The seed quality of Indonesian cowpea local varieties after storage

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Abstract. The optimum performance of the cowpea plant population in the field could be determined by their seed quality. The research aims to evaluate the seed quality of several cowpea local varieties after being stored in the form of seeds and pods. A total of 18 cowpea local varieties from East Java, West Nusa Tenggara, South Kalimantan, and West Sulawesi were evaluated for their seed vigor and viability after being stored for 30 and 60 days at room temperature using plant material in the form of seeds and pods. The seed multiplication was conducted in the field at Banyuwangi, and the seed quality test using sand media was carried out at the glasshouse of ILETRI Malang. The storage of cowpea in the form of pods and seeds for up to 30 days did not significantly affect the seed quality. Four local varieties (VU 0007, VU 0093, VU 0125, VU 0155) showed good viability at the storage of 30 and 60 days, respectively. The VU 0032 and VU 0076 have optimum viability after being stored for 60 days. The speed of the germination index (SGI) was not only describing the level of vigor but also able to show the type of plant material to be stored. The VU 0007, VU 0093, and VU 0155 were recommended to be stored in the form of seeds, meanwhile, the VU 0125 can be stored for up to 60 days in the form of seeds or pods. The morphological characters of sprouts, namely hypocotyl length, stem dry weight, and root dry weight, could be considered as a benchmark parameter for seed vigor of cowpea.

1. Introduction

The cowpea (Vigna unguiculata L. Walp) is thought to have originated from Africa [1] and it has now spread and been planted in several regions in Indonesia. One of the problems of cowpea cultivation in Indonesia is the absence of seed producers to produce certified cowpea seeds, hence the farmers get the planting material (seeds) from the market or produce from their cultivation. As a result, the use of those planting materials may cause the optimum plant population in the field cannot be reached, and then it will decrease the plant productivity.

The seed quality can be measured through the seed vigor and viability. Seed viability is a reflection of the ability of the embryo to grow, meanwhile, seed vigor is related to the ability of seeds to grow in sub-optimal conditions [2,3]. In the Phaseolus vulgaris, the use of seeds with a low vigor may result in a 20% decrease in the seed yield [4]. Other research showed that soybean seeds with high and intermediate vigor can compete against weeds, reduce the accumulation of weed dry mass, and produce similar seed yields between weeded and unweeded treatments [5]. This shows that vigorous seeds can compete against weeds.
The seed vigor and viability are determined by genetic and physiological factors, including storage conditions [3,6] and it is further explained that the important storage factors are temperature, moisture, seed characteristics, micro-organism geographical location, and storage structure. Long-term storage was reported could reduce the seed viability of cowpea by between 4 and 12% regardless of the temperature and relative humidity of the storage environment [7]. A study in the four cowpea cultivars (BRS Mazagao, UFRR Grão Verde, Pretinho Precoce 1, and BRS Guarib) during 3, 6, and 9 months of storage showed that the BRS Mazagao had better physiological quality up to 9 months of storage, meanwhile other cultivars show a reduction in physiological quality after three months of storage [8]. This suggests that the role of genetic factors also determines the storage period of cowpea genotype. In sorghum, the normal germination and speed germination after stored for 12 months was lower than the ten months storage [9]. The storage materials also affect the seed vigor, that the air-tight glass containers were better than sack containers to maintain the seed vigor and germinability of cowpea under ambient condition [10].

The quality of seed growth in the field is usually predicted through the result of the seed quality testing conducted in the laboratory. A significant correlation has been obtained between the germination performance test in the laboratory and seedling emergence in the field [11]. A study in the seed viability and in vitro shoot regeneration of soybean revealed that shoot induction was positively correlated with seed storage, and the nine months of storage decreased seed germination up to 50% [12]. A study related to the physiological and biochemical factors in cowpea seed showed that cultivars with high percent germination showing high sugar content, suggesting the important use of sugar within the seed germination process [13]. The seed size of cowpea was reported did not affect the seed germination and vigor, but the seedling dry weight was affected by the seed sizes [14]. A study on the effect of seed position on pods on seed viability of several local varieties of cowpea showed that the seeds in the middle and upper part had higher viability than those in the lower part of the pod, and the differences in the seed viability were more determined by the genetic factor [15].

The information on the different patterns of seed quality after being stored in the form of seeds and pods is important to identify the storage period of cowpea seeds for being used as planting material in the next season. The research aims to evaluate the seed quality of several cowpea local varieties after being stored in the form of seeds and pods. The results of this study will provide information about the seed quality of various local varieties of cowpea, and recommendations on tolerable storage period for each local variety.

2. Methods
The seed multiplication of cowpea local varieties was carried out during the dry season (April to July 2018) in Genteng Research Station, Banyuwangi (East Java, Indonesia) which located at 8°22′04.4″SL and 114°8′45.6″EL, 168 m above sea level, with soil type of Entisol.

2.1. Research materials
A total of 18 cowpea local varieties from East Java, West Nusa Tenggara, South Kalimantan, and West Sulawesi were used in this study (table 1).

| No | Accession code | Village | Regency | District | Province | Seed coat color |
|----|----------------|---------|---------|----------|----------|----------------|
| 1  | VU 0005        | Tanduk Kalua | Mimake | Mamasa   | West Sulawesi | Maroon        |
| 2  | VU 0007        | Pasirnai Lemah Patih | Wengin Anom | Gresik   | East Java   | Maroon        |
| 3  | VU 0022        | Muneng Kidul  | Sumberasih | Probolinggo | East Java   | White         |
| 4  | VU 0027        | Muneng Kidul  | Sumberasih | Probolinggo | East Java   | Maroon        |
| 5  | VU 0032        | Tatung       | Balong  | Ponorogo | East Java   | Maroon        |
| 6  | VU 0047        | Segala Anyar | Pujut  | Lombok Tengah | West Nusa Tenggara | Maroon spot |
| 7  | VU 0063        | Sumberbanteng | Kejayan | Pasuruan | East Java   | White         |
| 8  | VU 0076        | Rongtengah   | Sanoang | Sampang  | East Java   | Maroon        |
| 9  | VU 0093        | Segala Anyar | Pujut  | Lombok Tengah | West Nusa Tenggara | Black        |
| 10 | VU 0098        | Segala Anyar | Pujut  | Lombok Tengah | West Nusa Tenggara | White        |

Table 1. The origin and seed coat color of 18 cowpea local varieties.
2.2. Seed multiplication
The planting for seed multiplication was conducted in the paddy field after rice planting, and without soil tillage. Each local variety was planted in 5 single rows for 4.0 meters long, 0.75 meters between rows, plant spacing of 40 × 15 cm. Fertilizers of 50 kg/ha Urea, 100 kg/ha SP36, and 75 kg/ha KCl were applied entirely at the time of planting. The pests, diseases, and weeds were optimally managed.

After the plant reached maturity, 200 matured pods were randomly detached from each variety. Pods were dried under the sun on plastic tarps. For pod storage, 100 pods were used. A total of 100 pods were placed in two sealed plastic boxes (represent as two replicates) which contained 50 pods were for each plastic box. For seed storage, seeds from 100 pods were divided by two, and placed in two sealed plastic boxes (represent as two replicates). Those plastic boxes were stored at room temperature in the laboratory of Indonesian Legume and Tuber Crops Research Institute (ILETRI, Malang) for 30 and 60 days.

2.3. The seed quality testing
The seed quality test consists of seed viability and Speed of Germination Index (SGI). The seed viability test was using sterilized sand media in the glasshouse of ILETRI, and arranged in a randomized block design with three factors and two replications. The first factor was storage in the form of seeds and pods (storage material, S). The second factor was the storage period at ambient temperature, namely 30 and 60 days (storage time, T). The third factor was 18 cowpea local varieties (V). The seed multiplication in the field and seed quality testing using sand media of 18 cowpea local varieties were presented in figure 1.

The seed quality testing through the viability test was using 25 seeds from each treatment, and it was replicated two times. The percentage of normal and abnormal germination were counted every day. The observation was on ten days after seedling which consisted of seedling height, root length, hypocotyl length, epicotyl length, root dry weight, stem dry weight, and leaf dry weight. The SGI was defined according to the following formula of the Association of Official Seed Analyst [16].

![Figure 1. Seed multiplication in the field and seed quality testing using sand media of 18 cowpea local varieties](image)

3. Results and discussion
Analysis of variance of seed quality and seed morphological characters of 18 cowpea local varieties after being stored in the form of seeds and pods for 30 and 60 days showed that there were no significant three-level interactions, that is between storage material × storage time × local variety (table 2). The two-way interaction, namely between storage time × local variety showed significant interactions for seed viability, SGI, hypocotyl length, root dry weight, and stem dry weight. There was no significant
interaction between storage material × storage time, as well as between storage material × local variety on all observed characters. The effect of single factors, namely storage material was not significant for all characters, meanwhile, the effect of storage time was significant on the seed viability, SGI, hypocotyl length, root dry weight, stem dry weight, and leaf dry weight. The effect of local varieties was significant for all characters except leaf dry weight.

### Table 2. Analysis of variance of seed quality and seed morphological characters of 18 cowpea local varieties.

| No | Character                        | Mean square | S  | T  | V   | ST | SV | TV  | STV |
|----|----------------------------------|-------------|----|----|-----|----|----|-----|-----|
| 1  | Seed viability (%)               | ns          | *  | ** | ns  | ns | *  | ns  | ns  |
| 2  | Speed of Germination Index (SGI) | ns          | *  | ** | ns  | ns | *  | ns  | ns  |
| 3  | Seedling height (cm)             | ns          | ns | ** | ns  | ns | ns | ns  | ns  |
| 4  | Root length (cm)                 | ns          | ns | ** | ns  | ns | ns | ns  | ns  |
| 5  | Hypocotyl length (cm)            | ns          | *  | ** | ns  | ns | *  | ns  | ns  |
| 6  | Epicotyl length (cm)             | ns          | ns | ** | ns  | ns | ns | ns  | ns  |
| 7  | Root dry weight (g)              | ns          | *  | *  | ns  | ns | ns | **  | ns  |
| 8  | Stem dry weight (g)              | ns          | *  | ** | ns  | ns | ns | *   | ns  |
| 9  | Leaf dry weight (g)              | ns          | *  | ns | ns  | ns | ns | ns  | ns  |

ns = not significant, * = significant at 5% probability level (p < 0.05), ** = significant at 1% probability level (p < 0.01). S = storage material, T = storage time, V = local variety.

The mean of seed viability from the seed storage for 30 and 60 days was 75.97% and 78.06, respectively. The mean of seed viability from the pod storage for 30 and 60 days was 77.50% and 73.33%, respectively (table 3). The storage in form of seed as well as pods for 30 days was not affecting the seed viability. However, each local variety of cowpea had shown different seed viability after being stored for 60 days. A study reported that that high germination rates depended largely on seed viability and storage duration, and significantly differed according to genotypes. Furthermore, the seeds storage for more than 3 months had reduced moisture content and decreased germination percentages [12]. The loss in seed viability was reported causing problems in the production and expansion of recalcitrant legumes such as cowpea and soybean, which seed deterioration could be due to poor seed respiration, heating, and possible microbial infections [6,17,18]. Another study reported that soybean seeds deteriorate rapidly, with deterioration rates varying according to storage conditions and initial seed quality in addition to the genotype factor [19].

In this study, when the viability limit uses a minimum of 90%, then there were four local varieties of cowpeas, namely VU 0007, VU 0093, VU 0125, and VU 0155 which were showing high consistency in viability, both stored in the form of seeds and pods. Those varieties were still able to grow optimally until 60 days of storage. Meanwhile, VU 0032 and VU 0076 showed high viability after being stored for 60 days. The VU 0022 in the form of seeds showed 100% viability after being stored for 60 days. On the contrary, VU 0112 achieved high viability if stored in the form of pods for 60 days.

The speed germination index (SGI) reflects the process of rapid seed reactivating for optimum growth when the metabolic process is not inhibited. Seeds that have a high SGI value after undergoing the storage process indicate that the seeds still have high vigor. Four local varieties that had high viability have different SGI characters. The VU 0007 showed slow growth and was better stored in the form of a seed. The VU 0093 and VU 0155 have high SGI values after 60 days of storage and tend to be better when stored as seeds. A similar pattern was found in VU 0125, which has a consistently high SGI value after storage both in the form of seeds and pods. Local varieties with low viability, namely VU 0169 and VU 0173 showed low SGI values in the 30 and 60 days of storage. Based on those facts, SGI was more reflects in the seed vigor. The use of seeds with high vigor levels is important to support the achievement of yield productivity. Simultaneity and uniformity of early plant growth can be achieved.
by using high vigorous seed [20]. In the arid zone, rapid germination and seed longevity was reported to be varied among species [21].

Table 3. Seed viability of 18 cowpea local varieties on two level of storage time.

| No | Local variety | Seed viability (%) on storage time |
|----|---------------|-----------------------------------|
|    |               | 30 days | 60 days | Mean |
|    |               | Seed    | Pod     | Seed  | Pod   | Seed  | Pod   |
| 1  | VU 0005       | 67.50   | 65.00   | 65.00 | 87.50 | 66.25 | 76.25 |
| 2  | VU 0007       | 100.00  | 100.00  | 92.50 | 92.50 | 96.25 | 96.25 |
| 3  | VU 0022       | 75.00   | 80.00   | 100.00| 40.00 | 87.50 | 60.00 |
| 4  | VU 0027       | 70.00   | 57.50   | 87.50 | 57.50 | 78.75 | 57.50 |
| 5  | VU 0032       | 90.00   | 77.50   | 95.00 | 100.00| 92.50 | 88.75 |
| 6  | VU 0047       | 85.00   | 85.00   | 75.00 | 77.50 | 80.00 | 81.25 |
| 7  | VU 0063       | 67.50   | 50.00   | 82.50 | 75.00 | 75.00 | 62.50 |
| 8  | VU 0076       | 85.00   | 87.50   | 95.00 | 97.50 | 90.00 | 92.50 |
| 9  | VU 0093       | 90.00   | 97.50   | 97.50 | 97.50 | 93.75 | 97.50 |
| 10 | VU 0098       | 60.00   | 70.00   | 65.00 | 32.50 | 62.50 | 51.25 |
| 11 | VU 0106       | 65.00   | 57.50   | 82.50 | 82.50 | 73.75 | 70.00 |
| 12 | VU 0112       | 67.50   | 90.00   | 70.00 | 92.50 | 68.75 | 91.25 |
| 13 | VU 0125       | 95.00   | 95.00   | 90.00 | 95.00 | 92.50 | 95.00 |
| 14 | VU 0151       | 57.50   | 75.00   | 82.50 | 55.00 | 70.00 | 65.00 |
| 15 | VU 0155       | 100.00  | 95.00   | 100.00| 100.00| 100.00| 97.50 |
| 16 | VU 0159       | 57.50   | 55.00   | 70.00 | 80.00 | 63.75 | 67.50 |
| 17 | VU 0169       | 72.50   | 85.00   | 17.50 | 42.50 | 45.00 | 63.75 |
| 18 | VU 0173       | 62.50   | 72.50   | 37.50 | 15.00 | 50.00 | 43.75 |
| Mean |           | 75.97   | 77.50   | 78.06 | 73.33 | 77.01 | 75.42 |

Table 4. Seed vigor of 18 cowpea local varieties on two level of storage time.

| No | Local variety | SGI (% per day) on storage time |
|----|---------------|--------------------------------|
|    |               | 30 days | 60 days | Mean |
|    |               | Seed    | Pod     | Seed  | Pod   | Seed  | Pod   |
| 1  | VU 0005       | 1.35    | 1.30    | 2.38  | 2.08  | 1.87  | 1.69  |
| 2  | VU 0007       | 2.17    | 2.17    | 3.02  | 2.18  | 2.18  | 2.18  |
| 3  | VU 0022       | 1.58    | 1.60    | 2.67  | 1.47  | 2.13  | 1.53  |
| 4  | VU 0027       | 1.57    | 1.15    | 3.75  | 1.98  | 2.66  | 1.57  |
| 5  | VU 0032       | 2.22    | 1.63    | 3.57  | 4.50  | 2.89  | 3.07  |
| 6  | VU 0047       | 1.78    | 1.87    | 1.83  | 2.13  | 1.81  | 2.00  |
| 7  | VU 0063       | 1.35    | 1.00    | 2.40  | 1.75  | 1.88  | 1.38  |
| 8  | VU 0076       | 1.70    | 1.75    | 3.23  | 2.70  | 2.47  | 2.23  |
| 9  | VU 0093       | 1.80    | 2.03    | 3.78  | 3.95  | 2.79  | 2.99  |
| 10 | VU 0098       | 1.20    | 1.40    | 1.55  | 0.73  | 1.38  | 1.07  |
| 11 | VU 0106       | 1.30    | 1.15    | 2.40  | 2.23  | 1.85  | 1.69  |
| 12 | VU 0112       | 1.77    | 2.05    | 2.65  | 2.27  | 2.21  | 2.16  |
| 13 | VU 0125       | 2.90    | 2.57    | 3.55  | 3.48  | 3.23  | 3.03  |
| 14 | VU 0151       | 1.65    | 1.75    | 1.90  | 1.10  | 1.78  | 1.43  |
| 15 | VU 0155       | 2.75    | 3.23    | 4.00  | 3.67  | 3.38  | 3.45  |
| 16 | VU 0159       | 1.48    | 1.60    | 2.98  | 3.02  | 2.23  | 2.31  |
| 17 | VU 0169       | 2.03    | 2.28    | 0.68  | 1.68  | 1.36  | 1.98  |
| 18 | VU 0173       | 2.08    | 2.03    | 1.33  | 0.63  | 1.71  | 1.33  |
| Mean |           | 1.82    | 1.81    | 2.65  | 2.31  | 2.23  | 2.06  |
The difference in origin and also the genetic background of local varieties from several regions in Indonesia not only affect the seed quality and vigor but also affect several morphological characters of seedling (figure 2a to figure 2g), except that the characteristics of the leaf dry weight. In pinto beans, the seed weight determines the germination rate, germination percentage, and seedling dry weight [22].

![Figure 2. The performance of seedling morphological characters of 18 cowpea local varieties: (a) seedling height, (b) root length, (c) hypocotyl length, (d) epicotyl length, (e) root dry weight, (f) stem dry weight, (g) leaf dry weight.](image-url)
Genetic differences not only affect differences in the appearance of morphological characters that determine the seed yield but also affect the variability of seed vigor [22]. A study reported that the reflex of seed vigor in crop performance depended on the genotype and a function of seed vigor level [23].

In this study, the storage time of 30 and 60 days affected the seed quality and vigor, hypocotyl length, and dry weight of stems, roots, and leaves. The average performances of stem length after 30 days of storage were similar among storage materials, but the stem length derived from seed storage was longer than those stored in pods after 60 days of storage. However, the average performance of root length, epicotyl length, and leaf dry weight were not significantly different in terms of the storage time and the storage material. Another study in cowpea also obtained a greater sensitivity to the storage period, with a lower germination percentage and lower vigor, which affect the initial establishment of seedlings in the field through having less developed roots and lower initial biomass accumulation [24]. A study in other legumes such as soybean, it revealed that shoot growth proved to be supported and directly linked to seed quality, age, and genotype [12].

The characters of seed quality and vigor, and the morphological seedling characters (hypocotyl length, root dry weight, stem dry weight) were affected by the interaction between the storage time and local variety. The cowpea local variety with high seed quality, namely VU 0007, VU 0093, and VU 0125 was characterized by the consistent performance of those characters after a storage period of 30 and 60 days in form of seeds and pods. Those characters could be used as measure parameters of the quality of cowpea seeds.

4. Conclusion
Local varieties of cowpea vary in their shelf life, both in the form of seeds and pods. High seed quality will determine the vigor of the seed. Three local varieties of cowpea, namely VU 0007, VU 0093, and VU 0155 showed high vigor after storage for 60 days in form of seeds. Meanwhile, VU 0125 can be stored for up to 60 days in the form of seeds or pods. The storage of cowpeas for 30 and 60 days affects the variability of hypocotyl length, stem dry weight, and root dry weight of seedling, hence those characters may be used as indicators of cowpea seed vigor.

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