

# MORPHOLOGICAL AND RAPD VARIATIONS OF REGENERANTS DERIVED FROM CELL SUSPENSION CULTURE OF PANGOLAGRASS

C. S. Chen<sup>1</sup>, S. M. Wang<sup>1</sup> and Y. K. Cheng<sup>1</sup>

<sup>1</sup>Taiwan Livestock Research Institute, Heng-Chun Station, 1, Ranch Road, Ken-Ting, Heng-Chun, Ping-Tung, Taiwan 94606

## ABSTRACT

Pangolagrass cv. A254, a sterile strain, is one of Taiwan's major forage sources but recently has faced serious disease problems. Tissue culture was expected to generate variations in the breeding program of pangolagrass. The purpose of this study is to examine the somaclonal variation of regenerated plants by means of phenotypic characters survey and RAPD analysis. After long-term suspension culture and EMS mutagenesis, many regenerated plants with morphological variations have been found. Among the regenerants, 10 clones with obviously different characteristics were selected for further studies. Nine of them showed significant differences in morphological traits as compared to A254. By using 18 random primers and a total of 126 bands examined in RAPD analysis, a considerable degree of DNA polymorphism could be found between all of the 10 regenerants and A254. Their genetic similarities to A254 ranged from 0.48 to 0.93. The results indicate that long-term suspension culture and EMS mutagenesis did help to produce genetic variations of A254 in a breeding program.

## KEYWORDS

Pangolagrass, cell suspension, mutagenesis, variation, RAPD

## INTRODUCTION

Pangolagrass cv. A254 (*Digitaria decumbens*) is one of the major forage crops in Taiwan, but has faced substantial disease problems in recent years. It is difficult to improve A254 by conventional breeding methods because it is a triploid, is completely sterile and lacks natural variation. Production of genetic variation has played an important role in the breeding programs. A cell suspension system of A254 was established with young inflorescence as explants in our laboratory, and subjected to the mutagen, EMS (ethyl methanesulfonate) (Wang *et al.*, 1996). With this system, we have produced a substantial number of regenerated plants. RAPD's markers are an effective tool to find changes at the DNA level due to the abundance of markers. The purpose of this study is to examine the genetic variation produced by this system through a survey of phenotypic characters and RAPD analysis.

## METHODS

**Suspension culture and plant regeneration:** Explants taken from young inflorescences of A254 were cultured in modified MS medium (containing 2 mg/l 2, 4-D, 1 mg/l NAA, 1 mg/l kinetin) to induce compact calli. Then, the compact calli were transferred to modified liquid MS medium (containing 2 mg/l 2, 4-D, 5% coconut milk, 0.5% casein hydrolysate, 0.5% NaCl) for 13 months of suspension culture. Mutagenesis was conducted on the third day after subculture by treating suspension cells with 0.6% EMS for 4 hours and then transferring them to a fresh medium for compact callus induction and differentiation. From a batch of 202 regenerants, ten clones (TC1-TC10) with visual differences were selected and propagated for further studies.

**Measurements of phenotypic characters:** With exception of TC2, stolons of the other clones and A254 were cut and screened for a uniform mass then transplanted into 5 pots each. TC2 was too weak in vigor and no stolon could be found. After growth for 60 days, they were cut 5 cm above the ground, then cut again after an additional 40 days. After another 40 days, the number of stolons, number of

tillers, and plant height within each of the pots was measured. Three uniform tillers were selected from each pot for measurements of stem diameter, leaf number per tiller, leaf length and leaf width. Leaf length and leaf width were measured at the second fully expanded leaf from the top. Stem diameters were recorded at the third internode from the top.

**RAPD analysis:** Genomic DNA was extracted from young leaf tissues by using the procedure described in Chen *et al.* (1995). The PCR reaction contained 5-10 ng template DNA, 0.33-0.4 µgM 10-mer primer (Operon Technologies, dependent on the primer used), 0.4U Taq DNA polymerase (Boehringer Mannheim), 200 mM of each dNTP (Boehringer Mannheim), and buffer (50 mM Tris, 2 mM MgCl<sub>2</sub>, 20 mM KCl, 500 µg/ml BSA, pH 8.3) in 10 µl volume. The thermocycler was programmed for 35 cycles of 94 °C (20 sec), 34 °C (1 min), 72 °C (30 sec). After the last cycle, the samples were kept at 72 °C for an additional 4 minutes then cooled to 4 °C. Amplified fragments were electrophoresed on 1.4% agarose gel in 0.5X TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 2.5 mM EDTA, pH 8.3), stained with ethidium bromide and photographed under UV light. The presence or absence of specified fragments between each regenerant and A254 were scored and similarities were defined as follows:

$Sia = Cia / Tia$

Sia = the genetic similarity between regenerant i and A254.

Cia = the number of common fragments.

Tia = the total number of fragments scored on regenerant i and A254.

## RESULTS

The results of phenotypic examination showed that 9 clones had significant differences in traits as compared to A254 by t-test (Table 1). Their morphology could be roughly catalogued into three types. Type one, such as TC2, TC4, and TC8, had erect and thin leaves, and fewer stolons. TC2 was the clone with the weakest vigor. Type two, such as TC7 and TC9, were more creeping as compared to A254 with fewer erect stems and greater leaf width and stem diameter. Type three were similar to A254, but had significant differences in traits, with the exception of TC6.

Using RAPD analysis, the differences in RAPD fingerprints among clones could be easily detected (Fig. 1). Due to the low degree of similarity of TC2 to A254, only one primer out of the 18 primers used did not show polymorphisms among the 11 clones. All 10 regenerants, including TC6, had variations in banding patterns as compared to A254. By calculating genetic similarities from 126 bands, the genetic similarity of TC2 to A254 was only 0.48, while the other clones showed similarities ranging from 0.83 to 0.93 in comparison to A254 (Table 1). The results indicate that plenty of mutations at the DNA level existed in the regenerated clones.

## DISCUSSION

Somaclonal variations had been found in many species. But some of them were hard to detect (Royle et al., 1994; Hawbaker *et al.*, 1993). In pangolagrass, plants regenerated from somatic embryogenesis of cv. A24 were similar to their source plant and no obvious morphological difference could be found (Cheng and Lo, 1987; Cheng, 1993). However, in this study, about one third of the 202 regenerants had different appearances and the ten clones tested

showed changes at the DNA level.

Long-term culture might be an important factor for the production of somaclonal variation. A prolonged callus phase for plants may lead to changes in ploidy, single gene mutations, changes in DNA sequence copy number, DNA amplification, or chromosome breakage (Compton and Veilleux, 1991). The decrease of regeneration capacity in long-term culture is one problem that must be overcome. The longest time for regeneration was 15 months in our system. Treatment with EMS was likely another factor resulting in such high rates of variation. The variation of phenotypic characteristics of regenerants with EMS treatment were higher than those of regenerants with no mutagenesis (Wang, unpublished data). Bulk et al., (1990) also found that the mutation frequencies were higher after EMS treatment than those generated through tissue culture. The results of this study showed that both the long-term suspension and EMS mutagenesis were important in production of genetic variations of A254 and there might be some additive interactions between these two factors.

## REFERENCES

Bulk, R. W., H. J. M. Loffler, W. H. Lindhout and M. Koornneef. 1990. Somaclonal variation in tomato: effect of explant source and a comparison with chemical mutagenesis. *Theor. Appl. Genet.* **80**: 817-825.

Chen, C. S., S. M. Wang and Y. K. Cheng. 1995. Rapid method of RAPD analysis in Digitgrass. *Taiwan Livestock Res.* **28**: 235-244.

Cheng, Y. K. and K. T. Lo. 1987. Somatic embryogenesis and plant regeneration from stem segment cultures of pangolagrass. *Taiwan Livestock Res.* **20**: 69-79.

Cheng, Y. K. 1993. Somatic embryogenesis and plant regeneration from callus suspension culture of pangolagrass. *Taiwan Livestock Res.* **26**: 259-269.

Compton, M. E. and R. E. Veilleux. 1991. Variation for genetic recombination among tomato plants regenerated from three tissue culture systems. *Genome* **34**: 810-817.

Hawbaker, M. S., W. R. Fehr, L. M. Mansur, R. C. Shoemaker and R. G. Palmer. 1993. Genetic variation for quantitative traits in soybean lines derived from tissue culture. *Theor. Appl. Genet.* **87**: 49-53.

Roylance, J. T., N. S. Hill and W. A. Parrott. 1994. Detection of somaclonal variation in tissue culture regenerants of tall fescue. *Crop Sci.* **34**: 1369-1372.

Wang, S. M., Y. K. Cheng and C. S. Chen. 1996. Establishment of suspension culture and mutagenesis condition of pangolagrass cv. A254. *Taiwan Livestock Res.* (accepted).

**Table 1**

Means of morphological traits and genetic similarity to A254 of the 10 regenerated clones

| Clone | No. of Stolens | No. of tillers | Leaf no. /tiller | Stem diameter | Plant height | Leaf length | leaf width | Genetic similarity to A254 |
|-------|----------------|----------------|------------------|---------------|--------------|-------------|------------|----------------------------|
| TC1   | 7.2            | 74.5*          | 5.7*             | 0.92          | 29.8         | 16.3        | 0.42       | 0.93                       |
| TC2   | 0*             | 25.4*          | 4.5*             | 0.72*         | 14.5*        | 7.4*        | 0.34*      | 0.48                       |
| TC3   | 6.8            | 86.3           | 5.9*             | 0.92          | 32.2         | 15.8        | 0.41       | 0.93                       |
| TC4   | 4.3*           | 156.5*         | 5.1*             | 0.91          | 29.4         | 12.5*       | 0.35*      | 0.92                       |
| TC5   | 7.5            | 82.3           | 5.3*             | 0.95          | 29.3         | 14.2        | 0.43       | 0.93                       |
| TC6   | 6.9            | 95.7           | 6.4              | 0.92          | 28.5         | 15.6        | 0.42       | 0.95                       |
| TC7   | 10.2*          | 70.3*          | 6.5              | 1.03*         | 31.5         | 20.2*       | 0.47*      | 0.86                       |
| TC8   | 2.1*           | 53.2*          | 5.5*             | 0.86*         | 22.4*        | 8.5*        | 0.34*      | 0.87                       |
| TC9   | 11.3*          | 58.7*          | 6.2              | 1.02*         | 35.4*        | 17.2        | 0.45*      | 0.86                       |
| TC10  | 3.2*           | 115.5          | 6.2              | 0.90          | 31.5         | 12.3*       | 0.40       | 0.86                       |
| A254  | 7.8            | 95.3           | 6.4              | 0.94          | 30.7         | 16.4        | 0.41       |                            |

\* Indicate significantly different to traits of A254 at 5% level by t-test.

**Figure 1**

RAPD fingerprint of regenerants and A254. Amplification was primed with primer (5"GGGTAACGCC3")

