Journal of Animal & Plant Sciences, 2012. Vol. 14, Issue 1: 1855-1865
Publication date: 31/5/2012, http://www.m.elewa.org/JAPS; ***ISSN 2071 - 7024***

Determination of the optimum pollen germination medium for different fruit forms of oil palm (*Elaeis guineensis*)

K. A. Myint[1, 2], M.Y. Rafii[1, 3], S.A Sheikh-Abdullah[1], N. M. Lwin[2] A. Mohd Din[4] and M. A. Latif[1]

[1]*Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Myanmar Perennial Crops Enterprise, Ministry of Agriculture & Irrigation, Myanmar*
[3]*Institute of Tropical Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4] *Malaysian Palm Oil Board, P.O. Box 10620, 50720, Kuala Lumpur, Malaysia*

*Corresponding address: Dr. M.Y.Rafii, Institute of Tropical Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. , Email: mrafii@putra.upm.edu.my, Tel: +60389474825

Key words: pollen viability; *in vitro* germination; oil palm; germination media; sucrose solid medium

1 SUMMARY

The study was conducted to determine the optimum medium for *in vitro* pollen germination in African oil palm, *Elaeis guineensis* Jacq. based on germination percentages and length of pollen tube. The viability of pollens from three fruit forms of 20-year old *dura, pisifera,* and *tenera* palms was investigated at 60,120 and 180 min after incubation at 35°C using three kinds of medium: 11% of sucrose solid media, 11% of glucose solid media and 2.5 % of sucrose liquid media with 0.1% of boric acid. The longest mean pollen tube length was observed in *dura* pollen on all three media at 60, 120, and 180 min after incubation. The pollens germinated on sucrose solid medium had longer tube length than those germinated on glucose and sucrose liquid media. Adoption of sucrose solid medium as germination medium for the estimation of germinability and tube length of pollen in oil palm is suggested.

2 INTRODUCTION

Pollen plays a critical role in plant cycle, as viable pollen is crucial for efficient sexual plant reproduction (Bots and Mariani, 2005). The capacity of pollen to germinate and grow normally is termed as viability. High viable pollen will increase the percentage of fruit set and ultimately will produce high yield. Viability test helps in selecting pollen types that are highly viable. Pollen availability and its viability are important in oil palm production. Therefore, an estimation of pollen viability is essential for fruit setting. The fastest way of analyzing pollen viability is using vital stains that react with pollen enzymes, thereby indicating the presence of intact cellular content. Another frequently used method to access pollen viability is *in vitro* germination. Because the pollen grains of many species will easily germinate in a medium that contains boric acid and an osmoticum, this method is widely used (Taylor and Hepler, 1997). Despite the simple basic requirements of pollen tube growth media, the optimal composition may vary from species to species and the use of suboptimal media may underestimate pollen viability. Still pollen germination rates usually

provide more reliable data on pollen viability than vital stains. Finally, pollen viability may be measured after pollination, by analyzing germination on the stigma or seed set derived from that pollination. Both methods are time consuming and may lead to an overestimation of pollen viability if the pistil is over-pollinated (Bots and Mariani, 2005). Pollen germination and the growth of pollen tubes are, in principle, necessary for fertilization and seed formation in flowering plants. Studies on *in vitro* pollen germination and pollen tube growth are very useful for explaining the lack of fertility (Pfahler *et al*., 1997). *In vitro* pollen germination is one of the most convenient and reliable methods used to test the viability of fresh or stored pollen grains. It is a valuable tool to address basic questions in sexual reproduction because fertilization ability of pollen grains is a function of both germination as well as pollen tube growth (Gwata *et al*., 2003). Pollen viability, as determined by the *in vitro* germination assay, has already been mentioned as being correlated to fertilization success (Hicks *et al*., 1987). Because this criterion (germination %) generally follows that of the pollen-tube length, the relative importance of each one has not been discussed (Kuo *et al.,* 1981). As far as *in vitro* studies are concerned, the pollen-tube length criterion was very seldom used before 1980 (Heslop-Harrison *et al*., 1984). Nowadays it and the commonly used criterion of pollen germination percentage are frequently used in studies on the relationship between pollen quality tested *in-vitro* and fertilization results obtained *in vivo* (Kuo *et al*., 1981; Asif *et al*., 1983; Heslop-Harrison *et al*., 1984; Vasilakakis and Porlingis, 1985; Chichiricco and Grilli-Caiola, 1986; Know *et al*., 1987; Kerhoas and Dumas, 1987).

In vitro growth of pollen tube is adopted to evaluate pollen grain germinability. The development of the pollen grain tube is primarily influenced by temperature and air humidity and *in vitro* growing media, thus optimal conditions must be defined for different species. Different substances used in the germination media are needed to compensate for the difference between the *in vitro* environment and the natural conditions on stigma. Pollen grains are morphologically simple and the process of tube formation is a relatively uncomplicated example of growth and development. For these reasons, and because of the rapid rate of tube formation *in vitro* exhibited by some species, pollen tube formation has become a model system for studying growth and development in plants.

Oil palm pollen when moist rapidly losses its viability, with a marked drop in percentage germination being found in a moist sample within 48 hr after collection (Turner and Gillbanks, 2006). The quality of the pollen deteriorates with time and such pollen, if used for pollination purpose, can result in seeds with defective embryos (Chin, 1999). The assessment of viability of freshly collected as well as stored pollen is often desirable before using them for pollination. Germination only takes place under moist conditions, and various media and methods of their preparation have been suggested. Basically, the pollen grains are germinated in or on a simple medium of plain sucrose, with or without the presence of agar (Turner and Gillbanks, 2006). It was sometimes suggested that addition of boric acid might improve or accelerate germination rate, but tests showed that this addition was generally without effect on total germination (Turner and Gillbanks, 2006). There seem no doubts that the use of different stored pollen, or even of genetically different pollen similarly stored, may have different effect not only on germination but also on early seedlings growth (Hartley, 1988). The present study was undertaken to find out optimum pollen germination medium for different fruit forms of oil palm.

Journal of Animal & Plant Sciences, 2012. Vol. 14, Issue 1: 1855-1865
Publication date: 31/5/2012, http://www.m.elewa.org/JAPS; ***ISSN 2071 - 7024***

3 MATERIALS AND METHODS

Pollens from three fruit forms of 20-year old *dura, pisifera,* and *tenera* palms were collected from Malaysia Palm Oil Board/University Kebangsaan Malaysia (MPOB/UKM) Research Station. The pollen grains were taken from one inflorescence of each palm of *dura, pisifera* and *tenera* and viability test was carried out immediately. Pollen grains were rehydrated before culture by spreading them on a dry slide and maintaining the slide in petridish lined with moist filter paper (RH>95%) for 1h. Oil palm pollen usually commences emergence of tube at around 1h after incubation in oven at 35˚C. The percentage of germination (PG) and pollen tube length (PTL) was evaluated in 60 min interval up to 180 min. This experiment was conducted in completely randomized design with four replications. To determine an optimal *in vitro* testing medium, 3 types of media were prepared with two types of sugar (sucrose and glucose). The media were sucrose, glucose and sucrose liquid. Pollen germination percentage and tube length were recorded. The solid medium of sucrose was prepared with 1.2 g of agar and 11g of sucrose while the solid glucose medium was prepared with 1.2 g of agar and 11g of glucose. In both media, 100ml of distilled water was added and was heated up to boiling point. For preparation of the sucrose liquid medium, 2.5% sucrose solution, which contained 0.01% boric acid (100 ppm), was used. Pollen suspension was prepared in liquid solution and then hung from an ordinary microscope slide. After the drop was inverted and it was incubated in an oven at 35˚C for 3 hours, percentage germination was readily determined under microscope. Pollen germination was determined by direct microscopic observation. A pollen grain was considered germinated when pollen tube length (PTL) was at least equal to or greater than the grain diameter (Kakani *et al.*, 2002). Percentages of pollen germination were calculated by the following equation.

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated pollen grains per field of view}}{\text{Total number of pollen grains per field of view}} \times 100$$

Pollen tube lengths were measured using an ocular micrometer that was fitted to the eyepiece of Olympus microscope. Twenty pollen tubes were measured in each replication. The speed of germination of pollen grains (PGR) (%/min) and the rate of pollen tube growth (PTLR) (µm min^{-1}) were also measured in order to determine pollen vigor with the measuring time course of 60, 120, and 180 min after incubation. Means from all experiments were subjected to analyze of variance by SAS 9.2 (SAS Institute, Cary, NC). Tukey's studentized range (HSD) test was used for means comparison among three germination media.

4 RESULTS

4.1 Effect of different media on pollen germination percentage (PG) and rate (PGR): The present investigation determined the optimum germination medium for oil palm pollen viability. For germinability, analysis of variance indicated the significant effect on pollen germination percentage among the media except in *tenera* (Table 1).

Table 1: Mean squares of ANOVA for germination percentage (PG) of three oil palm fruit-forms on three germination media

S.V.	df	Mean Square of germination percentage (%)								
		Dura			***Pisifera***			***Tenera***		
		60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Germination medium	2	272.8**	186.7*	219.3*	721.8**	835.0*	845.3*	276.0^{ns}	93.8^{ns}	78.8^{ns}
Error	9	29.6	40.3	42.1	84.7	113.0	121.4	72.5	59.7	59.6

**, * and ns are significant at p≤0.01, p≤0.05 and non-significant at p>0.05, respectively, S.V.-Source of variation; df- Degree of freedom

The solid sucrose medium increased the germination percentage in *dura* and *tenera* pollen at 60, 120, and 180 min after incubation. The germination percentage of *tenera* on sucrose medium also increased but not statistically significant from other media. However, for *pisifera* pollen, the effect of sucrose liquid medium was more pronounced (Table 2).

Table 2: Means of germination percentage (PG) in three oil palm fruit-forms on three germination media at three different counting times

Germination Medium	*Dura*			*Pisifera*			*Tenera*		
	60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Sucrose	69.7a†	74.9a	75.8a	23.4b	26.8b	27.3b	78.2a	81.8a	82.7a
Glucose	56.5b	70.3ab	75.5ab	30.8b	35.5ab	36.5ab	68.0a	78.8a	82.7a
Liquid	54.5b	61.4b	62.8b	49.5a	55.0a	55.7a	61.8a	72.4a	75.0a

†Values within each column for each fruit-form followed by same small letters are not significantly different at $p \leq 0.05$ by Tukey's HSD.

The germination percentages was higher both in *dura* and *tenera* at sucrose solid medium on the other hand the germination percentages was higher in *pisifera* at sucrose liquid medium (Fig. 1, 2 and 3). The present study on pollen viability through *in vitro* pollen germination using different media revealed that the increase rate of germination percentage was the highest at 60 min after incubation for all 3 tested pollen sources (Table 3 and 4). The germination rate was discovered to be less significant at 120 and 180 min after incubation (Table 4).

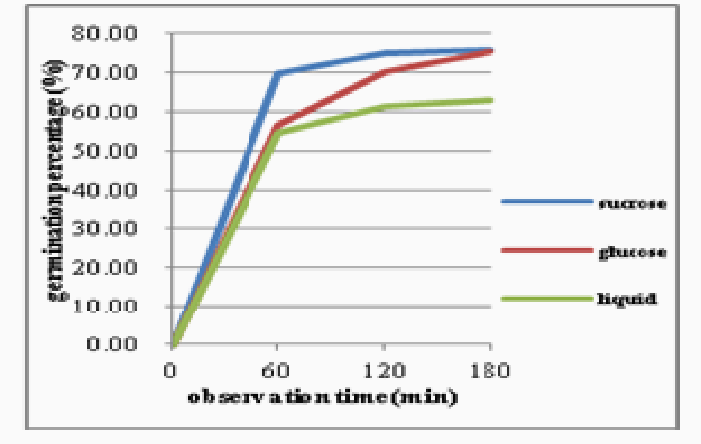


Figure 1: Germination percentage of *dura* pollen as a function of germination time

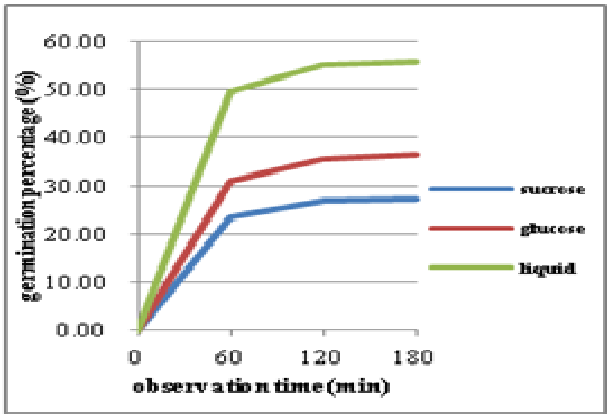


Figure 2: Germination percentage of *pisifera* pollen as a function of germination time

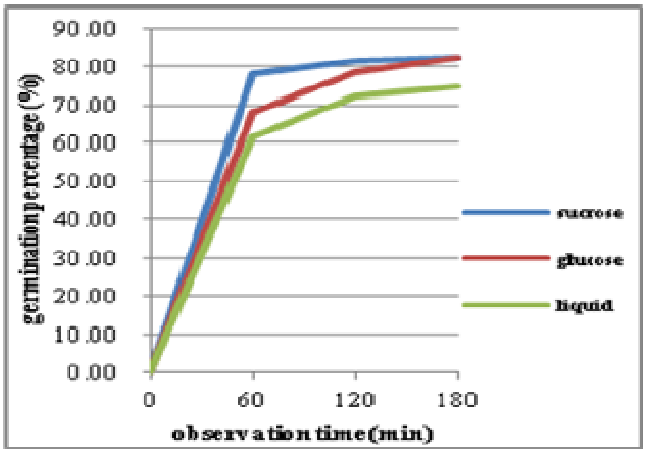


Figure 3: Germination percentage of *tenera* pollen as a function of germination time

Table 3: Mean squares of ANOVA for germination rate (PGR) of three oil palm fruit-forms at three different counting times

S.V.	df	Mean Square of germination rate								
		Dura			***Pisifera***			***Tenera***		
		60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Germination medium	2	0.077**	0.023**	0.006^{ns}	0.201**	0.001^{ns}	0.00003^{ns}	0.073^{ns}	0.018**	0.003^{ns}
Error	9	0.009	0.002	0.004	0.024	0.004	0.0002	0.020	0.001	0.001

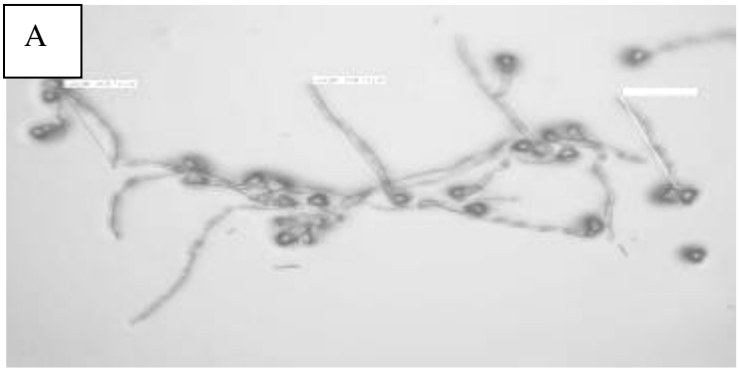
**, * and ns are significant at p≤0.01, p≤0.05 and non-significant at p>0.05, respectively, S.V.-Source of variation;
df- Degree of freedom

Table 4: Means of germination rate (PGR) in three oil palm fruit-forms on three germination media at three different counting times

Germination Medium	***Dura***			***Pisifera***			***Tenera***		
	60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Sucrose	1.163a†	0.088b	0.015a	0.390b	0.055a	0.008a	1.300a	0.063b	0.015a
Glucose	0.940b	0.230a	0.085a	0.513b	0.078a	0.013a	1.133a	0.180a	0.068a
Liquid	0.908b	0.118b	0.023a	0.825a	0.093a	0.013a	1.033a	0.180a	0.045a

†Values within each column for each fruit-form followed by same small letters are not significantly different at p ≤ 0.05 by Tukey's HSD.

4.2 Effect of different media on pollen tube length (PTL) and growth rate (PTLR): In pollen vigor assay study, the effect of medium on tube length was significant in all pollen sample sources (Table 5). In *dura* and *pisifera* pollen, the higher pollen tube lengths were obtained in solid sucrose and liquid sucrose media than that of glucose medium at 60,120 and 180 min after incubation. Among the tested media for *tenera* pollen, solid sucrose medium still showed the highest potential for tube length until 180 min (Table 6). The figures show *in vitro* germination of pollen on sucrose solid medium at 60, 120, and 180 min after incubation period (Fig. 4a, 4b and 4c). For the comparison, the average pollen tube length(µm) of each variety on different germination media at 60, 120 and 180 min after incubation are shown in Fig. 5a, 5b and 5c. Although liquid sucrose medium yielded the highest pollen germination percentages for *pisifera*, some pollen tubes ruptured at the end of incubation period (Fig. 6) whereas solid sucrose medium emitted longer pollen tube length with smooth and slender tubes without bursting (Fig. 7). Therefore, sucrose solid medium is the best germination medium for pollen vigor test in terms of tube length in all three oil palm fruit forms.



Journal of Animal & Plant Sciences, 2012. Vol. 14, Issue 1: 1855-1865
Publication date: 31/5/2012, http://www.m.elewa.org/JAPS; ***ISSN 2071 - 7024***

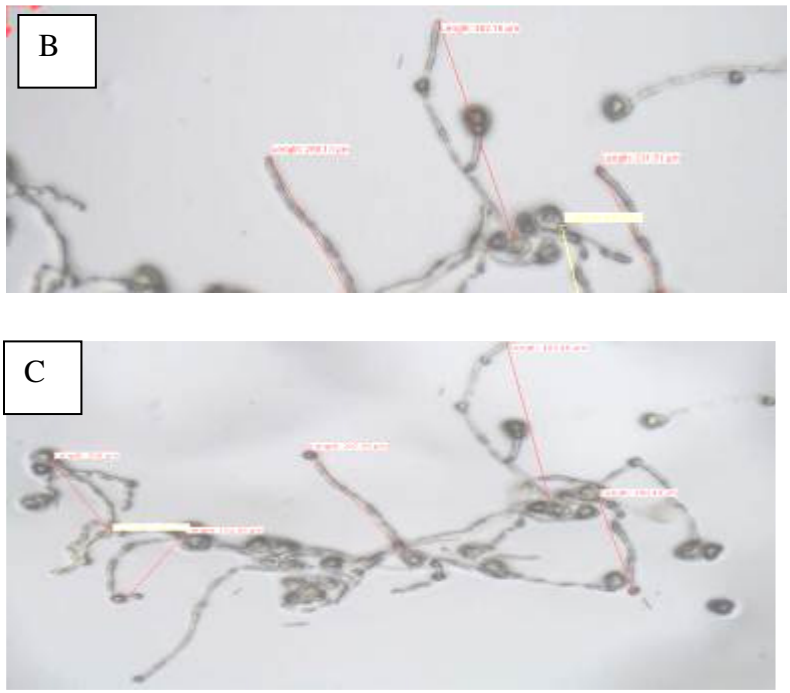


Figure 4: *In vitro* germinated pollen from 20 years old palm on sucrose solid media (a) 60 min (b) 120 min and (c) 180 min after incubation at 35˚C in oven

Table 5: Mean squares of ANOVA for pollen tube length (PTL) of three oil palm fruit-forms on three germination media

S.V.	df	Mean Square of tube length (µm)								
		Dura			***Pisifera***			***Tenera***		
		60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Germination medium	2	10486.2^{ns}	9600.7*	12941.7**	8501.6**	30309.4**	55233.6**	14738.7*	17694.1**	11825.3*
Error	9	2946.9	2121.9	1137.4	657.9	1043.8	2571.7	260.8	1516.9	2398.8

**, * and ns are significant at p≤0.01, p≤0.05 and non-significant at p>0.05, respectively, S.V.-Source of variation; df- Degree of freedom

Table 6: Means of pollen tube length (PTL) in three oil palm fruit-forms on three germination media at three different counting times

Germination Medium	***Dura***			***Pisifera***			***Tenera***		
	60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Sucrose	290.1a†	370.9a	406.20a	218.0a	330.2a	372.1a	250.0a	355.8a	375.2a
Glucose	197.7a	276.9b	315.9b	126.7b	172.0bA	196.5b	150.6ab	242.4b	269.1b
Liquid	205.7a	347.8ab	408.21ab	183.3a	314.0a	419.6a	139.9b	238.9b	308.0ab

†Values within each column for each fruit-form followed by same small letters are not significantly different at p ≤ 0.05 by Tukey's HSD.

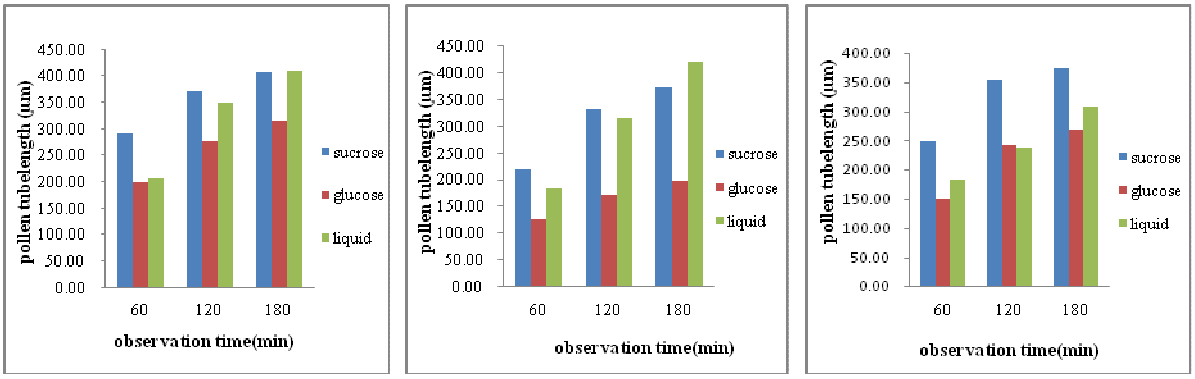


Figure 5: Germinated pollen tube length of (a) *dura* (b) *pisifera* and (c) *tenera* after different incubation periods at 35˚C

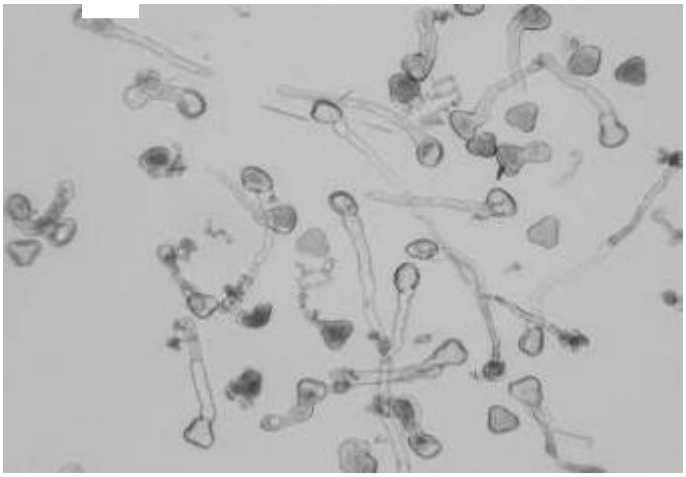


Figure.6: *Pisifera* pollen with ruptured tubes (indicated with arrow) in liquid sucrose medium

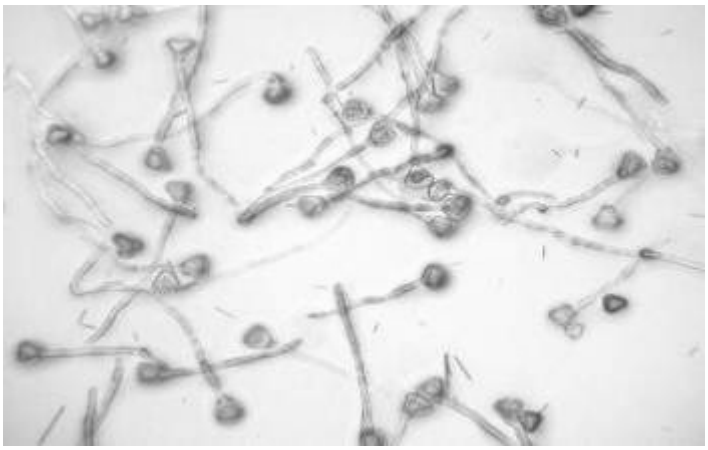


Figure 7: *Pisifera* pollen with unruptured tubes in solid sucrose medium

Pollen tube growth rate (PTLR) from all three tested germination media differed significantly except in *dura* at 60 and tenerra at 120 min after incubation (Table 7). Irrespective of germination media, the pollen tube growth rate was the highest at 60 min after incubation followed by 120 and 180 min. The solid and liquid sucrose media had significantly higher PTLR than solid glucose in

pisifera while solid sucrose provided higher PTLR than solid glucose and liquid sucrose in *tenera* at 60 and 120 min after incubation (Table 8).

Table 7: Mean squares of ANOVA for pollen tube growth rate (PTLR) of three oil palm fruit-forms on three germination media

S.V.	df	Mean Square of tube growth rate								
		Dura			***Pisifera***			***Tenera***		
		60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Germination medium	2	2.915^{ns}	1.429*	0.226*	2.362**	2.247**	2.010**	4.091*	0.054^{ns}	0.793**
Error	9	0.818	0.229	0.047	0.183	0.239	0.107	0.724	0.245	0.018

**, * and ns are significant at p≤0.01, p≤0.05 and non-significant at p>0.05, respectively, S.V.-Source of variation; df- Degree of freedom

Table 8: Means of pollen tube growth rate (PTLR) in three oil palm fruit-forms on three germination media at three different counting times

Germination Medium	***Dura***			***Pisifera***			***Tenera***		
	60 min	120 min	180 min	60 min	120 min	180 min	60 min	120 min	180 min
Sucrose	4.835a†	1.345b	0.580b	3.635a	1.870a	0.700b	4.165a	1.763a	0.325b
Glucose	3.293a	1.320b	0.650ab	2.113b	0.755b	0.408b	2.508ab	1.530a	0.448b
Liquid	3.430a	2.368a	1.023a	3.055a	2.180a	1.755a	2.333b	1.650a	1.150a

†Values within each column for each fruit-form followed by same small letters are not significantly different at p ≤ 0.05 by Tukey's HSD.

5 DISCUSSION

The germination percentage as well as pollen tube growth was higher in *dura* pollen in sucrose solid medium and lower in liquid medium. Even though, in some cases, liquid media gave longer pollen tube as the same with solid sucrose medium, but it showed ruptured pollen tubes. The higher germination percentages together with shorter pollen tubes were observed in glucose solid medium. *Pisifera* pollen behaved differently with *dura* pollen. The effect of liquid sucrose medium was more pronounced in *pisifera* pollen but some pollen tubes were ruptured at 180 min after incubation. A good germination percentage was obtained on solid sucrose media, while in liquid media, probably because the water uptake was unregulated, the grains generally busted. From this study, it was found that solid sucrose medium could increase the pollen tube length of three tested pollen sources. Tandon *et al.* (1999) suggested that the sucrose liquid medium containing 2.5% sucrose, 100-ppm boric acid and 10% polyethylene glycol (PEG) with 10000 M is the most suitable medium for viability of oil palm pollen (*Tenera*). However, Jayaprakash and Sarla (2001) stated that liquid media gave the inconsistent germinability results in *Cajanus cajan* (L.) pollen. We also found that all tested pollen sources emerged in the liquid sucrose in the tubes which bursted at the end of measuring period. For evaluation of pollen viability, pollen tube length criterion is more important than *in vitro* germinability. Priestley, (1986) found that the loss of vigor generally becomes evident well before the loss of germinability. To be able the pollen tube to grow, rapid synthesis of cell wall materials, and a high-energy supply are necessary. During pollen tube elongation, sugar is utilized as an energy sources for the synthesis of cell wall material such as pectins, cellulose and callose (Mascarenhas, 1993 and Derksen *et al.*, 1995). In pollen germinability assay *in vitro,* sucrose is generally used as energy in many plant species, because it usually stimulates germination and subsequent tube growth. One of the clarifications obtained from this study is that sucrose solid germination medium appeared to be

the optimum medium for determining pollen germinability and vigor (pollen tube length) in oil palm in comparison with glucose solid and sucrose liquid media. This is in agreement with the result obtained by Stadler *et al.,* (1999) who claimed that sucrose was the only one carbohydrate that support growth of *Arabidopsis* pollen. Sucrose has the functions in maintaining osmotic pressure of the germination medium to provide enough moisture for enhance germination without rupturing pollen tubes and acting as a substrate for pollen metabolism. In conclusion, there was a significant influence of germination medium on the pollen germinability and vigor. In addition, sucrose solid can be considered to be the best medium and to have the highest potential to check the viability of oil palm pollens.

6 REFERENCES

Asif, M.I., AL-Tahir, O.A., Farah, A.F. 1983. The effects of some chemicals and growth substances on pollen germination and tube growth of date palm. *Hort. Science* 18: 479-480.

Bots, M. and Mariani, C. 2005. *Pollen viability in the field.* Radboud Universiteit Nijmegen.

Chichiricco, G., and Grilli-Caiola, M. 1986. *Crocus sativus* pollen germination and pollen tube growth in vitro and after intra- and inter-specific pollination. *Can. J. Bot.* 64: 2774-2777.

Chin, C.W. 1999. Oil palm breeding techniques. *Proceeding of the symposium on the science of oil palm breeding,* pp. 50.

Derksen, J., Rutten, T., van AMstel, T., de Win, A., Doris, F. and Steer, M. 1995. Regulation of pollen tube growth. *Acta Bot. Neerl.* 44: 93 - 113.

Gwata, E.T, Wofford, D.S, Pfahler, P.L. and Boote, K.J. 2003. Pollen morphology and in vitro germination characteristics of nodulating and non-nodulating soybean (*Glycine max L.*) genotype. *Theor. Appl. Genet.* 106: 807-839.

Hartley, C. W. S. 1988. The oil palm (3rd edn). Longman group limited, Singapore.

Heslop-Harrisson, J., Heslop-Harrisson, Y. and Shivanna, K.R. 1984. The evaluation of pollen quality, and further appraisal of the fluorochromatic test procedure. *Theor. Appl. Genet.* 67: 36-375.

Hicks, C.B, Stephens, L.C. and Weigle, J.L. 1987. In vitro pollen germination and viability of a Java x New Guinea *Impatiens* interspecific hybrid. *Can. J.* Bot. 65:1967-1968.

Jayaprakash, P. and Sarla, N. 2001. Development of an improved medium for germination of *Cajanus cajan* (L.) Millsp. pollen *in vitro*. *Journal of Experimental Botany*, 52(357): 851 - 855.

Kakani, V.G., Prasad, P.V.V., Craufard, P.Q. and Wheeler, T.R. 2002. Response of in vitro pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. *Plant, Cell and environment* 25: 1651-1661).

Kerhoas, C. and Dumas, C. 1987. Nuclear magnetic resonance and pollen quality. In: Jackson, J.F., Linskens, H.F. (eds) *Mordern method in plant analysis*, vol 2. Springer, Berlin Heidelberg New York, Tokyo, pp 169-190.

Know, R.B., Williams, E.G., Kerhoas, C. and Dumas, C. 1987. Nuclear magnetic resonance and pollen quality. In: Dumas, C. (eds) Pollen, pistil and reproductive function in crop plants. *Plant Breed Rev.* 4 : 87-89.

Kuo, C.G., Peng, J.S. and Tsay, J.S. 1981. Effect of high temperature on pollen grain germination, pollen tube growth, and seed yield of chinese cabbage. *Hort. Science* 16: 67-68.

Mascarenhas, J. P. 1993. Molecular mechanisms of pollen tube growth and differentiation. *Plant Cell*, 5: 1303 – 1314.

Pfahler, P.L., Pereira, M.J. and Barnett, R.D. 1997. Genetic variation for in vitro sesame pollen germination and tube growth. *Theor. Appl. Genet*. 95: 1218-1222

Priestley, D. A. 1986. Seed aging: implications for seed storage and persistence in the soil. Ithaca, New york: Comstock.

Stadler, R., Truernit, E., Gahrtz, M. and Saure, N. 1999. The ASUC1 sucrose carrier may represent the osmotic drying force for anther dehiscence and pollen tube growth in *Arabidopsis*. *Plant J.*19: 269-278.

Tandon, R., Manohara, T. N., Nijalingappa, B. H. M. and Shivanna, K. R. 1999. Polyethylene

glycol enhances *in vitro* germination and tube growth of oil palm pollen. *Indian J. Exp. Biol.* 37: 169-172.

Taylor, T.P. and Hepler, P.K. 1997. Pollen germination and tube growth. Annual Review of Plant Physiology and Plant Molecular Biology 48 : 461-491.

Turner, P.D. and Gillbanks, R.A. 2006. *Oil palm cultivation and management*. Kuala lumpur: The incorporated society of planters.

Vasilakakis, M. and Porlingis, I.C. 1985. Effect of temperature on pollen germination, pollen tube growth, effective pollination period and fruit set of pear. *Hort. Science* 20:733-735