Pollen viability and seed set of interspecific hybrids between *Jatropha curcas* × *Jatropha integerrima*

Kitiya Amkul[1], Malee Panngam[2], Patcharin Tanya[2,3], Peerasak Srinives[2,3], Kularb Laosatit[4]*

[1]Plant Breeding Program, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
[2]Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
[3]Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand
[4]Postdoctoral Fellow, the project on Breeding to Accelerate Domestication of Novel Jatropha for Fuel and Feeds, Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand

**Corresponding author: pigirosa@hotmail.com*

ABSTRACT

Interspecific hybrids between *Jatropha curcas* × *Jatropha integerrima* are potential genetic resources for jatropha improvement. However, their pollen is normally partially sterile. This study was conducted to determine pollen viability status and seed set of the hybrids. Pollen viability was estimated by staining with 1% acetocarmine. The results showed that all interspecific hybrids had pollen viability falling between their parents. The pollen viability of interspecific hybrid derived from a cross between *J. curcas* (high phorbol esters accession) × *J. integerrima* ranged from 47% to 89% with an average of 72%. Similarly the viability of the hybrid *J. curcas* (low phorbol esters accession) × *J. integerrima* ranged from 44% to 90% with an average of 73%. The hybrid with low pollen viability also set less seeds. This information is useful for plant breeders to select the pollen donor in interspecific breeding programs.

Keywords: interspecific hybrids; acetocarmine; staining method; viability test

INTRODUCTION

Jatropha curcas L. is commonly known as jatropha or physic nut belonging to the family Euphorbiaceae, which is the same family as the other economically important crops such as cassava (*Manihot esculenta*), pararubber (*Hevea brasiliensis*) and castor bean (*Ricinus communis*). Jatropha is considered the most important non-edible oil seed crop as a feedstock for biodiesel production due to high seed oil content, rapid growth, easy propagation, and adaptation to wide agro-climatic condition. However, jatropha is still not profitable to grow because of its low seed and oil yield, non-synchronous fruit maturation, and the presence of toxic and carcinogenic compounds. Thus jatropha still needs genetic improvement for these characters. Many previous studies reported that jatropha had insufficient genetic diversity for developing elite cultivars (Xu *et al.*, 2012; Yue *et al.*, 2014), fortunately it has many related species to cross with. Interspecific hybridization between *J. curcas* and *J. integerrima* is expected to play a significant role in improving the desirable traits such as high number of female flowers, seed yield, oil content, and strong stem (Kumar *et al.*, 2009). Many studies were successful in producing interspecific hybrids between *J. curcas* and *J. integerrima* obtaining the F_1 plants which were more vigorous, longer in duration of flowering, bushy growth with more number of branches, etc. (Basha and Sujatha, 2009; Parthiban *et al.*, 2009a; 2009b). As expected from applying simple Mendelian rules, the F_2 generation phenotypically exhibits much more variation than the F_1 and BC_1 generations. In jatropha, all previous studies used backcross progenies as the starting population for selection (Liu *et al.*, 2011; Wang *et al.*, 2011; Sun *et al.*, 2012; Subashini *et al.*, 2014) because they could not produce F_2 seeds from the F_1 plants. Low or no seed set in plants is usually caused by poor pollen viability (Berjano *et al.*, 2006). Thus the viability in the F_1 plants should be investigated before use them as a parental material for further improvement. The staining technique (staining pollen with a dye such as acetocarmine) is the most widely used method for testing pollen viability due to its simplicity and low cost (Binder *et al.*, 1974).

The objective of this study was to evaluate pollen viability of the F_1 plants obtained from an

interspecific hybridization between *J. curcas* × *J. integerrima* using a staining technique. This information is expected to be useful for the plant breeders in developing segregating plants for further selection.

MATERIALS AND METHODS

Twenty interspecific F_1 plants derived from *J. curcas* × *J. integerrima* were used in this study. Four plants were obtained from across between *J. curcas* accession with high phorbol esters (PEs) × *J. integerrima*, while the rests 16 plants were from *J. curcas* accession with low PEs × *J. integerrima*. All interspecific hybrids were obtained only when *J. curcas* was used as the female parent. The parental and all 20 F_1 plants were grown in an experimental field of the Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Thailand. The pollen was tested for its viability during November to December 2014. A number of flowers were used for observing pollen viability while the others were left on the plant until maturity for recording fruit and seed set. The flowers from the tested plants were collected during 8.00-10.00 am and immediately fixed overnight in a fresh fixative solution (1:3 glacial acetic acid : absolute ethanol), washed once by tap water, transferred to 70% ethanol and kept in are refrigerator at 4 ºC until analysis (adapted from Nassar *et al.* (2000)).

Pollen viability was estimated by a staining method using 1% acetocarmine. All parts of the flower were removed, leaving only the mature anthers which were mounted in a drop of 1% acetocarmine on a glass slide. The anthers were gently crushed by a needle to release the pollen grains, mixed thoroughly, then gently placed with cover slip and observed under a light microscope (Olympus Co. Ltd.). Three slides were prepared from each plant and three randomly selected fields were observed in each slide under the 10X objective (100X magnification). The pollen viability was scored according to staining level. A round-shaped pollen with bold red or heavily stained was scored as fertile and viable, while that unstained or lightly stained was scored as sterile or non-viable. The percentage of pollen viability was determined as the ratio of number of viable grains to total grains. The percentage of pollen viability was subjected to an analysis of variance. If significant, their means were then compared by Duncan's multiple range test at the significant level of $P \leq 0.05$ using R statistical package (R Core Team, 2013). The experimental design used in comparing the percentage of pollen viability was a completely randomized design (CRD).

RESULTS AND DISCUSSION

Pollen viability has been evaluated by biologists using various staining techniques, such as 1% acetocarmine to detect cytoplasmic content, tetrazolium salts to detect dehydrogenase activity, aniline blue to detect callose in pollen walls and pollen tubes, iodine to determine starch content, fluorescein diacetate to determine esterase activity and intactness of the plasma membrane (Dafni and Firmage, 2000). The choice of method depends on crop species (Dafni and Firmage, 2000). In our study, pollen viability was determined using 1% acetocarmine which gave a sufficiently clear result as shown in Figure 1 and 2. Our result agreed well with that of Tiwari *et al*. (2014) who studied pollen viability in members of *Euphorbiaceae* family using various dyes and found that 1% acetocarmine gave clear results. Analysis of variance confirmed the significance of these differences suggesting high individual variation in pollen viability at a 95% confident interval. Percentages of pollen viability of the parents and their F_1 progenies are shown in Table 1. All of the interspecific F_1 plants had the percentage of pollen viability falling between the parents. Pollen viability of the high PEs *J. curcas* × *J. integerrima* ranged from 47 to 89% with an average of 72%. In this group, H1-T3 showed the highest pollen viability while H1-T1 was the lowest. Similarly, pollen viability of the cross between low PEs *J. curcas* × *J. integerrima* ranged from 44 to 90% with an average of 73%. The H2-M3 from this cross showed the highest viability, while H2-M12 was the lowest. Observation on fruits setting was performed in H2-M1, H2-M2, and H2-M3 and found 20%, 35% and 44% fruit setting, respectively. The other hybrids set less than 10 fruits during the whole season. Most inflorescences set many young fruits but only one can develop until maturity (Figure 3a). Yet, there was only one seed per fruit rather than 2-3 seeds as found in the normal jatropha (Figure 3b). We found a significant relationship between pollen viability and seed set (r = 0.998, $P \leq 0.05$). Since pollen viability relates to seed set, it is expected that this character would lead to an early detection of seed set in the F_1 plants, and eventually to the production of F_2 seeds for further use in genetic study or in improvement of jatropha.

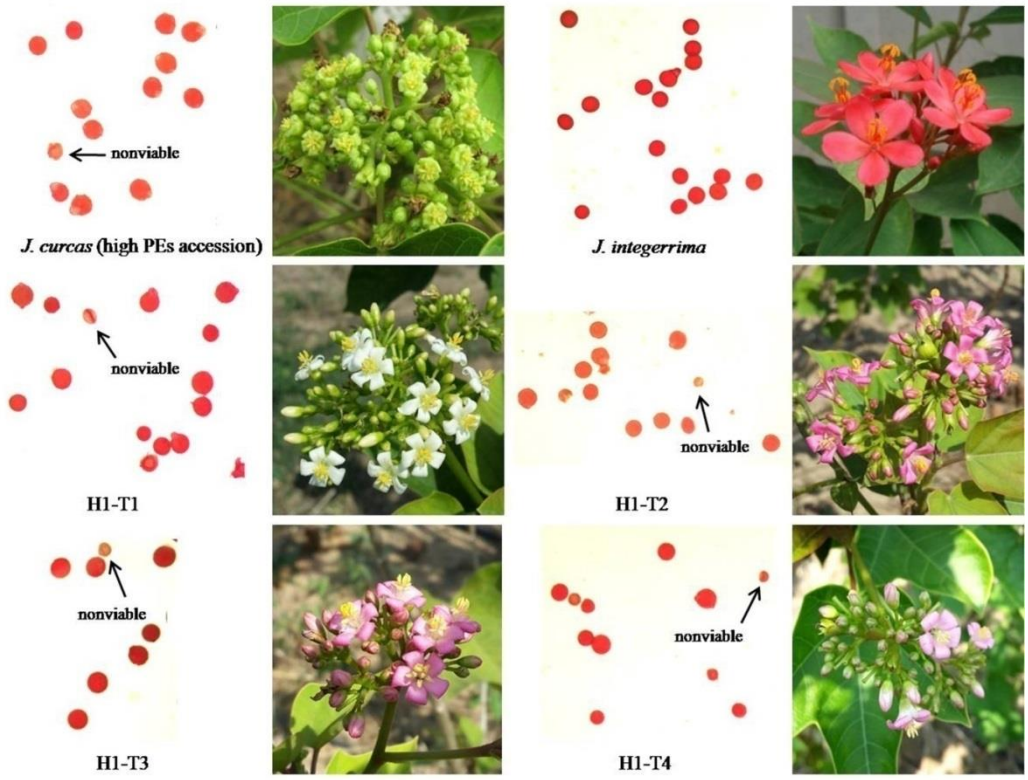

Figure 1 Pollen viability as tested by 1% acetocarmine on *Jatropha curcas* (high PEs accession), *Jatropha integerrima* and their interspecific hybrids.

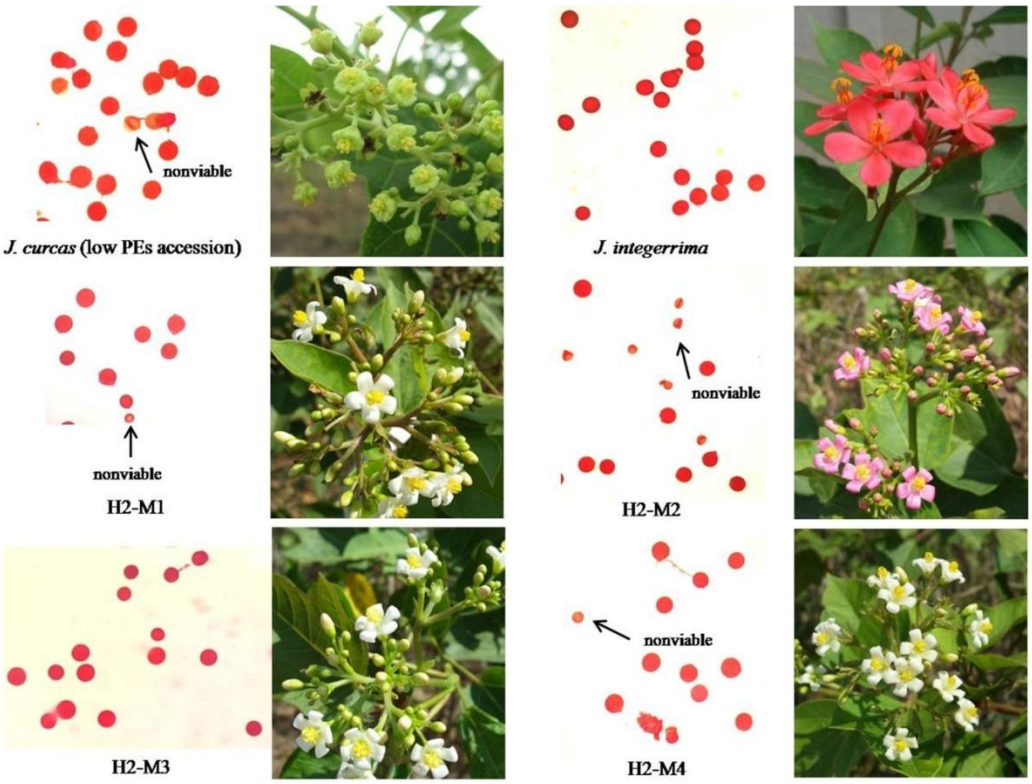

Figure 2 Pollen viability as tested by 1% acetocarmine on *Jatropha curcas* (low PEs accession), *Jatropha integerrima* and their interspecific hybrids.

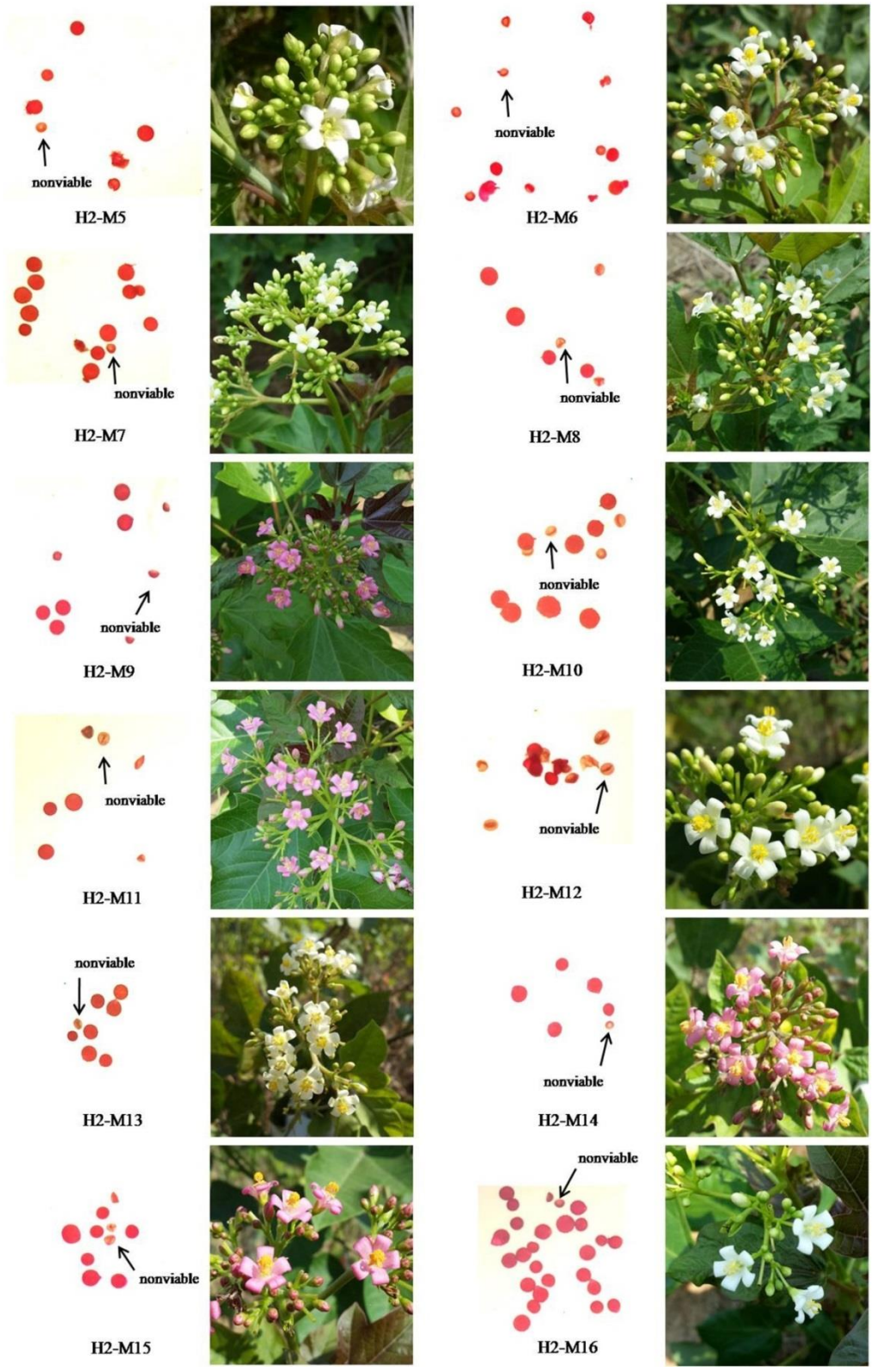

Figure 2 (continued).

Table 1 Duncan's multiple range test of pollen viability (%) in *Jatropha curcas*, *Jatropha integerrima*, and their interspecific hybrids, at $P \leq 0.05$.

Entries	Percentage of pollen viability*
J. curcas (High PEs)	92
J. curcas (Low PEs)	95
J. integerrima	98
J. curcas (High PEs) × *J. integerrima*	
H1-T1	47^{i}
H1-T2	72^{e}
H1-T3	89^{ab}
H1-T4	80^{cd}
J. curcas (Low PEs) × *J. integerrima*	
H2-M1	79^{d}
H2-M2	86^{b}
H2-M3	90^{a}
H2-M4	70^{e}
H2-M5	57^{g}
H2-M6	84^{b}
H2-M7	72^{e}
H2-M8	54^{h}
H2-M9	62^{f}
H2-M10	79^{d}
H2-M11	49^{i}
H2-M12	44^{j}
H2-M13	86^{b}
H2-M14	83^{bc}
H2-M15	83^{bc}
H2-M16	85^{b}

*Means followed by the same letters are not significantly different as compared by Duncan's multiple range test.

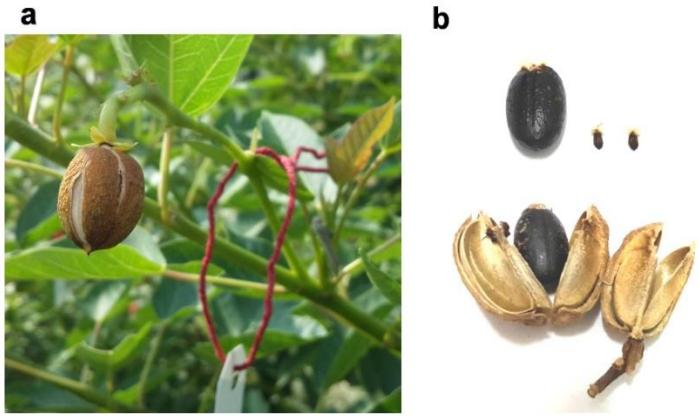

Figure 3 Fruit setting on an interspecific F_1 hybrid plant (a), and typical characters of seed set on interspecific F_1 hybrid (b).

CONCLUSION

Viability test is considered a fast and easy method to compare pollen germination; since the effects of external factors such as temperature, humidity, and germinating media were minimized. We used 1% acetocarmine to determine pollen viability of interspecific hybrid plants from *Jatropha curcas* × *J. integerrima* and found a significant relationship between pollen viability and seed set (r = 0.998, $P \leq 0.05$). The viability test may be used to identify the F_1 plants with a potential to produce high number of F_2 seeds in jatropha genetic and breeding.

ACKNOWLEDGEMENTS

This research was supported by the Chair Professor Project of Thailand's National Science and Technology Development Agency (Grant no. P-11-00599), and the Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok, Thailand.

REFERENCES

Basha SD, Sujatha M (2009) Genetic analysis of *Jatropha* species and some interspecific hybrids from India using nuclear and organelle specific markers. Euphytica 168: 197–214.

Berjano R, de Vega C, Arista M, Ortiz PL, Talavera S (2006) A multi-year study of factors affecting fruit production in *Aristolochia paucinervis* (Aristolochiaceae). Am J Bot 93: 599–606.

Binder WD, Mitchell GM, Ballantyne DJ (1974) Pollen Viability Testing, Storage and Related Physiology Review of the Literature with Emphasis on Gymnosperm Pollen. Department of the Environment, Canadian Forestry Service, Pacific Forest Research Centre, Victoria, B.C.

Dafni A, Firmage D (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Syst Evol 222: 113–132.

Kumar RS, Parthiban KT, Hemalatha P, Kalaiselvi T, Rao MG (2009) Investigation on cross-compatibility barriers in the biofuel crop *Jatropha curcas* L. with wild Jatropha species. Crop Sci 49: 1667–1674.

Liu P, Wang CM, Li L, Sun F, Yue GH (2011) Mapping QTLs for oil trails and eQTLs for oleosin genes in jartropha. BMC Plant Biol 11: 132–140.

Nassar NMA, Santos ED, David S (2000) The transference of apomixes genes from *Manihot neusana* Nassar to cassava, *M. esculenta* Crantz. Hereditas 132: 167–170.

Parthiban KT, Kumar RS, Thiyagarajan P, Subbulakshmi V, Sujatha M, Govinda MR (2009a) Jatropha hybrids: a promising development in biofuel research. Asia Pacific Agroforestry Newsletter 35: 7–8.

Parthiban KT, Kumar RS, Thiyagarajan P, Subbulakshmi V, Vennila S, Rao MG (2009b) Hybrid progenies in Jatropha – a new development. Curr Sci 96: 815–823.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Subashini G, Ibrahim SM, Paramathma M, Manivannan N (2014) Studies on genetic variability parameters in backcross population of jatropha (*Jatropha curcas*). Int J Trop Agric 32: 569–572.

Sun F, Liu P, Ye J, Lo LC, Cao S, Li L, Yue GH, Wang CM (2012) An approach for jatropha improvement using pleiotropic QTLs regulating plant growth and seed yield. Biotechnol Biofuels 5: 42–52.

Tiwari A, Kamble RB, Chaturvedi A (2014) Pollen biology of some members of Euphorbiaceae family. Asian J Plant Sci Res 4: 8–14.

Wang CM, Liu P, Yi C, Gu K, Sun F, Li L, Lo LC, Liu X, Feng F, Lin G, *et al.* (2011) A first generation microsatellite- and SNP-based linkage map of *Jatropha*. PloSOne 6: e23632.

Xu W, Mulpuri S, Liu A (2012) Genetic diversity in the *Jatropha* genus and its potential application. Plant Sciences Reviews 7: 254–268.

Yue GH, Lo LC, Sun F, Cao SY, Yi CX, Hong Y, Sun WB (2014) No variation at 29 microsatellites in the genome of *Jatropha curcas*. J Genomics 2: 59–63.