J. Myanmar Acad. Arts Sci. 2021 **Vol. XIX. No.4A**

SPORE- TETRAD AND POLLEN FERTILITY OF TEN *Pisum sativum* L. CULTIVARS

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Abstract

The studied ten *Pisum sativum* L. cultivars were commercially cultivated all over in various part of North Western Myanmar. They were grown abundantly in Kalay, Kalay-wa Township in Sagaing Region. The spore- tetrad and pollen fertility of ten *Pisum sativum* L. cultivars showed micronuclei per spore tetrad is less than 3 mean micronuclei per spore tetrad. The pollens normality have more than 69 % in all the cultivars and lines. These ten cultivars showed the percentage of normal pollen was greater than abnormal pollen. Thus, to improve their fertility and yield characters, it is essentially needed to understand pollen mother cell cycle that produces pollen and spore- tetrad.

Keywords: *Pisum sativum* L, Ten cultivars, spore- tetrad, pollen

Introduction

Pisum sativum L. was probably originated in South-West Asia, now cultivated in many temperate countires, as a cool-season crop in the subtropics and at higher altitudes in the tropics (Maesen, 1992).The pea is the oldest among of domesticated plants.

Currently the *Pisum sativum* L. is one of the world's most important grain legumes, serving a variety of roles as a source of both human food and animal feed. Its seed is harvested either as the dry mature form or immature state. The precise stage of maturity varying according to the end use. *Pisum sativum* L. is a diploid species 2n=14 which has been in cultivation for food since date back to 3000 years. Thus, it showed the basic chromosome of each set is 7 (Colin, 1956).

The spore- tetrad that has only one large nucleus and has no small nucleus was recorded as normal spore-tetrad. Abnormal spore-tetrad was observed many micronuclei and large normal nucleus. Number of micronuclei per spore-tetrad were also counted and recorded. Normal pollen has one generative nucleus and one vegetative nucleus in normal condition. Abnormal pollen were observed lack one or both tube nucleus and only the generative nucleus. Normal and abnormal pollen were also counted and recorded.

Peas cultuvars that grown in Myanmar shows variation in size, shaped, colour and other morphological forms. This work is tend to investigate the performance of spore-tetrad and pollen fertility.

Material and Methods

Materials

Ten *Pisum sativum* L. cultivars of family Fabaceae namely Pe-kyauk-sane, Kalay-sadaw-pe. Myitkyina-maw-lue, Shwebo-thann-lann, Ta- kaung,Kyun-lone-thae, Kanada, Ta-yoke-phwe-thae, Ta-yoke-phyu and Shwe-pe-thee were collected from Sagaing Township.

Methods

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The samples of pollen mother cells (PMCs) of the sample species were collected from ten collection cultivars. The flower buds start from the very beginning of the appearance till somewhat maturation buds were collected early in the morning round about 6.00 to 8.00 AM. The anther (s) from the collected flower were trace out and they were immediately fixed in freshly prepared 3:1 alcohol: acetic solution (Carnoy's solution), that filled in the brown glass vials were used in order to protect from the light that will give somewhat changes to PMCs. They were stored in the cool and dry place till they were used.

Cytological Analysis

Thirty tetrad cells were examined at the tetrad stage for numbering the number to micronuclei per tetrad cell. Hundred pollen grains for each cultivar were studied for normal and abnormal pollen. This was prepared by using 1% acetocarmine squarsh method and observed under the Olympus light microscope.

Slide preparation

After fixing for three days in the fixative solution, one of the flower bud were selected and place on the glass slide. By the help of needle pointer a pair of forceps, the anther from the flower removed and the remaining parts of the flowers were discarded, one to two drops of acetocarmine barax staining solution was added on the dissected anther. The anther were crushed by using specially prepared silver knife. The undesired materials were then again removed from the glass slide the sample was covered with thin glass cover slip. Using the unique thumb pressure, the cover slip was gently pressed to obtain well spread slide. In this way the slide was ready to observed the PMCs character under the microscope (Beeks, 1955).

Statistical analysis

Spore-tetrad studies were test with student 't' test as by Steel and Torrie (1960). The data of normal and abnormal pollen of 10 cultivars were compared using Chi-square test as stated by Steel and Torrie (1960).

Results

Tetrad Characters

All the cultivars possessed more than 0.87 mean micronuclei per spore-tetrad were recorded. The mean range of 0.87 – 2.2 in the occurrence of micronuclei per spore-tetrad of the ten *Pisum sativum* L. cultivars. They were not significantly differently from one to another but Shwebo-thann-lann possessed the highest mean number of micronuclei 2.200 ± 2.626 , thus cultivar Pe-kyauk-sane has significantly inferior to the remaining cultivars at 1 % and 5 % level respectively (Table 1).

Pollen Characters

Occurrence of normal pollen per cell studied among the 10 cultivars studied ranged from 69 % to 86 % normal but did not exhibited a single significantly superior or inferior number of pollen. Abnormal pollen was also observed non significant in any of the comparisons (Table 1).

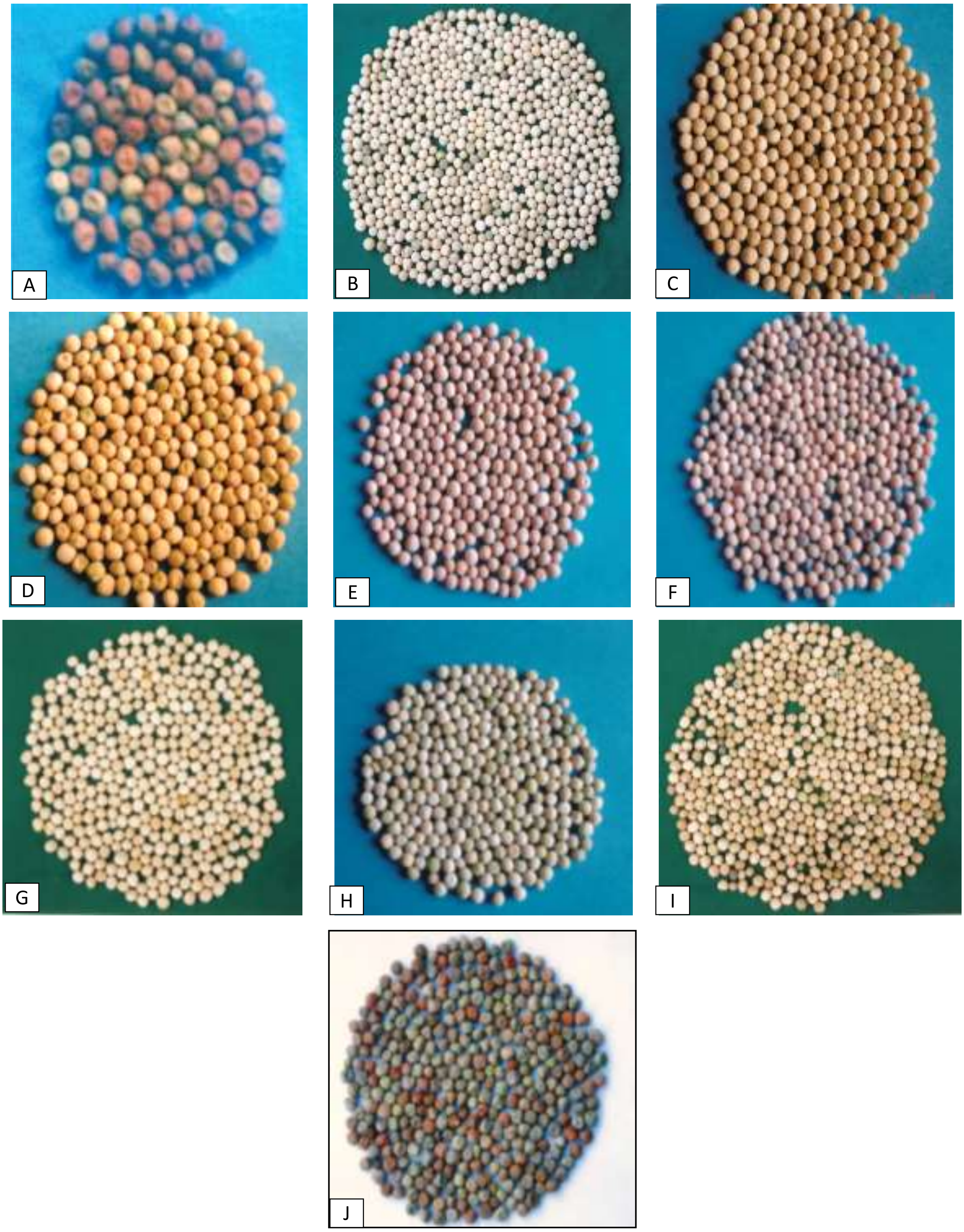

Figure 1 Seed characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane B. Kalay-sadaw-pe C. Shwebo-thann-lann

D. Myitkyina-maw-lue E. Ta-kaung-pe F. Kyun-lone-thae

G. Kanada H. Ta-yoke-phwe-thae I. Ta-yoke-phyu

J. Shwe-pe-thee

Table 1 Comparison on occurrence of micronuclei per spore-tetrad, pollen normality and abnormality of 10 *Pisum sativum* L. cultivars

Identity / Comparison	Micronuclei Mean ± S.D	Micronuclei 't' value	normal pollen %	Arc-sine value	abnormal pollen %	Arc-sine value	χ^2 value
Pe-kyauk-sane Vs Kalay-sadaw-pe	0.87 ± 1.454 1.77 ± 2.216	-1.828^{ns}	80 74	63.44 59.34	20 26	26.56 30.66	$0.251^{n\text{-}s}$
Pe-kyauk-sane Vs Shwebo-thann-lann	0.87 ± 1.454 2.20 ± 2.626	-2.386*	80 83	63.44 65.65	20 17	26.56 24.35	$0.117^{n.s}$
Pe-kyauk-sane Vs Myitkyina-maw-lue	0.87 ± 1.454 1.73 ± 1.672	-2.089*	80 83	63.44 65.65	20 17	26.56 24.35	$0.117^{n.s}$
Pe-kyauk-sane Vs Ta-kaung	0.87 ± 1.454 1.83 ± 1.894	-2.162*	80 74	63.44 59.34	20 26	26.56 30.66	0.251^{ns}
Pe-kyauk sane Vs Kyun-thone-thae	0.87 ± 1.454 1.17 ± 1.462	-0.783^{ns}	80 81	63.44 64.16	20 19	26.56 25.84	0.025^{ns}
Pe-kyauk-sane Vs Kanada	0.87 ± 1.454 1.67 ± 1.556	-2.230*	80 86	63.44 68.03	20 14	26.56 21.97	0.396^{ns}
Pe-kyauk-sane Vs Ta-yoke-phyu-thae	0.87 ± 1.454 1.87 ± 1.784	-2.339*	80 85	63.44 67.21	20 15	26.56 22.79	0.291^{ns}
Pe-kyauk-sane Vs Ta-yoke-phyu	0.87 ± 1.454 1.40 ± 1.705	-1.273^{ns}	80 69	63.44 56.17	20 31	26.56 33.83	0.597^{ns}
Pe-kyauk-sane Vs Shwe-pe-thee	0.87 ± 1.454 2.17 ± 1.675	-3.156**	80 73	63.44 58.69	20 27	26.56 31.31	0.248^{ns}
Kalay-sadaw-pe Vs Shwebo-thann-lann	1.77 ± 2.216 2.20 ± 2.626	-0.674^{ns}	74 83	59.34 65.65	26 17	30.66 24.35	0.686^{ns}
Kalay-sadaw-pe Vs Myitkyina-maw-lue	1.77 ± 2.216 1.73 ± 1.672	0.078^{ns}	74 83	59.34 65.65	26 17	30.66 24.35	0.686^{ns}
Kalay-sadaw-pe Vs Ta-kaung	1.77 ± 2.216 1.83 ± 1.899	-0.111^{ns}	74 74	59.34 59.34	26 26	30.66 30.66	0.004^{ns}
Kalay-sadaw-pe Vs Kyun-lone-thae	1.77 ± 2.216 1.17 ± 1.462	1.217^{ns}	74 81	59.34 64.16	26 19	30.66 25.84	0.414^{ns}
Kalay-sadaw-pe Vs Kanada	1.77 ± 2.216 1.67 ± 1.556	0.199^{ns}	74 86	59.34 68.03	26 14	30.66 21.97	1.275^{ns}
Kalay-sadaw-pe Vs Ta-yoke-phwe-thae	1.77 ± 2.216 1.87 ± 1.784	-0.139^{ns}	74 85	59.34 67.21	26 15	30.66 22.79	1.220^{ns}

Table 1 Continue

Identity / Comparison	Micronuclei Mean ± S.D	Micronuclei 't' value	normal pollen %	Arc-sine value	abnormal pollen %	Arc-sine value	χ^2 value
Kalay-sadaw-pe Vs Ta-yoke-phyu	1.77 ± 2.216 1.40 ± 1.705	0.713^{ns}	74 69	59.34 56.17	26 31	30.66 33.83	0.575^{ns}
Kalay-sadaw-pe Vs Shwe-pe-thee	1.77 ± 2.216 2.17 ± 1.675	0.775^{ns}	74 73	59.34 58.69	26 27	30.66 31.31	0.092^{ns}
Shwebo-thann-lann Vs Myitkyina-maw-lue	2.20 ± 2.626 1.73 ± 1.672	-0.775^{ns}	83 83	65.65 65.65	17 17	24.35 24.35	0.005^{ns}
Shwebo-thann-lann Vs Ta-kaung	2.20 ± 2.626 1.83 ± 1.899	0.615^{ns}	83 74	65.65 59.34	17 26	24.35 30.66	0.482^{ns}
Shwebo-thann-lann Vs Kyun-lone-thae	2.20 ± 2.626 1.17 ± 1.462	1.846^{ns}	83 81	65.65 64.16	17 19	24.35 25.84	0.013^{ns}
Shwebo-thann-lann Vs Kanada	2.20 ± 2.626 1.67 ± 1.556	0.935^{ns}	83 86	65.65 68.03	17 14	24.35 21.97	0.139^{ns}
Shwebo-thann-lann Vs Ta-yoke-phwe-thae	2.20 ± 2.626 1.87 ± 1.784	0.559^{ns}	83 85	65.65 67.21	17 15	24.35 22.79	0.062^{ns}
Shwebo-thann-lann Vs Ta-yoke-phyu	2.20 ± 2.626 1.40 ± 1.705	0.052^{ns}	83 69	65.65 56.17	17 31	24.35 33.83	0.255^{ns}
Shwebo-thann-lann Vs Shwe-pe-thee	2.20 ± 2.626 2.17 ± 1.675	0.213^{ns}	83 73	65.65 58.69	17 27	24.35 31.31	0.225^{ns}
Myitkyina-maw-lue Vs Ta-kaung	1.73 ± 2.892 1.83 ± 1.899	-0.213^{ns}	83 74	65.65 59.34	17 26	24.35 30.66	0.482^{ns}
Myitkyina-maw-lue Vs Kyun-thone-thae	1.73 ± 2.892 1.17 ± 1.462	1.358^{ns}	83 81	65.65 64.16	17 19	24.35 25.84	0.013^{ns}
Myitkyina-maw-lue Vs Kanada	1.73 ± 2.892 1.67 ± 1.556	0.141^{ns}	83 86	65.65 68.03	17 14	24.35 21.97	0.139^{ns}
Myitkyina-maw-lue Vs Ta-yoke-phwe-thae	1.73 ± 2.892 1.87 ± 1.784	-0.308^{ns}	83 85	65.65 67.21	17 15	24.35 22.79	0.062^{ns}
Myitkyina-maw-lue Vs Ta-yoke phyu	1.73 ± 2.892 1.40 ± 1.705	0.744^{ns}	83 69	65.65 56.17	17 31	24.35 33.83	1.124^{ns}
Myitkyina-maw-lue Vs Shwe-pe-thee	1.73 ± 2.892 2.17 ± 1.675	-1.001^{ns}	83 73	65.65 58.69	17 27	24.35 31.31	0.225^{ns}

Table 1 Continue

Identity / Comparison	Micronuclei		normal pollen %	Arc-sine value	abnormal pollen %	Arc-sine value	χ^2 value
	Mean $\pm$ S.D	't' value					
Ta-kaung Vs Kyun-lone-thae	1.83 $\pm$ 1.899 1.17 $\pm$ 1.462	1.483^{ns}	74 81	59.34 64.16	26 19	30.66 25.84	0.414^{ns}
Ta-kaung Vs Kanada	1.83 $\pm$ 1.899 1.67 $\pm$ 1.556	0.351^{ns}	74 86	59.34 68.03	26 14	30.66 21.97	1.275^{ns}
Ta-kaung Vs Ta-yoke-phwe-thae	1.83 $\pm$ 1.899 1.87 $\pm$ 1.784	-0.083^{ns}	74 85	59.34 67.21	26 15	30.66 22.79	1.220^{ns}
Ta-kaung Vs Ta-yoke-phyu	1.83 $\pm$ 1.899 1.4 $\pm$ 1.705	0.908^{ns}	74 69	59.34 56.21	26 31	30.66 33.83	0.092^{ns}
Ta-kaung Vs Shwe-pe-thee	1.83 $\pm$ 1.899 2.17 $\pm$ 1.675	-0.723^{ns}	74 73	59.34 56.17	26 27	30.66 31.31	0.574^{ns}
Kyun-lone-thae Vs Kanada	1.17 $\pm$ 1.462 1.67 $\pm$ 1.556	-1.259^{ns}	81 86	64.14 58.69	19 14	25.86 21.97	0.309^{ns}
Kyun-lone-thae Vs Ta-yoke-phwe-thae	1.17 $\pm$ 1.462 1.87 $\pm$ 1.784	-1.634^{ns}	81 85	64.14 67.21	19 15	25.86 22.79	0.203^{ns}
Kyun-lone-thae Vs Ta-yoke-phyu	1.17 $\pm$ 1.462 1.40 $\pm$ 1.705	-0.551^{ns}	81 85	64.14 56.17	19 31	25.86 33.83	0.782^{ns}
Kyun-lone-thae Vs Shwe-pe-thae	1.17 $\pm$ 1.462 2.17 $\pm$ 1.675	-2.422*	81 73	64.14 58.69	19 27	25.86 31.31	0.344^{ns}
Kanada Vs Ta-yoke-phwe-thae	1.67 $\pm$ 1.556 1.87 $\pm$ 1.784	-0.455^{ns}	86 85	68.03 67.21	14 15	21.97 22.79	0.003^{ns}
Kanada Vs Ta-yoke-phyu	1.67 $\pm$ 1.556 1.40 $\pm$ 1.705	0.629^{ns}	86 69	68.03 56.17	14 31	21.97 33.83	1.844^{ns}
Kanada Vs Shwe-pe-thee	1.67 $\pm$ 1.556 2.17 $\pm$ 1.675	-1.178^{ns}	86 73	68.03 58.69	14 27	21.97 31.31	1.143^{ns}
Ta-yoke-phwe-thae Vs Ta-yoke-phyu	1.87 $\pm$ 1.784 1.40 $\pm$ 1.705	-1.026^{ns}	85 69	67.21 56.17	15 31	22.79 33.83	1.573^{ns}
Ta-yoke-phwe-thae Vs Shwe-pe-thee	1.87 $\pm$ 1.784 2.17 $\pm$ 1.675	-0.660^{ns}	85 73	67.21 58.69	15 27	22.79 31.31	0.931^{ns}
Ta-yoke-phyu Vs Shwe-pe-thae	1.40 $\pm$ 705 2.17 $\pm$ 1.675	-1.735^{ns}	69 73	56.17 58.69	31 27	33.83 31.31	0.108^{ns}

n.s = non-significant S.D = Standard deviation
*,** = statistically significant at 5% and 1% level respectively

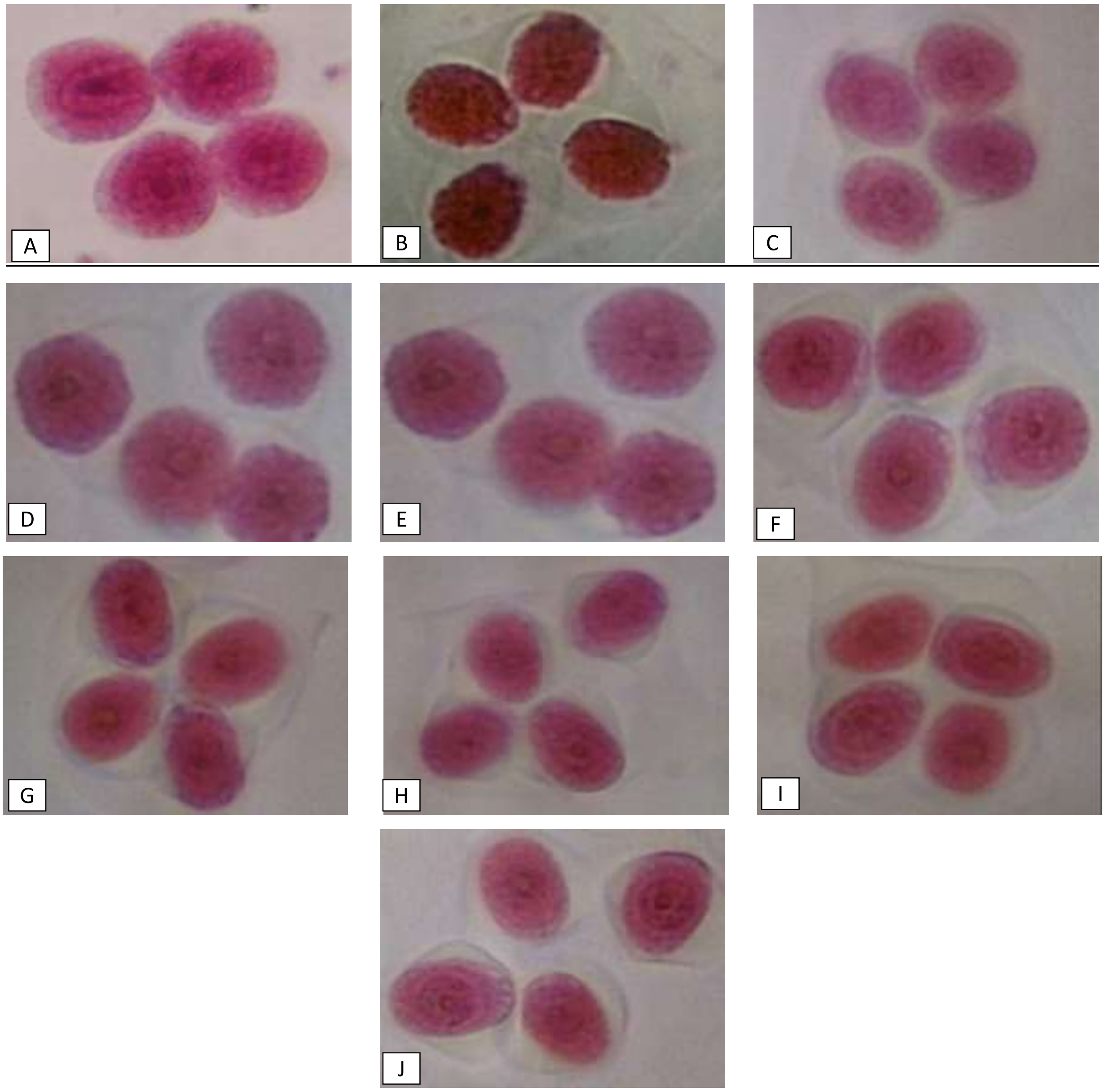

Figure 2 Spore-tetrad characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane B. Kalay-sadaw-pe C. Shwebo-thann-lann

D. Myitkyina-maw-lue E. Ta-kaung F. Kyun-lone-thae

G. Kanada H. Ta-yoke-phwe-thae I. Ta-yoke-phyu

J. Shwe-pe-thee

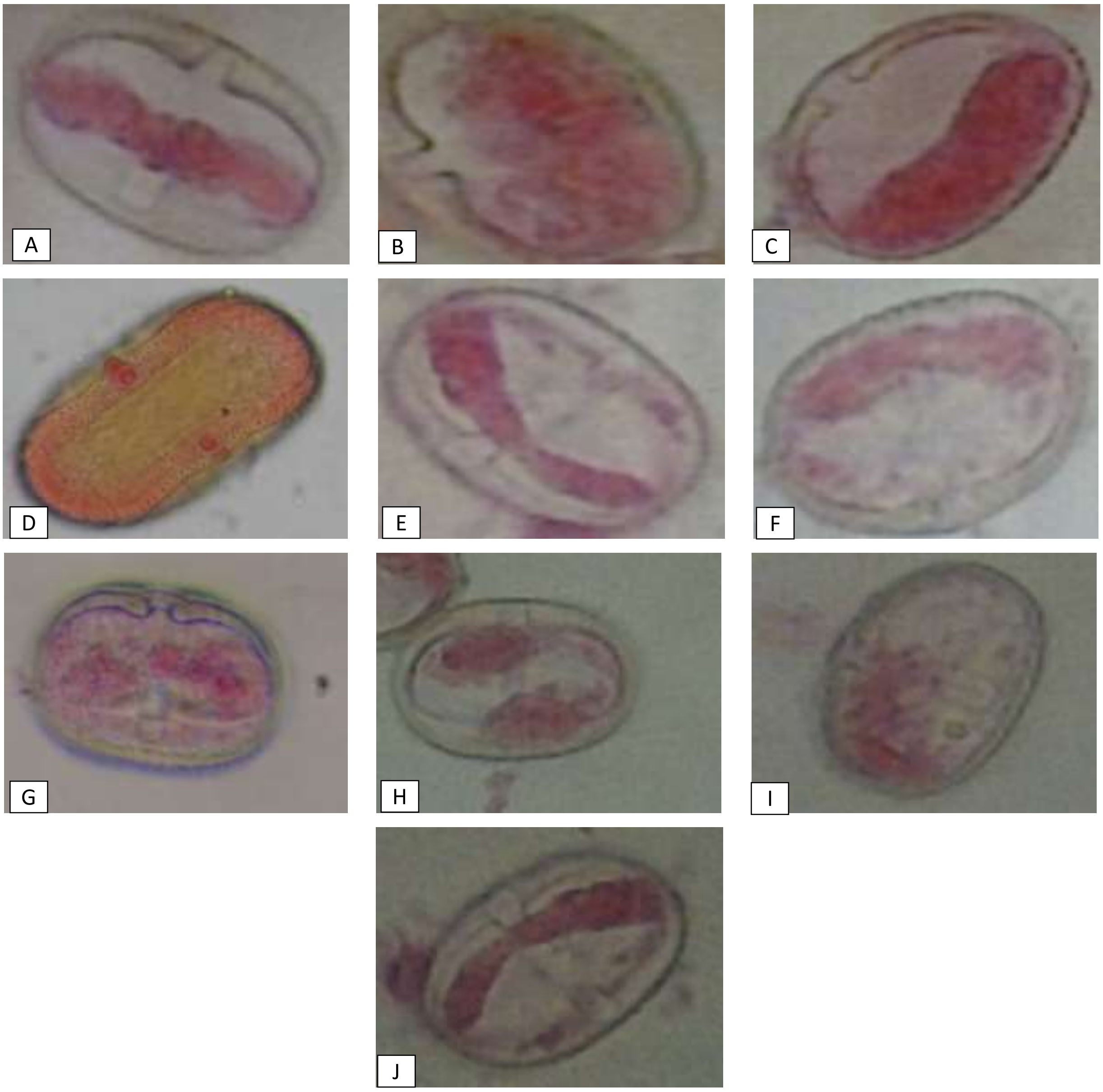

Figure 3 Normal pollen characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane B. Kalay-sadaw-pe C. Shwebo-thann-lann

D. Myitkyina-maw-lue E. Ta-kaung F. Kyun-lone-thae

G. Kanada H. Ta-yoke-phwe-thae I. Ta-yoke-phyu

J. Shwe-pe-thee

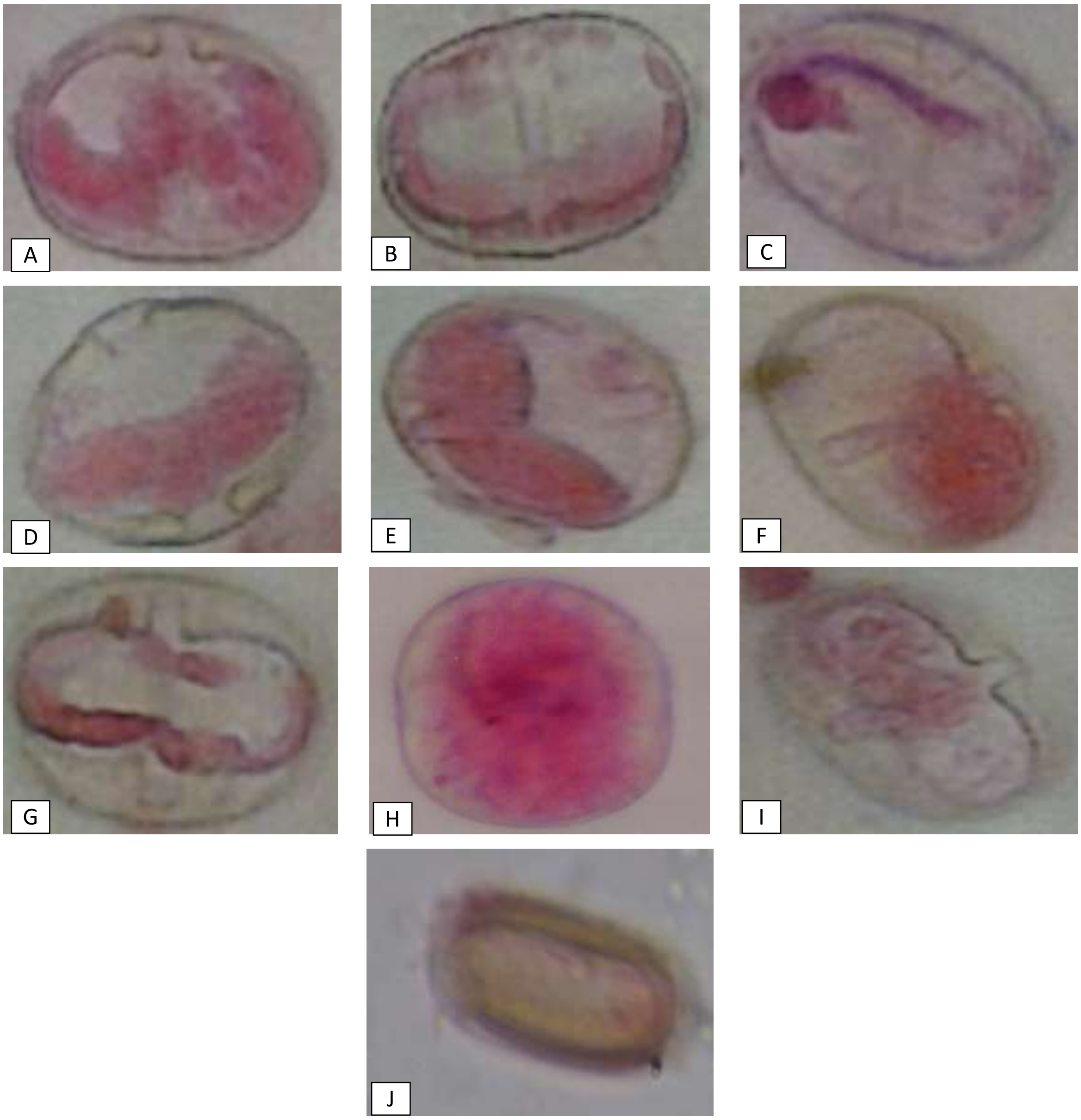

Figure 4 Abnormal pollen characters of *Pisum sativum* L. cultivars

A. Pe-kyauk-sane B. Kalay-sadaw-pe C. Shwebo-thann-lann

D. Myitkyina-maw-lue E. Ta-kaung F. Kyun-lone-thae

G. Kanada H. Ta-yoke-phwe-thae I. Ta-yoke-phyu

J. Shwe-pe-thee

Discussion and Conclusion

As peas are diploid and self- fertilizing, the breeding strategies adapted to improve the crop have been those conventionally adapted for such species. The extent of natural out crossing has been estimated to be less than 1%. Hybridization among cultivars or between cultivars, land races and primitive forms followed by pedigree, bulk or backcross methods of selection, has been traditionally used; more recently, single seed descent methods have also been evaluated (Bown, 1992) as three generations can be generated each year (Snoad, 1980).

In the present study, spore-tetrad formation and pollen fertility were observed and recorded.

Hockett (1984) stated that different genes for male sterility are responsible for different of pollen abortion that give rise low fertility and yield.

In the present study, it was observed that chance of the cccurrence of micronuclei per spore-tetrad is very low, only one cultivar i.e., Shwebo-thann-lann have more than 2.2 micronuclei in mean number, while the remaining cultivars were less than that. Pollen mother cells (PMCs) of each of the ten *Pisum sativum* L. cultivars were also collected from ten collections sites for spore-tetrad and pollen fertility study. Micronuclei per spore-tetrad, normal and abnormal pollen were also varied from cultivar to cultivar. These differences were also analysed by the help of acetocarmine squash method.

In the present study, among the 10 cultivars studied ranged from 69 % to 86 % normal but did not exhibited a single significantly superior or inferior number of pollen. Abnormal pollen was also observed non significant in any of the comparisons These results showed that the studied cultivars may be possessed genetic stability and highest pollen fertility. Thus, these cultivars and lines are good to conserved for long pea cultivation.

Acknowledgements

I would like to express my hearfelt thanks to Dr. Thar Htun Maung, Rector of Kalay University of giving permission and encouragement. I am very thankful to Dr. Khin Thida Soe, Professor and Head, Department of Botany, Kalay university for her various help and suggestion throughout this work.

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