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Anatomical Characteristics of the Formation of Crown Root Primordia in Unelongated Stems of Wheat

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Abstract : Anatomical observations were conducted to clarify some characteristics of the crown root primordia (CRP) formation in wheat stems. Unelongated portions of main stems were sampled from the plant at 3.2 and 7.2 plant age in leaf number, which were adopted as indexes because of the similarity to rice plants. Then, serial cross sections were made to investigate the position of CRP in the unelongated stem taking into consideration the running of vascular bundles in the stem. CRP were formed just outside tissues of the peripheral cylinder of longitudinal vascular bundles. The positions of CRP were not successive along the stem axis. They showed no definite relation to the running of vascular bundles. Diameters of CRP at the upper portion of the stems were larger than those at the lower portion. The positions of CRP along the stem axis were not distinguishable into nodal and internodal position. CRP and emerged CRs were not classified by the well-known 'nodal root' or 'shoot unit root', or the 'unit', which have been applied recently to rice plants. Further studies are necessary to clarify the factors controlling CRP formation anatomically and quantitatively.

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

Wheat (*Triticum aestivum* L.) is a monocotyledon of the family Gramineae. It has 5-6 seminal roots (Percival, 1921; Hoshikawa, 1980) and ca. 8-10 (Oyanagi, 1998) or 30 (Kawashima, 1998) crown roots (CRs) per stem. In addition, a hill, which comprises several stems, has a fibrous root system. It consists of not only seminal roots and CRs, but also branch roots. The CR number in root systems is 30-50 in wheat (Kawashima, 1998), which is much fewer than those in rice plants, which have ca. 500-1000 roots (Kawashima, 1998). Therefore, proper fertilizer and water management, and maintenance of high physiological activity and sound root systems during all growth stages are important for wheat cultivation. Thus, it is necessary to clarify the time of formation and emergence of each CR along the stem axis.

Very little information has been reported on the time and stage of CR formation and the position of CR along the stem axis. Percival (1921) and Fujii (1961) described the stem axis cross-section considering features of seminal roots and CR formation. However, their anatomical analyses of those aspects were limited. Recent studies have addressed the anatomical features of the unelongated stem of rice plant, indicating that CR primordia (CRP) are formed successively along the stem axis independent of node or internode portions inside the stem (Nitta et al., 1992, 1996). The present study describes morphological and anatomical features of CRP formation and their emergence in consideration of those in rice plants.

Materials and Methods

1. Plant materials

Soil (the loamy layer of the Kanto Region) used in this experiment was sampled from an experimental field of the College of Agriculture, Ibaraki University. To that soil, we added a basal dose of chemical fertilizers N, P_2O_5, and K_2O at the rate of 0.8, 0.7 and 1.2 g in 1/5000 a Wagner pot. Twelve wheat seeds (*Triticum aestivum* L., cv. Norin 61) were sown in six hills in each pot (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.

We used 'plant age in leaf number' (PALN) as a measure of plant age, which is commonly used for rice plants. At 3.2 (10 March 2003) and 7.2 (14 April 2003) PALN, plants that had grown uniformly among plants with the same position of emerged tillers were sampled being careful not to injure the shoot and roots. Then, unelongated stem portions of seven plants were fixed in an FAA solution (70% ethyl alcohol : formalin : acetic acid = 90 : 5 : 5 (v)) for more than 1 wk. The samples were preserved in a hydrofluoric acid (45%) - ethyl alcohol (1 : 1 (v)) solution for 8 to 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

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Abbreviations : CR, crown root; CRP, crown root primordia; PALN, plant age in leaf number; PV, peripheral cylinder of longitudinal vascular bundles.

Table 1. Shoot and root characteristics of samples.

PALN	Plant length[1)] (cm)	Nodal position of tillers emerged[2)]	Shoot dry weight[1)] (mg)	Root dry weight[1)] (mg)
3.2	9.4 ± 0.2	c, 1	39.8 ± 1.7	38.9 ± 1.6
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 mean coleoptile, 1st, 2nd and 3rd nodes, respectively.

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) a short time before section cutting. Thin 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 was observed using a light microscope (BX51; Olympus Optical Co., Ltd., Tokyo, Japan) at 200 μm intervals along the stem axis. Photographs were taken with a digital camera (Camedia C-4040 Zoom; Olympus Optical Co., Ltd., Tokyo, Japan). In these experiments, we counted the number of CRP in the sections with the thickest CRP among the neighboring serial sections without repetition.

The term 'CRP' was used not only for unemerged CRP, but also for the basal portion of emerged CRs in the stem, to clarify their formation position.

Results

1. Growth parameters

Uniformly and moderately grown plants with tillers emerged from the same nodal position were used for anatomical observations in this experiment (Table 1). The plant at 3.2 PALN was 9.4 cm in length and had a coleoptile and 1st nodal tiller. The plant at 7.2 PALN was 21.2 cm in length and had a coleoptile, 1st, 2nd and 3rd nodal tillers. The flag leaf appeared at the 9th nodal position under the same condition.

2. Formation of CRP in the stem

(1) Anatomical characteristics of the plant at 7.2 PALN

The position of CRP along the stem axis was examined under a light microscope.

In the basal 2 mm of the stem, six smaller root primordia (average diameter 560 μm; see Fig. 2) were formed successively along the stem axis. These primordia were formed apart from other CRP. Zee (1981) reported that the positions of seminal root primordia were separated from those of the CRP because of the epicotyl elongation in some cultivars. In our experiment also the six root primordia found in the basal 2 mm were identified as seminal roots.

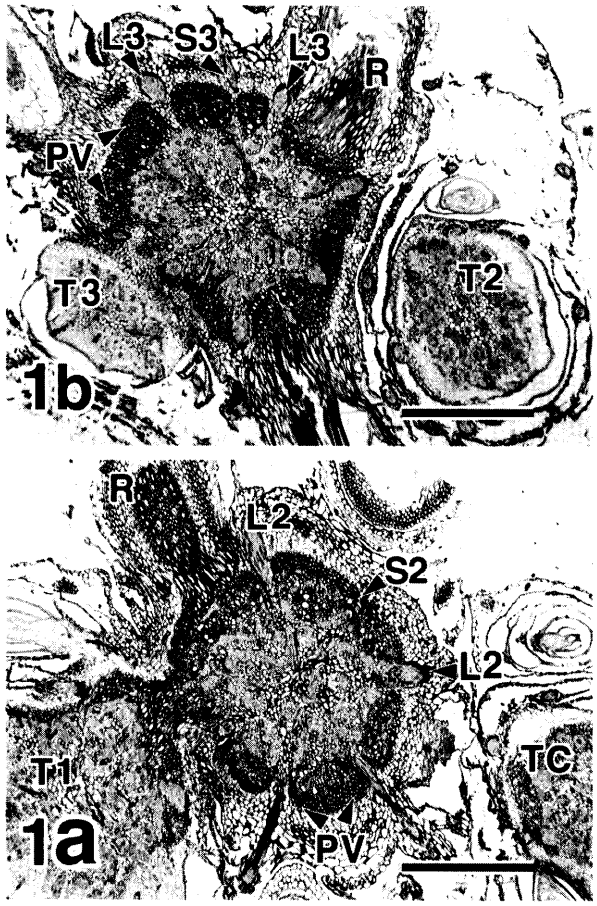

Fig. 1. Cross-section of unelongated stem of a plant at 7.2 PALN. a and b: cross sections at 4.0 and 5.8 mm from the stem base, corresponding to a and b in Fig. 2, respectively. Bars: 1 mm. L*n*, large vascular bundle coming from the *n*-th leaf sheath; PV, peripheral cylinder of longitudinal vascular bundles; R, CRP; S*n*, small vascular bundle coming from the *n*-th leaf sheath; Tc, tiller growing from coleoptile; T*n*, tillers growing from the *n*-th leaf axil.

Features of primordia formation of these seminal roots will be presented in another report. Here, we examined CRP formation in the stems more than 2 mm apart from the stem base.

Figs. 1a and 1b show the light-micrographs of cross sections at 4.0 and 5.8 mm from the base, respectively. Peripheral cylinder of longitudinal vascular bundles (PV) were formed in cortex portions inside several layers from the epidermis. They included large and small vascular bundles coming from the leaf sheath. PV

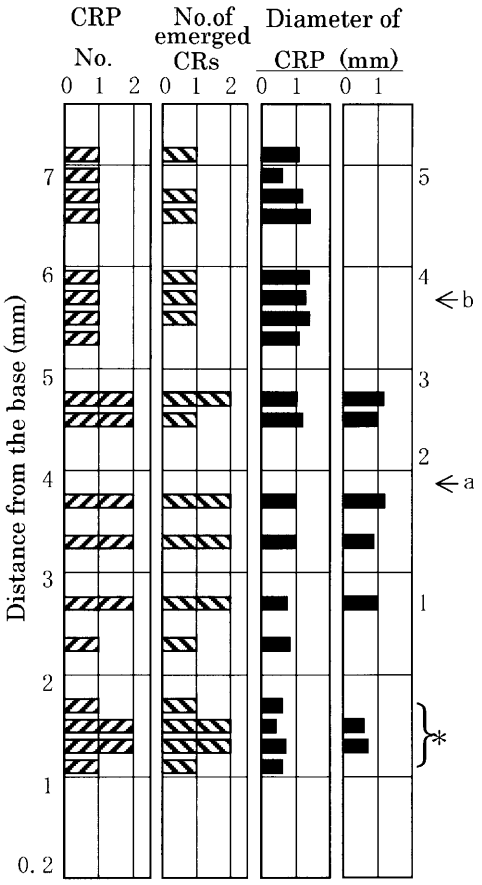

Fig. 2. Distribution of CRP and emerged CRs along the unelongated stem axis in a plant at 7.2 PALN. The total CRP number in this plant was 19, including 16 emerged CRs. *: Seminal roots. 1, 2, 3, 4 and 5 in the right margin indicate attached portions of 1st, 2nd, 3rd, 4th and 5th leaf sheath, respectively. a and b correspond to photographs of Fig. 1.

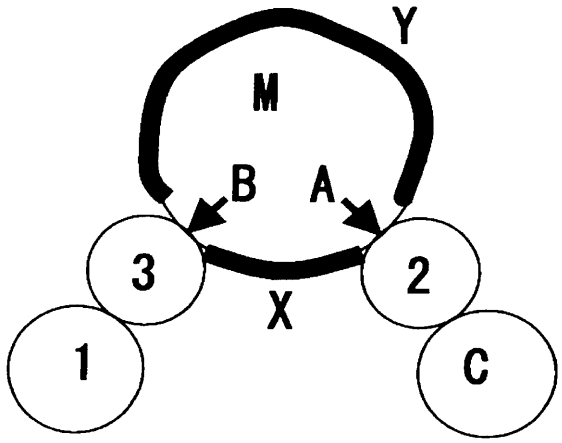

Fig. 3. Schematic diagram of a cross section of unelongated stem in the plant at 7.2 PALN. M: main stem. C: coleoptile tiller. 1, 2, and 3: 1st, 2nd and 3rd tiller, respectively. A and B show the directions of even (A) and odd (B) number tillers emerged, respectively. X and Y indicate the formation directions of CRP.

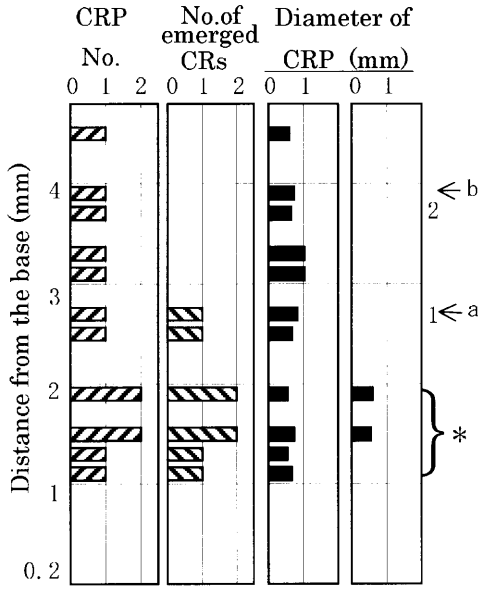

Fig. 4. Distribution of CRP and emerged CRs along the unelongated stem axis in a plant at 3.2 PALN. In this plant, the total CRP were seven, including two emerged CRs. *: Seminal roots. 1 and 2 in the right margin indicate attached portions of the 1st and 2nd leaf sheath, respectively to the stem. a and b correspond to photographs of Fig. 5.

was divided by the running of large vascular bundles coming from the leaf sheath in some stem portions, but the gaps (divided portions of PV) were not large. On one side, PV was not divided by the running of small vascular bundles coming from the leaf sheath. Just outside the regions of PV, CRP were formed (Fig. 1): CRP were formed in the PV even at the portions divided by large vascular bundles (Fig. 1b).

Fig. 2 shows the distribution of CRP and CRs together with that of seminal roots along the stem axis. The CRP were not distributed uniformly along the stem axis, and the position of CRP was unrelated to the position of leaf sheath attachment, the running of some vascular bundles, such as anastomoses, and large or small vascular bundles coming from the leaf sheath. The mean basal diameter of CRP was 1065 μm throughout the stem axis, but it was larger in the upper portion than in the lower portion.

Fig. 3 shows a schematic diagram of the direction of CRP formation in a cross sectional view. Tillers from odd and even numbered nodes face opposite directions. Therefore, CRP were formed in the inner portion between two directions of emerged tillers (X in Fig. 3) as well as in the outer portion (Y in Fig. 3). X (inner portion) is the side of seed (scutellum). The numbers of CRP throughout the stem axis were higher in the outer portion (Y)(13) than in the inner portion (X)(6).

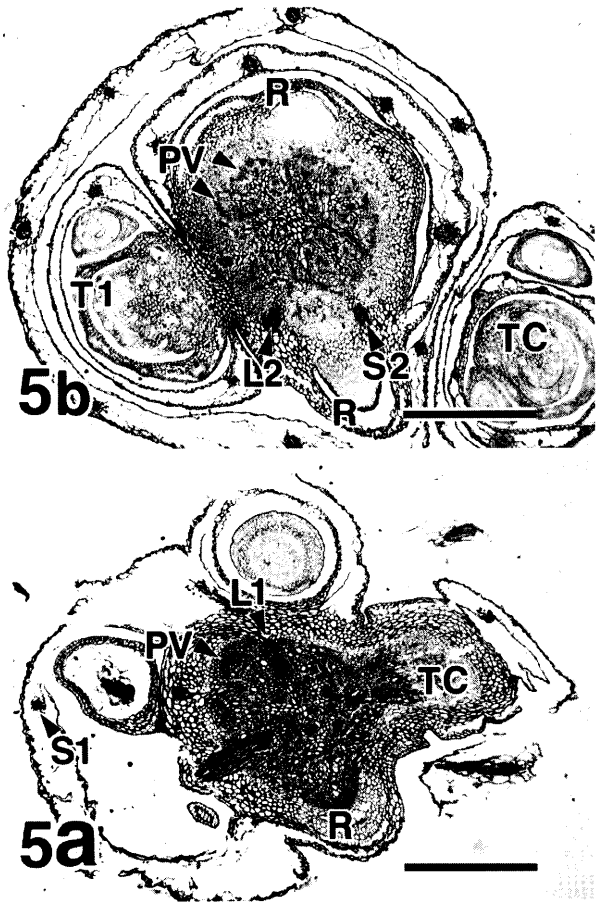

Fig. 5. Cross-section of unelongated stem of the plant at 3.2 PALN. a and b: cross-sections at 2.8 or 4.0 mm portion, respectively, from the stem base, corresponding to the portions of a and b in Fig. 4, respectively. Bars: 1 mm. For abbreviations see Fig. 1.

(2) Anatomical characters of the plant at 3.2 PALN

Fig. 4 shows the distribution of root primordia and emerged roots along the stem axis. Figs. 5a and 5b show light-micrographs of cross-sections at 2.8 and 4.0 mm from the base, respectively.

Within 2 mm from the stem base, similarly to the plant at 7.2 PALN, six small seminal root primordia (average diameter 615 μm) formed successively along the stem axis (Fig. 4). The distribution of CRP was not uniform along the stem axis, and there was no relation between the positions of CRP and leaf sheath attachment or some vascular bundle's running. The average basal diameter of CRP was 791 μm. The largest one was observed at 3.2 to 3.4 mm from the stem base.

The PV were formed in the cortex several layers inside the epidermis, including large and small vascular bundles coming from the leaf sheath (Figs. 5a and 5b). CRP were formed just outside the PV regions.

Discussion

1. Anatomical characteristics of the unelongated stem with CRP formed

In both plants at 3.2 and 7.2 PALN, there was no cavity in the unelongated stem, and the position of CRP along the stem axis was not distinguished between nodal and internodal positions. Therefore, CRP including CRs were not classified by the well-known 'nodal root' (Fujii, 1961) and the 'shoot unit root' classification (Kawata et al., 1963, 1972). Nemoto et al. (1995) reported that the rice plant is characterized as layers of phytomers, which consist of nodes and internodes, and that the CRP are formed anywhere in each phytomer irrespective of the nodal and internodal positions. However, their review article (Nemoto et al., 1995) included no anatomical observation of rice plants. We were unable to apply the 'phytomer' concept to wheat plants because the attached positions of the leaf sheath and vascular bundles were too close to each other. For this reason, our anatomical observation was insufficient to distinguish them into 'phytomers'.

The CRP were formed just outside PV regions. The PV was divided only by large vascular bundles 'running from the leaf sheath (Figs. 1 and 5), but the gaps were not so large. Nitta et al. (1992, 1996) revealed that the position of CRP in the rice plant can be distinguished based on the running feature of PV, which is largely divided by the running of large vascular bundles coming from the leaf sheath. This is called 'unit' classification, and is used for anatomical observations of rice plants. However, it seems to be inapplicable to the anatomical observation of wheat stems.

2. Anatomical characteristics of CRP formation

In most monocotyledonous plants, CRP seem to form in some specific positions along the stem axis. Nevertheless, few anatomical studies have been reported on the positions of CRP except for rice plants.

Our results showed that the CRP in wheat plants are formed just in the outside regions of PV. Features of CRP formation in wheat plants resemble those of rice plants, as reported by Kaufman (1959), Kawata et al. (1972), Nitta et al. (1992, 1996), and Nemoto et al. (1995). Moreover, the CRP diameter was larger in the upper portion of the stem than in the lower portion, indicating the same tendency as in rice plants (Nitta et al., 1998). Only one difference from rice plants is that the CRP are not formed successively along the stem axis. They also have no relation with the running of vascular bundles. The reasons for the lack of a mutual relationship remain unclear. A study using rice plants showed that supplies of assimilates and nutrition are needed for CRP formation (Kawata et al., 1978). In addition to these factors, some morphological

or quantitative factors may be necessary for CRP formation in wheat plants. For example, the thickness of CRP may offer some key to resolve this problem. CRP formation in wheat plants also seems to be related somewhat to the activity of dividing tissues, which comprise thin layers of cells compared with those in rice plants (Kawata et al., 1972; Nitta et al., 1992, 1996). Further research on the property of dividing tissues is needed. Those studies should consider water content, growth regulators such as plant hormones, and other physiological factors.

In addition to the number and position of CRP along the stem axis, we also examined the ratio of emerged CRs to total CRP. In our experiment, 2.7 out of 6.7 CRP formed within 4.6 mm from the stem base emerged out of the stems in the plants at 3.2 PALN. On the other hand, 14.0 out of 17.6 CRP formed within 7.2 mm from the stem base emerged in the plants at 7.2 PALN. Thus, the ratio of emerged CRs to total CRP was lower in the younger plants (40% in the plants at 3.2 PALN) than in the older ones (about 80% in the plants at 7.2 PALN). In addition, compared with rice plants, whose number of CRP is ca. 13 and 30 at 3.2 and 7.2 PALN, respectively (Nitta et al., 2003, 2004), wheat plants have far fewer CRP.

Our experiments show that the increase in the numbers of CRP and emerged CRs during four plant stage intervals (from 3.2 to 7.2 PALN) were 10.9 and 11.3, respectively. The average rate of increase (increased CRP per one PALN) was 2.7 for CRP and 2.8 for emerged CRs, respectively. These values correspond to the numbers of CRP and emerged CRs in a 'shoot unit root' (Kawata et al., 1963, 1972) and a 'unit' (Nitta et al., 1992, 1996) of rice plant. They are much lower than the values in rice plants, whose average number of CRP and emerged CRs in cv. Nipponbare are 10.3 and 9.8, respectively (Nitta et al., 1997).

In addition, during the four plant stage intervals (from 3.2 to 7.2 PALN) in this experiment, axial stem portions in which CRP were formed elongated by 2.6 mm acropetally. Thereby, the emerged CRs were found in a 4.4 mm stem axis from the base. Further approaches should measure axial stem features, such as size and volume, because the morphological conditions of the stem are thought to be an important factor for determining CRP formation and emergence of CRs.

3. Formation of seminal root primordia

In the plants at 3.2 and 7.2 PALN used in this experiment, six seminal root primordia were formed. They emerged successively in 2 mm from the base. Percival (1921) mentioned that seminal roots comprise five thin roots; i.e. a primary radicle, scutellum pairs and epiblast pairs; sometimes an additional one (sixth seminal root) emerges from the base of the plumule (coleoptile). Moreover, the coleoptile pairs appear at the portion the coleoptile is attached to the stem in Percival's figure (Percival, 1921). On the other hand, Hoshikawa (1980) called the coleoptile pairs a third seminal root pair and treated them as a component of seminal roots in addition to the six seminal roots shown by Percival (1921). Therefore, Percival and Hoshikawa named the coleoptile pairs differently. Still, both descriptions seem to lack the anatomical procedure as well as serial cross sectional views. Our anatomical observations were conducted using serial cross sections throughout the unelongated stem. However, the coleoptile pairs described by Percival (1921) and a third seminal root pair designated by Hoshikawa (1980) were not distinguishable in our samples. Moreover, we were unable to classify any roots that differed morphologically from six seminal or crown roots. Another experiment with young seedlings is needed to further investigate this matter.

Regarding the formation of seminal roots of Gramineae, Hoshikawa (1969) investigated 219 species of 88 genera. He reported that additional seminal roots were formed in some species including wheat (*Triticum aestivum* L.), in addition to the primary seminal root at the transitionary node (root node) or basal portion of the primary seminal root. Percival (1921) also reported that the sixth seminal root emerged just above the epiblast. We continue to investigate features of the formation of wheat seminal root primordia.

In conclusion, CRP were not formed successively along the stem axis. They also showed no definite relation to the running of vascular bundles. The position of CRP formed, could not be distinguished into nodal and internodal positions. Therefore, CRP and emerged CRs were not classifiable by the well-known 'nodal root' or the 'shoot unit root' classifications, or by the 'unit', recently applied to rice plants.

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