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Nitrogen Effects on Growth Responses and Carbohydrate Concentrations in Source and Sink Tissues of Two Rice Cultivars

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ABSTRACT: Two rice cultivars (*Oryza sativa* L.), Hwaseongbyeo of Japonica type and Taebackbyeo of Indica/Japonica type, were cultivated with hydroponic culture to examine nitrogen effects on the growth responses, contents and utilizations of carbohydrates, and the ripening velocity of grains with three different N levels. Plant height and tiller number were clearly increased to 80 ppm N level compared to 40 ppm N level and then they were slightly decreased in N level of 120 ppm. Higher dry weights were appeared with 80 ppm N level than did with other N levels, showing statistically differences in both cultivars and N levels, while dry weight of roots was heavier with decreasing the N levels. Therefore, T/R ratios were not significantly different among N levels, although there was statistically differences between rice cultivars. After the flowering stage, higher water-soluble carbohydrate (WSC) and water-insoluble carbohydrate (WISC) were contained in stem compared with other parts, showing that WISC of sheath and stem, unlike WSC, was significantly different among N levels. Starch of grain, WISC, was remarkedly increased from 3.0% at just after the flowering to 52.0% and 75.0% at 15 and 30 day after the flowering, respectively, showing that lower N application had faster accumulation of starch in rice grains. N would affect the contents of carbohydrates of each tissue, and starch accumulation in rice grains.

Keywords : rice, N fertilizer, water soluble carbohydrates, and starch.

Plants require a similar balance of resources-energy, water, and mineral nutrients to maintain optimal growth (Chapin III *et al.*, 1987) Carbohydrates, which are major energy source of plants, could be divided into WSC and WISC. They are translocated, stored, and utlized in plant systems to produce the ATP, C-skeletons, and cell wall materials for plant growth. Plant tissues have different contents of WSC although their contents are different depended on plant species and varieties. Generally, sink organs of the leaf and root growth zones stored higher WSC than did the source organ of the mature leaves (Spollen nad Nelson, 1988 : Volenec and Nelson, 1984). Additionally, nitrogen is the mineral nutrients that plants require the greatest quantity and that most frequently limits growth in both agricultural and natural systems (Raven *et al.*, 1986).

Carbohydrate concentrations and compositions differs among tissues and among plant species. C-3 plants have generally contained fructan as a major form of storage carbohydrate, while C-4 plants starch. Fructan occurs in abundant amounts in the growth zone of grass leaves, where cells are actively dividing and elongating (Spollen and Nelson, 1988; Song, 1998). Water-soluble carbohydrates were remarkedly higher in the growth zones of leaf and root than did other parts of rice (Song, 1995). The WSC of the leaf elongation zone of tall fescue was above 20% of dry weight, and contained a high proportion of low DP fructan relative to sucrose and hexoses. The root growth zones contained a much higher proportion of hexoses and sucrose than low DP fructan (Song, 1998).

Starch is the major storage carbohydrate in higher plants that is especially found in the seeds of cereals. It is generally stored in chloroplast and amyloplast of plant tissues and utilized for crop growth through its degradation (Stitt and Quick, 1989) and for crop productivity through its storage (Stark *et al.*, 1992). It is assumed that the ripening velocity of grains of cereal crops would be differed from the environmental conditions and cultural techniques. Especially, nitroge acquisition is a major carbon expense (Chapin III *et al.*, 1987). There is no doubts what carbon and nitrogen are very closely related in plant growth systems and product quality.

Tolerant genotypes of wheat for drought and salts stresses accumulated more water-soluble carbohydrates than did sensitive ones. Both ionic and non-ionic stresses increased the concentration of reducing sugars, sucrose and fructans. Drought tolerant varieties accumulated sucrose to a significantly great level than did sensitive ones under non-ionic stress condition (Kerepesi and Galiba, 2000). It also indicate that plant tissues contain and store non-structural carbohydrates differently dependent on the growth conditions.

The principal use of N in plants is in photosynthesis, because the reactions of photosynthesis require the large

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amount of N in the formation of Rubisco, chlorophyll, and related proteins. Approximately, 75% of the nitrogen in a leaf of C-3 crops is used in chloroplasts and in photosynthesis (Chapin III *et al.*, 1987). Therefore, N-use efficiency of photosynthesis is likely to be an important roles in vegetative and reproductive growths (Clark, 1990). The leaf N-use efficiency for photosynthetic carbon assimilation is closely related to leaf longevity. Leaf longevity was highly correlated with leaf N duration and significantly correlated with cumulative leaf CO_2 assimilation (Lynch and Rodriguez, 1994).

This study was initiated to understand N effects on carbohydrate utilization and partitioning by evaluating the plant growth responses and determining the content of carbohydrates. The specific objectives were to examine the N effects 1) on relative rates of leaf growth and tillering, 2) on the partitioning of dry weight to shoots and roots, 3) carbohydrate contents of plant tissues, leaves, stem, and panicle, and 4) on the velocity of ripening grain of rice.

MATERIALS AND METHODS

Plant materials and growth conditions

Two varieties of rice were used, one was Taebackbyeo selected for Indica/Japonica type and the other was the Hwaseongbyeo for Japonica type. Their seeds were sprouted in the incubator that was maintained at 28. When they have four or five leaves after sprouting, similar plants were selected and transplanted to hydroponic culture in the greenhouse. Each pot of fifteen liters volume was used of four tillers. Based medium was the IRRI's nutrient solution modified by nitrogen (Yoshida *et al.*, 1984). N levels were 20, 40, and 60 ppm in the vegetative growth stages and 40, 80, and 120 ppm in the reproductive growth stage with the ratio of $NH_4^+ : NO_3^-$ being 50 : 50. The solution was changed weekly.

Growth analysis and tissue sampling

Plant height and number of tillering was measured at several important growth stages. Growth of panicle was examined with the interval of 10 days after the flowering stage. Each plant tissue of leaf blades, leaf sheaths, stem, panicles, and roots was investigated and sampled to check for the partitioning of the dry weights. T/R ratios were calculated through dividing the root dry weight to the shoot dry weight including leaves, sheaths, stems, and panicles. Samples for determining the carbohydrates were taken from each plant tissue. Then, the samples were dried at 70°C for 48h and grounded and homogenized. Samples were stored at the freezer, which was maintained with below -20.0°C, and were determined for 30 days.

Analysis of carbohydrates

Dried tissue was ground with a motar and pestle. The WSC was extracted with distilled water and filtered through Whatman No. 2 paper. Total WSC in an aliquot was determined with the Anthrone procedure. The WISC, starch, which was contained in the residue following the WSC extraction, was hydrolyzed by perchloric acid. The residue was dried and put into the centrifuge tube. After adding 2 ml of distilled water, the tube was incubated in a boiling water bath for 15 min. and stirred occasionally. After allowing the centrifuge tubes to cool, 2 ml of 9.2N perchloric acid was added and the solution was stirred occasionally for 15 min. Then, the tube was centrifuge at 14,000 rpm for 20 min. and the supernatant was collected. The residue was extracted once more with 2 ml of 4.6N perchloric aacid. The supernatants were combined and made up to 25 ml with distilled water. The solution was used for the starch analysis with Anthrone procedure. The standard solution was made with glucose.

RESULTS AND DISCUSSIONS

Plant height and number of tillers at the flowering stage of two rice cultivars, Hwaseongbyeo and Taebackbyeo, cultivated with three different nitrogen levels, were shown on Table 1. Like we've known, plant height at the flowering growth stage was generally higher in Hwaseongbyeo of Japonica type than did in Taebackbyeo of Indica/Japonica type, showing the significant difference statistically. Com-

Table 1. Plant height and number of tillers at flowering stage of two rice cultivars, Hwaseongbyeo and Taebackbyeo, cultivated hydroponically with three different N levels.

Cultivar	N level (ppm)	Plant height (cm)	No. of tillers (No./Hill)
Hwaseongbyeo	40	97.2	14.8
	80	100.5	17.4
	120	93.3	16.6
	mean	96.9	16.3
Taebackbyeo	40	82.2	14.6
	80	88.5	16.2
	120	86.2	16.4
	mean	85.6	15.7
F-value	Cultivar	55.3**	0.17
	N level	2.8*	1.82
	C x N	2.1	0.12

*significant at p<0.05, **significant at p<0.01.

paring the plant height with three different N levels, it was highest in 80 ppm N level, compared to those in 40 and 120 ppm N levels. It was appeared significantly differences at $p<0.05$ among different N levels. Plant heights of Hwaseongbyeo and Taebackbyeo with treatment of optimum N level were about 100.5 and 88.5 cm, respectively, while they with treatment of surplus N level were 93.1 and 86.2 cm, respectively. These results were similar tendency to the previous report that the excess application of N could affect negatively to the vegetative growth of rice (Song, 1995).

Unlike the plant height, the number of tillers of both rice cultivars at the flowering growth stage were increased with raising the application amount of nitrogen, from 40 to 120 ppm, except for it of Hwaseongbyeo with the N level from 80 to 120 ppm, showing decreasing a little number of tillers. There were no significant differences in the number of tillers between two rice cultivars, although their numbers of tillers per hill of Hwaseongbyeo and Taebackbyeo were averagely about 16.3 and 15.7, respectively, appearing slightly differences between two rice cultivars.

These research results were different tendency from the report that the number of tillers of rice was increased with raising the application amount of N (Song, 1995).

Dry weights of different tissues and T/R ratio at flowering stage of two rice cultivars cultivated hydroponically with thre different N degrees were shown on Table 2. Comparing the dry weight of leaves with between cultivars and among three different N levels, they were statistically different at $p<0.05$ in between cultivars and in among N levels. Hwaseongbyeo of Japonica type had higher dry weight of leaves, the major part of plant systems in photosynthesis, than did Taebackbyeo of Indica type. It is assumed because Hwaseongbyeo had more tillers compared to that of Taebackbyeo.

Dry weight of sheath, stem, and panicle was also heavier in Hwaseongbyeo than that in Taebackbyeo. Comparing the dry weight with N levels, it was the heaviest in the treatment of 80 ppm N rather than did in 40 and 120 ppm N levels, showing that the dry weight was heavier in treatment of 40 ppm N than did in 120 ppm N. The dry weight of sheath, stem, and panicle was significantly different in between cultivars and among N levels.

Dry weight of roots of two rice cultivars at the flowering growth stage was a little different in between two cultivars and among three N levels without showing the differences statistically. However, it was slightly decreased with increasing the application amount of N fertilizer in both genotypes, which Hwaseongbyeo had heavier dry weight of roots than Taebackbyeo had.

Based on these results, the dry weight of shoot of two rice cultivars including the leaves, sheath, stem, and panicle was averagely about 47.6 and 38.8 g/hill in Hwaseongbyeo and Taebackbyeo, respectively. With three different N degrees, it of Hwasseongbyeo was about 53.5, 51.6, and 37.8 g/hill with treatment of 40, 80, and 120 ppm N, respectively. It of Taebackbyeo was about 41.1, 38.2, and 37.2 g/hill with treatment of 40, 80, and 120 ppm N, respectively. Especially, the dry weight of sheath and stem was heavier in treatment of lowest N level than those in treatment of optimum and excess N levels. The T/R ratios with between two cultivars and among three N levels were not clearly different because of the growth patterns with different N degrees, although the T/R ratio of Hwaseongbyeo was significantly higher than that of Taebackbyeo. It means that the partitioning of dry weight could be come to the tissues of sheat and stem compared to other plant tissues. It would be assumed that these tendency of dry weight partitioning could affect

Table 2. Dry weights of different tissues of source and sink and T/R ratio at flowering stage of two rice cultivars, Hwaseong byeo and Taeback byeo, cultivated with three different N levels.

Variety	N level (ppm)	Dry weight (g/hil)				T/R ratio
		Leaves	SSP†	Roots	Total	
Hwaseong byeo	40	13.4	35	5.1	53.5	9.4
	80	16.2	31	5.0	51.6	9.3
	120	12.1	22	3.8	37.8	9
	mean	13.9	29.3	4.7	47.6	9.2
Taebackbyeo	40	10.6	25.8	4.7	41.1	8
	80	11	22.9	4.2	38.2	8
	120	9.9	22.9	4.3	37.2	7.6
	mean	10.5	23.9	4.4	38.8	7.9
F value	Cultivar	15.04*	7.94*	0.64	7.39	29.63**
	N level	4.05*	3.78*	2.53	3.16	0.51
	C × N	1.14	1.85	1.52	1.53	0.01

*Significant at $p<0.05$, **significant at $p<0.01$.
†dry weight of sheath, stem, and panicle.

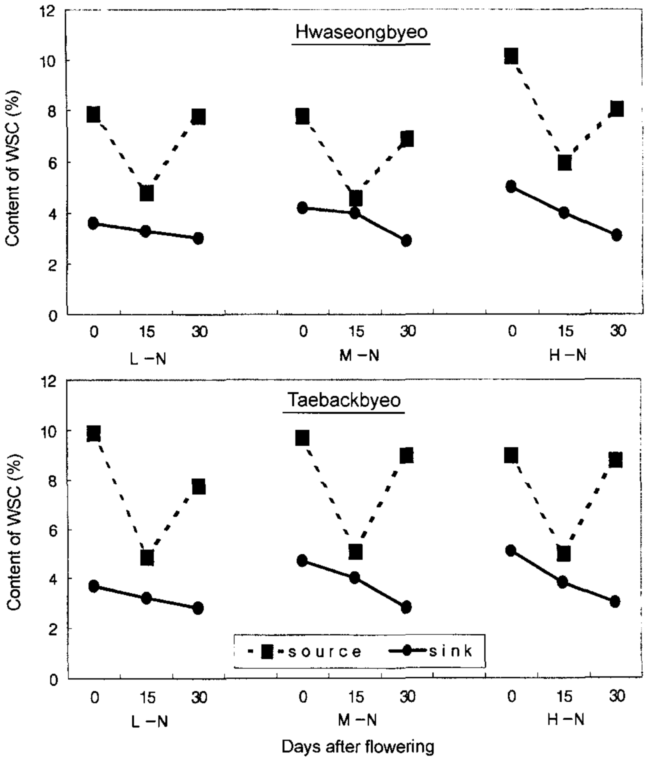

Fig. 1. WSC contents of source (leaf blade + leaf sheath + stem) and sink (panicle) tissues of two rice cultivars, Hwaseongbyeo and Taebackbyeo, at three different growth stages after flowering cultivated with different N levels. L-N : 40 ppm N, M-N : 80 ppm N, H-N : 120 ppm N levels.

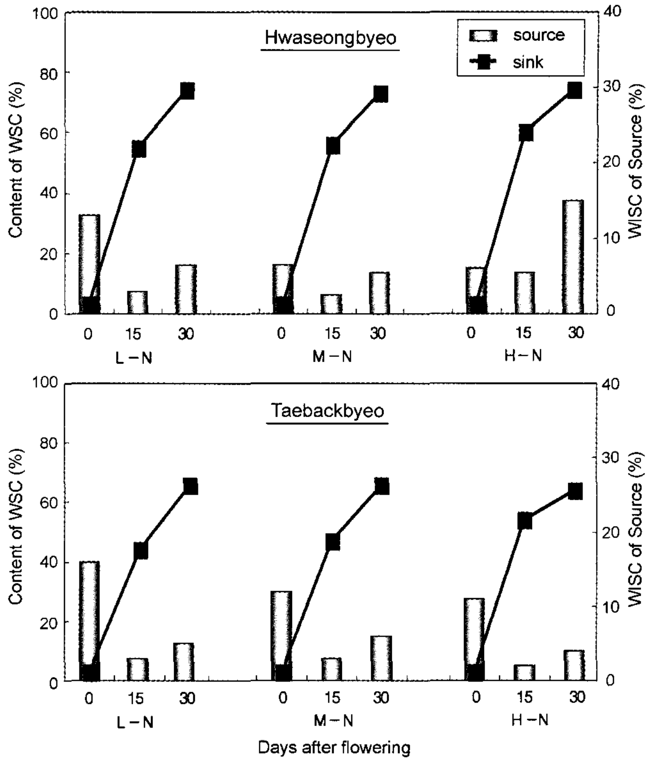

Fig. 2. WISC of contents of two different parts, source (leaf blade + leaf sheath + stem) and sink (panicle), at three different growth stages after flowering of two rice cultivars, Hwaseongbyeo and Taebackbyeo, cultivated with different N levels.

the lodging of rice.

Concentrations of WSC of source and sink tissues of two rice cultivars at three different growth stages after the flowering cultivated under three N levels by the hydroponic culture were shown on Fig. 1. The leaves, sheath, and stem were classified to the tissues of source, while panicles were to sink tissues.

The concentration of WSC in tissues of source and sink on two rice cultivars after the flowering stage was clearly different, even if the changing tendency of WSC content at different growth stages was similar between two cultivars.

In 40 ppm N level, the WSC content of source tissues in Hwaseongbyeo of Japonica type was about 8% based on dry weight at the flowering growth stage. It was remarkedly decreased to about 4.2% at 10 day after the flowering and then increased to about 8.0% again. This trend was appeared with treatment of 80 and 120 ppm N levels, although the WSC contents were a little different from the different ripening growth stages and with the N levels. Both the WSC contents of different tissues and the changes of WSC contents with different ripening growth stages were similar tendency in between two rice cultivars. It could be assumed from this results that the sink activity, i.e. the ripening velocity of rice grain, was the highest at 15 day after the flowering compared to either that at the flowering or that at 30 day after the flowering. On the another words, it means that the photosynthates of source would be translocated to the grain very fast at 15 day after the flowering, while they would be translocated slowly from source to sink at both the flowering and 30 day after the flowering.

The concentrations of WSC in the sink tissue of rice after the flowering growth stages showed different trends compared to those of the source tissues, including the leaves, sheath, and stem. The WSC was gradually decreased in both rice cultivars from at the flowering stage until at 30 day after the flowering. There were no significantly different in between two cultivars. With three different N levels, the concentration of WSC in sink tissues was slightly increased with raising the application amount of N fertilizer. What the WSC content of sink (panicle) has been decreased from at the flowering stage to at 30 day after the flowering would be related to the ability of sink to synthesize starch, water-insoluble carbohydrate, of rice grain and to the weak ability of sink at certain time after the flowering.

Table 3. Yield and yield components of two rice cultivars, Hwaseongbyeo and Taebackbyeo, cultivated with three different N levels.

Variety	N level (ppm)	No. of panicle/hill	No. of grain/ panicle	Ripening ratio (%)	1000 grain weight (g)	yield of rough rice (g/hill)
Hwaseong byeo	40	14.4	101.1	84.6	23.5	28
	80	16.8	106.5	75.9	21.7	28
	120	14.8	81.6	72.6	22.1	18.9
	mean	15.3	96.4	77.7	22.4	25
Taeback byeo	40	13	112.6	79.5	20.3	23.5
	80	14.6	93.5	79.8	19.7	20.9
	120	15	105.1	72.3	19.7	22.1
	mean	14.2	103.7	77.2	19.9	22.2
F value	Variety	0.48	0.57	0.02	113.5**	2.6
	N level	1.15	1.4	5.82*	5.4*	3.09
	VN	0.42	2.65	1.24	1.29	3.01

*Significant at $p<0.05$, **significant at $p<0.01$.

Concentrations of WISC of source and sink tissues of two rice cultivars at three different growth stages after the flowering cultivated under three N levels by the hydroponic culture were shown on Fig. 2.

WISC contents in source tissues of leaves, sheath, and stem were appeared differently with the ripening stages, from the flowering to 30 day after the flowering, showing that WISC at the flowering was maintained high, about 7.0 to 15.0% based on the dry weight, it was decreased low to about 2.5 to 5.0%, and then it was increased again to 7.0 to 15.5%, depending on both the ripening stages and three N levels. On the other hand, WISC contents (starch) in sink tissue of rice grain were remakedly increased after the flowring stages. especially, its increment was from at the just after the flowering to at 15 day after the flowering, compared to its increment after 15 day after the flowering, when the WISC content was gradually increased. With three different N levels, the stored starch was not significantly different, even if its storage was slightly different among three N degrees, showing that higher storage amount of starch was accumulated with lower N levels.

These tendency was appeared similarly in both two rice cultivars, except for the source tissues that the WISC content of source was maintained low in Taebackbyeo at 15 and 30 day after the flowering compared to that in Hwaseongbyeo. These trends of translocating photosynthates and utilizing N in plant system were similar and would be related to the previously reported research results that the nitrogen assimilation rate of rice was higher in the Japonica type than that in Indica type, although the rice of Indica type require more nitrogen than does Japonica type (Song, 1995).

Yield and yield components of two rice cultivars, Hwaseongbyeo and Taebackbyeo, cultivated with three different N levels were shown on Table 3. Number of panicle per hill was increased with raising N levels in Taebackbyeo of Indical type, while it was increased to the optimum N levels and then decreased with the excess N level. Number of panicle was not clearly different in between rice cultivars and among three N degrees.

Number of grain per panicle was not appeared differences in between rice cultivars and among three N degrees. The rate of ripened grain was increased with decreasing the application amount of N fertilizer; the ripening rates of grain in Hwaseongbyeo were about 84.6, 75.9, 72.6% with 40, 80, 120 ppm N levels, respectively. They in Taebackbyeo were about 79.5, 79.8, 72.3% with 40, 80, 120 ppm N levels, respectively. The ripening rates of rice grain were statistically different at $p<0.05$ among different N degrees.

1000 grain weight of two rice cultivars showed the tendency to decrease its weight with raising the N levels, showing that the 1000 grain weights were statistically different at $p<0.01$ between two cultivars and at $p<0.05$ among different N levels. It of Hwaseongbyeo was heavier than did Taebackbyeo. However, the yields of rough rice did not show a certain tendency in between cultivars and in among different N degrees.

SUMMARY

This study was conducted to examine the nitrogen effects on the growth responses, utilization and storage of carbohydrates, and yield on two rice cultivars cultivated with three different N levels. The research results were summarized as the follows;

Plant height and number of tillering were increased with raising N application from low N level to middle level. The N effects for them were not clearly appeared with above application of middle N levels. The T/R ratios were slightly decreased with raising N application. They were statistically different in cultivars, but the application amount of N. The

length of panicles was not significantly different between rice cultivars and among the N levels. Rice yields were generally highest in the middle N levels. After the flowering stage, higher water-soluble carbohydrate and water-insoluble carbohydrate were contained in stem compared with other parts, showing that WISC of leaf sheath and stem, unlike WSC, was significantly different among N levels. Starch of grain was remarkedly increased from 3.0% at just after the flowering to 52.0% and 75.0% at 15 and 30 day after the flowering, respectively, showing that lower N application had faster accumulation of starch in rice grains.

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