# SEED COAT COLOR AND QUALITY LOSSES IN COWPEA SEEDS DURING DELAYED HARVEST

*WARNA KULIT BENIH DAN KEMUNDURAN MUTU BENIH KACANG TUNGGAK SELAMA PANEN TUNDA*

**Marwanto**

*Lecturer of Seed Technology, Faculty of Agriculture, University of Bengkulu*

## ABSTRACT

Seed coat color may have an effect on the quality of cowpea seeds during delayed harvest. A field trial was conducted to evaluate the effect of seed coat color on quality losses of cowpea seeds during delayed harvest. Three local cowpea seeds with different seed coat colors (black, brown and white) were planted in research plots at Agriculture Faculty, Bengkulu University in September 2000 in a split-plot arrangement, with harvest stages as main plots and seed coat colors as subplots with three replications. Pods were hand-harvested at HM (harvest maturity = the first time the seed dried to less than 14% moisture content) and at a regular time (approximately 1 week) for up to 5 weeks after HM to provide seed samples that were used to determine seed viability (standard germination test) and vigor (accelerated aging and field emergence tests). Seed viability was high (more than 80%) and remained above 80% until 5 weeks after HM for black-seeded type, until 4 weeks after HM for brown-seeded type and only until 3 weeks after HM for white-seeded type. Accelerated aging germination was maintained at above 80% until 3 weeks after HM for black-seeded type and only until 2 weeks after HM for brown and white-seeded types. Field emergence declined rapidly, reaching levels that were significantly ($\propto$=0.05) less than those at HM within 3 weeks after HM for black and brown-seeded types and only within 1 week after HM for white-seeded type. These results demonstrate that quality losses in cowpea seeds during delayed harvest were affected by seed coat pigmentation. Black-seeded type was superior in quality to brown and white-seeded types during delayed harvest.

*Key words:*cowpea, delayed harvest, seed viability, seed vigor, seed coat color

## ABSTRAK

Kemunduran mutu benih kacang tunggak tidak dapat dihindari sewaktu benih ditunda panennya. Tujuan penelitian ini adalah untuk mengevaluasi pengaruh warna kulit benih kacang tunggak dalam memperlambat kemunduran mutunya selama panen tunda. Benih kacang tunggak lokal dengan warna kulit berbeda (hitam, coklat dan putih) ditanam di petak percobaan Fakultas Pertanian Universitas Bengkulu pada bulan September 2000 dalam rancangan petak terbagi dengan penundaan saat panen sebagai petak utama dan warna kulit benih sebagai anak petak dengan tiga ulangan. Pemanenan benih dilakukan pertama kali sewaktu benih telah mencapai matang panen (*harvest maturity* = HM) dan dilanjutkan sampai dengan 5 minggu setelah HM dengan selang waktu 1 minggu setiap panennya. Setiap kali dipanen, benih dievaluasi mutunya yang meliputi viabilitas dan vigornya. Viabilitas benih tetap bertahan di atas 80% sampai 5 minggu setelah HM untuk tipe benih hitam, sampai 4 minggu setelah HM untuk tipe benih coklat, dan hanya sampai 3 minggu setelah HM untuk tipe benih putih. Vigor benih yang diukur dengan uji penderaan dipercepat tetap bertahan di atas 80% sampai 3 minggu setelah HM untuk tipe benih hitam dan hanya sampai 2 minggu setelah HM untuk tipe benih coklat dan benih putih. Tiga tipe benih mengalami penurunan vigor benih (yang diukur dengan uji kemunculan lapang) secara tajam. Untuk tipe benih hitam dan benih coklat, penurunan vigornya hingga lebih kecil daripada vigor benih pada saat HM memerlukan waktu selama 3 minggu, sedang untuk tipe benih putih hanya memerlukan waktu selama 1 minggu. Hasil ini menunujukkan bahwa kemunduran mutu benih kacang tunggak selama panen tunda dipengaruhi oleh warna kulit benihnya. Selama panen tunda tersebut, tipe benih hitam bersifat lebih unggul dalam hal kualitas daripada tipe benih coklat maupun tipe benih putih.

*Kata kunci:* kacang tunggak, panen tunda, viabilitas benih, vigo benih, warna kulit benih

## INTRODUCTION

Producing and maintaining good quality cowpea [*Vigna unguiculata* (L.)] seed in the humid tropical regions for planting purposes is very difficult mainly due to its susceptibility to weathering damage. The seeds mature when dialy temperatures rise to 80 $^0$F (26.6 $^0$C) or more and the relative humidity (RH) may remain at 100% for several hours each day (Keigley and Muller, 1986; Mondragon and Potts, 1974; TeKrony et al., 1980). Under this condition seed vigor as well as viability deteriorates rapidly and the deterioration is usually refferred to as 'field weathering' (TeKrony et al., 1980).

In addition to physiological disruption by climatic factors, the cause of field weathering of seed is also associated with pathological infection such as *Phomopsis* infection (Dassou and Kueneman, 1984; Paschal and Ellis, 1978). However, the use of fungicides to control seed pathogens in the tropics including *Phomopsis* has met with a limited success (TeKrony et al., 1980).

One of the most promising solutions to the problems of field weathering appears to be the development of cultivars that resist 'weathering ' in the field. To provide genetic material for this purpose the identification of cowpea cultivars resistant to field weathering would be necessary. The common method used for this purpose is to delay harvest after plant maturity and then asses the seed quality (Dassou and Kueneman, 1984).

When working with soybeans, researchers have suggested that cultivars with lower susceptibility to weather damage has been associated with impermeable seed coat or hard seed character (Marwanto, 2003c, Miranda et al., 1980; Mondragon and Potts, 1974; Nugraha, 1987). This approach, however, is not commonly applied to cowpea seed because the seed belongs to a seed kind without impermeable seed coat character. One possible approach to the weathering problem in cowpea is based on the apparent resistance of seeds with pigmented seed coats to adverse field conditions. When working with soybeans, several reseachers reported that reported that soybeans with black seed coat were resistant to field weathering (Chuntirapongsa, 1993; Dassou and Kueneman, 1984; Marwanto, 2003b,c). However, the association of resistance to field weathering of cowpea seeds with its seed coat color is not well understood. The present study was conducted to evaluate the effect of seed coat color on quality losses of cowpea seeds during delayed harvest.

## MATERIALS AND METHODS

Seeds of local cowpea with black, brown and white seed colors were used in these studies. The seeds were planted in research plots at Agriculture Faculty, Bengkulu University on September 25, 2000 in a split-plot arrangement, with delayed harvest as main plots and seed coat colors as subplot with three replications. Each cultivar was planted in a plot consisting of a single raised bed, 65 cm wide and 4 meters long. Two rows were planted per bed. Row spacing was 35 cm between rows within beds and 65 cm between beds. Seeds were planted in hills 20 cm apart with 3-4 seeds per hill. N, P, and K fertilizer at a rate of 40, 60 and 80 kg ha$^{-1}$ was applied prior to planting.

Seeds were harvested by hand stripping of the pods at harvest maturity (the first time the seed moisture content decreased to a harvestable moisture, i.e. 13-15% and continued at regular intervals (approximately 1 week) for 5 weeks past harvest maturity. Pods were considered mature when they turned pale brown in brown-seeded cultivar and black in white and black-seeded cultivars. The harvested pods were then dried with heated air (<35 $^0$C) to reduce moisture content to 10-12% for threshing. The dried pods contained in jute bags were threshed by flailing and the seeds were separated from the pod walls and another plant parts by sieving. Sieving (round hole) was used to eliminate the small, immature and insect damaged seeds.

Seed quality evaluation of each cultivar harvested at each maturity stage included seed moisture content, viability, vigor, and field emergence.

Seed moisture was determined on seed fraction of the cowpea sample. Samples of about 20 g in duplicate from each treatment-replicate were placed in an oven at 105 $^0$C for 24 hours to obtain dry weight and determine the amount of moisture lost. Seed moisture content was calculated on a wet weight basis and expressed in %.

Table 1. Temperature (temp.) and rainfall for the period of November 1, 2000 through December 15, 2000 at the Agriculture Faculty Research Plot in Bengkulu.

| Date | Weather Conditiom | | Date | Weather Condition | | Date | Weather Condition | |
|---|---|---|---|---|---|---|---|---|
| | Temp ($^O$C) | Rainfall (mm) | | Temp ($^O$C) | Rainfall (mm) | | Temp ($^O$C) | Rainfall (mm) |
| Nov. 1 | 25.6 | Trace$^x$ | Nov. 17* | 24.3 | 45 | Dec. 2 | 26.7 | 0 |
| Nov. 2 | 27.1 | 4 | Nov. 18 | 25.1 | 11 | Dec. 3 | 28.0 | 0 |
| Nov. 3 | 25.5 | 4 | Nov. 19 | 24.8 | 6 | Dec. 4 | 28.0 | 0 |
| Nov. 4 | 26.3 | 9 | Nov. 20 | 25.6 | 56 | Dec. 5 | 27.0 | 0 |
| Nov. 5 | 26.0 | Trace | Nov. 21 | 24.4 | 51 | Dec. 6 | 26.0 | 4 |
| Nov. 6 | 26.6 | Trace | Nov. 22 | 26.0 | 39 | Dec. 7 | 26.7 | 0 |
| Nov. 7 | 26.9 | 5 | Nov. 23 | 28.3 | 11 | Dec. 8* | 26.5 | 5 |
| Nov. 8 | 26.2 | 37 | Nov. 24* | 26.5 | 13 | Dec. 9 | 27.2 | 5 |
| Nov. 9 | 26.8 | 5 | Nov. 25 | 27.1 | Trace | Dec. 10 | 26.1 | Trace |
| Nov. 10* | 27.3 | Trace | Nov. 26 | 27.3 | 1 | Dec. 11 | 26.6 | 12 |
| Nov. 11 | 27.3 | 0$^y$ | Nov. 27 | 27.2 | Trace | Dec. 12 | 27.1 | 54 |
| Nov. 12 | 27.0 | 15 | Nov. 28 | 25.4 | 9 | Dec. 13 | 26.4 | 0 |
| Nov. 13 | 27.2 | 0 | Nov. 29 | 28.3 | 29 | Dec. 14 | 28.0 | Trace |
| Nov. 14 | 25.4 | 22 | Nov. 30 | 26.8 | 1 | Dec. 15* | 28.1 | Trace |
| Nov. 15 | 25.8 | 95 | Dec. 1* | 27.8 | 0 | | | |
| Nov. 16 | 26.5 | 0 | | | | | | |

x : Trace means rainfall less than 1 mm, y : 0 means no rainfall * : weekly harvest dates

Seed viability was determined by the standard germination test. In this test, 50 seeds from each treatment-replicate were placed on mosit paper towels, which were rolled and placed inside plastic bags and kept at a room temperature. Germinated seeds were counted aftar 5 and 8 days. Dead seeds were removed after 5 days, while hard seeds after 8 days and counted with germinated seeds. The number of germinated seeds was expressed as a percentage of the total.

Seed vigor was determined by the accelerated aging test. In this test, 50 seeds from each treatment-replicate were subjected to a period of accelerated aging, 42 $^0$C and near 100% RH, for 48 hours prior to standard germination test. They were placed on a wire mesh tray of 20X5X2.5cm. The tray was placed inside a plastic box of 30X10X5cm and the box was filled with 100ml of water. A 10-mm gap was maintained between the water surface and the seed tray. The box was covered with airtight lid and kept in oven at 42 $^0$C for 48 hours. After aging, seeds were taken out of the aging box and subjected to standard germination test.

Field Emergence was determined by planting the seeds in the field. Three sets of 50 seeds for each seed type were sown in the field. Seeds were planted approximately 2 cm deep and the soil moisture was uniformly maintained. On the 4$^{th}$ and 10$^{th}$ day of sowing, counts were made to determine the number of shoots that had emerged from the soil. The count on the 10$^{th}$ day was considered as the final emergence. The number of emerged seeds was expressed as a percentage of the total.

Analysis of variance of each variable except seed moisture was conducted as a split plot design. When significant differences were revealed by use of the F test, comparasions of the means involved were made, using the Duncan's Multiple Range Test (DMRT) at the 0.05 level of probability.

## RESULTS AND DISCUSSION

Climatological data presented in Table 1 showed that less rainfall occurred on November 1 through November 9, 2000, several days before R8 of all seed types. Significant rainfall mostly occurred at one and two weeks after harvest maturity stage. Rainfall of equal or more than 5 mm occurred when most seeds were scheduled for harvest This less favorable condition was probably responsible for less maximum level of seed vigor of most seed types.

Table 2. Germination percentages of cowpea seeds with different seed coat color harvested at weekly interval beginning at harvest maturity (HM).

| Harvest | Seed Coat Color | | |
|---|---|---|---|
| | Brown | White | Black |
| HM | a 88.5 AB | a 87.5 A | a 93.0 A |
| HM+1 | a 90.0 AB | a 86.5 A | a 91.5 AB |
| HM+2 | a 93.5 A | a 91.5 A | a 96.0 A |
| HM+3 | ab 89.5 AB | a 87.5 A | a 95.5 A |
| HM+4 | a 80.0 B | b 49.5 B | a 87.0 AB |
| HM+5 | b 45.0 C | c 27.5 C | a 81.0 B |

Means not followed by the same small letter within row and the same capital letters within collumns differs significantly at the 0.05 level of probability acocording to DMRT

Delaying harvest date significantly reduced seed viability, vigor and field emergence. Averaged across cultivars, seed viability as measured by standard germination was 89.7%, 89.3%, 93.7%, 90.8%, 72.2% and 51.2% for the nondelayed, 1-week delayed, 2-week delayed, 3-week delayed, 4-week delayed and 5-week delayed harvests, respectively (Table 2). Seed vigor as measured by accelerated aging test was 83.5%, 84.0%, 87.2%, 78.7%, 57.8% and 45.7% for the nondelayed, 1-week delayed, 2-week delayed, 3-week delayed, 4-week delayed and 5-week delayed harvests, respectively (Table 3). The average of field emergence percentages in the nondelayed harvest was 87.5%, and it decreased to 79.2%, 66.0%, 48.0%, 40.5% and 44.0% in the 2, 3, 4 and 5-week delayed harvests, respectively (Table 4). Other researchers also reported the detrimental effects of delayed harvest on reduced soybean seed viability and vigor (Marwanto, 2003c,2004; TeKrony et al., 1980) and field emergence (Paschal and Ellis, 1978).

Seed performance in the field as measured by field emergence declined sooner and more rapidly than viability. The first significant decline in viability occurred in 4-week delayed harvest while in field emergence in 2-week delayed harvest. Thus, declines in field emergence proceeded seed viability by 2 weeks. In the 2-week delayed harvest, seed viability remained above 90% while field emergence had dropped to 66%.

The significant decline in seed viability and seed vigor (accelerated aging germination) occurred at the same time (4-week delayed harvest); however, the significant difference in seed viability and accelerated aging germination occurred in 3-week delayed harvest. In the 3-week delayed harvest, although the standard germination remained above 90%, the accelerated aging response was only 79%. This indicated that a definite reduction in the seed vigor had occurred in 3-week delayed harvest. The early decline in vigor reported here also emphasizes the value in using a vigor test such as accelerated aging in addition to the standard germination test when evaluating a seed lot's initial seed quality.

As a measure of seed vigor, the accelerated aging germination tended to be higher than filed emergence and their significant difference occurred from 2 to 4-week delayed harvest. Accelerated aging germination was 11.2, 30.7, and 17.8% higher than field emergence for 2-week delayed, 3-week delayed, and 4-week delayed harvest, respectively. Field conditions apparently provided a better environment for microorganism development and less than optimum moisture conditions while nearly optimum conditions were always maintained during germination for accelerated aging seed.

The analysis of variance revealed significant seed coat color differences and a significant seed coat color x delayed harvest interaction for seed viability, vigor and field emergence. The significant seed coat color x delayed harvest interaction for seed viability, vigor and field emergence was due to seed coat color differences in the rate of decrease of seed viability, vigor and field emergence with delays in harvest. For example, determination of seed germination in 4-delayed harvest indicated percentages above an acceptable level (>80%) for the three seed coat types except white-seeded type. Seed viability for the white-seeded type had already dropped to below 80% (near 50%) in 3-week delayed harvest. Thereafter, the drop in their seed germination percentages of all seed coat types except for black-seeded type was dramatic. White-seeded type showed the fastest decline from near 50% in 4-week delayed harvest to near 28% in 5-week delayed harvest. Brown-seeded type followed next with germination droping to 45% in 5-week delayed harvest. Black-seeded type maintained their germination percentage above 80% in 5-week delayed harvest when the percentages for seed of the other cultivars had decreased drastically. Marwanto (2004) also reported similar decreases in germination percentages with delayed harvest for soybeans.

The incidence of fungal growth on seeds in the germinator increased with delay in harvesting and a corresponding increase in abnormal seedlings and dead seeds was also noted (data not showed). This gave credence to the suggestion that with delayed harvest, there was an increased accumulation of fungi which probably contributed to further deterioration of the seeds which were already under environmental stresses.

Black-seeded type showed the greatest insensitivity to accelerated aging followed by brown and white-seeded types. Comparing the germination responses following accelerated aging observed for the seeds of the 3-week delayed harvest, black-seeded type showed a response higher than 90% while seed of the other two cultivars was less than 80%. For white and brown-seeded types, a notable feature for seed of 3-week delayed harvest was that although the standard germination percentage was near 90% for both seed types, the accelerated aging response was only 69% for brown-seeded type and 76% for white-seeded type. This indicated that significant reduction in their vigor had already occurred in 3-week delayed harvest.

There was a marked difference in accelerated aging response between white and brown-seeded types until 3-week delayed harvest. After this harvest date, a definite difference in accelerated aging response was observed and white-seeded type showed a greater sensitivity to accelerated aging than brown-seeded type.

Table 3. Accelerated aging germination of cowpea seeds with different seed coat color harvested at weeklyinterval beginning at harvest maturity (HM)

| Harvest | Seed Coat Color | | |
|---|---|---|---|
| | Brown | White | Black |
| HM | a 80.0 A | b 80.5 A | a 90.0 A |
| HM+1 | a 85.5 A | b 76.5 A | a 90.0 A |
| HM+2 | a 87.5 A | a 83.5 A | a 90.5 A |
| HM+3 | b 69.0 B | b 76.0 A | a 91.0 A |
| HM+4 | b 61.5 B | c 42.0 C | a 70.0 B |
| HM+5 | b 42.0 C | c 20.0 D | a 75.0 B |

Means not followed by the same small letter within row and the same capital letters within collumns differs significantly at the 0.05 level of probability acocording to DMRT

Table 4. Field emergence percentages of cowpea seeds with different seed coat color harvested at weekly interval beginning at harvest maturity (HM).

| Harvest | Seed Coat Color | | |
|---|---|---|---|
| | Brown | White | Black |
| HM+1 | a 83.0 AB | b 68.5 B | a 86.0 A |
| HM+2 | a 78.5 B | b 44.5 C | a 75.0 BC |
| HM+3 | b 51.5 C | c 23.0 D | a 69.5 C |
| HM+4 | b 43.0 C | c 13.5 D | a 65.0 C |
| HM+5 | b 41.0 C | c 18.5 D | a 72.5 BC |

Means not followed by the same small letter within row and the same capital letters within collumns differs significantly at the 0.05 level of probability acocording to DMRT

The pattern of decline in field emergence closely followed that observed in the accelerated aging responses. Black-seeded type significantly showed higher emergence percentages the other two seed types from 3 to 5 week delayed harvest. Between the two seed types, the white seeded type exhibited a faster decline in seed emergence than brown-seeded type. The most notable difference among the three seed types was in 5-week delayed harvest. In this harvest date, a 31.5% difference in the field emergence percentages existed between black and brown seeded types and a 54.0% difference between black and white-seeded types.

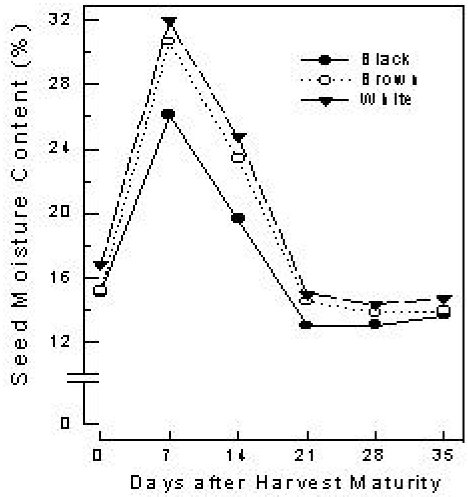


Fig. 1. Seed moisture trends following harvest maturity

Among seed coat types included in this study, black-seeded type showed a greater resistance to the unfavorable conditions during delayed harvest than brown and white-seeded types. The resistance to delayed harvest exhibited by the black-seeded type was probably a result of its seed coat permeability. Their seed coat permeability were 0.082 g $g^{-1}$ $hr^{-1}$ for black, 0.086 g $g^{-1}$ $hr^{-1}$ for brown and 0.104 g $g^{-1}$ $hr^{-1}$ for white-seeded types. The similar reason was also reported by others (Horlings *et al.*, 1991; Marwanto, 2003a, c) for soybeans. Black-seeded type with low seed coat permeability tended to absorb water at a slower rate than the others (Fig. 1) and this finally protect seed from deterioration in the field. Over the total sampling period, seed moisture of black-seeded type had a range of 13.11%, while the range in moisture contents of brown and white-seeded types was 16.84% and17.63%, respectively. Based on these data it appears that seed coat color affected the reduction of seed moisture fluctuation and black-seeded type was less subjected to moisture reabsoption, which occurs in the field, than brown and white-seeded types. As a result of this decresed response, mature seed of black-seeded type could be expected to have lower levels of moisture at any harvest date and higher level of seed viability and vigor than brown and white-seeded types.

Small seed size for black-seeded type was probably responsible for such resistance as well. One hundred seed weight were 8.01 g for black, 8.44 g for brown and 9.19 g for white-seeded types. When working with soybean seeds, Dassou and Kueneman (1984) and Marwanto (2003a, c) reported that soybean lines with small seed size were resistant to exposure of high humidity and temperature of tropical conditions. Small-seeded cultivar may gain their protection from a seed coat that tends to be less permeable while black-seeded cultivar from a seed coat that tends to be thicher than that of yellow-seeded cultivar (Mugnisjah *et al.*, 1987. Among less resistant seed type, white-seeded type was inferior in quality and exhibited its incapability to withstand the field conditions that favor deterioration. The less resistance exhibited by white-seeded type to field weathering was probably due to its large seed size. According to Calero *et al.* (1981), Marwanto (2003b) and Mugnisjah *et al.* (1987), one possible explanation for the relationship between large seed size and low seed qaulity is the positive correlation between seed size and seed coat permeability.

## CONCLUSIONS

Quality losses in cowpea seeds during delayed harvest were affected by seed coat pigmentation Cowpea seed with black seed coat color was superior in all the seed quality tests to brown and white-seeded types and exhibited its inherent capability to withstand field weathering during delayed harvest.

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