Effects of Picloram in Inflorescence Culture of Oil Palm

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ABSTRACT

Major factors governing tissue culture success include: 1) the laboratory and culture room environment, 2) media composition and 3) explant tissue. In addition, plant hormones have a major influence on tissue culture success as they are involved in the regulation of cell division and tissue and organ differentiation. In vivo *plant tissues have endogenous auxins that are tissue specific. In tissue culture it is often necessary to de-differentiate the ortet tissue to produce callus and then induce shoot regeneration or embryogenesis from the callus. These developmental processes are controlled by hormones. For example, the addition of exogenous auxins to the culture media is a common way of inducing callogenesis in many plant species. Synthetic auxins such as 2,4-D, IBA, NAA are commonly used for this purpose. The synthetic auxin, Picloram has rarely been used, but was recently reported to be successful in Arecanut palm tissue culture. Results at SumBio show that Picloram concentrations of 25 – 50 ppm have beneficial effects on callogenesis of oil palm inflorescence explant cultures.*

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a perennial monocotyledonous tree, native to West and Central Africa. It is one of the most important oil bearing crops in the world. Conventional breeding of oil palm often takes about 20 years per generation and is extremely slow and costly. This has made vegetative propagation of oil palm *via* tissue culture an important aspect in the development of oil palm industry to multiple elite commercial or parental genotypes. Several explant sources have been used in tissue culture of oil palm including mature seed embryos, seedlings, roots, young leaves, and inflorescences, however, there is still potential to further develop protocols for oil palm plant regeneration from these explants.

Plant hormones have a major influence on tissue culture since they are involved in the regulation of cell division, tissue and organ differentiation. Callogenesis is induced in many plant species by the addition of exogenous auxins such as 2,4-D, IBA and NAA to the culture medium. Both the type and level of auxins influence *in vitro* responses although the genotype and the developmental stage of the explants are also important factors governing success. Inflorescences are a target explant as their harvest is less damaging to the palm than immature leaf sampling and as a result there is the potential for more regular sampling. It will take a leaf sampled palm at least two years to recover before re sampling while an inflorescence sampled palm will recover quickly.

Most tissue culture studies of palms have focused on the effects of different auxin types and concentrations on various explants cultures as investigated in date palm (Othmani *et al.*, 2009, Abdul Soad, 2007, Eke *et al.*, 2005), peach palm (Steinmacher *et al.*, 2007, Valverde *et al.*, 1987), macaw palm (Moura *et al.*, 2009), rhapis palm (Daquinta *et al.*, 1997), and coconut (Verdeil *et al.*, 1994, Verdeil *et al.*, 1989). Picloram has recently been reported to be successful in *Arecanut* palm tissue culture in terms of callus and somatic embryo production. The continuous production of embryonic calli from the initial explants indicates the potential of the protocol for multiplication of palms (Karun *et al.*, 2004).

This current experiment was carried out to determine the effects of different picloram (4-amino-3,5,6-trichloropicolinic acid) concentrations on callogenesis and embryogenesis in oil palm tissue cultures using inflorescence explants.

MATERIALS AND METHODS

Inflorescences from five adult palms (0223, 0224, 0225, 0226, and 0227) which varied in sex and frond position were used for explants. The inflorescences were cut into pieces and incubated in a standard pretreatment liquid media supplemented with different concentrations of 25-50 ppm/l picloram for a week. Explants were further trimmed to about 2-4 mm and then cultured on sequences of solid MS medium containing various concentrations of picloram (1, 5, 15, 25 ppm/l) (*Table 1*). MS basal medium (Murashige and Skoog, 1962) supplemented with 170 mg/l $NaH_2PO_4.2H_2O$, 250 mg/l L-arginine hydrochloride, 1 g/l activated charcoal, 30 g/l sucrose and 7 g/l purified agar was used as solid media. The cultures were incubated in the dark at 25°C–30°C. Responses were recorded in terms of percentage callogenesis of explant cultures set up and the number palms (genotypes) that produced callus.

Table 1. Concentration of picloram in pre-treatment liquid and solid media.

Treatment	Picloram concentration (ppm)				
	Liquid	Solid media sequences			
1 (control)	0	0	0	0	0
2	0	25	15	5	1
3	25	25	15	5	1
4	50	25	15	5	1

RESULTS AND DISCUSSIONS

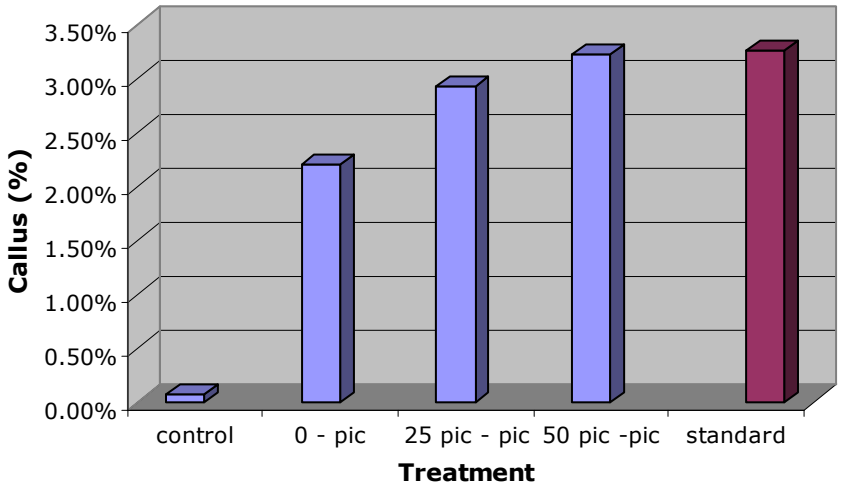

Figure 1. Effect of different treatments in terms of percentage callogenesis.

The effect of picloram supplemented liquid and solid media on the callogenesis percentages was observed. Before culturing on solid media sequences supplemented with picloram, explants were cultured in pre-treatment liquid medium that contained picloram (25 and 50 ppm) for a week. All treatments produced callus after 8 weeks incubation as an explant. *Figure 1* shows that the percentages of callus formed increased when the concentration of picloram in the pre-treatment liquid medium was increased from 25 ppm to 50 ppm. Compared to the control that was supplemented with 2,4-D and NAA (zero picloram), the effect of picloram was found to be greatly beneficial in increasing the rate of callogenesis. The SumBio standard treatment uses higher levels of 2,4-D in liquid media and higher levels of NAA in solid media compare to the control treatment. Figure 1 shows that picloram (treatment 4) has similar callogenesis rates with standard treatment. The callogenesis rate may further increase since this experiment is still on going. Furthermore, the picloram treatment started to produce embryoid cultures (*Table 2*) at 33 – 56 weeks time after sampling. The standard treatment has provided direct embryogenesis from explant culture but it has only produced two embryogenic cultures from 5366 explants cultures.

Table 2. Number of embryoid produced (embryogenesis) arising from the different pre-treatments and treatments.

Treatment	Embryo	Time embryogenesis (weeks)
control	0	Not achieved after 72 weeks
0 - pic	0	Not achieved after 72 weeks
25 pic - pic	2	56
50 pic -pic	1	33
standard	2	17

Both the type and level of auxins had effects on *in vitro* responses though the genotype and the developmental stage of the explants also influenced responses. However, the results show that the callogenesis rates were also determined by genotypes although this apparent variation could also be confounded with other

environmental factors (*Figure 2*). Palm 0223 was the most responsive to picloram supplementation in media. Palm 0226 gave no response to picloram treatment, while there were no significant differences for the other palms.

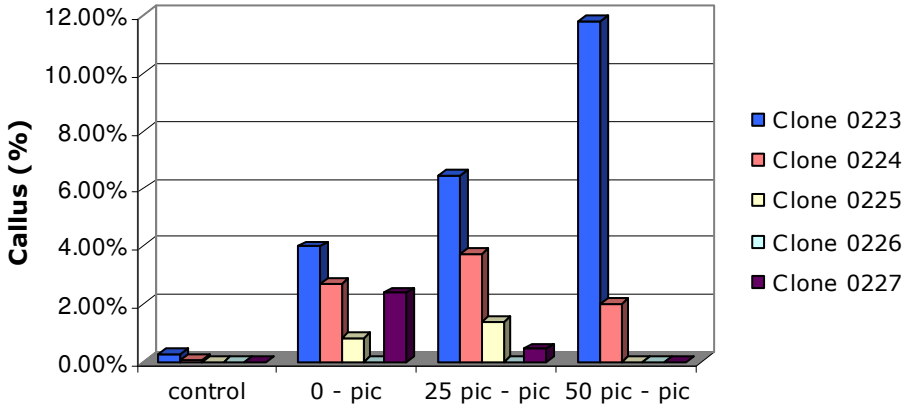

Figure 2. Callogenesis rates in cultures from different genotypes.

Inflorescences were harvested from eight different frond numbers (*Table 3*). *Figure 3* shows that frond number influenced the percentage callogenesis. Inflorescences from frond number 16 gave the best rates of callogenesis followed by frond number 17 and 15 in this experiment. Fronds 18 and 19 had lower rates of callogenesis rates. In addition, frond numbers 12, 13, and 14 showed negative responses to the addition of picloram. Verdeil *et al.*, 1994 reported that younger inflorescences as explants were more responsive than older inflorescences for coconut tissue culture. Different results were obtained with the standard treatment. Inflorescences explants of fronds 9 to 11 gave the higher callogenesis rate compared to old fronds. Younger fronds were more responsive using standard treatment.

Table 3. Frond number of inflorescence used in experiment and standard.

Treatment	Frond
standard	9,10,11,13,14,15,16,17,18
control	
0 - pic	12,13,14,15,16,17,18,19
25 pic - pic	
50 pic - pic	

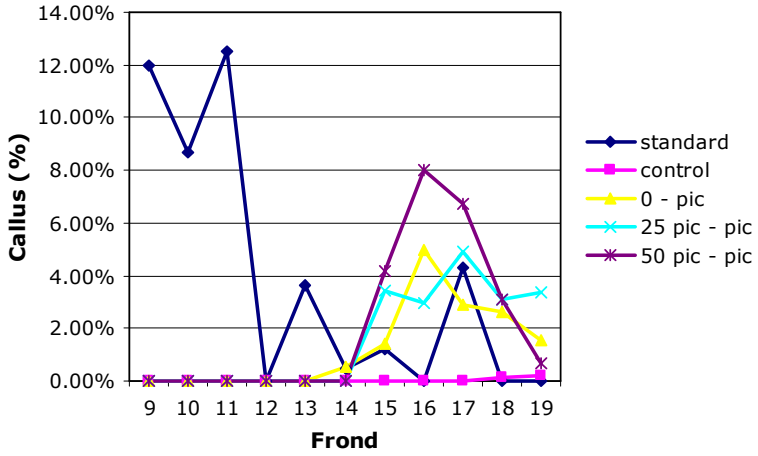

Figure 3. Callogenesis rates in terms of the responses of different frond number.

The percentages of callogenesis were also influenced by the type of inflorescence as shown in *Figure 4*. There was a different response between male and female inflorescences. The percentages of callogenesis increased when the concentration of picloram in the pre-treatment liquid medium was increased from 25 ppm to 50 ppm for female inflorescences. Female inflorescences were more responsive to picloram supplementation in media than male inflorescences. While in *Rhapis* palm culture similar percentages of callogenesis were achieved for male and female inflorescences (Daquinta *et al.*, 1997). The results from the current experiments are consistent in always showing greater response of the female inflorescences although the response is similar in the standard treatment.

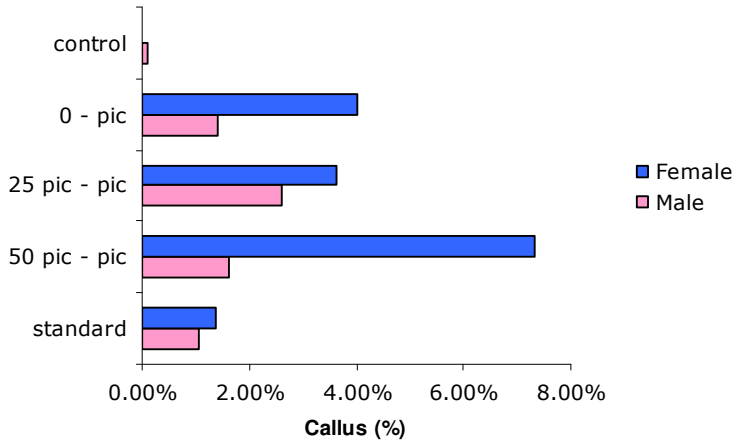

Figure 4. Callogenesis rates based on inflorescence type.

The current results provide an interesting basis for further experimentation into the potential use of picloram in the media for inflorescence cultures of oil palm. The treatments provide evidence of differential effects in regard to response of genotype, frond number and sex but generally show a positive effect of picloram. Clearly results need to be obtained about the later behaviour of the embryos produced. It would also be worthwhile testing further the potential of picloram to influence callogenesis and embryogenesis in early frond numbers where currently in the standard treatment shows the highest response.

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