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# Acropetal Lignification in Protective Tissues of Cereal Nodal Root Axes as Affected by Different Soil Moisture Conditions

Teresita O. GALAMAY, Akira YAMAUCHI, Toshihiro NONOYAMA* and Yasuhiro KONO
(*School of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan ;*
**Aichi College of Agriculture, Miai, Okazaki 444, Japan*)

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**Abstract** : This study aimed to examine the effects of different soil moisture conditions (waterlogging, moderate, drought) on acropetal lignification in protective tissues (hypodermis, cortical sclerenchyma, endodermis) in the lst nodal roots of Job's tears (waterlogging tolerant, drought susceptible), Japanese barnyard millet (walerlogging and drought tolerant), and pearl millet (drought tolerant, waterlogging susceptible). They were grown in root boxes for 41 days. For Job's tears, lignification in the hypodermis and the cortical sclerenchyma tended to be promoted as the soil moisture conditions became drier, and the reduction in shoot dry weight by drought was not very serious. For Japanese barnyard millet, lignification in the hypodermis tended to be promoted by wetter conditions, while in the cortical sclerenchyma, drought also tended to promote lignification. The shoot dry weight was only slightly reduced under both coditions. Conversely, in pearl millet, the waterlogging drastically inhibited not only the lignification in the hypodermis but even the differentiation of the cortical sclerenchyma itself. Accordingly, shoot as well as root system growth was severely restricted under the same conditions. In contrast, for pearl millet grown under drought, the lignification was promoted in all the three protective tissues, and the shoot growth reduction was relatively slight. These results strongly suggest that the acropetal lignification in these tissues, especially that of the cortical sclerenchyma, may be closely related to waterlogging and drought tolerances of the crops.

**Key words** : Lignification, Cortical sclerenchyma, Drought tolerance, Endodermis, Hypodermis, Protective tissue, Soil moisture condition, Waterlogging tolerance.

**イネ科作物の節根軸における保護組織の向頂的木質化の進行に及ぼす土壌水分条件の影響** : Teresita O. GALAMAY・山内　章・野々山利博・河野恭広（名古屋大学農学部）

**要　旨** : 異なる土壌水分条件（湛水区，適湿区，乾燥区）の，節根軸内の保護組織（下皮，皮層内厚壁組織，内皮）の発達に及ぼす影響を解剖学的に調べた．根箱で41日間生育させたハトムギ（耐湿性大，耐旱性小），ヒエ（耐湿性，耐旱性とも大），トウジンビエ（耐湿性小，耐旱性大）の第1節根軸の基部から2 cm毎の横断切片を作製し，光学および蛍光顕微鏡観察を行った．ハトムギでは，湛水，適湿，乾燥条件になるに従って，下皮および皮層内厚壁組織の向頂的な木質化が促進される傾向を示した．また，乾燥条件による地上部乾物重の減少はあまり大きくなかった(対適湿区比56%)．一方ヒエでは，乾燥，適湿，湛水条件になるに従って下皮での木質化がやや促進され，また，皮層内厚壁組織では湛水，乾燥両条件下で促進される傾向を認めた．地上部乾物重は湛水，乾燥区でそれぞれ適湿区の95%, 70%で，他種に比べて明らかに減少は少なかった．これらに対し，トウジンビエにおいては，湛水区では木質化は内皮のみに限られ，皮層内厚壁組織は分化そのものが抑制された．一方乾燥区では，3組織ともに向頂的木質化が顕著に促進された．それに伴い，地上部乾物重は湛水区で大きく減少した（対適湿区比18%）のに対し，乾燥区では減少割合は比較的小さかった(同64%)．これらの結果は，節根軸内のこれら3保護組織，とくに皮層内厚壁組織における向頂的木質化の進行が，その種にとって不適な土壌水分条件下での個体の発育に影響し，その作物の耐湿性・耐旱性を規定していることを示唆している．

**キーワード** : 下皮，耐旱性，耐湿性，土壌水分条件，内皮，皮層内厚壁組織，保護組織，木質化．

Crop plants are exposed to different soil environmental stresses during their growth frequently accompanying the development of various morphological protection mechanisms in their roots. The most well-known is lignification usually found in hypodermis, cortical sclerenchyma and endodermis. These tissues are believed to be important as protective tissue for the function and structure of the roots against stress enivironment[2,3,13] although the experimental evidence has been rarely presented.

Pardales et al. showed that in sorghum, seminal and nodal roots played different roles in plant growth under drought[10], watelogging[11] and high soil temperature stresses[12] and in growth recovery after these stresses were removed. Our previous investigation[4] also showed the histological difference between the two roots, i.e., the cortical sclerenchyma development was observed in the nodal roots, but not in the seminal root for many cereals. However, comparing the species, the developmental features of this tissue were almost similar to each other probably due to the fact that all the plants were grown equally under a moderate soil moisture condition. These crops, however, have been known to be different in their waterlogging and drought tolerances[7]. Considering this fact, it would be reasonable to assume that the response in the tissue development to various soil moisture conditions may be different among the species provided that this tissue bears the ecological significance in plant growth under such conditions and really functions as a protective tissue.

In this experiment, Job's tears, Japanese barnyard millet and pearl millet, which greatly differ in tolerance, were grown under different soil moisture conditions, and anatomical as well as histological observations of lignification were made in the cortical sclerenchyma, hypodermis and endodermis in the nodal roots of these species. Through the observation we aimed to examine if the specific plasticity in the development of these tissues under various soil moisture conditions can be related to their waterlogging and drought tolerances.

## Materials and Methods

We used the same cereal species as in our previous study[5] for test plants, i.e., Job's tears (*Coix lacryma-jobi* L., Kyoto local), which is waterlogging tolerant but susceptible to drought, Japanese barnyard millet (*Echinocloa utilis* Ohwi et Yabuno, cv. Hida-akabie), which is both waterlogging and drought tolerant, and pearl millet (*Pennisetum typhoideum* Rich. Miyazaki local), which is tolerant to drought but susceptible to waterlogging[7,14].

Root boxes (40 cm deep, 25 cm wide, 2 cm thick) were filled with the air-dried loamy sandy soil, to which compound fertilizer was applied at the rate of 0.25 g (equivalent to N, 30 mg ; P, 24 mg ; K, 30 mg)/kg soil. Three different levels of soil moisture conditions (waterlogging, moderate, and drought) treatments were prepared as described below, and four root boxes were prepared for each treatment. Prior to planting, root boxes for the waterlogging and the moderate treatments were totally submerged in water for 30 minutes and were allowed to drain for 24 hours. For the drought treatment boxes, 500 m*l* of water was poured from the soil surface. Three pregerminated seeds were planted at 2 cm depth in each root box on May 15, 1991, and then thinned to one plant per box five days after the planting.

The following treatments were then started : control (C-plot), moderate soil moisture condition was attained by totally submerging the root boxes for 30 minutes at a weekly interval (about 59.5% to the maximum water holding capacity of the soil) ; waterlogging (W-plot), waterlogged condition was maintained ; drought (D-plot), water was added weekly so that the original weight of the whole root box was maintained (about 29.8% to the maximum water holding capacity). The root boxes were placed in a growth chamber with natural light condition, in which air temperature was maintained at 25°C, and the plants were grown for 41 days.

At harvest, plant age in leaf number was determined as in the previous study[5], and then the root systems were sampled with the root box-pin board method[8], photographed, and preserved in FAA (formalin 1 ; acetic acid 1 ; 70% ethanol 18). The shoots were cut off from the root systems, oven-dried at 85°C for 72 hours, and weighed.

Using the identification method previously described[5], 10 to 15 lst nodal roots were collected from the plant of each species for every treatment. To further avoid contamination of other nodal roots, number of central metaxylem vessels were determined for the collected roots. Based on the previous study[5], the roots with seven to eight vessels for Job's tears, four to six for Japanese barnyard millet and pearl millet were chosen as the lst nodal root. As a result, three to five roots were obtained for each species from every treatment.

Cross sections were prepared with the ordinary paraffin method as well as the freezing microtechnique at 2-cm interval from the base acropetally towards the root apex, and the lignification in the hypodermis, cortical sclerenchyma and endodermis was examined. For lignin detection, the phloroglucinol-HCl test[6] was used and the sections were viewed with a light microscope. For the same purpose, epifluorescence microscopy was also used on the sections stained using the berberine-aniline blue procedure[1]. The sections were observed using the Olympus epifluorescent microscope with B excitation. All microphotographa were taken with Fuji Minicopy film (ASA 32), with exposure time set to 8 seconds with Nikon microflex HFX-II.

## Results and Discussion

### 1. Shoot and root growth

Table 1 indicates plant age in leaf number, shoot dry weight, and total root number of the plants grown for 41 days under the three soil moisture conditions, and photographs of the sampled root systems are shown in Figs. 1 to 9. As shown in Table 1, in all the species, the number of nodal roots was increased in W-plot as compared to C-plot (Figs. 2, 5, and 8). However, the root system development in W-plot differed according to the waterlogging tolerance of the species. Job's tears and Japanese barnyard millet, which are tolerant to waterlogging, showed relatively vigorous nodal root axes and lateral root development (Figs. 1 and 4) when compared to pearl millet (waterlogging susceptible), which formed a shallow root system (Fig. 7). Conversely, the droughted plants produced fewer nodal roots than control plants for all the species (Table 1). In lateral root development, however, an interspecific difference was found according to drought tolerance. Under drought conditions, Job's tears, which is relatively drought susceptible, showed apparently limited lateral root growth (Fig. 3), while Japanese barnyard millet and pearl millet, which are relatively tolerant to drought, developed laterals vigorously (Figs. 6 and 9).

The results on the shoot growth under the three moisture conditions and the nodal root production under waterlogged conditions (Table 1) are in good agreement with those in our previous studies[7,9]. On the other hand, in our series of root studies this is the first report that showed root system development with the spatial arrangement in soil profile for droughted and waterlogged plants (Figs. 1, 3, 4, 6, 7, and 9). Quantitative analysis on their components would elucidate the significance of the root system structure in relation to the waterlogging and drought tolerance of the species.

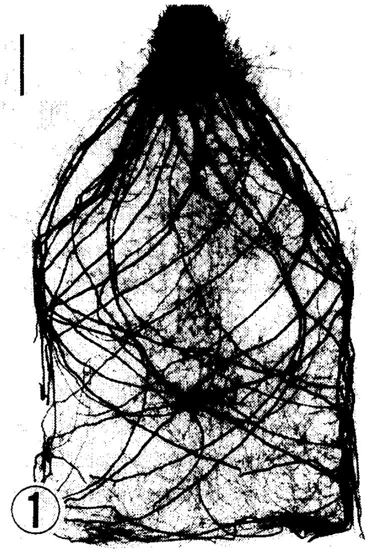
### 2. Acropetal lignification in hypodermis, cortical sclerenchyma, and endodermis

The ratios of the lignified tissue length to the whole root axis length for each species (lignification ratio) are shown in Table 2. In Job's tears and Japanese barnyard millet, the effects of the different soil moisture conditions on any lignified tissue development was not significant. In both species, approximately one-fourth in length of the endodermis was

Table 1. Plant growth at harvest (41 days after planting).

| Species | Plot | Plant age in leaf No. | Shoot dry weight (g/plant) | Total root number |
|---|---|---|---|---|
| Job's tears | W | 11.0±0.7 | 4.8±0.2 ( 96) | 40.0±4.0 |
| | C | 11.4±0.6 | 5.0±0.3 (100) | 32.5±5.4 |
| | D | 9.7±1.1 | 2.8±1.1 ( 56) | 15.3±4.2 |
| Japanese barnyard millet | W | 11.1±0.8 | 1.9±0.2 ( 95) | 76.3±8.0* |
| | C | 11.3±0.4 | 2.0±0.1 (100) | 33.0±1.0* |
| | D | 10.5±0.9 | 1.4±0.4 ( 70) | 25.0±1.4 |
| Pearl millet | W | 9.1±0.7 | 0.6±0.3 ( 18) | 28.0±5.0 |
| | C | 12.2±0.8 | 3.6±0.3 (100) | 18.0±2.0 |
| | D | 11.0±1.0 | 2.3±0.8 ( 64) | 12.0±3.0 |

Note : Data are shown in Mean±S.D. (n=3 to 5). *All samples measured had one tiller. Numerals in parentheses are percent to control plot (C) in mean value.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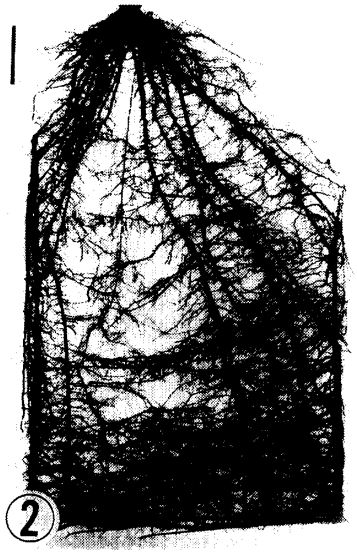
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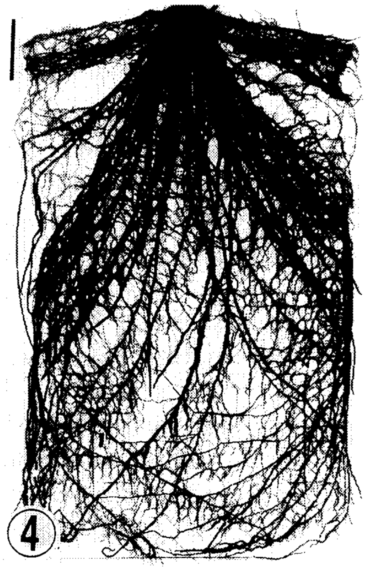
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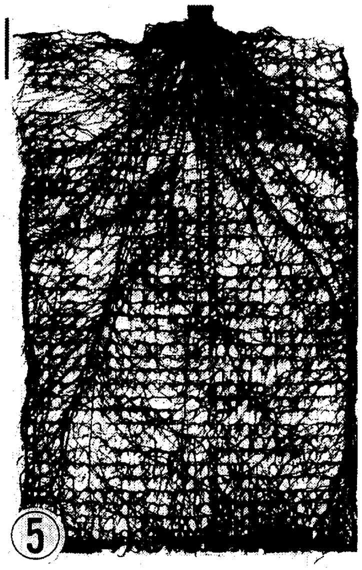
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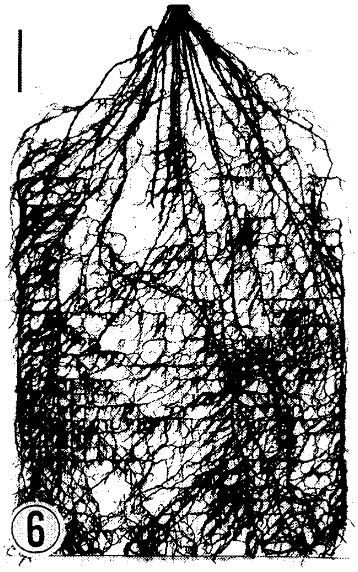
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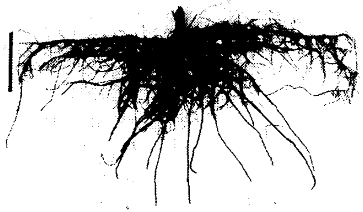
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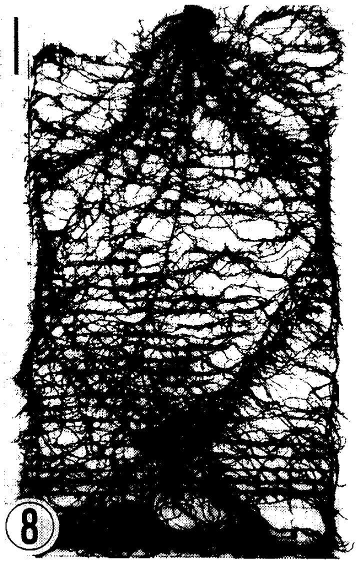
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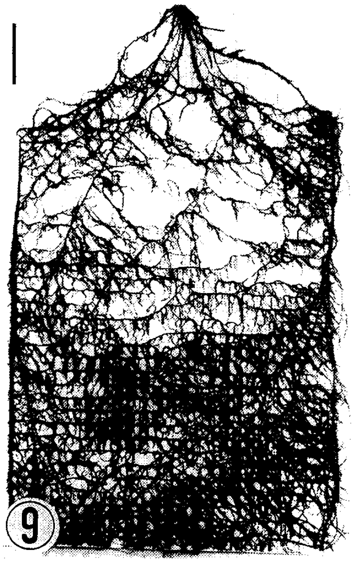
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Table 2. Acropetal development of lignified tissues in length percent of the 1st nodal root axis of three cereal species grown under three soil moisture conditions.

| Species | Plot | 1st Nodal root length (cm) | Hypodermis (%) | Cortical sclerenchyma (%) | Endodermis (%) |
|---|---|---|---|---|---|
| Job's tears | W | 11.0±4.0 | 20.6 | 15.5 | 24.7 |
| | C | 42.2±6.8 | 23.5 | 21.3 | 24.6 |
| | D | 37.0±1.7 | 28.8 | 23.3 | 26.1 |
| | LSD (0.05) | — | 10.5 | 11.2 | 6.2 |
| Japanese barnyard millet | W | 37.7±2.5 | 30.0 | 17.6 | 25.8 |
| | C | 44.3±4.0 | 27.9 | 13.6 | 24.3 |
| | D | 40.3±2.1 | 24.1 | 20.6 | 24.7 |
| | LSD (0.05) | — | 14.0 | 11.4 | 11.3 |
| Pearl millet | W | 8.3±1.5 | 0 | 0 | 72.0 |
| | C | 51.0±3.6 | 14.4 | 11.2 | 22.2 |
| | D | 38.7±2.1 | 19.8 | 27.7 | 29.2 |
| | LSD (0.05) | — | 2.3 | 3.3 | 4.6 |

lignified and the acropetal lignification in the hypodermis and the endodermis preceded that in the cortical sclerenchyma irrespective of growth conditions. The following trends were recognized, however, that for Job's tears the lignification ratio in the hypodermis and the cortical sclerenchyma tended to increase as growth conditions became drier (i.e., W-plot < C-plot < D-plot), while for Japanese barnyard millet, the ratio in the hypodermis increased slightly as conditions became wetter (i.e., D-plot < C-plot < W-plot), and the ratio in the cortical sclerenchyma tended to increase under both waterlogged and drought conditions.

In contrast, soil moisture conditions significantly affected the development of the lignified tissue for pearl millet (Table 2). Comparing the roots in W-plot (Figs. 10 and 11) with those in C-plot (Figs. 12 and 13) and in D-plot (Figs. 14 and 15), it was evident that in roots grown under waterlogging, ligification was recognized only in the endodermis but was completely absent in the tissues external to the endodermis. Moreover, the preceded acropetal lignification in the endodermis over the other two protective tissues was found not only in the roots of W-plot, but also in those of C- and D-plots, suggesting that this developmental pattern would be a specific feature for pearl millet. In addition, a comparison between the epifluorescent (Fig. 10) and the ordinary light micrographs (Fig. 11) of the basal parts of nodal roots grown under the waterlogging makes it clear that the waterlogging inhibited not only the lignification in the hypodermis but even the differentiation of the cortical sclerenchyma itself. On the other hand, clearly the drought in turn promoted the lignification in all the three tissues as shown in Table 2 and Figs. 14 and 15.

It is worth noting that although about 30 days had passed since the emergence of the lst nodal roots, lignification in the three tissues proceeded only up to approximately 10 cm

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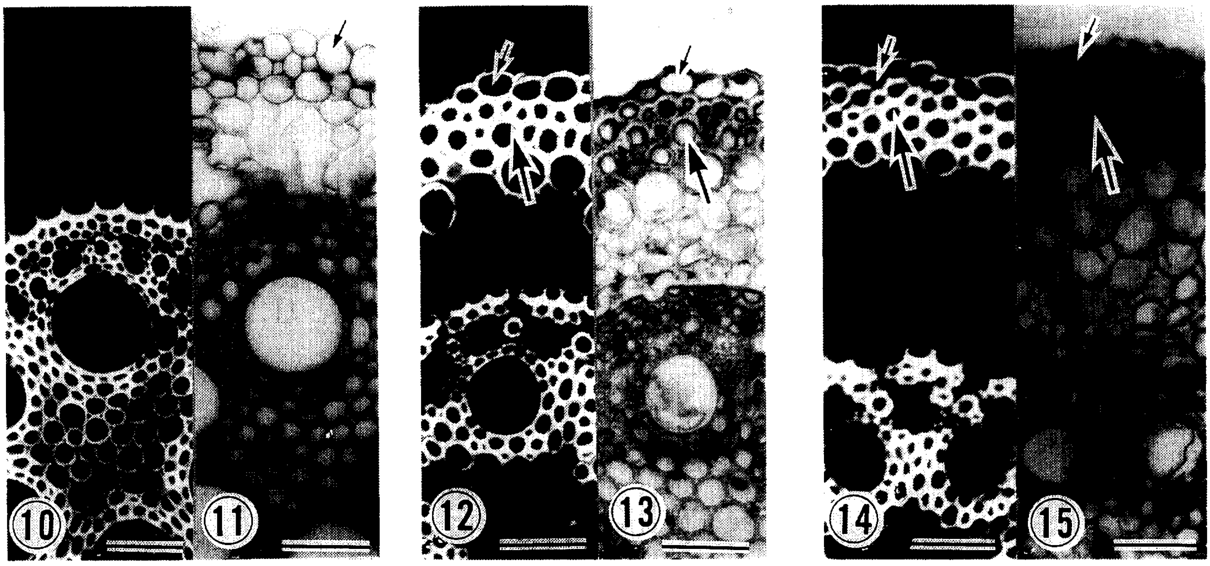
## Figure Legends

Figs. 1 to 9. Photographs of the sampled root systems of Job's tears (1 to 3), Japanese barnyard millet (4 to 6), and pearl millet (7 to 9) grown under waterlogged (1, 4, and 7), moderate moisture (2, 5, and 8), and drought (3, 6, and 9) conditions for 41 days. Bar is 5 cm.



Figs. 10 to 15. Transections of the basal parts of the lst nodal roots of pearl millet grown under waterlogged (10 and 11), moderate moisture (12 and 13), and drought (14 and 15) conditions for 41 days. Figs. 10, 12, and 14 are epifluorescent micrographs of the transections stained with berberin-aniline blue. Fig. 11, 13, and 15 is an ordinary light micrograph of the same transection in Fig. 10, 12, and 14, respectively. Small arrows indicate the hypodermis and thick ones the cortical sclerenchyma. Note the absence of epifluorescence in the tissues external to the endodermis in Fig. 10 in spite of the presence of the hypodermis and some cortical cells in Fig. 11. Bar is 100 $\mu$m.

away from the base of each root. This fact seems to suggest that a wide fluctuation in soil surface would have substantially influenced the development of the lignified tissue as was discussed in our previous report4). In other words, these lignified tissues can be characterized as protective tissue for the roots, whose development might be plastic in response to the surrounding, especially unfavorable environment. It would be also possible that lignification might have occurred only in the basal parts because of the young age of the lst nodal roots observed. Since we did not determine whether the roots had ceased their elongation at the time of sampling, a longer-term observation on more mature roots would answer this question.

In either case, we consider that the ecological implication of the varied development of the lignified tissue depending on growth conditions should be interpreted in the context of whole plant growth under such conditions. As shown in Fig. 7, in pearl millet, which is the most susceptible to waterlogging, the root system development in W-plot was poorest among the three species, and the protective tissue found in the lst nodal root was only the lignified endodermis. Similarly, as indicated by the ratio of the shoot dry weight in W-plot to that in C-plot (W/C ratio) (Table 1), the shoot growth was also largely restricted under the same conditions. In contrast to W-plot, in D-plot, the acropetal lignification in the three protective tissues was promoted, and the D/C ratio in the shoot dry weight was relatively high (64%) for this crop. For Job's tears, which is the most susceptible to drought among the three, drought conditions tended to promote lignification in all the three tissues. Although its D/C ratio (56%) was rather small when compared to the W/C ratio (96%) (Table 1), the shoot growth reduction of the droughted plants was not as serious as that of pearl millet. For Japanese barnyard millet, whose growth is relatively stable under both waterlogging and drought conditions, the lignification ratio in the three protective tissues tended to be increased by both waterlogged and drought conditions as mentioned above. Accordingly, shoot growth was only slightly affected by the two conditions (Table 1). These facts suggest that the acropetal lignification in the protective tissues, which may be intimately related to the function of the nodal root, would play an important role in the plants grown under adverse soil moisture conditions, and thereby would largely determine plant ability to withstand such conditions.

The hypothesis in this series of studies[4,5] has been that cortical sclerenchyma development is a sort of xeromorphism plants develop in response to dry soil conditions. This hypothesis is proven by the result that not only in pearl millet, which is drought tolerant, but even in Job's tears, which is drought susceptible, and Japanese barnyard millet, which is both waterlogging and drought tolerant, cortical sclerenchyma development was promoted by drought. However, the fact that the promoted acropetal development of this tissue was also observed in W-plot in Japanese barnyard millet indicates the possibility that the lignification of this tissue could be induced and/or promoted also by waterlogged conditions, and thus may play a certain role in the plant growth of some species. In this aspect, further study is required on the interspecific difference in the development of this tissue under various soil moisture conditions.

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