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# Variation of Peroxidase 8 in Maize, *Zea mays*, L.

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# 옥수수에 있어서 Peroxidase 8의 変異

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## ABSTRACT

As one way of evaluating polymorphism in maize, variation of peroxidase 8 (Px.8) in maize plants was investigated by means of horizontal acrylomide electrophoresis. The specific part of maize plants was stele and other parts of plants were also studied for 34 different maize lines and hybrids. The results obtained indicate that Px.8 has three distinct migrating patterns on gel such as fast, slow and fast-slow. The band pattern varied with materials used. Most hybrids were showing fast-slow band, indicating that those hybrids are heterozygous at least for Px.8 allele. Variation of band pattern was obseerved within a single inbred line. The Px.8 in stele tissue and in young leaf and nodal tissue was found to be identical, indicating the possible use of those tissues as an alternative tissue for Px.8 study. It was also found that there might be some definite relationship between Px.8 and Px.3 activities in the same tissue.


## INTRODUCTION

Thirteen major peroxidases of maize were previously reported by Brewbaker and Hasegawa. [4] They identified genetic loci governing nine peroxidase patterns. Also they reported clearly that most enzyme systems they found were tissue specific. Such a finding in maize will be used very usefully to identifying and classifying questionable maize lines. The use of electrophoretic techniques for identification, classification and for finding variability in germplasm of various species were reported in detail by many people.[9,6,7,5,2,3,1]

The use of various isoenzyme systems by electrophoretic procedures for genetic and breeding purposes may be not feasible where lab facilities are not well equipped. It many be not possible also to use all of the isozyme systems reported for either genetic or breeding purposes at one lab by one person. Therefore, one good isozyme system may be enough for some case. Brewbaker and Hasegawa.[4] reported that one of the enzyme systems they studied, peroxidase 8 (Px. 8), is quite specific to tissues of coleoptile, mesocotyl (cortex, stele), pith, stem apex, tassell and ear initial and 20 days old pericarp, embryo and endosperm, Of the tissues, stele may be the only tissue which doesn't show much environmental effects and complicated isozyme systems. Also stele is found to be absent in Px. 1, Px. 5 and Px. 9. We thought that use of one specific tissue such as stele for the polimorphism study will be necessary in certain lab condition. Therefore, the objectives of our study were to find any variants in Px. 8 in stele tissue, to observe the activities of Px. 8 and to discuss the

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possible use of other tissues for genetic or breeding purpose in maize.

## MATERIALS AND METHODS

Twenty one inbred lines and thirteen hybrids or synthetics from the Foundation Seed Stocks of the University of Hawaii were utilized. The inbreds and hybrids were soaked in water and germinated at 28°C. for five days. The stele was sampled from each coleoptile by opening the coleoptile with forcept. Abot 1 to 2 cm. long stele were used. Care was taken not to exposure the tissue to direct sun light much. All other techniques and procedures for running electrophoresis were previously described by Brewbaker et al.[2,3] In order to improve the resolution for Px.8, 5% of acrylamide gel was used instead of 7%. Gree leaf tissue was used as a standard reference for Px. 3 and relative position of Px. 8 was estimated based on the Px. 3 position.

## RESULTS

There were at least three distinct banding patterns in Px. 8 of maize stele studied. We called them as slow (S), fast (F), and slow/fast (S/F), respectively. The inbreds and hybrids showing such a different banding pattern is shown in Table 1 and Fig. 1. As shown in Table 1, some inbreds or hybrids were uncertain in their banding patterns. For example, inbreds such as Hi 27 and Hi 29 were showing both fast and slow bands. Most hybrids examined were showing either double bands, S/F or fast band. Such a simple variation of Px. 8 in stele tissue suggested that there may be only two alleles at one

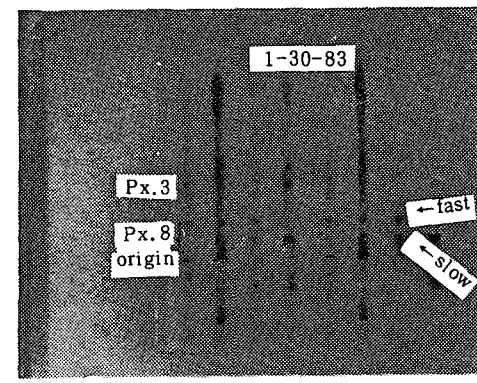


Fig. 1. Peroxidase 8 in stele tissue. Fast and slow bands are clearly visible. Four different lines were run with two replication. Notice that some line shows Px. 3 band.

**Table 1.** Different isozyme banding patterns of Px. 8 in maize stele of inbreds and hybrids.

| Banding patterns | Inbreds or hybrids | |
|---|---|---|
| Slow bands | Inbreds: | Hi 27, Hi 28, Hi 29, Hi 31<br>F44, A619 |
| Fast bands | Inbreds: | W 64, FR 20, Hi 29, Oh 07,<br>Hi 27, Hi 31, CI 64, MET,<br>F44, INV 138, INV 575, Oh 514<br>B73, B77 |
| | Hybrids: | H650, H948, H949, H945,<br>Hi 27 x Hi 29, $Moo_2$ #2, $Moo_2$ #3,<br>CMS, CIMMYT A21, CIMMYT 103. |
| Fast and slow | Inbreds: | Hi 27, Oh 07, Hi 29, Hi 31,<br>INV 575, INV 138, CI64, F44,<br>CI66, MET, Hi 28, B73, B37,<br>B77, C 103, CM 104, A619,<br>Hi 34-1, Mo 26W. |
| | Hybrids or synthetics: | Hi 27 x Hi 29,<br>H 632, H 649, H 946, H 949,<br>CIMMYT A21, CIMMYT 103, $Moo_2$ #3, CMS. |

locus involved. This results are agreed upon the previous report by Brewbaker and Hasegawa.[4] Figure 1 also shows that tissue, stele, was absent in other peroxidase such as Px. 3, Px. 4, or Px. 6. These were also agreed upon the previous report. Since stele tissue is composed of the first leaf and other meristematic tissue, peroxidase 8 was observed along with other isoenzymes like Px. 3 in some case (Fig. 2).

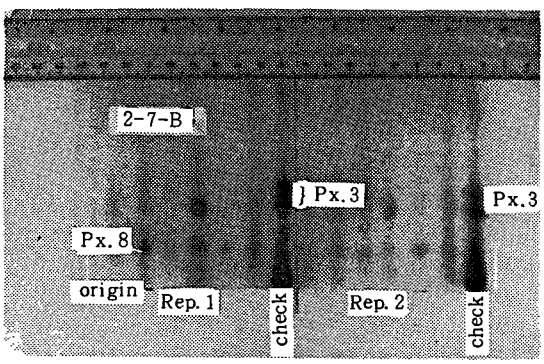


Fig. 2. Seven maize lines including a check line were with two replications. Px. 3 with double bands are distinct in the check line and Px. 3 with single band are shown in some lines.

The relative position of slow and fast bands for Px. 8 was estimated based on the Px. 3 position. Many previous reports described the distance reports described the distance of each isozyme in different terms such as Rm or Rf. In our study the fast band of Px. 8 was always position-ed half way between the origin and the slow band of Px. 3. and the slow band of Px. 8 was positioned at the ¼ distance from origin to slow band of Px. 3 (Fig. 3). The Px. 3 band which we used as standard reference shows either single or double bands depending upon the leaf materials. Hi 27 inbred was a good leaf sample to show a distinct single band.

Brewbaker and Hamill[2] had reported that all the maize tissue shows various number and activities of isozyme during development. They also emphasized that critical comparisons of peroxidase isozyme activities during ontogeny must be done on an isonitrogenous basis of samples. They found that

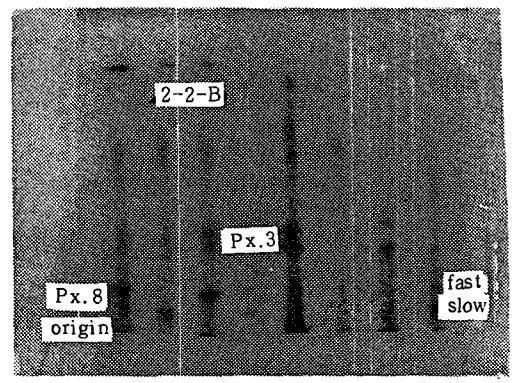


Fig. 3. Fast band of Px. 8 is located about half way between slow band of Px. 3 and origin.

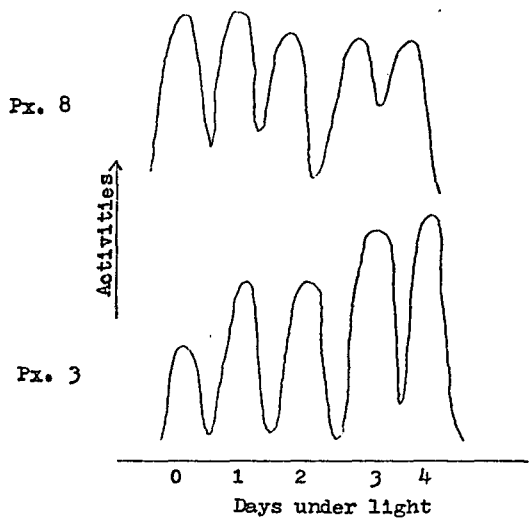


Fig. 4. Densitometric readings of Px. 8 and 3 in the stele tissue subjected to the different days under light.

Px. 1, 4 and 5 decreased markedly during lignification of the stem. From our study it was also found that the Px. 8 was quite dependent on the developmental stages. Fig. 4 shows that activities of px. 8 decreased as the stele gets older. But there were no prominent changes in activities before the stele bursted out the coleoptile. Px. 3, however, increased as the stele becomes greener. We also found that Px. 8 was also active in any young leaf tissues as far as the leaves are low in photosynthetic activities (Fig. 5). The interrelationship between Px. 8 and Px. 3 in a randomly chosen leaf sample was shown in Fig. 5 and the activities of Px. 8 and Px. 3 in the same leaf tissue was measured by

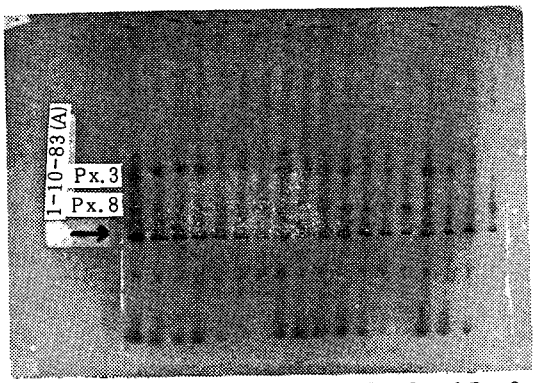


Fig. 5. Inter-relationship between Px. 3 and Px. 8 in a single leaf tissue.

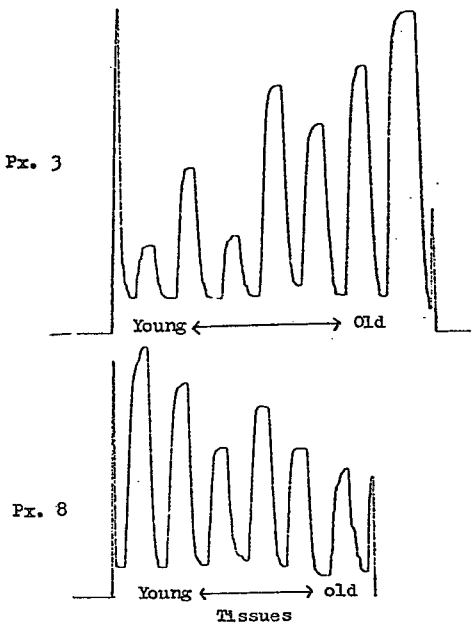


Fig. 6. Densitometric readings of Px. 3 and Px. 8 activities in the same leaf tissues.

densitometer (Fig. 6). As shown in Fig. 5 and 6, the activities of Px. 3 and Px. 8 in the leaves appeared to be reversible each other. As Px. 8 activity goes up, the Px. 3 activity goes down. The close relationship between these two peroxidases was found also in any meristematic parts of nodes, which include ear initials also. Px. 8 was reportedly known as very active in the stem apex or ear and tassell initials.[4] We examined the portion of every nodes of a plant at knee high stage and found interesting relationship exists between Px. 8 and Px. 3. Fig. 7 shows that

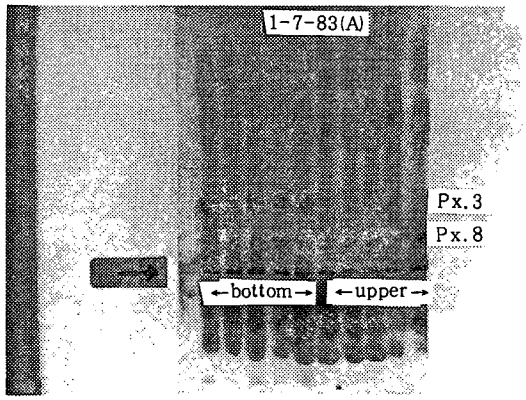


Fig. 7. Bottom nodes show Px. 3 and Px. 8, while upper nodes only Px. 8.

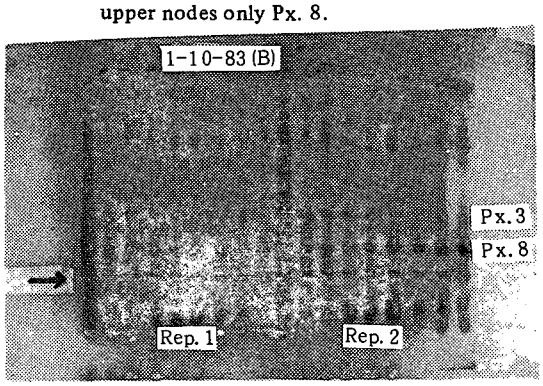


Fig. 8. A randomly taken young node shows different activities of Px. 3 and Px. 8 depending upon the small areas in the node.

the nodes of bottom parts of plants had both Px. 3 and Px. 8 isozymes, while the nodes of upper parts are lacking Px. 3. We also noticed changes of Px. 3 and Px. 8 activities in the same node (Fig. 8). Px. 3 was very active where leaf was attached, while the Px. 8 was active almost in all parts of nodes including some parts of internode. We should indicate here that all the plants or parts of plants examined were taken before flowering stage.

## DISCUSSION

Studies on a specific isozyme may be useful where facilities are not quite feasible. Information from such a specific isozyme study can be used for genetic and breeding purposes in certain areas. In order to obtain such a information we tried to run the stele of maize as our specific tissue for Px. 8

and found that two alleles at one locus are involved in fast, slow, and fast/slow bands. We used only limited number of maize samples. We may have to examine more maize samples to verify further Px. 8 variation. As Shumaker[8] indicated electrophoresis performed under a single set of conditions may be not enough to detect enzyme variability.

As Brewbaker et al.[4] indicated, the stele was useful material since the tissue was least affected by environments and furthermore the tissues were known as having little enzymatic variation except Px. 8. Small problems associated with the stele might be that we may have to sacrifice the plants. In order to find some alternative tissue, we tried to locate Px. 8 in other tissues and found that very young leaf tissue from any nodes had the Px. 8 as far as they are young and yellow in color. We also found Px. 8 was active in the young nodes. Such a leaf tissue or node can be sampled from either tiller or from leaf whorl by peeling off carefully. The dependency of Px. 8 activities on lignification of tissue or on the photosynthetic activities should be further studied.

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## 摘　　要


옥수수에 있어서 多形現象에 關한 硏究의 一環으로 Peroxidase 8에 對하여 水平電氣 泳動法에 依한 變異를 본 結果 中心柱의 Peroxidase 8은 옥수수의 系統이나 品種에 따라 Fast, Slow, Fast/slow 밴드를 보여 주어 Peroxidase 8은 單一 遺傳因子에 依하여 左右된다고 생각되며 특히 本硏究에서 同一한 自殖系統에 있어서도 相異한 밴드의 패턴을 보여 주었는데 이는 使用한 系統이 同質接合體가 되지 못하였거나 混種된 것이 아닌가 생각되어진다. 그러나 대가 自殖系統의 옥수수는 Fast나 Slow의 單一 밴드 패턴을 보여주었는데 反하여 單交雜 내지는 合成品種은 여러가지 형태의 패턴을 보여주었다. 그리고 Peroxidase 8은 대개 初葉(中心株)이나 木化되지 않은 組織에서 흔히 觀察되나 Peroxidase 3와도 關係를 가지고 있는 것으로 생각되어진다. 특히 옥수수 줄기의 節에서 觀察되는 Peroxidase 3과 8의 패턴으로 보아 이 두 酵素는 서로 相反되는 作用을 한다고 생각한다.


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