Omega-3-Fatty Acid Concentration in *Portulaca oleracea* is Altered by Nitrogen Source in Hydroponic Solution

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**ABSTRACT.** Purslane (*Portulaca oleracea* L.) has been identified as an exceptionally rich source of α-linolenic acid (LNA), an essential fatty acid that is beneficial in reducing the incidence of coronary heart disease and certain cancers. In general, about two thirds of the LNA in terrestrial plants is associated with chloroplasts. A green-leafed unnamed cultivar of purslane and a golden-leafed cultivar ‘Goldberg’ were grown hydroponically in a complete nutrient solution with 14.3 mM nitrogen provided as nitrate (NO$\text{}_3^-$) and ammonium (NH$\text{}_4^+$) forms to yield NO$\text{}_3^-$-N: NH$\text{}_4^+$-N ratios of 1:0, 0.75:0.25, 0.5:0.5, and 0.25:0.75. Young leaves, harvested 18 days after treatment initiation, were analyzed to determine the fatty acid composition and concentrations, and thylakoid protein and chlorophyll concentrations. Although the leaves of plants grown with a NO$\text{}_3^-$-N: NH$\text{}_4^+$-N ratio of 0.5:0.5 contained 239% and 114% more LNA than plants grown with ratios 1:0 and 0.75:0.25, respectively, they contained only 41% and 26% more chlorophyll. The green-leafed cultivar had higher (39%) chlorophyll concentration than ‘Goldberg’, but both cultivars had similar LNA concentrations (per g dry weight (DW)). In general, α-linolenic acid usually occurs as a critical constituent in chloroplasts (Tremolieres et al., 1979), making up about two thirds of the total fatty acids (TFA) in chloroplasts of photosynthetic tissues (Allen et al., 1966; Crombie, 1958; Wolff, 1966). The high concentration of LNA in purslane is due to its high concentration of total fatty acids and not to an unusual fatty acid composition (Simopoulos et al., 1992). If LNA production in purslane is to be optimized, we need to understand the effects of cultural practices such as mineral nutrient application on LNA accumulation. Nitrogen (N) nutrition of plants could affect chloroplast development with the resultant impact on LNA concentration of leaves.

**PLANT MATERIAL.** Twenty-five-day-old seedlings of two purslane cultivars, a) an unnamed green-leafed cultivar (Valley Seed Service, Fresno, Calif.) and b) ‘Goldberg’, a golden-leafed cultivar, (Johnny’s Selected Seeds, Albion, Maine) were transplanted into a closed hydroponic system in the greenhouse. The cultivars were selected for their upright growth habit and the apparent difference in leaf chlorophyll concentration.

**MATERIALS AND METHODS.**

Plants were grown in individual 8-L containers capped with styrofoam platforms (2.5 cm thick × 30 cm wide × 30 cm long). The nutrient solution in each container was aerated for 1 min every 15 min, using a timer-controlled air bubbler. Six purslane seedlings were transplanted onto each styrofoam platform and each 8-L container held a complete nutrient solution.
(Table 1) with 14.3 µmol total N in one of four NO₃⁻:NH₄⁺:N ratios (1:0, 0.75:0.25, 0.5:0.5, and 0.25:0.75). During the experiment, nutrient solution was monitored twice weekly for chemical changes. Due to the large solution volume and small plant mass, solution nutrient content and water volume were not adjusted during the short experimental period. However, pH adjustments were made whenever the pH deviated 0.4 units from the set point range (6.6 to 6.8) by adding 0.5 M HCl or NaOH as needed.

The four N-form treatments were arranged in a randomized complete block design with five replications of each treatment for each cultivar. The experiment was conducted two times.

**Plant growth environment.** The initial study began 31 May and plants were harvested 18 June 1996. The second experiment began 27 June and ended 15 July 1996. Environmental conditions were recorded on a Campbell Scientific 21X Datalogger (Campbell Scientific, Logan, Utah) at 1-min intervals using quantum sensors (LI-190-SA; LI-COR, Inc., Lincoln, Neb.) to monitor photosynthetic photon flux (PPF) and copper–constantan thermocouples to monitor air and solution temperatures. The average daily PPF was 9.25 ± 0.66 mol·m⁻²·d⁻¹ during the first experiment and 9.30 ± 0.58 mol·m⁻²·d⁻¹ during the second experiment. Canopy air temperature averaged 24.8 ± 1.6 °C during the first study and 25 ± 1.3 °C during the second. Nutrient solution temperature averaged 24.3 ± 1.4 °C and 24.6 ± 1.4 °C for two studies, respectively. Plants were maintained under ambient CO₂.

Purslane plants were harvested after 18 d (12 to 14 true leaf stage) of growth in the N-form treatment solutions. One plant from each treatment replicate was used to determine whole plant fresh weight, dry weight (DW), leaf area (using a LI-3100 area meter, LI-COR, Inc., Lincoln, Neb.), and the number of side shoots. From the remaining five plants in each treatment replicate, fully expanded young leaves from the third, fourth, and fifth nodes (counting down from the shoot tip), were harvested and divided into two subsamples. One subsample was used to determine whole leaf chlorophyll, and thylakoid chlorophyll and protein concentrations, and the other subsample was used to determine fatty acid composition and concentrations.

**Thylakoid recovery.** Thylakoid membranes were recovered from leaf samples by grinding 4 g leaf tissue in 20 mL of a 50 mM phosphate buffer (pH 7.6) containing 0.4 M sorbitol, 20 mM NaCl, 20 mM HEPES, and 3 mM MgCl₂·6H₂O (Berkowitz and Gibbs, 1985; Chapman et al., 1983). The filtrate was centrifuged at 3000 g for 4 min, and the pellet was washed in 20 mL of a 50 mM phosphate buffer (pH 7.6) containing 20 mM NaCl, and then centrifuged at 5000 g for 10 min. The thylakoid pellet was recovered following centrifugation at 10,000 g for 10 min.

**Chlorophyll analysis.** Thylakoid chlorophyll was determined by suspending a 50-µL sample of thylakoid in 4 mL of 80% acetone and recording the absorbance at 645 nm and 663 nm (Arnon, 1949). Whole leaf chlorophyll was determined by extracting chlorophyll from leaf discs with N, N-dimethyl formamide and measuring absorption values at 647 nm and 664 nm (Inskeep and Bloom, 1985). The ratio of whole leaf chlorophyll to thylakoid chlorophyll was used to determine percent thylakoid recovery for each sample. The recovery factor was used to estimate thylakoid protein on a leaf DW basis.

**Protein analysis.** Protein concentration was determined using the spectrophotometric procedure of Lowry et al. (1951). A 50-µL sample of thylakoid was suspended in 150 mL 0.85% NaCl and 2.2 mL Biuret reagent was added. After 30 min, 100 µL phenol reagent was added, mixed well, and absorbance was recorded at 750 nm. Thylakoid protein was expressed as mg·g⁻¹ leaf DW.

**Fatty acid extraction, preparation, and analyses.** Lipids were extracted using the dry column method as follows (Maxwell et al., 1980). Leaf tissue (~4 g) was homogenized for 30 s in 25 mL dichloromethane (DCM):methanol (9:1). The homogenate was mixed with 4 g anhydrous sodium sulfate and then flushed through a 22 mm × 30 cm glass column packed with 1.9 calcium phosphate:celite with 150 mL DCM:methanol (9:1). The filtrate was collected and vacuum dried in a rotary evaporator. The dried lipid fraction was dissolved in 1 mL DCM and transferred to 2 mL glass screw cap vials, flushed with N₂ gas, and stored under refrigeration until transesterification.

The transesterification procedure of Lepage and Roy (1986) was used to methylate the fatty acid fraction. A 100-µL aliquot of extracted lipid was mixed with 2 mL of an internal standard [100 µL·mL⁻¹ of heptadecanoic acid (a 17:0 fatty acid) in 4 methanol : 1 hexane] and 200 µL acetyl chloride in a screw cap test tube. Test tubes were sealed tightly with teflon thread seal tape and heated for 1 h at 100 °C. After cooling, 5 mL 6% K₂CO₃ was added slowly, mixed, and then centrifuged at 5000 g for 10 min. The lipid layer was removed, completely dried under N₂ gas, and then dissolved in 50 µL DCM. Finally, the resulting fatty acid methyl esters were injected into a gas chromatograph (Varian 6000, Palo Alto, Calif.) for fatty acid separation and analysis. Fatty acid peak areas were determined with an HP 3395 integrator (Hewlett Packard, Wilmington, Del.) and fatty acids were identified by comparing retention times with standards separated under similar chromatographic conditions [Poly Unsaturated Fatty Acid (PUFA-2), Matreya, Inc., Pleasant Gap, Pa.].

**Chromatographic conditions.** The gas chromatograph was fitted with Supelcowax capillary column [10 stationary phase, 1 µm film thickness, 0.53 mm ID × 30 m (Supelco, Bellefonte, Pa.)] and a flame ionization detector. Air and hydrogen flow rates at the detector were maintained at 300 and 30 mL·min⁻¹, respectively, and the N₂ carrier gas flow rate was 1 mL·min⁻¹. Column temperature gradient increased from 190 to 235 °C at a rate of 2 °C·min⁻¹ and ion temperature was held at 240 °C. Analysis time was 30 min per sample.

**Data analysis.** Bartlett’s test of homogeneity (Little and Hills, 1978) was used to confirm the homogeneity of variances in data from repeated experiments. The variances of the treatment observations were comparable in the two experiments and there were no differences in the treatment effects between experiments. Therefore, results from the two experiments were combined and the data were analyzed using SAS General Linear Models procedure (SAS Inst. Inc., 1994).

**Results**

Regardless of the N-form supplied to the purslane, the growth characteristics remained similar (Table 2). However, there were cultivar differences in growth with the green-leafed cultivar being taller, heavier, more branched and having a larger leaf area per plant than ‘Goldberg’.

The leaf fatty acid composition of both purslane cultivars was similar and unaffected by N-form supplied. The mean fatty acid composition (all expressed as percent of TFA ± se) averaged across all N-form treatments and both purslane cultivars was 14.4 ± 0.7 for palmitic (16:0), 3.2 ± 0.3 for palmitoleic (16:1), 1.1 ± 0.1 for stearic (18:0), 2.5 ± 0.1 for oleic (18:1), 11.1 ± 0.2 for linoleic (18:2), and 67.7 ± 0.8 for linolenic (18:3). LNA was the major fatty acid constituent making up two-thirds of the TFA. Further, the 16:3 fatty acid hexadecatrienoic acid was not detected in the leaves confirming
the report (Simopoulos et al., 1992) that purslane is an 18:3 plant.

N-form had a pronounced influence on the concentrations of fatty acids in purslane leaves, but did not affect plant growth or fatty acid composition. The LNA and TFA concentrations of both purslane cultivars were lowest in plants exposed to NO$_3^–$ as the sole N source (Table 3). Increasing NH$_4^+$ in the nutrient solution from 0% to 50% of total N increased the average LNA 3.4-fold and TFA 3.1-fold across both cultivars. However, increasing NH$_4^+$ in the nutrient solution from 0% to 75% of total N only increased LNA 2.2-fold and TFA 2.1-fold in purslane leaves.

Increasing NH$_4^+$ in the nutrient solution from 0% to 50% of total N only increased the leaf chlorophyll 47% in the green-leafed cultivar and 35% in 'Goldberg' (Table 3). The green-leafed purslane consistently had higher chlorophyll content than the golden-leafed 'Goldberg'. There was a highly significant positive correlation [TFA = 3.89(Chl) – 40.7, $R^2 = 0.77$, ($P \leq 0.001$)] between leaf chlorophyll concentration (mg·g$^{–1}$ DW) and leaf TFA content (mg·g$^{–1}$ DW) for the green-leafed cultivar, but a similar relationship was not observed with 'Goldberg'. Increasing NH$_4^+$ in the nutrient solution from 0% to 50% of total N only increased the thylakoid protein content 47% in the green-leafed cultivar and 63% in ‘Goldberg’. However, the ratio of thylakoid protein to chlorophyll ratio ($\text{TP/Chl}$) averaged for both cultivars were not altered by N form.

Discussion

Nitrogen form dramatically altered the fatty acid concentration in purslane leaves without altering fatty acid composition or thylakoid protein/chlorophyll ratio. These results show that the relative constancy of lipid molecular species in the leaf is essential for chloroplast function and hence preserved. The presence of NH$_4^+$-N in the nutrient solution greatly increased leaf TFA, and modestly increased chlorophyll, and thylakoid protein concentrations, possibly by providing readily assimilable N that could stimulate chloroplast development. Raab and Terry (1994) reported that NH$_4^+$-N grown compared to NO$_3^–$-N grown sugar beets (Beta vulgaris L.) had doubled chloroplast volumes and greatly increased (4.3-fold) soluble leaf protein, but chlorophyll content increased by only 54%. Golvano et al. (1982) reported greater length of internal plastid membranes of etioplasts of wheat (Triticum vulgare L. ‘Ye’cora’) seedlings given NH$_4^+$-N compared to NO$_3^–$-N nutrition. Perhaps one could attribute the increase in TFA observed in our study to further

Table 2. Concentration of inorganic nutrients (mM) used to prepare nutrient solutions at NO$_3^–$-N:NH$_4^+$-N ratios of 1:0, 0.75:0.25, 0.5:0.5, and 0.25:0.75. All nutrient solutions contained micronutrients in the following concentrations (all in µM): B, 25; Mn, 2; Zn, 2; Cu, 0.5; Mo, 0.5; FeEDTA, 50.

| N treatment | NO$_3^–$:NH$_4^+$ ratio in nutrient solution [concn (mM)] |
|-------------|-----------------------------------------------------|
| Ratio of nitrate-N to ammonium-N in nutrient solution | 1:0 | 75:25 | 50:50 | 25:75 |
| KNO$_3$ | 4.3 | 5.3 | 5.3 | 5.3 |
| Ca(NO$_3$)$_2$ | 5.0 | 2.7 | 3.6 | 1.8 |
| MgSO$_4$ | 2.0 | 2.0 | 2.0 | 2.0 |
| KH$_2$PO$_4$ | 1.0 | 1.0 | 1.0 | 1.0 |
| NH$_4$H$_2$PO$_4$ | 1.0 | 1.0 | 1.0 | 1.0 |
| NH$_4$Cl | 2.6 | 6.2 | 9.7 | 9.7 |
| CaCl$_2$ | 2.3 | 1.4 | 3.2 | 3.2 |
| KOH | 5.3 | 5.3 | 5.3 | 5.3 |

Table 3. Plant growth characteristics of an unnamed green-leafed cultivar and a golden-leafed cultivar ('Goldberg') of Portulaca oleracea (purslane) grown in nutrient solutions with four nitrate (NO$_3^–$) to ammonium (NH$_4^+$) ratios. Data represent means of 10 observations.

| NO$_3^–$:NH$_4^+$ ratio in nutrient solution | Shoot fresh wt (g) | Shoot dry wt (g) | Leaf dry wt (g) | Plant ht (cm) | No. of shoots |
|------------------------------------------|-------------------|-----------------|----------------|--------------|-------------|
| Green-leafed cultivar | | | | | |
| 1:0 | 42.4 | 1.7 | 0.8 | 39.8 | 13 |
| 0.75:0.25 | 41.4 | 1.7 | 0.8 | 39.8 | 12 |
| 0.5:0.5 | 38.9 | 1.6 | 0.7 | 37.6 | 11 |
| 0.25:0.75 | 39.4 | 1.6 | 0.7 | 37.9 | 12 |
| Golden-leafed cultivar | | | | | |
| 1:0 | 32.2 | 1.4 | 0.6 | 25.7 | 6 |
| 0.75:0.25 | 33.6 | 1.4 | 0.6 | 26.0 | 7 |
| 0.5:0.5 | 32.5 | 1.3 | 0.6 | 25.3 | 7 |
| 0.25:0.75 | 30.6 | 1.2 | 0.6 | 23.5 | 7 |

Source of variation | df | F value | F value | F value | F value | F value |
|-------------------|----|---------|---------|---------|---------|---------|
| NO$_3^–$:NH$_4^+$ | 3 | 0.5** | 1.7*** | 1.1** | 3.1* | 0.4** |
| Cultivar | 1 | 25.1*** | 31.8*** | 18.1*** | 510.5*** | 276.8*** |
| NO$_3^–$:NH$_4^+$ x cultivar | 3 | 0.2** | 0.2** | 0.1** | 0.6** | 1.9** |

**,*****Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.
enlargement of purslanes already large chloroplasts by NH₄⁺-N nutrition. However, Palaniswamy (1998) found that, under certain environmental conditions, the majority of LNA recovered in purslane leaves was not associated with thylakoid membrane.

Although others (Tchang et al., 1985; Tremolieres, 1972; and Tremolieres et al., 1973) found that LNA was synthesized in a fixed ratio to chlorophyll in *Pisum sativum*, this relationship was not observed with purslane. Furthermore, ‘Goldberg’ and the green-leafed cultivar had consistently lower TFA and LNA concentrations, but ‘Goldberg’ had consistently lower chlorophyll concentration than the green-leafed cultivar. These data suggest that the relatively short-term (18 d) exposure of purslane to NH₄⁺-N in the nutrient solution stimulated fatty acid synthesis in excess of that associated with thylakoid development.

The uncoupling of TFA accumulation from chlorophyll and thylakoid protein synthesis by NH₄⁺-N in the nutrient solution suggests that accumulation of TFA is not associated solely with thylakoid membranes in the fatty acid-rich species *Portulaca oleracea* (purslane). Indeed, we observed the presence of osmophilic lipid globules in purslane chloroplasts indicating the capacity for fatty acid storage (Palaniswamy, 1998). Perhaps purslane stores significant amounts of TFA in its large chloroplasts, which could account for its exceptionally high concentration of TFA compared to other green leafy vegetables.

The relatively low fatty acid concentration in purslane plants exposed to NO₃⁻ as the sole N source may result from impaired assimilation of NO₃⁻ to ammonium. Ammoniacal N can be used directly by plants in the synthesis of amides and amino acids, whereas NO₃⁻ has to be reduced by processes that command up to 25% of either photosynthetic or mitochondrial electron transport capacity (Bloom et al., 1989). Perhaps the reduction of NO₃⁻ proceeds too slowly to maximize chloroplast development in young leaves. The relative decline in TFA of young leaves of plants grown in the 75% NH₄⁺-N solution compared to those in the 50% NH₄⁺-N solution could be an adverse effect of accumulation of some unassimilated ammonia. Or perhaps the modest decline in leaf TFA in plants grown with 75% NH₄⁺ (0.25:0.75) compared to the 0.5:0.5 NO₃⁻:NH₄⁺ ratio could be due to preferential catabolism of the fatty acids, over protein. An increase in catabolism of lipids, carbohydrates, and proteins (as respiratory substrates) had been observed in NH₄⁺-N grown radish (*Raphanus sativus* L.) (Goyal et al., 1982) and white mustard (*Sinapis alba* L.) (Mehrer and Mohr, 1989).

Lack of a growth response in purslane to increased NH₄⁺-N fertilization may be related directly to the relatively high metabolic investment associated with increased lipid anabolism as well as the effects of N-form on photosynthesis. Raab and Terry (1994) also found that the photosynthetic rate per chlorophyll molecule was reduced for enlarged chloroplasts and suggested this was due to increased CO₂ diffusion resistance within the chloroplasts. However, at *PPF* levels above 1000 μmol·m⁻²·s⁻¹ and elevated carbon dioxide levels, they found that the photosynthesis rate per unit leaf area was higher in NH₄⁺-grown than NO₃⁻-grown plants. In our study, purslane was exposed to N-form treatments for only 18 d and under ambient carbon dioxide and relatively low *PPF* conditions. Marschner (1995) indicated that the optimum proportion of N-form for best growth is dependent on root zone temperature, light intensity (irradiance), and total concentration of N supplied. Therefore, the optimum proportion of N-form for purslane growth will probably vary with environmental conditions; however, it is apparent that NH₄⁺-N in the nutrient solution dramatically stimulates TFA accumulation in a relatively short period of time, and hence improves the nutritional value of the harvested product with respect to essential fatty acids.

### Table 3. Concentrations (all in mg·g⁻¹ leaf DW) of whole-leaf chlorophyll (Chl), total fatty acids (TFA), α-Linolenic acid and and thylakoid protein, and the ratios TFA/Chl and protein/Chl in leaves of an unnamed green-leafed cultivar and a golden-leafed cultivar (‘Goldberg’) of *Portulaca oleracea* (purslane) grown in nutrient solutions with four nitrate (NO₃⁻) to ammonium (NH₄⁺) ratios. Data represent means of 10 observations.

| NO₃⁻:NH₄⁺ ratio in nutrient solution | Chlorophyll (mg·g⁻¹ DW) | Total fatty acids (mg·g⁻¹ DW) | α-Linolenic acid (mg·g⁻¹ DW) | Thylakoid protein (mg·g⁻¹ DW) | TFA/Chl ratio¹ | Protein/Chl ratio |
|-------------------------------------|-------------------------|-----------------------------|-----------------------------|-----------------------------|----------------|-----------------|
| 1:0                                 | 13.6                    | 11.7                        | 7.8                         | 143                         | 0.9            | 10.5            |
| 0.75:0.25                           | 15.3                    | 19.7                        | 13.2                        | 156                         | 1.3            | 10.2            |
| 0.5:0.5                              | 20.0                    | 41.5                        | 29.5                        | 210                         | 2.1            | 10.5            |
| 0.25:0.75                           | 17.6                    | 25.3                        | 17.4                        | 194                         | 1.4            | 11.0            |
| 1:0                                 | 10.1                    | 13.8                        | 8.4                         | 105                         | 1.4            | 10.4            |
| 0.75:0.25                           | 11.2                    | 18.9                        | 12.4                        | 131                         | 1.7            | 11.7            |
| 0.5:0.5                              | 13.6                    | 37.2                        | 25.5                        | 171                         | 2.7            | 12.6            |
| 0.25:0.75                           | 13.1                    | 27.8                        | 18.6                        | 178                         | 2.1            | 13.6            |

¹Statistical analyses were not conducted on TFA/Chl or protein/Chl ratios.

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