

Relationship between Apical Dome Diameter at Panicle Initiation and the Size of Panicle Components in Rice Grown under Different Nitrogen Conditions during the Vegetative Stage

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Abstract : A pot experiment was conducted to analyze the relationship between the size of each apical dome (AD) and the numbers of differentiated primary rachis-branches (PBs) and spikelets. Two rice cultivars were used : one was a heavy-panicle type 'Akenohoshi' and the other was a many-tillering type 'Nipponbare'. Rice plants were applied nitrogen (N) at various rates (75–600mg N pot⁻¹ week⁻¹) during the vegetative stage. The base diameter and the height of ADs were measured at the panicle initiation (PI) stage. At heading, the numbers of differentiated PBs, secondary rachis-branches (SBs), and spikelets were counted. The N treatment increased shoot N concentration in both cultivars. The N treatment increased the base diameter of AD in Akenohoshi at the PI stage, but not in Nipponbare. The ADs in Akenohoshi had a base diameter about 6.2% larger on average than that in Nipponbare. The N treatment did not affect AD height. In Akenohoshi, the plants with a larger AD base diameter differentiated more PBs per panicle and then differentiated more SBs and spikelets than did those plants with a smaller AD diameter. However, in Nipponbare, the N treatments did not affect the number of differentiated PBs per panicle. These results suggest that the AD size at the PI stage, which is enlarged by higher N nutrition in the vegetative stage, is a determinant of the number of differentiated PBs and spikelets and that a larger AD size is responsible for a higher number of PBs and spikelets in Akenohoshi.

Key words : Apical dome, Nitrogen, Primary rachis-branch, Reproductive stage, Rice, Spikelet number, Vegetative stage.

The relationship between the size of the apical dome (AD) and the final size or the number of aboveground organs derived from its division has been intensively researched. The AD size increased and the leaf divergence angle decreased with the growth in *Ambrosia artemisiaefolia* var. *elatior* (Soma and Kuriyama, 1970). This morphological change increased the number of leaves differentiated on an AD per spiral rotation and changed the decussate phyllotaxis of *Ambrosia* into a spiral form. Yamazaki (1963a, b, c) researched in detail the relationship between AD size during the vegetative stage and the length and width of leaves under various conditions. The final size of rice leaves successively increased as they ascended the nodes and was proportional to the AD size at the time those leaves differentiated (Yamazaki, 1963a). Plants under favorable conditions such as high nitrogen (N) treatment or sparse planting had larger ADs, and these larger ADs when dissected and grown in in vitro tissue culture produced larger leaves (Yamazaki, 1963b, c).

It is probable that the larger the AD size, the higher the number of spikelets produced. Fukushima (1999) found a correlation between the AD size at the panicle initiation (PI) stage and the number of differentiated

primary rachis-branches (PBs) per panicle in several cultivars. Mu et al. (2000) reported a varietal difference in the number of differentiated PBs with AD volume. Kobayasi et al. (2001) detected a relationship between AD diameter which was changed by short-day treatment and the number of differentiated PBs. Nitrogen treatment during the vegetative stage enlarged ADs and leaf size (Yamazaki, 1963b). These results suggest that ADs enlarged by N treatment during the vegetative stage produce more spikelets per panicle.

Under the hypothesis that the increase in spikelets per panicle due to N treatment during the vegetative stage is caused by AD enlargement, we applied N at various rates during the vegetative stage and examined the relationship between AD size at the PI stage and spikelet number per panicle in this experiment. We analyzed the relationship between the size of ADs at PI and morphological panicle characteristics such as the numbers of differentiated PBs and spikelets.

Materials and Methods

A pot experiment was conducted outdoors at Shimane University, Matsue, Japan. Heavy-panicle type rice cultivar 'Akenohoshi' and many-tillering type 'Nippon-

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Abbreviations : AD, apical dome; N, nitrogen; PB, primary rachis-branch; PI, panicle initiation; SB, secondary rachis-branch.

Table 1. The rates (g pot⁻¹ week⁻¹) of nitrogen, phosphorus, and potassium applied to two rice cultivars (Akenohoshi and Nipponbare) during the vegetative stage.

Treatment	N	P ₂ O ₅	K ₂ O
1N	0.075	0.065	0.125
2N	0.150	0.065	0.125
4N	0.300	0.065	0.125
8N	0.600	0.065	0.125

As nitrogen ammonium sulfate, as phosphorus sodium biphosphate, and as potassium potassium chloride were applied. Solutions were applied weekly.

bare' were used. Surface-sterilized seeds were germinated at 32°C for 24h. Selected seeds were sown for uniformity on May 30 (Nipponbare) and June 9 (Akenohoshi), 1999. Twenty germinated seeds were planted in each Wagner pot (1/5000 a) containing 3.6 kg equivalent of oven-dry soil (air-dried Andosol and a granitic saprolite Cambisol mixture, 1:1 volume) using the circular dense-culture method (Satake, 1972). The pots were watered at field capacity level for ten days after sowing, and later kept flooded with 2 to 3 cm of water. Nitrogen treatments are described in Table 1. Ten pots were used per treatment.

Tillers were removed every week by cutting at the base of tillers with scissors and only main culms were grown to obtain shoots uniform in panicle development and panicle size. This is because (1) tillers differentiate panicles a few days later than main culms (Matsushima and Manaka, 1956), (2) plants grown under pot experiments usually linger over producing tillers for a long time, and panicle developmental stages of such tillers vary considerably, and (3) the number of spikelets per panicle on tillers, particularly on secondary tillers, is smaller than that on main culms (Kuroda et al., 1999). Although all the emerging tillers were removed, the effects of ADs of tillers were not completely eliminated because tiller buds inside leaf sheaths of main culms were not removed. The possibility that removing tillers changes the physiology of the plant or causes stress to the plant cannot be denied, but in this experiment, observation of ADs was confined to main culms.

One plant per pot was sampled at PI for measurements of AD size. At sampling, the plant age in leaf number was recorded, with an incomplete leaf preceded by the coleoptile as the first. The roots were removed, and a shoot section containing an AD (approximately 3 cm long) was cut from the base of the main culm. The sections were fixed in formalin-acetic-alcohol (70% ethanol : acetic acid : formalin = 18 : 1 : 1), and then dehydrated in an alcohol series ranging from 40% n-butanol and 25% ethanol in water to absolute n-butanol. The sections were then embedded in paraffin. Serial, longitudinal sections (approximately 10 μm thick) were cut, stained with 0.05% toluidine blue O,

and examined under a light microscope. The median sections were selected from these serial sections and then used for measurements of AD diameter and height. For details of the method of measuring AD diameter and height see Kobayasi et al. (2001).

At heading, after measuring flag leaf blade length, one to three plants per pot were sampled from each treatment group to measure panicle length, rachis length, and morphological panicle components (the numbers of PBs, secondary rachis-branches (SBs), and spikelets). The panicle length was defined as the length from the neck node to the panicle tip. The rachis length was defined as the length from the neck node to the base of the terminal PB (the knot-like vestige of the rachis tip). The method of counting the numbers of differentiated PBs, SBs, and spikelets were the same as that of Kobayasi et al. (2001).

Every week during the late vegetative stage and early reproductive stage, the chlorophyll content of the penultimate fully-expanded leaves in four plants per pot was measured with a chlorophyll meter (SPAD502, Minolta). At the PI stage, three plants per pot were collected, dried at 80°C for 48 hours, ground, and shoot N concentration was measured using the Kjeldahl method.

Results

1. Apical dome size

The N treatment during the vegetative stage increased the shoot N concentration (Table 2). The shoot N concentrations at the PI stage were 22.1 and 21.0 mg g⁻¹ respectively, in Nipponbare and Akenohoshi plants treated with 1N (1N group) and 43.6 and 39.8 mg g⁻¹, respectively, in the 8N group.

As the shoot N concentration at the PI stage increased, the AD diameter in Akenohoshi at the PI stage increased significantly (Table 2), that in the 1N and 8N groups being 90.1 and 98.4 μm (9.2% larger), respectively (Fig. 1). The diameter of ADs in Nipponbare was increased by 4.5% in the 8N group, but the increase was not significant. These results are in agreement with the results described by Yamazaki (1963b) in which N treatment enlarged vegetative AD. This experiment showed that N treatment during the vegetative stage enlarged even the AD at the PI stage. The diameter of ADs in Akenohoshi was larger by about 6.2% than that in Nipponbare on the average. However, N treatment showed little effect on the AD height (Table 2).

2. Morphological panicle components and characters as affected by N treatment

In Akenohoshi, there was a positive correlation between the diameter of ADs and the number of differentiated PBs per panicle (Fig. 2). The number of differentiated PBs in Akenohoshi was 9.6 in the 1N group and 11.6 in the 8N group (about 20% higher, Table 2). An increase in the number of differentiated PBs resulted in

Table 2. Effects of nitrogen treatments during the vegetative stage on the shoot nitrogen concentration, the size of apical domes determined at the stage of panicle initiation, and the morphological characteristics of panicles and flag leaves.

Cultivar	Treatment	Shoot N concentration (mg g ⁻¹)	Size of apical domes		Flag leaf blade length (cm)	Panicle length (cm)	Rachis length (cm)	The number of differentiated		
			Diameter (μm)	Height (μm)				PBs per panicle	SBs per panicle	Spikelets per panicle
Nipponbare	1N	22.1±0.1	85.0±3.8	49.4±5.9	26.7±0.5	20.0±0.2	14.5±0.2	9.8±0.1	18.5±0.3	107.4±1.6
	2N	24.9±0.5	84.7±1.9	55.2±2.8	27.1±1.0	20.2±0.2	14.3±0.3	10.0±0.1	18.3±0.4	107.2±1.2
	4N	36.0±0.8	90.4±2.8	59.7±4.7	23.6±0.6	19.5±0.2	13.6±0.3	10.1±0.1	18.3±0.3	107.8±1.3
	8N	43.6±0.7	88.8±1.9	52.2±3.6	27.2±0.7	19.9±0.3	13.9±0.2	9.9±0.1	21.0±0.4	117.6±1.6
Akenohoshi	1N	21.0±0.6	90.1±3.0	54.4±2.7	27.2±0.7	21.8±0.3	15.1±0.2	9.6±0.2	30.9±0.7	161.2±4.0
	2N	25.5±0.3	89.7±2.7	43.8±3.4	29.8±0.8	22.1±0.3	15.4±0.2	10.1±0.1	35.1±0.7	187.1±3.4
	4N	32.5±0.3	92.4±3.1	60.9±8.5	28.8±0.8	22.4±0.2	15.9±0.2	10.9±0.3	39.0±0.9	202.4±4.9
	8N	39.8±0.1	98.4±2.1	48.7±3.4	27.6±1.2	21.9±0.3	16.1±0.2	11.6±0.3	39.4±2.0	204.0±10.7

Values are means of 8–10 samples ± S.E.

PBs, Primary rachis-branches; SBs, Secondary rachis-branches.

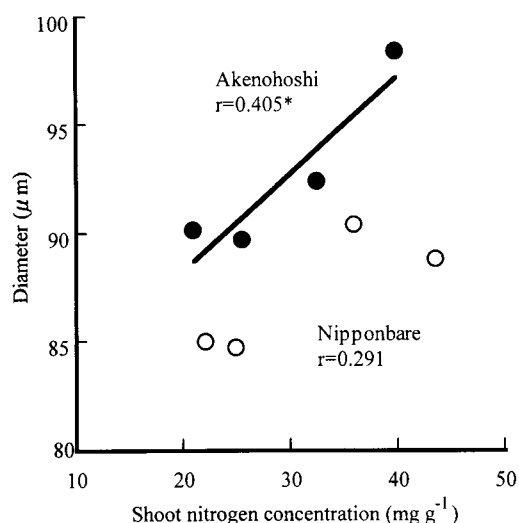


Fig. 1. The relationship between the diameter of apical dome and shoot nitrogen concentration determined at the panicle initiation stage in two rice cultivars (Nipponbare, ○; Akenohoshi, ●) applied 1N, 2N, 4N or 8N during the vegetative stage.

* indicates significant at 5% level of probability.

an increase in the numbers of differentiated SBs and spikelets per panicle in Akenohoshi (Fig. 3). However, in Nipponbare, the N treatments did not affect the number of differentiated PBs, SBs, and spikelets per panicle (Fig. 3).

3. Leaf growth and panicle morphological characters

The N treatments during the vegetative stage did not change the heading date of either of the cultivars, but increased the total leaf number on the main culm (data not shown). The increase in total leaf number was caused by rapid leaf emergence induced by high N application during the vegetative stage (Fig. 4). Although the number of differentiated PBs in Akenohoshi was increased by the N treatment, panicle and flag leaf blade lengths were not (Table 2). A positive correlation was seen between rachis length and the number of

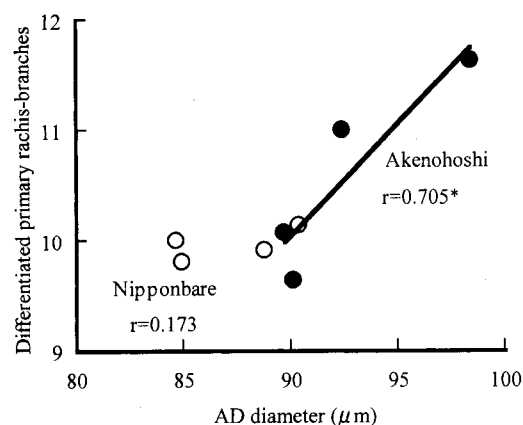


Fig. 2. The relationship between the apical dome diameter determined at the panicle initiation stage and the number of differentiated primary rachis-branches per panicle in two rice cultivars (Nipponbare, ○; Akenohoshi, ●) applied 1N, 2N, 4N or 8N during the vegetative stage.

* indicates significant at 5% level of probability.

differentiated PBs ($r=0.642^*$) in Akenohoshi. However, the correlation coefficient was only 0.346 in Nipponbare.

Discussion

Nitrogen treatment during the vegetative stage increased the diameter of ADs at the PI stage and the number of differentiated PBs in Akenohoshi plants. The increase in the number of differentiated PBs resulted in the numbers of differentiated SBs and spikelets per panicle. However, in Nipponbare, the N treatment hardly increased the diameter of ADs, and did not affect the quantitative morphological characteristics such as the number of differentiated PBs. Several studies indicated that the final size or the number of aboveground organs in rice was related to AD size at the time when those organs differentiated (Yamazaki, 1963a, b, c; Fukushima, 1999). Yamazaki (1963a) showed that the final size of rice leaves on a culm successively increased as they ascend the nodes, and found that the final size was proportional to the AD size at the time when those leaves differentiated. However, in this experiment, no

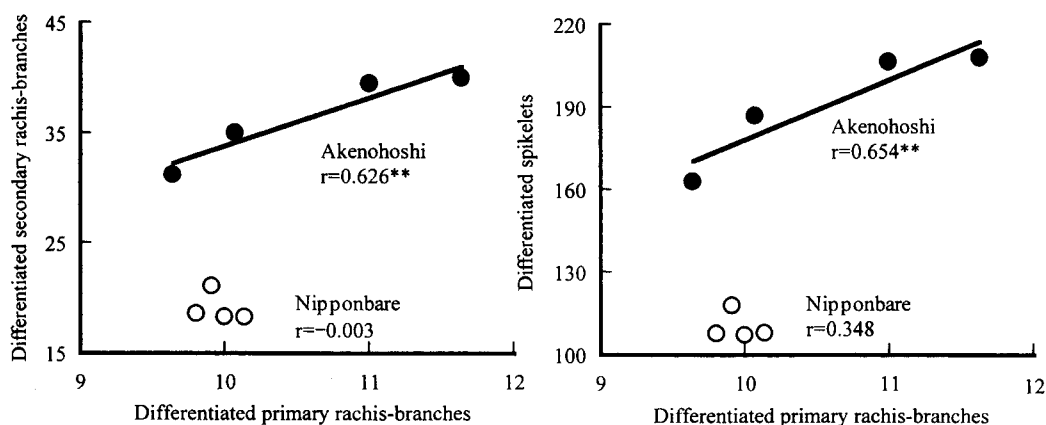


Fig. 3. Correlation of the number of differentiated primary rachis-branches with the numbers of differentiated secondary rachis-branches (left) and spikelets (right) per panicle in two rice cultivars (Nipponbare, ○; Akenohoshi, ●) applied 1N, 2N, 4N or 8N during the vegetative stage.

** indicates significant at 1% level of probability.

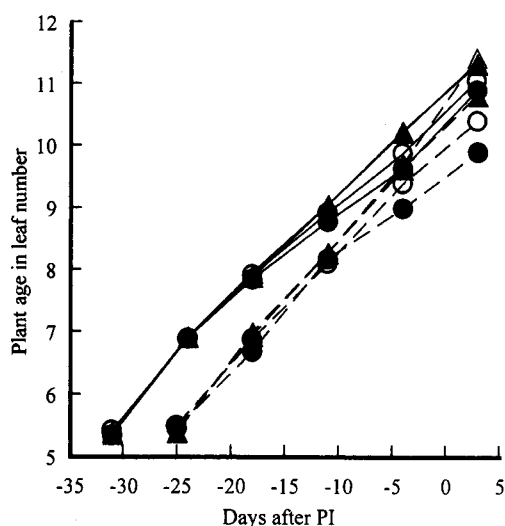


Fig. 4. The change of plant age in leaf number with the lapse of time in two rice cultivars (Nipponbare, solid line; Akenohoshi, broken line) applied 1N(●), 2N(○), 4N(▲) or 8N(△) during the vegetative stage.

relationship was seen between AD size at the PI stage and the length of the flag leaf blade. This is probably because final leaf size was influenced not only by the size of ADs but by environmental conditions (Yamazaki, 1963b). The panicle length was also unaffected by the AD size in this experiments. In Akenohoshi, the rachis length had a positive correlation with the number of differentiated PBs. Kondo and Futsuhara (1980) also reported this correlation. Panicle length is the sum of rachis length and terminal PB length, and might be more affected by environment.

In the present experiment, the number of differentiated PBs on a panicle in Akenohoshi increased with the increase in the AD diameter at the PI stage in agreement with the previous studies on varietal differences in spikelet number per panicle (Yamagishi et al., 1992; Fukushima, 1999; Mu et al., 2000). Matsuba (1991)

speculated that the size of ADs at the PI stage determines the theoretical maximum number of differentiatable PBs per panicle. What determines the number of differentiated PBs in terms of the diameter of ADs at the PI stage? Primary rachis-branches differentiate by means of periclinal division at the flank meristem of the corpus (Shimizu et al., 1968). This fact suggests that the number of differentiated PBs depends on the circumference of or the cell number in the flank meristem of the corpus. However, N treatment in the vegetative stage increased the layer number of tunica and decreased the relative size of the corpus (Shimizu, 1960). The relationship between panicle morphological characteristics and AD size would be more complex and could be controlled through tunica-corporis structure and size.

A high rate of cell division during the vegetative stage due to the N treatment would cause rapid leaf emergence and thus increase in AD size. Nitrogen treatment will promote the activity of phytohormones such as gibberellins and cytokinins and increase the cell division rate. In rosette plants, cell division is activated before stem elongation, and it is related to the action of gibberellins in the apical meristem (Sachs, 1965). *Xanthium* treated with GA₃ showed a higher cell division rate, with doubling of AD size (Maksymowych et al., 1976). These results indicate that active cell division increases AD size during the vegetative stage.

In the present experiment, the AD diameter of Nipponbare was little affected by high N treatment while that of Akenohoshi was increased by 10% (8N). It is possible that apical cells are more partitioned to tiller ADs at leaf primordium differentiation in Nipponbare than in Akenohoshi. Although all the emerging tillers were removed, the effects of ADs of tillers were not completely eliminated because tiller buds inside leaf sheaths of main culms were not removed. However, Hanada (1977) reported that dense seeding did not decrease the AD size of tillers. The primordium of a tiller

is initiated below the end of the margin of a leaf primordium (Hoshikawa, 1989), and this indicates that a tiller AD is not directly divided from an AD of a mother stem. Another hypothesis is that the size of ADs might be proportional to the plant's ability to absorb assimilates and other substances. With floral induction, the respiration rate of AD is increased and the synthesis and accumulation of RNA and protein are activated (Lyndon, 1998). It is possible that tiller ADs in Nipponbare have a relatively high absorbing ability and that this characteristic is linked with its high tillering ability and small panicle size. In the present study, tillers were removed to obtain uniform shoots in development and size, but in a further study, we need to take into account the quantitative relation in spikelet number between main culms and tillers.

Several factors such as N, assimilates, phytohormones, and AD size may affect the morphological panicle characteristics (Matsuba, 1991; Kobayasi and Horie, 1994). The increment in shoot N concentration during the period between the PI stage and the early spikelet differentiation stage increases the ratio of the number of differentiated SBs to that of PBs (Kobayasi and Horie, 1994). Gibberellins sometimes increase not only the number of differentiated SBs but also the number of differentiated PBs, and Matsuba (1991) speculated that gibberellins delay the termination of PB differentiation and increase the number of differentiated PBs. The size of ADs at the PI stage is thought to determine the theoretical maximum number of differentiable PBs (Matsuba, 1991), and the actual number of differentiated PBs and spikelets would be determined by nutritional conditions such as the amount of N and assimilates under the control of phytohormones such as gibberellins and cytokinins. The organogenesis related to N and assimilate 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 relationship between AD size and spikelet number should be performed in regard to the effect of N and phytohormones.

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*In Japanese.

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