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Physiological and Ecological Studies in Environmental Adaptation of Plants

III. *Altitudinal Variation in Some Characters of Cytochrome Oxidase Isozymes in Polygonum cuspidatum Sieb. et Zucc.*

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Summary

The number and the activity of cytochrome oxidase isozymes in *Polygonum cuspidatum* Sieb. et Zucc. were investigated on the mature plants growing in a few different altitudes and on these seedlings grown under various levels of a constant temperature.

The number of the isozymes in the mature plants was 3 at low altitude and 2 at high altitude, but that in the plants transplanted from high to low altitude was 4. The number and the activity of the isozymes in the seedlings derived from the plants in high altitude and from the transplanted plants were remarkablly changed by growth temperature for them and staining temperature for the isozymes. Especially, the seedlings from high altitude had a few specific isozymes which were inactivated at a high staining temperature but were activated at a low staining one.

Genetic difference among the seedlings with the altitudinal difference in their parent plants' habitats seems to be detected on a regulation ability of cytochrome oxidase activity rather than on the isozyme composition, but the difference seems to be undeveloped in the mature plants.

Introduction

It is well known that plants in the same species grown at different habitats show some physiological or morphological differences to each other. Analytical experiments, however, have often led such differences to different causes in spite of the same kind of the differences.

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On *Typha latifolia*, McNAUGHTON[9] has reported that the activity of some enzymes differs genetically between different habitats on temperature, but JONES et al.[4] and MASHBURN et al.[7] have concluded that a variation of enzymatic function is not genetical. On a difference in the growth form of *Spartina alternifolia* between different habitats on a water condition, STALTER and BATSON[16] have reported to be genetical because the growth form was not changed by a reciprocal transplantation between these habitats, while from an electrophoretical study taken on these plants showing the different growth forms, SHEA et al.[12] have concluded that their growth forms express a difference in their physiological condition for the reason why any isozyme variation was not found between the different growth forms.

SHIBATA et al.[15] have found that *Polygonum cuspidatum*—was referred to as *Polygonum reynoutria* in the previous paper—has differences in photosynthetic or respiratory abilities between the plants from different habitats on altitude, and these differences have been nearly maintained after a reciprocal transplantation. This paper will describe an electrophoretical study on cytochrome oxidase taken to ascertain whether the differences are genetical.

Materials and Methods

Leaves of mature *Polygonum cuspidatum* Sieb. et Zucc. (mature leaves) which were growing in the field and leaves of their seedlings grown under a constant temperature (seedling leaves) were used as materials to investigate the composition and activity of cytochrome oxidase isozymes.

The mature leaves were collected from the plants in Matsumoto, Nagano Prefecture (600m altitude) and in mountain area near Matsumoto (1500m, 2000m, and 2250m altitudes), and from the plants grown in the field for 10 years after a transplantation from 1500m to 600m altitude. These mature plants were referred as 600m plants, 1500m ones, 2000m ones, 2250m ones, and transplanted ones, respectively. For the seedling leaves, seeds of *P. cuspidatum* Sieb. et Zucc. were collected from the 600m plants, the 2000m ones, the 2250m ones, and the transplanted ones, and were germinated at 25°C. After these seedlings were grown for a certain period with a nutrient solution (hyponex as a commerical reagent) at 5°, 15°, and 25°C under a continuous illumination (5000 lux), their leaves were used to analyze the isozymes. When the leaves of the seedlings were derived from the seeds of the 600m plants, these were referred as 600m seedling leaves. To grow the seedlings to about the same developmental stage having 2 foliage leaves, growth period was for about 30 days at 5°C, for about 25 days at 15°C, and for about 15 days at 25°C.

The mature leaves collected in the field were kept in an ice box, and were

carried to the laboratory. Although the detached leaves being supplied with water did not decrease cytochrome oxidase activity for 48 *hrs* after the collection, the leaves as well as the seedling leaves were received with following process as quick as possible. About 3*g* fresh weight of the leaves was homogenized with 0.067M phosphate buffer, pH 7.0, in an ice bath, and the homogenates were stocked at −20°C untill just before electrophoresis. Under the temperature at which the homogenates were stocked, cytochrome oxidase activity was not decreased for 30 days.

After the frozen homogenates were thawed, these were centrifuged for 10 *min.* at 7000 r.p.m. with 0.5ml of 0.4M sucrose in a cold room (3°C). These supernatants were directly applied to polyacrylamide gel for electrophoresis, and the electrophoresis was taken at 3°C for about 4 *hrs* with 200V-15mA. Analysis of cytochrome oxidase isozymes was taken according to LAWERANCE et al.[6]. In order to know the isozyme activity under relatively low temperature that will be experienced by the plants in mountain area, the enzyme was stained at 5°, 15°, and 25°C to each of the gels divided into 3 parts.

Results

Climatic conditions of the field in which the mature leaves and seeds were collected are referred to the previous paper[14].

Cytochrome oxidase in the mature leaves was consisted of from 2 to 4 isozymes, and the zymograms obtained were classified into 7 types with the migration pattern of the isozymes and these activities shown by staining degree

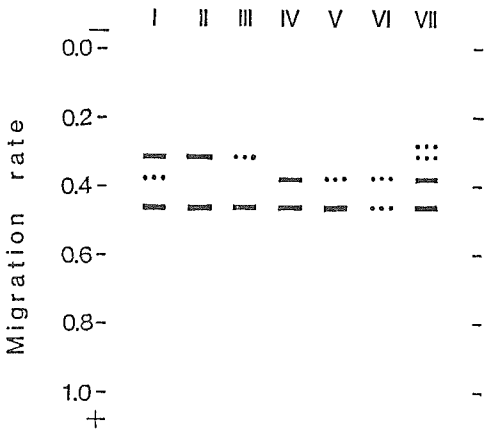

Fig. 1 Migration patterns of cytochrome oxidase isozymes in mature leaves of *Polygonum cuspidatum* Sieb. et Zucc. in various altitudes. Dotted lines show the isozymes which have lower activity than these shown by solid lines.

(Fig. 1). All of the 600m plants growing in a mild climate showed type I (3 isozymes), and all of the 1500m plants were found to be type V (2 isozymes) althpugh all of the transplanted plants, having the original habitat in 1500m altitude, were in type VII (4 isozymes). Most of the 2000m and 2250m plants, growing in a severe climate, showed type IV (2 isozymes) although some of these plants were found within a variation from type I to VI. Thus, the number of the isozymes in the mature plants was a fewer at higher altitude than at 600m.

The isozymes in the mature plants were intensively stained with the increase of staining temperature without a relation to the altitudinal difference

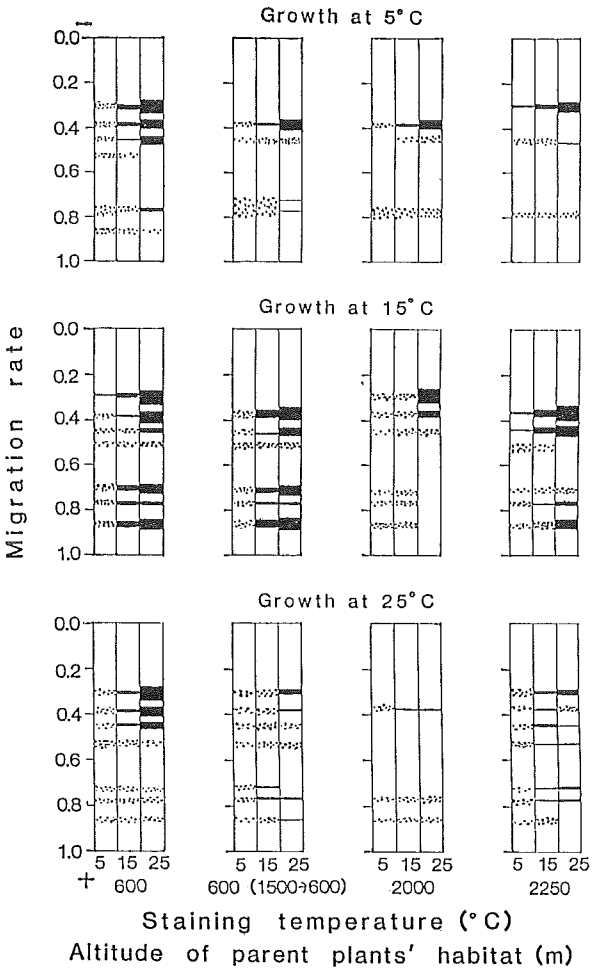

Fig. 2 Compositions and activities of cytochrome oxidase isozymes in seedling leaves of *Polygonum cuspidatum* Sieb. et Zucc. affected by habitat's altitudes for the parent plants, growth temperatures for the seedlings, and staining temperatures for the isozymes.

of their habitats. That is, the activities of all the isozymes were intensified with the increase of the temperature.

The zymograms for the isozymes in the seedlings were summarized in Fig. 2. The number of the isozymes in the 600m seedlings was changed a little by growth temperature, but that in the other seedlinggs was remarkablly changed by the temperature, especially at 5°C and at 25°C too for the 2000m seedlings. Accordingly, the variation of the number of the isozymes with the difference of growth temperature was specific for the seedlings from higher altitude than 600m. From these results, it was considered that the basic number of the isozymes in the seedlings was shown in the 600m seedlings, and the number was 7 consisting of 3 isozymes migrated rapidly and 4 ones migrated slowly.

The number of the isozymes in the seedlings was greatly varied by not only growth temperature but also staining one when the seedlings were derived from the plants in higher altitude than 600m and from the transplanted plants. Such variations caused by the difference of the staining temperature were resulted from the fact that some of the isozymes were inactive at 25°C of the temperature although the somes were active at 5° and 15°C. On the other hand, the isozyme veriation in the 600m seedlings was little on the number, but one or two isozymes were lowered on their activeties at 25°C more than at 5°C or 15°C of the staining temperature when the seedlings were grown at 5°C.

On the isozyme variation with their growth, the isozymes in the mature plants were characterized by the lack of all of the isozymes migrated rapidly and one or two of the isozymes migrated slowly found in the 600m seedlings grown at 15° or 25° C, and were homologous to someone of the 3 isozymes migrated slowly in the seedlings. The isozyme which made the slowest migration in the transplanted plants, however, was never found to be homologous to any isozyme in the seedlings.

Discussion

Photosynthetic or respiratory activity in plants has been well known to be different among growth ages or habitats. On the difference of the activities, some workers[1,8,10,15] have suggested that it may be genetical among the different habitats. On the other hand, an isozyme composition of some enzymes has been reported to be changed by season[3] or by variable single environmental facter[2].

On *P. cuspidatum Sieb.* et Zucc., it is suggested that the number of cytochrome oxidase isozymes changes with their growth from 7 in the seedlings

to 3 in the mature plants. In this case, the isozymes in the mature plants are characterized by the lack of all of the isozymes migrated rapidly and one or two of the isozymes migrated slowly found in the seedlings, and have a tendency to decrease these number with the increase of their habitat's altitude.

In general, it is known that a genetic variation decreases from the distribution center to its peripheral area, and, on *Oryza sativa* L., the decrease in the veriation has been shown as the decrease in the number of esterase isozymes[11]. A specialized speciation, also, has been sgugested in the same consideration[17]. Recently, the freezing resistance of *Abies sacharinensis* being intensified with the altitudinal increase in habitat has been suggested to result from the decrease in genetic variation[3]. On *P. cuspidatum* Sieb. et Zucc who has the original habitat in lowland area, the subalpine zone may be a peripheral area of their distribution, and a climatic condition in the zone seems to be too severe for them. For the reason, it can suppose that the decrease in the number of cytochrome oxidase isozymes with increasing altitude has a relation with a genetic specialization in higher altitudes. On the other hand, *P. cuspidatum* Sieb. et Zucc. transplanted from the highland to the low has increased the isozyme number, although the plants transplanted adversely were not able to investigate because they died within a few years after a growth making gradually weakness[15]. This result seems to suggest that the isozyme activity is regulative to environmental facters, and is in agreement with a little variation of photosynthetic ability by transplantation[15]. For these contrast data, it appears to be reasonable that the mature *P. cuspidatum* Sieb. et Zucc. used here as the plants in high altitudes has been partially differentiated on cytochrome oxidase. This suggestion is agreement with a taxonomical fact that some populations of the plants in more higher altitudes have been calssified into several variaties.

On the isozymes in the transplanted plants, an interesting fact was found. The 3 of 4 isozymes in the transplanted plants are homologous to these in the 600m plants, but the residual isozyme is never found to be homolougous to anyone. At the present, it is not clear why the isozyme is specifically activated in the transplanted plants alone.

The activity and the number of the isozymes in the 600m seedlings were changed a little by the level of growth temperature, and these activities were intensively increased with the increase of the staining temperature at all the growth temperature levels. However, the seedlings derived from the plants in high altitudes and from the transplanted plants have a few specific isozymes showing higher activity under a low growth temperature than under a high

growth one, and the number of the isozymes in the seedlings except for the 600m ones is greatly varied by the level of growth temperature or staining one. Accordingly, such regulation of the isozyme activity seems to be specific for the seedlings having the original habitat in higher altitude than 600m. These facts may suggest a possibility that the regulation ability of cytochrome oxidase activity is genetical.

The specific variation of the isozymes found in the seedlings alone seems to have a relation with a cold climate during seed germination season at high altitudes. It has been reported by SHIBATA and ARAI[13] that the germination of *P. cuspidatum* Sieb. et Zucc. seeds in a low temperature after stratification is more quick on the seeds from high altitudes than from low ones, and the seedlings which made a quick germination in the field will be required on a way to avoid the cold climate for their survival. The specific variation of the isozymes in the seedlings from high altitudes may be resulted from the requirement described above.

On a difference in the possibilities described above for the mature plants and their seedlings, it is likely that the variable range of cytochrome oxidase isozyme activity in *P. cuspidatum* Sieb. et Zucc. is genetical in the seedlings while is regulative in the mature plants. These plants in subalpine zone seems to contain various stages in the intraspecific differentiation, and the differentiation may be cuased by the altitudinal difference of environmental temperature.

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