10. Assimilation of the newly absorbed nitrate in response to water-deficit stress intensity estimated by 15N tracing in whith clover

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화이트 클로버에서 15N 추적에 의한 수분결핍 스트레스 강도에 따른 질산태 질소의 흡수와 동화

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Key words : Amino acid synthesis, Ammonia, Continuous ^{15}N feeding, Protein, Water-deficit.

<Objective>

During prolonged periods of drought, the decrease in water availability for transport-associated processes leads to changes in the concentrations of many metabolites followed by disturbances in amino acid and carbohydrate metabolism. In particular, changes in the concentration and composition of the N-soluble fraction in response to water-deficit (Girousse et al., 1996; Lazcano-Ferrat and Lovatt, 1999) have been demonstrated in a wide range of species. Despite extensive research attempting to elucidate the roles of compatible N-solute accumulation in ameliorating the detrimental effects of abiotic stresses on plant growth and yield, the physiological significance of their accumulation remains equivocal. The present work was designed to investigate the kinetics of *de novo* synthesis of amino acid and protein as ammonia concentration varied in response to the intensity of water-deficit stress.

<Materials and Methods>

White clover (*Trifolium repens* L. cv. Regal) was regularly watered to field capacity until full vegetative stage (about 18 weeks after sowing). At this stage, water-deficit stress was imposed by decreasing the volume of water supply per day during 7 day. For ^{15}N feeding for the well-watered (control) treatment, 25 mL of ^{15}N solution (1 mM $K^{15}NO_3$ with 8.34 ^{15}N atom % excess) were administered evenly through three tubes placed on each pot at 10:00 h and 16:00 h, respectively. For the water-deficit treatment, 2.5 mL of ^{15}N solution, containing same N amount applied to control pot (corresponding 0.7 mg N per pot per day) with same ^{15}N atom %, was fed as described for control plants. The $^{15}NO_3^-$ feeding was done everyday throughout the entire sampling period (during 7 days).

<Results>

The physiological relationship between ammonia concentration, in response to the change in leaf water parameters, and *de novo* synthesis of amino acid and protein was also assessed. Leaf and root dry mass was not significantly affected for the first 3 days, whereas metabolic parameters such as total N and ammonia were significantly affected within the first day of water-deficit treatment. Inhibitory effect of water-deficit on N acquisition from the soil was significant throughout the experimental period. Water deficit induced significant increase in ammonia concentration in leaves during the first 3 days, and in roots for only the first day. In both leaves and roots, an increase in *de novo* amino acid synthesis was observed, which peaked in leaves within the first 3 days of water-deficit treatment ($\Psi_w = -1.18$ MPa). The rate of decrease in *de novo*

protein synthesis gradually accelerated as the duration of the water-deficit treatment increased. There was a significant positive relationship between ammonia production and the increase in *de novo* amino acid synthesis during the first 3 days period, but not during the latter period (day 3 to day 7). This experiment clearly indicates that the increase in *de novo* amino acid synthesis caused by water-deficit is a transient adaptive response occurring during the first few days and that it is associated with the increased ammonia concentrations which in turn arise in response to a decrease in *de novo* protein synthesis.

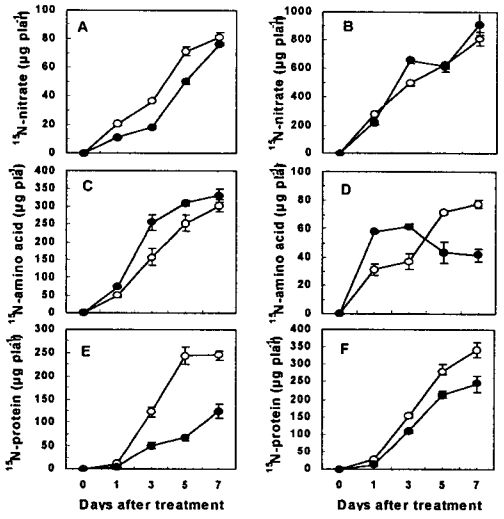

Fig. 1. Changes in the amount of ^{15}N in the nitrate, amino acid and protein fractions of leaves(A; C; E) and roots(B; D; F) under well-watered(○, control) or water-deficit(●) conditions.

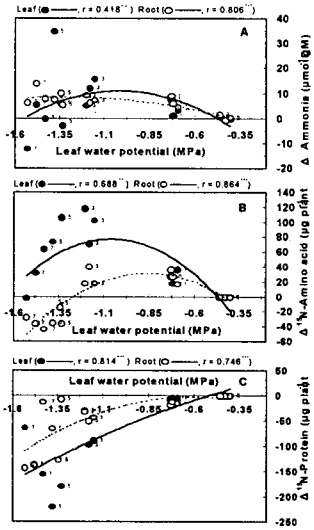

Fig. 2. Relationships between leaf water potential in the water-deficit stressed plants and NH_3-NH_4^+ concentration(A), ^{15}N amount in amino acid(B) and ^{15}N amount in protein fraction(C) as affected by water-deficit treatment in leaves(ciosed symbol) and roots(open symbol).

Table 1. Water-deficit effects on dry mass, total N and NH_3-NH_4^+ concentration in leaves and roots during 7d of experimental period.

Measurement	Organ	Days after treatment				
		0	1	3	5	7
Dry weight (% of control)	Leaves	98.9^{a}	98.4^{a}	95.4^{ab}	89.0^{b}	93.1^{ab}
	Roots	99.8^{a}	109.9^{a}	102.6^{ab}	89.6^{c}	85.3^{cd}
Total N (% of control)	Leaves	100.4^{a}	89.5^{b}	89.0^{b}	88.6^{b}	83.7^{bc}
	Roots	99.6^{ab}	84.2^{c}	91.8^{bc}	107.2^{a}	108.7^{a}
NH_3-NH_4^+ (% of control)	Leaves	101.8^{c}	108.3^{b}	126.1^{a}	128.4^{a}	96.4^{cd}
	Roots	99.9^{c}	124.0^{ab}	125.3^{ab}	131.8^{a}	133.2^{a}

Table 2. Linear correlation between NH_3-NH_4^+ concentration and ^{15}N amount incorporated into amino acid and protein fraction from the newly absorbed N, as affected by water-deficit treatment in leaves and roots during different periods of water-deficit stress

	△ NH_3-NH_4^+ concentration (mol g^{-1} DM) Period of water-deficit stress		
	0 - 3 d (n = 9)	3 - 7 d (n = 9)	Full period (n = 15)
△ ^{15}N-amino acid (g plant-1)			
Leaves	r = 0.732 *	r = 0.466 $^{n.s}$	r = 0.536 *
Roots	r = 0.786 **	r = 0.214 $^{n.s}$	r = 0.206 $^{n.s}$
△ ^{15}N-protein (g plant-1)			
Leaves	r = - 0.909 ***	r = - 0.516 $^{n.s}$	r = 0.458 $^{n.s}$
Roots	r = - 0.731 *	r = - 0.455 $^{n.s}$	r = 0.665 **