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The Effect of Seed Position in Pod on The Seed Viability of Cowpea (*Vigna unguiculata*)

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Abstract

In Indonesia, cowpea (*Vigna unguiculata* L. Walp) is widely cultivated in the dry climate area. Famers used the seeds from previous plants or traditional markets. The aims of the study were to determine the effect of seed position in the pods on seed viability and to study the seed and seedling characteristics regarding the genetic variation and seed position. The cowpea used in this study was from 18 Indonesian local varieties. Twenty-five matured pods were randomly detached from each variety. Each pod was divided into three parts, i.e. the lower third (base), the middle third, and the upper third (tip). The seeds of each part were germinated using sand media to evaluate their viability. Each local variety has different response to the percentage of seed viability in each seed position in the pod. The highest abnormal germination occurred in the seed from lower part of the pod, followed by seed in the middle of the pod and the tip of the pod. The variation of seed viability in pods was more determined by genetic differences in local varieties. The average seed viability from three parts of the pods was highest for seeds originated from local varieties of Lumajang and Sampang. Therefore, it is recommended that the seeds of both local varieties can be mixed to be used for optimum cowpea cultivation.

How to Cite

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INTRODUCTION

Cowpea is a legume plant that widely grown in various tropical and sub-tropical regions. This plant has high adaptation to various environments and considered as drought tolerant (Mahalakshmi et al., 2007). In Indonesia, cowpea is cultivated in various agroecosystems, including in arid climates. One of the problems of cowpea productivity is the low plant population in the field. This is due to the absence of seed producers, hence the farmers use seeds from previous plants or buy seeds in the local market.

In Indonesia, the widest cowpea cultivation is carried out in the dry season or at the beginning of the rainy season in dry climate area. The use of high-quality seed is important, which not only to reduces production costs, but also to obtain uniform crop performance and ideal plant populations. In general, there are two indicator of seed quality, namely seed viability and seed vigour. Seed viability is defined as the ability of the embryo to germinate, and is affected by a number of different conditions, whereas seed vigour is the sum of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence (Tekrony, 2003; Shaban, 2013; El-Abady, 2014). The seed quality is reflected by their ability to grow simultaneously, uniformly and have a vigorous plant especially at the beginning of growth (Nautiyal et al., 2000; Chachalis et al., 2008; Hapsari & Trustinah, 2017).

Seed size is an important physical indicator of seed quality that affects vegetative growth and is frequently related to yield, market grade factors and harvest efficiency (Ambika et al., 2014). Baysah et al., (2018) studied the effect of seed size of four cowpea varieties and the result showed that seed size did not affect the germination, although the seedling dry weight was determined by its seed size. However, Massimi (2018) reported that seed size had potential impact in seed viability and vigour in barley. Previous research on lentil seeds revealed that germination parameters were significantly related to the seed weight (Hojjat, 2011). Lambat et al. (2014) studied the effect of storage on seed germination, seedling vigour and mycoflora on cowpea, and they concluded that the medium size seeds showed significantly higher seed viability, higher field emergence percentage and lesser invasion of fungal flora during storage up to coming kharif (cropping) season sowing time.

Compared to other major legume plants in Indonesia such as soybeans and mungbeans, the pod size of cowpea is relatively longer. Trustinah et al., (2017) characterized 150 accessions of cowpea in Indonesia and obtain an average pod length of 15.7 cm and the number of seeds/pods is 12.4 seeds. Another study by Setyowati & Sutoro (2010) stated that pod length of cowpea is between 14.8-15.6 cm and number of seeds/pods is between 12.2 - 13.5 seeds. This shows the variation in the pod as well as seed size which may affect in the process of the seed formation and maturation in a pod. With such conditions, the position of seeds in a pod may affects the viability and vigor of the seed in cowpea. The seed quality is important related to the provision of cowpea seeds for planting which is done by processing all pods into seeds.

The aims of the study were to determine the effect of the seed position in pods on the seed viability of some cowpea varieties originated from Indonesia. The information obtained from this study is expected to be able to be used as a guide in producing a high quality cowpea seeds.

METHODS

The research material consisted of 18 local varieties of cowpea. Those local varieties were collected from provinces of West Sulawesi, East Java, West Nusa Tenggara, and South Kalimantan on 2016. The field experiment was carried out in Malang (Indonesia) from March to June 2017. The characteristics of the research site were at 445 m above sea level, the climate type (Oldeman) was C2, and the soil type was heavy Entisol. The seed viability test was carried out at the Indonesian Legume and Tuber Crops Research Institute's greenhouse in Malang on July 2017.

The field experiment was arranged in a randomized block design. The treatment was consisted of 18 cowpea varieties, with three replications. Soil processing was carried out optimally. Irrigation channel was made for each treatment. The seeds were planted with 2-3 seeds per hole, and at 12 days after planting it was thinned into 1 plant per hole. The plant spacing was 50 cm x 15 cm. Plant was fertilized by 50 kg Urea, 100 kg SP36 and 75 kg KCl, which was given entirely at the time of planting. Pest, disease and weed control were carried out intensively.

The seed viability test

Twenty-five matured pods were randomly detached from plant body of each variety. Pods were dried under the sun, and after the pods were dried, they were stored at room temperature for 15 days. A day before seed viability test, each pod was divided into three parts, i.e. the lower third (base), the middle third, and the upper third (tip). Then the seeds derived from each part of the pods were mixed into one bag.

The seed viability test was using sterilized sand media, which was conducted in glass house, and arranged in a randomized block design with three replications. Twenty-five seeds from each part of the pod germinated in sand media.

Observation and data analysis

Observations that were made on normal and abnormal germination every day included: 100 seeds weight, seedling length, and root length. The observations on the pod length and number of seeds in each pod were carried out on intact pods. All observed data were subjected to analysis of variance (ANOVA). Correlation analysis was used to determine the relationship between the seed viability along with other observed characteristics with seed positions in the pod (Prematunga, 2012).

RESULTS AND DISCUSSION

The origin of the local variety

The used local varieties were originated from East Java (11 accessions), West Nusa Tenggara (four accessions), South Kalimantan Selatan (two accessions), and West Sulawesi (one accession) (Table 1). Most of accessions had white seed coat (nine accessions), and the others had brown (one accession), black (three accessions), dark reddish brown (four accession), and white spot (one accession).

Analysis of variance (ANOVA)

The results of ANOVA on several germination characteristics of local cowpea varieties showed an interaction between the seed position and the seed viability (normal and abnormal seedling). Seed position affects the seed viability and weight of 100 seeds. The local varieties were significantly affect all observed characters (Table 2). Significant different interactions showed that

Table 1. The origin of 18 Indonesian local cowpea varieties

Code	Origin				Seat coat color
	Village	District	Regency	Province	
VU 0005	Bumiayu	Wonomulyo	Pole Wali Mandar	West Sulawesi	Dark reddish brown
VU 0007	Pasinan Lemah Putih	Wringin Anom	Gresik	East Java	Black
VU 0020	Muneng Kidul	Sumberasih	Probolinggo	East Java	Brown
VU 0022	Muneng Kidul	Sumberasih	Probolinggo	East Java	White
VU 0027	Muneng Kidul	Sumberasih	Probolinggo	East Java	Black
VU 0032	Tatung	Balong	Ponorogo	East Java	Dark reddish brown
VU 0047	Segala Anyar	Pujut	Lombok Tengah	West Nusa Tenggara	Dark reddish brown
VU 0063	Oro-oro Pule	Kejayan	Pasuruan	East Java	White
VU 0076	Gunung Sekar	Sampang	Sampang	East Java	Dark reddish brown
VU 0093	Segala Anyar	Pujut	Lombok Tengah	West Nusa Tenggara	Black
VU 0098	Gunung Makmur	Takisung	Tanah laut	South Kalimantan	White
VU 0106	Segala Anyar	Pujut	Lombok Tengah	West Nusa Tenggara	White
VU 0112	Segala Anyar	Pujut	Lombok Tengah	West Nusa Tenggara	White spot
VU 0125	Gunung Makmur	Takisung	Tanah laut	South Kalimantan	White
VU 0151	Banaran Wetan	Bagor	Nganjuk	East Java	White
VU 0155	Kaliboto Lor	Jatiroto	Lumajang	East Java	White
VU 0164	Gunung Sekar	Sampang	Sampang	East Java	White
VU 0173	Birowo	Binangun	Blitar	East Java	White

Figure 1. Collection of local cowpea varieties obtained from the field, farmers, and market

Table 2. The analysis of variance for viability and morphological seedling characters of local cowpea varieties originated from different seed position in the pod

Character	Mean Square		
	Seed position (P)	Local variety (V)	P × V
Viability of normal seedling (%)	146.6016*	8.5323*	3. 0149**
Viability of abnormal seedling (%)	12.9706*	60.4824*	29.6147*
100 seeds weight (g)	17.9981*	44.6016**	4.8414^{ns}
Stem length (cm)	35.2287^{ns}	72.6878*	8.2100^{ns}
Root length (cm)	5.6536^{ns}	10.3555**	0.7328^{ns}
Pod length (cm)	-	39.8711**	-
Number of seed/pod	-	3.8818*	-

* = significant at 5% probability level ($p < 0.05$), ** = significant at 1 % probability level ($p < 0.01$), ns = not significant

(A) (B) (C)

Figure 2. (A) Example of local cowpea accession, (B) Field experiment, and (C) Seed viability test

seed viability in cowpea was influenced by the seed position on the pods, and also determined by the genetic characteristics of each local variety of cowpea.

Pod length and number of seeds per pod

The pod length of 18 varieties varied from 11.30 to 24.80 cm (average 17.00 cm) (Figure 3). There were eight accessions with pod lengths above the average and the remaining accessions were below average. Each region has a variety of pod lengths indicated by the pod lengths above 20 cm that were originating from various provinces in Indonesia. Number of seed/pod vary from 10.50 to 20.10 seeds/pod with an average of 12.97 seeds /pod (Figure 4). Three accessions with pod lengths of above 20 cm, apparently not followed by more number of seeds/pod.

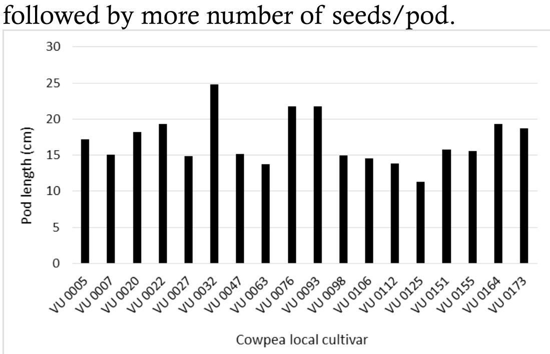

Figure 3. Pod lengths of 18 local cowpea varieties

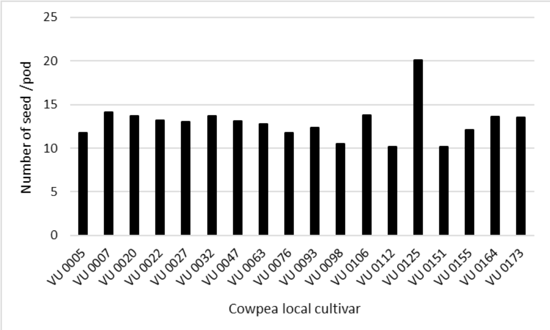

Figure 4. Number of seeds per pod of 18 local cowpea varieties

Seed viability

Seeds viability originated form the three seed positions in the pods of the 18 local cowpea varieties is presented in Table 3. The average seed viability of upper third of the pod was 64.17% (range 25-95%), while the middle third average was 65% (range 35-90%) and the lower third was 60.28% (range 25-90%). Seeds obtained from upper third and middle third of pod have equal viability, while those from the lower third showed the lowest seed viability. The research by Krisnawati & Adie (2018) on yam bean resulted that both seed viability and vigour are not affected by the position of seeds in the pod. In maize, as reported by Bahiyah (2012), seeds originated from the middle part of corn cob have higher viability and vigour compared to ones from the upper and lower of corn cob.

The average seed viability from three seed positions was 63.15% with a range between 28.33 - 83.33%. Local varieties VU 0155 and VU 0164 had similar value of seed viability (83.33%). The significant interaction between the seed position and local varieties shows the different response of each local variety to the seed position in each local variety of cowpea. The seed originated from the upper third of the pod namely VU 0020 had the highest seed viability, whereas on VU 015, the highest viability was come from the seeds from the middle third position. Furthermore, VU 0164 had the best seed viability of seeds originated from the lower third part of the pod. Local varieties of cowpea were said to have high viability if there was consistency in seed viability from all parts of the pod. This condition was showed by varieties VU 0155 and VU 0164. In this study, black seed coat seed was tended to show a low seed viability (VU 0007 and VU 0093), except the VU 0027 with viability of 75% (Figure 5).

Table 3. Viability of normal seedling of local cowpea varieties which derived from different seed position in the pod

Local variety	Viability of normal seedling from three seed positions in the pod (%)			
	Upper	Middle	Lower	Mean
VU 0005	70.00	75.00	80.00	75.00
VU 0007	60.00	30.00	55.00	48.33
VU 0020	95.00	35.00	25.00	51.67
VU 0022	70.00	80.00	60.00	70.00
VU 0027	65.00	80.00	80.00	75.00
VU 0032	50.00	80.00	60.00	63.33
VU 0047	70.00	85.00	30.00	61.67
VU 0063	45.00	80.00	80.00	68.33
VU 0076	35.00	35.00	65.00	45.00
VU 0093	50.00	45.00	65.00	53.33
VU 0098	25.00	35.00	25.00	28.33
VU 0106	70.00	70.00	50.00	63.33
VU 0112	75.00	80.00	70.00	75.00
VU 0125	65.00	50.00	50.00	55.00
VU 0151	75.00	55.00	55.00	61.67
VU 0155	80.00	90.00	80.00	83.33
VU 0164	75.00	85.00	90.00	83.33
VU 0173	80.00	80.00	65.00	75.00
Mean	64.17	65.00	60.28	63.15

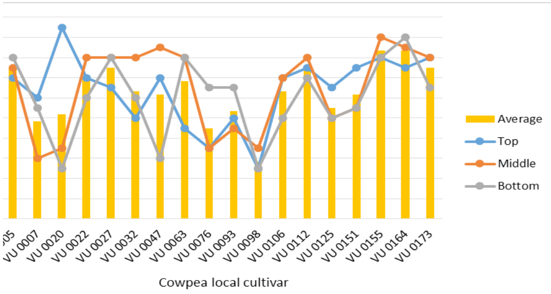

Figure 5. Viability of normal seedling of local cowpea varieties which originated from different seed position in the pod

Viability of abnormal seedling

Abnormal seedling can be defined as a seedling that does not have all essential structures or is damaged, deformed or decayed that prevents normal development. The average viability of abnormal seedling was highest on the seed position originated from the lower third of the pod (20.56%), and then followed by seed from the middle third (18.33%) and the lowest was from the seeds at the upper third of the pod (Table 4). This could be associated with the process of seed formation in each pod, where the process of seed formation starts from the lower pods and continues to tip (upper) of the pod. In other words, the seeds which formed in the lower pods are older than the seeds in the middle or upper part of the pod. Martinez et al. (2007) stated that the assimilate distribution starts from the base to the tip of the pod, this will result in differences in seed size in each part of the pod.

Table 4. Viability of abnormal seedling of local cowpea varieties based on seed position in the pod

Local variety	Abnormal seedling based on seed positions in the pod (%)			
	Upper	Middle	Lower	Mean
VU 0005	10.00	25.00	15.00	16.67
VU 0007	20.00	20.00	30.00	23.33
VU 0020	0.00	50.00	35.00	28.33
VU 0022	30.00	15.00	40.00	28.33
VU 0027	15.00	20.00	10.00	15.00
VU 0032	50.00	20.00	30.00	33.33
VU 0047	15.00	15.00	20.00	16.67
VU 0063	20.00	20.00	20.00	20.00
VU 0076	5.00	5.00	20.00	10.00
VU 0093	25.00	25.00	20.00	23.33
VU 0098	0.00	10.00	0.00	3.33
VU 0106	15.00	10.00	40.00	21.67
VU 0112	0.00	20.00	20.00	13.33
VU 0125	20.00	25.00	15.00	20.00
VU 0151	20.00	10.00	15.00	15.00
VU 0155	5.00	10.00	20.00	11.67
VU 0164	0.00	15.00	5.00	6.67
VU 0173	15.00	15.00	15.00	15.00
Mean	14.72	18.33	20.56	17.87

This research obtained a significant interaction between the seed position and local varieties, showed that the genetic factor of each local variety plays role in determining the viability of abnormal seedling. Accession of VU 0032 has the highest percentage of abnormal seedling from the upper part of pod. The VU 0020 has the highest percentage of abnormal seedling from the middle seed position, but the seed positioned at the upper part of the pod resulted in 0% of abnormal seedling. At the lower part of the pod, VU 0022 and VU 0106 had the highest percentage of abnormal seedling.

Weight of 100 seeds

Seed size is one of the genetic characters of cowpea. In some commodities, it was reported to determine the viability and vigour of the seed. In this study, weight of 100 seeds was not influenced by the interaction between the seed position and genetic variation. Thus the difference in seed size was caused more by genetic factors of each local variety.

Weight of 100 seeds in the middle part of the pods was 13.18 g. It was not significantly different from the upper position (12.87 g), but it was significantly different compared to the results obtained from lower part of the pod (12.35 g) (Table 5). The average weight of 100 seeds from 18 cowpea accessions was 12.80 g with a range between 7.23 - 18.29 g/100 seeds. VU 0112 had the smallest seed size while the biggest seed size was obtained from VU 0020

Table 5. Weight of 100 seeds of local cowpea varieties originated from different seed position in the pod

Local variety	100 seed weight from three seed positions in the pod (g)			
	Upper	Middle	Lower	Mean
VU 0005	14.92	15.80	15.06	15.26
VU 0007	10.25	9.75	9.40	9.80
VU 0020	18.05	19.37	17.44	18.29
VU 0022	12.30	13.10	11.87	12.42
VU 0027	10.47	10.83	10.23	10.51
VU 0032	15.40	15.14	17.08	15.87
VU 0047	10.34	10.48	10.20	10.34
VU 0063	13.87	14.79	14.74	14.47
VU 0076	13.33	14.80	4.08	10.74
VU 0093	17.90	17.42	17.57	17.63
VU 0098	15.55	16.37	14.43	15.45
VU 0106	10.21	10.00	10.22	10.14
VU 0112	7.09	7.44	7.14	7.22
VU 0125	11.49	11.80	11.38	11.56
VU 0151	10.85	10.61	11.53	11.00
VU 0155	11.58	12.07	11.61	11.75
VU 0164	14.63	14.89	15.41	14.98
VU 0173	13.46	12.60	12.93	13.00
Mean	12.87	13.18	12.35	12.80

The stem length of seedling

The average stem length of seedling originated from seeds of the upper part was 10.84 cm, equivalent with those from the middle (11.05 cm) and the lower part of the pod (11.07 cm) (Table 6). The difference in stem length was more determined by the genetic differences of each variety of cowpea. The stem length of 18 accessions ranged from 7.75 - 13.93 cm (average 10.99 cm). VU 0020 have the shortest stem length (7.75 cm), whereas the longest was VU 0164 (13.93 cm).

Research conducted by Sebayang et al., (2014) in papaya seeds revealed that the treatment of seeds was able to increase the several morphological properties of seedling including the seedling fresh weight. In yam bean seed, it was reported that the diversity of morphological characteristics of seedling was more determined by differences in the varieties used (Olasoji et al., 2011). Krisnawati & Adie (2018) based on the their research on yam bean seeds also reported that the root length and stem length of the seedling were not affected by differences in the position of seeds on the pod.

Table 6. The stem length of seedling of local cowpea varieties originated from different seed position in the pod

Local variety	The stem length of seedling of local cowpea varieties originated from different seed position in the pod (cm)			
	Upper	Middle	Lower	Mean
VU 0005	10.80	11.30	10.60	10.90
VU 0007	8.90	8.32	8.26	8.49
VU 0020	5.70	8.42	9.12	7.75
VU 0022	11.32	11.80	10.74	11.29
VU 0027	11.30	12.80	11.60	11.90
VU 0032	13.10	11.90	12.00	12.33
VU 0047	10.50	11.02	9.90	10.47
VU 0063	9.28	12.10	11.60	10.99
VU 0076	9.40	11.20	10.58	10.39
VU 0093	12.24	13.50	12.00	12.58
VU 0098	10.80	12.60	11.58	11.66
VU 0106	9.40	11.00	12.40	10.93
VU 0112	8.30	8.14	10.10	8.85
VU 0125	8.94	8.04	9.60	8.86
VU 0151	12.40	11.10	11.60	11.70
VU 0155	12.70	10.48	11.30	11.49
VU 0164	14.40	13.70	13.68	13.93
VU 0173	15.70	11.50	12.60	13.27
Mean	10.84	11.05	11.07	10.99

Root length

The root length of seedling from was more determined by the influence of genetic differences compared to the effect of the seed position in the pods as well as the interaction between the seed position and genetic variation. The seedling root originated from the seed of the upper part was longer (11.87 cm) than those from the middle part (10.88 cm) and the lower part (10.29 cm). However, the difference in root length in all accessions was not significantly different. The average root length in all accessions varied from 8.13 cm to 14.63 cm (average 11.02 cm) (Table 7). VU 0112 had the longest root (14.63 cm) and there were four accessions with root lengths below 10 cm.

Table 7. Root length of seedling of local cowpea varieties obtained from different seed position in the pod

Local variety	Root length from three seed positions in the pod (cm)			
	Upper	Middle	Lower	Mean
VU 0005	10.40	7.70	13.00	10.37
VU 0007	9.30	9.98	9.00	9.43
VU 0020	7.50	8.60	8.30	8.13
VU 0022	10.20	9.40	9.70	9.77
VU 0027	10.90	12.44	9.00	10.78
VU 0032	11.10	12.60	10.10	11.27
VU 0047	11.80	11.30	9.80	10.97
VU 0063	12.24	13.20	10.50	11.98
VU 0076	10.50	12.30	9.90	10.90
VU 0093	11.40	12.40	11.90	11.90
VU 0098	14.70	15.40	11.20	13.77
VU 0106	12.50	9.70	9.20	10.47
VU 0112	19.60	11.80	12.50	14.63
VU 0125	12.60	10.60	10.10	11.10
VU 0151	12.60	9.20	11.20	11.00
VU 0155	14.20	10.60	9.10	11.30
VU 0164	10.40	7.90	8.70	9.00
VU 0173	11.80	10.80	12.10	11.57
Mean	11.87	10.88	10.29	11.02

Relationship between characters

The correlation between seed and seedling characteristics with viability of normal seedling from each seed position on the pods was shown in Table 8. Correlation coefficient between normal seedling with several germination characters (100 seed weight, pod length, and number of seeds/ pods) showed that only 100 seed weight that have a correlation value r = -0.322** with the seed viability of the seeds originating from the middle of the pod. This shows that the larger size of the seeds resulted in the lower the seed viability.

Table 8. Correlation between seed viability with germination characteristics of three seed positions in the pod

Seed and seedling characteristics	Correlation with normal seed viability (%)		
	Upper	Middle	Lower
100 seed weight (g)	-0.142	-0.322**	0.227
Pod length (cm)	-0.138	-0.027	0.119
Number of seed/ pod	0.148	-0.073	-0.097

** = significant at 1 % probability level ($p < 0.01$)

A research conducted by Baysah et al. (2018) that found that seed size did not affect the germination rate of cowpea, but the fresh weight of seedling was more influenced by the seed size than differences in varieties. In other studies, it was revealed that seed size plays an important role in determine the seed viability and vigor. Larson et al. (2015) stated that seed size had an effect on the performance of cowpea germination. It was also confirmed by Wang et al. (2017) that the difference of seed sizes determines the germination and germination rates.

Research to determine the relationship between the position of the seeds in the pods and the quality of seeds in cowpea has not been reported in Indonesia. Furthermore, the relationship between cultivars and the seeds position in the pods also has never been previously studied. Results of this study show that the position of seeds in the pods determines the viability of the seeds, and what needs to be considered is the importance of genetic role in determining the quality of the seeds. Information derived from this study can be used as a guide in producing a high quality cowpea seeds. The use of seeds with high quality will indeed reduce the production costs and also creating opportunity to increase the yield of cowpea because the plant population will be in optimal condition.

CONCLUSION

Viability of cowpea seeds originated from seeds in the middle and upper parts of pods was higher than those from the lower part of the pod. Seed size only negatively affects the germination in seeds from the middle part of pod. The average seed viability from three parts of the pods was highest for seeds originated from local varieties of VU 0155 Lumajang and VU 0164 from Sampang, hence the seedsof both local varieties can be mixed from all parts of the pod for planting material.

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