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Effects of Potassium Deficiency on C and N Metabolism during Regrowth of Italian Ryegrass (*Lolium multiflorum* L.)

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칼륨 결핍이 이탈리안 라이그라스 재생기간동안의 탄소와 질소의 대사에 미치는 영향

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ABSTRACT

To investigate C and N metabolisms in response to potassium-deficient stress during regrowth of Italian ryegrass(*Lolium multiflorum* L.), C and N metabolites were analyzed at day 0 (cutting date), 6, 12 and 24 days after defoliation. K-sufficient (control, +K) and K-absent (−K) nutrition solutions were applied from 7 days before defoliation, and continued for one cycle of 24 days-regrowth period. During 24 days of regrowth dry matter of regrowing shoots and remaining tissues were not significantly different between +K and −K treatment. In remaining stubble, all C compounds in both +K and −K treatment largely decreased (69% to 84% of the initial level) during the first 6 days of regrowth, and then rapidly recovered. The decline of soluble sugars and fructan in roots for the first 6 days much less in the −K medium. Amino acids, soluble and insoluble proteins in stubble also fell down during the first 6 days, thereafter actively replenished in both +K and −K treatment. The decline of nitrate in stubble prolonged to 12 days of regrowth. Initial amounts of all N compounds in roots were significantly lower in the −K medium. Higher accumulation of amino acids and soluble protein in roots in the −K medium was observed after 12 days of regrowth. In regrowing shoots, 3 all carbohydrates increased with a very similar pattern for both treatments. Nitrate was not significantly different between two treatments. Depress of soluble protein accumulation in −K medium was noteworthy after 12 days of regrowth. These results indicated that an active utilization of organic reserves occurred to support regrowth even under K deficient condition with a similar extent with K sufficient condition.

(**Key words** : *Lolium multiflorum* L., Potassium deficiency, Regrowth, C and N metabolism)

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I. INTRODUCTION

Studies on the contribution of organic carbohydrate and nitrogen reserves to regrowth after defoliation have reported in forage species. Adaptation to defoliation in many grass species involved a capacity for mobilization of N compounds stored in perennial tissues such as roots and stubble, allowing N to be supplied to the growing zones despite the decline of N uptake by roots that usually occurred as a response to defoliation (Volenec et al., 1996). Also, N reserves are mobilized to regrowing shoots in *Medicago sativa* (Ourry et al., 1994; Kim et al., 1991), especially during the first 10 days of shoot regrowth, when the utilization of exogenous N (mineral N and atmospheric N_2) is severely limited.

Enough K nutrition is very important for regrowing shoot after defoliation and for nodule number, nodule mass and N_2-fixation of alfalfa (Duck et al., 1980) and for photosynthetic rates of soybean(Chevalier, 1977). Potassium deficiency reduces the CO_2 assimilation rate in many higher plants. Alleviation of K deficiency increases photosynthesis (Collins and Duck, 1981), and carbohydrate assimilation (Matches et al., 1963). Ajayi et al.(1970) demonstrated that inadequacy of K nutrition occurred sever stem lesions. They conclued that potassium enhanced NH_4^+ assimilation into amino acids preventing accumulation of NH_4^+ to toxic concentration. Less is known about how K nutrition effects the C and N metabolism in remaining organs (stubble and roots) after defoliation. Mainly in intact plants, the influence of K nutrition on C and N metabolism has been examined in various crop species. However, K nutrition with respect to the accumulation and utilization patterns of C and N reserves has not been thoroughly studied in regrowing Italian ryegrass (*Lolium multiflorum* L.).

The objectives of this study were 1) to determine pool size of main C and N metabolites in remaining organs (stubble and roots) after defoliation 2) to explore the partitioning of organic reserves during regrowth in response to K nutrition.

II. MATERIALS AND METHODS

1. Plant material and growth conditions

Italian ryegrass (*Lolium multiflorum* L.) seeds were sterilized and germinated in a sand bench. When three leaf stage was developed, 5 seedlings were grown hydroponically on 3 L pot with continuous aeration. The nutrient solution was prepared as described by Kim et al. (1991), and renewed every 6 days. Plants were grown in growth chamber with a 18/6 h of light/dark photo period and a 25/20℃ of thermo-period. When the plants were a full-vegetative stage (98 day after planting), nutrient solutions modified to give 3 mM K (K-sufficient, +K) and 0 K-free (K-deficient, −K) were supplied from 1 week before defoliation. Plants were then cut at a height of 6 cm above the roots base and regrowth allowed for 24 days under +K and −K medium. Samplings were carried out on 0, 6, 12 and 24 days after defoliation with separating regrowing shoots, stubble and roots. Tissue samples were frozen with liquid nitrogen, lyophilized, finely ground and stored at vacuum desiccators for further analysis.

2. Carbohydrate analysis

Soluble sugars were extracted with 92% ethanol. Tubes were shaken for 10 min at room temperature, centrifuged at 14,000 × g for 10 min at 4℃ and supernatant was retained. The ethanol extraction was repeated more than twice, and combined supernatant. The soluble sugars contents from the supernatant were determined with anthrone reagent (Van Handel, 1968) using glucose as a standard. The residue was dried at 80℃ to remove ethanol. Deionized water was added, and heated to gelatinize the starch. The pH of the solution was adjusted to 5.1 by adding 0.2 N Na-acetate buffer. Starch was digested by adding amylo-glucosidase (Sigma A3514) and α-amylase (Sigma A0273) in the acetate buffer to each sample. Tubes were incubated at 55℃ for 24 h with occasional shaking. Tubes were centrifuged as

described and glucose in the supernatant was determined using glucose oxidase (Glucose Trinder, Sigma 315-100). Starch concentrations were estimated as 0.9 × glucose concentration. Fructans present in the starch extracts was hydrolyzed with 0.1 N H_2SO_4 and fructose released quantified using resorcinol (Davis and Gander, 1967). Glucose liberated from the fructan was determined as descrived, and fructan concentration was calculated as the sum of fructan glucose and fructose × 0.9.

3. Nitrogenous compounds analysis

About 200 mg of finely ground freeze-dried sample was extracted with 80% ethanol while heated on a hot plate for 5 min. The 80% ethanol-soluble fraction was filtered over a filter paper (Toyo-rhoshi No. 5a), centrifuged, and passed through a H^+ column (Dowex 50W × 8). The solution collected from the H^+ column was adjusted to pH 7.0 and concentrated to a final volume of 0.5 mL (nitrate fraction). Amino acids were eluted from the Dowex 50W × 8 column with 25 ml of 0.5 N HCl and concentrated to 1.0 mL. Concentration was done by drying each collected solution by rotary vacuum evaporation and re-dissolving the residues in distilled water to obtain the final volume of each fraction as described above. Ethanol-insoluble fraction on the filter paper after filtration were dried at 60°C for 24 h to obtain dry weights and used for the insoluble protein determination. Soluble protein was extracted with 100 mM $NaPO_4$ buffer (pH 6.8) according to the method described by Li et al. (1998), and concentrated to 1.0 mL. N determination of all fractions was performed using N single mode analysis on a ANCA-SL mass spectrometer (Europa Scientific, Crewe, UK).

III. RESULTS

1. Dry matter accumulation

Shoot regrowth of potassium-sufficient (+K) and potassium-deficient (−K) plant was not significantly different (Fig. 1A). Dry weight in regrowing shoots increased slowly for the first 6 days, thereafter increased rapidly from 12 d to 24 d. After 24 days of regrowth, dry weight of regrowing shoot (2.11 mg/plant) completely recovered the initial level (2.13 mg/plant, day 0). Dry weight of roots was less varied without significant difference between +K and −K treatment during entire regrowth period (Fig. 1B).

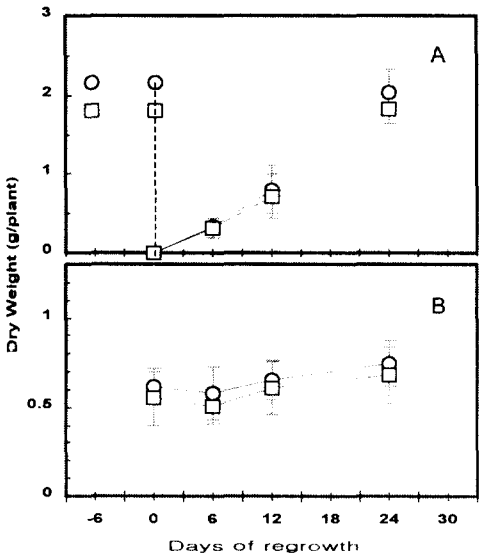

Fig. 1. Changes in dry weight in the regrowing shoots (A) and remained roots (B) during 24 days of regrowth. K-sufficient (+K) and P-deficient (−K) treatment were applied from 6 day before cutting, and renewed every 6 days. Dashed line indicates before defoliation. Each value is the mean ±S.E. for n=5.

2. Changes in organic reserves in remaining organs

At day 0 (cutting date), when +K or −K treatment was applied previously during 1 week, total amount of carbohydrate compounds (soluble sugars, starch and fructan) in stubble was not significantly

different between +K (139.7 mg/plant) and −K (135.3 mg/plant) treatment. More than 79% of total carbohydrate in two remaining tissues (stubble and roots) was stored in stubble. During the first 6 days of regrowth, a remarkable decrease (69% to 91% decline of the initial level at day 0) in stubble occurred in all 3 carbohydrates fractions. When compared with the absolute amounts decreased during this regrowth period, the highest decrease appeared in soluble sugars, the secondly in starch, followed by fructans. After 6 days of regrowth, re-accumulation of 3 carbohydrate fraction in stubble actively occurred in both +K and −K medium. The increasing rate of polysaccharide fraction (starch and fructan) during this period was higher in the +K medium on than in the −K medium. In consequence, the contents of polysaccharide at day 24 were significantly higher in the +K medium.

In roots (Fig. 2B), the decrease of soluble sugar and fructan in the +K medium was remarkable (62% and 57% decline of the initial level at day 0) during the first 6 days. After 24 days of regrowth, the content of most carbohydrate compounds (except fructan in the +K medium) completely recovered or exceeded the initial content (day 0) in both +K and −K medium.

At day 0 (cutting day), the contents of all N compounds analyzed in stubble were not significantly different between +K and −K treatment (Fig. 3A). Sum of N compounds in this organ was 48.5 and 44.8 mg/plant, respectively, in the +K and −K medium. This indicated that N utilization and its partitioning into reserve organs were not significantly depressed by −K treatment for 1 week before defoliation. Nitrate content in stubble in the +K and −K medium significantly decreased for the first 12 days and then highly increased in the +K medium, while slightly increased in the −K medium until day 24 (Fig. 3. A1). Amino acids in both +K and −K medium greatly decreased to about half of the initial (day 0) during the first 6 days, and then rapidly recovered (Fig. 3. A2). At day 24, the content of amino acids was largely exceeed the initial level in both two treatments. Soluble proteins also largely fell down (46.1% in +K and 39.3% in −K of the initial level) for the first 6 day (Fig. 3. A3). Insoluble proteins in both +K and −K medium followed a similar pattern (sharp decrease for 6 days

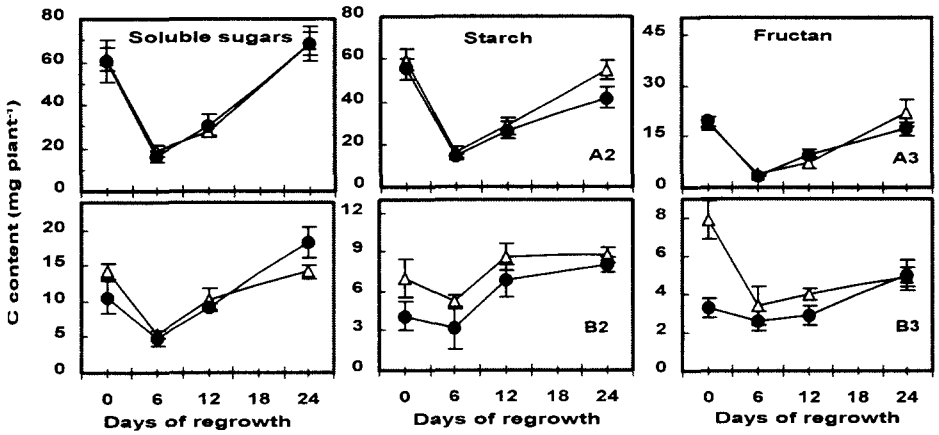

Fig. 2. Changes in soluble sugars (A1), starch (A2) and fructan (A3) contents in stubble remained after cutting at 6 cm above root base and roots (B1-B3, same order) during 24 days of regrowth. K-sufficient (+K) and K-deficient (−K) treatment were applied from 6 day before cutting, and renewed every 6 days. Each value is the mean ± S.E. for n=5.

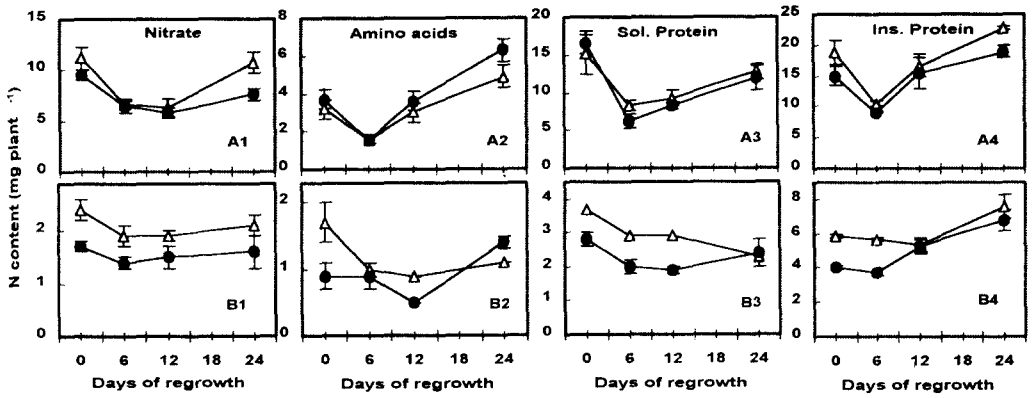

Fig. 3. Changes in nitrate (A1), amino acids (A2), soluble protein (A3) and insoluble protein (A4) contents in stubble remained after cutting at 6 cm above root base and roots (B1-B4, same order) during 24 days of regrowth. K-sufficient (+K) and K-deficient (−K) treatment were applied from 6 day before cutting, and renewed every 6 days. Each value is the mean±S.E. for n=5.

and increase afterward) (Fig. 3. A4).

In roots, at day 0, sum of N compounds examined in both +K and −K treatment was 13.7 and 9.4 mg/plant, respectively. Nitrate in this organs was less varied in both +K and −K medium, and kept on a lower level in −K medium throughout regrowth period (Fig. 3. B1). Amino acids in the +K medium significantly decreased for the first 6 days and then maintained continuously constant level until day 24, while in the −K medium decreased until day 12 (Fig. 3. B2). Soluble proteins in the +K medium gradually decreased during entire regrowth (Fig. 3. B3). A sharp increase of insoluble proteins in the −K medium was remarked after 6 days of regrowth (Fig. 3. B4).

3. Carbohydrates and nitrogenous compounds in regrowing shoots

In regrowing shoots, the accumulation of soluble sugars was remarkably higher than that of polysaccharides. Soluble sugars were not significantly affected by K treatment during entire regrowth (Fig. 4A). Starch contents also were not affected by

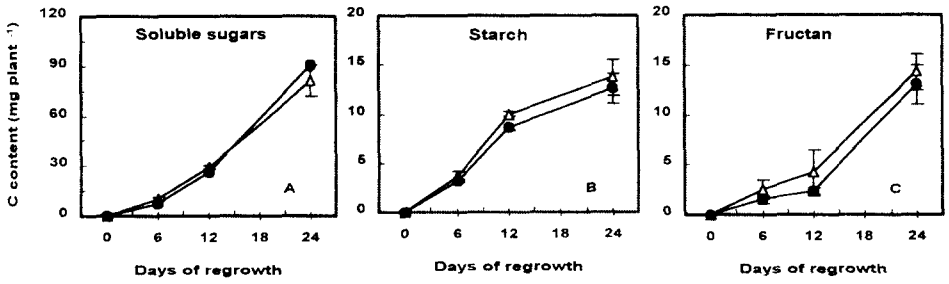

Fig. 4. Changes in soluble sugars (A), starch (B) and fructan (C) contents in the regrowing shoots during 24 days of regrowth. K-sufficient (+K) and K-deficient (−K) treatment were applied from 6 day before cutting, and renewed every 6 days. Each value is the mean±S.E. for n=5.

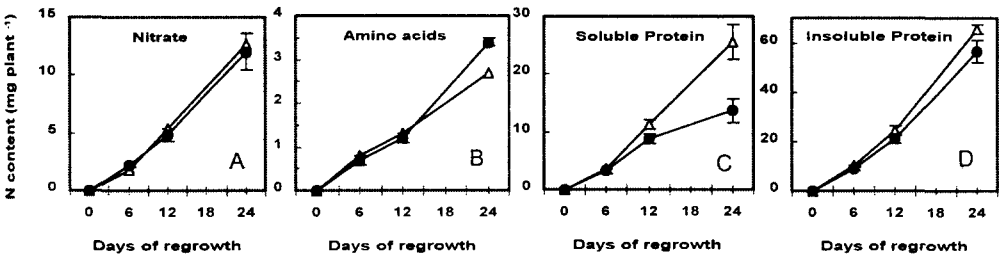

Fig. 5. Changes in nitrate (A), amino acids (B), soluble protein (C) and insoluble protein (D) contents in the regrowing shoots during 24 days of regrowth. K-sufficient (+K) and K-deficient (−K) treatment were applied from 6 day before cutting, and renewed every 6 days. Each value is the mean±S.E. for n=5.

K-treatment during for the first 6 days, thereafter they were slightly higher in the +K medium. At day 24, starch contents in both +K and −K medium were 13.7 and 12.6 mg/plant, respectively (Fig. 4B). Fructan contents remained at low level within 12 days and then sharply increased. At day 24, fructan contents in both +K and −K medium were 14.3 and 13.0 mg/plant, respectively (Fig. 4C).

Nitrate content in regrowing shoots was not significantly different between +K and −K treatment throughout 24 days of regrowth (Fig. 5A). Amino acids were the smallest pool of N compounds examined. Amino acids were not significantly different between +K and −K treatment within 12 days, and then the increasing rate in the −K medium was largely higher (Fig. 5B). Soluble proteins were much sensitively responsed to K treatment. After 6 days of regrowth, their accumulation was largely depressed in the −K medium. At day 24, the content of soluble protein in the −K medium was about half of that of +K treatment (Fig. 5C). Insoluble proteins were the largest pool of N metabolites examined. There is no significant difference for the first 12 days, and then the increase in the +K medium was significantly higher (Fig. 5D).

Ⅳ. DISCUSSION

Non-significant difference in dry weight between +K and −K treatment was observed in either regrowing shoots or roots (Fig. 1). These results suggest that K-deficiency did not affect regrowth dynamics for one regrowth cycle of 24 days, with accordance of the results of Duck et al. (1980) and Barta (1982) who showed that K-deficiency did not significantly reduced root weight. However, some results with alfalfa was contrary, presenting the reduction of root weight with poor plant persistence (Kitchen et al., 1990) and depress of plant growth and decrease of nodules number under low K nutrition (Collins and Duke, 1981). These results suggest that plant growth response to K nutrition involves in other environmental and management factors.

At day 0, more than 79% of total carbohydrate and more than 67% of total N compounds were stored in stubble in both treatments (Fig. 2 and 3). The decrease of organic reserves for the early regrowth period was also higher in stubble than in roots. These results suggests that stubble is a primary storage site of organic reserves. High concentration of organic reserves has been reported in stubble of perennial ryegrass (Ourry et al., 1988) and tall fescue (Volenec, 1986), and timothy and switchgrass (Smith and Greenfield, 1979). All carbohydrate compounds examined in remaining tissues (stubble and roots) greatly decreased during the first 6 days of regrowth. During this period, a higher decline in soluble sugar, starch and fructan in

stubble occurred in both +K and −K medium. The fluctuation of 3 carbohydrate compound in the −K nutrition was very simialr with +K treatment and the absolute content also was not significantly different in most measuring time (Fig. 2. A1, A2 and A3). These results suggested that the utilization of carbohydrate reserves actively occurred to support the regrowth even under −K nutrition. Li et al. (1997) reported that low K reduced root starch and total non-structure carbohydrate utilization after shoot removal. They demonstrated that total amylase activity increased between day 0 and 6 for both 0 and 6.0 mM K treatment.

Non-significant difference between +K and −K medium in all three C compounds in regrowing shoots for 24 days of regrowth (Fig. 4) indirectly indicate that the quantitative equilibrium of C metabolites, even under K-deficient stress, possibly due to an active degradation of carbohydrate reserves during the first 6 days. This suggestion is well supported by the results of Caldwell et al. (1984) who gave an evidence, by ^{14}C labeling, of the translocation of carbohydrate reserves to growing shoot after the first 14 days of regrowth.

At day 0 (cutting day), all N compounds in stubble were not significantly different between +K and −K medium (Fig. 3). This indicated that a short term (7 days) treatment of K nutrition did not modify the N partitioning into plant tissues, especially into reserve organs. Under K-sufficent and K-dificent condition, the decrease of all nitrogenous compounds in stubble remarked for the first 6 days of regrowth (Fig. 3. A1-A4), when the uptake of external N was extremely low (Kim et al., 1991). Soil N sources (exogenous N) and organic N reserves in plant tissues (endogenous N) are both effective sources for regrowth of shoots. Kim et al. (1993) suggested that shoot removal stimulated N-flow from reserve organs.

In the −K medium, nitrate in stubble decreased as a similar rate with +K treatment until day 12, and then much slowly reconstituted (Fig. 3 A1). A significantly higher decrease of soluble proteins in this organ occurred in the −K medium for the first 6 days (Fig. 3. A3). And consequently amino acids in regrowing shoots at 24 day of regrowth significantly higher in the −K medium. These results suggest that under K-deficient condition nitrate assimilation in stubble much actively occurred, and that mobilization of reduced N to regrowing shoots might be accelerated to meet the demand of amino acids in regrowing shoots. Kim et al. (1993) gave an evidence of active remobilization of amides and amino acids derived from N reserves via xylem for the first 10 days of regrowth of alfalfa.

In conclusion, an active utilization of organic reserves under K-deficient condition successfully support for one cycle of regrowth, showing non-significant difference of dry matter production between +K and −K medium. However, this experiment does not distinguish the direct effects of K-deficiency on mobilization of organic reserves and utilization of intake mineral N, and does not estimate the limitation of organic reserves utilization for the subsequent defoliation-regrowth cycles.

V. 적 요

Potassium의 결핍하에서 이탈리안 라이그라스의 재생동안 질소와 탄소의 이용성이 재생활력에 미치는 영향을 조사하고자, 수경재배 조건하에서 예취 7일전 potassium의 공급구 (+K)와 potassium 결핍구 (−K)로 처리하고 재생 24일 동안 동일한 K 처리조건에서 예취일(0 일), 재생 6일, 12일 그리고 24일차에 각각 3부위의 주요 식물조직을 분리, 수확하여 화학적 성분 및 생산성을 각각 비교하였다. 재생 건물수량은 +K 및 −K 처리구 공히 잎의 경우 재생초기 6일 동안 낮은 수준을 유지하다가 12일차 이후 급격히 증가하여, 재생 24일차 처리간에 유의적인 차이를 나타내지 않았다. 뿌리의 건물수량의 변화폭은 지상부에 비해 매우 낮으며, 재생 24일차 처리간 유의적인 차이가 나타나지 않았다. 그루터기내 모든 carbohydrate 함량은 재생 6일 동안 초기수준(0일)의 69%에서 84%의 감소 후 빠르게 재축적 되었다. 급격한 회복을 나타냈다. 뿌리에서 soluble sugar와 fructan의 감소는 재생 6

일 동안 −K 처리구에서 더 낮았다. 그루터기내 amino acids, soluble protein, insoluble protein 함량은 재생 6일 동안 감소한 후 증가 되었으며, nitrate의 감소는 재생 12일차까지 지속되었다. 뿌리내 모든 질소화합물의 초기 함량은 −K 처리구에서 유의적으로 더 낮았으며, 재생 12일차 이후 amino acids와 soluble protein의 재축적이 상대적으로 높았다. 재생중인 잎 조직내의 모든 탄수화물의 함량은 재생기간중 유사한 경향으로 증가하였으며 절대적인 함량 역시 두 처리간 유의적 차이가 없었다. Nitrate 함량은 처리간에 유의적인 없었으며, −K 처리구에서 재생 12일차 이후 soluble protein의 재축적이 두드러지게 억제되었다. 이러한 결과는 −K stress 조건에서도 불구하고 +K 조건과 유사한 수준으로 저장 탄수화물 및 질소가 이용됨으로써 재생 활력을 유지할 수 있음을 잘 보여준다.

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