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Embryonal shoot tip multiplication in peanut: Clonal fidelity and variation in regenerated plants

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ABSTRACT

A high yielding, early maturing, semi dwarf variety of peanut (*Arachis hypogaea* L.) was multiplied *in vitro* by embryonal shoot tip culture on Murashige and Skoog (MS) medium supplemented with phytohormones. Of the different phytohormones tested, BA (5 mg L^{-1}) in combination with NAA (0.1 mg L^{-1}) produced the best results in terms of average number of shoots produced per culture. The regenerants (R_1) showed a decrease in plant height, leaflet size, number of pegs and seeds, and seed weight. They showed an increase in the number of primary branches in comparison with the seed-derived control plants. No significant change in the number of secondary branches and hundred seed weight (HSW) was observed. In R_2, although a low percentage of variants (< 1%) was observed, pod yield was comparable to that of the seed-derived control. The variants obtained in the R_2 were evaluated in the R_3 and R_4 and the characters were found to be heritable.

Key words: *Arachis hypogaea* L., clonal fidelity, field evaluation, micropropagation, peanut, somaclonal variation.

INTRODUCTION

The technique of micropropagation using shoot tips and axillary buds has been applied to a broad range of crops (Debergh and Zimmerman 1991) including ornamentals (Pillai and Hildebrandt 1968), fruit crops (Norton and Skirvin 1997, Sink and Reynolds 1986), food legumes (Kartha *et al* 1981; Griga and Novak 1990), oil crops (Robinson and Everett 1990) and cereals (Finch *et al* 1992). Probability of genetic changes in plants obtained by direct regeneration from meristematic cells of shoot tip is low (Bhojwani 1980) in comparison with callus and cell suspension culture (Oono 1978), thus sustaining the genetic fidelity of the regenerants.

Peanut (*Arachis hypogaea* L.) is one of the most important crops of the semi-arid tropics not only as a source of high quality cooking oil, but also as a source of proteins for both humans and animals. TAG-24 is a new high yielding, early maturing, semi-dwarf peanut cultivar developed at the Bhabha Atomic Research Centre, Mumbai, India in collaboration with Punjabrao Krishi Vidyapeeth, Akola (S.H.Patil *et al*, unpublished work). Large scale seed multiplication of this new cultivar was essential to meet the demand for seeds and *in vitro* shoot tip multiplication was attempted to study if this technique could yield uniform plants and supplement seed multiplication by the conventional methods. Data on shoot tip multiplication of peanut cultivar TAG-24 and agronomic evaluation of the regenerants in the field are presented in this paper.

Abbreviations: BA - Benzyladenine; MS - Murashige and Skoog medium; NAA - 1-napthalene acetic acid; HSW - Hundred seed weight.

MATERIAL AND METHODS

Peanut cv. TAG-24 was used as the source material. Shoot tips from immature seeds were used since our preliminary studies had shown that they produce more multiple buds in comparison with shoot tips from mature seeds and field-grown plants of peanut (Eapen and George, unpublished data). Immature pods collected 3-4 days prior to harvest were sterilised with 70% (v/v) ethanol for 1 min followed by 0.1% mercuric chloride for 10 min. The pods were washed five times with sterile distilled water and under aseptic conditions they were cut open to remove the embryonal axes. The shoot tips (1 mm) excised from embryonal axes were used for culture. The medium used for culture was that of Murashige and Skoog (1962) supplemented with 1, 2 or 5 mg L^{-1} benzyladenine (BA) in combination with 0.1 mg L^{-1} 1-naphthalene acetic acid (NAA). The shoots which proliferated were separated after 4 weeks and subcultured on fresh medium of the same composition. The

number of shoot buds formed were recorded at the end of the first subculture. Twenty four shoot apices cultured on BA (2 mg L^{-1}) and NAA (0.1 mg L^{-1}) were maintained on the same medium and at the end of the 4th passage, 400 well-developed shoots were excised and cultured on half strength MS medium (macro and micro nutrients) supplemented with NAA (0.2 mg L^{-1}) for rooting. Rooted plants were first transferred to sterilized soil in paper cups and after 2 weeks 277 plants were transplanted to field along with 100 seed raised control plants. At harvest, 26 plants each were selected at random from the R_1 as well as the control, and agronomic characters viz. plant height, number of primary and secondary branches, leaflet size (length and breadth), number of pegs, number of pods, pod weight and hundred seed weight (HSW) were studied. Statistical analysis was carried out using 't' test.

Seeds collected from 233 R_1 plants were progeny tested (R_2) and were scored for chlorophyll and other visible mutations. Pod yield of 3810 R_2 plants was compared with that of 3000 control (seed raised) plants. Only the two mutant plants found in R_2 progeny were used for raising R_3 and R_4 progeny to study the stability of variant characters.

RESULTS

Shoot tip culture

When shoot tips were cultured on MS medium supplemented with phytohormones, they enlarged considerably and new shoot buds developed from the axils accompanied by slight callussing at the base. The results are summarized in Fig. 3. There was an enhancement in shoot multiplication with increasing concentrations of BA, either alone or in combination with NAA (Fig. 3). However, at high concentration of BA (5 mg L^{-1}) the shoots were small and therefore, 2 mg L^{-1} BA with 0.1 mg L^{-1} NAA was used to maintain the cultures. Out of 400 shoot buds transferred to rooting medium, 95% produced roots.

Evaluation of plants

R_1 generation

Out of 277 regenerants (R_1) transplanted to the field, 233 plants survived up to maturity, which were evaluated for different characters. The R_1 plants were shorter with reduced leaflet size (length and breadth) in comparison with the control (Table 1).

A reduction in the number of pegs and seeds was also noticed in the R_1. No significant difference between R_1 and control plants was observed with respect to the number of secondary branches and HSW. However, the regenerants showed an increase in the number of primary branches (Table 1). Other morphological characters of the regenerants were comparable to those of the control with a few exceptions. Two regenerants showed morphological variation for extended inflorescences in the axils and one showed partial sterility with reduced number of pods.

R_2 generation

All 233 regenerants (R_1) were progeny tested in R_2 and evaluated for chlorophyll and visible mutations. No chlorophyll mutant was observed in R_2. The two regenerants which showed variation for extended inflorescence in the axils produced normal progenies. However, two normal looking regenerants gave rise to progenies which showed variation. The progeny of plant No.140 was shorter with smaller pods. Progeny from regenerant number 20 had constricted pods and were taller. Rest of the 231 R_2 lines produced plants which were normal in all morphological characters in comparison with the control. The pod yield per R_2 plant was 22.7 g as against 24.3 g in the control.

Table 1. Agronomic evaluation of R_1 and control plants.

Characters	Control	R_1	't' value
ant height, cm	39.1	25.9*	13.8
No. of primary branches	5.4	6.9*	2.2
No. of secondary branches	2.2	1.7	1.2
Leaflet length, cm	4.9	4.1*	8.3
Leaflet breadth, cm	2.4	2.2**	2.3
No. of pegs	45.9	77.1*	6.1
No. of seeds	91.5	59.4*	6.1
Seed weight, g	37.8	20.4*	5.8
HSW, g	40.2	37.0	1.8

* - Significant at 5%, ** - Significant at 1%
't' test was carried out on the basis of observations taken on 26 plants. Table value of 't' at 5% is 2.060 and 1% - 2.787.

R_3 and R_4 generations

Only the two variant plants in R_2 were grown in subsequent generations (R_3 and R_4) to study the behaviour of the progenies: Progeny of plant number 20 which had deeply constricted pods and were taller segregated for deep constriction and plant height. Out of 39 plants, 37 were normal in height and pod shape while two had deeply constricted pods and were taller (Figs. 1 & 2). The plants with deeply constricted pods were further studied in R_4 and they again segregated for normal and deeply constricted pods. Further studies are in progress to understand the inheritance of these

traits.

The progeny of plant No.140 bred true for plant height and pod size in R_3 and R_4. The plants were

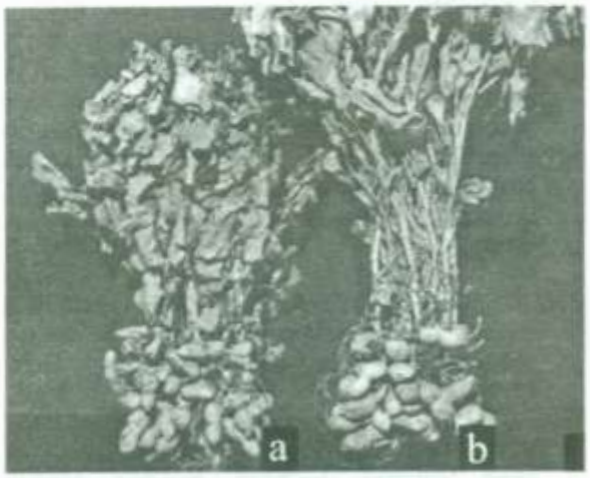

Fig. 1 a. Seed-raised control.
1 b. Somaclonal variant showing increased height and constricted pods in R_2 generation.

shorter and produced smaller pods (Fig. 2).

DISCUSSION

The present studies have shown that it is possible to multiply peanut plants through shoot tip culture from immature embryos. Although other food legumes such as chickpea, lentil, pea, black and green gram have been multiplied through shoot tip culture (Bajaj and Dhanju 1979; Kartha *et al.* 1981), information is not available on agronomic

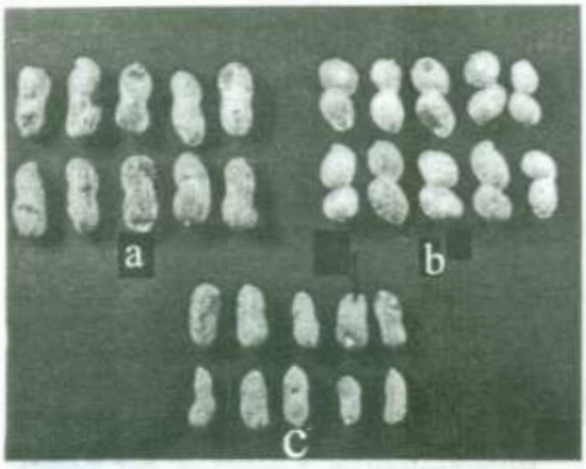

Fig. 2 Variation in pod size and shape in R_2 generation.
a. Control.
b. constricted pod.
c. small pod.

evaluation of micropropagated plants. In the present study, most of the agronomic characters in the micropropagated peanut regenerants (R_1) were inferior in comparison with the control. This may

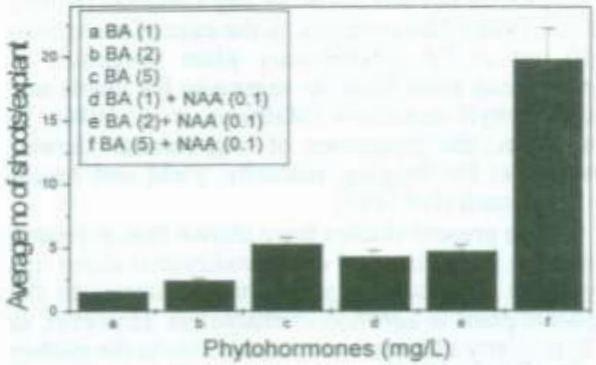

Fig.3 Effect of BA and NAA on shoot multiplication of peanut on MS medium

be due to the physiological changes in micropropagated plants due to continuous exposure to hormones *in vitro* in comparison with seed raised plants. However, shoot tip multiplication is known to yield normal plants in diploid and tetraploid watermelon (Compton *et al.* 1993). Micropropagation is routinely carried out for ornamental and fruit crops (Debergh and Zimmerman 1991). The R_1 regenerants of peanut although agronomically inferior, gave rise to normal R_2 progenies showing that variation was due to epigenetic changes rather than genetic. In R_2, the progenies of two plants showed variation and their further evaluation in R_3 and R_4 showed that these characters were heritable. Apart from these two variants, rest of the R_2 plants were normal and yield was comparable to that of the control.

In vitro culture of plant cells is known to produce changes called somaclonal variation (Larkin and Scowcroft 1981) and shoot tip culture is no exception to this (Vuylsteke *et al.* 1991). Irrespective of whether it is derived from shoot tip or callus, plant cells in culture is in a stressful environment in contrast to the highly integrated and balanced environment of the whole plant. Rapid genomic changes may occur from mechanisms of genetic instability in culture (McClintock 1984; Karp and Bright 1985) leading to variant cells. Mutations may be expressed in the first or second generation of plants depending on whether they are dominant or recessive. Variants have been reported in shoot-tip propagated plantains and bananas (Vuylsteke *et al.* 1991; Israeli *et al.* 1991) and the frequency varied from 1% to 30% in different experiments depending on the genotype (Reuveni *et al.* 1984; Hwang and Ko 1987). In *Musa* spp. variants were obtained for different phenotypic characters such as inflorescence morphology, fruit shape, pseudostem, petiole, bract colour, leaf shape and plant stature (Vuylsteke *et al.* 1991; Israeli *et al.*

1991). In the present study on shoot tip derived plants of peanut, 2 out of 233 regenerants (0.9%) produced variant progenies. However, peanut plants obtained from cotyledons without employing tissue culture techniques, but by the addition of BA did not show any variation (Bhatia *et al.* 1986). Nevertheless, in the case of mungbean 10 out of 70 cotyledonary plant progenies of mungbean were found to segregate for viable and chlorophyll mutations (Mathews *et al.* 1986). In soybean, the progenies of regenerants showed variation for lodging, maturity, yield and height (Graybosch *et al* 1987).

The present studies have shown that in peanut *in vitro* multiplication using embryonal shoot tips yielded plants (R_1), which were inferior to the parent plant in agronomic characters. However, in R_2 majority of them were comparable to the mother plant, although a low frequency (<1%) of variants were observed. Hence, even in shoot tip multiplication which is expected to maintain clonal fidelity, somaclonal variants do occur in peanut at a low frequency, and hence care has to be taken to avoid the variants to obtain uniform plants.

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