Differentiation of Starch Property in Perisperm of Grain Amaranths

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Grain amaranths which include three species, *Amaranthus hypochondriacus* L., *A. cruentus* L. and *A. caudatus* L. were domesticated in highlands of tropical and subtropical America. The first two species are indigenous to Mexico and central America, while the third one to the Andes. Some workers recognize the fourth species of grain amaranths, *A. edulis* Speq. (*A. mantegazzianus* Pass.), which has commonly been known as a mutant of *A. caudatus*. Hanelt[2] proposed that the fourth species may better be treated as *A. caudatus* ssp. *mantegazzianus*. The probable native regions of the three cultivated species are summarized by Sauer.[13]

Grain amaranths are one of the most ancient crops which are still cultivated in the Americas, Asia and Africa. However, the crop has declined to disappear in its original regions and is now going to be produced more in Asia than in the Americas. Grain amaranths distribute widely in northern India and Nepal.

Amaranths have agronomic potential because of high protein content of the leaves and seeds, and possibility of improving composition of essential amino acids, lysine in particular.[1,6,9,17] They possess the C_4 photosynthetic pathway which confers high yielding ability. It was reported that the field plot yield amounted to 3000 kg/ha with nitrogen application of 170 kg/ha.[3]

On the other hand, grain amaranths have been analyzed for the property of starch as a new type of cereals.[7,9,17] These earlier studies showed that the perisperm in each species of the genus *Amaranthus* contained glutinous starch granules. Starch is mainly stored in the endosperm in common cereals, while it is stored in the perisperm in grain amaranths. This may be of particular interest because the perisperm is a diploid structure derived from the nucellus, whereas the endosperm is produced as a result of fusion between the male generative nucleus and two polar nuclei to form a triploid structure.

We have cultivated grain amaranths to evaluate them as a new crop in Japan since 1979. The present report deals with the characteristics of grain amaranths, especially for the first time with the differentiation of glutinous and nonglutinous starch types in dicotyledons.

Agronomic characteristics of grain amaranths

Origins of two species of grain amaranths used in this study are shown in Table 1. Eight lines of *A. hypochondriacus* and seven lines of *A. caudatus* were cultivated to examine the morphological characteristics in Tsukuba, Ibaraki Prefecture, Japan.

Plants of grain amaranths were vigorous in germination and growth habit. They had monoecious inflorescences in the main stem and several branches. The grain shape was circular and the grain size was very small, amounting to less than one gram in the weight of 1000 grains. There were seeds of a pale ivory, yellow, yellowish brown, black or brightly red color. The flowering occurred from July to September and plant height ranged from 0.5 to 3.4 m. Color of the leaves, stems and inflorescences also varied a great deal. The lines of *A. hypochondriacus* were classified into two types based on their

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Table 1. Starch properties of grain amaranths

Species	Origin	Number of lines			
		Glutinous	Nonglutinous	Segregating	Total
A. hypochondriacus	Mexico	4	0	1	5
A. hypochondriacus	Nepal	1	1	1	3
A. caudatus	Nepal	0	3	0	3
A. caudatus	China	0	3	0	3
A. caudatus	Japan	0	1	0	1

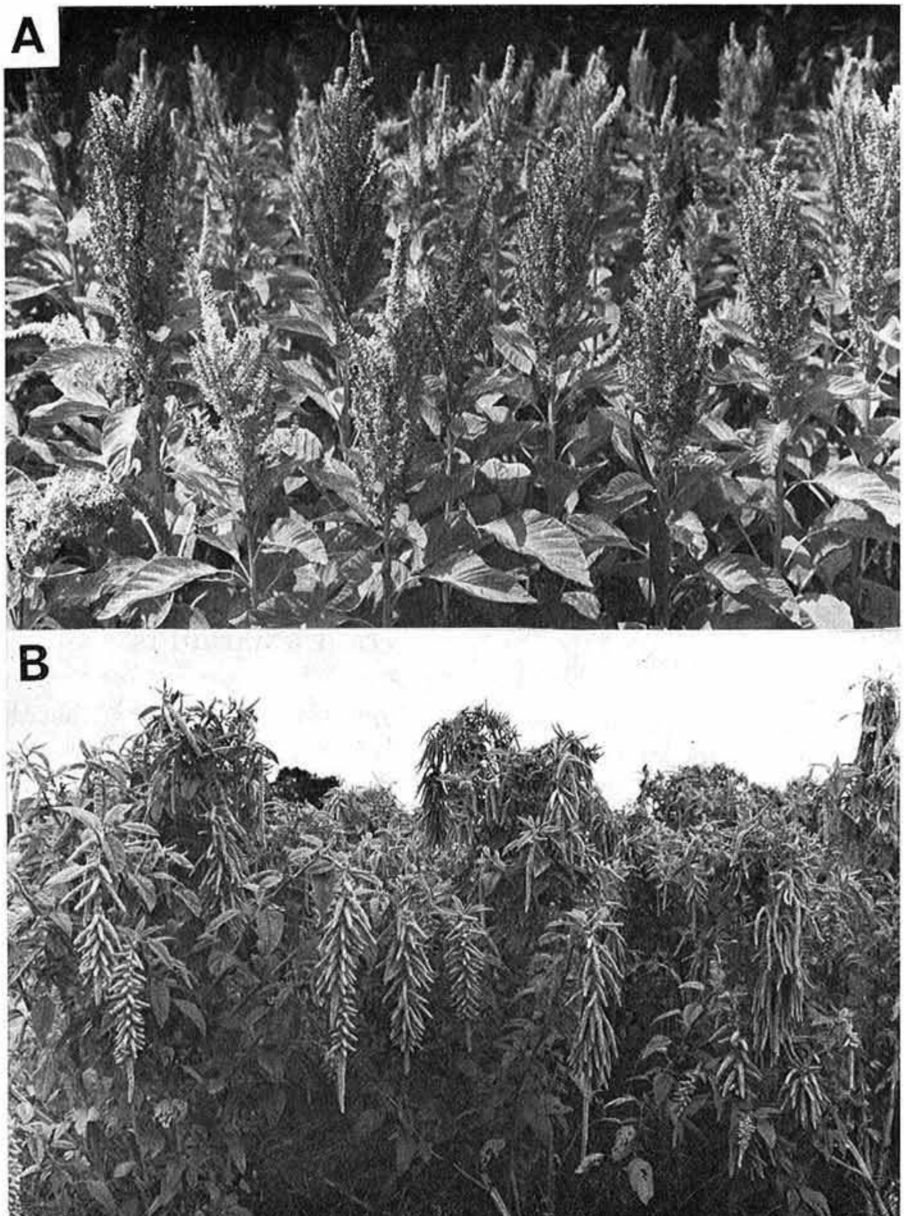

Plate 1. Characteristics of grain amaranths, *Amaranthus hypochondriacus* (A) and *A. caudatus* (B)

Table 2. Properties of perisperm starches of grain amaranths

Species	Grain appearance	Starch type	Iodine staining	λmax (nm)	Amylose content (%)	Gelatinization temperature (°C)
A. hypochondriacus	opaque	glutinous	reddish brown	540.0	0.0	64
A. hypochondriacus	opaque	glutinous	reddish brown	537.5	0.3	62
A. hypochondriacus	translucent	nonglutinous	blue	572.5	6.7	65
A. caudatus	translucent	nonglutinous	blue	552.5	4.4	54

morphological characteristics. *A. caudatus* was characterized by the presence of drooped inflorescences, differing from *A. hypochondriacus* (Plate 1), and relatively late flowering time.

Glutinous and nonglutinous starches in perisperm of grain amaranths[10]

1) *Experimental methods*

The grains were squashed and stained with I_2–KI solution to distinguish glutinous from nonglutinous starches. The starch granules were prepared from whole grains of three plants of *A. hypochondriacus* and one plant of *A. caudatus* using a modification of the alkali digestion method.[18] Size and shape of starch granules were observed microscopically. To estimate the amount of amylose in perisperm starch, iodine amperometric titration was performed using the dead stop endpoint method.[5] A total amount of carbohydrate was determined by the phenol sulfuric acid method[16] with glucose as a standard. The property of iodine-starch complexes was analyzed by a modification of the method of McCready and Hassid.[8] The gelatinization temperature was determined by photopastegraphy.[4]

2) *Property of perisperm starch*

Preliminary examination of the squashed grains stained with I_2-KI solution indicated that there are two different types of perisperm starches, glutinous and nonglutinous, in *A. hypochondriacus* (Table 1). All of the *Amaranthus* grains introduced from Mexico stained reddish brown, indicating glutinous starch type, while the grains introduced from Nepal stained reddish brown and blue, indicating glutinous and nonglutinous starch types, respectively. One of the lines from Mexico and that from Nepal were segregating lines. These results showed that the perisperm in *A. hypochondriacus* produces different types of starch, unlike the findings previously reported. Grains of *A. caudatus* stained blue, indicating nonglutinous type of starch.

Size of starch granules was 1 μm in diameter and about one-fifth of starch granules in the endosperm of rice, *Oryza sativa* L., as shown in Plate 2. Amylose content in the four plants of grain amaranths varied from 0.0 to 6.7% (Table 2). This suggests that there is a differentiation of starch types in the perisperm of *A. hypochondriacus*, while nonglutinous starch has so far been observed in *A. caudatus*. The absorption maximum of iodine-starch complexes ranged from 537.5 to 572.5 nm, suggesting a change in the unit chain length of amylose and amylopectin

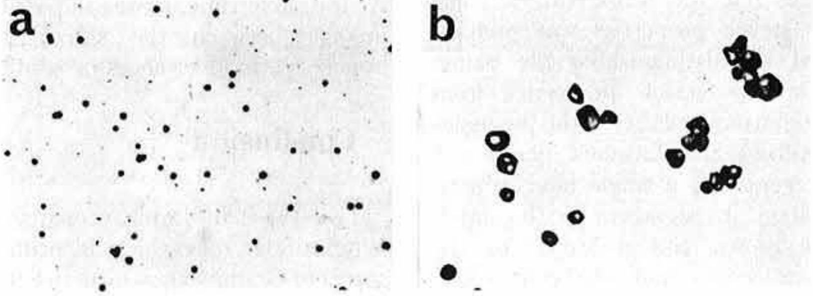

Plate 2. Starch granules from *A. hypochondriacus* (a) and from rice, *Oryza sativa* (b).

molecules. There were differences among the four plants with respect to the photopastegrams. The initial gelatinization temperature was 54°C in *A. caudatus* and higher than 60°C in *A. hypochondriacus* (Table 2). Wolf et al.[17] reported that the starch of *A. leucosperma* (=*A. hypochondriacus*) gelatinized within about the same range of temperature as that of maize and wheat starches. Moreover, it was reported that the initial gelatinization temperature was 63°C and the gelatinization range was 63–74°C in the starch of *A. paniculatus* (=*A. cruentus*).[9] Our results showed that the gelatinization temperature for the starch of *A. hypochondriacus* was similar to that of *A. paniculatus* as against *A. caudatus* which had a temperature comparable to that of *A. leucosperma*.

As far as we know, there is no report on the difference in starch types, glutinous and nonglutinous, not only in a species of grain amaranths but also in dicotyledons. We revealed for the first time a differentiation of perisperm starch in *A. hypochondriacus*, like in the endosperm starch of monocotyledonous Gramineae.[10,14,15]

Inheritance of starch characteristics in perisperm of *A. hypochondriacus*[11]

1) Experimental methods

One of the five lines introduced from Mexico was heterogeneous for the perisperm starch properties. The heterogeneous line was assumed to be derived from the natural hybridization between glutinous and nonglutinous plants of *A. hypochondriacus*. The nonglutinous plants from the heterogeneous line was self-fertilized. The segregation for starch properties was preliminarily examined by distinguishing the plants heterozygous for the starch properties from homozygous nonglutinous plants in the progenies of the self-fertilized nonglutinous plants. A total of 500 progenies of a single nonglutinous plant that was found to segregate for the starch properties were grown and recorded for the hypocotyl, inflorescences and seed-coat color. Mature seeds were individually harvested and examined for starch properties by staining with I_2–KI solution. The glutinous and nonglutinous starch granules stained reddish brown and blue, respectively.

Table 3. Segregation for glutinous and nonglutinous types of perisperm starch of *A. hypochondriacus*

Number of plants			χ^2	Probability
Nonglut.	Glut.	Total	(3 : 1)	
354	138	492	2.439	0.1—0.2

2) Segregation for glutinous and nonglutinous starch properties

Grains of *A. hypochondriacus* with a colorless seed-coat were classified into translucent and opaque groups based on the grain appearance. The translucent and opaque grains stained blue and reddish brown, respectively, with I_2–KI solution. Therefore, the starch granules stored in the perisperm of translucent grains are of the nonglutinous type, while those of opaque grains are of the glutinous type. These differences in grain appearance and iodine staining were closely associated with the amylose content of starch granules (Table 2).

The segregation for starch types in the perisperm of *A. hypochondriacus* was examined using F_3 seeds of the progenies of a single nonglutinous plant heterozygous for starch properties. The segregation of nonglutinous and glutinous starch types was 354 : 138, fitting the expected 3 : 1 ratio (Table 3). From the results, it was considered that starch properties in the perisperm of *A. hypochondriacus* are controlled by a single major gene, with the glutinous allele recessive to the nonglutinous allele. The mode of inheritance in the perisperm of *A. hypochondriacus* is similar to that recorded in the endosperm of Gramineae. At the same time, it was impossible to establish linkage between the starch properties and hypocotyl, inflorescences or seed-coat color.

Conclusion

Two types of starch property, glutinous and nonglutinous, have been identified in the endosperm of Gramineae such as rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.). sorghum (*Sorghum bicolor* (L.) Moench), foxtail millet (*Setaria italica* Beauv.) and proso millet (*Panicum miliaceum* L.). In these monocotyledons, starch granules are mainly stored in the endosperm which is a triploid structure.

Grain amaranths (genus *Amaranthus*) have been analyzed for starch properties.[7,9,17] These earlier studies indicated that the perisperm of grain amaranths produces glutinous starch granules. Recently, several workers, including the authors, determined that both glutinous and nonglutinous types of perisperm starches exist in the same species of grain amaranths, *A. hypochondriacus*.[10,11,12,14,15] In grain amaranths, starch granules are stored in the perisperm of diploid cells derived from the nucellus. Therefore, the starch property in the perisperm of grain amaranths is maternally inherited. The evidence obtained in the present studies substantiates the differentiation of glutinous and nonglutinous starch properties in the perisperm of *A. hypochondriacus*. This finding demonstrates for the first time the presence of the differentiation in the properties of perisperm starches in dicotyledons.

Sakamoto[12] assumed that the glutinous perisperm of *A. hypochondriacus* originated from the nonglutinous perisperm during the early domestication process in Mesoamerica and was later brought to Asia. Besides, he pointed out the possibility of the absence of nonglutinous starch type in cultivated grain amaranths of the New World. However, our results suggest that the two types of starch property at least in *A. hypochondriacus* differentiated in Mexico which is the original region of this species.[11] The results obtained in the present studies also make it possible to clarify whether or not *A. hypochondriacus* with both glutinous and nonglutinous perisperm starches was propagated from Mexico to Nepal and India.

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