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EFFECT OF GLUCOSE CONCENTRATION ON THE FORMATION OF TERMINAL INFLORESCENCES AND RIGID SHOOTS IN OIL PALM CULTURES

Keywords: Glucose, oil palm polyembryogenic cultures, terminal inflorescences, rigid shoots.

TARMIZI, A H*

Oil palm cultures respond to external factors such as high osmoticum. It was observed that a high concentration of glucose (0.55 M to 0.82 M or 10% to 15% w/v) induced terminal inflorescences (TI) and rigidity in in vitro *shoots. The occurrence of TI and rigid shoots varied from clone to clone. TI and rigid shoots also differed in their morphology within the same flask. This indicated that cultures responded differently in their development to glucose treatment. Thus, sensitivity of the cultures to high concentrations of glucose has potential to be used as a technique for* in vitro *indexing of the stability of oil palm clonal materials.*

INTRODUCTION

Studies on phenotypic variability related to the tissue culture process have been reported widely in many plantation crops such as plantain (Vuylsteke and Ortiz, 1996), banana (Tang, 1995) and white spruce plants (Isabel *et al.*, 1996). Morphological variation in oil palm cultures has also been reported extensively since the mid seventies when the first oil palm tissue culture techniques were established (Jones, 1974). However, there are many other aspects of phenotypic variation that have not been described and understood. Jones (1993), in reviewing tissue culture problems, mentioned the need to look into various morphological changes in oil palm cultures so as to improve the overall understanding of problems in oil palm tissue culture procedures.

Variation in *in vitro* shoot morphology is quite common in oil palm tissue cultures. Besides the formation of stunted, swollen, wrinkled and rigid shoots, the formation of TI from the shoots was also observed (Tarmizi, 1997). Paranjothy *et al.* (1990) also reported that formation of TI could be induced by supplementing the medium

* Palm Oil Research Institute of Malaysia, P.O. Box 10620, 50720 Kuala Lumpur, Malaysia.

with high concentrations of benzylaminopurine (BAP). Since the formation of TI in oil palm cultures is quite unique, attempts were made to identify treatments that can induce TI with a view to use them as indicators of sensitivity to stress conditions in oil palm cultures. Glucose was chosen to induce TI as it is known to affect the normal development of oil palm polyembryogenic (PE) culture (Tarmizi and Paranjothy, 1990).

MATERIALS AND METHODS

PE cultures from three PORIM clones of different genotypes, and from the same subculture zone (P90, P126 and P150) were grown on MS media (Murashige and Skoog, 1962) + 0.7% agar (Sigma) + 5 µM indole-butyric acid (Paranjothy *et al.*, 1990), supplemented with glucose at 0 (control on normal multiplication media), 0.25, 0.55 and 0.82 M, and incubated at normal conditions (27°C-29°C, 12 hr photoperiod) and 1000 lux light intensity fluorescent illumination: 90 µmol m^{-2} s^{-1} PAR. The cultures were then observed for production of TI and rigid shoots over a period of nine months. The data obtained from at least five replicates (three clumps of embryoid/replicate) were analysed by analysis of variance (PORIM in-house programme).

RESULTS AND DISCUSSION

Glucose concentrations of 0.55 M and 0.82 M induced formation of TI (*Table 1*) at a significantly greater frequency ($P<0.05$) than the lower concentration and control. Generally, cultures started to form TI after more than five months in the flasks. The number of TI produced varied in the clones tested and clone P126 did not produce any TI. There was a statistically significant difference in the production of TI in clones P90 and P150 compared to clone P126 ($P<0.05$). This indicates genotype dependent variation in response to high glucose concentrations. Genotype also influenced callusing and embryogenesis in oil palm cultures (Rajanaidu *et al.*, 1997). Cultures of other species, such as peanut, have also shown significant differences between genotypes for somatic embryo formation, subculture capacity and plant regeneration (Ozias-Akins *et al.*, 1992; Chengalrayan *et al.*, 1998). Clone P126 might be more stable to external factors, and this stability may be

TABLE 1. EFFECTS OF VARIOUS LEVELS OF GLUCOSE ON PRODUCTION OF TERMINAL INFLORESCENCES FROM PE CULTURES
(data represent the mean of five replicates ±S.E.)

Duration	Months								
	3			6			9		
Clone	P90	P126	P150	P90	P126	P150	P90	P126	P150
Glucose concentration									
*Control	0	0	0	0	0	0	0	0	0
0.25 M	0	0	0	0	0	0.2 ±0.20	0	0	0
0.55 M	0	0	0	1.8 ±1.36	0	0.8 ±0.38	2.4 ±1.44	0	3.2 ±1.32
0.82 M	0	0	0	0	0	0.2 ±0.20	3.4 ±1.01	0	0.2 ±0.20

Note: *Normal multiplication medium.

maintained further until establishment in soil. However, this needs to be confirmed. Another interesting observation was that even in the same flask there was variation in the morphology of TI (*Figure 1*). This variation might have resulted from differences in the early development of shoots caused by other physiological or genetic factors. This showed that different shoots in the same flask responded differently to the glucose treatment.

Figure 1. The influence of glucose treatment (0.82 M) on inducing various types of terminal inflorescences within the same flask of clone P90 (7.5X).

Glucose at the lower concentration of 0.25 M did not induce TI formation (only one inflorescence was formed in clone P150). This TI might not have been induced by the treatment as TI are occasionally found in normal multiplication media as well (Tarmizi, 1997).

Besides the TI, the glucose also induced formation of rigid shoots (stunted, stiff leaves pointing straight up) with levels of 0.55 M and 0.82 M significantly more effective ($P<0.05$) in the induction. The number of rigid shoots varied clonally (*Table 2*) as clones P90 and P150 significantly produced more than P126 ($P<0.05$). The rigid shoots also differed in morphology, even within a flask (*Figure 2*). One of the factors in this phenomenon might be the complexity in origin of the shoot apex itself which can arise from a wide range of locations within embryoid tissues (Burgess, 1985), and so may differ in sensitivity towards the glucose treatment. Work is in progress to monitor further development of these rigid shoots.

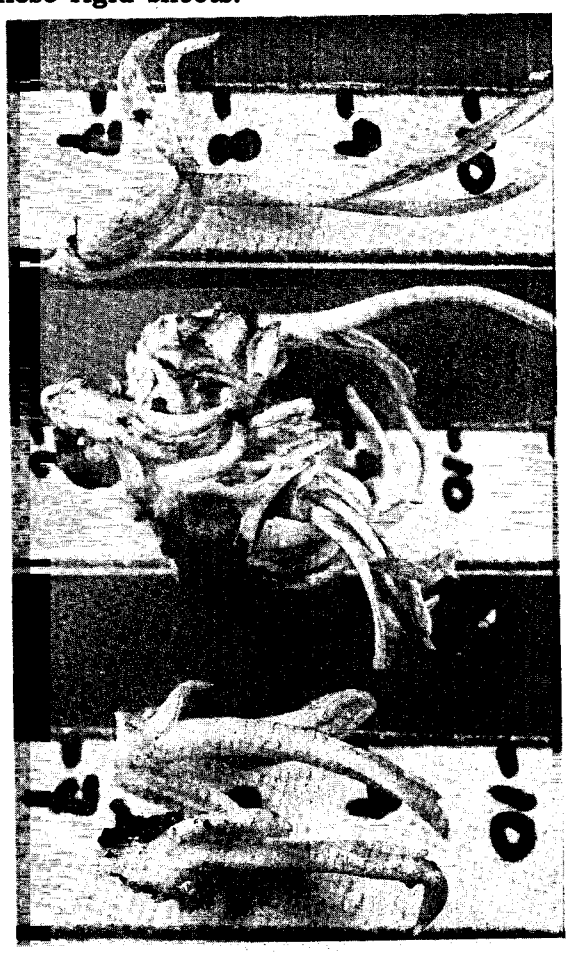

Figure 2. The influence of glucose treatment (0.82 M) on inducing various types of rigid shoots within the same flask of clone P90 (3X).

Oil palm cultures are sensitive to high concentrations of glucose. The formation of rigid shoots and also the production of TI by glucose at concentrations of 0.55 M to 0.82 M may be valuable tools to investigate the basis of *in vitro* shoot development. This is the first report of TI

TABLE 2. EFFECTS OF VARIOUS LEVELS OF GLUCOSE ON PRODUCTION OF RIGID SHOOTS FROM PE CULTURES
(data represent the means of five replicates ±S.E.)

Duration	Months								
	3			6			9		
Clone	P90	P126	P150	P90	P126	P150	P90	P126	P150
Glucose concentration									
*Control	0	0	0	0	0	0	0	0	0
0.25 M	0	0	0	0	0	0	0	0	0
0.55 M	0	2.4 ±0.40	3.4 ±0.40	17.4 ±0.51	4.4 ±1.72	16.6 ±0.51	18.4 ±0.68	4.8 ±1.91	19.8 ±0.66
0.82 M	0	0	0	10.4 ±1.21	1.0 ±0.76	9.40 ±2.25	13.8 ±1.28	1.0 ±0.76	10.60 ±1.96

Note: *Normal multiplication medium.

induction by glucose and the mechanism causing it remains unknown. BAP at high concentration also produces TI and this poses a question on the relationship between glucose and BAP. The effects of osmotic stress on redifferentiation of other cultures have been reported. Tremblay and Tremblay (1995), in their study on maturation of black spruce somatic embryos, reported that a lower concentration of glucose (than sucrose) was required to increase the osmotic potential in tissue culture medium to inhibit growth of embryos. Colmer *et al.* (1996) suggested that glucose may play an important role as an osmoticum in younger leaf blades of *Spartina alterniflora.* Thus, the stress induced by glucose in the other crops mentioned, could have been the same mechanism that induced abnormal shoots and TI in oil palm cultures.

As manifested by TI or rigid shoots, *in vitro* indexing of clonal stability can be tested for by exposing clones to stress conditions such as high concentrations of glucose. This selection may reveal sensitive or stable clones of oil palm. However, the sensitivity of cultures will have to be correlated with future field performance of the palms.

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