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RESEARCH PAPER

Callus Induction from Inflorescences of Oil Palm

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ABSTRACT

Callus was induced from inflorescence collected from different axils of palms on a modified MS media with 0.3% activated charcoal and 475µM 2, 4-D. The opened inflorescences of both male and female responded poorly to callus induction. Immature inflorescence collected from 9th, 10th, 11th and 12th axils of four different palms gave a callus induction percentage ranging from 23 to 72%. There was a genotypic effect in response of different inflorescences which was shown as the differences in response to the same media. A gradient callus induction response towards the shoot apex was observed; Immature and internal inflorescence could be used as explants as the callus induction percentage and time taken for callus induction was less.

Key words: Callus induction, inflorescence, oil palm

Abbreviations : 2, 4-D: 2, 4- Dichlorophenoxyacetic acid, MS: Murashige and Skoog

INTRODUCTION

Tissue culture in oil palm has been reported from several explants. But the reports of tissue culture from explants from mature trees are very few in number. Most of the reports are tissue cultures from embryos. To get a true to type plant, we need to collect explants from mature trees. Out of the different explants like spear, root and inflorescence, which can be collected from mature trees inflorescence is one of the explants, which can be collected with limited damage to the tree. Another advantage with unexposed inflorescence is that they are protected by leaf like sheaths which prevent infestation by fungi and bacteria and hence can avoid the chemical damage from sterilization. However the only literature available on tissue culture of inflorescence was that of Texeira *et al*. (1994). Hence the present study was undertaken to find out the response for callus induction of inflorescence collected from different leaf axils.

MATERIALS AND METHODS

Three different types of inflorescence were used in this experiment. Mature male and female inflorescence was collected from fully opened inflorescence of five palms. The details of the palms are given in Table 1. Immature inflorescences of both male and female were collected from 9th, 10th, 11th and 12th leaf axil from four different palms including one pisifera, one dura palm from Zambian accession and two *tenera* palms (264 and 114). Internal inflorescence (male and female) nearer to the spear from the 1st leaf, 0 leaf and below 0 collected from three tenera palms (Tenera 433, 505 and one tenera from Palode) and inoculated onto the callus induction media. The fully opened and immature inflorescences were surface sterilized using 20% sodium hypochlorite for 20 minutes and rinsed three times in sterile distilled water and sliced into 1 to 2 mm sections before inoculating into the media. In this case sterilization was not required and hence the outer most leaf sheath was removed and they were sliced into 1 to 2mm sections and inoculated.

Explants were inoculated onto a previously reported media (Texeira *et al.,* 1994) for callus induction from inflorescence. The media used was a modified Murashige and Skoog (1962) media containing half strength major salts, full strength minor salts and iron

and several other additives. The auxin used was 2,4-dichlorophenoxyacetic acid at 475µM and 3% activated charcoal and 3% sucrose. The pH was adjusted to 5.8 with either 0.1N NaOH or 0.1 N HCl prior to adding 0.8% (w/v) agar and sterilized at 121°C at 1.06 kg/cm^2 for 15 min. Explants were cultured in dark at 27-29°C and subcultured in the same media after 12 weeks. The observations for callus induction were taken at the end of 24 weeks. Each experiment was conducted with 15 explants and three replications. The observations are presented as the average of three replications.

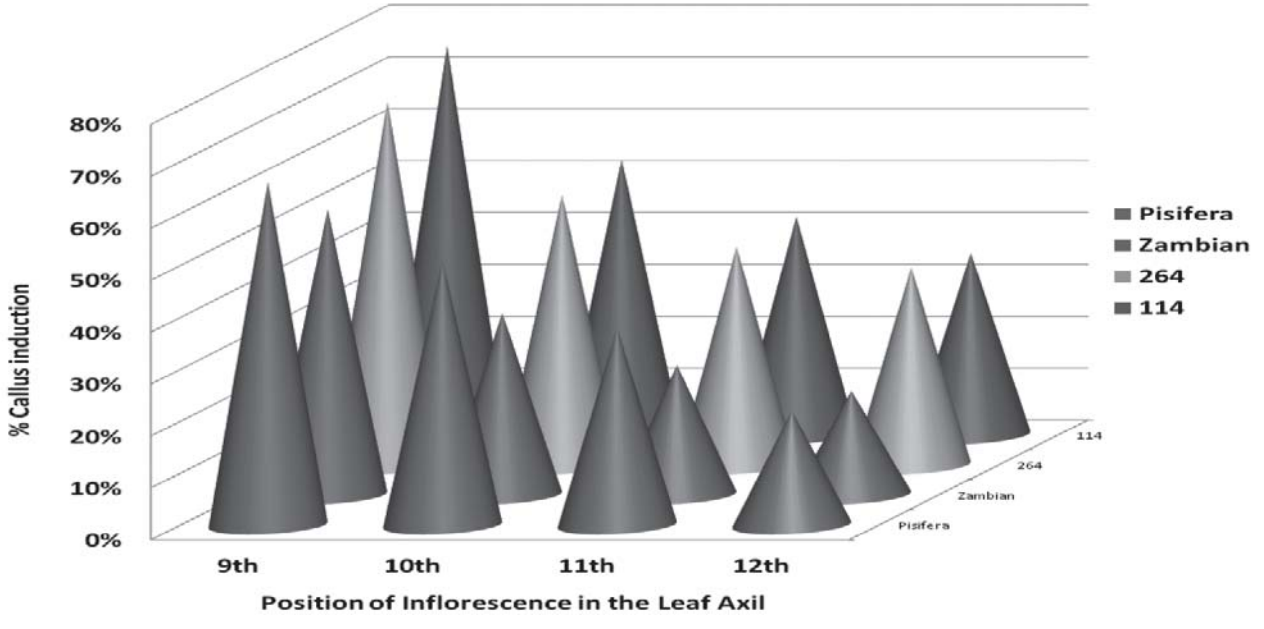
RESULTS AND DISCUSSION

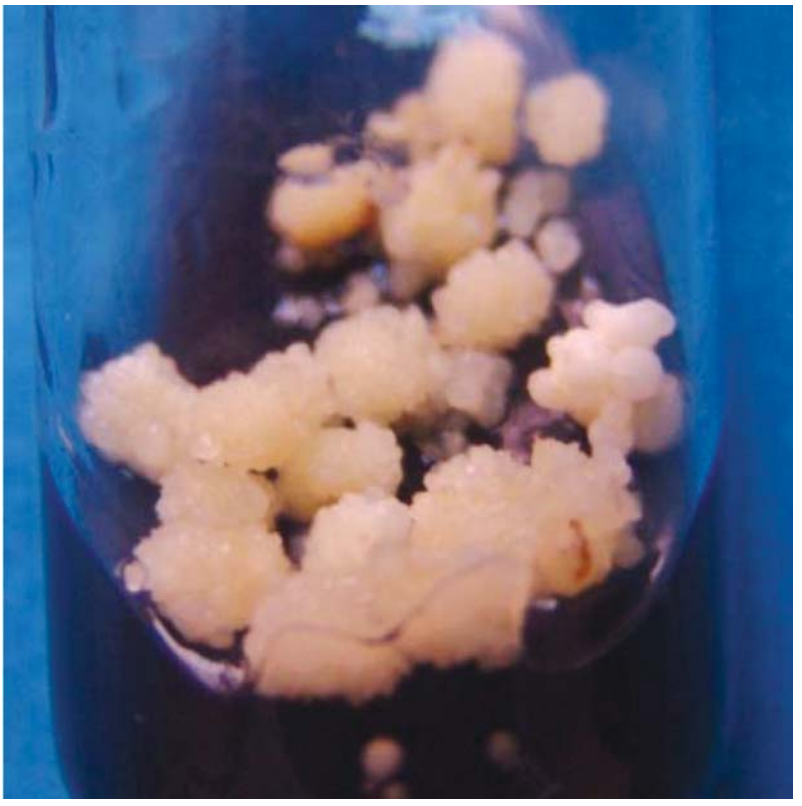
There was no response for callus induction in opened inflorescence from palm no. 182, 156 and 199 (Table 1). The phenolic exudates were seen and the explants turned black and died. The response of inflorescence of palm no. 395 (22 %) and 414 (11 %) was seen as white globular structures arising from inflorescence after a period of 12 weeks. Further subculture, they turned brown and failed to respond further.

The inflorescence collected from 9th, 10th, 11th and 12th leaf axil from four different palms swelled enormously at the end of 12 weeks and responded differently to callus induction after the first subculture. The inflorescence collected from the 9th leaf axil of palm no. 114 gave the maximum callus induction response (72%) followed by palm no 264 (70%). Among the four different inflorescences, inflorescence collected from 9th leaf axil give the maximum response in all the palms. Inflorescence collected from the four different axils are consistently showing a lesser response in pisifera palm compared to other palms, depicting the genotypic influence.

The maximum response for callus induction was obtained with internal inflorescence i.e., at the end of 12 weeks, it was found that there was enormous swelling and after the second subculture, the callus induction was faster. They swelled enormously and the edges started producing callus. The callus induction percentage obtained with internal inflorescences ranged from 84-91%. The callus obtained was of two types non embryogenic (a hard hairy nature and does not proliferate further) and Embryogenic callus (nodular and growing faster and on subculture, it further proliferates).

The tissue culture with inflorescence explants was during 1970's by Smith and Thomas (1973) and Ong (1977) and they reported poor response of inflorescence tissues. Texeira *et al*., (1994) reported successful culture of immature inflorescence. Embryogenic callus can be induced and multiplied from inflorescence explants of oil palm and plants can be regenerated. The success in obtaining callus formation was likely due to the use of very young inflorescences, and the presence of suitable concentration of 2, 4-D in association with activated charcoal. In the present study also, it was found that immature and internal inflorescence responds well in culture, due to meristematic nature of the explants. The non responsive nature of mature inflorescence may be due to excessive amount of phenolics present in them. It has been reported in other palms like date palm that, blackening and death was a common feature (Tisserat.,





Callus formation in oil palm

1981). Oxidation and phenolic accumulation has been a common problem in palm tissue cultures (Blake and Eeuwens, 1991; Jones, 1974; Nwankwo and Krikorian, 1983). Texeira *et al*. (1994) reported that the problem of oxidation of inflorescence explants of oil palm was high and could be overcome by use of an adequate level of activated charcoal and PVP-40, associated with an optimal concentration of 2,4-D. The use of PVP-40 was also helpful in reducing charcoal flocculation during medium preparation. Our results showed two important factors affecting the response. One is a genotypic effect and other is the position of the explants in different leaf axils on the palm has an influence on callus induction. This could be a kind of age-related response, because the position is related to the physiological age of explants. A gradient callus induction response towards the shoot apex was observed, and the closer (younger) to the apex the higher the response. This kind of response has been demonstrated in other monocot plants like *Hordeum* (Becher *et al*., 1992), *Miscanthus* (Holme and Petersen, 1996), and *Avena* (Chen *et al*., 1995). To conclude, the present study demonstrates that inflorescence tissues especially, immature and internal inflorescence could provide better explants due to better and faster callus induction.

Table 1: Callus induction percentage from inflorescence of different palms

Palm no	Inflorescence type	Response obtained
182	Male (opened)	0%
156	Male (opened)	0%
199	Female (opened)	0%
395	Male (unopened)	22%
414	Male (unopened)	11%
Palode	0, -1,-2 and -3 (Internal inflorescence)	84%
433	0,-1,-2 and -3	86%
505	0, -1,-2 and -3	91%

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