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Effect of Nitrate:Ammonium Nitrogen Ratio on Oxalate Levels of Purslane

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INTRODUCTION

Purslane (*Portulaca oleracea* L., Portulacaceae) is eaten extensively as a potherb, and added in soups and salads around the Mediterranean and tropical Asian countries (Fig. 1). There is a newer interest in cultivation of purslane as a crop since its identification as one of the best sources in the plant world for omega-3 fatty acid, α-linolenic acid (LNA). LNA is a precursor of a specific group of hormones (prostaglandins) and may offer protection against cardiovascular disease, cancers and a number of chronic diseases and conditions throughout the human life. Research indicates that omega-3 fatty acids will eventually receive the attention and broad recommendations now given to fiber (Philipson et al. 1985). Purslane is also reported as an excellent source of the antioxidant vitamins α-tocopherol, ascorbic acid, and β-carotene as well as glutathione, and the amino acids isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine, and valine (Miller et al. 1984). Purslane has been described as a "power food of the future" because of its high nutritive and antioxidant properties (Levey 1993). Purslane has potential as an animal feed (Bosworth et al. 1980), in aquaculture (Simopoulos et al. 1995), and in the food processing industry (Wenzel et al. 1990).

Although purslane is listed as a commercially cultivated vegetable of the world (Kays and Dias 1995), in many parts of the world it is still regarded as a "weed with nutritive potential." Despite its nutritive value in the human diet its acceptance as a green leafy vegetable is limited to a large extent because of reported accumulation of oxalic acid in large amounts, and the harmful health effects associated with oxalic acid in the diet. Studies report that nitrate to ammonium ratios in plant mineral nutrition can have an influence in the oxalate levels in New Zealand spinach (*Tetragonia tetragonioides* Pallas. Kunz.) (Ahmed and Johnson 2000). In a previous study we reported the influence of nitrate to ammonium ratios in hydroponics on the omega-3 fatty acid concentrations in purslane leaves (Palaniswamy et al. 2000). The objective of this study was to study the effect of NO_3^--N: NH_4^+-N ratio on the oxalic acid concentrations and determine the NO_3^--N: NH_4^+-N ratio in hydroponics that would minimize the oxalic acid concentrations in purslane leaves.

METHODOLOGY

Plant Material and Growing Conditions

Twenty-one days old seedlings of purslane (Valley Seed Service, California) were transplanted into a closed hydroponic system in the greenhouse. Nitrogen at 200 µg mL^{-1} was provided as NO_3^- and NH_4^+ forms to yield NO_3^--N: NH_4^+-N ratios of 1:0, 0.75:0.25, 0.5:0.5, and 0.25:0.75. The solutions also contained macronutrients (in µg mL^{-1}) 31 P, 207 K, 200 Ca, 48 Mg, and 64 S and the micronutrients (in µM) 2 Na, 50 Cl, 25 B, 2 Mn, 2 Zn, 0.5 Cu, 0.5 Mo and 50 FeEDTA. The nutrient solutions in the hydroponic systems were aerated for 1 min every 30 min using a time-controlled air bubbler. The solution pH was monitored at 4 d intervals and maintained at 6.6 to 6.8 by adding 0.5 M HCl or NaOH as needed. Treatments were arranged in randomized complete blocks design with five replications. There were six plants in each treatment replication.

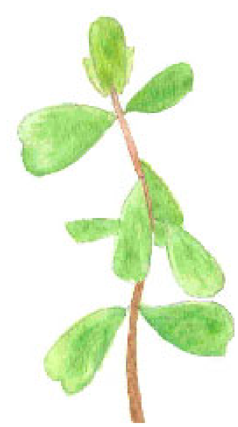

Fig. 1. Purslane (Illus. Archana Sambandan).

Harvest and Data Collection

The plants were harvested at the 8-true leaf stage. Fully expanded young leaves (leaves from the 3rd, 4th, and 5th nodes from the shoot tip) were harvested and the leaves and stems were dried separately at 60°C for determining the oxalic acid concentrations. At harvest the whole plant fresh weight, and leaf area were determined. The leaf area was determined using a planimeter (LI 3100, LI-COR Inc., Lincoln, Nebraska). The shoots were dried at 60°C for 24 hr and the shoot dry weight determined. The data was analyzed using SAS General Linear Models (SAS, Inc. 1996, Cary, North Carolina).

Oxalic Acid Determination

Oxalic acid concentrations of the leaf and stems were determined using the procedure described by Ilarslan et al. (1997). An oxalate kit (Oxalate urinalysis diagnostic kit: procedure No. 591, Sigma, St. Louis, Missouri) was used for the determination of the purslane leaf and stem oxalic acid concentrations.

RESULTS

The oxalic acid concentrations were the highest in the leaves and stems that were grown in nutrient solutions with no ammonium and the lowest in leaves and stems that were grown with 75% ammonium in the nutrient solution (Table 1). There was a strong negative correlation between the ammonium concentrations in the nutrient solution and the oxalic acid concentrations of the leaf ($Y = -1.55x + 12$; $R^2 = 0.9856$) and the stem ($Y = -0.86x + 8.85$; $R^2 = 0.958$). Oxalic acid concentrations were lowest in leaves and stems that were grown in nutrient solutions with 75% ammonium nitrogen and 25% nitrate nitrogen. The oxalic acid concentrations in the leaves and stems grown in nutrient solutions with a ratio of 0.25 NO_3^--N: 0.75 NH_4^+-N were 40% and 37% lower respectively compared to the leaves and stems of plants grown in nutrient solutions with no ammonium. The leaves had up to 40% more oxalic acid concentrations than the stems. The nitrate to ammonium ratios in the nutrient solutions did not influence the dry weight, fresh weight, and the leaf area of purslane plants at harvest.

DISCUSSION

The results of this study showed that the oxalic acid concentrations in purslane leaves and stems decreased with increasing ammonium levels in the nutrient solutions. Our results are in agreement with the earlier reports on the effects of ammonium nutrition on oxalic acid in New Zealand spinach (Ahmed and Johnson 2000).

Plants can absorb nitrogen both as NO_3^- and NH_4^+. Ammoniacal-N can be directly used by plants in the synthesis of amides and amino acids, whereas NO_3^--N has to be reduced by processes that command up to 25% of either photosynthetic or mitochondrial electron transport capacity (Bloom et al. 1989). However, ammoniacal-N as a sole source of N acidifies the rhizosphere due to the excretion of H^+ from plant roots, and can be deleterious to plant growth (Weir et al. 1972). A combination of these two forms in an appropriate ratio is generally beneficial in plant growth as reported by other researchers (Gashaw and Mugwira 1981; Ikeda and Osawa 1983; Salsac et al. 1987). The optimal nutritional balance in crop cultivation depends on the specific response desired (phytochemical composition or the dry mass production) of a plant species. Apparently when nitrogen is provided in a nitrate form, the nitrate has to be reduced in the shoots (nitrate reduction by nitrogen reductase) before the N can be used by the plant. This reaction results in the production and accumulation of organic acids such as oxalic acid (Libert and Franceschi 1987) in the leaves and stems. They also proposed that nitrate ions inhibited the oxalic acid oxidase activity preventing the breakdown of oxalic acid, and resulting in the accumulation of oxalic acid in the leaves and stems. The higher concentrations of

Table 1. Plant growth characteristics and oxalic acid concentrations in purslane grown in nutrient solutions with four NO_3:NH_4 ratios. Data represent means of five replications.

NO_3^-:NH_4^+ ratio	Oxalic acid (mmols/g DW)		Shoot fresh weight (g)	Shoot dry weight (g)	Leaf area (cm^2)
	Leaf	Stem			
1:0	1.0	0.8	26.7	1.2	254
0.75:0.25	0.9	0.7	24.8	1.2	225
0.5:0.5	0.7	0.6	23.9	1.1	224
0.25:0.75	0.6	0.5	19.0	0.9	218
Significance linearity	**	**	NS	NS	NS

** Significant at 1% level. [NS] Not significant oxalic acid that we observed in purslane leaves and stems grown with nitrate as the sole source of nitrogen may be due to such an accumulation of oxalic acid.

Oxalic acid concentrations in food crops have long been a concern in the human diet, because of the negative health effects associated with high intake of oxalic acid-occurrence of kidney stones, low plasma levels of iron and calcium, occurrence of hyposideremia, and hypocalcemia, that correspond highly with the intake of oxalic acid which acts as an absorption inhibitor. While purslane is an excellent source of the omega-3 fatty acids, amino acids, and vitamins, its use as a popular vegetable crop has been diminished by the oxalic acid contents and reported cases of illnesses associated with vegetarians and high oxalic acid intake via plant foods in omnivores. Thus it is desirable to find cultural practices that would reduce the oxalic acid concentrations in purslane. A nutrient solution with 75% of total nitrogen provided as ammonium may decrease the oxalic acid concentrations in purslane leaves thus making purslane a more desirable food crop. According to our earlier study (Palaniswamy et al. 2000) the omega-3 fatty acids in purslane leaves were enhanced when grown in nutrient solutions with ~65% of N provided as ammonium. A combination of NO_3^- and NH_4^+ nitrogen at a ratio of 0.35:0.65 in hydroponic cultivation of purslane would optimize the nutritional value of leaves (lower oxalic acid and higher omega-3 fatty acid concentrations).

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