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Isozymes of α-Amylase, Esterase and Peroxidase in Aged Rice Seed

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Abstract

The germination of aged rice seed was stimulated by pretreatment of the seed with 1 ppm. of abscisic acid plus 1 ppm. of α-naphthylacetic acid. Isozymes of α-amylase, esterase and peroxidase of the seedling were analyzed by a gel-isoelectric focusing method. The seedling which normally germinated from an aged seed, had grown also done with normal profile of isozymes.

Introduction

Rice seeds have a relatively long period of viability compared with some other crops such as bean or soyabean seeds. The percentage of normal germination falls substantially after storage of rice seeds for more than about 5 years, and the percentage of abnormal germination increases. These seeds germinate do so at a slower rate.

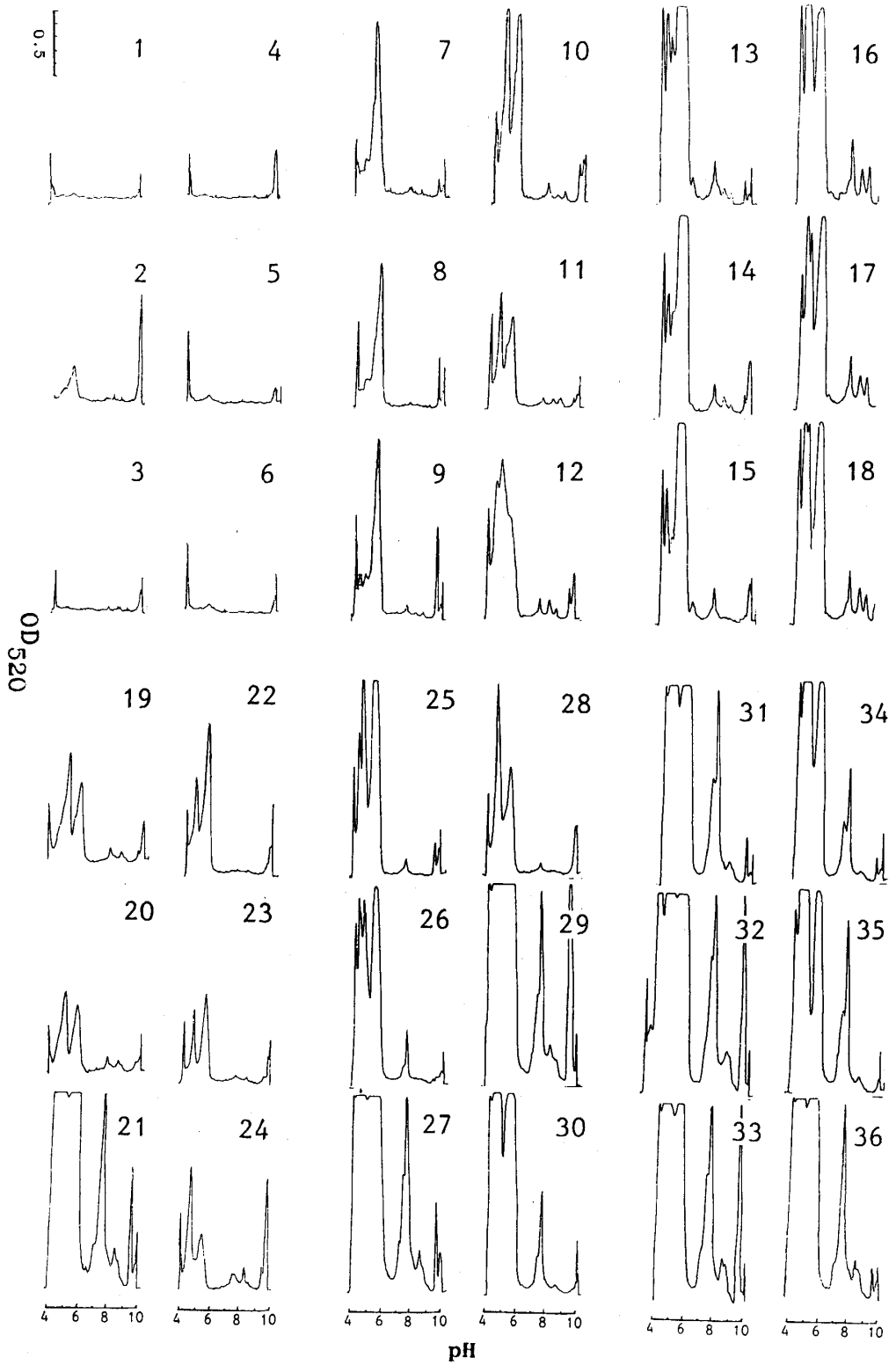
On the hypothesis that less of capacity for normal germination might be related to change in plant growth regulator balance within the seed[1,2], it was decided to study stimulation of the germination of aged rice seeds and their responses to externally applied growth regulators[1,3,4]. And also present, the hypothesis will be confirmed to study banding profiles of isozymes of α-amylase, esterase and peroxidase, because they are sensitive to the hormonal regulation[5–9] and the extent of tissue differentiation[10–13].

Materials and Methods

Rice (*Oriza sativa* L. cv. *Sasanishiki,* cv. *Norin No. 22* and cv. *Biwaminori*) of 22, 12 and 2 years old seeds were used respectively. Seeds were hulled carefully by hand, rinsed in 70% ethanol for 5 sec. and sterilized by immersion for 12 min. in the supernatant of 8% suspension of calcium hypochlorite. After through washing with sterile water seeds were soaked in 2 ml of medium with 1 ppm. of abscisic acid (ABA) plus 1 ppm. of α-naphthylacetic acid (NAA) in 10 ml tubes. After 3 days incubation in the dark, seeds were sown singly on to the surface of 15 ml of agar medium in 100 ml tubes respectively. After 3 or 7 days incubation in the dark at 30°C, seeds were counted and abnormal germination were investigated[1,3,4]. Roots, leafsheathes and leaves of seedlings were measured and then, these seedlings were stocked in a freezer at −20°C.

The most important method of this experiment was *in vitro* germination, because most of all aged rice seeds were generally contaminated.

The preparation of enzymes, the separation of them by gel-isoelectric focusing method and the detection of isozymes were revealed in previous papers[5,7–9].



Figures from 1 to 36: Densitometer scanning records of the peroxidase stain of electrolysis gels. From 1 to 18: incubated for 3 days. From 19 to 36: for 7 days. 1–3, 7–9, 13–15, 19–21, 25–27 and 31–33: treated with ABA plus NAA, the rest: not treated control. From 1 to 6 and 19 to 24: 22 year old rice seeds. From 7 to 12 and 25 to 30: 12 year old seeds. From 13 to 18 and 31 to 36: 2 year old seeds of cv. *Sasanishiki*.

Results and Discussions

Five bands of peroxidase isozymes were detected on the gel of electrolysis from the seedling of 22 year old seed which germinated by treatment with ABA plus NAA for 3 days, but not from the control of same aged seed which not germinated. They are shown in figures 2 of germinated and 1 or 3 of not germinated by the treatment and in figures 4, 5 and 6 of controls.

After 7 days incubation, these 5 bands were detected even from those seeds which germinated did so at a slower rate as shown in figures 22, 23 and 24 (controls). Usually 10 bands including above 5 bands were detected from a seedling as shown in figures 19 or 20. Among them, two bands on pH 6.1 and pH 5.2 (apparent pH of isoelectric point) were intensely stained. Totally 12 bands were detected from a seed which germinated did so at a normal rate by the treatment with ABA plus NAA (fig. 21).

Nine bands were detected from a 12 year old seed which germinated for 3 days incubation (fig. 9). The seedling had less than 7 mm long of leafsheath. Among these 9 bands, 6 bands were stained and the rest 3 bands were scarcely stained. These 9 bands were also detected from most of all 12 year old seeds as shown respectively in figures from 7 to 12.

After 7 days, more 3 bands were detectable from a seedling as shown in figures from 25 to 30 respectively. Two bands on pH 8.2 and pH 7.8 were intensely stained in a case of seed which germinated did so at a normal rate (fig. 27 of treated, or fig. 29 of not treated). The intensity of these two bands was rather parallel to the length of roots.

All of 12 bands were detected from samples of 2 year old seeds which germinated for 3 days incubation with ABA plus NAA and also from those without treatment too as shown in figures from 13 to 18, and also from samples of 7 days incubation too (figures from 31 to 36). Thus the banding profile and the intensity of stain vaguely correlate with the extent of seedling growth.

Intensities of corresponding bands among those seeds of three sort of ages were compared. These intensities are similar in a comparison among seeds which had germinated and grown in similar extent. They are shown in figures 21 of 22 year, 27 of 12 year and 36 of 2 year old seeds.

The stain of a band on pH 6.1 in the case of treatment with ABA plus NAA for 3 days was intenser than that of control. The treatment lowers those intensities of allmost bands of 12 and 2 year old seeds except the enhancement of pH 6.1 band.

Thus, the seedling of aged seed which stimulated by the treatment with ABA plus NAA, normally germinated and grew had grown also done with normal profile of enzyme activities of peroxidase isozymes.

Enzyme activities of α-amylase and esterase isozymes were parallel with the extent of germination of those seeds, since these activities are owing mainly to α-amylase and esterase of endosperm. It was suggested that the endosperm of aged seed retains normal responsibility to the growth of seedling even if it was 22 year old.

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