

Cortical Sclerenchyma Development in Axile Roots of Cereal Crops

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Abstract : A cereal root system consists of different axile roots that develop sequentially, i.e. (a) seminal root(s) (SR), mesocotylar roots (MR), nodal roots which originate from coleoptilar node (coleoptilar nodal root (CNR)), those from the 1st node (1 NR) and so on. The main objective of this study was to examine the histological features of basal parts of these roots with special reference to the occurrence of heterorhizy in cortical sclerenchyma development. Job's tears, Japanese barnyard millet, and pearl millet were planted in root boxes and grown in a growth chamber, with air temperature maintained at 25°C. The root systems were sampled at the 7th leaf expansion stage. In SRs and MRs, cortical sclerenchyma development was not observed among the three species. Some of the MRs in Japanese barnyard millet and pearl millet lacked the central metaxylem vessel. In all the species examined, it was CNRs that first developed cortical sclerenchyma in the process of root system formation. In half of the CNRs observed for pearl millet, however, cortical sclerenchyma development was absent, suggesting that its development is quite plastic. Subsequently, its development was also recognized in 1 NRs and 2 NRs. Histological characteristics of this tissue were less distinct in the CNRs, while they were most distinct in the 2 NR. This result suggests that cortical sclerenchyma development in the axile roots of the species proceeded with the progress of ontogeny.

Key words : Axile root, Coleoptilar nodal root, Cortical sclerenchyma, Heterorhizy, Mesocotylar root, Nodal root, Seminal root.

イネ科作物の主軸根における皮層内厚壁組織の発達 : Teresita O. GALAMAY・山内 章・巽 二郎・河野恭広 (名古屋大学農学部)

要 旨 : イネ科作物の根系は、その個体発生において、順次発生してくる種子根、中茎根、および鞘葉節から発根する鞘葉節根、第1節から発根する第1節根などの主軸根から構成される。本研究の主な目的は、これらの主軸根の組織学的特徴を、とくに皮層内厚壁組織の発現に関する異形根性に注目し検討することである。ハトムギ、ヒエ、トウジンビエを、室温 25°C に設定した人工気象室内に設置した根箱で生育させ、第7葉展開期にそれらの根系を採取した。いずれの種においても、種子根および中茎根では皮層内厚壁組織の発達は認められなかった。また、ヒエとトウジンビエの中茎根の中には、後生木部中央大導管を欠くものも観察され、これも一種の異形根性と考えられた。3種とも、それぞれの根系の形成過程において、最初に皮層内厚壁組織の形成発達が観察されたのは鞘葉節根であった。すなわち、皮層内厚壁組織の発達に関する異形性は、種子根・中茎根と鞘葉節根・第1節根・第2節根の間に存在することが明らかとなった。しかし、トウジンビエでは、供試した鞘葉節根の内の半数に、皮層内厚壁組織の発達を欠くものが観察され、このことは本組織が可塑性に富む性格の組織であることを示唆するものと解された。また、第1節根、および第2節根でもこの組織の形成発達が認められたが、その組織学的特徴は、上位節から発根した節根ほど明確であった。この結果は、皮層内厚壁組織の形成発達が個体発生の進行にともなって確立することを示している。

キーワード : 異形根性、種子根、主軸根、鞘葉節根、節根、中茎根、皮層内厚壁組織。

In the process of ontogeny, cereal crops sequentially develop different axile roots¹⁾, i.e. (a) seminal root(s) that originate from embryo, adventitious roots from mesocotyl (mesocotylar roots (MR)), and nodal roots (NR) from coleoptilar node (coleoptilar nodal root (CNR)), those from the 1st node (1st nodal root (1 NR)), and so on to form a root system. In our previous study²⁾, we compared the anatomy of the basal parts of seminal roots and nodal roots emerged from the highest

node of nine cereal species at heading stage. The most interesting finding was that heterorhizy in terms of cortical sclerenchyma development was recognized in all the species examined except rice, i.e. rice developed cortical sclerenchyma in both the seminal and the nodal root axes, while development of cortical sclerenchyma was found only in the nodal root axes for the other species. We discussed the possibility that heterorhizy might be related to different roles between seminal roots, which

support plant growth mainly for seedling establishment, and nodal roots for the rest of plant life. Therefore, it is very interesting to know from which axile root heterorhizy begins to occur in the process of root system formation.

We further speculated that the well-developed cortical sclerenchyma provides a protection mechanism for the vascular system, especially in the basal parts of the nodal root axes, which are frequently exposed to a wide fluctuation of soil environment in moisture and temperature, for example. This speculation is supported in recent works by Pardales et al.^{13,14,15)} which showed different roles played by seminal and nodal roots in the growth of sorghum under drought, waterlogging, and high soil temperature stresses, and growth recovery after these stresses were removed.

Since the cereal species have been found to differ in waterlogging and drought tolerances⁸⁾, the features of occurrence of heterorhizy may be different among the species. Therefore, choosing three cereal species which greatly differ in the tolerances, this study aimed to investigate the developmental processes of different axile roots in relation to shoot development and to examine the anatomical features of the roots with special reference to the occurrence of heterorhizy.

Materials and Methods

Based on the studies by Kono et al.⁸⁾ and Yamauchi et al.¹⁶⁾, we used Job's tears, (*Coix lacrima-jobi* L., Kyoto local) which is waterlogging tolerant, Japanese barnyard millet (*Echinocloa utilis* Ohwi et Yabuno, cv. Hidakabie) which is both waterlogging and drought tolerant, and pearl millet (*Pennisetum typhoideum* Rich, Miyazaki local) which is drought tolerant. Root boxes (40 cm deep, 25 cm wide, 2 cm thick) were filled with air-dried loamy sandy soil, to which compound fertilizer were applied at the rate of 0.25 g (equivalent to N, 30 mg ; P, 24 mg ; K, 30 mg)/kg soil and the fifteen root boxes were prepared for each species. Five seeds of each species were planted in each root box at 4 cm intervals. Preliminary experiments were conducted to determine the seeding depth that promotes the mesocotyl elongation so that MRs can develop well, and as a result, the seeds were planted at

a 5 cm depth. The plants were grown in a growth chamber with natural light condition, in which air temperature was maintained at 25°C. Irrigation was done weekly by totally submerging the root box in water for 30 min. and allowing it to drain.

The root systems were sampled using the root box pin board method of Kono et al.⁹⁾ and the sampled root systems were fixed and preserved in FAA (formalin 1 ; acetic acid 1 ; 70% ethanol 18). One root box was sampled for each species at every emergence of a new leaf to determine the relationship between root and leaf emergence until the 7th leaf completed its expansion. The rest of the root boxes were sampled when more than half of the plants in a box emerged the 7th leaf. These roots were used for anatomical observations.

Since the examined species expand a few leaves at any given time, the identification of the leaf number was not as simple as for rice or wheat, in which the n -th leaf will emerge from the lamina joint of the $(n-1)$ -th leaf only when the $(n-1)$ -th leaf has completed its expansion. Therefore, in this study the plant age in leaf number was obtained for convenience as follows : the length of the youngest leaf (the n -th leaf) that emerged from the uppermost lamina joint (usually that of the $(n-2)$ -th or $(n-3)$ -th leaf) was divided by the final leaf-blade length and $(n-1)$ was added to the value obtained.

For the anatomical observation of the axile roots, three to four root systems were selected from those sampled at the stage mentioned above. A basal 0.5-cm segment was cut off from every SR and NR, and twenty MRs selected at random. Their cross sections were prepared with the ordinary paraffin method, the specimens were stained with hematoxylin, and examined under a light microscope. In addition, the phloroglucinol-HCl test⁵⁾ was used for lignin detection. Since it was difficult to identify CNR, 1 NR and 2 NR based on the node from which they originated, the identification was accomplished based on the position and timing of emergence from the stem, diameter, and developmental features of their axes and lateral roots.

Results and Discussion

1. Relationship between leaf emergence and root development

Leaf emergence proceeded similarly among the three species. The relationship between leaf emergence and root development basically coincided with that reported by Hoshikawa⁴⁾. Nodal roots emerged first, one root for Japanese barnyard millet and pearl millet, two roots for Job's tears when the 3rd leaf was emerging and expanding (8 to 9 days after planting (DAP)). Subsequently, at the beginning of the 4th leaf emergence (11 to 12 DAP), one root for Japanese barnyard millet and pearl millet, and one or two roots for Job's tears emerged. Based on the root axis length, the diameter, and their emerging position, these roots were judged as CNRs. When the 5th leaf was expanding (15 DAP), the emergence of relatively thick roots started, and ceased with the emergence of the 6th leaf (19 DAP). These roots were judged as 1 NR based on work done by Yamazaki and Nakamoto¹⁷⁾. At the 7th leaf expansion stage (24 DAP), further, thicker roots started to emerge, which were judged as 2 NRs. MRs started to emerge acropetally (towards the shoot apex) from the base of mesocotyl at the same time when the 1st leaf started to expand and the 1st order lateral roots started to emerge acropetally (towards the root apex) on the SR axis.

Table 1 summarizes the number of axile roots that formed a root system of the plant at the 7th leaf expansion stage and Figs. 1 to 3 show the root system for each species sampled at the 5th leaf expansion stage.

2. Anatomical examination on the root axes

Cross sectional views of the SR, MR, CNR, 1 NR, and 2 NR are shown in Figs. 4 to 22. Our bases in the identification of the cortical

sclerenchyma were as follows: the tissue that 1) was located between the hypodermis and the cortical parenchyma, 2) consisted of relatively small cells that were aligned continuously, and 3) showed clearer positive reaction in phloroglucinol-HCl test with aging.

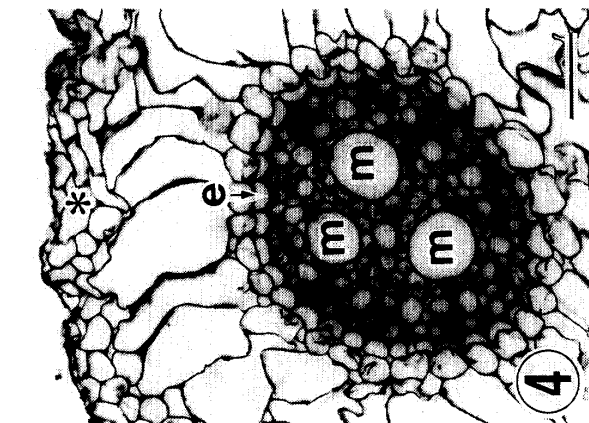
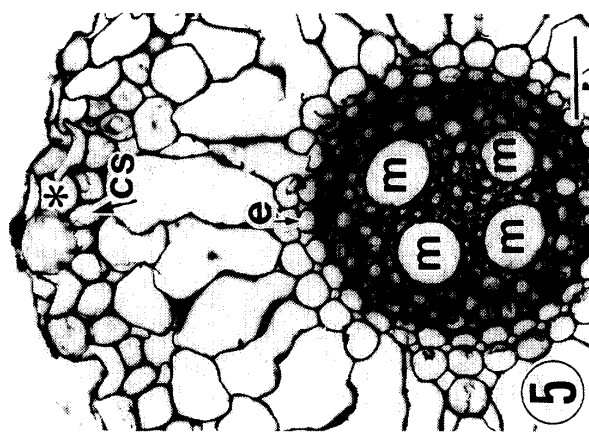
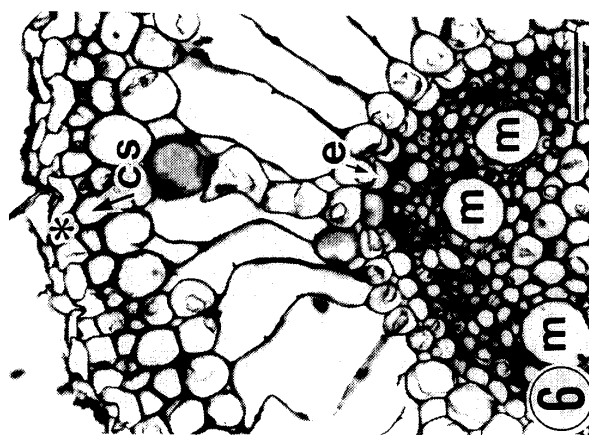
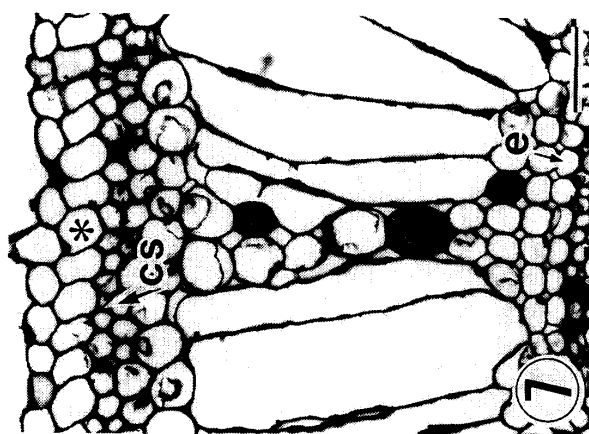
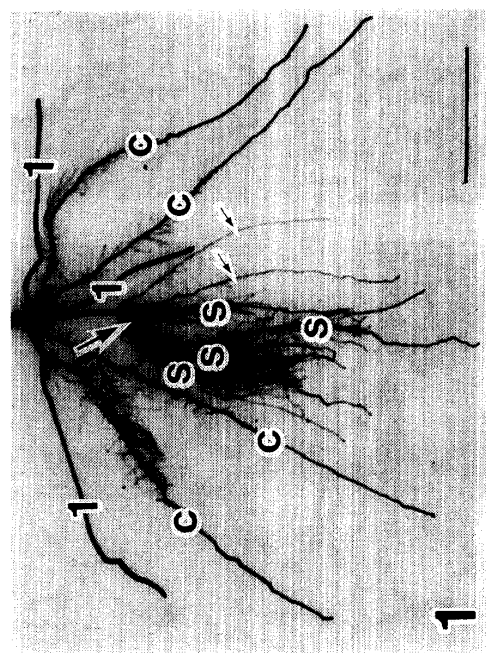
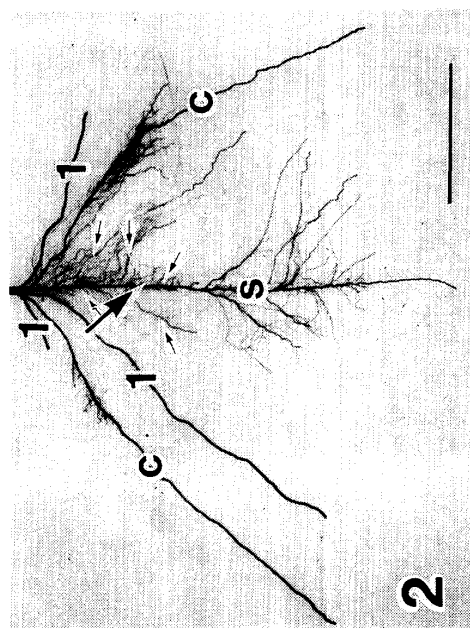
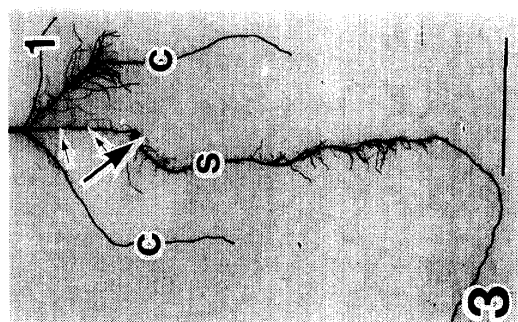
For the SR, the cortical sclerenchyma was not observed in all the species as previously reported²⁾ (Figs. 4, 8, and 12). Additionally, in pearl millet, tissues external to the endodermis were all shed, with the endodermis forming the root surface in three among the four roots examined (Fig. 12). Such a decorticated seminal root axis has been observed by McCully¹¹⁾ for other cereal species. In those roots, the central cylinder was encircled by the endodermis, whose cell wall was heavily thickened, and the pericycle consisted of double layers of cells also with heavily thickened walls.

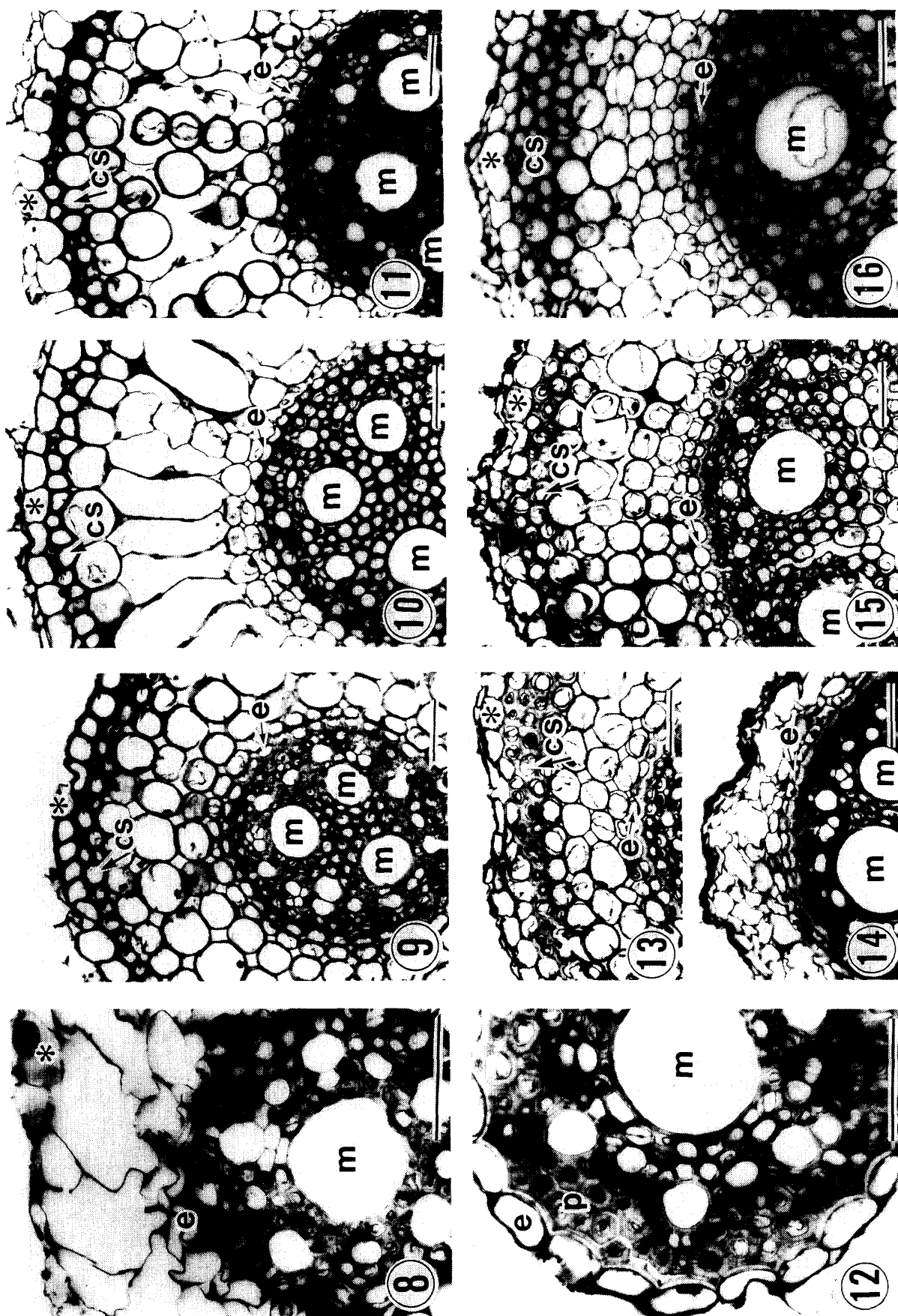
For MRs, Japanese barnyard millet produced the most number of roots, followed by pearl millet and Job's tears (Table 1, Figs. 1 to 3). The MRs of all the species in common possessed basically the same structure with the SR in the points that, though approximately 20 days had passed since the emergence, the cortical sclerenchyma development was absent, and hypodermal cells showed no wall thickening and reacted negatively in the phloroglucinol-HCl test (Figs. 17, 19 to 22). In the structure of the central cylinder, however, two different types of MRs were found in pearl millet and Japanese barnyard millet; one had a central cylinder that had one central metaxylem vessel (CMXV) like the SR (Figs. 19 and 21), while the other had a central cylinder without any CMXV and thereby the structure was similar to that often found in lateral roots of rice^{6,7)} (Figs. 20 and

Table 1. Comparison of plant age in leaf number, mesocotyl length and number of axile roots of three cereal species at the 7th leaf expansion stage (24 days after planting).

Species	Plant age in leaf No.	Mesocotyl length (cm)	No. of				
			SR	MR	CNR	1NR	2NR*
Job's tears	6.3±0.1	3.9±0.3	3.8±1.5	7.3± 4.1	3.0±0.8	3.1±0.7	2.0±0.8
JB millet**	6.5±0.1	4.8±0.4	1.0±0.0	31.3±10.4	2.0±0.0	4.0±0.3	2.4±0.2
Pearl millet	6.3±0.1	4.2±0.4	1.0±0.0	13.7± 2.1	1.8±2.0	2.7±0.0	1.8±2.0

Note: Data are shown in Mean±S.D. (n=3 to 5). **Japanese barnyard millet, *still increasing. SR, seminal root; MR, mesocotylar root; CNR, Coleoptilar nodal root; 1NR, 1st nodal root; 2NR, 2nd nodal root.





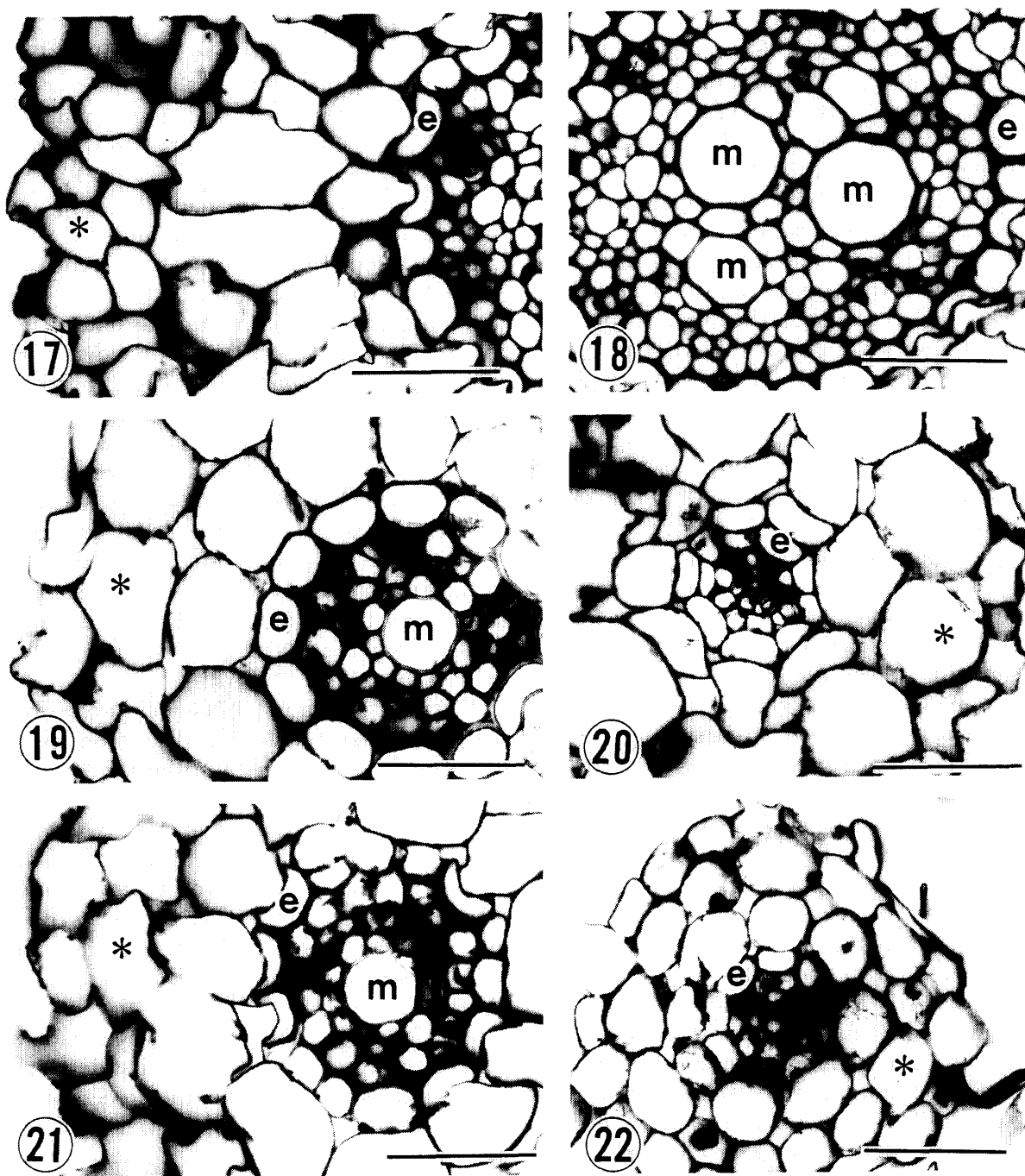


Figure Legends

Figs. 1 to 3. Photographs of the sampled root system of Job's tears (1), Japanese barnyard millet (2) and pearl millet (3) at the 5th leaf expansion stage. Thick arrows indicate the seed position and small arrows, the mesocotylar root. Bar is 5 cm. c, coleoptilar nodal root; l, 1st nodal root; s, seminal root.

Figs. 4 to 7. Transections of the basal part of seminal root (4), coleoptilar nodal root (5), the 1st nodal root (6), and the 2nd nodal root (7) of Job's tears. cs, cortical sclerenchyma; e, endodermis; m, central metaxylem vessel. Star marks indicate hypodermis. Bar is 100 μ m.

Figs. 8 to 11. Transections of the basal part of seminal root (8), coleoptilar nodal root (9), the 1st nodal root (10) and the 2nd nodal root (11) of Japanese barnyard millet. Symbols and the scale size in each figure are the same as in Figs. 4 to 7.

22). As shown in Table 2, the two types also differed in diameters of the root axis and the central cylinder, i.e., the former type was relatively thick (T-type), while the later was relatively slender (S-type). The T-type MRs accounted for about 60% of the MRs for Japanese barnyard millet and about 40% for pearl millet. The occurrence of two such types in the MRs is also considered to be a sort of heterorhizy. It is necessary to further study the features on the elongation and branching habit of the two types of the MRs to more specifically characterize their morphology.

In contrast to Japanese barnyard millet and pearl millet, Job's tears developed only a few MRs that were mostly localized in the basal portion of mesocotyl (Fig. 1). All of the MRs had one to three CMXVs, and were relatively thick, and thus heterorhizy was not recognized (Figs. 17 and 18). In all the species, the ratio of the root axis diameter to that of the central cylinder was greater in MRs as compared to those in the other axile roots (Table 2).

The mesocotyl is a portion of the shoot, which develops between the basal part of the SR and coleoptilar node, and is the site in which the root vascular bundles are connected with those of the shoot. MRs can therefore be characterized as adventitious roots in this aspect. On the other hand, they can also be characterized as lateral roots considering that the arrangement of vascular bundles in the basal parts of the mesocotyl is similar to that in the roots³⁾. Arber¹⁾ and Hoshikawa⁴⁾ pointed out that the MR may play an important role in seedling establishment under dry conditions. Furthermore, the MRs may be analogous to the basal roots reported by Lyford¹⁰⁾ and Zobel¹⁸⁾. More attention needs to be paid to the functional and morphological character-

istics of this root.

For CNRs, in all the species examined, the diameters of the root axes and the central cylinder were smaller and the number of CMXVs was fewer when compared with those of the higher nodal roots (Table 2). Histologically, however, they had a basically identical structure with the 1 NR and the 2 NR, and developed the cortical sclerenchyma immediately underlying the hypodermis (Figs. 5, 9, and 13). It is interesting to note that in pearl millet the cortical sclerenchyma was absent in two out of the four CNRs observed, which was the same with the SR in this aspect, although the two CNRs had three to four CMXVs (Fig. 14). In Job's tears the cortical sclerenchyma showed a clear positive reaction in the phloroglucinol-HCl test. But the degree of cell-wall thickening was apparently less compared to that of the higher NRs in spite of the fact that the CNR should have been more mature than the 1 NRs and 2 NRs at the time of sampling (Fig. 5).

For the 1 NRs and 2 NRs, a clear trend was recognized commonly for the three species that the stratification feature of the hypodermis and the cortical sclerenchyma was more distinct in the 2 NRs than in the 1 NRs (Figs. 6, 7, 10, 11, 15, and 16). Similarly, the diameters of the root axis and the central cylinder and the number of the CMXVs were greater in the higher NRs (Table 2), and this result concurs with the findings of Nakamoto and Yamazaki¹²⁾.

As described above, in all the species examined, it was the CNR that first developed the cortical sclerenchyma in the process of root system formation. Therefore, it was concluded that the heterorhizy in the cortical sclerenchyma development was recognized between

Figs. 12 to 16. Transections of the basal part of seminal root (12), coleoptilar nodal roots (13 and 14), the 1st nodal root (15), and the 2nd nodal root (16) of pearl millet. Note that a coleoptilar nodal root of pearl millet (Fig. 14) has no cortical sclerenchyma. Symbols and the scale size in each figure are the same as in Figs. 4 to 7.

Figs. 17 to 22. Transections of the basal part of mesocotylar root of Job's tears (17 and 18), Japanese barnyard millet (19 and 20), and pearl millet (21 and 22). Fig. 17 and 18 indicates the tissues external to the endodermis, and the central cylinder of Job's tears, respectively. Figs. 19 and 20 shows the two types of MR for Japanese barnyard millet, respectively, and Fig. 21 and 22 for pearl millet. The roots shown in Figs. 19 and 21 were named T-type, and Figs. 20 and 22, S-type. See details in text. Symbols and the scale size in each figure are the same as in Figs. 4 to 7.

Table 2. Comparison of root diameter (R.D.), central cylinder diameter (C.C.D.), number of central metaxylem vessels (C.M.X.V.), and R.D./C.C.D. ratio in each axis of the different axile roots among three cereal species at the 7th leaf expansion stage.

Species	Axile root	R.D. (μm)	C.C.D. (μm)	R.D./C.C.D. ratio	No. of C.M.X.V.
Job's tears	SR	430 b	150 b	2.9 ac	3.3 b
	MR	241 a	73 a	3.5 bc	1.4 a
	CNR	545 b	172 b	3.2 bc	5.2 c
	1NR	748 c	278 c	2.7 a	7.5 d
	2NR	1260 d	523 d	2.6 a	12.7 e
Japanese barnyard millet	SR	343 c	147 b	2.3 a	(1.0)
	MR- (T-type)	156 b	49 a	3.2 b	(1.0)
	(S-type)	99 a	29 a	3.6 b	(0.0)
	CNR	563 d	207 c	2.7 a	2.3 a
	1NR	730 e	274 d	2.6 a	4.8 b
	2NR	930 f	346 f	2.7 a	8.0 c
Pearl millet	SR	—	203 b	—	(1.0)
	MR- (T-type)	193 b	50 b	3.9 b	(1.0)
	(S-type)	89 a	23 a	3.8 b	(0.0)
	CNR	455 c	240 b	1.9 a	3.3 a
	1NR	697 d	352 d	2.0 a	4.9 b
	2NR	900 e	460 e	1.9 a	6.7 c

Note : Data followed by the same letter in a column are not significantly different by Duncan's multiple range test ($p=0.05$). Numerals in parentheses were not treated by the statistical analysis due to the zero variance ($n=3$ to 20). SR, seminal root ; MR, mesocotylar root ; CNR, Coleoptilar nodal root ; 1NR, 1st nodal root ; 2NR, 2nd nodal root.

the SR and MR, and the CNR, 1 NR and 2 NR in the three species examined.

In this study, we characterized histologically the cortical sclerenchyma as the tissue that was located between the hypodermis and the cortical parenchyma, consisting of relatively small cells of regular size, and showed clearer positive reaction in the phloroglucinol-HCl test with aging. These characteristics were more clearly recognized in the higher NRs for Job's tears and pearl millet, and became most distinct in the 2 NR (Figs. 7 and 16), suggesting that this tissue developed from a primitive form to the established one with the progress of ontogeny. Additionally, the fact that pearl millet lacked the cortical sclerenchyma development in half of the CNRs observed suggests that the development of this tissue is quite plastic. Although the species that differ in waterlogging and drought tolerances were compared in this experiment, the difference was not reflected in the specific development

in the cortical sclerenchyma for each species. This could be due to their growth conditions, i.e., the plants were grown under moderate soil moisture conditions. Therefore, further examination of the cortical sclerenchyma development in the axile roots of these species under different soil moisture conditions is necessary to elucidate the ecological significance of the tissue in relation to the stress tolerance of the plant species.

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References

- Arber, A. 1965. The Gramineae, a Study of Cereal, Bamboo, and Grass. Wheldon & Wesley, New York. 222—247.
- Galamay, T.O., Y. Kono., A. Yamauchi and M.

- Shimizu 1991. Comparative root anatomy of seminal and nodal root axes of summer cereals with special reference to the development of hypodermis and cortical sclerenchyma. *Jpn. J. Crop Sci.* 60 : 184—190.
3. Hoshikawa, K. 1967. On the morphological characteristics of the mesocotylar root in sorghum. *Proc. Crop Sci. Soc. Japan* 36 : 532—533**.
 4. ——— 1969. Underground organs of the seedlings and the systematics of Gramineae. *Bot. Gaz.* 130 : 192—203.
 5. Jensen, W.A. 1962. *Botanical Histochemistry*. W. H. Freeman and Company, San Francisco. 175—208.
 6. Kawata, S. and H. Shibayama 1966. On the lateral root formation in the crown roots of rice plants. *Proc. Crop Sci. Soc. Japan*. 33 : 423—431*.
 7. Kono, Y., M. Igeta and N. Yamada 1972. Studies on the developmental physiology of the lateral roots in the rice seminal roots. *Proc. Crop Sci. Soc. Japan*. 41 : 192—204*.
 8. ———, A. Yamauchi, N. Kawamura, J. Tatsumi, T. Nonoyama and N. Inagaki 1987. Interspecific differences of capacities of waterlogging and drought tolerances among summer cereals. *Jpn. J. Crop Sci.* 56 : 115—129.
 9. ———, ———, T. Nonoyama, J. Tatsumi and N. Kawamura 1987. A revised experimental system of root-soil interaction for laboratory work. *Environ. Control Biol.* 25 : 141—151.
 10. Lyford, W.H. 1975. Rhizography of non-woody roots of trees in the forest floor. In Torrey, J.G. and D.T. Clarkson eds., *The Development and Function of Roots*. Academic Press, London. 179—196.
 11. McCully, M.E. 1987. Selected aspect of the structure and development of field-grown roots with special reference to maize. In Gregory, P.J., J.V. Lake and D.A. Rose eds., *Root Development and Function*. Cambridge University Press, Cambridge. 53—70.
 12. Nakamoto, T. and K. Yamazaki 1988. Quantitative relationship among vegetative organs and their conductive tissues in several millets. *Jpn. J. Crop Sci.* 57 : 490—495*.
 13. Pardales, J.R.Jr. and Y. Kono 1990. Development of sorghum root system under increasing drought stress. *Jpn. J. Crop Sci.* 59 : 752—761.
 14. ———, ——— and A. Yamauchi 1991. Response of different root system components of sorghum to incidents of waterlogging. *Environ. Exp. Bot.* 31 : 107—115.
 15. ———, A. Yamauchi and Y. Kono 1991. Growth and development of sorghum roots after exposure to different periods of hot root-zone temperature. *Environ. Exp. Bot.* 31 : 397—403.
 16. Yamauchi, A., Y. Kono and J. Tatsumi 1987. Comparison of root system structures of 13 species of cereals. *Jpn. J. Crop Sci.* 56 : 618—631.
 17. Yamazaki, K. and T. Nakamoto 1983. Primary root formation in the growing shoots of several cereal crops. *Jpn. J. Crop Sci.* 52 : 342—348*.
 18. Zobel, R.W. 1975. The genetics of root development. In Torrey, J.G. and D.T. Clarkson eds., *The Development and Function of Roots*. Academic Press, London. 261—275.

* In Japanese with English summary.

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