EFFECTS OF BAP (6 - BENZYLAMINO-PURINE) IN SHOOT REGENERATION OF PINEAPPLE *(ANANAS COMOSUS* (L) MERR) CULTURED IN-VITRO

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ABSTRACT

Growth and development in plants are regulated by the interactions between promotive and inhibitory hormones [1], Synthetic chemicals with similar physiological activities to these endogenous substances with an ability to modify plant growth are termed plant growth regulators [2]. Tissue culture (micropropagaion), which is a modern tool in Agriculture for rapid clonal (vegetative) propagation employs some of these synthetic chemicals to bring about desirable changes in plants. The paper reports of the effects of the growth regulator BAP (6 - BENZYL AMINOPURINE), a cytokinin, on shoot regeneration. The regulator was investigated for its effect on pineapple plantlets cultured in-vitro on morphogenesis and subsequent ex-vitro. The concentration of BAP in the culture medium significantly affected morphogenesis. A mean total value of 108.5 shoots were regenerated within four weeks from 20 original shoots cultured on medium containing 1 mg/l BAP.

Keywords: *Plant Growth Regulators, Culture, In-vitro, Morphogenesis, Establishment Ex-vitro.*

INTRODUCTION

Cytokinins are known to promote shoot regeneration in most plant species with or without auxins applied exogenously in culture media [3]. Amongst the plant growth regulators with cytokinin-like activity, BAP (6 - BENZYL AMINOPURINE) is known to be one of the most effective in enhancing shoot regeneration [4]. Apart from being effective as a regulant in shoot proliferation, the concentration in the medium is equally important.

At least three axillary shoots from bud cultures [5] of pineapple were obtained on 0.5 or 1 mg/l BAP within 30 days. Further multiplication was obtained by separation of the axillary shoots and sub-culture four times on half strength MS medium with BAP. They extimated that by aplication of tissue culture techniques, at least five hundred plantlets can be produced in 12 months.

MATERIALS AND METHODS

Aseptic shoots of pineapple produced on MS (Skoog and Murashige) medium containing 0.5 mg/l BAP were sub-cultured onto MS medium containing four concentrations of BAP, i.e. 0.1, 0.5, 1.0 and 2 mg/l. Each of the media contained 30 g/l sucrose and 7 g/l sigma agar.

Individual shoots, approximately 3 cm in height were trimmed and sub-cultured singly into small glass jars containing 15ml of medium. They were incubated at 28 - 30°C 16 hr day length provided by warm white fluorescent tubes for four weeks.

The experimental design was a randomised complete block (RCBD) replicated four times. There were five cultures at each of the four BAP concentrations giving a total of 80 cultures for the experiment. Parameters recorded were the total number of shoots regenerated at each concentration and the general morphology of the plantlets.

RESULTS

The number and conditions of the regenerants were influenced by the concentration of BAP in the media after incubation for four weeks.

Regeneration on 0.1 mg/l BAP

Regeneration began after the second week of incubation and callus formation was quite regular. New shoots proliferated mainly at the base of the original shoots. The new shoots were fairly uniform in terms of size and shoot extension.

Approximately, 40% of the cultures showed primary shoot dominance. Browning of shoots was evident. After incubation for four weeks, the treatment exhibited the second highest number of shoots of 108 (Table 1). The mean shoot height from ten randomly selected cultures after four weeks of incubation was 2.3 cm.

Regeneration on 0.5 mg/l BAP

The regeneration of plantlets on 0.5 mg/l BAP also began after two weeks of incubation. Most of the shoots cultured on this medium passed through a callus phase (about 70%). Regenerated shoots were more uniform in size and in extension rate than those in the 0.1 mg/l BAP treatment. Most of the shoots were brown and dying-back. By the fourth week about 90% (almost all) of the shoots had died and had been superceded by proliferating shoots. The average number of shoots produced per culture was 3.6 with a mean shoot extension of 1.8 cm.

Regeneration on 1.0 mg/l BAP

Plantlets regenerated on 1.0 mg/l BAP remained green and were more robust than those on both 0.1 and 0.5 mg/l BAP. Some callus was produced after the shoots had already regenerated. Primary shoot dominance was highest in this treatment. Rooting was regular and leaf browning absent. Generally, the development of plant-lets in this treatment was good. Nearly hundred percent of all shoots were healthy.

The leaves were broader than in any other treatment and the highest mean number of 12.1 shoots (plantlets) per vessel was obtained in addition to shoot extension of 4.3 cm. The mean number of shoots produced over the 4-week period was 108.5 (Table 1).

Table 1: Effect of BAP concentration on the Number of Shoots Proliferated

BAP Concentration (mg/l)	0.1	0.5	1.0	2.0
Mean Number of Shoots	108.0	37.7	108.5	19.2

VR 9.61
S.e.d = 21.29
** Significant at 5%*

Regeneration on 2.0 mg/l BAP

Shoot proliferation from 2.0 mg/l media was low after four weeks of incubation. Browning of shoots was frequent. Very little callus was produced and the mean shoot extension was limited (1.7cm). It took about two weeks before shoot regeneration occurred. Probably, the shoots severed and cultured contained differentiated cells and tissues which had to be redifferentiated before subsequent morphogenesis [6].

DISCUSSION

Apical dominance observed in 60% of the cultures on 0.1 mg/l BAP may indicate that organogenesis of bud formation could not necessarily supercede the original shoot growth and that both promotive and inhibitory regulators had an effect on extension (apical dominance) and branching (bud growth). Callus proliferation could also have been due to an interplay between the BAP applied and the endogenous auxin levels, possibly IAA [1]. The browning of tissues which occurred could have been due to the presence of tannins or other hydroxyphenols which could inhibit shoot proliferation as well. Uniformity in the regenerants on 0.5 mg/l is an indication of suppression of apical dominance. This behaviour naturally could cause little or no growth to the main/original shoot. Again this level (0.5 mg/l) of the cytokinin could possibly have favoured "branching" or bud formation more than the original shoot extension.

Frequent callus production probably delayed shoot growth as more shoots were recorded on 0.1 mg/l than on 0.5 mg/l while the 1.0 mg/l concentration yielded the highest as recorded earlier. The 1.0 mg/l concentration could be a good balance between promotive and inhibitory regulators. It could also mean a possible balance for cytokinins and auxins. This is due to the fact that there were more shoot regeneration, healthier looking plantlets and apical dominance (original shoot growth). The number of shoots regenerated and the condition of cultures, such as frequent browning on 2.0 mg/l BAP suggested an inhibitory level and that lower concentrations

could be more appropriate.

CONCLUSION

Callus formation which could be important for varietal development (ultra structure study of callus shows) is obtained at lower concentrations (0.5 mg/l), where- as more plantlets are obtained at slightly higher levels of 1.0 mg/l. It may be necessary to obtain both (callusing and plantlets) through changes in concentrations and/or liquid media.

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