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Preliminary Study on the Grouping of Taro [*Colocasia esculenta* (L.) Schott] by Isoelectricfocusing

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ABSTRACT

Isoelectricfocusing (IEF) of the prolamin and albumin fractions from nine taro cultivars from American Samoa and Hawaii was conducted. Based on the distribution of the major prolamin and albumin bands and the uniqueness of some of these bands, the nine cultivars were categorized into three groups. Group I consisted of Niu'e, Manu'a, and Lehua cultivars, Group II, Matagi Fanua, Pula Sama Sama, Samoa, Apii and an unknown (yellow) cultivar, and Group III, Palagi cultivar.

INTRODUCTION

Taro [*Colocasia esculenta* (L.) Schott] is one of the most widespread root crops in the tropics. It is high in carbohydrates and low in proteins and fat. It is one of the major diet components of Samoans and other Pacific islanders. The National Academy of Sciences in 1975 considered taro as one of the underexploited tropical plants with promising economic value (Anon., 1975). Grouping of taro corms based on the traditional taxonomical methods is still confusing (Whitney *et al.*, 1939; Merrick & Togiva, 1976; Areta, 1987; Coats *et al.*, 1988). No systematic studies on the chromosome numbers on these cultivars have been conducted. Based on the isoelectric points (pIs) of different proteins (Wilson, 1984; Guo *et al.*, 1985; Sastry *et al.*, 1986) isoelectricfocusing (IEF) has been applied in the classification of rice, sorghum grain and zein in corn in recent years. The purpose of this report was to attempt the grouping of nine taro cultivars by IEF.

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MATERIALS AND METHODS

Materials

Taro corms of Manu'a, Niu'e, Matagi Fanua, Palagi, Pula Sama Sama and Samoa cultivars were obtained from American Samoa. Corms of Lehua, Apii and an unknown (yellow) cultivar were obtained locally in Hawaii.

Protein extraction

Taro corm samples were hand-peeled, cut into 5 mm thick slices, freeze-dried at 60°F shelf-temperature and powdered before extraction. Extraction of prolamins and albumins from various cultivars of taro corms was based on the procedure of Padhye & Salunke (1979) with minor modifications. Because of the low protein content in these samples, 35 g samples were used. The concentration of protein samples was conducted using the Bio-gel P-6DG (Bio-rad Laboratories, Inc., Richmond, CA) and the Minicon-15B concentrator (Amicon Corp., Denver, MA). Figure 1 shows the different steps in the extraction of taro prolamins and albumins.

Isoelectricfocusing (IEF)

Thin-layer gels (0·2 mm × 100 mm × 125 mm) of polyacrylamide-containing ampholytes of pH 3-10 were prepared according to the Bio-rad instructions. Gels and samples were used immediately after preparation, and the samples (about 200 μg protein each, according to the Bio-rad Protein Assay) were applied on to the gel, about 1·8 cm from the cathode. A standard protein mixture (pI values 4·2 to 10·2, from Bio-rad Lab.) was also applied to the gel as reference. Isoelectricfocusing was performed in a Bio-rad horizontal electrophoresis cell at 0°C. The electrolytes were 1M sodium hydroxide at the cathode and 1M phosphoric acid at the anode. The run was carried out at a constant power of 4 W and voltage limited to a maximum of 1500 V for 60 min.

Staining and destaining of gels

Gels were immersed in fixing reagent (4% sulfosalicylic acid, 12·5% trichloroacetic acid, and 30% methanol) for 35 min and stained with coomassie brilliant blue R 250 solution (72% isopropanol, 10% acetic acid, 0·04% coomassie brilliant blue R 250, and 0·5% copper sulphate) for 2 h. The first destaining stage was accomplished by soaking the gels in a solution of 12% isopropanol, 7% acetic acid, and 0·5% copper sulphate with at least

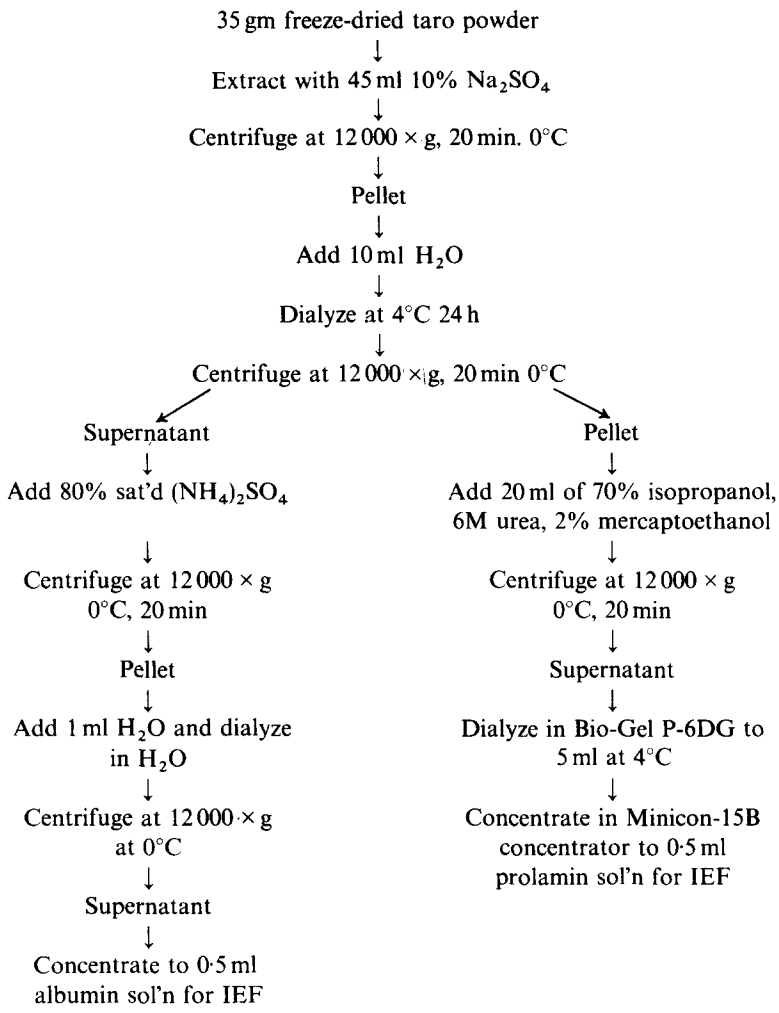

Fig. 1. Schematic procedure on the extraction of taro albumins and taro prolamins.

three changes of solution for 5 min each, and the second destaining stage was in several changes of 12% isopropanol and 7% acetic acid until the background became clear.

RESULTS AND DISCUSSION

Extraction and concentration of prolamins and albumins

Because taro corms contain only small amounts of protein and mainly starch and gums, preparation of prolamins and albumins is more difficult

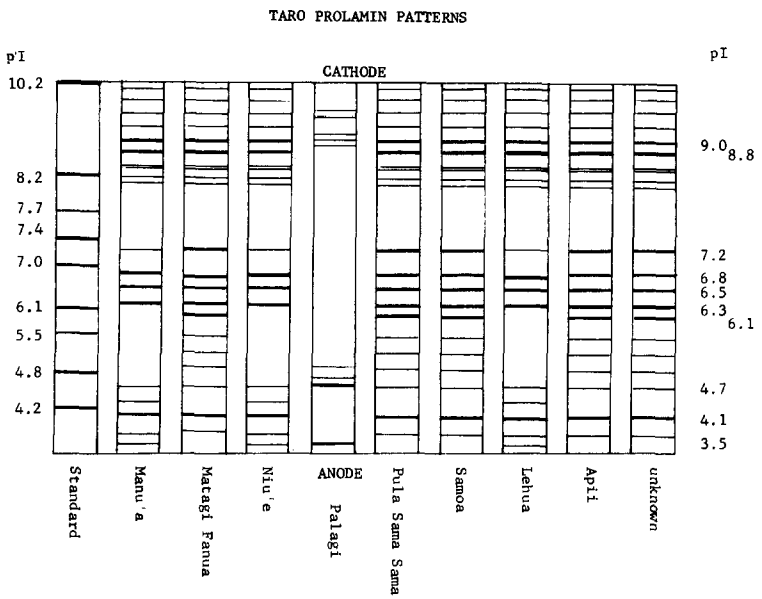

Fig. 2. Representative tracings of taro prolamine IEF patterns. Heavy lines indicate major protein bands. Lighter lines indicate presence of traces of protein bands.

than the extraction of other plant materials. A large sample (35 g) has to be used. Concentration of prolamin was accomplished directly by burying dialysis bags of prolamin extract solution in Bio-gel P-6DG at 4°C instead of freeze-drying (Padhye & Salunke, 1979). This procedure prevented denaturation of the extracted proteins during freeze-drying. The quantity of albumin solution was less than 5 ml after centrifugation so it could be concentrated with an Aminco Minicon-15 B concentrator without difficulty.

IEF of taro prolamins

Among the nine cultivars tested, Palagi prolamins showed a few unclear bands above pI values of 8·0, the other eight varieties have distinct bands at pI values of 8·8 and 9·0 (Fig. 2). Manu'a, Niu'e and Lehua cultivars had only three distinct bands at pI values of 6·3, 6·5 and 6·8. Matagi Fanua, Pula Sama Sama, Samoa, Apii, and an unknown yellow cultivar showed the same main prolamin bands at pI values of 4·1, 6·1, 6·3, 6·5, 6·8 and 7·2. However, Palagi prolamin did not possess these bands; instead, it showed two unique prolamin bands at pI values of 3·5 and 4·7.

IEF of taro albumins

Taro albumin patterns on IEF gels were less complex than taro prolamins

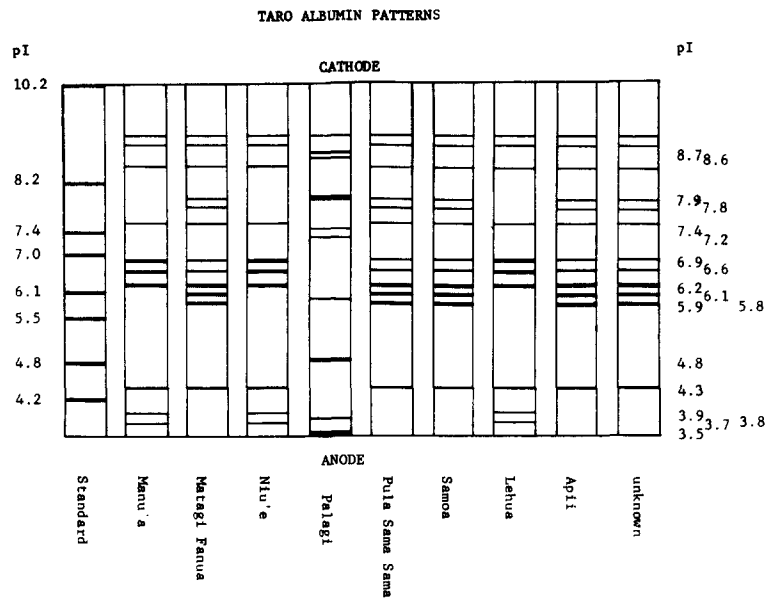

Fig. 3. Representative tracings of taro albumin IEF patterns. Heavy lines indicate major protein bands. Lighter lines indicate presence of traces of protein bands.

(Fig. 3). Based on the difference of major albumin bands in the nine taro cultivars tested, Manu'a, Niu'e and Lehua cultivars possessed unique bands at pI values of 3·7 and 3·9, whereas Matagi Fauna, Pula Sama, Samoa, Apii and the unknown yellow cultivars showed unique bands at pI values of 5·8, 6·1 and 7·8. All cultivars tested except Palagi showed common bands at 4·3, 6·2, 8·5, 9·0 and 9·1. Palagi cultivar possessed unique bands at pI values of 3·5, 3·8, 4·8, 5·9, 7·2, 7·4, 8·6 and 8·7.

Grouping of cultivars

Since Manu'a, Niu'e and Lehua cultivars showed similar prolamin and albumin IEF patterns, these cultivars together formed Group I. Based on the same principle, Matagi Fanua, Pula Sama Sama, Samoa, Apii, and an unknown yellow cultivar together formed Group II. Palagi by itself formed Group III. Since the Palagi cultivar has completely different IEF prolamin and albumin patterns, the classification of this cultivar as taro is questionable. This cultivar is not listed in the 'Key to the Taro Cultivars of American Samoa' and 'Taro Cultivars in American Samoa' (Merrick & Togiva, 1976; Areta, 1987), and the word 'palagi' in the Samoan language means 'foreign'. It is possible that this Palagi cultivar should belong to another aroid which is closely related to 'Colocasia'. Classification of this cultivar warrants further investigation.

From differences in the major IEF prolamin and albumin patterns obtained in this study, it is clear that corms of various taro cultivars can be grouped separately based on their IEF properties. Cultivars which do not contain the common IEF prolamin and albumin bands in their corms are probably not true taro and should be re-classified. IEF analysis of cultivars available should be conducted in conjunction with traditional taxonomy and chromosomal counting in order to re-classify the cultivars of this under-utilized crop of potential economic value.

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