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STUDIES ON IDENTIFICATION OF CHESTNUT SPECIES AND VARIETIES BY ISOZYME ANALYSIS

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Abstract

Peroxidase, alkaline phosphatase and esterase isozymes in the barks from shoot tips of chestnut trees were analyzed by the disc-isoelectroforcusing method. Sixteen varieties belonging to Japanese, Chinese and European species were used in this study. A total of 10 bands were distinguishable for the peroxidase and alkaline phosphatase isozymes. In both the isozymes, however, no species-specific bands were found. A difference in staining intensity of a band was observed among species or varieties. For esterase, no clear zymograms were obtained.

Introduction

The chestnut has a long history of culture and is widely distributed in the temperate zones of the world. Major varieties are belong to the three species ; *Castanea crenata* SIEB. et ZUCC. (Japanese species), *C. mollissima* BLUME (Chinese species) and *C. sativa* MILL. (European species). Resistance to disease, insect or cold varies among these species[3].

Recently, the electrophoresis of protein and isozyme has been conducted for the identification of the phylogenetic relationships in a variety of the plant. In woody plants, SAITO[1] reported the presence of a interspecific difference in the soluble protein extracted from the pollen of five species of genus *Alnus*. MIYAZAKI and SAKAI[7] demonstrated that peroxidase zymography is useful for testing genuineness of the clones of *Cryptomeria japonica* D. DON.. AOKI et al.[2] determined the phylogenetic relationship between cultivars of mume and apricot by analyzing peroxidase isozyme in their foliage. On the basis of the results of serodiagnostic investigation, HYUN[5] has found a clear difference between Japanese and Chinese chestnuts. However, no information is available on the zymogram in the chestnut. The present study was carried out to examine whether or not the isozyme method could be applied to the identfication of the chestnut species.

Materials and Methods

Sixteen varieties including two clones were used in this study, ten, three, two and one of which belonging to Japanese, Chinese, Japanese Chinese hybrid and European species, respectively. Shoots were collected from the trees grown in the experimental farm of Faculty of Agriculture, Kobe University. Barks from the shoot tip were used for determination of isozyme pattern. The barks, approximately 1 g in fresh weight, was finely diced with a razor blade, and ground in a mortar at 5 °C with 4 ml of 0.2 M Tris-HCI buffer (pH 8.5) and a small amount of quartz sand (150 - 200 mesh). After the slurry had been centrifuged, the supernatant was stored in a freezer prior to electrophoretic analysis.

Electrophoresis was carried out by the method[8] of gel isoelectrofocusing. The gel was prepared from the mixture of solution A (30 g acrylamide, 0.8 g BIS and 0.2 g TEMED in 100 ml water), solution B (40 ppm aqueous solution of riboflavine), solution C (20 ml of 40% Ampholine, pH 3-10 and 80 ml water), and water

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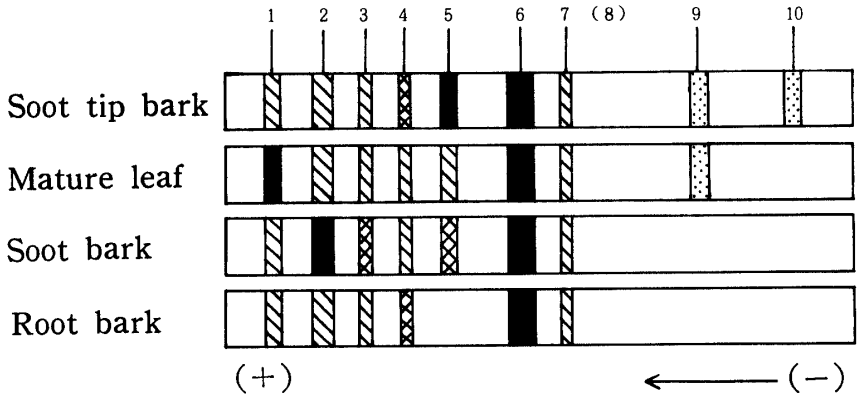

Fig. 1. Schematic zymogram showing peroxidase patterns of various tissues from two-year-old seedling of Japanese chestnut 'Gin-yose'. Sampled on July 22, 1981. Arrow shows the direction of migration toward the anode.

stained densely, stained moderately,
stained lightly, stained very lightly.

in a volume ratio of 4 : 2 : 3 : 15. After the gels had been polymerized in glass tubes, 0.1 ml of the bark extracts (ca. 20 mg fresh weight) were loaded on the gels. The gel size was 9 cm long and 5 mm in diameter. The tubes were set in a ordinary disc electrophoretic apparatus. The upper chamber was filled with 0.2 M ethylenediamine (cathode), and the bottom chamber with 0.05 M HCI (anode). After exposure to stabilized electric current of a 200 volt for three hours at 5 ℃, the gels were removed from the glass tubes and incubated into the stain solutions.

Peroxidase[10] was stained by incubating for 10 min. at 4℃ in solution I (100 mg *o*-dianisidine in 0.84 ml acetic acid, 70 ml water and 30 ml 0.2M acetate buffer, pH 4.9), followed by transferring into solution II (98 ml 0.2M acetate buffer, pH 6.5, and 2ml 3 % H_2O_2). Alkaline phosphatase[9] was stained with solution containing 100ml 0.1 M Tris-citric acid buffer (pH 8.5), 100mg α-Naphthyl acid phosphate-Na salt, 100mg Fast Blue RR salt, 10 drops of 10% aqueous $MgCl_2$ and 10 drops of 10% aqueous $MnCl_2$. Esterase[4] was stained with solution containing 7% α-naphthyl acetate in 0.5 ml acetone, 12.5 mg Fast Blue RR salt, 1 ml 0.2 M Tris HCI buffer (pH 7.1) and 23.4 ml water.

After staining, the bands were designated according to their mobilites and numbered in sequence from anode to cathode.

Results

1. *Tissue specificity of peroxidase isozyme.*

Peroxidase isozymes wers preliminary analyzed for the various tissues from a two-year-old seedling of Japanese chestnut 'Gin-yose'. The peroxidase zymograms showed a large variability among different tissues (Fig. 1). The largest number of the isozyme bands were observed for the extracts from barks of the growing shoot tip. The mature leaves lacked Band 10, the shoot barks Band 9 and 10, and the root barks Band 5, 9 and 10.

2. *Variation of isozyme pattern among varieties or species.*

In the present study, isozyme pattern among the varieties were compared by using the extracts from barks of shoot tips.

(1) *Peroxidase* The zymograms of 16 varieties (3 species) are shown in Fig. 2. A total of 10 bands were distinguishable. Seven bands from Band 1 to 7 were common to all the varieties and some varieties of Japanese species lacked one to three bands which located in 8 to 10 position. However, no species-specific bands observed. Staining intensity of the bands varied among varieties. Band 5 stained more densely in Chinese and European than in Japanese species. The low intensity of the Band 6 was observed

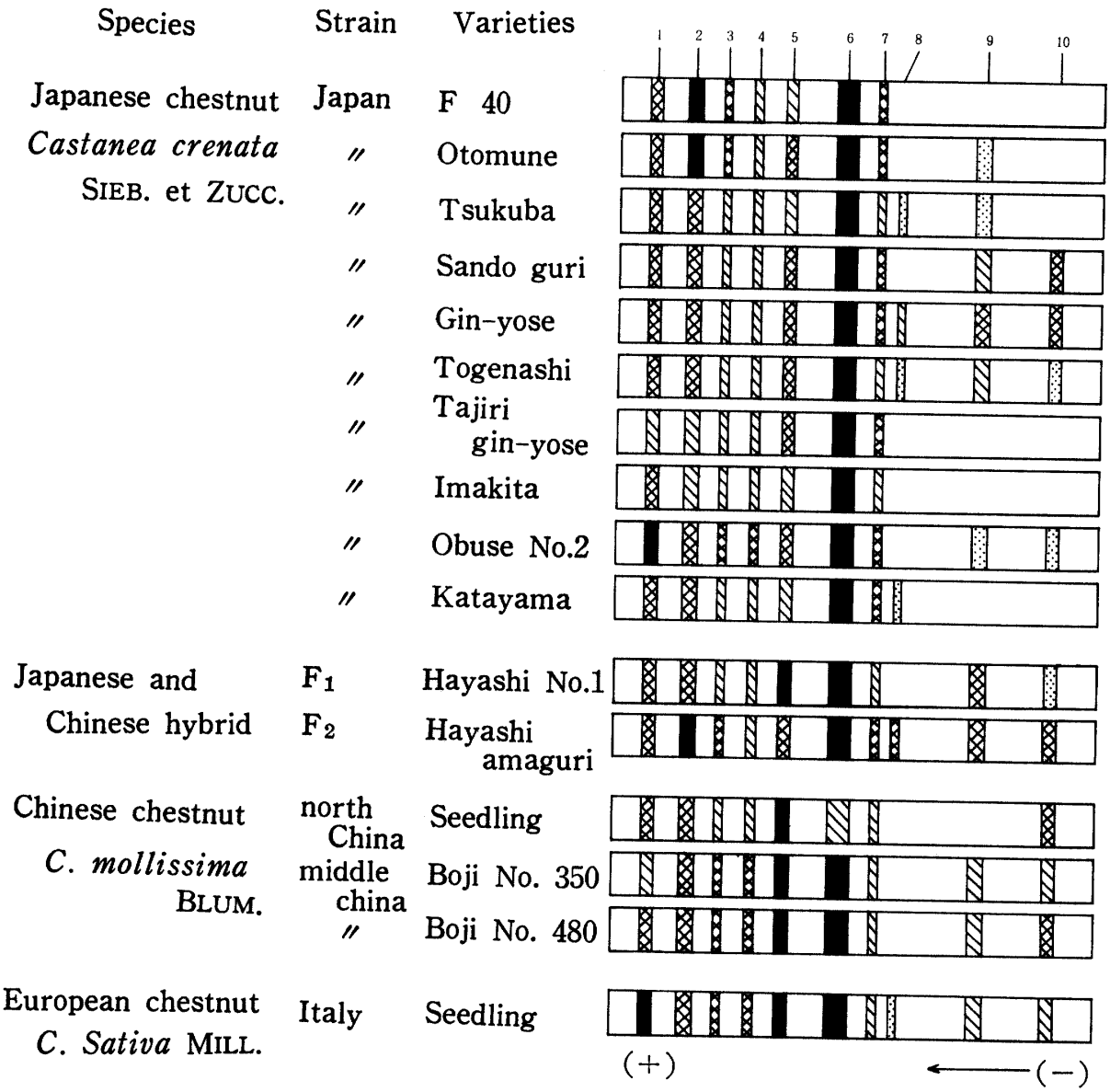

Fig. 2. Schematic zymogram showing peroxidase patterns of 16 varieties belong to Japanese, Chinese and European species. Sampled on Dec. 3, 1981. Arrow shows the direction of migration toward the anode.

stained densely, stained moderately,
stained lightly, stained very lightly.

in a seedling of north China strain.

(2) *Alkaline phosphatase* As shown in Fig. 3, the zymograms consisted of 10 bands in 14 varieties, and samples prepared from F 40 of Japanese and a seedling of European species lacked Band 3 of 10 bands. No species-specific bands were detected as was the case with peroxidase isozymes. Thus, the zymograms showed less dramatic defferences among varieties.

(3) *Esterase* No clear zymograms were obtained for esterase (data not given).

Discussion

In many plants, peroxidase is known to exist in the form of isozymes which are genetically regulated. MIYAZAKI and SAKAI[7] reported that the electrophoretic variation of peroxidase was quite useful for testing genuineness of *Cryptom-*

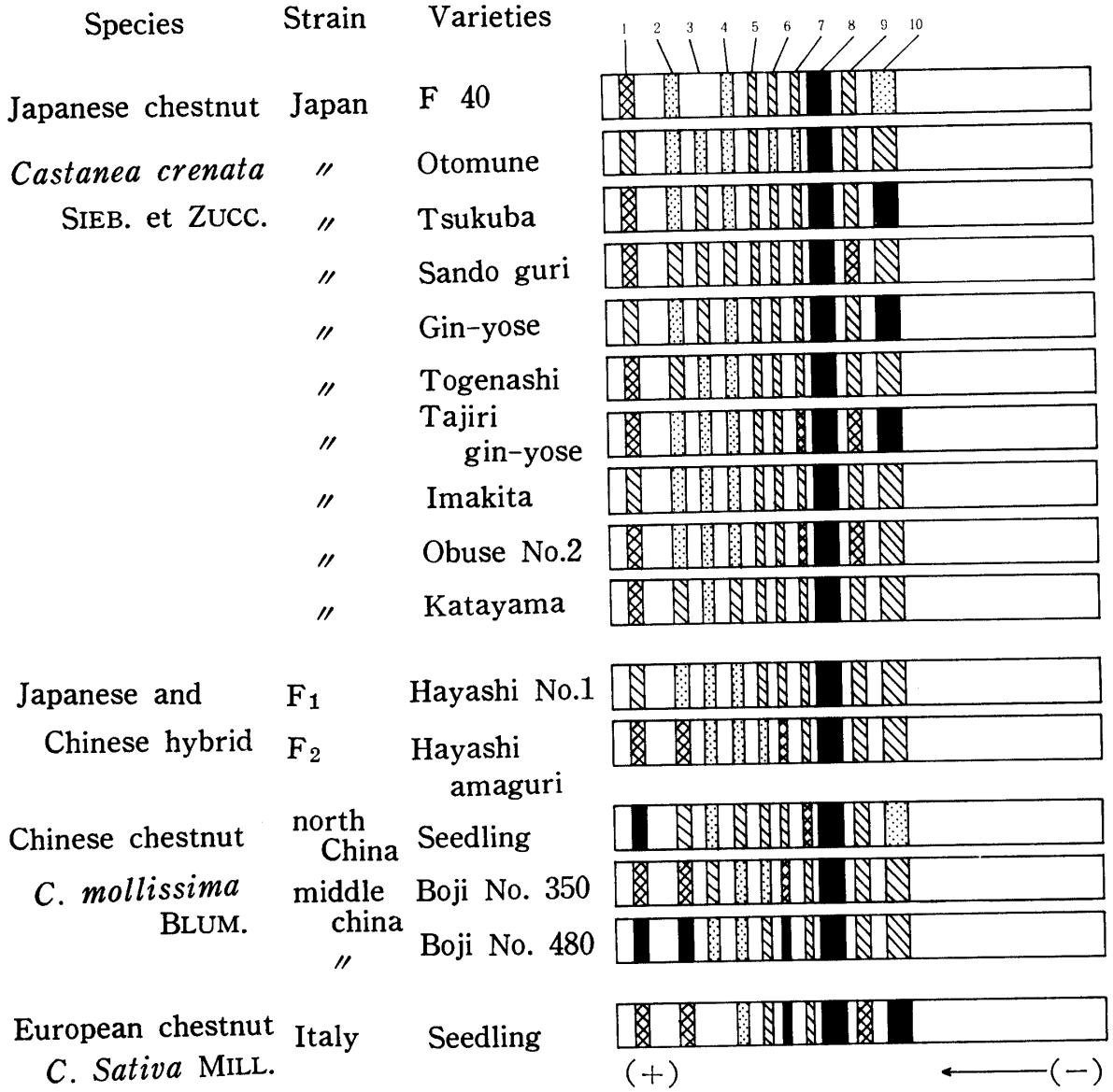

Fig. 3. Schematic zymogram showing alkaline phosphatase patterns of 16 varieties belong to Japanese, Chinese and European species. Sampled on Dec. 3, 1981. Arrow shows the direction of migration toward the anode.

stained densely, stained moderately,
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eria japonica D. DON.. AOKI et al.[2] found that the cultivars of pure mume and apricot stocks had a species-specific band of peroxidase isozyme. In the present study with chestnut, the peroxidase zymogram consisted of a total of 10 bands, the number ranging from a minimum of 7 to a maximum 10. However, no species-specific bands were found among Japanese, Chinese and European species, indicating that there is no special differentiation of peroxidase isozyme among *Castanea* species. This is true for alkaline phosphatase.

Differences in density of common bands, on the other hand, were found among the species. In order to a certain whether the density comparisons may be useful as a means of identification, further expeiments must be conducted on more varietes of Chinese and European species.

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アイソザイムによるクリ品種の鑑別

澤野　稔・一井隆夫・中西テツ・小寺善幸

要　　約

アイソザイム法がクリ品種の鑑別に有用であるかどうかを検討するために，ゲル等電点焦点法により，パーオキシダーゼ，アルカリ性フォスファターゼ及びエステラーゼ・アイソザイムを分析した。材料は日本グリ，中国グリ及びヨーロッパグリの3種16品種の枝條先端部の樹皮を用いた。

エステラーゼでは明瞭なバンドが得られなかったが，パーオキシダーゼ及びアルカリ性フォスファターゼでは，共に合計10本のアイソザイムバンドが識別された。比較したクリ3種の間に，種特有のバンドは確認できなかったが，パーオキシダーゼ・アイソザイムにおいて，バンド活性に差異が認められた。