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The effect of nitrate and plant size on nitrate uptake and *in vitro* nitrate reductase activity in strawberry (*Fragaria* × *ananassa* cv. Selva)

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Abstract

Nitrogen plays a major role in the growth and yield of strawberry. For optimizing nitrogen fertilizer application, it is necessary to understand the response of strawberry to nitrogen supply. The objective of this study was to determine the effect of nitrate supply and plant size on strawberry nitrate uptake and nitrate reductase activity (NRA). Strawberry plants cv. Selva were grown under growth chamber conditions in nutrient solutions containing 0, 0.1, 0.25, 0.7 or 4 mM nitrate. Cumulative nitrate uptake increased with the increase in nitrate supply. Increasing nitrate concentrations from 0 to 0.25 mM decreased leaf NRA; however, further increases to 4 mM nitrate restored NRA to activities observed at 0 mM nitrate. The activity of NADH- and NADPH-dependent NR was similar at every external nitrate concentration. Activity of NR was greater in the smallest plants and decreased as plant size increased. However, increasing external nitrate concentration increased nitrate uptake, but had not the same effect on nitrate reductase activity, showing that NRA and nitrate uptake are not necessarily correlated.

Keywords: *Fragaria* × *ananassa*; Hydroponic culture; Nitrate uptake; Nitrate reductase; *In vitro* activity; Nitrogen

1. Introduction

As in many other plants, nitrogen plays a key role in strawberry growth and yield. Too much nitrogen can cause poor fruit quality, excessive plant growth and an increase in number of runners (Voth *et al.*, 1967; Welch and Quick, 1981). Nitrogen management in strawberry plantations is difficult because its shallow roots need regular irrigation and it leads to high nitrate leaching. For minimizing nitrogen fertilizer applications, decreasing production costs and diminishing underground water contaminations, it is necessary to understand the response of strawberry to nitrogen supply. This necessitates the development of an understanding of the factors that regulate nitrogen uptake and assimilation in strawberry.

The effect of nitrogen supply on strawberry yield in field experiments has been the subject of many studies (Locascio and Saxena, 1967; Keefer *et al.*, 1978; Albregts and Howard, 1986; Lamarre and Lareau, 1997). For understanding the effect of nitrogen (uptake and assimilation) in strawberry, plants need to be grown in nutrient solutions (Darnell and Stutte, 2001). There are a few experiments concerning the response of strawberry to nitrogen fertilizers in hydroponic cultures. Ganmore-Neumann and Kafkafi (1983, 1985) have studied the effect of nutrient solutions containing 7 mM nitrogen with different nitrate:ammonium ratios on nitrogen uptake, growth and development of strawberry. Darnell and Stutte (2001) reported the effect of different NO_3^- concentrations (3.75–15 mM) on nitrate uptake, nitrate reduction, yield and growth of strawberry. They concluded that NO_3^- uptake rate increased in response to increasing external NO_3^- concentrations. However, NRA, growth and fruit yield were not affected by treatments. They believed that strawberry growth and yield was limited by its ability to reduce and assimilate nitrate into the tissue and not by its ability to uptake nitrate.

Nitrate is a signal for a series of reactions, which regulate its uptake and subsequent reduction by nitrate reductase (Stitt, 1999; Haba *et al.*, 2001). Recent research showed that signals derived from nitrate are involved in triggering widespread changes in gene expression, resulting in a reprogramming of nitrogen and carbon metabolism to facilitate the uptake and assimilation of nitrate (Stitt, 1999). Nitrate reductase (EC 1.6.6.1) (NR) is the first enzyme in nitrate assimilation in higher plants (Godlewska and Clark, 1996). Investigations concerning this enzyme have been

Abbreviation: NR(A), nitrate reductase (activity)

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reported for many plant species (Campbell, 1990; Oaks, 1994), but there is little information on strawberry (Darnell and Stutte, 2001; Claussen and Lenz, 1999). In this experiment, strawberry plants cv. Selva were grown under different NO_3^- concentrations, lower than those employed by Ganmore-Neumann and Kafkafi (1983, 1985) and Darnell and Stutte (2001), to study the effect of external NO_3^- concentrations ranging between 0 and 4 mM on NO_3^- uptake as well as *in vitro* NRA in the plant. Darnell and Stutte (2001) reported that strawberry NR activity, growth and fruit yield did not increase in nitrate concentrations more than 3.75 mM; therefore, low nitrate concentrations (<4 mM) were applied in this experiment. There is no report on strawberry growth and nitrate uptake or reduction in nutrient solutions with less than 3.75 mM nitrate; therefore, nitrate concentrations were chosen according to previous experiences by Aslam *et al.* (1992). They concluded that nitrate uptake by barley roots follows the Michaelis–Menten kinetics up to an external nitrate concentration of about 0.1 mM. The uptake rates of nitrate then increased linearly at external concentrations between 0.1–0.25 and 0.7–1.0 mM, with a saturation phase occurring between 0.25 and 0.7 mM. Therefore, external NO_3-N concentrations of 0, 0.1, 0.25, 0.7 and 4 mM were employed in this experiment.

Our preliminary experiments showed that NR activity was higher in smaller plants. Schrader *et al.* (1974) also reported higher rate of decay of NR activity in larger leaves of corn, oat and tobacco. However, there is no survey on the effect of plant size on NR activity in strawberry. In this experiment we studied the effect of different nitrate concentrations on strawberry plants in four weight ranges to survey whether bigger plants uptake or assimilate less nitrate and thereby whether we can reduce the supply of nitrate to the plants.

2. Materials and methods

2.1. Plant material

Strawberry runners cv. Selva were rooted in 200 ml beckers filled with a modified one-fourth Hoagland solution with 1 mM nitrate (KNO_3) as the only nitrogen source (Hoagland and Arnon, 1950) for a period of 1 month. The plants were placed in a growth chamber with a 16 h light (photon flux density = 500 μmol m^{-2} s^{-1}) and 8 h dark cycle (Basra *et al.*, 2002). The temperature and relative humidity during growth period were 20/17 °C day/night and 70–75%, respectively. These environmental conditions were applied for growing plants in other stages of the experiment. The solutions were aerated continuously with a tube connected to a central air pump in the laboratory and the pH was closely monitored and maintained at 5.8, being adjusted as needed with H_3PO_4. Old solutions were replaced by new ones every week and deionized water was added regularly to make up for the water loss via evapo-transpiration (Zornoza and Gonzalez, 1998).

2.2. Experimental design

The experiment was conducted in a complete randomized design with two factors (plant size and nitrate concentration) and four replicates. Rooted daughter plants, which were in vegetative stage, were weighted and grouped in four different size ranges (1–3, 3–5, 5–7 and 7–10 g). Five different nitrate concentrations (0, 0.1, 0.25, 0.7 and 4 mM) were prepared by adding appropriate amounts of nitrate (KNO_3) to one-fourth Hoagland solutions. Potassium sulphate and calcium chloride were added to 0, 0.1, 0.25, 0.7 mM nitrate solution to ensure the same level of potassium as in the 4 mM nitrate solution. Solution pH was checked regularly and maintained at 5.8. All plants were kept in the same environmental conditions as explained earlier. One plant was placed in a 2-L bucket. Before initiation of the experiment, the plants were kept in the respective nitrogen concentrations for 1 week to achieve steady state with the new nitrate concentrations in the solutions. The experiment was then initiated by refreshing the nutrient solutions and continued for 3 more days.

2.3. Nitrate uptake measurements

Nitrate concentrations were monitored three times a day (every 8 h) by using HPLC method (Thayer and Huffaker, 1980). The HPLC system used in this study comprised a pump (model LC-6A), a UV spectrophotometric detector (model SPD-6A), a data system (model CR-5A) (all Shimadzu Scientific Instruments, Columbia, MD, USA) and an injector (Rheodyne, Cotati, CA, USA). The HPLC column was packed with Partisil-10 SAX (Whatman, Clifton, NJ, USA) and 30 mM KPO_4 (pH 3.0) was used as the eluant.

Adequate nitrate was added to keep the nitrate concentration constant. Deionized water was added regularly to compensate for evapo-transpiration losses. Solutions were aerated continuously for oxygen supply and thorough mixing. Extreme care was exercised to maintain the nitrate concentration as constant as possible. Nitrate uptake was measured as the amount taken up from the nutrient solution. Total uptake of nitrate was assessed as the total of added nitrate plus the difference between initial and final concentration of nitrate, multiplied by the volume of the solutions (Simonne *et al.*, 1992).

2.4. In vitro NRA

2.4.1. Enzyme extraction

At the end of the experiment, plants that were in vegetative stage had two to three young leaves. Fresh weight of root, crown, and leaves averaged 2.0, 0.6 and 1.4 g, respectively. All leaves (except petioles) were quickly washed with distilled water, blotted with paper towel, and then frozen in liquid N_2 in a precooled mortar. As the leaves were young, the veins have not been removed. After evaporation of the liquid N_2, the tissue was rapidly ground to a powder, while still in a frozen state. Insoluble polyvinylpolypyrrolidone (PVP) was quickly added and mixed with the leaf powder. Then the extraction buffer (pH 8.2) containing 0.2 mol Tris–HCl, 1 mmol EDTA, 5 μmol FAD and 2 mmol β-mercaptoethanol per liter, plus 0.025% (w/v) casein milk powder was added and quickly mixed, while being frozen in the mortar. The insoluble PVP and extraction medium