Heat Treatment for Enhanced Responsiveness of Dormant Axillary Buds of Pineapples[1]

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ABSTRACT

A method has been developed which utilizes heat treatment to enhance shoot emergence from dormant axillary buds of pineapples. Dormant buds excised from the crown of the pineapple fruit were immersed in hot water (50 °C - 60°C) for six or eight minutes prior to transfer to liquid or semi-solid nutrient medium. Results indicate that, compared to the non-heat treated buds, a larger proportion of shoots emerged at four weeks after initiation when buds were immersed in hot water. This is less than the time of six to twelve weeks reported in other studies. Additionally, the release of buds from dormancy was synchronized to within a three– week period, i.e., from the end of the first week after initiation to the beginning of the fourth week.

Key words: Dormant axillary buds, heat treatment, hot water, micropropagation, pineapple, responsive buds, shoot emergence.

RESUMEN

Se ha desarrollado un método que, por medio del tratamiento por calor, incrementa la emergencia del brote de los capullos axilares latentes. Capullos latentes extirpados de la corona de la fruta de la piña se sumergieron en agua caliente (50°C - 60°C) durante seis a ocho minutos antes de transferirlos a un medio nutritivo líquido o semisólido. Los resultados indicaron que al compararlos con los capullos no tratados, una proporción mayor de brotes emergió cuatro semanas después de que los capullos fueran sumergidos en agua caliente. Esto contrasta con períodos de seis y doce semanas mencionados en otros estudios. Además, el tiempo transcurrido mientras los capullos emergían de su latencia fue medido en tres semanas, desde el fin de la primera semana hasta el comienzo de la cuarta semana.

Palabras clave: Capullos latentes axilares, tratamiento por calor, agua caliente, micropropagación, piña, capullos sensibles, emergencia del brote.

INTRODUCTION

Selections of elite plants have been propagated vegetatively in order to preserve, and transfer to progeny, unique genetic complexes and heterogeneity which confer superior fruit characteristics (Litz 1987). Previous reports (Mathews and Rangan 1979, 1981; Rangan 1984; Wakasa *et al.* 1978; Zepeda and Sagawa 1981) have indicated that plant tissue culture techniques could be used for vegetative propagation of pineapple (*Ananas comosus* L. Merr.), and thus transfer desired characteristics to progeny. Both callusing and non-callusing systems have been used.

It has been noted by Litz (1987) that use of cell and tissue culture for crop improvement is limited by evidence of variation in plants developed from callus cultures. For pineapple; such variation has been observed (Wakasa *et al.* 1978). To reduce this variation, a non-callusing system of micropropagation should be used, for example, shoot emergence from dormant axillary buds or apical meristems.

Generally, the process of releasing pineapple buds from dormancy is slow. Mathews and Rangan (1979) and Zepeda and Sagawa (1981) reported that shoot buds did not appear in lateral bud explants until eight to ten weeks after initiation. Cote *et al.* (1992) obtained initial shoots after two to three months of culture. Further, in some instances shoots may emerge from initiated buds at six to eight weeks, while other buds, initiated at the same time, remain dormant until transferred to fresh medium (Wakasa *et al.* 1978). The failure of buds to be released from dormancy could be attributed to either microbial contamination of cultures (Leifert and Waites 1990; Van der Linde and Hol 1990; Wakasa *et al.* 1978; Zepeda and Sagawa 1981), low responsiveness of non-contaminated cultures (Mapes 1973; Wakasa *et al.* 1978; Zepeda and Sagawa 1981), or the presence of toxic substances (Ramírez 1978).

In order to reduce the time to the breaking of dormancy, and consequently increase the production of pineapple shoots during a given period, methods were

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sought to promote shoot emergence. The synchronized release from dormancy of axillary buds will enhance shoot multiplication. Thus, an investigation was initiated to examine the influence of heat treatment, the physical form of the medium, and wounding of the explant on the responsiveness of dormant axillary buds of pineapples.

MATERIALS AND METHODS

Crowns of the pineapple (*A. comosus* L. Merr.) were kept with their bases immersed in a suspension of Benlate (2 gdm^{-3}) for three days prior to use. The crowns were defoliated to reveal stems approximately 3 cm in length. For each stem, the upper and lower 1 cm regions were discarded, and the remainder sterilized with 15% household bleach (5.25% sodium hypochlorite) for 30 minutes. Explants 3 mm - 5 mm in diameter, consisting of dormant axillary buds and adjacent stem tissue, were excised from the stem region of the crown, sterilized with 10% bleach for 30 minutes, then rinsed five times with sterilized, distilled water. Disinfected axillary buds were then placed in sterilized distilled water (60°C) for six or eight minutes. By the end of the period of heat-treatment, the temperature of the water had decreased to 53°C and 50°C respectively. Heat-treated axillary buds were then transferred to the defined nutrient medium. For the control, buds were not immersed in hot water; instead, they were placed in the appropriate nutrient medium immediately after disinfection.

To determine the effect of wounding on responsiveness, buds were partially split in the uppermost 1 mm region after heat treatment and before transfer to the nutrient medium. The nutrient medium consisted of MS Medium (Murashige and Skoog 1962) supplemented with 0.5 $mgdm^{-3}$ 6-benzylamino-purine (BAP) and 0.1 $mgdm^{-3}$ gibberellic acid (GA_3). The pH of the medium was adjusted to 6.0 before autoclaving. In the semi-solid medium, agar at 3.0 g dm^{-3} was used. Liquid-medium cultures were kept stationary and were not continuously aerated during the period of culture.

Cultures were maintained under a 16-hour photoperiod with a photon flux density of 75-95 µmol m^{-2} s^{-1} provided by fluorescent tubes. Six replicates, each with six buds, were used for the liquid medium, while four replicates, each with four buds, were used for cultures of the semi-solid medium.

RESULTS

Responsive buds were those which changed their appearance from an off-white or cream colour to green or pale-green. The results presented in Fig. 1 indicate that immersion of pineapple buds in hot water prior to initiation increased their responsiveness during culture in a liquid medium. After one week of culture, as many as 44% of the heat-treated buds were responsive, while none was responsive in the control, i.e., no heat treatment before culture. By the third week after initiation, more than 60% of the heat-treated buds in the liquid medium were responsive, compared to only 6% for non-heat-treated buds. At all times, other than the first week, there was a larger proportion of responsive buds when heat was applied for eight instead of six minutes. In the control, there was no increase in the proportion of responsive buds between the third and sixth week. The culture of heat-treated buds in the liquid medium resulted in at least a ten-fold increase in responsiveness after six weeks.

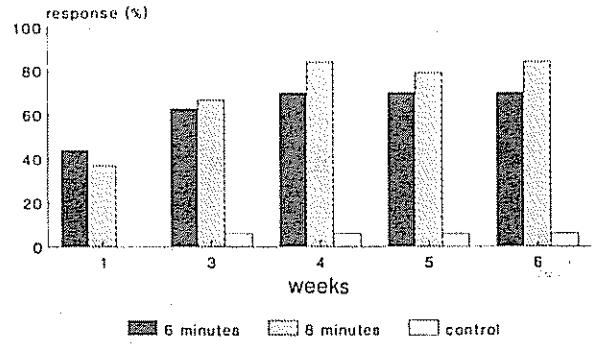

Fig. 1. Responsiveness of axillary buds pineapple cultured in liquid medium as influenced by heat.

The responsiveness of buds cultured in the semi-solid medium is shown in Fig. 2. After one week of culture, there were 19% and 17% of responsive buds for the six-and eight-minute heat treatments respectively, but no responsive bud was seen in the control that received no heat treatment. There were more responsive buds following immersion in hot water for six instead of eight minutes. At six weeks, the proportion of responsive heat-treated buds in the semi-solid medium was twice that of the control.

The results in Figs. 1 and 2 indicate that buds which were not heat-treated were less responsive, even after monitoring of cultures for a longer period. For example, at six weeks, shoots had emerged from only 13% and 6% of cultures in the semi-solid medium and liquid

mediums respectively. In general, the breaking of dormancy occurred within the first four weeks of culture. Thereafter, fewer additional buds were responsive, and a higher proportion was observed with those placed in the semi-solid medium.

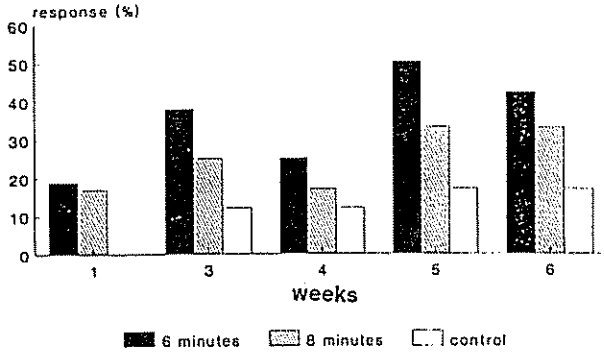

Fig. 2. Effect of heat on responsiveness of pineapple axillary buds cultured in semi-solid medium.

Table 1 shows that, regardless of the heat treatment and the physical form of the medium, wounding of dormant buds, by cutting the upper region, adversely affected the response of buds. Responsiveness was inhibited when heat-treated buds were wounded before placing them in a semi-solid medium, and only approximately 25% were responsive after culture in the liquid medium.

DISCUSSION

In this investigation, use was made of heat treatment, modification of the physical form of the medium, and wounding of the plant tissue. The results indicate that each method influenced the responsiveness of explants. However, whereas enhanced responsiveness was obtained with heat treatment and the use of liquid medium, wounding of buds had an adverse effect. The factor that made the greatest contribution to improved responsiveness of dormant pineapple buds was the application of heat. The increased responsiveness of heat-treated buds was at least twelvefold for those buds cultured in liquid medium and approximately twofold when buds were placed in the semi-solid medium.

Table 1. Responsiveness of partially split pineapple buds heat-treated for six or eight minutes.

Time after initiation (weeks)	Responsive buds (%)			
	Semi-solid medium		Liquid medium	
	6 min	8 min	6 min	8 min
1	0	0	14	8
3	0	0	28	33
4	0	0	22	17
6	0	0	22	25

Low responsiveness of dormant axillary buds of pineapple could be attributed to microbial contaminants (Wakasa *et al.* 1978; Zepeda and Sagawa 1981) or the presence of toxic substances (Ramírez 1987; Zepeda and Sagawa 1981). Hot water has been used to reduce such contamination; for example, Van der Linde and Hol (1990) used hot water on narcissus buds to reduce endogenous contamination by *Fusarium* sp. To reduce the levels of toxic substances, activated charcoal (Ramírez 1987) and frequent transfer of explants to fresh medium (Zepeda and Sagawa 1981) are two methods which have been used in previous studies. In this investigation, however, the cream-coloured precipitate observed in the non-heat-treated cultures, but not in the heat-treated cultures, was non-microbial. Examination of the precipitate with a light microscope did not show the presence of either bacterial or fungal organisms.

Axillary bud explants of the cultivar used in this investigation did not become brown, i.e., oxidized, in the absence of heat treatment. Although activated charcoal has been used to remove contaminants (Kohlenbach and Wernicke 1978) and substances secreted by plant tissue (Fribdorg *et al.* 1978; Wang and Juang 1976), it is also known to suppress bacterial growth (Vuylsteke 1989) and reduce the growth of *in vitro* cultures (Constantin *et al.* 1977; Fribdorg *et al.* 1978). It seems likely, therefore, that the promotor effect of heat was not by way of reducing endogenous bacteria or fungi, but by degradation of inhibitory substance(s).

As a result of this investigation, it is now possible to obtain a large proportion of responsive buds without the addition of activated charcoal nor the frequent transfer of explants to the nutrient medium.

In the present study, wounding was combined with heat treatment to determine the likelihood of further increasing responsiveness of pineapple axillary buds. Responsiveness of buds was adversely affected by the wounding of buds during excision, to the extent that none of the heat-treated axillary buds cultured in the semi-solid medium was responsive. When buds were cultured in liquid medium, wounding reduced the responsiveness of buds by approximately 70%. This

adverse effect of wounding is the reverse of that reported by Mathews and Rangan (1979). The difference in response, however, may be attributed to variation in the type of explants; shoots were used in the earlier investigation, while axillary buds were used in the present study.

Regardless of which one of the two periods of heat treatment was used, buds in the liquid medium were more responsive than those in the semi-solid medium. The liquid medium may provide more readily-available components to explants. In general, with both the six and eight minute heat treatments, there were twice as many responsive buds in the liquid medium compared to those in the semi- solid medium. In the control, however, the reverse occurred, i.e., there were more responsive buds when the semi-solid medium was used.

This investigation has demonstrated that heat treatment increases the proportion of buds released from dormancy and reduces the time taken for that release to occur. Continuous aeration of liquid cultures, as reported by other researchers (Mathews and Rangan 1979; Zepeda and Zagawa 1981), was not required. Immersion of buds in hot water prior to the initiation of cultures would result in more rapid multiplication of pineapple shoots cultured *in vitro*. Such shoots can be used for subsequent shoot multiplication and rooting of pineapple plants derived from *in vitro* cultures.

Variation in pineapple plants produced from *in vitro* cultures is a likely source of additional material for a breeding programme (Mathews and Rangan 1981; Wakasa *et al.* 1978). However, where the aim is to propagate plant material and not to breed an improved cultivar, the most appropriate system should be non-callusing and have a high level of responsive explants. Increased responsiveness of axillary buds would make it unnecessary for callus cultures to be used for micropropagation. The production of a large quantity of pineapple plants using micropropagation could, therefore, be obtained by the immersion of dormant axillary buds in hot water for eight minutes followed by culture in a liquid nutrient medium.

CONCLUSIONS

— Heat treatment promotes responsiveness of axillary buds of pineapple and synchronizes the release of buds from dormancy.

— Heat treatment reduces the time required for releasing pineapple buds from dormancy.

— A liquid medium, not a semi-solid medium, facilitates greater response of heat-treated axillary buds.

— Partial splitting of heat-treated buds, at the time of initiating cultures, adversely affects responsiveness.

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