Localization of Cells Containing Sedimented Amyloplasts in the Shoots of Normal and Lazy Rice Seedlings

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Abstract We have examined the localization of the cells containing sedimented amyloplasts (putative statocytes) and its relation to the graviresponse sites in the shoots of normal and lazy rice seedlings. All graviresponsive organs of the shoots of normal rice seedlings, the mesocotyl, the coleoptile and the leaf-sheath base, were found to possess the statocytes. This is the first indication that mesocotyl senses gravity by its own cells in inducing gravi-tropic bending in rice seedlings. In lazy-Kamenoo, although the shoots lost their gravitropic response with the advance of age, sedimentation of amyloplasts itself might not be attributable to the agravitropic growth of the shoots, because, including those of the leaf-sheath bases that had lost their response to gravity, sedimented amyloplasts appeared to be identical to those of normal Kamenoo and of younger seedlings of lazy-Kamenoo whose gravitropism is still apparent.

Key words : Amyloplasts, Coleoptile, Gravitropism, Lazy, Leaf-sheath, Mesocotyl, *Oryza sativa* L., Rice.

Introduction

A shoot of rice seedling consists of different organs such as the mesocotyl, the coleoptile, and the true leaves, in which most actively growing site shifts from organ to organ as the seedling grows. The site of graviresponding organ in rice seedlings thus transposes from the mesocotyl/coleoptile region to the leaf-sheath base as they grow (Abe et al. 1994). However, gravitropism studies of rice seedlings have mainly dealt with those of the coleoptiles. One may raise a question, therefore, as to whether each graviresponsive organ possesses its own gravisensing apparatus in inducing gravitropic bending in the shoots of rice seedlings. In higher plants, sedimentable amyloplasts are considered to be statoliths that somehow function for graviperception mechanism (Sack 1991). This is also the case in gravi-tropic response of the shoots of cereal grasses including rice plant (Kutschera et al. 1990, Sato 1961, Song

et al. 1988) although Sato and Shibata (1981) observed the presence of calcium oxalate crystals as possible statoliths in rice leaf pulvinus.

We have accordingly examined the localization of the cells containing sedimented amyloplasts and its relationship to the sites of graviresponse in the shoots of rice seedlings. On the other hand, rice seedling shoots of lazy-Kamenoo are known to show abnormal response to gravity (Goto 1978, Yamazaki 1985) and become gravitropically incompetent due to the diminished graviresponse of newly developing leaf-sheath bases (Abe et al. 1994). Because several mutant plants are reported to show less graviresponsiveness due to the reduced amount of sedimentable amyloplasts (Hertel et al. 1969, Kiss and Sack 1990, Sæther and Iversen 1991), we also examined the presence of sedimented amyloplasts in the shoots of lazy-Kamenoo seedlings.

Materials and Methods

The glumes of seeds of *Oryza sativa* L. cv. Kamenoo (japonica type) and its lazy line, lazy-Kamenoo, were removed prior to imbibition. Then, the seeds without the glumes were soaked in distilled water and incubated at 28 °C for 20 h.

Received October 26, 1994.

Accepted November 16, 1994.

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The germinated seeds were placed, horizontally with the embryo side up, on 1% agar contained in plastic cells (1 x 1 x 4.5 cm; one seed per cell). Those germinated seeds in the cells were enclosed in a glass container (14 cm in diameter and 12 cm in height) containing distilled water, 5 mm in depth, and incubated for 48 or 72 h at 28 °C in the dark.

As we previously reported, shoots of lazy-Kamenoo seedlings grown in the conditions described above respond gravitropically at their early stage of growth but lose their graviresponse by 92 h after seed imbibition (Abe et al. 1994).

Fig. 1 Photographs showing age-dependent graviresponsiveness in lazy-Kamenoo (right hand) in comparison with gravitropic response of normal Kamenoo (left hand) seedlings. Photographs were taken 24 h after the start of gravistimulation at 28 °C under darkness. Arrow (g) indicates the direction of gravity.

Shoots of normal Kamenoo seedlings, on the other hand, respond fully to gravistimulation even 92 h after imbibition (Abe et al. 1994). To observe sedimented amyloplasts in the shoots of both normal Kamenoo and lazy-Kamenoo seedlings, therefore, whole shoots of 68-h-old and 92-h-old seedlings were fixed with FAA (formalin : acetic acid : 50% ethanol, 5 : 5 : 90, v/v/v) solution for light microscopy. During 48 h of the fixation, the shoots were kept in a vertical position with the shoot tips pointing upward. The fixed materials were then cut into 5-mm-long sections and dehydrated with an ethanol series. Paraffin sections of approximately 10 µm in thickness were prepared and stained first with Gentian violet (1g per 100 ml) and then with I₂-KI (3g KI and 1g I₂ per 100 ml) solutions.

Results and Discussion

From the measured gravicurvature of the shoots 24 h after the start of gravistimulation, we classified the stage of seedling growth into three groups for further study (Abe et al. 1994). At Stage I (44-h-old), the shoots of lazy-Kamenoo were still strongly responsive to gravistimulation; at Stage II (68-h-old), the response to gravistimulation substantially decreased; and at Stage III (92-h-old), the shoots became totally agravitropic (Fig. 1). In all these stages from I to III, normal Kamenoo responded to gravistimulation (Fig. 1).

Localization of the cells containing sedimented amyloplasts in the shoots of normal Kamenoo seedlings is shown in Fig. 2. In the photographs A to F in Fig. 2, sedimented amyloplasts are seen in mesocotyl cells (A), coleoptile cells (B) as well as in the first leaf cells (C) at Stage II. Sedimented amyloplasts are, likewise, seen in those mesocotyl (D), coleoptile (E) and the first leaf cells (F) at Stage III, though amyloplasts in the mesocotyl have somewhat different morphology from those of Stage II. These organs are gravitropically responsive, although the gravitropic bending commences at the mesocotyl and coleoptile region and transposes to the base of the leaf sheath as the seedlings grow (Abe et al. 1994). We did not examine whether the sedimented amyloplasts in the tissues re-sediment upon horizontal exposure of the seedlings. But, number of adjoining cells containing sedimented amyloplasts in the tissues led

Fig. 2 Sedimented amyloplasts in the shoots of the vertically-grown seedlings of normal Kamenoo. A, B and C indicate mesocotyl, coleoptile and the first leaf, respectively, at Stage II. D, E and F indicate mesocotyl, coleoptile and the first leaf, respectively, at Stage III. Arrowheads indicate typical sedimented amyloplasts. Arrow (g) indicates the direction of gravity.

us to consider those to be statocytes.

In the studies of the shoots of rice seedlings, coleoptile has been considered to be a graviresponding organ (Hoson et al. 1992, Kutschera et al. 1990). Since the seedling consists of different graviresponsive organs, however, it is necessary to consider the age or stage of growth and each organ for its gravitropism study (Abe et al. 1994).

The present study indicates that in the shoots of rice seedling all graviresponsive organs, mesocotyl, coleoptile and leaf-sheath possess their own gravisensing apparatus. Those organs, therefore, may respond to gravity at different stages of growth independently from one another. This is, to our knowledge, the first indication that mesocotyls in rice seedlings have cells containing sedimentable amyloplasts that could function as

statocytes in inducing gravitropic response. Sato (1961) reported that in rice seedlings amyloplasts were present in the mesocotyl, the coleoptile and the leaf-sheath base and more abundant than those of other cereal plants such as barley and wheat, but he did not examine whether the amyloplasts were sedimentable or responsible for the gravitropic response in rice seedlings.

Because the seedlings of lazy-Kamenoo show

nearly normal response to gravity at their early stage of growth and lose it in an organ-dependent fashion as they grow (Abe et al. 1994), we examined the cells containing sedimented amyloplasts in different organs of the shoots of lazy-Kamenoo seedlings that had lost their gravitropic response (Fig. 3). The agravitropic shoots of lazy-Kamenoo at Stage III were found to have sedimented amyloplasts in the cells of the mesocotyl (D), the co-

Fig. 3 Sedimented amyloplasts in the shoots of the vertically-grown seedlings of lazy-Kamenoo. Otherwise as for Fig. 2.

leoptile (E) and the leaf-sheath (F), which appeared to be nearly identical to those of normal Kamenoo and those of gravitropic shoots of lazy-Kamenoo at Stage II (A, B and C).

Thus, shoots of lazy-Kamenoo likely have normal sedimentable amyloplasts, though some studies have shown that starchless mutants of *Arabidopsis thaliana*, *Nicotiana sylvestris* and *Zea mays* are gravitropically less sensitive (Hertel et al. 1969, Kiss and Sack 1990, Sæther and Iversen 1991). The rate of the sedimentation still needs to be clarified in both normal and lazy-Kamenoo. Nevertheless, it is probable that the sedimentation of amyloplasts itself is not involved in the agravitropism of the leaf-sheath base in lazy-Kamenoo.

Amyloplasts localized at inner parenchyma adjacent to vascular bundle has been considered as statoliths. As shown in Figs. 2 and 3, however, sedimented amyloplasts are rather found in numerous cells of mesocotyls, coleoptiles and the first leaf cells in rice seedlings. This may have some relations, as reported by Sato (1961), to the fact that rice plants accumulate starch more abundantly than other cereal crops.

Acknowledgments

This work was supported by grants from the Institute of Space and Astronautical Science (Sagamihara, Kanagawa, Japan) and by a Grant-in-Aid (No. 05454044) from the Ministry of Education, Science and Culture of Japan.

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和文抄録

イネの正常およびもつれ系統の幼植物における沈降アミロプラストを有する細胞の局在

イネの正常およびもつれ系統の幼植物を用いて、沈降アミロプラストを有する細胞の局在を、幼植物における重力反応部位との関連において研究した。正常なイネ幼植物のすべての重力反応器官、すなわち、中茎、幼葉鞘および葉鞘基部は重力感受細胞 (statocytes) を保持していた。イネ幼植物の重力によって誘起される屈曲反応の中で、中茎については中茎それ自身の細胞が重力を感受していることが、本研究で初めて示された。もつれ亀ノ尾系統の幼苗は、エージが進むと重力屈性を示さなくなるが、その時期においても、正常な亀ノ尾系統やもつれ亀ノ尾のまだ重力屈性を示すエージの若い苗と同様に、アミロプラストは沈降していることが見出された。アミロプラストの沈降それ自身はこのもつれ系統の重力非感受の原因となっていないものと考えられる。

キーワード：アミロプラスト、幼葉鞘、重力屈性、もつれ系統、葉鞘、中茎、イネ