Indian J. Genet., **59**(1): 47-52 (1999)

ISOZYME CHANGES DURING MORPHOGENESIS IN THE *IN VITRO* CULTURES OF *PANICUM MAXIMUM* J.

S. ALARMELU*, THEYMOLI BALASUBRAMANIAM AND C. PADMANABHAN

School of Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003

(Received: September 11, 1996; accepted: November 13, 1998)

ABSTRACT

Callus cultures derived from mature embryos and inflorescence explants of guinea grass [cv. GG2], *Panicum maximum J.* at different stages of development were used to study the patterns of iso esterases and iso acidphosphatse, Isozyme patterns were found to vary with all developmental stages studied. Twelve isoesterases were detected in the embryogenic calli. With the germination of embryoids into plantlets, only two of these isozymes were retained. Acid phosphatase was found to be organ and tissue specific. The novel isozymes that appeared in the embryogenic and organogenic calli can be used as marker to distinguish between embryogenic and non-embryogenic calli.

Key words: Guinea grass, acid phosphatase, esterase, isozyme marker, embryogenic calli.

Guinea grass is a widely used livestock herbage. While there is much genetic variation in this crop, its apomictic mode of reproduction makes cultivar improvement unfeasible through conventional breeding. Somaclone culture technique could potentially be used to induce variation in apomictic biotypes and variants could be identified by biochemical markers thus permitting selection at molecular and cellular levels [1, 2].

Isozymes are valuable tools in identifying genetic and epigenetic variation. [2]. Isozyme patterns have been used to detect changes during somaclonal variation as they play a vital role in development and differentiation [3, 4]. Identification and characterization of cultivars by the isozymes of esterase and acid phosphatase in tobacco callus cultures has been reported [5]. Novel acid phosphatases were observed during cytodifferentiation in callus cultures of *Vigna* [6]. The esterase isozymes were found to vary between embryogenic and shoot forming cultures of maize [7]. Distinctly different esterases and acid phosphatase isozymes were observed in both wheat and barley calli during differentiation [8]. Isozymes as markers in guinea grass callus differentiation are reported for the first time.

*Present address: Division of Crop Improvement, Sugarcane Breeding Institute, Coimbatore 641 007

MATERIALS AND METHODS

Plant material

Panicum maximum J. cv GG2 was obtained from the Forage Crop Unit, Coimbatore. Mature embryos (seed) and unemerged inflorescence were used as explants. Callus cultures were initiated in modified Murashige Skoog's [9] (MS) medium supplemented with 2, 4-dichloro phenoxyacetic acid 2.5 mg l^{-1} for the inflorescence and 5.0 mgl^{-1} for embryos [10] and kinetin 0.5 mg l^{-1} and yeast extract 1.0 g l^{-1}. For induction of embryogenesis, the white compact callus was transferred to MS medium supplemented with 1.0 mg l^{-1} 2, 4-D and was maintained for 30 days [10]. The seed callus and the embryoids were transferred [11] to regeneration media supplemented with gibberellic acid 1.0 mg/1, L-asparagine 150 mg l^{-1} and yeast extract 1.0 g l^{-1} after 30 days. Observations on shoot regeneration and germination of embryoids were taken within 30-35 days of culture.

Gel Electrophoresis

Isozymes were analysed from seed, seed calli, organogenic calli and young leaf tissues from both *in vitro* plantlets and *ex vitro* plants. One gram of callus tissue was homogenised in 3.0 ml of cold 0.05 m Tris-glycine (pH 8.3) buffer and centrifuged for 20 minutes at 4°C at 10,000 rpm. The protein content of the supernatant was estimated [12]. Enzyme extract equivalent to 70 µg of protein was loaded on the gel and electrophoresis was carried out in 7.5% polyacrylamide gel in a buffer system using Tris-glycine (pH 8.3) [6] at a constant current of 20 mA till the tracking dye reached the anode end of the gel. Acid phosphatase and esterases were stained by standard methods [6, 13].

The R_n values of the bands were calculated by measuring distance traveled by the bands relative to that of the tracking dye.

RESULTS AND DISCUSSION

Esterase

A total of seventeen isoesterases were detected with R_n values ranging from 0.02-0.86 (Table 1). Esterases of undifferentiated explants were markedly different from those in morphogenic calli (Fig 1). The number of isoesterases in the calii stage (lane 3, Fig 1) were more than those in the regeneration stage (lane 1, Fig 1). EST-1, EST-2 and EST-5 were monomorphic and were resolved at R_n of 0.02, 0.03 and 0.19 respectively. EST-3 (R_n 0.13) was found exclusively in the seed calli,

Table 1. R_n values of isozymes: esterase and acid phosphatase

S. NP.	Esterase		Acidphosphatase	
	Isozymes	Relative mobility R_n	Isozymes	Relative mobility R_n
1.	EST-1	0.02	AP-1	0.04
2.	EST-2	0.03	AP-2	0.07
3.	EST-3	0.13	AP-3	0.17
4.	EST-4	0.17	AP-4	0.30
5.	EST-5	0.19	AP-5	0.36
6.	EST-6	0.30		
7.	EST-7	0.35		
8.	EST-8	0.40		
9.	EST-9	0.44		
10.	EST-10	0.45		
11.	EST-11	0.47		
12.	EST-12	0.51		
13.	EST-13	0.53		
14.	EST-14	0.67		
15.	EST-15	0.73		
16.	EST-16	0.84		
17.	EST-17	0.86		

non-embryogenic calli and embryogenic calli. EST-6 and EST-7 was present in the explant inflorescence, seed calli and embryogenic calli. EST-6 and EST-7 can thus serve as reliable markers of embryogenesis. EST-8 (R_n 0.40) was exclusive to non-embryogenic calli. EST-10 (0.45) was detected only in inflorescence derived non-embryogenic and embryogenic calli and seed derived organogenic calli. Non-embryogenic calli and embryo genic calli showed the appearance of EST-11 which was absent in other tissue extracts. The band EST-15 (R_n 0.73) was distinctly present only in embryogenic calli which could be distinguished from organogenic calli, non-embryogenic calli and *in vitro* plantlets. Isozymic pattern showed EST 16 (0.84) only in non-embryo genic calli and EST-17 (0.86) in embryogenic calli. EST-17 was found to show higher activity at R_n 0.86 than other isoesterases present in embryogenic calli. Esterase exhibits broad substrate specifications with diverse physiological functions [14]. The undifferentiated callus from seeds behaved like a unique tissue that expresses lesser isozymes than the differentiated calli. The presence of EST-15 and EST-17 in embryogenic calli and EST-8 and EST-16 in differentiating

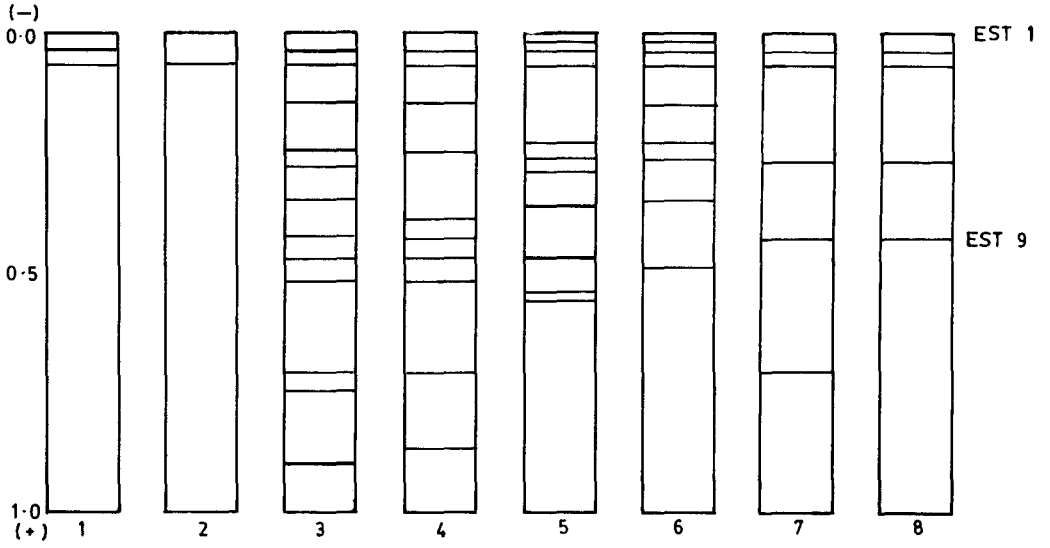

Fig. 1. The electrophoretic patterns of Esterases of undifferentiated explants and morphogenic calli

(non-embryogenic) calli and EST-11 in dedifferentiating and differentiating (embryogenic) calli indicates that this enzyme may be related to differentiation. The absence of these enzymes in differentiated cultures (*in vitro* plantlets) suggest the varied gene involvement in the developed cells that might have suppressed the synthesis of these isozymes [15]. The detection of esterase can thus be used to distinguish embryogenic cells from non-embryogenic cells in guinea grass as suggested in maize cultures by Everett *et al.* [7].

Acid phosphatase

The electrophoretic pattern of acid phosphatase (Fig. 2) revealed a total of five isozymes with R_n values ranging from 0.04-0.36 (Table 1). Two isozymes, AP-1 and AP-2, with R_n values 0.04 and 0.07 of equal intensity were found in all the tissue extracts. AP-3 (R_3 0.17) was detected only in organogenic (shoot forming calli) and non-embryogenic calli. The explant inflorescence was found to have two additional bands AP4 and AP-5 located at R_n values 0.30 and 0.36 respectively. These isozymes were absent in their calli and regeneration stage.

FIG. 2

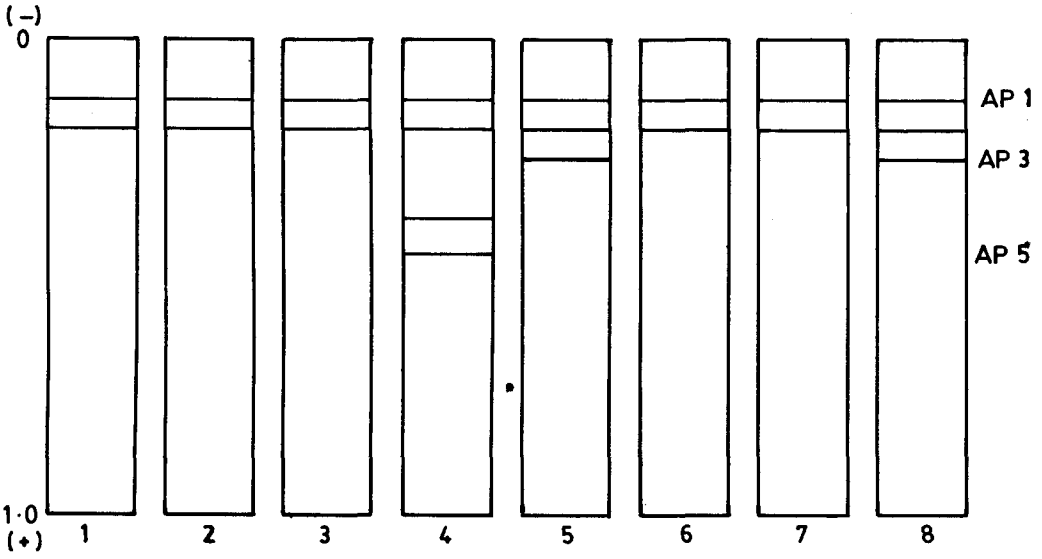

Fig. 2. The electrophoretic patterns of Acid phoophatase in organogenic and non-embryogenic calli

Acid phosphatase is commonly present in the cell and has been detected in association with the cell wall [16]. The appearance of a new acid phosphatase AP-3 on organogenic calli and non- embryogenic calli shows the importance of phosphate metabolism during differentiation. It makes it possible to discriminate between undifferentiating or dedifferentiating calli from differentiating calli. The occurrence of AP-3 in organogenic calli and non-embryogenic calli and AP-4 and AP-5 in inflorescence suggests that the acid phosphatase pattern is organ (inflorescence) and tissue (organogenic and non-embryogenic calli) specific as suggested in many grass tissues [17].

Isozyme variation in cultured tissue suggest that they are differentially activated in development [8]. Isozymes serve as a natural marker system at genetic, physiological and biochemical levels. Acid phosphatase and esterases can be used independently to distinguish the explants and differentiating tissues. A combination of the two

isozymes provides an even more useful marker system for characterizing tissue response at molecular and cellular levels in guinea grass.

REFERENCES

1. B. D. Metzinger, C. M. Taliaferro, B. B. Johnson and E. D. Mitchelli. 1987. *In vitro* regeneration of apomictic blue stem grasses. Plant Cell Tissue Organ Culture., **101**: 31-38.
2. J. Michel. 1975. Isozymes and strategy for their utilization in plant genetics Genetic and Epigenetic control. *In*: L. Lucein. Genetic manipulation with plant material. Plenum Press, New York and London, 368-378.
3. T. K. Orton. 1983. Spontaneous electrophoretic and chromosomal variability in callus cultures and regenerated plants of celery. Theor. Appl. Genet., *67*: 17-24.
4. D. A. Evans, W. A. Sharp and Medina-Felho. 1984. Somaclonal and gametoclonal variation. Amer. J. Bot., **71**: 759-774.
5. A. Bassiri and P. S. Carlson. 1979. Isozyme patterns in tobacco plant parts and their derived calli. Crop Sci., **19**: 909-914.
6. K. K. De and S. Roy. 1984. Role of an acid phosphatase isoenzyme in callus tissue during cyto differentiation. Theor. Appl. Genet., **68**: 285-287.
7. N. P. Everett, M. J. Wach and D. J. Ashworth. 1985. Biochemical markers of embryogenesis in tissue cultures of the maize inbred B 73. Plant Sci., **41**: 133-140.
8. H. S. Chawla. 1988. Isozyme modifications during morphogenesis of callus from barley and wheat. Plant Cell, Tissue Organ Culture., **12**: 299-304.
9. T. Murashige and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant., **15**: 473-497.
10. C. Lu and I. K. Vasil. 1982. Somatic embryogenesis and plant regeneration in tissue cultures of *Panicum maximum J.* Amer. J. Bot., **69**: 77-81.
11. C. Lu and I. K. Vasil. 1981. Somatic embryogenesis and plant regeneration from freely suspended cells and cell groups of *Panicum maximum in vitro*. Ann. Bot., **47**: 543-548.
12. O. H. Lowry, N. J. Rosebrough., A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., **193**: 265-275.
13. G. Nielsen. 1977. Esterase isozyme variants in barley. Hereditas., **87**: 155-162.
14. J. G. Scandalios and J. C. Sorenson. 1977. Isozymes in plant cell culture. *In*: J. Reinert and Y. P. S. Bajaj (Eds)., Applied and fundamental aspects of plant cell, tissue and organ culture, Springer-Verlag, Berlin, New York, 719-730.
15. S. Bandyopadhyay, J. L. Das and V. K. Dubey. 1988. Tissue differentiation and buffer-soluble protein pattern in wheat and triticale. Curr. Sci., **57**: 20-23.
16. P. G. Arnison and W. G. Boll. 1978. The effect of 2,4-D, kinetin on the activity and isozyme pattern of various enzymes in cotyledon cell suspension cultures of bush bean *Phaseolus vulga* cv. Contender Can. J. Bot., **56**: 2185-2195.
17. I. Lorenc-Kubis and B. Morawiecka. 1985. Post-synthetic modifications of proteins change during development. Acid phosphatases from different grass tissues. *In*: Lectins, Walter de Gruyter and Co, Berlin Vol. IV. 259-266.