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Effects of Nitrate to Ammonium Ratio on Yield and Nitrogen Metabolism of Strawberry (*Fragaria x Ananassa* cv. Selva)

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ABSTRACT

Strawberry plants cv. Selva was grown with four NO_3^-: NH_4^+ ratios, (7:0, 6.5:0.5, 6:1, 5.5:1.5 mM) at constant nitrogen concentration of 7 mM/L and constant pH of 5.8. Other essential elements were prepared for the plants adequately. The effect of different NO_3^-: NH_4^+ ratios on, yield, number of fruits, total nitrogen and nitrate content of different parts of the plants and leaf nitrate reductase activity was determined. Nitrate reductase activity, during the vegetative growth, was affected by the nitrogen sources and maximum activity was shown in the ratio of 6:1. The strawberry plants also showed higher nitrogen content in this ratio compared to the treatment lacking ammonium. Yield and number of fruits are higher in nutrient solutions having both nitrate and ammonium. The strawberry plants need more ammonium than nitrate in the vegetative stage, but ammonium must not exceed more than 50% of total nitrogen in the nutrient solution. Ammonium also improves yield and nitrogen content of strawberry tissues. Plant developmental stages and environmental condition will determine the best ratio of nitrate to ammonium

Key Words: *Fragaria x ananassa;* Soilless culture; Nitrate reductase; *In vivo* activity; Nitrogen

INTRODUCTION

The strawberry is an important commercial fruit in Iran and many other countries. The use of trickle irrigation and fertilization is increasing rapidly. For having a good yield, it is necessary to determine the level of nutrients concentration for fertilization. The mineral nutrition of strawberry has been reviewed by Matlock (1954). Nitrogen is the major nutrient taken up by strawberry. Several scientists have studied the effect of different nitrogen levels on strawberry growth and fruit yield (Ganmore-Neumann & Kafkafi, 1985; Albregts & Howard, 1986; Lamarre & Lareau, 1997; Darnell & Stutte, 2001), but very little has been reported on the effect of source of nitrogen on growth and yield of strawberry (Ganmore-Neumann & Kafkafi, 1983; Ganmore-Neumann & Kafkafi, 1985; Claussen & Lenz, 1999).

In most plants it has been shown that both NO_3^- and NH_4^+ forms are taken up and metabolized by plants. The preferential source has been shown to be dependent on plant species, cultivar and environmental factors. Most species grow best if they have access to both nitrate and ammonium nitrogen (Errebhi & Wilcox, 1990). Strawberry is a plant, which prefers NO_3 (Darnell & Stutte, 2001), but total nitrogen uptake rate and growth was higher when both nitrogen sources were present in the solution (Ganmore-Neumann & Kafkafi, 1985).

Nitrate reductase (NR) is the most important enzyme responsible for nitrate assimilation in plants. Its activity is considered to be a limiting factor for growth, development, and protein synthesis by plants (Solomonson & Barber, 1990), and was reported to be highly correlated with plant growth and yield (Shen *et al*., 1993).

There is a little work on the effect of nitrogen source on nitrate reductase activity in strawberry (Claussen & lenz, 1999). Further research on nitrate reductase activity is focused on nitrate nutrition (Darnell & Stutte, 2001). The nitrate reductase activity might increase when NH_4^+ is added to a solution having nitrate. The objectives of the present study were to determine the effects of different NO_3^-: NH_4^+ ratios on nitrate reductase activity, on nitrate and total nitrogen concentration in various plant parts and to compare changes in nitrate reductase activity at different stages of growth.

MATERIALS AND METHODS

Uniform strawberry plants (*Fragaria x ananassa* cv. Selva), which got sufficient chilling were brought in November from a commercial field nursery to a greenhouse. Each plant was mounted in a 4x7 inch plastic pots containing 70% perlit: 30% peat moss (by volume). The greenhouse conditions were: 20/15°C (day/night) temperature and the relative humidity was 60-70%. The plants were irrigated with a balanced nutrient solution by drip irrigation system. The total N content was 7 mM in all treatments. Treatments were consisted of 4 different NO_3^-: NH_4^+ ratios (7:0, 6.5:0.5, 6:1, 5.5:1.5 mM), respectively.

The concentration of other macronutrients was K^+, 2.5; Ca^{2+}, 1.55; Mg^{2+}, 0.37; PO_4^{3-}, 0.55 mmol/L. Micronutrients and Fe concentration were as suggested by Hoagland and Arnon (1950). The pH was adjusted to 6 with H_2SO_4.

Experimental setup was consisted of four different irrigation systems using non-recirculating system. Each treatment had 3 replicates, and there were 10 pots in each experimental plot. At bloom, flowers were tagged and hand pollinated. Ripe fruits were harvested and number and fresh weight (FW) were determined. Fruits were dried at 70°C for 96 h for further analysis.

Shoot NR (nitrate reductase) activity was measured during two different stages of growth and development, including vegetative and reproductive stages, using an *in vivo* procedure (Jaworski, 1971; Villora *et al.*, 2002). Leaf blades from young mature leaves were cut into 5 mm sections and the samples (0.3 g) were placed in 5 ml of incubation buffer (100 mM K-phosphate buffer pH 7.5, 0.1 M KNO_3, containing 1% (v:v) propanol). The samples were then vacuums infiltrated (5 mm Hg) for 2 min and incubated in the dark in a water bath for 30 min at 30°C. The amount of nitrite produced was determined as a measure of NR activity by developing the Azo color complex in a reaction mixture containing 1 ml extract, 2 ml 1% (w/v) sulfanilamide in 1.5N HCl and 2 ml 0.02% N-NEDA (naphthylethylenediamine dihydrochloride). After 30 min for full color development, absorbance was determined at 540 nm. The NR activity was expressed as nmoles NO_2^- produced g^{-1} fresh weight h^{-1}.

The experiment was terminated after 24 weeks and plants were harvested at the end of the experiment. The plants were separated into leaves and roots. Total N in leaves, roots and fruits was determined using Leco method (Dumas, 1981). Samples were ignited in an induction furnace at approximately 900°C, in helium and oxygen environment in a quartz combustion tube. An aliquot of the combustion gases was passed through a copper catalyst to remove oxygen and convert nitrous oxides to N_2, scrubbed of moisture and carbon dioxide, and nitrogen content determined by thermal conductivity.

Total extractable NO_3-N in leaves and fruits was determined by flow injection analyzer method (Carlson *et al.*, 1990; Switala, 1997; Wendt, 1999). Nitrate is determined by reduction to nitrite via a copperized cadmium column. The nitrite is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediaminie dihydrochloride. The absorbance of the product is measured at 520 nm.

The entire experiment was repeated the following growing season. Results from the two experiments were subjected to combined analysis. Data were analyzed as a randomized complete design with 3 replicates and 10 plants per treatment in each replication. Data were subjected to analysis of variance and mean separation, using Duncan multiple range tests.

RESULTS AND DISCUSSION

The effect of NO_3^-: NH_4^+ ratios on fruit fresh weight (yield) and number of fruits were significant ($p \leq 0.01$). The highest yield was obtained in the NO_3^-: NH_4^+ ratio of 5.5:1.5 and other treatments were not different from each other statistically (Fig 1). It shows that adding ammonium to the nutrient solution will increase the strawberry yield. It agrees to the results of Ganmore-Neumann and Kafkafi (1983), which showed that, adding ammonium to the nutrient solution less than 50% of total nitrogen (based on the temperature & plant growth stage) would increase the yield of strawberry. Changes in fruit number had the same pattern as yield. The better growth of strawberry plants in a mixture of NO_3^-: NH_4^+ is maybe because of the neutralization of H^+ and OH^- production (Van Tuil, 1965) in the nitrogen metabolism of NH_4^+ and NO_3^-, respectively. It is probable that minimum stress is inflicted on the cell when minimal pH changes occur.

The total nitrogen concentration in leaves, roots, and fruits of strawberry are significantly higher in the nutrient solutions containing 1 or 1.5 mM NH_4^+ than the solution lacking ammonium (Fig 2). It suggests that in the ratios of 6:1 or 5.5:1.5 (NO_3^-: NH_4^+), plants can assimilate more nitrogen as compared to solutions with very low or null ammonium. Total nitrogen content in leaves and fruits in the ratio of 6:1 (nitrate: ammonium) is the highest while in roots the highest N content was found in the ratio of 5.5:1.5, which shows a big difference with the ratio of 6:1 (Table I). It has been seen in ryegrass that nitrogen uptake rates for plants supplied with NO_3^-+NH_4^+ were similar or higher than those plants provided with NO_3^- alone (Sagi & Lips, 1998). In strawberry total uptake of nitrogen was higher when both nitrogen sources were present in the solution (Ganmore-Neumann & Kafkafi, 1983). Leaf N concentration at NO_3:NH4 ratios of 6:1 and 5.5:1.5 are averaged 34 mg.g^{-1} DW, which is near the 30 mgg^{-1} considered to be sufficient for strawberry (Albregts & Howard, 1981).

The NO_3^- content of leaves and fruits showed fluctuations in the treatments. We found an opposite trend in NO_3^- content changes in leaves and fruits of strawberry. Our data showed that increasing leaf nitrate would decrease fruit nitrate content. The highest leaf NO_3^- concentration was found in the nitrate: ammonium ratio of 5.5:1.5 but in this treatment fruit nitrate concentration is in the lowest category among the treatments (Fig 3).

Table I. The nitrogen concentration of various strawberry tissues under different NO_3: NH_4 ratios

NO_3^-: $NH4^+$	Total N concentration (%)		
	Leaf	Root	Fruit
7:0	3.33b	1.25c	1.42d
6.5:0.5	3.32b	1.36b	1.45c
6:1	3.42a	1.37b	1.51a
5.5:1.5	3.41a	1.63a	1.47b

Different letters are significantly different at $p \leq 0.01$

The activity of leaf NR in the vegetative stage is affected by differences in external NO_3^-: NH_4^+ ratios (Fig. 4). Leaf NR activity in the ratio of 6:1 showed the most activity while the activity of nitrate reductase in the ratio of 5.5:1.5 (NO_3^-: NH_4^+) showed the least activity and similar to the ratio of 7:0. It suggests that increasing the amount of ammonium in nutrient solutions will increase the NR activity but it has a threshold and increasing the ammonium more than the critical level will not increase the leaf NR activity (Fig. 4). Therefore with choosing a proper ratio of different nitrogen sources, the NR activity will increase and this will increase the nitrogen assimilation and nitrogen efficiency of strawberry. The activity of NR in reproductive stage was not affected by external NO_3^-: NH_4^+ ratios.

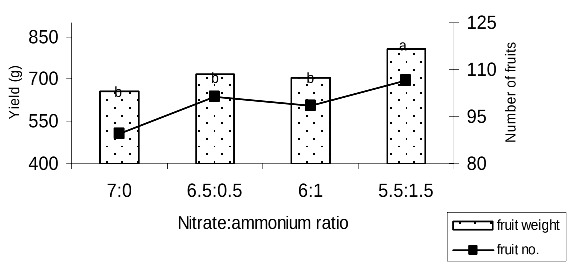
Although Darnell and Stutte (2001) showed that in strawberries grown in nitrate as the only nitrogen source, external NO_3^- concentrations more than 3.75 mM did not increase NR activity, but our data suggest that with adding the NH_4^+ in the nutrient solution not more than 15%, the activity of NR will increase. Our results confirm the results of Zornoza and Gonzalez (1998b). They showed an increase in leaf NR activity in spinach (cv. Giant) with increasing external ammonium. In the ratio of 6:1 the increased activity of nitrate reductase in strawberry cv. Selva is related inversely to the amount of NO_3 in leaves. Our results agree to the results of Zornoza and Gonzales (1998a). They also observed fewer nitrates in spinach cultivars having more NR activity. But they did not show any differences in leaf NO_3 content in different nitrate: ammonium ratio supply.

The NR activity in different stages of growth showed that this activity is higher in the vegetative growing stage than the reproductive stage (Fig. 4). Our data is similar to the results of Darnell and Stutte (2001), which showed the higher NR activity during the vegetative growing stage than the reproductive stage. It is maybe due to the higher potential of strawberry for NO_3 uptake in the vegetative stage compare to fruiting stage. Nitrate reductase activity in leaves was 341 and 244 nmolg^{-1} FW/h, during vegetative and reproductive stages respectively. Similar activities were reported by Darnell and Stutte (2001) in strawberry leaves (360 and 236 nmolg^{-1} FW/h in vegetative and reproductive stages, respectively).

CONCLUSION

Strawberry growth, yield and activity of nitrate reductase increased when plants fed with a mixture of

Fig. 1. The effect of NO_3^-: $NH4^+$ ratios on fruit fresh weight (yield) and number of fruits in strawberry cv. Selva



Numbers followed by different letters are significantly different at $p \leq 0.01$.

Fig. 2. The effect of NO_3^-: $NH4^+$ ratios in nutrient solutions on leaf, root, and fruit total nitrogen content of strawberry cv. Selva

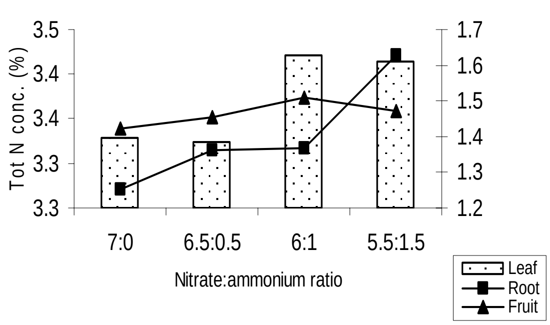
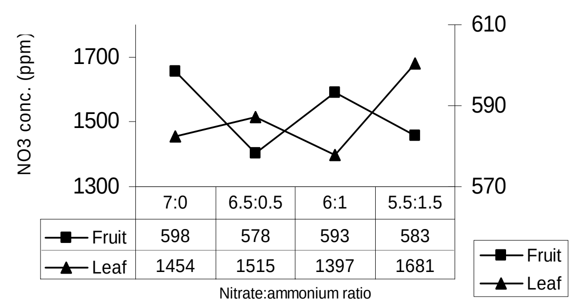
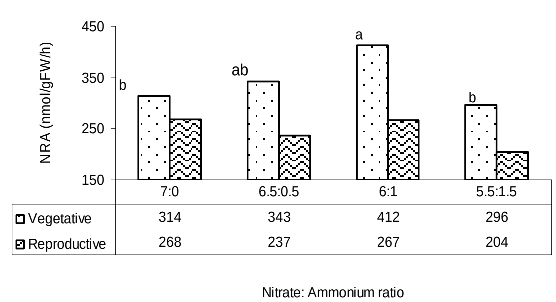


Fig. 3. The effect of NO_3^-: $NH4^+$ ratios in nutrient solutions on nitrate concentration in leaves and fruits of strawberry cv. Selva



The different letters are significantly different at $p \leq 0.01$

Fig. 4. The effect of nitrate: ammonium ratios on leaf NR activity of strawberry cv. Selva during vegetative and reproductive stages



* Different letters are significant at $p \leq 0.05$.

nitrate and ammonium. The leaf nitrate reductase activity affected by different nitrate: ammonium ratios and growth stages. The strawberry plants need more ammonium than nitrate in the vegetative growth stage. But at bearing stage their need for ammonium will decrease and the NR activity is less affected by external nitrate: ammonium ratios.

The growth and plant developmental stages and the environmental conditions influence the response of strawberry plants to different nitrate: ammonium ratios in the nutrient solutions. Generally ammonium must not exceed more than 50% of total nitrogen present in the nutrient solution. But increasing ammonium to 16% (6:1 ratio of NO_3^-: NH_4^+) has a marked effect on nitrogen status of strawberry plants.

Leaf and fruit total nitrogen concentration and fruit nitrate content of strawberry cv. Selva were higher in the ratio of 6:1 while total yield, root total nitrogen and leaf nitrate were higher in the ratio of 5.5:1.5 (NO_3^-:NH_4^+). The addition of ammonium improved yield and nitrogen content of strawberry tissues. The level of added ammonium must be regulated according to cultivars and environmental conditions.

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