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SOMACLONAL VARIATION IN THE APOMICTIC PASTURE GRASS PASPALUM DILATATUM

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ABSTRACT

The most productive biotypes of *Paspalum dilatatum* are obligate apomicts (2n = 50) and produce uniform progeny. Poor seed set and susceptibility to ergot are two problems in this grass, but apomixis and the lack of useful sexual forms have prevented improvement by conventional breeding methods. Recently, somaclonal variation has emerged as a possible new option for plant improvement and the aim of this study is to asses its potential for the improvement of *P. dilatatum*. We used immature embryos of Grasslands Raki (2n = 50) to initiate embryogenic callus cultures and batches of somaclones were subsequently regenerated at various times during long term culture. In the field, 213 somaclones exhibited extensive phenotypic variation for vigour, growth habit, leaf width, and leaf chlorophyll concentration. Only limited variation in leaf dry matter digestibility was detected and no resistance to ergot was found. As the culture period was lengthened there was an increase in the frequency of extreme dwarfism and inflorescence abnormalities. The seed produced by many somaclones germinated poorly and although this has hampered progeny testing, seedlings from 43 somaclones are under evaluation. Preliminary results indicate that some somaclones have given rise to highly variable progeny.

KEYWORDS

Tissue culture, embryogenic callus, plant uniformity, apomixis.

INTRODUCTION

Agriculturally useful forms of *Paspalum dilatatum* are obligate apomicts with 50 chromosomes and produce uniform offspring genetically identical to the maternal parent (Burson, 1983). The apomict is a valuable summer growing grass but poor seed set and ergot are major problems for commercial seed production. Apomixis can be a useful breeding tool in grasses, provided there is a sexual cross-compatible form available for hybridization with the apomict (Bashaw, 1980). In *P. dilatatum* a relatively low vigour sexual biotype with 40 chromosomes is available, but it is almost completely incompatible with the apomict and the few hybrids produced have been of no value (Bashaw *et al.*, 1983). Similar results have been obtained with interspecific crosses so that hybridisation has not produced useful genetic variation. Radiation breeding has also failed to produce new improved forms.

The recent development of somaclonal variation — enhanced variability in plants derived from tissue culture — (Scowcroft and Larkin, 1982) offers fresh hope for the improvement of 'difficult' species like *P. dilatatum*. In 1981, a project was initiated at Plant Physiology Division, DSIR, to assess somaclonal variation in *P. dilatatum* and its potential for improving seed production in the apomictic New Zealand selection Grasslands Raki. Compared with other paspalum types, Raki is large, erect, and late flowering. An efficient procedure for regenerating plants from embryogenic callus culture has been developed. This paper describes phenotypic variation present amongst the primary regenerants from long term callus culture.

MATERIAL AND METHODS

Inflorescences were collected from a Raki plant growing in a greenhouse on 8 March 1981. Immature embryos (0.5-1.0 mm long) were dissected asceptically and placed with scutellum uppermost on a Murashige and Skoog (MS) medium with 2,4-D at 5.0 mg/1. Callus developing on the scutellum was transferred twice more at approximately monthly intervals onto the same medium. Two types of callus could be distinguished; a coarse white callus which later became necrotic, and a more compact pale yellow callus which began to form globular structures. Only the second type of callus was selected for subculture.

At the fourth transfer, some of the callus was placed on an MS medium with 2,4-D at 2 mg/l and kinetin (K) at 0.2 mg/l for callus multiplication. Some callus was also placed on a range of regeneration media with varying levels of NAA and K to induce somatic embryo development. This procedure was repeated three more times over four months. Best somatic embryo development occurred on an MS medium with NAA at 0.2 mg/l and K at 0.2 mg/l. Somatic embryos arising on the surface of callus can be seen in Fig. 2(a). Sections of the callus with developing embryos were subcultured in batches onto an MS Media without hormones to induce root and leaf growth. In time, seven different batches of plantlets were obtained as detailed in Table 1. All subcultures were carried out in 9 cm plastic petri dishes incubated at 26 °C at a light level of 35 $\mu Em^{-2}s^{-1}$.

For transfer to the greenhouse, individual plants were not separated, to avoid damage to the root systems. After four weeks the shoot clumps were well established and the individual plants could be readily separated and planted into propagating tubes. As this stage tillering was not evident, but it is possible that some embryo derived plants may have multiplied in culture.

Individual plants were later transferred to 15 cm pots and observations of vegetative and floral characters were made outside during the summer of 1981/82. These observations are summarised in Table 1. All plants, with the exception of extreme dwarfs, were planted in the field at Palmerston North in November 1982 on a 75 cm grid. Raki was represented by 18 seedlings within the field trial and was also planted in buffer rows around the trial. Raki, Natsugumo (Japan), DR780 (Australia), and a southern European selection were used as standard lines. Each somaclone was represented by a single ramet, while the four standards were replicated as eight seedlings using an augmented block design. During 1983-85 all plants in the field were evaluated for the following: productivity (plant yield) in summer and in winter-spring, scored on a 1-5 scale; growth habit, scored on a 1 (prostrate) to 4 (very upright) scale; inflorescence number and abnormalities; ergot resistance; and leaf width. Leaf chlorophyll concentration was determined using a portable chlorophyll-sensitive photometer (Hardacre *et al.*, 1984). In addition, leaf dry matter digestibility was determined on leaf laminae from 110 somaclones and 11 Raki plants by an enzymatic method (Roughan and Holland, 1977).

All plants were cut back to a 6 cm stubble in May (autumn) and November (spring) each year.

Open pollinated seed was harvested from selected somaclones in March-April of the three years 1983-85. Variation in seedlings from these harvests is being assessed.

RESULTS

Pot observations

Plants showing abnormal inflorescence development were detected in all batches of somaclones (Table 1). Abnormalities ranged from infertile pollen (pale anthers cf. purple anthers in Raki), through reduced floret formation (short inflorescence branches) and floret abortion (shrivelled flower parts), to the complete absence of inflorescences. Extremely dwarf plants were noted with increasing frequency in the later batches. Some of these plants did not reach 15 cm tall. In general, the proportion of 'normal' plants became less with later batches of regenerants. Within a batch the same abnormalities often occurred many times. This result would be expected if a variation arose in the early stages of callus proliferation leading to mutant sectors and individual mutant cells developed into embryos.

Field trial

Summer growth assessments of 213 somaclones in February 1983 and 1984 showed that where variation occurred it was almost entirely in the direction of reduced vigour. None of the somaclones were significantly more productive than the Raki parent. Similar results were obtained from winter-spring growth assessments made in November 1983. Within initial batches A to D, up to 30% of the plants had growth scores significantly lower ($P = 0.05$) than the Raki mean. Thereafter the percentage of low vigour plants greatly increased and in batch G only one

Table 1. Details of seven batches of somaclones: time in culture, and frequency of extreme dwarfism and floral abnormalities as observed in summer pot culture.

Batch	Culture period (days)		Total somaclones	Number of extreme dwarfs	Number of somaclones with floral abnormalities
	Callus	Regeneration			
A	121	28	13	0	3
B	121	51	22	0	4
C	121	91	55	3 (3)*	7
D	152	42	43	1	17
E	152	61	52	0	31
F	201	98	29	8 (8)*	23
G	260	52	20	19 (10)*	20

* Dwarfs not included in field trial.

plant approached the vigour of Raki.

Although Raki is an erect type, 31 somaclones had an even more erect growth habit. These very erect plants were scattered through batches C to G and most had floral abnormalities. A change to a more prostrate habit was found in 29 plants scattered through batches A to E.

Almost all somaclones had narrower leaves than Raki plants and variation in leaf width was evident in most batches (Fig. 1). Leaf width was moderately correlated with summer productivity ($r = +0.48$). Most somaclones developed yellower leaves than Raki under cool temperatures in winter-spring (Fig. 1). Loss of green colour was especially prevalent in later batches — C to G. Four of the most chlorotic plants in batch C were frost killed in winter 1983. Differences in leaf colour were less obvious during summer. Small but significant differences in leaf dry matter digestibility (DMD) were found between batches. Low-vigour batch G plants had improved DMD (mean = 65%) compared with Raki (mean = 61%), whereas the DMD of batch C plants was significantly lower (mean = 59%).

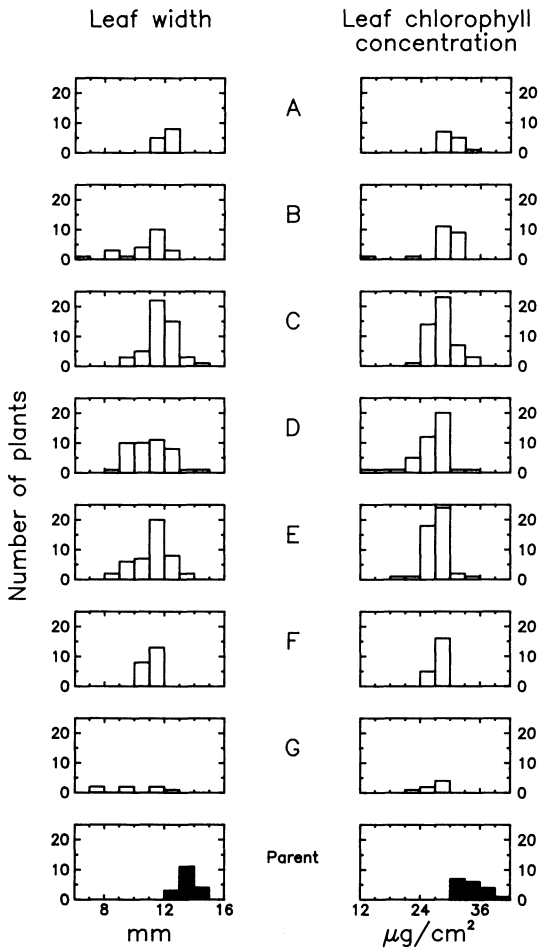

Figure 1. Frequency distributions of leaf width and leaf chlorophyll concentration for somaclones grouped in batches and for Raki parent plants. Leaf width and chlorophyll determinations were made in early December and late September, respectively, on plants growing in the field in 1984.

Plant survival in the field has been high as only 13 of the 213 somaclones have been lost after three years.

Plants which had shown floral abnormalities in pot culture (Table 1) also flowered abnormally in the field in autumn 1983. No inflorescences were produced by 25 somaclones, while 41 showed floret abortion and 28 had short inflorescence branches. Some normal and aberrant plants are shown in Fig. 2 (b)-(f). Inflorescence emergence was often markedly delayed in somaclones with floral abnormalities. Dwarf plants in the field were nonflowering except for two dwarfs of Batch G which produced many perfectly formed miniature flowering tillers. When inflorescences were examined in autumn 1983 there was no evidence of ergot resistance in any somaclone.

Approximately 55% of the somaclones flowered normally, but seed from them did not germinate as well as seed from Raki. In 1983 seed from unbagged inflorescences was collected from 30 somaclones for detailed purity and germination tests. None of this seed germinated and seed from six Raki plants germinated erratically. In 1984 and 1985 inflorescences were bagged soon after anthesis to collect seeds as they matured and shattered. Seed samples (2.5 g, >1500 seeds) from 14 Raki plants gave rise to 50-150 seedlings each. Similar seed samples from four of 60 somaclones gave more than 50 seedlings, 13 gave 11-45 seedlings, 26 gave less than 10 seedlings and the remaining 17 samples failed to germinate. Preliminary observations of these seedlings in the field (1985/86) showed that some somaclones have produced highly variable progeny.

DISCUSSION

In this study, plants regenerated from embryogenic callus cultures of a uniform apomictic cultivar have proved to be highly variable. Other than small differences in vigour, none of the 140 Raki seedlings in and around the trial block showed the aberrant phenotypes described in the somaclones. Only 5% of somaclones conformed to Raki in leaf width and chlorophyll. When vigour and flowering behaviour are also considered, only six out of 213 somaclones were comparable to Raki and none were agronomically superior.

Plants have been regenerated from callus cultures of many cereals and grasses including the apomicts *Poa pratensis* (McDonnell and Conger, 1984) and *Panicum maximum* (Hanna *et al.*, 1984). In some cases, extensive variation has been reported; in other cases regenerants were

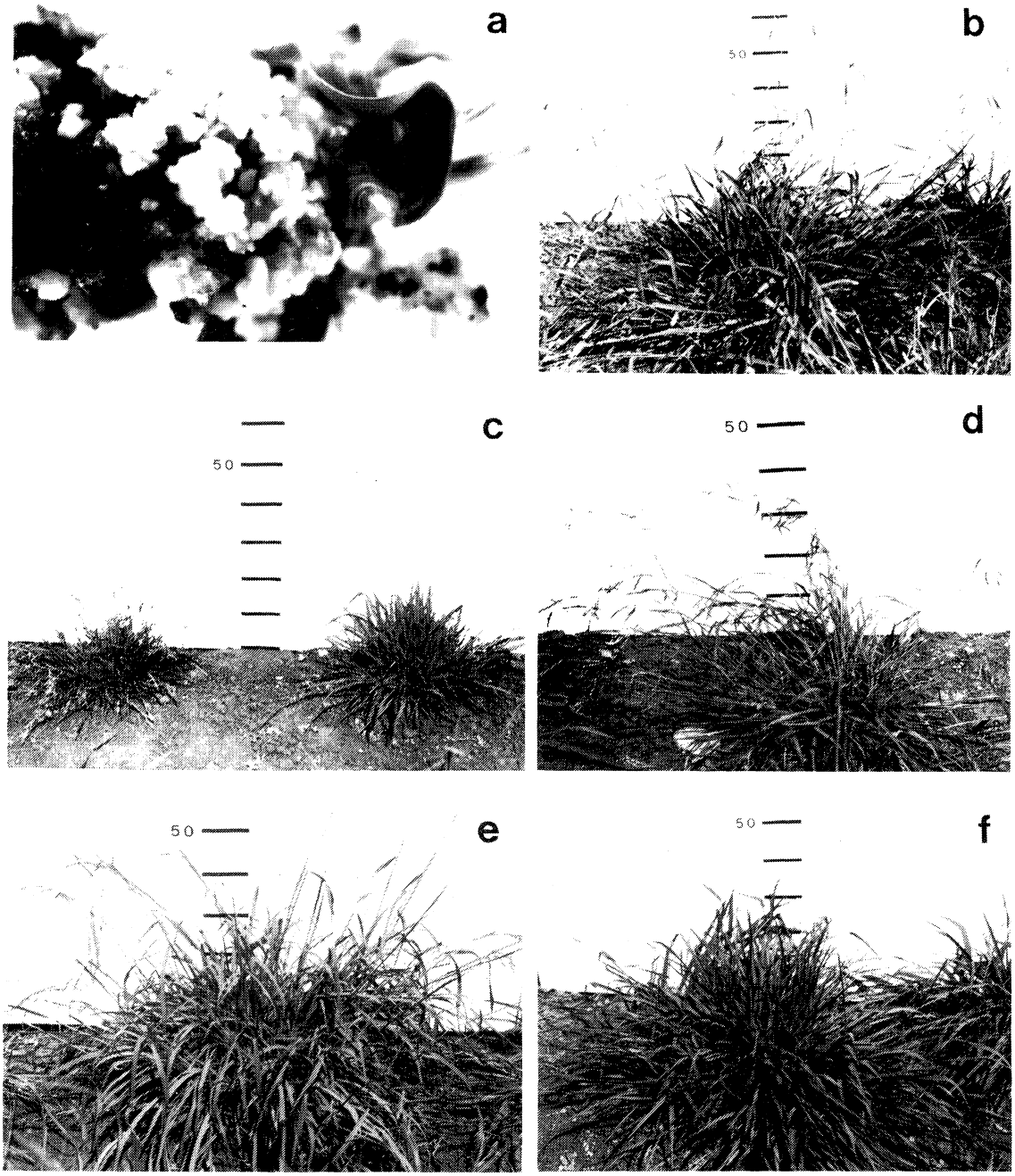

Figure 2. (a) embryos arising on the surface of callus; (b) Raki plant (parent); (c)-(f) representative somaclones. (c) two extreme dwarfs (flowering and nonflowering); (d) and (e) prostrate and very erect growth habits respectively; (f) vigorous nonflowering plant.

uniform (see Hanna *et al.*, 1984 and Swedlund and Vasil, 1985 for references and discussion). The amount of variability obtained may depend on the explants used, the nature of the tissue cultures and the method of regeneration.

In the present study, callus derived from immature embryos was subcultured three times before being transferred to a medium allowing embryo development. The mode of regeneration was by somatic embryogenesis because scutellum, coleoptile, and roots were clearly recognisable on the callus. When embryogenic callus was maintained on callusing medium further embryos arose on the scutellum of somatic embryos.

Variation was found in plants of batch A and increased in later batches. In subsequent work (Cohen and Davies unpub. data) embryos were induced either directly, or after one callus subculture, from immature inflorescence explants and immature embryos. These plants have proved to be more uniform. The variation described in this paper appears to have been induced during the prolonged period of embryogeneic callus culture.

It was anticipated that the somaclones (SC_1 generation) would reproduce uniformly by apomixis. The variation observed in their seedling progeny (SC_2) was, therefore, unexpected. It may have arisen from enhanced cytological instability in somatic tissues of the somaclones, but no morphological variation between tillers in individual plants was noted. Alternatively, variation may have arisen from a breakdown in apomixis leading to sexual reproduction. Seed will be collected from SC_2 variants in autumn 1986 to determine whether the SC_3 progeny are uniform or continue to express variability.

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SYMPOSIUM DISCUSSION

Mr H.K. Hall, Crop Research Division, DSIR

I have been working with apomixis in some *Rubus* species (blackberries) and we find that something like 93% of the seedlings that we grow from a seed population are apomictic and the other 7% are not. Are you finding a similar situation with *Paspalum?*

Davies

We have never looked at the Raki material in that much detail. The type of apomixis in *Paspalum* is such that all progeny should be genetically identical to the parent. We have looked at quite a few hundred *Paspalum* plants without finding any off-types — they are exceedingly uniform. There is a bit of variation in vigour, but that is probably environmental. There are a number of different types of apomixis, so it is possible that the situation in blackberries is slightly different — perhaps it is a facultative apomictic situation.

Mr L. Decourtye, INRA

In your mutation experiment, which part of the plant did you irradiate?

Davies

We took immature embryos, from flower heads. We have also used young flowering stem material, the results were fairly similar.

Dr A.D. Thomson, Botany Division, DSIR

Does *Paspalum* grow in cooler environments in N.Z.?

Davies

It seems to be spreading — Grasslands Division of DSIR have a collection of seed from Central Otago, and I have seen it growing at Lake Waitaki, in a cool temperate situation.

Dr A.D.H. Brown, CSIRO

Is it possible to define a tissue culture regime where you will not generate variation?

Davies

We did have two later series of somaclones, where we regenerated plants after only 1-3 months and they showed very little variation — there were a few plants with yellow variegated leaves, but no obvious sign of any other variations. This particular series — from what is quite a long period of callus culture — were extremely variable. There is no problem regenerating plants that are uniform, particularly if you stick to the very first plants that regenerate.

Mr G.J. Piggott, Ministry of Agriculture & Fisheries

Could the same technique be used with other sub-tropical grasses?

Davies

I see no reason why not. There are quite a number of papers that show regeneration in tissue cultures of several C4 grasses, although there is some controversy about the amount of variation obtained.