



Effect of Nutrient Starvation on Some Aspects of Nitrogen Metabolism In Substrate-Grown Strawberry Plantings Cv. Nyoho

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Summary: Three-week nitrogen (N) supply restriction is a way to stimulate early and uniform flower induction in forced June-bearing nursery strawberry production in Japan. In the first weeks after planting into excessively-fertigated substrates, N-starved plantings undergo drastic changes in their N utilization and N metabolism, often facing symptoms of interveinal chlorosis in their young expanding leaves. Interveinal chlorosis is reflected in delayed plant development, and consequently in yield reduction. Characterizing variations in main parameters of N metabolism in plant tissues in N-fed and N-starved strawberry plantings cv. Nyoho, this report portrays the pattern of changes in N metabolism during the nursery period. Results show that foliar $\text{NH}_4\text{-N}$ and petiole $\text{NO}_3\text{-N}$ concentrations and N-assimilating enzymes activities in plant tissue were significantly reduced due to the N-starvation, making young plants unlikely to be adaptive to N rich nutrition after planting, and that subsequent $\text{NH}_4\text{-N}$ accumulation in plant tissues can lead to sever interveinal chlorosis.

Key words: chlorosis, nitrogen metabolism, plant tissues, strawberry

Introduction

Melons, watermelon, and strawberries are all classified as vegetables in Japan, and constitute 30% of total greenhouse vegetable area (Nichols & Christie 2008). For a large portion (6360 ha) of greenhouse space in this country, 25000 farms produce strawberry (Yoshida 2013), mainly forced and semi forced. Forced June-bearing strawberry production involves flowering induction in autumn by a number of artificial methods, growing plants under heated greenhouse to avoid the induction of dormancy and dwarfing, and keeping them in a semi-dormant state to allow continued harvesting (Yamasaki 2013). As reported by Petrovic et al. (2009), one method of stimulating early and uniform flower induction in forced June-bearing strawberry production is restriction of Nitrogen (N) supply for pot- or tray-grown nursery plants in late August. Hence, N-starved nursery plants must

undergo drastic changes in their N utilization and N metabolism shortly after planting into highly fertigated substrates in early autumn. Petrovic & Yoshida (2013) reported that Japanese strawberry growers often face problems of interveinal chlorosis in young expanding (immature) leaves in such plants 10-20 days after planting. In 'Nyoho' strawberry, a cultivar that often suffers from these chlorotic symptoms, this is reflected in a delay in plant development, and consequently in yield reduction. Accompanied by observations from growing practice, this led to a hypothesis that interveinal chlorosis phenomenon in strawberry leaves may be due to drastic changes in plant N nutrition and subsequently excess $\text{NH}_4\text{-N}$ accumulation in plant tissues, just after planting (Petrovic et al. 2009, Petrovic & Yoshida 2013).

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Petrovic et al. (2009) and Petrovic & Yoshida (2013) thoroughly investigated changes that occur in the absorption and metabolism of N in substrate-grown strawberries immediately after transplanting. An insight into the modifications of N metabolism in strawberry plants during the nursery period, when N supply for nursery plants is stopped, is of great importance for obtaining a complete picture of the interveinal chlorosis phenomenon (ICP) in strawberry plants, and potentially in other crops. Characterizing variations in the main parameters of N metabolism in plant tissues (ammonium ($\text{NH}_4\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$) leaf concentrations and leaf and root activities of nitrate reductase (NR) and glutamine synthetase (GS)) in N-fed and N-starved strawberry plants, this report illustrates that the pattern of changes in N nutrition of strawberry plants is a priori for planting, and furthers our understanding of the possible mechanisms controlling the ICP.

Materials and Methods

Experimental strawberry plants (*Fragaria x ananassa* Duch. cv. Nyoho) were transplanted to a greenhouse and planted into 16 l peat bags (eight plants per bag) on 20 September 2008 (Fig. 1). They were supplied with 30%-50% (v/v) 'Ohtsuka A' nutrient solution (Tab. 1) and grown as strawberries for commercial purposes. The bags were fertigated using a drip irrigation system comprising two drippers with an output of 2 l h^{-1} each 0.80 m peat bag (Petrovic & Yoshida 2013).

On May 26th 2009 runner tips from mother 'Nyoho' plants were cut (Fig. 2A) and rooted into tray containers (cell volume=130 ml, Suku-suku Tray, Marusan Sangyo, Tochigi, Japan) (Fig. 2B), and manually supplied with 30 ml of 30% (v/v) 'Ohtsuka A' nutrient solution, once every two days, until July 9 2009 when they were divided into two experimental treatments: (i) plants continued to



Figure 1. Substrate-grown strawberry plants (*Fragaria x ananassa* Duch. cv. Nyoho) just after planting into peat bags. Photo Y. Yoshida.

Graf. 1. Supstratski gajeni živići jagoda (*Fragaria x ananassa* Duch. Sorta „Nyoho”) neposredno nakon sadnje u kese punjene tresetom. Foto J. Jošida.

Table 1. Composition of 'Ohtsuka A' nutrient solution (mmol l^{-1}) (Yoshida et al. 2002).

Tabela 1. Sastav „Ocuka A“ hranjivog rastvora (mmol l^{-1}) (Yoshida et al. 2002).

N			H_2PO_4^-	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}
NO_3^-	NH_4^+	Total					
8.0	0.85	8.85	0.85	3.9	2.05	0.93	0.93



Fig. 2. Greenhouse nursery production of strawberries. Panel A, mother 'Nyoho' plants runners. Panel B, rooted strawberry plantings on tray containers. Photo Y. Yoshida.

Graf. 2. Plastični proizvodnja živića jagode. Slika A, stoloni matičnih biljaka jagode sorte "Nyoho". Slika B, u kontejnerima ukorenjeni živići. Foto J. Jošida.



Figure 3. Strawberry plant habit: a, newly-expanding (immature) leaf. b, immature leaf petiole. c, fully-developed (mature) leaf. d, mature leaf petiole. e, runner (stolon). f, daughter plant. (Hemelrick et al. 1996).

Graf. 3. Habitus biljke jagode. a, novonastali (mladi) list. b, lisna drška mladog lista. c, potpuno razvijeni list. d, lisna drška potpuno razvijenog lista. e, izvodnica (stolon). f, živić (nova biljka). (Hemelrick et al. 1996)

be supplied with 30% (v/v) 'Ohtsuka A' nutrient solution (non-starved control), and (ii) plants supplied only with tap water (N-starved plants).

Substrate moisture of both treatments was maintained via watering on a regular basis. In seven

day intervals starting from day-8, roots, mature and immature leaf and petiole samples (Fig. 3) from ten replicates were removed to determine $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ content respectively, as well as to determine activities of N-assimilating enzymes-NR and GS. Samples were also taken on the day when starvation started for half of the plants (day-1). Each replicate contained three plants ($n=30$).

Determination of $\text{NH}_4\text{-N}$ concentrations in leaf tissues and $\text{NO}_3\text{-N}$ in petiole tissues

Fresh immature and mature leaf and petiole samples were extracted, and concentration of $\text{NH}_4\text{-N}$ in the leaf tissue and $\text{NO}_3\text{-N}$ concentration in petiole tissues determined as described previously by Petrovic et al. (2009). Ammonium concentrations were expressed as $\mu\text{moles NH}_4\text{-N g}^{-1}$ fresh weight ($\mu\text{mol g}^{-1}$ FW) while nitrate concentrations were expressed as $\mu\text{moles NO}_3\text{-N g}^{-1}$ dry weight ($\mu\text{mol g}^{-1}$ DW).

Nitrate reductase (NR) extraction and activity assays

The roots and leaves were removed from plants and composited to form a sample from each treatment. The sample was immersed immediately in cold (2°C) deionized water and carried to the laboratory. These were blotted, weighed, cut into small pieces, and ground in a chilled pestle and mortar with a grinding medium composed of

0.1 M potassium phosphate buffer (pH 7.5), 10^{-3} M cysteine and 10^{-3} M EDTA. Three ml of the grinding medium were added to each gram of the sample. The homogenate was squeezed through nylon cloth and the filtrate was centrifuged for 30 min at 10,000 x g. The supernatant was used as a crude enzyme extract. All procedures were carried out below 5°C. NR was assayed according to Sasakawa & Yamamoto (1977) and expressed as $\mu\text{moles NO}_2^- \text{g}^{-1}$ fresh weight h^{-1} ($\mu\text{mol NO}_2^- \text{g}^{-1} \text{FW h}^{-1}$).

Glutamine synthetase (GS) extraction and activity assays

Fresh leaf tissue and root samples (1 g) were homogenized with four volumes of grinding buffer containing 100 mM Tris-HCl (pH 7.8), 5 mM MgSO_4 , 1 mM DTT, and a small quantity of Polyclar-AT (0.5 g) as an absorbent. The homogenate was squeezed through nylon cloth and the filtrate was centrifuged for 15 min at 15 000 x g at 4°C. The supernatants were collected, kept in ice and used for GS assays. GS was assayed following O'Neal & Joy (1973). Activity of GS was expressed as $\mu\text{moles GH}$ formed per g fresh weight per min ($\mu\text{mole GH g}^{-1} \text{FW min}^{-1}$).

Data are presented as means and standard errors. All statistical analyses were performed using the statistical software package SPSS Version 12.0 (MapInfo Corporation, Troy, NY, USA).

Results and Discussion

N is one of the most limiting factors for plant growth and was assumed to be heavily involved in the flower bud initiation process in strawberries (Fujimoto 1972). N assimilation begins with the uptake of inorganic N, nitrate (NO_3^-) or ammonium (NH_4^+), from the soil. NO_3^- is subsequently reduced to NH_4^+ by the sequential action of NR and nitrite reductase (Oliveira et al. 2002). Further assimilation of NH_4^+ to glutamine is catalysed by the enzymes, GS and glutamate oxoglutarate, acting in concert (Srivastava & Dwivedi 2000).

In the current study, only a week after nutrient supply was stopped, NO_3^- -N and NH_4^- -N concentrations and related NR and GS activities in root and immature leaf tissue in N-starved strawberry plantings were undetectable (Table 2). In the mature leaves of N-starved plantings, these

parameters rapidly decreased so that at the time of the last sampling they could not be detected. In mature leaves, NO_3^- -N and NH_4^- -N concentrations and the enzymes activities of N-starved plants were significantly lower compared to those in non-starved control plantings. Petrovic et al. (2009) reported severe interveinal chlorosis in cv. Nyoho in plants that exhibited drastic increase in foliar NH_4^- -N concentrations, induced by the supply of NH_4^- -N rich nutrient solution or by GS inhibitor treatments. Regardless of the treatment applied, foliar NH_4^- -N concentrations followed a uniform pattern in the post-planting period, peaking 9 to 11 days after the transplantation, before dropping steadily (Petrovic et al. 2009, Petrovic & Yoshida 2013). Raab & Terry (1995) reported similar daily patterns in NH_4^- -N changes in leaves of NH_4^- -N-fed sugar beet. The increase in NH_4^- -N within the first ten days after planting (Petrovic & Yoshida, 2013) suggests that lower NR and GS activities occurred and thereby disturbed the synthesis of glutamine from glutamate and ammonium (Manderscheid & Wild 1986). Drastic changes in N nutrition in nursery N-starved 'Nyoho' plants may be an initial cause of such imbalances (Petrovic & Yoshida 2013).

In the control group, non-starved strawberry plants petiole NO_3^- -N and foliar NH_4^- -N concentrations, though low, (Table 2) were detected. NO_3^- -N and NH_4^- -N concentrations in mature leaves, and activities of the N assimilating enzymes, were significantly higher compared to the values measured in immature leaves and roots. The values of examined parameters were more similar in roots and immature leaves.

The results show that during starvation in the nursery period, foliar NH_4^- -N and petiole NO_3^- -N are undetectable and N-assimilating enzymes activities are low already after only a week of stopping nutrients. Under such conditions after the planting into peat bags, NR and GS activity cannot respond fast enough to the extreme increase in NH_4^- -N concentrations in the plant tissues, causing an imbalance in N-assimilation. If a plant fails to assimilate the NH_4^- -N taken up or generated in leaves, concentrations can increase to toxic levels (Mifflin & Lea 1980). Numerous authors attribute NH_4^- -N toxicity to its accumulation, especially when it occurs in photosynthetic tissues (reviewed in Marques et al. 1983). The reported NH_4^- -N toxicity symptoms vary widely, and often

Table 2. Foliar $\text{NH}_4\text{-N}$ ($\mu\text{mol g}^{-1}\text{FW}$) and $\text{NO}_3\text{-N}$ ($\mu\text{mol g}^{-1}\text{DW}$) concentration and NR ($\mu\text{mol NO}_2\text{ g}^{-1}\text{FW h}^{-1}$) and GS ($\mu\text{mol GH g}^{-1}\text{FW min}^{-1}$) activity in leaves and roots of 'Nyoho' plants supplied with tap water (N-starved plants) and with a 30% 'Ohtsuka A' nutrient solution (non-starved plants, control) for three weeks (sampling day-1, -8, -15 and -22). The values are mean \pm SE for 30 replicates plants.

Tabela 2. Sadržaj $\text{NH}_4\text{-N}$ ($\mu\text{mol g}^{-1}\text{FW}$) i $\text{NO}_3\text{-N}$ ($\mu\text{mol g}^{-1}\text{DW}$) u listovima i aktivnosti NR ($\mu\text{mol NO}_2\text{ g}^{-1}\text{FW h}^{-1}$) i GS ($\mu\text{mol GH g}^{-1}\text{FW min}^{-1}$) u lišću i korenu jagoda sorte "Nyoho" snabdevanih samo vodom (izgladnjivane biljke) i prihranjivanim biljkama (kontrola) tokom tri nedelje (prvi, osmi, petnaesti i dvadeset drugi dan uzorkovanja). Predstavljene vrednosti su proseci \pm SE za 30 replika.

Plant part /Biljni organ	Parameter Parametar	N-starved plants Izgladnjivane biljke						Non-starved plants (control) Prihranjivane biljke (kontrola)					
		Days of treatment Dan ogloda						Days of treatment Dan ogloda					
		1	8	15	22	1	8	15	22	1	8	15	22
Immature leaf Mladi list	$\text{NH}_4\text{-N}$	0.25 \pm 0.03	*	-	-	-	0.21 \pm 0.04	0.24 \pm 0.03	0.21 \pm 0.03	0.23 \pm 0.01			
	$\text{NO}_3\text{-N}$	9.54 \pm 0.47	-	-	-	9.57 \pm 0.52	10.06 \pm 0.69	9.68 \pm 0.12	9.78 \pm 0.37				
	NR	<0.05	-	-	-	<0.05	<0.05	<0.05	<0.05				
Mature leaf Potpuno razvijeni list	GS	9.7 \pm 0.33	-	-	-	10.2 \pm 0.40	11.1 \pm 0.38	9.9 \pm 0.51	10.0 \pm 0.26				
	$\text{NH}_4\text{-N}$	0.86 \pm 0.3	0.12 \pm 0.01	0.03 \pm 0.02	-	0.83 \pm 0.3	0.73 \pm 0.4	0.81 \pm 0.3	0.92 \pm 0.25				
	$\text{NO}_3\text{-N}$	17.01 \pm 0.23	5.81 \pm 0.03	1.48 \pm 0.07	-	19.26 \pm 0.17	17.43 \pm 0.21	16.95 \pm 0.33	15.65 \pm 0.19				
Root Koren	NR	0.79 \pm 0.13	0.28 \pm 0.03	0.13 \pm 0.01	-	0.81 \pm 0.11	0.7 \pm 0.09	0.8 \pm 0.09	1.0 \pm 0.32				
	GS	53.2 \pm 0.17	3.7 \pm 0.02	1.2 \pm 0.05	-	56.2 \pm 3.72	60.7 \pm 2.85	49.8 \pm 3.07	54.2 \pm 2.66				
	NR	<0.05	-	-	-	<0.05	<0.05	<0.05	<0.05				
	GS	12.1 \pm 0.6	-	-	-	11.5 \pm 0.1	13.7 \pm 0.28	9.8 \pm 0.12	11.3 \pm 0.33				

*Not detected/Nije detektovan

include interveinal chlorosis (Claussen & Lenz 1995, Claussen & Lenz 1999). According to Puritch & Baker (1967), degeneration of the ultrastructure of chloroplasts caused by NH_4^+ toxicity and manifested by NH_4^+ accumulation, decreases photosynthesis and causes foliar chlorosis and necrosis in the tomato. Having low photosynthetic activity soon after planting, strawberry plants are unlikely to adapt to N rich nutrition, and thus it can be argued that accumulated $\text{NH}_4\text{-N}$ leads to the expression of ICP in leaves in 'Nyoho' plants.

Preliminary investigations of Petrovic & Yoshida (2013) showed severe interveinal chlorosis occurrence in Nyoho plants grown in excessively-fertigated peat bags and frequently fertigated substrates. Such plants recovered after less severe yellowing due to a 4-7 day restraint in the supply of nutrients. These observations led to a hypothesis that ICP in strawberry leaves may be due to drastic changes in plant N nutrition and subsequently excess $\text{NH}_4\text{-N}$ accumulation in plant tissue just after planting (Petrovic & Yoshida 2013).

Conclusions

As a result of three-week nutrient-starvation in strawberry plants during their nursery period, NR and GS enzymes activities in plant tissue are significantly reduced, and it takes time to balance N assimilation process following planting into excessively-fertigated substrates. Hence, interveinal chlorosis phenomenon in young strawberry leaves due to drastic changes in plant N nutrition and subsequently excess $\text{NH}_4\text{-N}$ accumulation in plant tissues is not rare (Petrovic et al. 2009, Petrovic & Yoshida 2013). However, the exact mechanisms of the ICP in substrate-grown strawberries are yet to be addressed.

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Efekat gladovanja na različite aspekte metabolizma azota u supstratski gajenom rasadu jagoda sorte Nyoho

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Izvod: Dinje, lubenice i jagode u Japanu pripadaju povrtarskim vrstama. Poslednja dekada avgusta je period kada prestaje svako đubrenje, a pogotovo đubrenje azotnim đubrivima sadnica rasada jednorodnih (junskih) jagoda namenjenih gajenju u plastenicima u uslovima forsirane proizvodnje. Ovako "izgladnele" biljke se krajem septembra presađuju u supstratske podloge (plastične kese punjene tresetom) koje se zatim intenzivno đubre, čime se podstiče ranije i uniformnije zametanje cvetova. Rezultati ove studije su pokazali da tokom gladovanja u fazi rasada koncentracije amonijačnog i nitratnog jona i aktivnosti enzima koji učestvuju u asimilaciji azota u biljnom tkivu značajno opadaju. Nakon presađivanja u supstratske podloge, ove "izgladnele" mlade biljke su teško adaptibilne na uslove intenzivne prihrane azotom, te u svom tkivu akumuliraju amonijačne jone što neretko rezultira intervenalnom hlorozom mladog lisnog tkiva. Hlorotične biljke zaostaju u razvoju i daju smanjene prinose.

Gljučne reči: hloroza, metabolizam azota, biljna tkiva, jagode