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Effects of Non-Structural Carbohydrates on Spikelet Differentiation in Rice

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Abstract : Spikelet number per unit area is a strong determinant of rice yield, and spikelet differentiation must be promoted to increase spikelet number. Nitrogen has been considered to be the most critical factor in promoting spikelet differentiation and the role of non-structural carbohydrates (NSCs) in spikelet differentiation is unclear. To reveal the relation between NSCs and the number of differentiated spikelets, we conducted a field experiment in Matsue, Shimane, Japan, using two japonica cultivars Koshihikari and Nipponbare. The NSC content was changed by shading and thinning during the early reproductive stage. These treatments did not change the amount of nitrogen per hill in the leaves and stems. The number of differentiated spikelets, which was defined as the sum of the numbers of surviving spikelets and vestiges of degenerated spikelets, was not influenced by NSC content in rice plants; neither was the number of primary rachis-branches, which is considered to determine spikelet number per panicle. The ratio of dry matter allocation to leaves and the rest of the plants was changed, but the morphological characteristics such as tiller number and plant length were not influenced by the treatments. It is concluded that the differentiation of spikelets was not influenced by the NSC content within the range examined in the present experiment. The role of NSC in the mechanism of spikelet differentiation is discussed.

Key words : Dry matter production, Nitrogen, Non-structural carbohydrate, Rice, Shading, Spikelet differentiation, Spikelet number, Thinning.

Spikelet number per unit area is a strong determinant of rice yield (Senanayake et al., 1996). The number of spikelets is the difference between the number of differentiated and degenerated spikelets (Matsushima, 1957), and usually there is a strong positive correlation between the numbers of differentiated and degenerated spikelets (Matsushima, 1957; Wada, 1969). This means that the promotion of spikelet differentiation results in the increase in spikelet number.

There is a strong positive correlation between the numbers of differentiated secondary rachis-branches (SBs) and differentiated spikelets, so that the stage of the differentiation of SBs is critical in determining spikelet number (Wada, 1969; Kobayasi and Horie, 1994). The period from the beginning of panicle differentiation to the stage of SB differentiation is only about five days (Matsushima, 1957), and rice-growing conditions during this short period is important to increase spikelet number through SB differentiation.

Nitrogen (N) is considered to be the most effective factor promoting spikelet differentiation. Wada (1969) demonstrated the relationship between the number of differentiated spikelets and the total amount of N accumulated in shoots until the late spikelet differentiation stage. Kobayasi and Horie (1994) revealed that an increase in shoot N concentration from panicle initiation to the early spikelet differentiation stage results in the promotion of spikelet differention. Hasegawa et al. (1994) demonstrated an association between N concentration in the plant at the time of panicle formation and spikelet number per unit area.

Non-structural carbohydrates (NSCs) are used as a structural and respiratory substrate, and are therefore necessary to differentiate rachis-branches and spikelets. However, their role in spikelet differentiation is unclear. Wada (1969) speculated that the demand for carbohydrate in the early reproductive stage would be small because rice panicles hardly grow during this stage. No clear relationship between assimilate concentration and spikelet development could be detected among three cultivars (Mohapatra and Sahu, 1991). However, 90% shading during the stage between panicle initiation and spikelet differentiation decreased the spikelet number per panicle (Matsushima, 1957). In the previous studies by Wada (1969) and others on the relation between carbohydrate and spikelet differentiation, dry weight increased during the reproductive stage. However, it is probable that in the previous studies NSC was not exhausted and was sufficient for spikelet differentiation. The heavily shaded plants lose dry weight, exhaust stored NSC, and may not supply NSCs for spikelet differentiation.

Our objectives in this experiment are to determine whether heavy shading, under which NSC may be exhausted, affects the spikelet differentiation in rice plants and to quantify the relationship between the amount of NSCs and the number of differentiated spike-

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Abbreviations : N, nitrogen; NSC, non-structural carbohydrate; PB, primary rachis-branch; SB, secondary rachis-branch.

lets. We conducted shading and thinning treatments during the early reproductive stage, when most spikelets differentiate. Under the heaviest shading (82% shading), rice plants could not increase dry weight and consumed most of the NSCs stored in the plants. To obtain a wide range of NSCs under a field condition, we added a thinning treatment. We also counted the numbers of differentiated primary rachis-branches (PBs) and differentiated SBs because the number of differentiated PBs is thought to determine the theoretical maximum number of spikelets per panicle (Matsuba, 1991) and because the number of differentiated SBs is the most critical morphological factor in determining spikelet number and is responsive to the environment (Matsushima, 1957). In this paper we describe the effects of shading and thinning treatments on spikelet differentiation and discuss the role of NSC in spikelet differentiation.

Materials and Methods

1. Plant material and culture method

Two cultivars of japonica rice (Koshihikari and Nipponbare) were used. The experiment was conducted at the experimental paddy field of Shimane University in Matsue, Shimane Prefecture, Japan (35°27′ N latitude, 133°43′ E longitude, and 17 m altitude). The soil was alluvial and sandy clay. Thirty-day-old seedlings grown in nursery boxes were transplanted on May 15, 1996. Planting density was 22.2 hills m^{-2} (two seedlings per hill; a hill spacing was 15 cm and row spacing 30 cm). Basal application and topdressing were done according to the standard for plain fields in Shimane Prefecture (Table 1). During shading and thinning treatments, N was not applied because N was the most effective factor in spikelet differentiation. The experimental design was a split plot design with two replicates. The area of each plot ranged from 4 m^2 (shaded) to 20 m^2 (thinned).

Table 1. The amount (g m^{-2}) and the time of basal application and top-dressing in two cultivars (Koshihikari and Nipponbare).

	Basal	Tillering*	Panicle Formation**	Booting***
Koshihikari				
N	3	0	1.5	1.5
P_2O_5	3	0	0	0
K_2O	3	0	3	0
Nipponbare				
N	4	1	2.5	2.5
P_2O_5	4	0	0	0
K_2O	4	1	5	0

* Active tillering stage (Nipponbare, June 1).
** Panicle formation stage (Koshihikari, July 18; Nipponbare, July 18).
*** Booting stage (Koshihikari, July 30; Nipponbare, July 28).

2. Shading and thinning treatments

To change NSC content and dry matter production, we applied shading and thinning treatments. The plants were shaded from the stage of neck-node differentiation (Koshihikari, July 1; Nipponbare, July 8) to the stage of spikelet differentiation (for eight days) by 34% (light shading), 58% (moderate shading), or 82% (heavy shading), using two types of shade cloth (34 and 58% shading) singly or in combination. Most of the tillers in the same hill started panicle development in a few days after the time of panicle initiation on the main culms (Matsushima and Manaka, 1956). Therefore, the shading period in this study covered the period between panicle initiation and the stage of SB differentiation of most of the tillers. About ten plants were sampled at about three-day intervals and the developmental stages of the main culms were examined under a binocular microscope. For thinning, the plants in alternate rows were cut about 5 cm above the soil surface using a hand sickle (thinning percentage was 50%).

3. Measurements of NSC and nitrogen content

The plants from eight hills per replicate were sampled at the beginning and at the end of the shading treatments. After measurement of plant length, leaf area and tiller number, the samples were divided into three parts (leaf blades, roots and the rest). After removal of roots, leaf blades (leaves) and the rest (stems) were dried at 80 °C for more than 48 h to measure dry weight for each part. Each plant part was ground and then used for chemical analysis for N and NSC. Nitrogen concentration in each plant part was determined by the semi-micro Kjeldahl method.

The content of NSC in the stems was shown as the sum of the contents of soluble sugar and starch. Soluble sugar was extracted with 80% ethanol, and its content was measured by the anthron sulfuric acid method. The samples after the extraction of soluble sugar were hydrolyzed with amyloglucosidase, and starch content was measured by using Food Analysis Starch (Boehringer Mannheim Co., Ltd., Mannheim, Germany).

4. Determination of the number of differentiated spikelets

The number of differentiated spikelets was defined as the sum of the numbers of surviving and degenerated spikelets. Panicles from eight hills per replicate were sampled a few days before heading. Surviving spikelets were defined as final spikelets used for a yield component in this study. The number of degenerated spikelets were determined by counting the number of the vestiges on the panicles with the naked eye or a binocular microscope (Matsushima, 1957). The numbers of differentiated PBs and SBs were also counted similarly. The numbers of differentiated PBs, SBs, and spikelets on unproductive tillers were not counted.

Table 2. The effects of shading and thinning on the number of differentiated spikelets, primary rachis-branches (PBs), and secondary rachis-branches (SBs) per hill.

Cultivar	Treatment	Differentiated spikelets	Differentiated PBs	Differentiated SBs
Koshihikari	Heavy shading	1512a	143a	248a
	Moderate shading	1414a	130a	237a
	Light shading	1687a	147a	297a
	Thinning	1656a	135a	304a
	Control	1593a	137a	281a
Nipponbare	Heavy shading	1427a	143a	223a
	Moderate shading	1511a	148a	242a
	Light shading	1317a	130a	211a
	Thinning	1656a	141a	290b
	Control	1332a	121a	225a

Means in the same cultivar within a column followed by the same letter are not significantly different at 5% level of LSD.

Results

1. The numbers of differentiated spikelets, primary rachis-branches, and secondary rachis-branches

Shading and thinning had no significant effect on the numbers of differentiated spikelets and PBs (Table 2). However, the number of differentiated SBs in Nipponbare was significantly increased by thinning. These treatments did not affect the panicle number in these cultivars (data not shown), so that we considered that any error in counting spikelet number differentiated on unproductive tillers was negligible.

2. Dry matter production and distribution

The shading treatments decreased dry matter production in Koshihikari plants during treatment (Fig. 1). The dry weight of shoots (leaves+stems) in Koshihikari rice was 12.51 g per hill at the beginning of the treatments, and increased by 3.84 g per hill during the subsequent eight days in the control group, but decreased by 0.85 g per hill under heavy shading. The thinning treatment increased the dry weight by 4.45 g per hill. The effect of shading on dry matter production in Nipponbare rice was obscure, but thinned Nipponbare plants gained 7.78 g per hill during the treatment. In both cultivars, dry weight of the stems was influenced by the treatments more strongly than that of the leaves. In particular, stem dry weight was increased by thinning treatment. Although the treatments changed the ratio of dry matter allocation to the leaves and stems, they did not affect morphological characteristics such as leaf area index, plant length and tiller number (data not shown).

3. The content of non-structural carbohydrates (NSCs)

The shading treatments significantly decreased NSC content in the stems of both cultivars (Table 3). Heavy shading in particular decreased NSC content in both

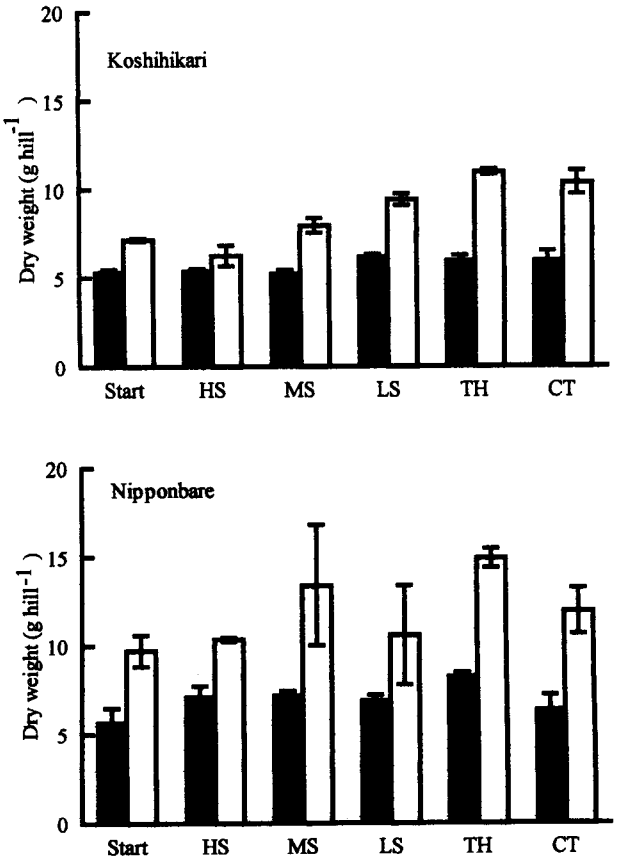

Fig. 1. Dry weight of leaves and stems in Koshihikari (upper) and Nipponbare (lower) eight days after the start of shading and thinning.
Leaf weight (black bar) is the weight of leaf blades. Stem weight (white bar) is the weight of the rest of aboveground parts. Bars indicate standard errors (n=2).
Start: at the start of the treatments, HS: heavy shading (82% shading), MS: moderate shading (58% shading), LS: light shading (34% shading), TH: thinning, CT: control.

cultivars. The NSC content in heavily-shaded Koshihikari and Nipponbare plants, was 21% and 46%, respectively, of that in control plants. Light shading decreased NSC content to 65% of the control in Koshihikari plants, but only to 94% of the control in Nipponbare plants. The shading treatments decreased the NSC amount per hill in both cultivars, but the decrease in Nipponbare was slight because the change in dry weight was slight. By thinning, NSC content in the stems of Koshihikari plants increased by 13%, but it decreased a little in Nipponbare plants. However, thinning increased the amount per hill of NSC in the stems of both cultivars, though not significantly, because of their increased dry weight.

At the beginning of the treatments, the amounts per hill of NSC in the stems of Koshihikari and Nipponbare plants were 0.71 and 1.59 g, respectively. Although the heavy shading considerably reduced NSC content in the plants, the heavily-shaded Nipponbare plants still had 0.91 g NSC per hill. This result implies that NSC in the plants was not fully exhausted by the heavy shading because of the abundant storage of NSC before the treatments.

Table 3. The effects of shading and thinning on the content of non-structural carbohydrates (NSCs) in the stems.

Cultivar	Treatment	The content of NSC in stems (mg g^{-1})	The amount of NSC in stems (g $hill^{-1}$)
Koshihikari	Heavy shading	34.3a	0.22a
	Moderate shading	105.3b	0.84b
	Light shading	104.4b	0.99b
	Thinning	181.1c	1.98c
	Control	159.8c	1.65c
Nipponbare	Heavy shading	87.7a	0.91a
	Moderate shading	128.3b	1.78a
	Light shading	181.0c	1.91a
	Thinning	177.7c	2.65a
	Control	192.1c	2.29a

Means in the same cultivar within a column followed by the same letter are not significantly different at 5% level of LSD.

4. The content of nitrogen

Shading and thinning had no significant effect on the amount of N in the shoots per hill (Table 4). The amount of shoot N in Koshihikari plants ranged from 210.8 to 263.2 mg per hill and that in Nipponbare plants from 215.0 to 294.3 mg per hill. Although stem weight was decreased by shading and increased by thinning, the amount of N in the stems per hill and the allocation ratio of N to the leaves and the stems were not influenced by these treatments.

The shading treatments raised the N concentration in both cultivars, but the thinning treatment did not. The N concentration in the heavily-shaded plants (leaves + stems) was 22.6 mg g^{-1} in Koshihikari (64% higher than that of the control) and 16.3 mg g^{-1} in Nipponbare (40% higher than the control). The effects of shading on the N concentration were stronger in the stems than in the leaves in both cultivars. In particular, the N concentration in the stems of Koshihikari plants was increased by 76% by heavy shading. Although the shading treatments raised the N concentration, the effect was not significant statistically

Discussion

The number of differentiated spikelets was little affected by the shading and the thinning treatments although the treatments surely changed the content of NSC in the plants. Heavy shading (82% shading) reduced the NSC content in the stems in both cultivars by more than 60% of control plants. The results suggested that, within a usual range of NSC in field conditions, the direct effects of NSC content during the early reproductive stage on spikelet differentiation are negligible. Wada (1969) reported that the amount of carbohydrate stored in the plants did not affect the spikelet differentiation. In wheat, kernel number per m^2 was related to the photothermal quotient (Fischer, 1985), which is the ratio of solar radiation to cumulative effective tempera-

Table 4. The effects of shading and thinning on nitrogen content in rice.

Cultivar	Treatment	Nitrogen amount (mg $hill^{-1}$)			Nitrogen concentration (mg g^{-1})		
		Leaves	Stems	Shoots	Leaves	Stems	Shoots
Koshihikari	Heavy shading	171.2a	90.8a	261.9a	31.5a	14.8a	22.6a
	Moderate shading	134.1a	76.7a	210.8a	25.4a	9.7a	16.0a
	Light shading	167.4a	95.8a	263.2a	26.9a	10.2a	16.9a
	Thinning	138.2a	89.1a	227.3a	23.0a	8.1a	13.4a
	Control	139.5a	87.1a	226.6a	23.2a	8.4a	13.8a
Nipponbare	Heavy shading	181.6a	104.9a	286.4a	25.3a	10.1a	16.3a
	Moderate shading	177.6a	116.7a	294.3a	24.6a	9.0ab	14.6a
	Light shading	145.9a	74.3a	220.9a	21.0a	7.1bc	12.8a
	Thinning	183.3a	103.3a	286.6a	22.1a	6.9c	12.4a
	Control	123.8a	91.2a	215.0a	19.4a	7.6bc	11.7a

Means in the same cultivar within a column followed by the same letter are not significantly different at 5% level of LSD.

ture in the critical preanthesis period. Increase in the photothermal quotient in wheat is thought to increase NSC in the plants through increase in dry matter production. The reproductive stage in wheat is much longer than in rice, so that kernel production in wheat plants may be more tightly linked to dry matter production. Hasegawa et al. (1994) proposed a model for determining spikelet number per unit area based on a linear function of plant N concentration and an asymptotic function of the aboveground biomass at the panicle formation stage. They showed the relation of asymptotic response of spikelet number per unit area to aboveground biomass with soluble carbohydrate content of the crop. On the other hand, it is reported that there are no causative relationships between assimilate content and spikelet development in rice (Mohapatra and Sahu, 1991) and in wheat (Mohapatra et al., 1983). An extremely small amount of NSC might be needed for spikelet differentiation, and in field conditions, NSCs might not be fully exhausted even under heavy shading treatments. After the treatments, the heavily-shaded Koshihikari plants had 0.22 g NSC per hill, and such a small amount of NSCs would be enough for spikelet differentiation. However, this study cannot deny the possibility that spikelet differentiation is inhibited by a smaller amount of NSCs obtained in artificially controlled conditions than in field conditions. Further research on the relation between spikelet number and NSCs is needed since the rate of carbohydrate flow has indeed been used as a determining factor in organ proliferation in some deterministic models (Dayan et al., 1981; Hasegawa et al., 1994).

Although the shading and thinning treatments changed the ratio of leaf to stem dry weight, they did not affect spikelet differentiation. Decrease in leaf weight by shading was smaller than that in stem weight. Shading and thinning treatments probably changed the dry-matter allocation to leaves, stems, roots, and young panicles. It is possible that young panicles and leaves have priority to acquire dry matter, compared to other organs at the spikelet differentiation stage, but young panicles are small, and the need for carbohydrate in panicles is negligible (Wada, 1969).

The number of differentiated PBs was not affected by the shading and thinning treatments. The number of PBs per panicle is a morphological factor determining the theoretical maximum number of spikelets per panicle and is determined at the time of panicle initiation (Matsuba, 1991). Other morphological characteristics such as tiller number, leaf area index, and plant length were not reduced even by heavy shading, which decreased dry matter production and NSC content during the treatments. These facts suggest that morphological characteristics in rice are less related to assimilation than in wheat.

Matsushima (1957) revealed that 90% shading during the reproductive stage decreased both panicle number and spikelet number per panicle, but that at the tillering stage or just before the panicle initiation stage decreased only panicle number. In our study, the shading treatments did not change panicle number and the number of differentiated spikelets. However, shading before panicle initiation would change spikelet number through a decrease in panicle number. The shading at the early reproductive stage decreased panicle number and spikelet number per panicle (Matsushima, 1957). This decrease in spikelet number was probably caused by degeneration of spikelets, and suggested that the indirect effects of NSCs on spikelet number are not negligible.

The shading treatments increased N concentration in the stems of Nipponbare, but N concentration did not promote spikelet differentiation. In contrast, N application during the period between panicle initiation and spikelet differentiation promotes the differentiation of spikelets and SBs (Kobayasi and Horie, 1994). There are two possibilities. One is that the effect of decreased NSC on spikelet differentiation was canceled by the increased N concentration. In this experiment, N was not applied during the treatments because N is the most effective factor in spikelet differentiation and the amount of N at the late stage of spikelet differentiation is highly correlated with the number of differentiated spikelets (Wada, 1969). The shading treatments in this study did not change N absorption and decreased dry matter production, so that they increased the N concentration in the plants. It is impossible to change the content of NSC by shading without changing both the concentration and the amount of N in the plants. Interrelation between N and NSC contents on spikelet differentiation has little been studied and further study is needed. The other possibility is N application affected hormonal regulation in spikelet differentiation through altering the balance between N and NSCs. The role of plant hormones in determination of spikelet number was little revealed. The content of gibberellic acid in rice plants is influenced by shading (Osada et al., 1973), and exogenous gibberellin increases spikelet number per panicle (Matsuba, 1991). Patel and Mohapatra (1992) speculated that spikelet development in rice is regulated by hormonal balance among auxin, gibberellin, and cytokinin. The organogenesis related with N and NSC dynamics is thought to be controlled by a hormonal balance between auxin, gibberellin, and cytokinin (Woolley and Wareing, 1972; Patel and Mohapatra, 1992). Further research on the interaction between N and NSC through hormonal regulations in spikelet differentiation is needed.

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