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USE OF ISOENZYMES FOR THE DETERMINATION OF GENETIC VARIABILITY FROM TISSUE CULTURE AND BETWEEN CULTIVARS OF TARO, COLOCASIA ESCULENTA

(Utilisation des isoenzymes pour la détermination de la variabilité génétique à partir de culture de tissus et de cultivars de taro, Colocasia esculenta)

Robert WARNE and Michael S. STRAUSS

Department of Biology, Northeastern University
360 Huntington Avenue, Boston, MA 02115 U.S.A.

SUMMARY

A frequent problem with plants produced from tissue culture is the presence of genetic alterations. This is a particular problem where such procedures are undertaken for the purpose of clonal propagation of material. While some of these changes are morphologically visible, it is possible for minor changes in the primary sequence of a protein to leave the whole plant largely unaffected. These changes could, in many cases, be detected by alterations of protein migration in an electrophoretic system. Starch gel electrophoretic techniques have been adapted to monitor the changes in several isoenzyme groups in plants from tissue cultured taro, *Colocasia esculenta*. These techniques are also useful in the development of isoenzyme profiles for specific cultivars of taro. This data is important in producing characterizations of taro gemplasm collections ; particulary where similar cultivars are found in two or more collections. The reliability of isoenzyme analysis for tissue culture and cultivar distinction is discussed.

RESUME

Un problème fréquent chez les plantes issues de cultures de tissus est la présence d'altérations génétiques, en particulier lorsqu'il s'agit de propagation clonale du matériel. Si certains de ces changements sont morphologiquement visibles, des changements mineurs dans la séquence primaire des protéines peuvent dans beaucoup de cas, être détectés par l'altération de la migration d'une protéine en électrophorèse. Des techniques d'électrophorèse sur gel d'amidon ont été adaptées au contrôle de la variation de plusieurs groupes d'isoenzymes chez des plantes provenant de culture de tissus du taro, Colocasia esculenta. Ces techniques rendent également services dans l'établissement

des profils enzymatiques de cultivars particuliers du taro. Cette donnée est d'importance pour la caractérisation des cultivars appartenant à deux ou plusieurs collections. La fiabilité de différents systèmes d'isoenzymes est discutée.

INTRODUCTION

Taro, *Colocasia esculenta*, is a major subsistence crop of the Pacific and an important secondary crop of Asia, West Africa, and many parts of the humid tropics. As is common to clonally propagated crops, taro is in need of research to develop techniques which will allow establishment of genebanks of important material. Also like many clonal crops, the most practical method for storage of taro is by tissue culture. Techniques have been published which allow for tissue culture of many taro cultivars (ARDITTI and STRAUSS, 1979). However, none of these has addressed the problem of possible genetic changes occurring during the culture process. Further, no mechanism exists for monitoring the presence of such possible change, apart from grow-out of cultured materials.

Soon after tissue culture was applied to clonal propagation of plants it became obvious that not all plants resulting from a culture were phenotypically identical to the original. Such variation is not unusual to vegetatively propagated crops and can result from somatic mutation (BEAUCHESNE, 1982 ; EVANS, SHARP and MEDINA-FILHO, 1984 ; HUSSEY, 1978 ; SCOWCROFT and LARKIN, 1982 ; SKIRVIN, 1978). These mutant plants are usually rogued out or, rarely, saved for an improved character they display. Other mutations may bring about morphological or physiological changes not readily obvious. Thus, it is suggested that such crops consist of a mixture of slightly differing clones (HUSSEY, 1978). Long term culture and repeated subculture, particularly of callus, increases the likelihood of genetic changes occuring. Polyploid or aneuploid lines often result from callus cultures (CHALEFF, 1983 ; HUSSEY, 1978, 1980 ; MURASHIGE, 1977 ; SKIRVIN, 1978). Consequently, it is necessary that any method for propagating plant material be designed to minimize induced genetic alterations. Since minor biochemical changes may have little effect on growth characteristics or yield of the crop, general phenotypic variation should be of first consideration. However, a mechanism for characterizing of even subtle changes will allow for detection of genetic changes at an early stage.

Isoenzyme analysis has been applied to a number of crop species (TANKSLEY and ORTON, 1983). A large amount of the variation seen in these isoenzymes has been attributed to genetic change (SCANDALIOS and SORENSON, 1977). Often the biological properties of the molecule are unaltered by small primary sequence changes. Consequently, these changes may be undetected at the phenotypic level. This study was initiated to investigate the use of isoenzymes to monitor genetic stability of <u>in vitro</u> maintained taro cultivars. It is part of a larger study to develop techniques of tissue

culture and cryogenic storage for taro. Detailed reports on this and other aspects of this study will be published elsewhere.

Finally, isoenzymes can be useful in the distinguishing of similar cultivars (BROWN and CLEGG, 1983 ; TANKSLEY and ORTON, 1983 ; WEEDEN, 1983). Presently taro cultivars can be characterized using standardized descriptor lists. However, collections frequently contain numerous accessions which, though they appear similar, have differing origins. Further, seemingly identical cultivars have different names in different collections (STRAUSS, unpublished). Isoenzyme analysis was investigated for use as a tool to distinguish between similar cultivars of taro.

MATERIALS AND METHODS

Plant material was obtained from the Fiji (UNDP/FAO-SPC Root Crops Protection Projece), Hawaii (University of Hawaii, Kauai Branch Station). All plants were maintained under greenhouse conditions at the Burlington Botanical Research Facility, Northeastern University. Selected plants were also cultured in environmental chambers under 16h. photoperiods at 25 to 30 C and 70 per cent relative humidity.

Protein extracts were prepared by freezing 250-300 mg of leaf material liquid nitrogen, and grinding with a mortar and pestle. Upon evaporation of the liquid nitrogen, 3.0 ml Tris-HCl grinding buffer-PVP solution (GOTTLIEB, 1981) was centrifuged for ten minutes at 10,000 Xg and the resultant supernatant used for electrophoresis.

Isoenzyme analysis was by starch gel electrophoresis and enzyme activity staining. The details of techniques for taro will be available in a forthcoming manual. However, general reviews upon which our techniques are based, are available (BREWER, 1970 ; CONCKLE et al., 1982 ; FERREL and BERGMAN, 1976 ; GORDON, 1983 ; SHIELDS, ORTON and STUBER, 1983 ; SOLTIS et al., 1983 ; VALLEJOS, 1983).

RESULTS AND DISCUSSION

The first task of this study was to determine which classes of isoenzymes would be useful in assessing tissue culture induced genetic change in taro. Many isoenzyme systems are found to be conservative and show no variation between different cultivars or different age/size plants from the same cultivar. The transferase group as a whole (aspartate aminotransferase, phosphoglucoisomerase, and phosphoglucomutase) fit into this category. These isoenzyme profiles do not vary between different cultivars or different ages/sizes of the same cultivar (transferases are used as conser-

Table 1. Variation of esterase isoenzyme bands in plants of *Colocasia esculenta* cv. Lehua maoli of different average leaf sizes

Ave. Leaf width, cm[a]	Bands, No.	Rf values
1.0	2	0.13 - 0.25
5.0	3	0.06 - 0.13 - 0.15
10.0	4	0.14 -0.27 - 0.72 - 0.83

a-increasing average leaf size is indicative of relative age from culture.

Table 2. Variation of peroxidase isoenzyme bands and Rf values in *Colocasia esculenta* cv Lehua maoli plants assayed at different times of the year

Assay date	Bands, No.	Rf values
3 February, 1984	1	0.13
1 May, 1984	2	0.13 - 0.25
25 May, 1984	2	0.13 - 0.25
19 June, 1984	3	0.25 - 0.40 - 0.73
22 August, 1984	5	0.25-028-0.40-0.73-0.81
18 October, 1984	3	0.28 - 0.40 - 0.73
18 January, 1985	2	0.20 - 0.38

vative markers to eliminate the possibility of the results being a product of the electrophoretic technique). This finding leaves the hydrolases and oxidoreductases as potential isoenzyme systems for assessing genetic change in taro. In fact, these two groups of isoenzymes show significant variation between different cultivars. However, they also show variation due to age/size of plants (Table 1) and variation due to seasonal change (Table 2).

To illustrate age/size variation esterase isoenzymes of different sizes of the cultivar "lehua maoli" were examined (Table 1). Young/Small plantlets will typically produce three to five bands in an esterase profile. An adult/large plant of the same cultivar may produce as many as eight bands in the same profile. Therefore, it becomes imperative to establish a standardized age/size or set of ages/sizes when conducting these essays.

Seasonal variation, can best be demontrasted using the peroxidase system (Table 2). The same adult plant will show a variation from two to six bands in a peroxidase profile in assays taken over the course of a year. This so called "seasonal variation" has been observed for a number of the other oxidoreductase systems (alcohol dehydrogenase, catalase, malate dehydrogenase, shikimate dehydrogenase). It should be emphasized that these two forms of variation are not limited to change in the number of bands present in a profile. Variation in the distance bands migrate (Rf values) also becomes an important factor.

With the seasonal and age/size factors in mind, the isoenzyme systems that have been found to serve as potential genetic markers are : esterase, peroxidase, malate dehydrogenase, catalase, peptidase, aminopeptidase.

One cultivar, "Lehua maoli", was been taken out of tissue culture at various and grown in soil. Isoenzyme profiles indicate that the tissue culture technique utilized did not affect the isoenzyme profiles of the cultivar (Table 3). We suggest that this is an indicator of genetic stability of this cultivar under our tissue culture protocol. As a further test, plants from this culture (which has been maintained by subculture for over three years) have been planted in the field at the University of Hawaii, Kauai Branch Station, along with field grown material. As of this writing no visible differences were noted betwenn tissue cultured and field grown plants (DE LA PENA, personal Communication).

We conclude that selected isoenzyme systems can be used to monitor the genetic stability of tissue cultured material. However, it should be noted that such techniques are not capable of detecting all possible genetic change, but rather only change with confers altered electrophoretic mobility on the isoenzymes assayed. Thus, while possibly useful in detecting significant changes, the technique is only useful when combined with other tests. This, as well

Table 3. Isoenzyme bands and Rf values for *Colocasia esculenta* cv. Lehua maoli plants taken from tissue cultures at various dates. Plants of similar size and age were used for each system.

Enzyme System Date	Band N°.	Rf values
CATALASE		
15 nov., 1984	1	0.25
19 Feb., 1984	1	0.25
26 Sep., 1984	1	0.25
19 Feb., 1984	1	0,25
ESTERASE		
15 Nov., 1984	4	0.14 - 0.27 - 0.72 - 0.83
19 Feb., 1984	4	0.14 - 0.27 - 0.72 - 0.83
26 Sep., 1984	4	0.14 - 0.27 - 0.72 - 0.83
19 Feb., 1984	4	0.14 - 0.27 - 0.72 - 0.83
MALATE DEHYDROGENASE		
15 Nov., 1984	2	0.13 - 0.56
19 Feb., 1984	2	0.13 - 0.56
26 Sep., 1984	2	0.13 - 0.56
19 Feb., 1984	2	0.13 - 0.56
PEPTIDASE		
15 Nov., 1984	2	0.66 - 0.77
19 Feb., 1984	2	0.66 - 0.77
26 Sep., 1984	2	0.66 - 0.77
19 Feb., 1984	2	0.66 - 0.77
LEUCINE AMINOPEPTIDASE		
15 Nov., 1984	2	0.43 - 0.55
19 Feb., 1984	2	0.43 - 0.55
26 Sep., 1984	2	0.43 - 0.55
19 Feb., 1985	2	0.43 - 0.55

Table 4. Variation in isoenzymes of different cultivars of *Colocasia esculenta*

Cultivar (source[a])	Catalase	Esterase	Malate dehydrogenase	Peptidase[a]
Lehua maoli (KBS)	.25	.14,.27,.72,.83	.13,.56	.66,.77
Tsurunoko (KBS)	.28	.13,.19,.31,.40	.15,.44,.50	.75,.88,.94
Akado (KBS)	.25	.10,.15,.31,.41,.51	.14,.33,.49	.69,.80,.89
Kakakura-ula (KBS)	.33	.13,.19,.35,.45	.11,.35,.55	.65,.74,.83
Lauloa Palakea-Keokeo (KBS)	.31	.06,.19,.31,.38	.14,.36,.39	.69,.94
Papapueo (KBS)	.33	.20,.34,.43,.50,.59	.33,.43,.56	.75,.81,.90
Keone (KBS)	.44	.18,.25,.40,.56	.21,.41,.60	.76,.86,.95
Vavai Ding (Fiji)	.38	.06,.15,.18,.28,.38	.21,.46,.66	.81,.88,.94
Vavai Loa (Fiji)	.36	.06,.15,.28,.38	.13,.44,.61	.71,.85,.91
Toakula (Fiji)	.34	.06,.15,.28	.13,.38,.56	.85,.88,.95
Tausala ni mumu (Fiji)	.21	.09,.25,.39,.53	.14,.39,.51	.74,.86
Vutikota (Fiji)	.18	.14,.30,.43,.55	.13,.34,.50	.69,.78.,90
Mumu (Fiji)	.29	.06,.19,.28,.38	.19,.44,.63	.75,.88,.98

[a]-KBS, University of Hawaii, Kauai Branch Station.
Fiji, UNDP/FAO-SPC Root Crops Development in the Pacific Programme

as other studies, suggest that such data, while useful, should be cautiously interpreted (LASSNER and ORTON, 1983 ; ORTON, 1982).

To demonstrate the a detection of genetic variability among cultivars, five isoenzyme systems were examined for several cultivars (Table 4). The data clearly establishes the potential of isoenzyme profiles in producing characterizations of taro gemplasm collections ; particularly where similar cultivars are found in two or more collections. In essence, this electrophoretic system is able to detect the differences between cultivars on a biochemical scale and provide the researcher with a reference suitable for a wide range of studies.

In conclusion, the starch gel electrophoretic technique developed in this study to monitor the changes in isoenzyme groups in taro is a quick and easy way to establish many of the genetic differences in related cultivars, and in plants from tissue culture. However, isoenzymes while a powerful indicator, should not be the sole determinant of genetic stability of *in vitro* produced plants. The information produced by isoenzyme techniques must be combined with additional data, such as morphologic and agronomic descriptors. Care must be taken to utilize only those isoenzyme systems that have been confirmed as reliable for genetic stability studies.

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