorimetry and analyzed K, Ca, Mg, Na, Fe, Cu, Mn, Zn by ICP (Integra XL Dual. GBC Scientific Equipment).

RESULTS

Growth rates

Growth rates were measured at 3 weeks after shifting from normal nutrient solution to unbalanced nutrient solution. There was not difference between K levels at growth rates (leaf number, leaf length, and leaf width)(Fig. 1). Plant in cultured under 0K level was highly wilted compared with 2 K level.

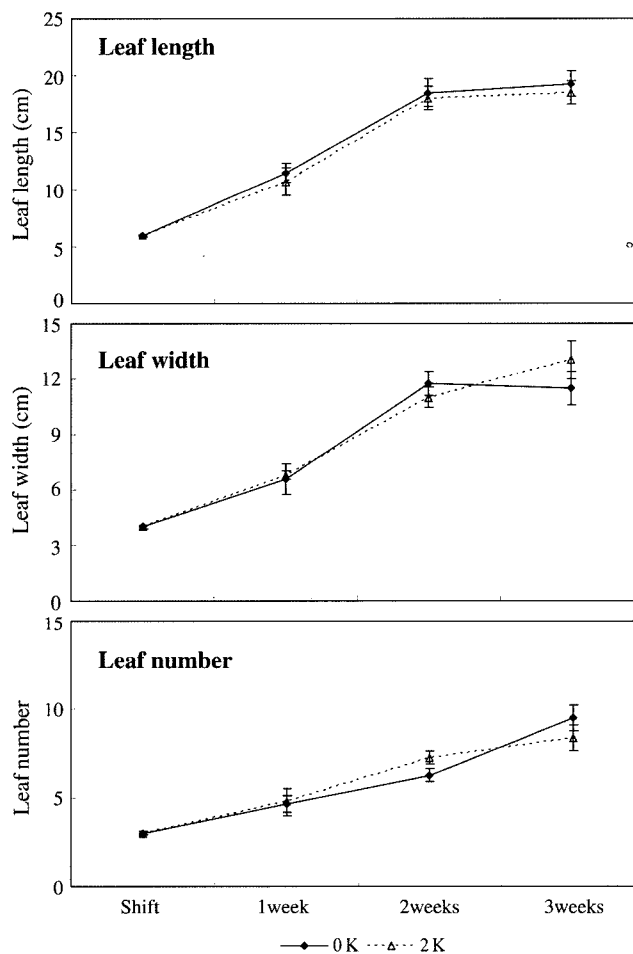


Fig. 1. Difference of leaf length, leaf width and leaf number by different K levels.

Nitrate content

Nitrate content of 0K level was higher than that of 2 K level at new leaf, old leaf and root (Fig. 2). Nitrate content of 0K level was 5 times higher than that of 2 K level in new leaf, and nitrate content of old leaf was higher than that of new leaf both of 0K and 2 K level. Nitrate content of root was higher than that of old leaf and new leaf when 2 K level.

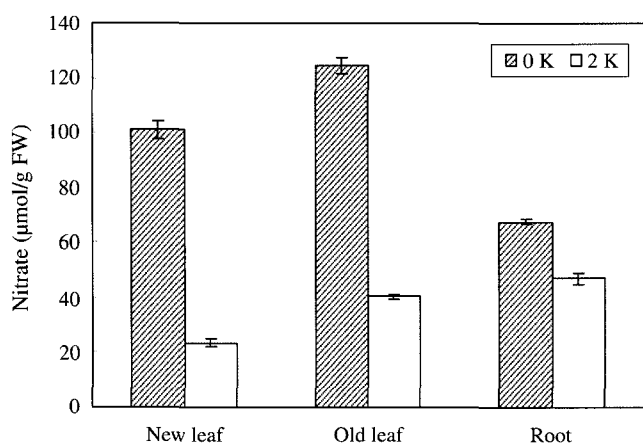


Fig. 2. Difference of nitrate content by different K levels in lettuce.

Ascorbic acid content

Ascorbic acid, total ascorbic acid, AsA(reduced form) and DHA(oxidized form), content of 0K level was lower than that of 2 K level at in old and new leaf. Ascorbic acid of new leaf was higher than that of old leaf both of 0K and 2 K levels. Ascorbic acid form was almost reduced form (AsA) except root under 0 K level.

Nitrogen, phosphorus, and potassium contents

Nitrogen content of 0K level was a little higher than that of 2K level in new leaf, old leaf and root. There was no difference of phosphorus content between 0 K level and 2 K level in new leaf, old leaf and root. Potassium content of 2 K level was higher than that of 0K level. It could be observed in root, old leaf and new leaf (Table 2). K/N ratio of 2 K level was 2-3 times higher than that of 0K level in new leaf, old leaf and root (Fig. 4).

Mineral content

There were no difference between 0K and 2 K level at Mg, Na, Fe, Cu, Mn, and Zn content (Table 4). But Ca content of 0 K level was higher than that of 2 K level (Fig. 5).

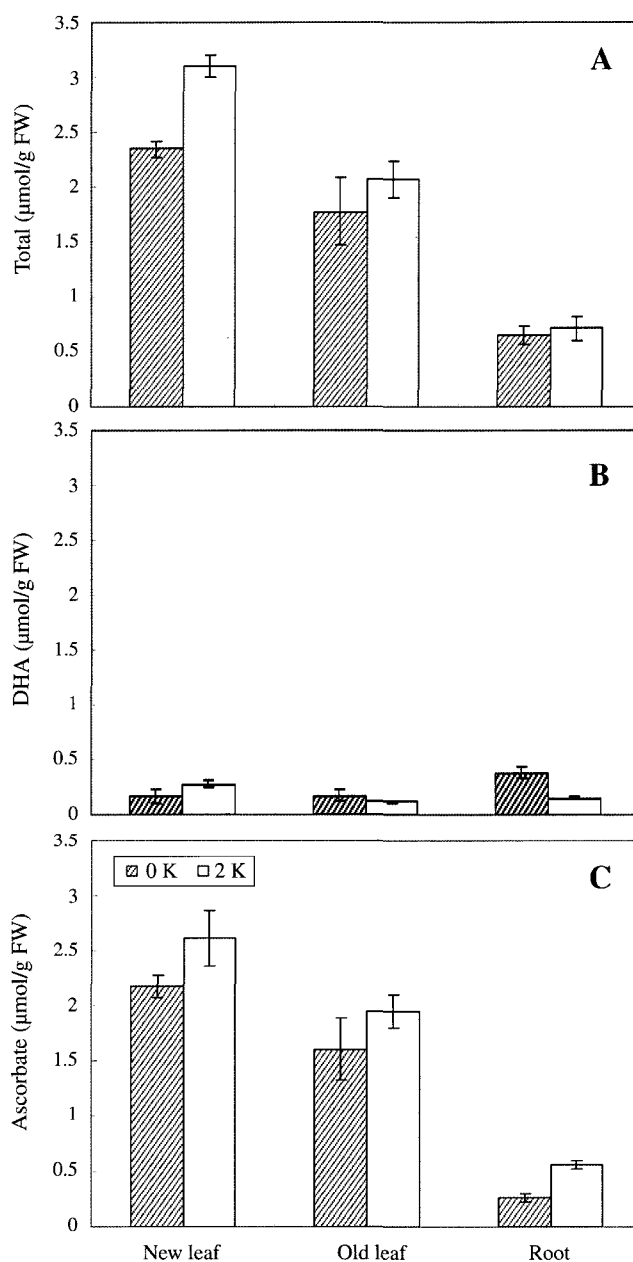


Fig. 3. Difference of ascorbic acid content by different K levels in lettuce. Total ascorbate is A, dehydroascorbate (oxidized form) is B, and ascorbate(reduced form) is C.

Table 2. Difference of nitrogen, phosphorus and potassium contents by different K levels in lettuce.

		Nitrogen	Phosphorus	Potassium
			(%)	
New leaf	0K	5.21±0.02	0.81±0.02	2.44±0.01
	2K	5.00±0.09	0.81±0.01	3.88±0.01
Old leaf	0K	4.35±0.02	0.54±0.01	2.38±0.03
	2K	3.99±0.03	0.55±0.02	4.59±0.05
Root	0K	3.56±0.03	0.76±0.01	1.10±0.01
	2K	3.02±0.08	0.68±0.03	3.49±0.02

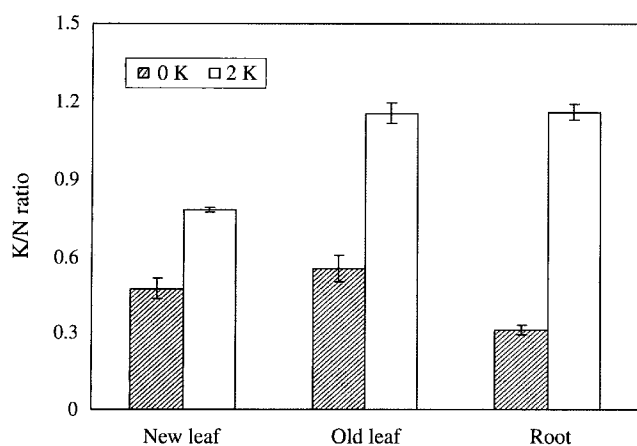


Fig. 4. Difference of K/N ratio between 0K level and 2K level in lettuce.

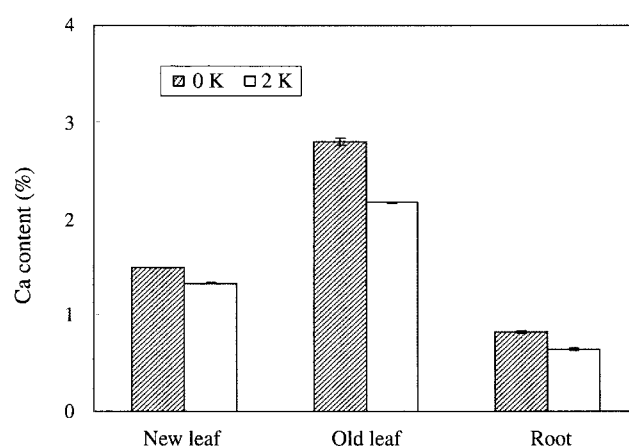


Fig. 5. Difference of Ca content between 0K level and 2K level in lettuce.

Table 3. Changes of mineral element contents by different K levels in lettuce.

		Mg	Na	Fe	Cu	Mn	Zn
		(%)					
New leaf	0K	0.27±0.00	0.26±0.00	234±11	5±0.3	110±0.9	87±34
	2K	0.23±0.00	0.23±0.01	215±2	3±0.5	91±1.1	49±5
Old leaf	0K	0.50±0.01	1.17±0.01	711±168	15±0.5	221±2.2	45±18
	2K	0.34±0.00	0.81±0.02	407±35	4±0.5	157±1.4	72±28
Root	0K	0.15±0.00	2.07±0.03	483±14	6±0.4	24±0.8	19±4
	2K	0.09±0.00	1.41±0.01	344±11	1±0.7	34±0.3	47±31

DISCUSSION

All things being equal, nitrate concentrations in plant can be expected to bear some relationship to the availability of nitrate in the root to the amount of fertilizer-N applied. There was report about straightforward relationship between the nitrate concentration of head lettuce leaves the amounts of fertilizer-N applied within a given planting data and nitrogen. This report said nitrate content increased by increasing Nitrogen (Maynard D. N *et al.*, 1976). Also we could get same result (Fig. 2) that nitrate concentration increased by increasing nitrogen which was absorbed from nutrient solution. We treated same level of nitrogen but absorbed nitrogen was different (Table 2). This result can explain that Cushnahan *et al.* (1995) reported that under low K situations increased in N uptake as a result of Na application were due to the K-sparing effect of Na, in that Na⁺ had replaced K⁺ in the vacuoles of root cells thereby freeing the latter ion(K⁺) to fulfil its vital role as the co-transporter cation for nitrate translocation to shoot tissue. They considered that in the absence of Na⁺ insufficient K⁺ would have been made available to drive the nitrate transport mechanism, and hence nitrate would either have accumulated roots hindering

further N uptake by feed back regulation.

Some report showed that Nitrogen was not relationship with ascorbic acid or due to increasing ascorbic acid but nowadays believe that heavy use of nitrogen fertilizers on plants along with decreasing their ascorbic acid content may also increase the concentration of nitrate in their edible parts (Lorenz & Weir, 1974). So decreasing ascorbic acid content in 0 K level can explain above reason.

This report can demonstrate that absorption of nitrogen is to prevent by potassium, that nitrate accumulation in plant is increased by add nitrogen and that ascorbic acid content in plant can decrease by increasing nitrogen.

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