Relation between Crown Root Primordia Formation and Stem Size in Unelongated Stems of Wheat (*Triticum aestivum* L.)

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Abstract: The number and thickness of crown root primordia (CRP) were examined with special reference to the size of the peripheral cylinder of longitudinal vascular bundles (PV), in which CRP are formed. Unelongated parts of main stems were sampled from the plant at 3.2, 5.2 and 7.2 plant age in leaf number; this index was adopted because of the morphological similarity to rice plants. Serial cross sections were made to investigate the position and the basal diameter of CRP in the unelongated stem. No relationship was observed between the CRP number and the PV side area in each growth stage. In contrast, the basal diameter of CRP increased with the increment of the circumference length of PV at each stage. Taken together, the number of CRP is not related to the PV size, whereas the CRP thickness shown by the basal diameter depend largely upon the PV size. Investigation using physiological approaches is necessary for further understanding of factors that determine CRP frequencies.

Key words: Crown root primordia, Peripheral cylinder of longitudinal vascular bundles, *Triticum aestivum* L., Unelongated stem, Vascular bundle, Wheat.

Wheat (*Triticum aestivum* L.) has 5-6 seminal roots (Percival, 1921; Hoshikawa, 1980) and ca. 8-10 (Oyanagi, 1998) or ca. 30 (Kawashima, 1998) crown roots per stem. Therefore, the number of crown roots per plant is ca. 30-50, which is much fewer than that in rice plants (ca. 500-1000 roots; Kawashima, 1998). Klepper et al. (1984) reported that the number of emerged roots could be presumed by the number of emerged leaves. Consequently, great attention was paid to the formation of crown root primordia (CRP) and their emergence along the wheat stem axis, for the basis of accounting for the water and nutrient supply as well as plant support (Morita, 2000).

For monocotyledonous plants, many studies on CRP formation were conducted in the 1960s to 1970s, especially in Japan. In those reports, studies of rice plants occupied the majority, as represented by Fujii (1958, 1961), Inosaka (1962) and Kawata et al. (1963). Nonetheless, a few reports were published on studies of wheat plants (Percival, 1921; Zee, 1981). In recent years, CRP formation in rice plants have been studied actively with consideration of stem inner structures (Nitta and Hoshikawa, 1992; Nitta et al., 1996a, 1998a). Their works revealed that precedent theories related to the position of CRP formation were not applicable to rice plants. A new method to understand the position of CRP formation was also proposed (Nitta and Hoshikawa, 1992; Nitta et al., 1996a, 1998a), and has been used in many experiments (Nitta et al., 2003, 2004, 2005a). However, for wheat plants, only a few studies have reported CRP formation. A previous study (Nitta et al., 2005b) investigated the position and number of CRP in wheat stem axes at 3.2 and 7.2 plant age in leaf number (PALN), which has been similarly adapted for rice plant studies. The results showed that CRP were not formed successively along the stem axis. Moreover, no definite relation to the running of vascular bundles was observed. However, morphological structures within a stem were inferred to be an important factor for determining CRP formation and emergence (Nitta et al., 2005b). The size and running features of the peripheral cylinder of longitudinal vascular bundles (PV) must be clarified to determine those CRP characteristics because CRP are formed immediately outside the region of the PV. In this study, we investigated morphological features of CRP formation and their emergence with regard to the PV structure in the unelongated stems of wheat.

Materials and Methods

1. Plant materials

Wheat (*Triticum aestivum* L., cv. Norin 61) plant materials used in this experiment were grown similarly to those in previous experiments (Nitta et al., 2005b). Soil (the loamy layer of the Kanto Region) was sampled from the experimental field of the College of Agriculture, Ibaraki University. Chemical fertilizer was applied at 0.8 g N, 0.7 g P₂O₅, and 1.2 g K₂O per 1/5000 a Wagner pot (inside dimension: 159 mm in diameter, 190 mm in depth). In each pot, 12 wheat seeds were sown (two seeds per hill) at a depth of 2 cm on 10 January 2003. Plants were thinned to six plants per pot after shoot emergence. Plant age was measured as plant age in leaf number.
(PALN); it is commonly used for rice plants (Hoshikawa, 1989). At 3.2 (10 March 2003), 5.2 (28 March 2003) and 7.2 (14 April 2003) PALN, plants that had grown uniformly tillering from the same position were sampled. Then, unelongated stems of seven plants were fixed in an FAA solution (70% ethyl alcohol : formalin : acetic acid = 90 : 5 : 5 (v)) for more than 1 wk. Samples were preserved in a hydrofluoric acid (45%) - ethyl alcohol (1:1 (v)) solution for 8-12 d to soften the fixed materials. The samples were then dehydrated through a graded series of ethyl and butyl alcohol, and embedded in paraffin. Then, to soften the materials again, we submerged the materials exposed by razor trimming on the stubs into a 30% (v v\(^{-1}\)) glycerin solution for half a day or one day (Kaufman et al., 1965). Then, thin cross sections (10 µm thickness) were cut with a rotary microtome (EDR-88; Yamato Kohki Industrial Co. Ltd., Asaka, Japan) and stained with 0.05% (w v\(^{-1}\)) toluidine blue O solution (Sakai, 1973).

2. Light microscopy
Every serial cross section of seven plants at each PALN was observed under a light microscope (BX51; Olympus Optical Co. Ltd., Tokyo, Japan) at 200 µm intervals along the stem axis. In this study, we observed the stem axes in cases where PV were formed. Photographs were taken using a digital camera (Camedia C-4040 Zoom; Olympus Optical Co. Ltd., Tokyo, Japan). In these experiments, we counted CRP in the sections with the thickest CRP among the neighboring serial sections without repetition. Using the same section, the basal diameter of CRP and a curved-line immediately outside of the PV (circumference of PV; see Fig. 1a) were measured with a personal computer equipped with the image analyzing software (WinROOF; Mitani Corp., Tokyo, Japan).

In this experiment, only four serial cross sections were available at 7.2 PALN, because the other serial cross sections were not successive throughout the unelongated stem due to their hardness when sectioning. It was revealed in our previous study (Nitta et al., 2005b), that the primordia of six seminal roots were formed in the basal portion of the stem. Therefore, for quantitative analysis on the stem inner structure in this study, six seminal roots are also included in CRP. Moreover, the term ‘CRP’ was used not only for unemerged CRP, but also for the basal part of emerged crown roots in the stem, to clarify

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**Table 1. Shoot and root characteristics of samples.**

| PALN | Plant length\(^1\) (cm) | Nodal position of emerged tillers\(^2\) | Shoot dry weight\(^1\) (mg plant\(^{-1}\)) | Root dry weight\(^1\) (mg plant\(^{-1}\)) |
|------|------------------------|----------------------------------------|------------------------------------------|----------------------------------------|
| 3.2  | 9.4 ± 0.2              | c, 1                                   | 39.8 ± 1.7                               | 38.9 ± 1.6                              |
| 5.2  | 13.1 ± 0.3             | c, 1, 2, 3                             | 134.2 ± 5.9                              | 137.6 ± 3.3                             |
| 7.2  | 21.2 ± 0.6             | c, 1, 2, 3                             | 363.4 ± 23.9                             | 280.0 ± 25.8                            |

1: Values are means ± S.E. 2: c, 1, 2 and 3 indicate the coleoptile, 1st, 2nd and 3rd nodes, respectively.

Fig. 1. Cross-section of an unelongated stem of a plant at 7.2 PALN. a and b: respective cross sections at 2.2 and 6.4 mm from the stem base; bars, 1 mm. Solid lines indicate the measured part of the CRP measured for basal diameter. Broken-curved lines indicate the measured part of PV circumference. L\(n\), large vascular bundle coming from the \(n\)-th leaf sheath; PV, peripheral cylinder of longitudinal vascular bundles; R, CRP; S\(n\), small vascular bundle coming from the \(n\)-th leaf sheath; T\(c\), tiller growing from the coleoptile; T\(n\), tillers growing from the \(n\)-th leaf axil.
their formation position.

Results

1. Plant growth

We used uniformly and moderately grown plants with tillers emerged from the same nodal position in this experiment (Table 1). The plant at 3.2 PALN was 9.4 cm long and had a coleoptile and a 1st nodal tiller. The plant at 5.2 PALN was 13.1 cm long and had a coleoptile, along with the 1st, 2nd and 3rd nodal tillers. The plant at 7.2 PALN was 21.2 cm long and had a coleoptile, along with the 1st, 2nd and 3rd nodal tillers. The flag leaf appeared at the 9th nodal position under the same condition. In addition, it was revealed by our previous observation of dissecting the stem under stereoscopic microscope, that the plants at 5.2 PALN had already reached reproductive stage.

2. CRP formation, stem size and their relations

Figures 1a and 1b show light-micrographs of cross sections at 2.2 and 6.4 mm, respectively, from the unelongated stem base of 7.2 PALN. The PV were formed in cortex areas inside several layers from the epidermis. Large and small vascular bundles coming from the leaf sheath were included in PV. The CRP were formed immediately outside PV regions. The PV were larger in the upper stem (Fig. 1b) than in the lower stem (Fig. 1a). Respective numbers of CRP in these cross sections are 1 and 4.

The side area of PV, the number of CRP and emerged crown roots increased with the advance of PALN (Figs. 2a and 2b). Concurrently, the CRP diameter increased before 5.2 PALN; subsequently, it did not change until 7.2 PALN (Fig. 2c). No relations existed between the side area of PV and the number of CRP in each PALN (Fig. 3). On the other hand, when the circumference was measured at the same cross section of each CRP, it was closely correlated with the CRP diameter (Fig. 4).

We also calculated the percentage of the sum of basal area of CRP to the side area of PV. Results indicated that this value increased from 43.7% at 3.2 PALN to 57.0% at 5.2 PALN, followed by a decrease until 7.2 PALN (42.3%, as shown in Table 2).

Discussion

We reported in a previous paper that CRP were not formed successively along the stem axis in wheat plants.
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these reports, Nitta et al. (1996a, 1996b, 1997, 1998b) reported that such positive correlations are observed not only in the main stem and tillers, but also in all cultivars. Moreover, the ‘formation rate of CRP’, which is indicated by the slope of the regression line in these positive correlations, appeared to be a useful tool for comparing CRP formation frequencies among tillers and cultivars.

In this experiment, the CRP diameter closely correlated with the PV size (Fig. 4). This fact supports the report of Yamazaki and Nakamoto (1983), who showed that the stem diameter closely related with the basal CRP diameter. However, no relations were apparent between the side area of PV and the number of CRP on each PALN in a wheat plant (Fig. 3). This result shows that the number of CRP was not dependent on the size of the PV where CRP were formed. In addition, no definite relation exists between CRP formation and vascular bundles’ running in wheat plants (Nitta et al., 2005b). These facts, therefore, indicates not only the PV size, but also running features of vascular bundles are not related to the CRP formation. Why is that so?

In rice plants, root pruning of the seedlings at 3.2 PALN decreased the CRP thickness and the size of PV after transplanting (Nitta et al., 2005a). This implies that the CRP formation was affected by physiological conditions of the plants. The percentage of the sum of the basal area of CRP to the PV side area, ranged from 42 to 57% depending on PALN in this experiment (Table 2). The fluctuation of this index was also reported among cultivars (Nitta and Yamamoto, 1996) and root-pruning treatments (Nitta et al., 2005a). These facts indicate that the CRP formation is largely influenced not only by the PV size, but also by physiological conditions of the wheat plant, or by cultivar properties. In addition, compared to rice and other summer crops, wheat plants have low growth rates of leaves and stems (Takahashi and Nakaseko, 1994; Suge et al., 1997), suggesting that the rates of CRP differentiation, division and growth are also low. Therefore, if water and nutrients are supplied sufficiently from neighboring vascular bundles, immediate formation of CRP might not occur because of their inactive differentiation, division and growth. These facts suggest that several factors

Table 2. Percentage of the sum of the basal area of CRP to the PV side area.

| PALN | Side area of PV (mm²) | Sum of basal area of CRP (mm²) | Percentage of basal area of CRP to side area of PV (b/a) |
|------|----------------------|-------------------------------|-------------------------------------------------------|
| 3.2  | 8.2 ± 0.5            | 3.6 ± 0.4                     | 43.7 ± 2.1                                            |
| 5.2  | 21.1 ± 1.3           | 12.0 ± 0.8                    | 57.0 ± 2.0                                            |
| 7.2  | 40.3 ± 4.9           | 16.9 ± 0.7                    | 42.3 ± 1.9                                            |

1: Values are means ± S.E.
such as physiological conditions of the plant, cultivar differences, differentiating ability, division and growth, might regulate CRP formation during growth.

In addition, as in rice plants, factors of differentiation, division and growth for CRP appear to include fertilizer amounts and its application timing, water status within a plant or in soil, and temperature during growth (Kawata et al., 1978; Nitta et al., 1996b). Moreover, as Jin and Hanada (1993) reported in water culture experiments of rice, application of kinetin suppresses the increase of emergent roots, suggesting that plant hormones influence CRP formation. Exact understanding of CRP formation awaits future studies on physiological and hormonal investigations.

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