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VARIATION OF ESTERASE ISOENZYMES IN DIFFERENT PLANT TISSUES OF *AGROSTIS STOLONIFERA* L.

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Previous studies (Wu, 1976) showed that esterase isoenzyme variability existed in the leaf tissue of different genotypes in the populations of *Agrostis stolonifera* L. Attempts were made to further investigate the variation of esterase isoenzyme in different tissues of *A. stolonifera* L. The existance of a particular isoenzyme in different tissues has been shown to be genetically controlled (Efron, 1973). Thus it is worthwhile looking in more detail to have a better understanding of causes of the variability of the esterase isoenzyme in *A. stolonifera*.

The tillers of the copper tolerant (obtained from the Old lawn whose copper tolerance was 0.86) and non-tolerant (from the Freshfield sand dune with a tolerance of 0.05) genotypes (Wu, 1976) were grown in $7\times7\times10\,cm^3$ plastic pots in a glass house for 10 weeks. The uppermost two or three leaves of each shoot and the internodes of each stolon were then cut off. The roots of both tolerant and non-tolerant genontypes were produced by growing the tillers in nutrient solution for two weeks at 21°C and 24 hr light (8×80 W fluorescent light). A 30-mm piece of root tip was then cut. These three tissues were then used for enzyme preparation.

In addition to these three tissues, calli induced from the root tips and the shoot tips of the plants were also used. The root tip callus was induced from a short (2 cm) length of stolon containing a node. These segments of stolon were washed and sterilized in 1% hypochlorite for 15 mins and then rinsed several times with sterilized distilled water. The sterilized stolon sections were inserted vertically into a culture medium "A" leaving the node about 0.5 cm above the surface of the medium. Medium "A" was prepared by adding 2,4-dichlorophenoxy acetic acid (2.4-D) 0.5 ppm to a basic culture medium. The basic medium was modified from the Linsmaier and Skoog (1965) medium which contained the following substances (mg/1): NH_4NO_3, 1000; $Ca(NO_3)_2\cdot$

$4H_2O$, 1000; KNO_3, 800; KCL, 65; $MgSO_4 \cdot 7H_2PO$, 370; NaH_2PO_4, 16.5; $FeSO_4 \cdot 7H_2O$, 2.5; Fe-EDTA $\cdot H_2O$, 13.5; $ZnSO_4 \cdot 7H_2O$, 4.5; glycine, 3; thiamine, 0.1; pyrioxine, 0.1; nicotinic acid, 0.5; sucrose, 20,000; yeast extract, 2,500; agar, 8,000; Kinetin, 2; Indole-acetic acid (IAA), 2.

The cultures were grown in darkness at 20°C for eight weeks, before they were transplanted on to medium "B", which contained 2,4-D 4 ppm in the basic medium for another eight weeks.

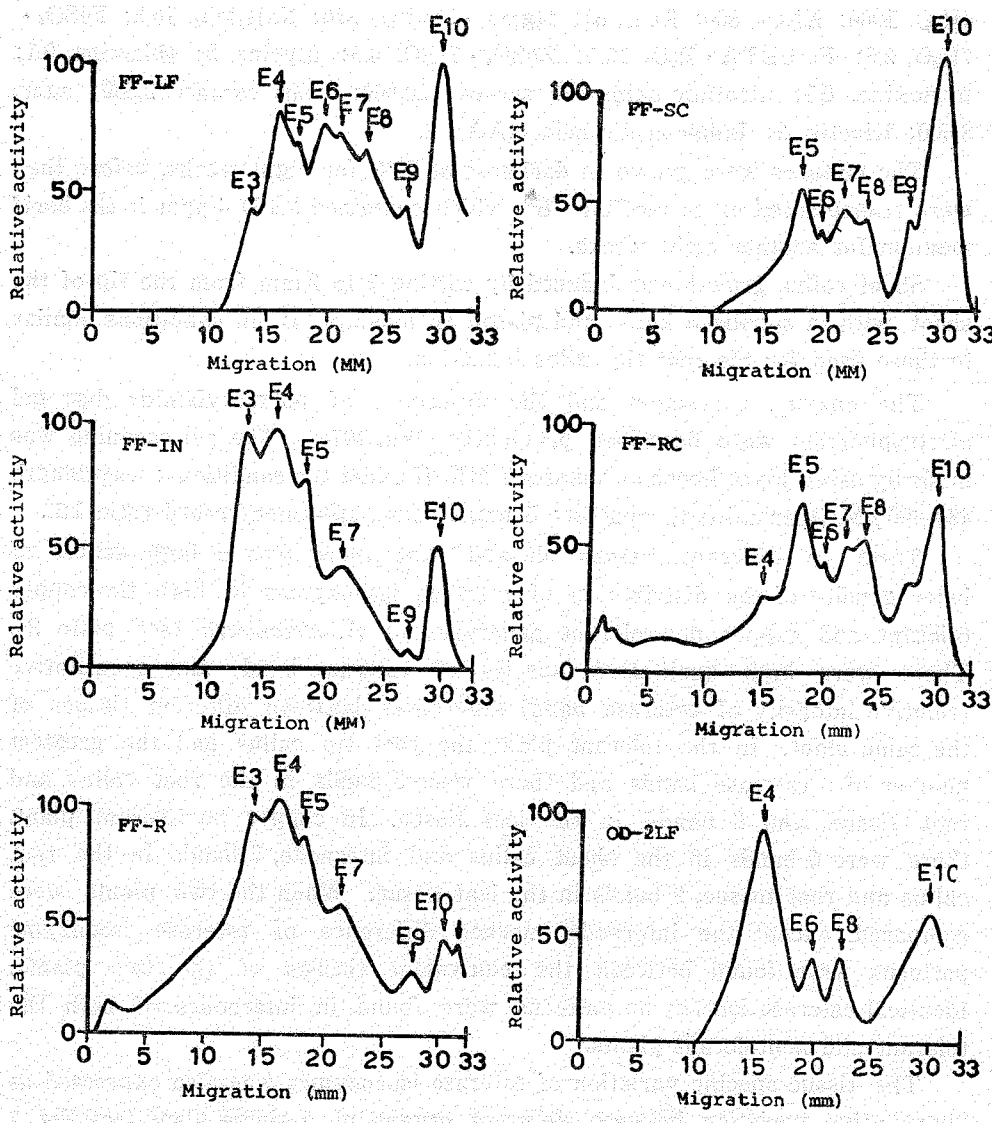
Shoot callus growth was induced by cutting 1 to 2 mm from the tip of the shoot with a sterilized knife, and placed on medium "B" in conditions similar to those used for the root tip callus induction.

The enzyme extraction and the procedure of polyacrylamide disc gel electrophoresis were described previously (Wu, 1976). The gel scanning was made by using Joyce Loebe chromoscan MK. II under the conditions: wavelength 480–490 μM (transmitted), aparture 5 mm.×1 mm., specimen/record ratio 1:3.

Tests on different tissues showed that there was a high degree of heterogeneity of the distribution of esterase isoenzymes in both the copper tolerant and copper non-tolerant genotypes of *A. stolonifera* (see plate 2). There were both qualitative (species of esterase band) and quantitative (relative intensity of esterase band) differences between different tissues of the same plant. In the tolerant plant, the root tip callus had the greatest number of 9 esterase bands and there were 5 bands in the root callus, and root tissue, and 6 bands in the leaf tissue. In copper nontolerant plant, there were 6 bands in the shoot callus and internode, 7 bands in the root callus and root tossue, 9 bands in the leaf tissue. When the two plants were compared, except the internode, marked difference of esterase isoenzyme patterns were found between the equivalent tissues of the two plants. Identical esterase isoenzyme patterns were found in internodes of both the tolerant and nontolerant plants.

The tissue specific variation of esterase isoenzymes was also expressed as quantitative variation between different organs of a single plant (see Fig. 1 and 2). For instance, the ratio of the activity of band E3 (migration rate 0.04) /E9 (migration rate 0.83) in the five different organs of the Old lawn genotype is nearly 0/25 in leaf, 95/5 in internode, 95/40 in root, 95/20 in root tip callus, 100/20 in shoot tip callus, and the esterase activity of these two bands in the nontolerant genotype are nearly 40/40, 100/5, 95/30, 0/45 and 0/40 respectively.

The relative activity of the bands E4 and E10 in zymogram 2 of leaf extracts of the two Old low samples, OD–2 and OD–3, showed 95/65 and 98/64 respectively. This result indicates that the quantitative variation did happen between the plants having identical zymograms.



FF: Freshfield grassland nontolerant genotype OD: The Old Lawn tolerant genotype

Fig. 1. Relative activities of tissue specific esterase isoenzymes of *Agrostis Stolonifera*

From the results of the present studies, it is reasonable to assume that the expression of esterase isoenzymes in different tissues of a particular genotypes is genetically regulated. Different genotypes may have different esterase isoenzyme patterns not because they differ in the genes determining the basic presence of the isoenzymes, but because they differ in genes determining their occurrence in particular tissue. In other words observed variation between genotypes in the esterase isoenzymes in a particular plant organ is not necessary an indication of variation in structural genes controlling esterase isoenzyme production in

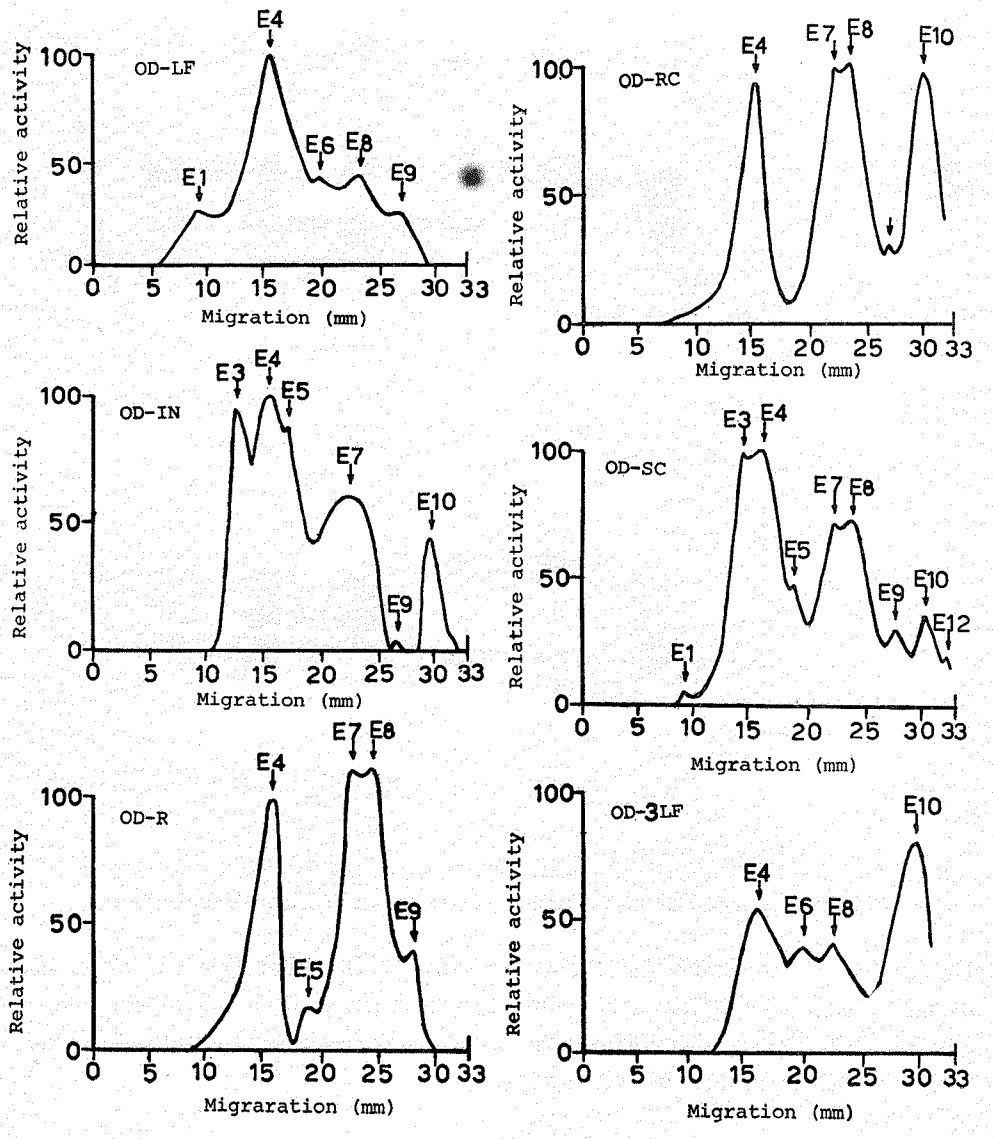


Fig. 2. Relative activities of tissue specific esterase isoenzymes of *Agrostis Stolonifera*

the organism. However, in the previous studies I am interested to determine the variability and the genotypical structure of the plant populations. It is not important what genetic factors were involved. Any genes whatever they determine are indicaters of the genetic variability of the plant populations and the characteristics of plant genotypes.

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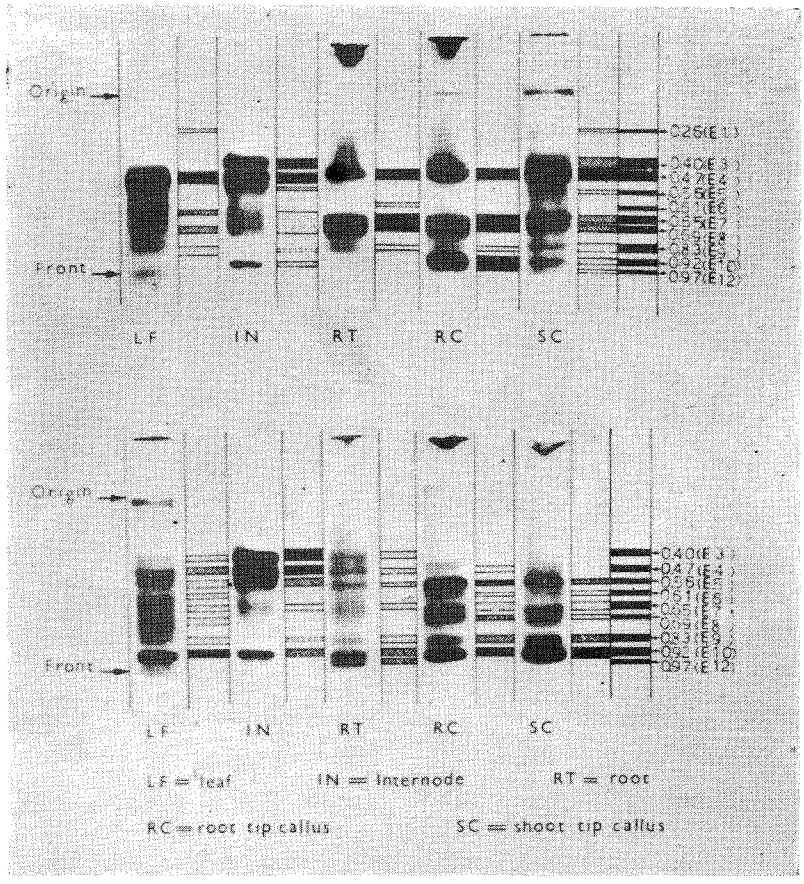

Plate 2. Zymograms of Tissue Specific Esterases of *Agrostis Stolonifera*

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在匍匐剪股穎 (*Agrostis Stolonifera* L.) 不同組織中脂化酵素同位酵素之變異

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經同一因子型匍匐剪股穎不同組織中，脂化酵素同位酵素的分析。顯示在不同組織間，同位酵素的分離圖（zymogram），以及各同位酵素的種類和相對的活性（activity）均有相當的變異。但在探討族羣無性繁殖系統和遺傳結構的目的下，這種遺傳變異仍不失爲探討植物族羣遺傳結構的依據。