International Journal of Oil Palm, **8 (1&2)** : 9-12, June and December, 2011

RESEARCH PAPER

Efficacy of Dyes and Media on Pollen Viability and Germinability in Oil Palm (*Elaeis guineensis* Jacq.)

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ABSTRACT

The hybrid nature of commercially cultivated oil palm necessitates artificial hybridization between proven parent palms for development of planting material. A successful hybridization greatly depends on the quality of pollen *viz.* viability and germination capacity. Five established dyes and eight different media were used to assess the pollen viability and germinability. 2, 5-diphenyl Tetrazolium bromide (MTT) had high correlation of viability with *in vitro* germination. The media consisting of 2.5 % Sucrose; 100 ppm H_3BO_3 and PEG 10% showed maximum germination with pollen tube length of 317.88 µm.

Key words : Oil palm, pollen, viability test, *in vitro* germination.

INTRODUCTION

Oil palm is a major oil producing crop in the world and it is reported to yield 5 tonnes of oil per hectare. The hybrid nature of crop necessitates artificial hybridization between proven dura and pisifera palms for development of commercial planting material. A successful hybridization greatly depends on the quality of pollen *viz.*, viability and germination capacity (Beyhan 1993; Beyhan and Odabas, 1995). Pollen viability and vigour are important for successful pollination and is generally considered to indicate the ability of the pollen grains to perform their function of delivering the sperm cells to the embryo sac following compatible pollination (Shivanna *et al.*, 1991).The viability is greatly affected by the original quality of the pollen as well as the storage conditions. In date palm, pollen grain was reported to have a direct effect on size, shape and weight as well as time of ripening (DeMason and Sekhar, 1988).

For assessing the pollen viability and germinability, various methods were reported in horticulture crops and a large variety of dyes and media have been used. Many previous reports established the differential response of pollen to media composition and it also dramatically affects pollen metabolism (Taylor and Hepler, 1997). Moreover, it was established that the rate of *in vitro* pollen germination depends largely on the success of finding the optimal medium. Pollen grains of many species would easily germinate in a medium that contains boric acid and an osmoticum (Taylor and Hepler, 1997). Despite the simple basic requirement of pollen tube growth media, the optimal composition may vary from species to species and the use of suboptimal media could underestimate pollen quality.

In addition, pollen germination rates usually provide more reliable data on pollen viability than vital stains. In general, a linear relationship is expected between viability and germination ability. It is reasonable to assume that, pollen which cannot be made to germinate by usual means or which on germination, show poor tube growth, is likely to be ineffective in causing fertilization. Hence eight different media were screened for *in vitro* germination to assess the germinability of oil palm pollen by evaluating the parameters like pollen tube length (PTL) and germination percentage.

MATERIALS AND METHODS

Collection, processing and handling of pollen

The male inflorescence selected for pollen collection was bagged five days before anthesis. The inflorescences were surface sterilized with disinfectant mist-sprays of 2% formalin solution to kill any pollinating agents such as weevils, insects, thrips and earwigs. The inflorescences were later isolated *in situ* with a pollen collection bag, usually of 74 x 86 cm size, having a celluloid or plastic observation window of 16 x 16 cm at the front. During the bagging process, the stalk at the bottom of the bag was wrapped with a layer of rolled cotton wool dusted with insecticide and then secured with a strong fine wire.

Male inflorescence was harvested along with the bag after 80 - 90% of the flowers have anthesized. The bag is first left for air-drying and then dried for 24 h in the heating room at 38 - 40°C for preliminary drying of pollen grains. Upon completion, the bag was well shaken to dislodge pollen grains from the spikelets. When all the pollen grains have been detached, they were transferred to paper bag and sieved using 150 µm brass-sieve and contaminant free pollen grains were poured into an envelope. Pollen envelopes were then put in oven at 38-40°C for 24 h to ensure that they were totally dry. Drying temperature was maintained at not more than 40°C to avoid damage to the pollen grains. Thus the moisture was maintained below 7%. While testing for *in vitro* germination, the pollen was rehydrated by spreading on clean cavity slide and keeping the slide in a petri dish with moist filter paper (RH >95%) for one hour at ambient temperature.

Fresh pollen was collected from dwarf progeny field at Directorate of Oil Palm Research, Pedavegi, Andhra Pradesh, India. Three kinds of pollen samples were used - fresh pollen, dead pollen (pollen heated to 100°C for 24 hours to kill the pollen) and stored pollen (pollen stored for 10 months in deep freezer at -20°C).

Two methods for raising pollen cultures namely sitting drop culture and surface culture were selected for the study.

RESULTS AND DISCUSSION

1. Screening of media for *in vitro* germination

Response of oil palm pollen to different media composition in terms of germination percent and pollen tube length is presented in Table 1. Among eight media combinations used, germination of oil palm pollen was poor in case of M1, M2, M4, and M6. The tube length in M8 was good, but germination was low. The response was fairly good in M3 and M5 media but the pollen tube length was short in both the cases. The M7 media produced maximum germination with pollen tube length of 317.88 µm. The media thus selected was employed for assessing the efficiency of the dyes for pollen viability. Many previous reports established the differential response of pollen to media composition (Taylor and Hepler, 1997).

2. Selection of dye for viability test

The results of viability test using the five dyes are shown in Table 2. Dyes in the first group (Baker's and X-Gal) always stained dead pollen. X-Gal has positively

The following eight treatments were tried

Media code	Composition
M1	8% Sucrose; 200 mg/L $MgSO_4$ 7 H_2O; 250 mg/L Ca (NO_3) 4H_2O; 100 mg/L KNO_3; 100 mg/L H_3BO_3 (Rosell *et al.*, 1999)
M2	0.8% Agar; 10% Sucrose; 50ppm Citric acid (Ateyyeh *et al.*, 2000)
M3	10% Sucrose; 2 x 10^{-3} M H_3BO_3; 6 x 10^{-3} M Ca $(NO_3)_2$ (Shivanna and Rangaswamy, 1992)
M4	15% Sucrose; 2 x 10^{-3} M H_3BO_3; 6 x 10^{-3} M Ca $(NO_3)_2$ (Shivanna and Rangaswamy, 1992 (modified))
M5	1.2% Agar; 11% Sucrose (Turner and Gilbanks, 1974)
M6	1% Agar; 200 mg/L $MgSO_4$; 100 mg/L KNO_3; 100 mg/L H_3BO_3; 200 mg/L Ca NO_3 (Brebaker and Kwack,1964)
M7	2.5% Sucrose; 100 ppm H_3BO_3; PEG 10% (Tandon *et al.*, 2001)
M8	2.5% Sucrose; 100 ppm H_3BO_3 (Tandon *et al.*, 2001 (modified))

Table 1. Effect of media on *in vitro* pollen germination

Media	Germination (%)	Pollen Tube Length (μm)
M1	0.51(0.94)[c]	152.50 (4.59)[d]
M2	0.00 (0.70)[c]	0.00 (0.70)[d]
M3	47.57(6.91)[b]	140.78 (11.86)[c]
M4	2.68 (1.45)[c]	107.45 (3.94)[d]
M5	65.44 (7.99)[b]	146.73 (12.13)[bc]
M6	0.00 (0.70)[c]	0.00 (0.70)[d]
M7	97.94 (9.93)[a]	317.88 (17.78)[b]
M8	3.80 (2.09)[c]	584.00 (24.13)[a]
CD(0.05)	1.50	5.84
F test	**	**

Figures in parenthesis indicate transformed values
Figures with same superscript do not differ significantly

stained more than a third of the dead polyads in *Acacia* species. In oil palm, Baker's dye stained stored pollen faster than fresh pollen, and X-Gal stained fresh and stored pollen at the same rate. In both the cases, as there is no colour differentiation between fresh, stored and dead pollen, it was impossible to differentiate between fresh and dead pollen. Hence, it was decided that Baker's and X-Gal are not suitable for testing pollen viability in oil palm.

The second group dyes (TTC, MTT, p-Phenylenediamine) never stained dead pollen. In chestnut genotypes, TTC did not give a reliable estimation for pollen germinability. Similar was the case with oil palm pollen, as it showed only 48.87% viability for fresh pollen and 23.12% viability in stored pollen. It's performance in the present study is very low with low correlation of viability with *in vitro* germination. However MTT performed better with high correlation of viability with *in vitro* germination. MTT recorded 98.51% of viability for fresh pollen and 85.99% viability for stored pollen and did not stain dead or aborted pollen. Rodriguez-Riano and Dafni (2000) reported that the use of p-Phenylenediamine as the most reliable method to distinguish between fresh and dead pollen by showing high correlation with *in vitro* germination of fresh pollen for many species. Though it did not stain dead or aborted pollen in oil palm, it showed very poor response with low viability for fresh (43.02%) and stored pollen (38.93%).

Hence, the use of MTT was the most reliable method to distinguish between fresh and dead pollen in oil palm, since dead pollen was never stained and also strongly contrasted with the colour of fresh pollen.

CONCLUSION

In this study, MTT dye was most effective in determining the exact amount of viable pollen in oil palm. The media suggested by Tandon et *al.,* (2001) consisting of 2.5% Sucrose; 100 ppm H_3BO_3 and PEG 10% was found most effective for *in vitro* germination test in oil palm.

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Table 2. Efficacy of various dyes in assesing pollen viability in oil palm

Dye	Viability percent of different pollen samples		
	Fresh pollen	Stored pollen	Dead pollen
Bakers	78.19 (8.84)[b]	90.63 (9.51)[a]	65.11(8.09)[b]
X-Gal	89.63 (9.46)[ab]	88.12 (9.38)[a]	77.43 (8.82)[a]
TTC	48.87 (6.96)[c]	23.12 (4.81)[c]	0.00 (0.70)[c]
MT T	98.51(9.92)[a]	85.99 (9.27)[a]	0.00 (0.70)[c]
p-Phenylenediamine	43.02 (6.55)[c]	38.93 (6.23)[b]	0.00 (0.70)[c]
CD (0.05)	0.71	0.53	0.22
F test	**	**	**

Figures in parenthesis indicate transformed values
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