Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Preliminary study on a gravity-insensitive rice mutant*

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Received Dec. 3, 2002; revision accepted Apr. 15, 2003

Abstract: A gravity-insensitive mutant was isolated from rice (*Oryza sativa* L. cv. Zhonghua 11) transformed by *Agrobacterium tumefaciens*. The mutant's shoot growth (prostrate growth) was insensitive to gravity; whereas root growth displayed a normal positive gravitropism. Histological observation of root caps and leaf sheaths indicated that there was no significant difference in the number and size of amyloplasts in cells of the mutant and cells of the wild type.

Key words: *Oryza sativa* L., Gravity-insensitive mutant, Gravitropism, Amyloplast
Document code: A **CLC number:** Q945.3; Q945.4

INTRODUCTION

The most obvious manifestation of gravity's effect on plants is the downward bending of roots and the upward growth of shoots. Gravity is an important factor in plant development (Barlow, 1995). The starch-statolith hypothesis proposed by Haberlandt and Němec (Audus, 1962) is the most widely accepted view of how plants perceive gravity. Physiological data suggested that the amyloplast containing starch granules act as the statolith in higher plants. Sedimented amyloplasts are located in root-cap columella cells and the endodermal or bundle sheath cells in stems (Tasaka *et al*., 1999; Kiss, 2000; Johannes *et al*., 2001). Three classes of evidences support the starch-statolith theory. First, mobile and starch-filled amyloplasts are usually present in gravitropic organs. Second, accumulation of starch is often correlated with the development of gravitropic competence (Wright, 1986). Third, low starch content or low amyloplast mobility is correlated with impaired gravitropism in a number of mutants (Caspar and Pickard, 1989; Kiss *et al*., 1989). Results of recent spaceflight studies are consistent with the statolith-based model for gravity perception in plants (Kiss *et al*., 1999).

To uncover the molecular mechanisms of plant gravitropism, genetic analysis of mutants is thought to be a useful approach. To date, only starch-less or starch-reduced mutants in maize, tobacco and *Arabidopsis* have been reported as showing reduced gravitropism in the shoot and the root (Kiss *et al*., 1989; 1996; 1997; Kordyum and Guikema, 2001; Yoder *et al*., 2001). We isolated a novel mutant from the rice mutant pool constructed by efficient transformation technique mediated by *Agrobacterium tumefaciens* (Zhu *et al*., 2001), which showed gravity-insensitive behavior in shoot growth but normal behavior in root growth (Fig.1, see the next page). Histological observation revealed that the mutant had normal amyloplasts in cells of both root cap and shoot sheath. Genetic analysis indicated that the mutation was probably caused by a monogenic and recessive gene.

* Project supported by the National Basic Research Program (973) (No. G1999011600) and Prophase Special Program of National Basic Research Priorities Program (No. 2001CCA0800), China

(a) (b)

Fig.1 A gravity-insensitive Mutant of rice (a) and the Wild type (b)

MATERIALS AND METHODS

Mutant screening

Seeds of *Oryza sativa* L. cv. 'Zhonghua 11' were transformed with *Agrobacterium tumefaciens*. Transgenic seeds (T_1) were germinated and transplanted for mutational determination (Zhu *et al*., 2001). Plants with prostrate growth trait were selected and seeds from a single plant were harvested as T_2 seeds. T_2 plants were observed for segregation analysis.

Gravitational treatment

Seeds were surface sterilized, sown in petri dishes, and allowed to germinate in the dark at 25 ºC −28 ºC for 2 days. Then the germinating seeds were transplanted to rectangular boxes (100×20×200 mm^3) containing 1.6% (W/V) agar medium, one row with 10−12 seeds and 6 boxes for each sample. These boxes of seedlings were divided into two groups: one in darkness and the other with a 12-h photoperiod of homogeneous light. Active carbon (0.5%, W/V) was added to the agar medium in order to eliminate the effect of light on root growth. When roots of the plants in darkness reached 10 to 15 mm, 3 boxes were reoriented by 90 degrees for observing the response of roots to gravity at intervals of 1, 2, 2.5, 5.5, 11 h. Similar treatments were applied to the remainder of the boxes in both groups when shoot reached 10 to 15 mm for observing the response of shoots to gravity at intervals of 24, 36, 45 and 72 h.

Cyto-histological observation

The root and shoot sheaths of the wild type and the mutant were fixed vertically with Carnoy fixative. After 30 min for roots and 3 h for seedlings, 5 μm thick sections were cut using section cutter (Microm GMBH Type HM 325, Germany) and stained with Schiff 's reagent described by Li (1987). Sections were viewed and photographed with a microscope (Axiovert 200 invert Germany).

RESULTS

Isolation and genetic characterization of rice mutant with little gravitropism in leaf sheath

The genetic properties of the mutant were examined by determining the segregation of the leaf sheathes gravitropism traits in T_1 and T_2 progeny. In the T_1 and T_2, all of the progeny of the mutant showed abnormal gravitropism. In the T_1 progeny of the normal plant, the leaf sheath gravitropism traits segregated about 3:1 (normal plant=372, mutant=121, χ^2=0.03, P>0.05) (Table 1), indicating that the mutation was monogenic and recessive.

Table 1 Genetic analysis of T_1 and T_2 plants in transgenic lines

	No. normal plant	No. mutant plant	χ^2 (3:1)	P
T_1	67	14	2.18	0.01
T_2 (Mutant progeny)*	0	70	–	–
T_2 (Normal progeny 1)**	372	121	0.03	0.05
T_2 (Normal progeny 2)***	107	0	–	–
Wild-type	60	0	–	–

*T_2 progeny derived from mutants;
** Lines derived from normal T_1 plants segregated at T_2 progeny;
*** Lines derived from normal T_1 plants that did not segregate at T_2 progeny

Root and leaf sheath morphology

In the wild-type and mutant, the roots began to elongate vertically after germination (Fig.2a). When the roots were placed horizontally, they curved

about 51.2º±9.5º (Wild-type) and 50.0º±9.5º (Mutant) downward within 11 h in darkness, respectively, exhibiting positive gravitropic responses (Fig.2b).

When they were placed horizontally, the leaf sheaths of the wild-type curved about 42.3º±4.7º upward within 45 h in darkness, whereas the mutant curved about only 3.5º±3.9º upward within 45 h under the same condition. Similar results were observed in light (Table 2 and Fig.3).

Amyloplasts in columella and bundle sheath cells

Sedimentable amyloplasts were observed in the columella cells of wild-type and the mutant. These amyloplasts were sedimented towards the gravity vector (Fig.4). The diameters of sedimentable amyloplasts were about 2 μm and their number was 3−5 per cell (Table 3). There was no difference in the number and size of amyloplasts in the root caps of the wild-type and the mutant; there was no difference in the length and diameter of the root caps of the wild-type and the mutant.

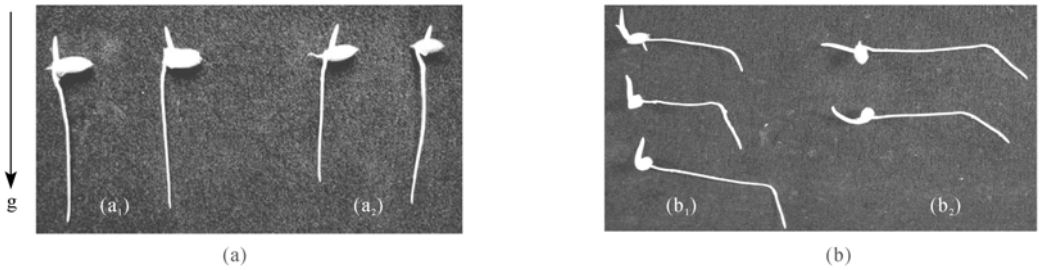


Fig.2 Growth responses of grown in darkness roots to gravity
(The arrow marked g indicates the direction of gravity)
(a) Upright roots: (a_1) Wild-type; (a_2) Mutant;
(b) Direction of the roots after rotating 90º: (b_1) Wild-type; (b_2) Mutant

Table 2 Comparisons of roots and leaf sheaths between the mutant and wild-type

Item	Wild-type	Mutant
The root length grown 11 h after reorientation (mm)	12.0±2.0 a	11.8±2.9 a
The curving angle of roots grown 11 h after reorientation	51.2º±9.5º a	50.0º±9.5º a
The curving angle of leaf sheaths grown 45 h after reorientation in light	41.5º±7.5º A	3.3º±4.1º B
The curving angle of leaf sheaths grown 45 h after reorientation in dark	42.3º±4.7º A	3.5º±3.9º B

Results are expressed as the mean±standard deviation;
Values followed by the same small or capital letter are not different at the 0.05 or 0.01 level

Table 3 Amyloplasts in the special cells of roots and leaf sheaths from mutant and wild-type

	Item	Wild-type	Mutant
Amyloplasts in the columella cells of root caps	Length of the root cap (μm)	181±32 a	195±34 a
	Diameter of the root cap (μm)	187±24 a	196±22 a
	Number of amyloplasts (per cell)	4.65±1.2 a	3.85±1.3 a
	Diameter of the amyloplasts (μm)	2.2±0.8 a	2.1±0.7 a
Amyloplasts in the bundle sheath cells	Number of amyloplasts (per cell)	7.0±2.1 a	5.7±2.3 a
	Diameter of amyloplasts (μm)	2.9±0.8 a	2.8±1.1 a

Results are expressed as the mean±standard deviation;
Values in the same row followed by the same letter are not different at the 0.05 level

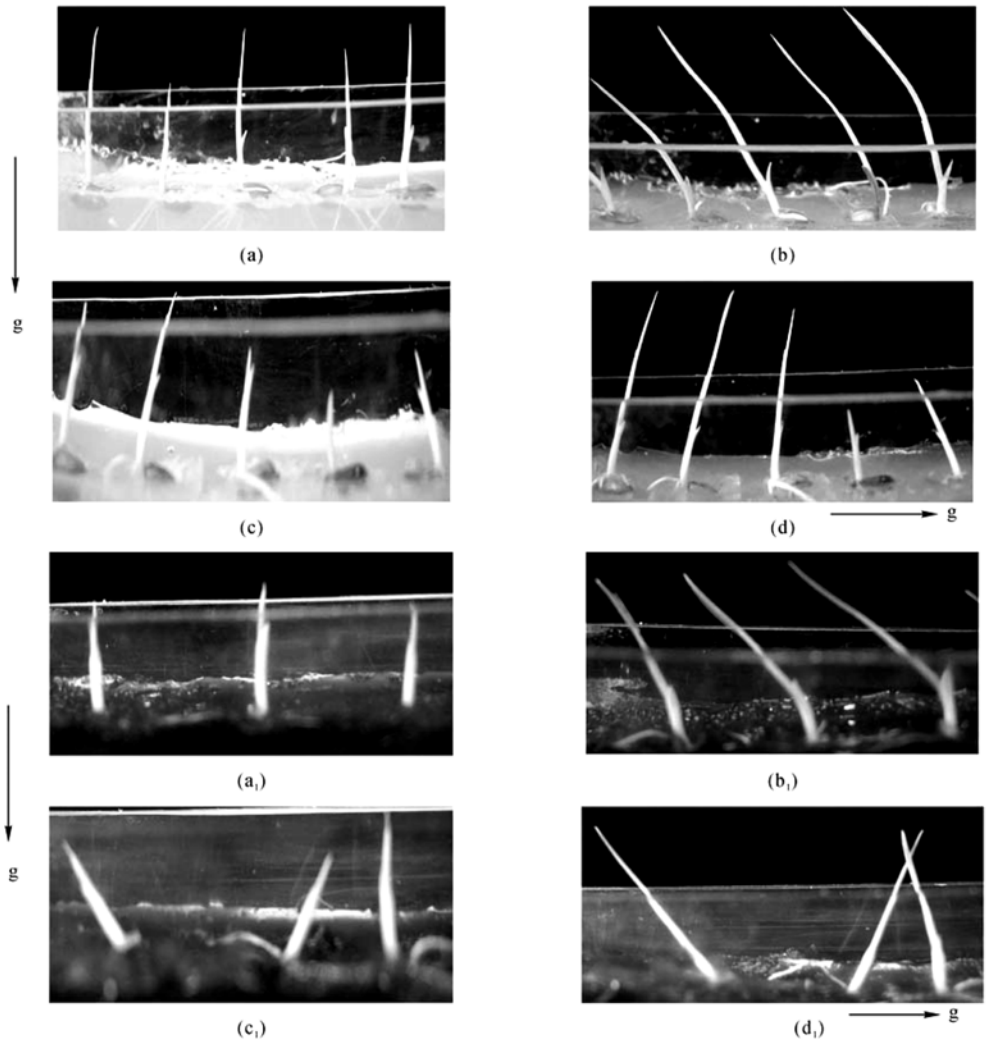


Fig.3 Growth responses of seedlings to gravity grown in dark (a−d) and in light (a_1−d_1)
(The arrow marked g indicates the direction of gravity)
(a) Wild-type; (b) Mutant; (c) Wild-type grown 45 h after rotating 90º; (d) Mutant grown 45 h after rotating 90º;
(a_1) Wild-type; (b_1) Mutant; (c_1) Wild-type grown 45 h after rotating 90º; (d_1) Mutant grown 45 h after rotating 90º

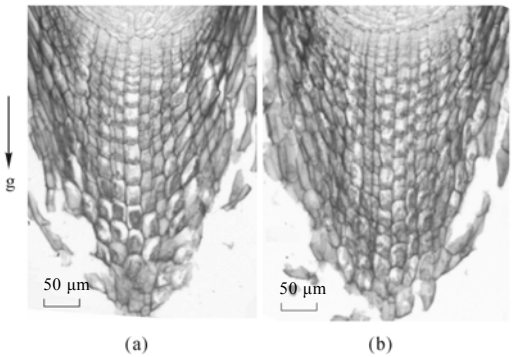
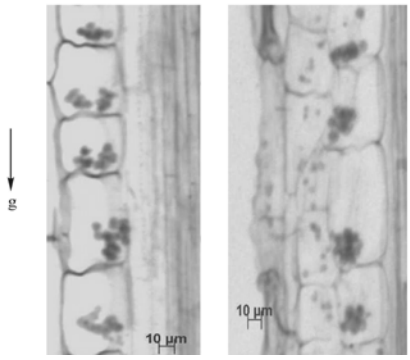


Fig.4 Amyloplast in root cap cells
(The arrow marked g indicates the direction of gravity)
(a) Wild type; (b) Mutant (Scale bar=50 μm)

Longitudinal sections of fixed leaf sheaths were also examined. Large, starch-filled amyloplasts were present in the wild-type and the mutant (Fig.5). The number of amyloplasts was 5−9 per cell in the wild-type and 4−8 per cell in the mutant (Table 3).

DISCUSSION

It is accepted generally that starch-containing amyloplasts function as statoliths in gravity perception because they sediment in the direction of gravity in specialized cells (Sack, 1991; 1997). In



(a) (b)

Fig. 5 Amyloplast in bundle sheath cells
(The arrow marked g indicates the direction of gravity)
(a) Wild-type; (b) Mutant (Scale bar=10 μm)

Arabidopsis roots and shoots, sedimenting amyloplasts are found in the columella and starch sheath or the endodermis, respectively. In our study, however, the amyloplasts in bundle sheaths of the mutant seemed to play no role in gravity perception. These results indicated the mechanism of gravity perception is probably different between the starch containing rice mutant and the starchless or starch-deficient *Arabidopsis* mutant. Are the amyloplasts in leaf sheaths of the mutant mobile like those of the roots? Further study on the distribution of amyloplasts in these two plants growing in clinostat simulating microgravity is on going. Maybe the results can shed light on the possible of gravity-receptors. The conversion of physical stimulus such as gravity vector to chemical signals in the cells is the least characterized event in gravitropism. According to the results of our study, we suggest that the gene controlling the signal transduction and/or differential growth probably mutated in the mutant. The difference between the roots, positive gravitropism, and the leaf sheaths, agravitropism, also indicate at least some elements of gravitropic mechanisms in different parts of a rice mutant (Tasaka *et al*., 1999). Some authors suggested Ca^{2+} and phosphoinositides acted as second messengers in the gravity signal transduction pathway (Sanders *et al*., 1999; Fasano *et al*., 2002).

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