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# Colchicine Induced Morphological Variants in Pineapple

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## Abstract

In pineapple compact nodular callus was induced from various plant parts that showed high regenerative ability. Colchicine was used to obtain some variants. In colchicine-treated callus about 5% of the regenerants were variants/albinos which showed significantly low chlorophyll **a** and chlorophyll **b**. Shoot and root regenerating potentiality and other growth behaviour were poor in variants compared to normal plants. Shoot tip culture of the variants and callus from the same source continuously produced variant progenies for a number of (4 - 5) *in vitro* cycles. Compared to normal, two important peroxidase bands were missing in the variant pineapple.

## Introduction

Pineapple is an important fruit crop that belongs to the family Bromeliaceae. It is cultivated mainly in Thailand, Indonesia, Brazil, Peru, Mexico, USA, India and other regions of the tropical world. Beside fruit and juice, the plant contains a variety of compounds of which bromelain, a proteolytic enzyme deserves special mention because it aids digestion. The use of bromelain for dental, general surgery and as an antibody is also not uncommon (Murachi 1972). In this fruit crop the improvement is generally made by mutation and selection of sports. However, information to improve the yield of pharmaceutical compounds in this crop is inadequate. Somaclonal variation and induced mutations have been considered useful to create genetic variability. Somaclonal variants with novel characters occur spontaneously but infrequently in *in vitro* cultures. Some of these traits of agronomic importance have been utilized in the past in improving crop plants (Bouharmont 1994; Karp 1995; Hammerschlag et al. 1995). Duncan (1997); Veilleux and Johnson (1998) have recently reviewed the application of somaclonal variation in bringing about such improvements. The *in vitro* use of mutagens on undifferentiated tissues and organs were also made (Novak 1991) and some successful reports of mutant selection are available (Predieri et al. 1997; Yang and Schmidt 1994; Brunner and Keppl 1991; Mujib and Jana 1995). More recently colchicine has been applied widely in cultures to alter ploidy level (Van Harten 1998) and recoveries of polyploid regenerants were achieved (Hamill

et al. 1986; Kaeppler et al. 2000). In the present paper, a colchicine-induced variant of altered morphology is reported.

## Materials and Methods

Pineapple (*Ananus comosus*) var. Queen was selected as an experimental material. Fast growing callus was obtained from a variety of explants especially from the base of the crown. Among hormonal supplements 5.4 - 10.8 μM NAA and 2.22 μM BAP were useful. The detailed procedure for explant sterilization, establishment and maintenance of callus and regeneration protocol have been earlier described (Mapes 1973). All media were adjusted to pH 5.8 prior to sterilization. Cultures were kept at diffuse light (2.03 $w/m^2$) at 24 ± 2°C.

For colchicine experiment, 0.01% (w/v) was prepared by dissolving colchicine in water. Colchicine treatment was given for 7, 8 and 10 days. The flasks containing 25 - 30 callus pieces per flask immersed in the liquid medium were placed on a rotary shaker at 120 rpm in a growth room. A few calli showed necrosis and early death. After cultivation for a varying lengths of time, surviving calli were washed several times with sterilized distilled water and transferred to solid regeneration medium that contained BAP (8.90 μM) + NAA (0.54 μM).

Plant pigment i.e. chlorophyll **a** and chlorophyll **b** were measured following Arnon method (1949). Fresh leaves from both normal and the variant were homogenized in 80% acetone. The supernatant was filtered through Whatman No. 42 filter paper. This extraction procedure was repeated several times until the leaves became colourless. Chlorophyll content was measured spectrophotometrically.

Polyacrylamide gel electrophoresis was performed according to Davis (1964) for isoenzyme analysis. Samples containing 40 μg protein each were loaded to each gel consisting of 28% acrylamide. The gel buffer contained 0.05 Tris, 0.038 M glycine at pH 8.9. An electric current of 3 MA at 20 volts was applied. The ruff was continued until the bromophenol front reached the end of the gel tube. The enzyme was stained following Smith (1972) method. The staining solution contained a mixture of saturated benzidine, 30% ammonium chloride and 0.4% $H_2O_2$ in the proportion of 50 : 10 : 2. Each treatment contained 3 - 5 replica and each experiment was repeated at least twice. Statistical analyses; i.e., analyses of variance were made for both mean and standard error.

## Results and Discussion

The colchicine treated callus produced normal green plants on the regenerating media; however, phenotypic variation was also observed among the regenerated plantlets (Plate 1). Table 1 shows that of the 133 calluses transferred, seven gave

variant lines with a frequency of 5.26%. On the callus induction media (MS + 2,4-D at 4.52 μM) the albino shoots regularly callused. The colour and growth rate of variant-callus was a little different to that of the normal callus (Plate 1a,b). The variant callus developed into albino shoots on transfer to the shoot regeneration

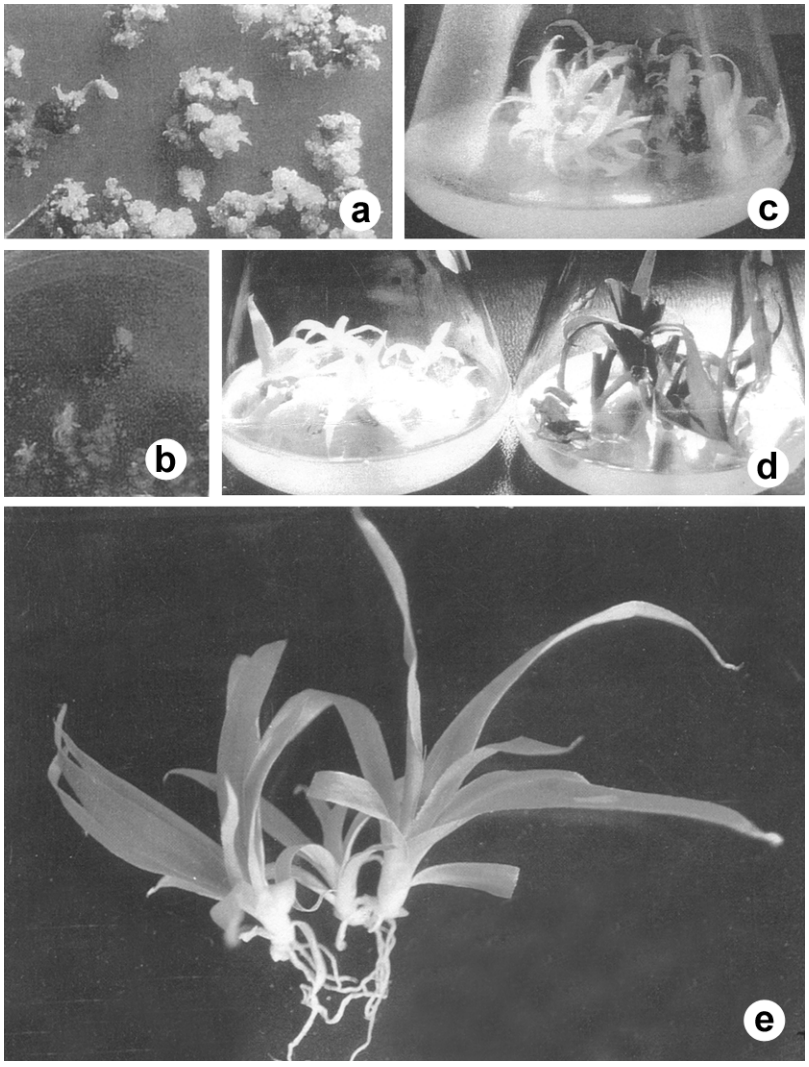


Plate 1. Mutagen effect on *in vitro* cultivated pineapple. (a) Normal callus of pineapple. (b) Callus type 2, mostly produced variant regenerants. (c, d) Variant and normal regenerants grew along and separately in *in vitro* condition. (e) Rooted plants before transplantation.

media. Likewise, when variant shoot tips were cultured on the regenerating media containing NAA (2.69 μM), the newly generated multiple shoots also became variants. No cases of green or chimeras were found in the succeeding generations.

The regenerated variant lines grew well along with the normal plants (Plate 1c,d) and easy *in vitro* cloning for variant regenerants was possible; however its growth suffered heavily compared to that of normal plants (Plate 1e). Although variation in plant height was less at the initial first four weeks, the difference became remarkably high at the later phases of growth. Table 2 shows that on an average 1.84 ± 0.08 cm of plant height was observed in the variants, while it was 4.58 ± 0.17 cm in normal green plants. Leaf morphological variation was also noted among the regenerated progenies. In normal plants length/breadth (L/B) ratio of leaf was significantly higher compared to that of the variant leaf where L/B ratio was recorded to be 5.52 (Plate 1e, Table 3). As regards roots, induction and further growth of it was severely inhibited in variant lines. In the latter roots attained a length of 0.88 cm while in normal plants the average root length was 10.9 cm in six months' cultures. Surprisingly, the variation range in the number of adventitious shoots was smaller between the variant lines and normal green plants.

**Table 1. Mutagen effect on callus cultivated on regeneration medium.**

| | | Total No. of shoots | Frequency (%) | No. of variants | Frequency |
|---|---|---|---|---|---|
| No. of non-treated (control) callus on regeneration medium | 126 | 112 | 88.8 | 0 | 0 |
| No. of treated callus transferred on regeneration medium | 133 | 80 | 60.1 | 7 | 5.25 |

**Table 2. Variation in differentiation and growth.**

| | No. of | Shoot length (cm) | | No. of | Root length (cm) | |
|---|---|---|---|---|---|---|
| | shoots | I | II | shoots | I | II |
| Normal | 5.8 ± 0.37 | 2.06 ± 0.10 | 4.58 ± 0.17 | 5.75 ± 0.47 | 5.45 ± 0.34 | 10.97 ± 0.65 |
| Albino | 4.2 ± 0.48 | 1.20 ± 0.11 | 1.84 ± 0.08 | 4.20 ± 0.08 | 0.54 ± 0.08 | 0.88 ± 0.06 |

All values are expressed as in mean ± standard error I and II represent 1 and 6 months' culture.

Chlorophyll analysis further demonstrated that in the variant lines, total chlorophyll was about 15 times less than that of normal plants. Similarly, chlorophyll **a** and chlorophyll **b** were significantly higher in normal plants compared to those in the variant lines (Table 3).

In the present investigation isoenzyme variation of both normal and regenerated albinos were studied. In most cases, differences were noticed in the occurrence and absence of isoenzyme bands. Normal calluses exhibited a total of five isoenzymes of peroxidase bands compared to three in the albino type. Both

the normal and albino had three common bands (Rf 0.05, 0.37 and 0.64). In other words, the normal type showed two additional bands of Rf 0.10 and 0.26 and a faint band of Rf 0.40 which was absent in the variant. Besides, distinct differences in band intensities were also observed. The regenerated variant grew very slowly; it grew for three years or more without any regenerative loss. The detailed evaluation of the variant lines may prove helpful in utilizing any of its novel trait in the improvement of this popular crop.

**Table 3. Leaf physical index and chlorophyll content (mg/l).**

| | Length (cm) | Breadth (cm) | Length/ breadth (L/B) | Total chlorophyll | Chlorophyll | |
|---|---|---|---|---|---|---|
| | | | | | **a** | **b** |
| Normal | 6.78 | 0.52 | 13.03 | 1.8774 ± 0.22 | 1.226 ± 0.183 | 0.065 ± 0.04 |
| Albino | 2.1 | 0.38 | 5.52 | 0.126 ± 0.015 | 0.084 ± 0.019 | 0.38 ± 0.05 |

In the present investigation, phenotypic variation particularly in the synthesis of pigment and growth pattern was noticed among the regenerated clones. The variants showed significantly low chlorophyll compared to that of normal plants. Besides, leaf size, the rate of shoot and root growth were substantially reduced in the variants. They grew with less vigour and produced identical clones without further segregation when cultured *in vitro*. The altered phenotype was stable and it maintained its usual high regenerative potential for over three years.

The incidence of altered phenotype in plant culture accompanied by various genetic modifications such as resistance to diseases, herbicides, antibiotics has been reported (Brar and Jain 1998). The mechanism of altered phenotype still remains unknown. Evans and Sharp (1986) reported that it could be due to the change of chloroplast DNA. In pineapple, stable variants were noted in leaf colour, wax secretion, foliage density and spines through cell culture from various sources (Wasaka 1979). The variants showed distinct altered morphology. Further *ex vitro* studies to isolate other variants were difficult because of high mortality rate. Alteration of isoenzymes particularly of peroxidase, amylase, alcohol dehydroxygenase and other isoenzymes were earlier reported in the regenerated variants (Heinz and Mee 1971; Davies et al. 1986). Sung et al. (1993) reported that the alteration of chromosomal number, protein profiles and phenotype may also arise by the use of high level plant growth regulators and sometimes by heterogeneous cell populations.

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