## Short Report

# Morphological Observation of the Rice Cultivar with Six Coleoptilar Node Roots\*

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Received September 24, 1996

**鞘葉節冠根数6本の品種におけるイネ幼植物の形態的観察**:渡邊肇・高橋清(東北大学農学部) キーワード:イネ,鞘葉節冠根,中茎,根,幼植物。

Key words: Coleoptilar node root, Mesocotyl, Rice, Root, Seedling.

The number of coleoptilar node roots (CNRs) are usually five, and very occasionally six. Sugiura<sup>5)</sup> found that six nodal roots were formed at the cotyledonary node\*\*\* when treated with the  $\alpha$ -naphthaleneacetic acid (a-NAA). Fujii2) and Hoshikawa3) also reported that six CNRs were formed in rice seedling. However, the frequency of occurrence of seedling with six CNRs was very small in these reports. Furthermore, there are inconsistencies concerning the position of an additional CNR so far. We have found a unique cultivar ('JC 91') which has six CNRs at a high frequency. In this paper, we clarify the percentage of occurrence of seedlings with six CNRs and also discuss the position of occurrence of an additional CNR using this cultivar.

#### Materials and Methods

Seeds of the cultivar 'JC 91' of rice (*Oryza sativa* L.) were used in the experiments. This is an Indica type cultivar and originated from India. Uniform size dehulled seeds were selected and sterilized in 70% (v/v) ethanol for 30s, then in 1% (v/v) sodium hypochlorite for 20 min. Sterilized seeds were promptly washed with sterilized water several times. Six seeds were placed in a test tube ( $3\times30$  cm) which contained 30 mL of a culture medium. The medium was consisted of 8 gL<sup>-1</sup> agar. The test tubes were sealed with Milli Wrap<sup>TM</sup> (Millipore Japan, Tokyo, Japan). The culture was incubated in the darkness at  $30^{\circ}$ C for 14

\* This work was presented at the 199th Meeting of the Crop Science Society of Japan, held in April, 1995.

days. After that, plant height and the length of the seedling organs were measured. The number of CNRs at the coleoptilar node was counted by use of a stereoscopic microscope. From these data, the frequency of occurrence of seedlings which have six CNRs among the investigated plants was calculated. Experiments were repeated 10 times. Each experiment was consisted of 3 to 4 replications, respectively. For anatomical studies, 5 mm segments, including the coleoptilar node, were obtained from 10 seedlings as shown in Fig. 1 (right). These segments were fixed in FAA (ethanol: acetic acid: formaldehyde: H<sub>2</sub>O =70:5:5:20 v/v, dehydrated in a tertiary butanol series, embedded in paraffin wax, sectioned serially at 10  $\mu$ m, and stained with haematoxylin.

#### Results and Discussion

We are able to clearly distinguish the CNRs from first roots because mesocotyl and the first internode had elongated. The frequency of occurrence of seedling with 6 CNRs varied from 41.7% to 72.7% (41.7, 41.7, 50.0, 53.0, 60.9, 61.1, 61.1, 66.7, 70.6, 72.7%). The average was 58.0%. Sugiura<sup>5)</sup> reported that treatment with  $\alpha$ -NAA produced seedlings with 6 CNRs, but the percentage of occurrence was very low even in these treatments (2 to 6%). We observed the seedlings with 6 CNRs without any hormone treatment and the frequency of occurrence of seedlings with 6 CNRs was very much higher comparison with Sugiura's experiment. Furthermore, there are inconsistencies between reports so far concerning the position of an additional CNR. Sugiura<sup>5)</sup> reported that an additional CNR emerged on the seminal root side. In contrast, Hoshikawa<sup>3)</sup> reported that it developed on the scutellum side. In this paper, we show the precise position where this additional root

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<sup>\*\*\*</sup> The term of 'cotyledonary node' described in Sugiuras' report corresponded to that of the 'coleoptilar node'. Accordingly, we use the term of coleoptilar node in this report.

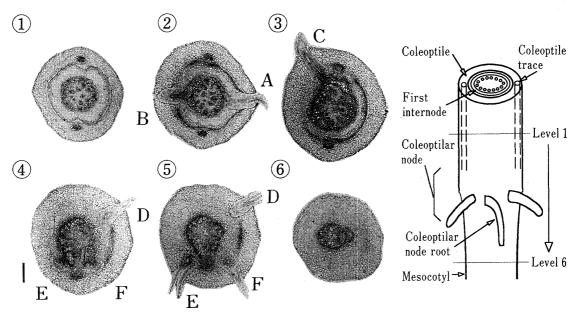


Fig. 1. Left: Transverse section near the coleoptilar node including the CNRs that have emerged. Right: The sketch illustrates the position of cutting regions at different levels of the seedling. The cross-sections from 1 to 6 in the photos correspond to the respective levels in the right-hand sketch. Level 1 indicates the basal part of the first internode. Levels 2 to 5 show the positions very close to the coleoptilar node including the CNRs which have emerged. Level 6 indicates the upper part of the mesocotyl. The alphabetical letters, A to F in the photos represent each of the 6 CNRs. Same alphabetical letters (D, E and F) correspond to the same roots. Bar=0.1 mm.

develops. Fig. 1 (left) shows a transverse section near the coleoptilar node which includes the CNRs that have emerged. In this figure, it can be observed that the roots develop in two opposite directions; 3 of 6 CNRs emerge at points B, C and E on the seminal root side, and the 3 other CNRs develop at points A, D and F on the scutellum side. In addition, we observed that in 9 other seedlings, 6 CNRs emerged in a similar way; i.e. 3 CNRs emerged in two opposite directions from each other. It is acknowledged from other studies that 3 CNRs emerge on the seminal root side and another 2 CNRs emerge at the scutellum side where there are 5 CNRs present<sup>2,3)</sup>. From these observations, we conclude that an additional root develops on the scutellum side.

Nitta et al.<sup>4)</sup> have reported that there are cultivar differences in the number of nodal root primordia in rice seedling. Bell et al.<sup>1)</sup> also reported that considerable variation exists from seedling to seedling in the details of the vascular pattern, and that this is coupled with additional variation in the number and location of CNR primordia in Italian ryegrass. Therefore, more studies on the numbers and location of emergence of root primordia are needed. These studies are currently underway

in our laboratory. Since this study was conducted *in vitro*, research concerning morphology of seedling grown under soil conditions is warranted. We also suggest that 'JC 91' rice cultivar is valuable material to investigate such diversity and the morphology of an additional nodal root that emerges from the coleoptilar node.

### Acknowledgments

The authors thank Prof. Peter B. Kaufman at the University of Michigan for this helpful comments and critical reading of the manuscript. The authors also thank Dr. K. Nemoto at Tokyo University and Mr. Y. Nitta at Kochi University for helpful and fruitful discussions.

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- \* In Japanese.
- \*\* In Japanese with English summary.