Oxalic Acid Concentrations in Purslane (*Portulaca oleraceae* L.) is Altered by the Stage of Harvest and the Nitrate to Ammonium Ratios in Hydroponics

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Abstract

Oxalic acid (OA) occurs naturally in a large number of plant species. When present in the human diet, OA may combine with essential minerals such as calcium, iron, magnesium, and potassium to form less soluble salts known as oxalates and hinder the bio-availability. Although purslane (*Portulaca oleracea*, Portulacaceae) is an excellent source of omega-3 fatty acids and antioxidant vitamins, its consumption as a green vegetable, is limited by high concentrations of OA. In previous studies we reported the influence of nitrate to ammonium ratios in hydroponics on the leaf omega-3 fatty acid concentrations. In this study we determined the NO_3^--N: NH_4^+-N ratio in hydroponics and the stage of harvest that would minimize the oxalic acid concentrations in purslane leaves. Fully expanded leaves were harvested at 8-true leaf and at 16-true leaf stages from purslane plants grown in hydroponic systems containing Nitrogen (200 μg. mL^{-1}) as nitrate (NO_3^-) to ammonium (NH_4^+) ratios (1:0, 0.75:0.25, 0.5:0.5, and 0.25:0.75), and analyzed for OA concentrations. Results indicate that at both stages of harvest the OA concentrations were ≈ 40 to 50 % lower in the leaves grown in solutions containing ammonium compared to the leaves grown with no ammonium. Leaves harvested at 16-true leaf stage had ≈ 36 to 45 % lower OA concentrations compared to the leaves harvested at 8-true leaf stage. The dry weight (DW), fresh weight (FW) and leaf area (LA) were greater at 16-true leaf stage than at 8-true leaf stage, but were not influenced by the NO_3^-: NH_4^+ ratios in hydroponics.

INTRODUCTION

High oxalic acid (OA) concentrations in leaves of plant species, especially those used as green leafy vegetables in the daily diets, have been of concern because of the harmful health effects associated with the intake of high OA. Presence of high OA also hinders the acceptance of newly identified sources of otherwise nutritious vegetables. Although purslane (*Portulaca oleracea*) is identified as an excellent source of omega-3 fatty acids, anti-oxidant vitamins and essential amino acids (Miller et al., 1984; Simopoulos and Salem Jr., 1986; Simopoulos et al., 1992) and 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 (Mathams and Sutherland, 1952), and the harmful health effects associated with oxalic acid in the diet because a diet high in oxalate can increase the risk of kidney stones and may affect calcium absorption (Bataille

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and Fournier, 2001). 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*) (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). Here, we demonstrate that NO_3^--N: NH_4^+-N ratio in hydroponics as well as the stage of harvest can influence the oxalic acid concentrations and determine the NO_3^--N: NH_4^+-N ratio and the stage of harvest that would minimize the oxalic acid concentrations in purslane leaves.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Twenty-one days old seedlings of purslane (*Portulaca oleracea*) (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 (Table 1). 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.

Harvest and Data Collection

Plants were harvested at 8 true-leaf stage and 16 true-leaf stages. 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

The oxalic acid concentrations of the leaf and stems were determined using the procedure as described by Ilarslan et al. (1997). 0.01 g of dry leaf or stem sample was ground with 5 mL de-ionized water. 5 mL EDTA (1M) was added and filtered with Whatman No. 1 filter paper. The oxalate kit purchased from Sigma (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: oxalate reagents were warmed to 37 ^oC; test tubes were labeled for blank, control, standard and sample; 1mL oxalate reagent A (DMAB (30dimethylamino) benzoic acid + MBTH (3-methylk-2-benzothiazolinone hydrazone), pH=3.1) was added to each tube; 50 µL of sample were added to each sample tube; 50 µL deionized water were added to the blank and control tubes; 50 µL oxalate standard were added to the standard tube; 0.1 mL of oxalate reagent B (oxalate oxidase and peroxide) was added to all tubes and immediately mixed by gentle inversion. All tubes were incubated at 37 ^oC for 5 min. The absorbances of blank, control, standard, and sample were determined at 590 nm in a Thermo Spectronic UV/VIS spectrophotometer (Rochester, NY). Measurements were taken twice to obtain consistent absorbances. Corrected absorbances were determined by subtracting the blank absorbance from absorbance readings of standard, control and the sample. The oxalate concentration in mg per 100 g of fresh weight was determined as per the SIGMA Urinalysis Diagnostic Kit.

RESULTS

The shoot fresh mass, leaf dry mass, shoot dry mass, leaf area and the plant height were only affected by the stage of harvest and not influenced by the nitrate to ammonium ratio in nutrient solutions (Table 2).

OA concentrations were up to 40 % higher in the leaves and stems that were grown in nutrient solutions with no ammonium than in the leaves and stems that were grown with ammonium in the nutrient solution (Table 3). The oxalic acid concentration of the stems and leaves were up to 45 % lower at 16-true leaf stage than at 8-true leaf stage. Oxalic acid concentrations were the lowest in the 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 and harvested at 8-true leaf stage were 39 % and 56 % lower respectively compared to the leaves and stems of plants grown in nutrient solutions with no ammonium. 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 and harvested at 16-true leaf stage were 40 % and 34 % lower respectively compared to the leaves and stems of plants grown in nutrient solutions with no ammonium.

There was a strong negative correlation between the ammonium concentrations in the nutrient solution and the oxalic acid concentrations of the leaf and the stem. Regression analysis was performed for the oxalic acid of the leaves harvested at 8 true-leaf stage ($Y = 19.8x2 - 146.62x + 520.5$; $R^2 = 0.9842$) and 16 true-leaf stage ($y = 19x2 - 182.94x + 797.1$; $R^2 = 0.9464$). The leaves had up to 45% more oxalic acid concentrations than the stems.

DISCUSSION

The results of this study showed that the OA concentrations in purslane leaves and stems decreased with increasing ammonium levels in the nutrient solutions without significant decrease in fresh and dry weight yield (Figure 1). Our results are in agreement with the earlier reports on 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 nitrate 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 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. The fact that presence of oxalates in plants does not seem to affect the normal plant growth suggests that the synthesis of oxalates may have a significant function or adaptation or serve as a possible defense mechanism against predation or other adverse environmental conditions. Although the presence of significant amounts of oxalates during the different stages of growth of many plants and does not seem to be detrimental to normal plant development, 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 and harvesting at 16-true leaf stage 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 and when harvested at 14 to 16 true leaf stage. A combination of NO_3^- and NH_4^+ nitrogen at a ratio of 0.35:0.65 in hydroponic cultivation of purslane and harvesting the produce at 16-true leaf stage would optimize the nutritional value of leaves (lower oxalic acid and higher omega-3 fatty acid concentrations) without significant decrease in fresh and dry weight yield.

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Tables

Table 1. Concentrations of inorganic nutrients (mM) used to prepare nutrient solutions at NO_3-N:NH_4-N ratios of 1:0, 0.25:0.75, 0.5:0.5, 0.25:0.75. All nutrient solutions contained micronutrients in the following concentrations (all in μM): B,25; Mn, 2; Cu 0.5; Mo,0.5; FeEdTA,50.

Inorganic nutrient	N Treatment			
	Ratio of nitrate-N to ammonium-N in nutrient solution (conc (mM)			
	1:0	0.25:0.75	0.5:0.5	0.25:0.75
KNO_3	4.3	5.3		
$Ca(NO_3)_2$	5.0	2.7	3.6	1.8
$Mg SO_4$	2.0	2.0	2.0	2.0
$KH_2 PO_4$	1.0			
$NH_4H_2PO_4$		1.0	1.0	1.0
NH_4Cl		2.6	6.2	9.7
$CaCl_2$		2.3	1.4	3.2
KCl			5.3	5.3

Table 3. Oxalic acid concentrations in purslane grown in nutrient solutions with four NO_3:NH_4 ratios and harvested at 8-true leaf stage and 16-true leaf stage. Data represent means of five replications.

	Oxalic acid (mg/100 g FW)			
	8-Leaf Stage		16-Leaf Stage	
NO_3^-:NH_4^+ ratio	Leaf	Stem	Leaf	Stem
1:0	622.5	492.2	396.9	226.0
0.75:0.25	539.2	325.9	296.8	180.9
0.5:0.5	387.3	224.3	247.6	149.3
0.25:0.75	380.1	216.4	238.5	148.8

Sources of Variation	df	Leaf F Value	Stem F Value
NO_3^-:NH_4^+	3	6.81***	28.13***
Stage of harvest	1	36.47***	102.48***
NO_3^-:NH_4^+ x Stage of Harvest	3	0.54 NS	8.11***

NS, *** Differences not significant, or significant at P≤ 0.001 respectively

Table 2. Plant growth characteristics of purslane grown in nutrient solutions with four NO_3:NH_4 ratios and harvested at 8-true leaf stage (8-LS) and 16-true leaf stage (16-LS). Data represent means of five replications each.

NO_3^-:NH_4^+ ratio	Shoot FM[Z] (g)		Leaf DM[Y] (g)		Shoot DM (g)		Leaf Area (cm^2)		Plant Height (cm)	
	8-LS	16-LS	8-LS	16-LS	8-LS	16-LS	8-LS	16-LS	8-LS	16-LS
1:0	23.9	77.3	0.6	2.2	1.1	4.1	254	578	28.9	43.0
0.75:0.25	26.7	93.1	0.6	2.2	1.2	4.2	225	630	30.7	44.1
0.5:0.5	19.0	83.6	0.5	2.1	0.9	3.8	226	621	27.4	44.3
0.25:0.75	24.8	64.8	0.7	1.9	1.2	3.9	218	608	29.2	41.3

Sources of Variation

	df	F Value	F Value	F Value	F Value	F Value
NO_3^-:NH_4^+	3	2.59 NS	0.34 NS	0.45 NS	0.14 NS	0.81 NS
Stage of harvest	1	211.32***	128.6***	179.41***	455.96 ***	194.00***
NO_3^-:NH_4^+ x Stage of Harvest	3	2.5 NS	0.69 NS	0.09 NS	1.09 NS	1.0 NS

[Z] Fresh Mass, [Y] Dry Mass. NS, *** Differences not significant, or significant at $P \leq 0.001$ respectively

Figures

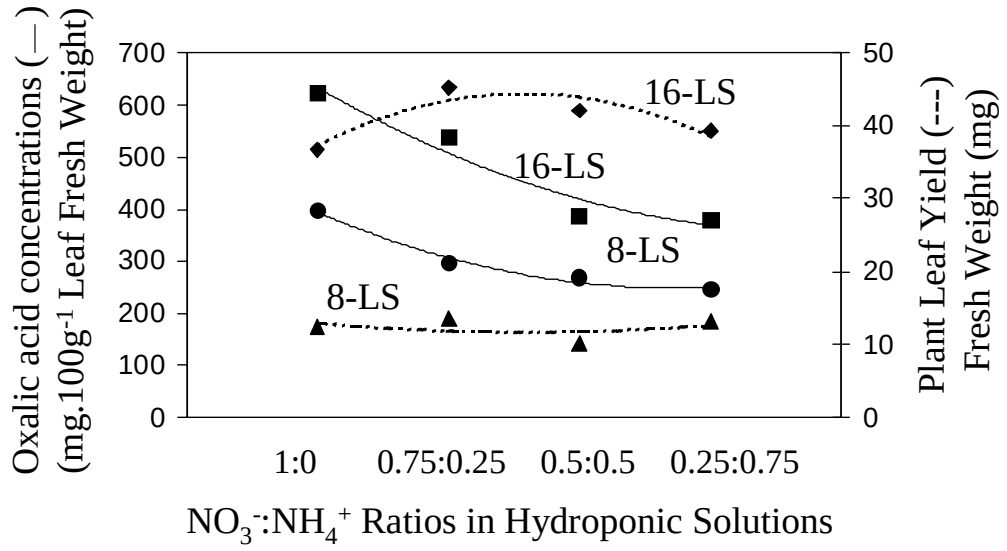

Fig. 1. Oxalic acid concentrations and fresh leaf weight of purslane grown in nutrient solutions with four NO_3:NH_4 ratios and harvested at 8-true leaf stage and 16-true leaf stage. Data represent means of five replications.