Application of the Electrophoretic Isozyme Method to Muskmelon Breeding

I. A Search for Available Enzymes

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Summary

In order to explore the possibility of application of isozyme analysis to melon breeding, several enzymes of melon seedlings were studied using the polyacrylamide gel isoelectrofocusing technique. The plants examined included 10 cultivars of 3 botanical varieties and 3 F1 hybrids between them.

As to the zymograms of glutamate dehydrogenase, malate dehydrogenase, and esterase, there was no significant difference among cultivars. In the peroxidase isozymes there was a slight difference in zymograms between powdery mildew resistant cultivars and susceptible ones: that is, the former had 22 bands and the latter 21 bands. In the acid phosphatase isozymes remarkable differences in banding patterns expressed by optical density were shown not only among varieties, but also within a variety, that is, between the American type and the British one in var. reticulatus. It is concluded that acid phosphatase isozymes seem useful for the discrimination of varietal differences among plants, and accordingly it may be applied to the selection in melon breeding.

Introduction

Recently the electrophoretic method of isozymes has been shown to be useful for genetic and phylogenetic studies of plants (7, 13, 15, etc.). As to melon plants, electrophoretic studies of isozymes were reported by Eguchi and Fujieda(4), Loy (6), and Asahira and Ooi(1).

The present study was carried out, using some melon cultivars in which the British and American types of var. reticulatus were included, to explore the possibility of application of isozyme analysis to melon breeding: i.e., to search for 1) the enzymes which can reflect genetic differences among varieties and cultivars and 2) the enzymes by which the plants resistant to diseases can be selected at their seedling stage.

Materials and Methods

The materials used in the investigation were composed of 10 cultivars and 3 F1 hybrids: i.e., 3 cultivars of the British type, 5 cultivars of the American type, and 3 F1 hybrids between the two types (Cucumis melo var. reticulatus; Hao Qen (var. cantaloupensis); and Honey Dew (var. inodorus). Materials and their some characters are shown in Table 1. All cultivars included in the British type are susceptible to powdery mildew and resistant to necrotic spot (probably identical to crown blight, Whitaker and Davis(14)), and show high sugar content. On the contrary all cultivars of the American type have a resistance to powdery mildew and a high susceptibility to necrotic spot, and show low sugar content and orange flesh colour. The other two varieties are susceptible to powdery mildew and resistant to necrotic spot.

PMR No.45(5) and PMR No.5(8) are powdery mildew resistant cultivars which have been bred in America using the varieties secured from India, and they were introduced to our country by Tamai(10). Ms-1 and Ms-2, both of which are male sterile strains, were obtained from Dr. G.W. Bohn of U.S. Department of Agriculture, La Jolla, California, U.S.A. Ms-1 was derived from the
Isozymes examined were glutamate dehydrogenase, malate dehydrogenase, esterase, peroxidase, and acid phosphatase. For isozyme extraction, the first and second, fully expanded leaves at young seedling stage or the top part of the growing stem at topping stage was collected and stored at -70°C. They were homogenized in trisglycine buffer at pH 8.7 (tris 6 g, glycine 28.8 g in 1,000 ml of distilled water) for peroxidase and esterase, and in 0.1M tris-HCl buffer at pH 8.7 for the other enzymes. Then they were centrifuged at 25,000g for 20 min. Supernatants were again stored at -30°C and prepared for subsequent procedures. The ratios of the samples to the buffers are 2g to 30ml for peroxidase, 2g to 0.5ml for esterase, and 2g to 1ml for the other enzymes.

Electrophoresis was carried out using the polyacrylamide gel isoelectrofocusing technique (pH 3.5–10 carrier ampholite) on the disc electrophoretic apparatus. The methods of electrophoresis and staining of gels (esterase, peroxidase, and acid phosphatase) are the same as described by Tokumasu et al. (12). The staining of the other enzymes was carried out as described in the paper of Sako and Stahmann (9).

The seedlings of which the first and second leaves were chopped for isozyme extraction were grown in sand beds in a vinyl house, and their main characters were investigated.

Results

Table 2 shows the organs used for the isozyme extraction, the kind of enzymes, and the number of bands detected.

Table 2. Enzymes examined and the number of isozyme bands detected.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Nos. of bands</th>
<th>Nos. of examined cultivars or hybrids</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Young leaves*</td>
<td>Top**</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>2</td>
<td>--</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>4–5</td>
<td>--</td>
</tr>
<tr>
<td>Esterase</td>
<td>--</td>
<td>20</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>21–22</td>
<td>22</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>8–11</td>
<td>--</td>
</tr>
</tbody>
</table>

* The first and second, fully expanded leaves at young seedling stage.
** Top part of growing stems, which includes immature leaves, at topping stage.

1. Glutamate dehydrogenase and malate dehydrogenase.
Earl, PMR No. 5, Ms-2, and Honey Dew were examined for glutamate dehydrogenase. Two bands were detected in all cultivars, and there was no difference among them.

In the malate dehydrogenase isozymes 4–5 bands were detected in Ms-2, Earl, and their F₁ hybrid, but significant difference was not found among them.

2. Esterase.

Earl, PMR No. 5, Ms-2, Honey Dew, and 3 F₁ hybrids (Iyo No. 1 × Earl, Barnett × PMR No. 5, and Ms-2 × Earl) were examined using the top part of growing stems. Twenty bands were detected, and the number of bands and band density were uniform in these cultivars. Fig. 1 shows the zymogram and banding pattern expressed by optical density in Earl.

3. Peroxidase.

All cultivars and hybrids listed in Table 1 were examined using the leaves. Five cultivars susceptible to powdery mildew had 21 bands. However, 5 cultivars and 3 F₁ hybrids, all of which had resistance to this disease, had 22 bands. To say more exactly, as shown in Fig. 2, there was no band between band Nos. 6 and 7 in susceptible cultivars, but a faint band was detected there in resistant ones. Nevertheless, this band was also detected in the susceptible cultivars when these cultivars were infected with this disease. Moreover, the density of this band did not differ between resistant parents (homozygous) and F₁ hybrids (heterozygous).

The zymogram of the top part of growing stems showed 22 bands, and there was no difference among varieties and cultivars, independently of susceptibility or resistance to powdery mildew.

4. Acid phosphatase.

Seven cultivars of 3 varieties and 1 F₁ hybrid were examined. Fig. 3 shows their zymograms and banding patterns. Earl and Pearl in the British type resembled closely each other, and there were many similarities among PMR No. 45, PMR No. 5, and Ms-2. However, there were remarkable differences in banding patterns among vars. reticulatus, cantaloupensis, and inodorus, and, moreover,
Fig. 3. Acid phosphatase zymograms of the leaves and their banding patterns expressed by optical density in 7 cultivars of 3 varieties and F₁ hybrid.
between the American and the British types. For example, Earl showed high optical density in band Nos. 5 and 6, and low density in band Nos. 3 and 4. In contrast with this, Ms-2 showed high density in band No.3, and low density in band Nos. 5 and 6. F₁ hybrid between Ms-2 and Earl showed an intermediate banding pattern between both parents.

The stability of acid phosphatase isozymes within Ms-2 and within Earl was investigated using 4 plants each, and the result is shown in Table 3. The rates of their common bands

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Percentage of common bands*</th>
<th>Variation of band density**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms-2</td>
<td>97.2%</td>
<td>23.1%</td>
</tr>
<tr>
<td>Earl</td>
<td>100.0</td>
<td>25.5</td>
</tr>
</tbody>
</table>

* No. of pairs of common bands
** The mean of coefficients of variation of the optical densities (relative percent in a gel) of each band.

were 97% and 100%, respectively. Furthermore, the variation of band density of both cultivars was as low as 23 and 26%.

Discussion

Eguchi and Fujieda (4) made a phylogenetic study of *Cucumis melo* by means of esterase zymograms of germinating seeds, using starch gels. They reported that there were differences among varieties, but no difference among cultivars in the same variety. Asahira and Ooi (1), using the polyacrylamide gel isoelectrofocussing technique, described that the classification of melon plants by esterase isozyme patterns of germinating seeds was impossible. In the present study 6 cultivars in var. *reticulatus* and 1 in var. *inodorus* were examined. As a result, no difference was found in esterase zymograms of the top part of growing stems, like in those of germinating seeds, among the cultivars.

In the peroxidase isozymes there was a slight difference in the number of bands between powdery mildew resistant cultivars (22 bands) and susceptible ones (21 bands), but this difference disappeared when the susceptible cultivars were infected with this disease. The band which is originally absent in the susceptible cultivars and appears with the infection of the disease may be regarded as an inducible isozyme. This enzyme may be available for the discrimination between the resistant and susceptible seedlings, but in practice it is not so effective for the following reasons: 1) the faint band is inducible, not being a constant marker, 2) the faint band shows no difference in its density between homozygous and heterozygous genic conditions, 3) in practical cultivation it is very difficult to grow the seedlings without infection of powdery mildew, and 4) the simplicity of the inoculation test of powdery mildew at the seedling stage cannot be replaced by the complicated procedure of the isozyme test. Loy (6) found no significant difference in peroxidase activities or isozyme patterns between bush and vine types of muskmelons.

In banding patterns of acid phosphatase isozymes expressed by optical density, remarkable differences were shown not only among varieties, but also between the American type and the British one. Furthermore, F₁ hybrids between Ms-2 and Earl showed an intermediate banding pattern. Within each cultivar, variation was scarcely shown in both zymograms and banding patterns. Asahira and Ooi (1) described that acid phosphatase is useful for the estimation of genetic relationships among varieties of *Cucumis melo*.

In the present study, the isozymes available enough for the selection of the plants resistant to powdery mildew and necrotic spot could not be found. One of the purposes of this investigation, therefore, failed to be achieved as far as these two diseases are concerned. However, acid phosphatase is expected to reflect genetic differences among varieties and cultivars. Therefore, hereafter we will investigate the relationships between banding patterns of acid phosphatase of seedlings and main characters (e.g., flesh colour, sugar content, fruit shape, resistance to diseases, etc.) of adult plants, using F₂ and B₁ generation plants between the American type and the British one.

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Literature Cited


