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BRIEF COMMUNICATION

Content of oxalate in *Actinidia deliciosa* plants grown in nutrient solutions with different nitrogen forms

C. RINALLO* and G. MODI**

Dipartimento di Biologia Vegetale, Università degli Studi di Firenze,
*P. le delle Cascine 28, I-50144 Firenze, Italy**
*Dipartimento Provinciale ARPAT, via Ponte alle Mosse 22, I-50144 Firenze, Italy***

Abstract

Kiwifruit plants (*Actinidia deliciosa* cv. Hayward) were grown in Hoagland nutrient solution with calcium nitrate, potassium nitrate, ammonium nitrate or ammonium chloride as the nitrogen source. Plants grown in the solution with nitrate nitrogen displayed a higher oxalate content, greater shoot length and leaf area, and higher content of ascorbic acid and NO_3^- ions in the leaves. Plants grown in the solution with ammonium nitrate, and particularly with ammonium chloride, showed low oxalate content, low content of ascorbic acid and NO_3^-, high content of Cl^- and Na^+, low shoot length and leaf area. Oxalate formation appeared to be connected with the assimilation of nitrate, more precisely with nitrate reduction, while ammonium nitrogen assimilation did not induce the synthesis of oxalic acid.

Additional key words: ammonium, ascorbic acid, nitrate, oxalate, plant nutrition.

Oxalates occur in many plants as free oxalic acid, soluble salts of potassium and sodium, or insoluble salts of calcium and magnesium. They are usually confined to the vacuoles of idioblast cells in angiosperms (Foster 1956, Franceschi and Horner 1980). In *Actinidia deliciosa* cv. Hayward, whose fruits are a good source of ascorbic acid, idioblasts containing oxalate are present in the roots, stems, leaves and fruits (Ferguson 1984, Lemon and Considine 1993, Rinallo 1997). Kiwifruit raphide crystals of oxalate are considered antinutrients as well as toxins (Vityakon and Standal 1989).

Oxalic acid formation in the higher plants is still unclear. Bush (1995) and Webb *et al.* (1995) state that calcium is essential for biological growth and development, but at high concentrations is toxic to cells, and that oxalate formation may help to reduce its toxicity. Raven and Smith (1976) reported that the synthesis of oxalic acid occurs during nitrate and sulphate assimilation. Sugiyama and Okutani (1996) found on spinach leaves that nitrate reduction was not a prerequisite for oxalate synthesis. Osmond (1967) reported that calcium absorption and oxalate synthesis varied independently. Sutherland and Sprent (1984) found oxalate crystal accumulation in some plants even when grown without nitrate. Saito (1996) showed that ascorbic acid is a major precursor of oxalate in plants.

There is a lack of studies on the effect of different nitrogen forms on oxalate formation of *Actinidia deliciosa*. The purpose of the present work was to determine oxalate content in leaves of kiwi plants growing in a standard Hoagland solution or in the same solution but with the nitrogen in form of calcium nitrate, potassium nitrate, ammonium nitrate or ammonium chloride. In addition accompanied variations in contents of ions and ascorbic acid, and in leaf area and shoot length were followed.

One hundred 1-year-old female *Actinidia deliciosa* cv. Hayward plants grown from rooted cuttings were placed in a greenhouse and transplanted to twenty containers each containing 20 dm^3 pure fine quartz sand. Before transplanting, the root systems were washed carefully with deionised water to remove any soil; each plant was then pruned to two buds, one of which was cut by hand at the onset of sprouting. The twenty containers, each with five plants, were divided into five groups of four containers, and each group was divided into four

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*Address for correspondence; fax: (+39) 055 360137, e-mail: rinallo@unif.it

replications. Plants received half-strength nutrient solution. Hoagland solution (HS), which contains 4 mM calcium nitrate and 6 mM potassium nitrate as nitrogen source, served as the control. In the remaining solutions 10 mM calcium nitrate (variant CN), 10 mM potassium nitrate (KN), 10 mM ammonium nitrate (AN), or 10 mM ammonium chloride (AC) were used as the nitrogen source. All solutions were prepared with deionised water and adjusted to a pH of 6.2. Two dm^3 of solution was given once every two days, once daily, or twice daily, depending on the ambient temperature and the growth stage, using immersion pumps that were connected to the tanks and fitted with a timing device to regulate the amount dispensed.

Sixty days after sprouting shoot length of each plant and leaf area, measured on the four basal leaves of each plant (20 leaves per replication) with an *LI-3100* area meter (*Li-Cor,* Lincoln, USA) were examined. Each set of 20 leaves was bisected to give two sample-sets of 20 half-leaves each. Twenty of these half leaves (five solutions per four replications) were used to determine dry mass and total amounts of Ca^{2+}, Na^+ and K^+ using the method reported by Rinallo *et al.* (1993). The other twenty half leaves were weighed and ground to a slurry in 50 cm^3 double-distilled water at room temperature for 5 min. Part of this slurry was centrifuged at 16 800 *g* at 4 °C, and used to determine pH of the leaf cell sap, and contents of ascorbic acid, soluble oxalate and total oxalate (using test kits from *Boehringer Mannheim* GmbH), and NO_3^- and Cl^- (using a *Dionex 2000I* basic chromatography module, Norwalk, USA).

Leaf cell sap pH values did not differ statistically among plants grown in different nutrient solutions. Ascorbic acid contents in plants grown on KN, AN and AC had significant reductions of 22, 43, and 49 %, respectively, compared with plants grown on HS or CN. Content of total oxalate did not differ between HS and CN grown plants, but decreased by 3 % in KN, by 53 % in AN and by 56 % in AC (Table 1). Soluble oxalate content did not differ between HS and CN, but values increased significantly (about 17 %) on KN, AN and AC-grown plants over HS and CN. Plants grown on HS, CN and KN had higher levels of NO_3^- than plants grown on AN and AC. The NO_3^- content was 68 % lower in AN grown plants and 89 % lower in AC-grown plants. The Cl^- content was similar in HS, CN and KN grown plants, but in AN and AC grown plant it was twice and two and half times as high, respectively (Table 1). HS, CN and KN grown plants had higher contents of calcium and potassium than AN and AC grown plants. Sodium content in KN, AN and AC grown plants were 4, 19, and 32 %, respectively, higher than in HS and CN grown plants. Plants grown on HS and CN did not differ in shoot length, but plants grown on KN, AN and AC produced shoots that were 15, 25, and 28 %, respectively, shorter (Table 1). Leaf area was significantly (13 %) lower in plants grown on KN, and more than 20 % lower in plants grown on AN and AC, than in plants grown on HS and CN. Leaf dry mass/fresh mass ratio did not differ among nutrient solutions.

Table 1. Effect of nitrogen form on pH of cell sap from leaves, contents of ascorbic acid, total oxalate and soluble oxalate, NO_3^-, Cl^-, Ca^{2+}, and Na^+ [mg g^{-1}(d.m.)] in leaves, shoot length [cm], leaf area [cm^2], and dry mass [% of fresh mass] of *Actinidia deliciosa* grown in Hoagland solution with varying nitrogen forms: HS - calcium nitrate + potassium nitrate; CN - calcium nitrate; KN - potassium nitrate; AN - ammonium nitrate, and AC - ammonium chloride. Mean are based on four replications. Means within rows followed by different letters differ significantly at $P = 0.01$ by the LSD test.

Source of nitrogen	HS	CN	KN	AN	AC
pH	3.36a	3.34a	3.33a	3.18a	3.09a
ascorbic acid	0.94d	0.90d	0.73c	0.53b	0.48a
total oxalate	2.57d	2.61d	2.50c	1.21b	1.12a
soluble oxalate	0.60a	0.58a	0.68b	0.71b	0.72b
NO_3^-	7.72c	8.38e	7.63c	2.45b	1.02a
Cl^-	5.39a	5.22a	5.32a	11.80b	13.38c
Ca^{2+}	1.58c	2.41d	0.88b	0.68a	0.60a
K^+	3.22c	2.54b	3.99d	1.29a	1.13a
Na^+	3.32a	3.20a	3.45b	3.99c	4.39d
shoot length	24.04c	23.86c	20.34b	18.12a	17.29a
leaf area	64.64c	62.08c	56.06b	50.42a	48.69a
dry mass	14.14a	14.11a	14.09a	13.33a	13.42a

The different nitrogen forms induced significant modifications in the growth and biochemistry of *Actinidia* plantlets. Ammonium (ammonium nitrate or ammonium chloride) as the nitrogen source led to significant reductions in the content of NO_3^-, ascorbic acid, total oxalate, and to a higher content of sodium and chlorine in *A. deliciosa* leaves. In plants grown with these solutions shoots were shorter and leaves were smaller. Lower growth could be due in part to the lower content of ascorbic acid, which has a role in cell division and expansion and in photosynthesis (Foyer 1993) or it could be caused by the sensitivity of kiwifruit to ammonium toxicity, as reported by Lionakis and Schwabe (1985). The nitrate nitrogen form significantly increased shoot length, leaf size, ascorbic acid, oxalate and NO_3^- in the leaves. These findings confirm the statement of Joy (1964) that nitrate increased the oxalate content while ammonium reduced it. *Actinidia deliciosa* synthesises oxalate in response to nitrate reduction in order to maintain both the cation-anion balance and intracellular pH. It is now generally accepted that hydroxyl ions generated during nitrate reduction are neutralised by oxalic acid (Raven and Smith 1976, Davies 1986). The oxalic anions caused the formation of oxalate in order to restore the ionic balance (Raven and Smith 1976). Selvaray *et al.* (1995) also reported high nitrate reductase activity in tissues where there was an accumulation of calcium oxalate crystals. In plants grown with ammonium, the lower oxalate content could be attributed to ammonium assimilation favouring a slight acidification of the cytoplasm (Raven and Smith 1976, Schubert and Yan 1997). The high chlorine and sodium contents in these plants could likewise play a role in restoring the cation-anion balance disrupted by the assimilation of ammonium and by the absence of calcium or potassium in the nutrient solution.

The findings of the present study suggest that ascorbic acid is not a precursor of oxalic acid since the contents of both acids increased or decreased with the increase or decrease of plant growth, and that *Actinidia deliciosa* normally produces oxalic acid whose levels vary with the nitrogen forms: the plants growing with ammonium nitrate or ammonium chloride (without calcium) produced soluble and calcium oxalate reutilising calcium from raphides in the older tissues (Rinallo, unpublished data).

References

Bush, D.S.: Calcium regulation in plant cells and its role in signaling. - Annu. Rev. Plant mol. Biol. **46**: 95-122, 1995.

Davies, D.D.: The fine control of cytosolic pH. - Physiol. Plant. **67**: 702-706, 1986.

Ferguson, A.R.: Kiwifruit: a botanical review. - Hort. Rev. **6**: 1-64, 1984.

Foster, A. S.: Plant idioblast: remarkable examples of cell specialization. - Protoplasma **46**: 184-193, 1956.

Foyer, C.H.: Ascorbic acid. - In: Alscher, R.G., Hess, J.L. (ed.): Antioxidants in Higher Plants. Pp. 31- 58. CRC Press, Boca Raton 1993.

Franceschi, V.R., Horner, H.T., Jr.: Calcium oxalate crystals in plants. - Bot. Rev. **46**: 361- 427, 1980.

Joy, K.W.: Accumulation of oxalate in tissues of sugar beet and the effect of nitrogen supply. - Ann. Bot. **28**: 689-701, 1964.

Lemon, C.W., Considine, J.A.: Anatomy and histochemistry of the root system of the kiwifruit vine *Actinidia deliciosa var. deliciosa*. - Ann. Bot. **71**: 117-129, 1993.

Lionakis, S.M., Schwabe, W.W.: Some effects of mineral nutrient deficiencies on the growth of *Actinidia chinensis* Planch. - J. hort. Sci. **60**: 411- 422, 1985.

Osmond, C.B.: Acid metabolism in *Atriplex*. I. Regulation of oxalate synthesis by apparent excess cation absorption in leaf tissue. - Aust. J. biol. Sci. **20**: 575-587, 1967.

Raven, J.A., Smith, F.A.: Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. - New Phytol. **76**: 415-431, 1976.

Rinallo, C.: Effects of rain acidity on oxalate content of leaves of *Actinidia*. - J. hort. Sci. **72**: 101-108, 1997.

Rinallo, C., Modi, G., Ena, A., Calamassi, R.: Effects of rain acidity on the chemical composition of apple fruit. - J. hort. Sci. **68**: 275-280, 1993.

Saito, K.: Formation of L-ascorbic acid and oxalic acid from D-glucosone in *Lemna minor*. - Phytochemistry **41**: 145-149, 1996.

Selvaray, J.K., Hemalatha, C., Gandhi, J.S.: Differential nitrate reductase activity in nitrate grown cluster bean, *Cyamopsis tetragonoloba* (L.) Taub. and green gram, *Vigna unguiculata* (L.) Wilezeb. Inhibitory effects of ammonia, amino acids and urea. - Indian J. exp. Biol. **33**: 456-458, 1995.

Schubert, S., Yan, F.: Nitrate and ammonium nutrition of plants: effects on acid/base balance and adaptation of root cell plasmalemma H^+ ATPase. - Z. Pflanzenernähr. Bodenk. **160**: 275-281, 1997.

Sugiyama, N., Okutani, I.: Relationship between nitrate reduction and oxalate synthesis in spinach leaves. - J. Plant Physiol. **149**: 14-18, 1996.

Sutherland, J.M., Sprent, J.I.: Calcium oxalate crystals and crystals cells in determinate root nodules of legumes. - Planta **161**: 193-200, 1984.

Vityakon, P., Standal, B.: Oxalate in vegetable amaranth (*Amaranthus gangeticus*). Forms, contents and their possible implication for human health. - J. Sci. Food Agr. **48**: 469- 478, 1989.

Webb, M.A., Cavaletto, J.M., Capita, N.C., Lopez, L.E., Arnott, H.J.: The intravacuolar organic matrix associated with calcium oxalate crystals in leaves of *Vitis*. - Plant J. **7**: 633-648, 1995.