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Research Note

SOLUBLE STARCH SYNTHASE IIa *(SSIIa)* ENZYME EXPRESSION IN ENDOSPERM AND ALKALI DISINTEGRATION IN SEEDS OF MYANMAR RICE CULTIVARS

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Rice (*Oryza sativa* L.) is grown all over the world and consumed by human beings worldwide. It is the most important crop in Myanmar because it is the main staple food of the entire nation. Starch is the end product of carbon fixation in photosynthesis and is accumulated in storage organs as an energy source. It comprises 90% of the total dry matter in the rice grain and can mainly affect grain quality. Starch biosynthesis in cereal plants is catalyzed by four classes of enzymes, namely; ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), starch-branching enzyme (BE), and starch-debranching enzyme (DBE) (Smith *et al.*, 1997; Myers *et al.*, 2000; Nakamura, 2002; Fujita *et al.*, 2006). Starch synthase proteins from rice and other plant species can be grouped into five classes, soluble starch synthase I (*SSI*), soluble starch synthase II (*SSII*), soluble starch synthase III (*SSIII*), soluble starch synthase IV (*SSIV*) and granule-bound starch synthase (*GBSS*). One gene for *SSI,* three genes for *SSII (SSIIa, SSIIb, SSIIc*) and, two genes for *SSIII (SSIIIa, SSIIIb*), *SSIV (SSIVa, SSIVb*) and *GBSS* can be found in rice (Hirose and Terao, 2004). The physiochemical property of cooked rice is mainly determined by the amylose content. However, amylopectin structure also greatly influences the physical properties of starch and their functionalities. Nakamura *et al*., (2002) suggested that *SSI* is mainly involved in the synthesis of short chain of degree of polymerization (DP) with ≤12 amylopectin in rice endosperm. *SSIIa* gene is responsible for the structural difference between amylopectin of *japonica* type and *indica* type rice and the amount of *SSIIa* in *japonica* rice cultivars are lower than that in *indica* (Umemoto *et al.,* 2002). They further suggested that *SSIIa* is a candidate gene for controlling differences in alkali disintegration of rice grains. However, the information on correlation between *SSIIa* enzyme activity and alkali disintegration in Myanmar local rice cultivars is not available. Therefore, a study was carried out to detect *SSIIa* enzyme expression in Myanmar rice cultivars in comparison with *japonica* and *indica* types of rice cultivars.

Rice cultivars Nanjing 11 (*indica*), Yamasenishiki (*japonica*), MMR1, MMR2, MMR3, MMR4 and MMR5 were used in these experiments.

Immature endosperms of rice grain were used to extract the enzymes. Native-Page gel electrophoresis method was carried out as reported by Okamoto *et al.,* (2002). It was performed on slab gel system prepared with 7.5% (w/v) resolving gel containing 0.4% (w/v) rabbit liver glycogen (TypeIII; Sigma). After electrophoresis, the gels were incubated with 10ml buffer of 100mM N,N-bis (2-hydorozyethyl) glycine (Bicine) (pH8.5), 500mM tri sodium citrate (pH8.5), 1mM EDTA, 100mlL^{-1} glycerol, 2mM DTT for 15 min twice on ice by gentle shaking and another 24 hours at 27°C in 10ml buffer of 100mM N,N-bis (2-hydorozyethyl) glycine (Bicine) (pH8.5), 500mM sodium citrate (pH8.5), 1mM EDTA, 100mlL^{-1} glycerol, 2mM DTT either in the presence or in the absence of 1mM ADP-glucose. *SSI* and *SSIIa* enzymes activities in the gels were detected in iodine buffer containing 1% potassium iodide (KI) and 0.2% iodine (I_2).

Three grains of polished rice were used for each cultivar in alkali test. They were soaked in 10ml of 1.7% potassium hydroxide (KOH) in a petri dish (6 x3 cm, 1 cm deep) for 24 hours at room temperature. Alkali disintegration for each grain was scored according to Little *et al.,* (1958) and repeated three times.

The *SSI* activity in the developing endosperm was detected in all cultivars (Fig.1a). The *SSIIa* activity was detected in Nanjing 11 (Fig.1b) but not

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in Yamsenishiki, a *japonica* type. This may be due to reduced activity of *SSIIa* or functional capacity relative to the activity of this enzyme in *indica* type rice. The expression of *SSIIa* of Myanmar rice cultivars in endosperm was absent in this experiment compared to Nanjing 11cultivar.

Alkali spreading value of Nanjing 11 cultivar showed that it is resistant to disintegration while Yamasenishiki was very sensitive in 1.7% KOH solution. Myanmar rice cultivars (MMR1, MMR2, MMR3, MMR4 and MMR5) can be seen as intermediate sensitive to alkali solution (Fig.2). Nanjing 11 showed alkali disintegration score of 3 while Yamasenishiki cultivar showed susceptibility in alkali disintegration score (7.8) and Myanmar rice cultivars seemed to have intermediate alkali disintegration score of 4-6 (Fig.3).

SSII plays a role in synthesizing of intermediate chains of amylopectin from the analysis of the *SSII* deletion mutant of Chlamydomonas (Fontaine *et al*., 1993). In the case of rice, *SSIIa* in *japonica* and *indica* rice causes the phenotypic difference between the two rice cultivars in amylopectin structure. It might be different either in the amount of *SSIIa* or in the functional capacity of *SSIIa* in the two types of rice (Umemoto *et al.*, 2002). Umemoto and co-

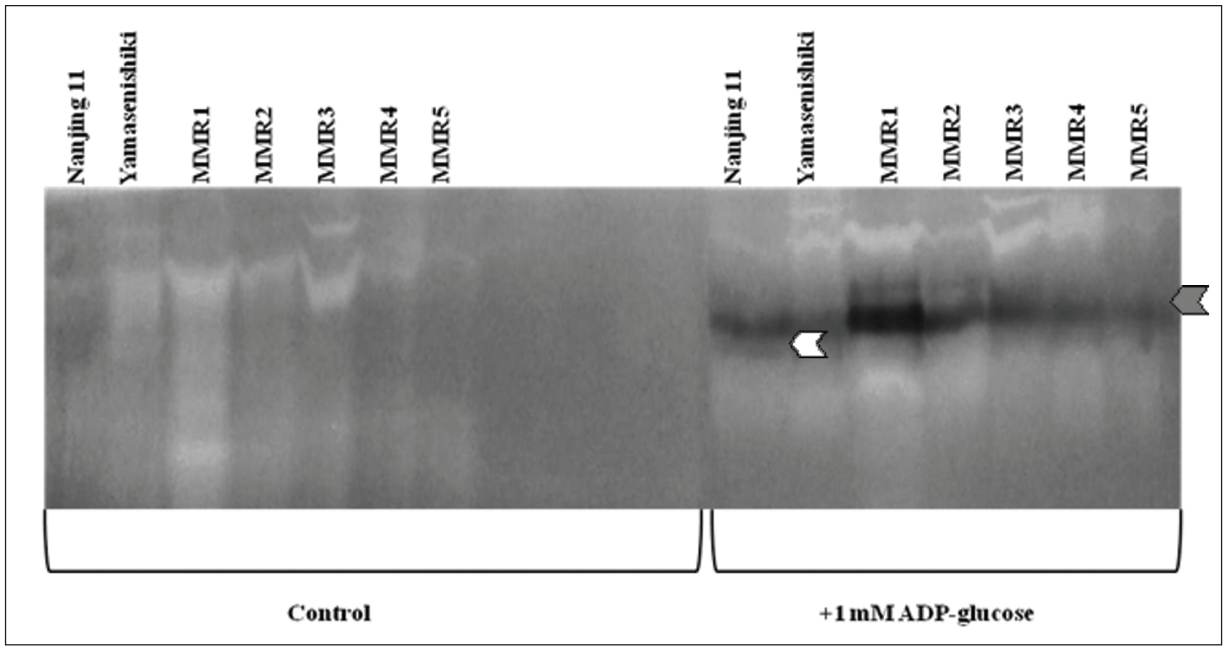

Fig. 1. Native-PAGE activity staining for starch synthase activity in soluble fraction.
SSI activity in rice endosperm in all cultivars.
SSIIa activity in Nanjing 11 cultivar.

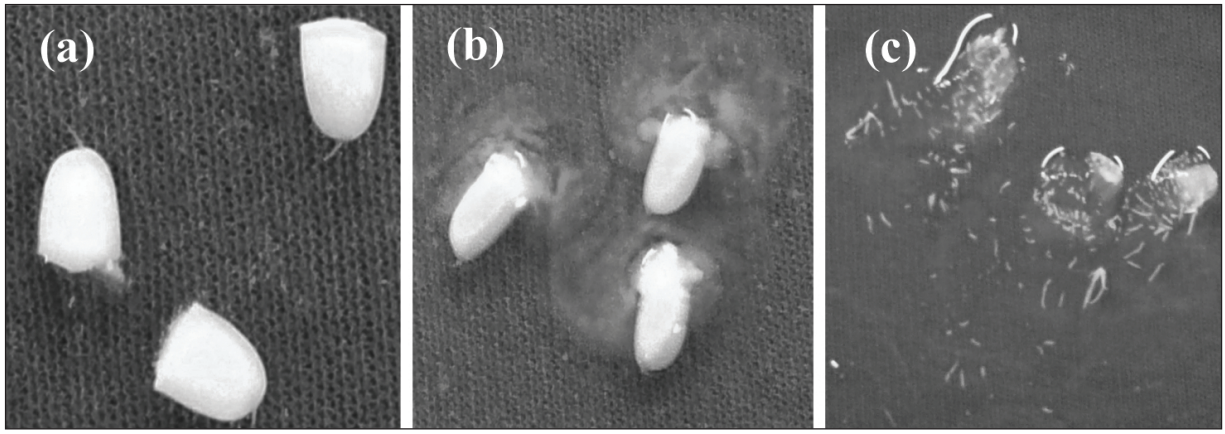

Fig. 2. Different disintegration of starch granules in 1.7% KOH solution in a) Nanjing 11; b) Myanmar cultivar; c) Yamasenishiki.

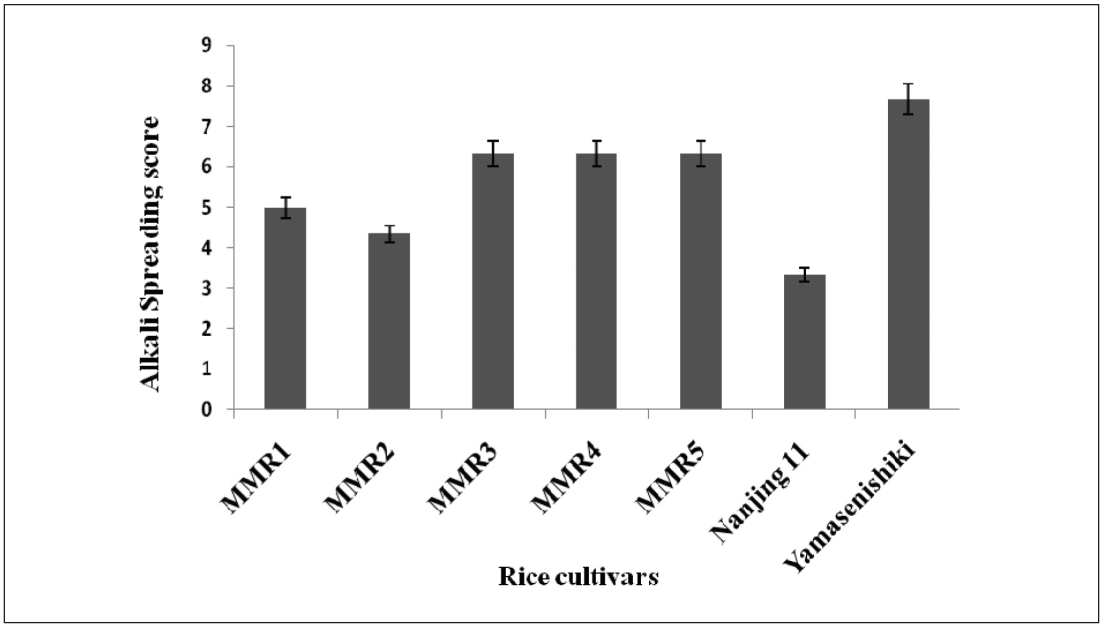

Fig. 3. Distribution of alkali disintegration in 1.7 % KOH solution at room temperature for different rice cultivars.

workers (2004) provided strong evidence that the role of *SSIIa* in amylopectin biosynthesis was distinct and could not be substituted by *SSIIb* or *SSIIc*, or that *SSIIa* was the only *SSII* enzyme expressed in developing rice endosperm.

This study shows that *SSI* activity is expressed in all but *SSIIa* activity is lacking in Myanmar rice cultivars. It can be generally assumed that *SSIIa* enzyme expression level was reduced in their endosperm. Okamoto *et al.,* (2009) also suggested that *SSIIa* activity is usually very weak in zymogram analysis compared to *SSI*, sometimes only faint band can be detected even with long chain of amylopectin in rice cultivars (Umemoto *et al.,* 2004). It might also be a reflection of chain length distribution of amylopectin in these Myanmar rice cultivars. Further studies are needed in order to confirm whether there is *SSIIa* activity in related chain length distribution of amylopectin in Myanmar rice cultivars.

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