Causes of differences in seed quality among cowpea (*Vigna unguiculata* (L.) Walp) cultivars

E. A. ASIEDU, P. AKOWUAH-ADUSEI, B. ASAFO-ADJEI, H. ADU-DAPAAH & O. A. DANQUAH
CSIR-Crops Research Institute, P. O. Box 3785, Kumasi, Ghana

ABSTRACT

Three cowpea cultivars differing in seed coat colour, IT83S-818 (white, black-eye), TVX 2724-01F (brown) and IT82D-32 (dark brown), were aged using the controlled deterioration method (20% seed moisture content and incubated at 40 °C) for 0, 1, 2 and 4 days. Time to 50 per cent germination (t_{50}) and final germination percentage were determined to assess the vigour differences that exist among these cultivars. In unaged seeds, the white cultivar germinated quicker than the pigmented cultivars as a result of the characteristically higher rate of water uptake, which enhanced rapid hydration of the embryonic cells for quicker germination. When ageing progressed, however, the white cultivar germinated more slowly with a lower final germination percentage since the embryonic cells possibly became weaker and leaky leading to reduced vigour and eventually, loss of germinability. In contrast, the pigmented cultivars germinated more slowly before seeds were aged due to their slower rates of water uptake; germination became quicker as ageing progressed due to increasing softening of the seed coats. Thus, the pigmented cultivars showed decreases in the time to 50 per cent germination during ageing. They, however, retained high germination percentages throughout the ageing period, which is an indication of high vigour. These three cultivars, together with two other unpigmented cultivars, IT81D-1137 (white) and TVX 3236 (cream and brown) were subsequently examined for the incidence of disease pathogens. It was observed that the unpigmented cultivars had higher levels of the importasnt seed pathogens such as *Fusarium* species and *Aspergillus flavus* which cause reduction in germination. These seeds also showed susceptibility to mechanical damage caused by hand-beating of dry pods in sacs. These differences in behaviour between the two groups of cowpea cultivars, pigmented and unpigmented, were attributed to differences in tannin and lignin contents in their seed coats.

NARS edition; revised 30 Jun 06.

Introduction

Differences in storage potential between seeds of different species and also between cultivars within the same species may be related to genetic differences. For example, genetic differences in storage potential of seeds have been observed among cultivars of several crop species such as rice (Juliano, Perez & Chang, 1990; Krishnasamy & Seshu, 1990), soybean (Kueneman, 1983; Harwig & Potts, 1897), long bean (Abdullah, Powell & Matthews, 1992), and cowpea (Legesse & Powell, 1992). Cultivar differences in seed coat colour have also been related to differences in seed storage potential. Among six cultivars of long bean (*Vigna sesquipedalis*), unpigmented cultivars that imbibed water more quickly in free water also took up moisture more rapidly from their storage environment, which led to increased moisture contents to levels ranging between 22.04 and 24.66 per cent when seeds were stored at 42 °C and 100 per cent relative humidity for 5 days. This contributed to rapid deterioration in the unpigmented cultivars which was evident by the reduced vital staining of the embryos, as well as reduced germination.

On the contrary, the pigmented cultivars, which showed delayed imbibition in free water and also took up moisture more slowly from their storage environments, deteriorated at a slower rate and, therefore, germinated well after the storage period with high percentage (Abdullah, Powell & Matthews, 1992). Similarly, differences have also been found in *Phaseolus* bean in which white and speckled-seeded cultivars stored at 40 °C and 100 per cent relative humidity deteriorated more rapidly than pigmented cultivars (Abdullah, Powell & Matthews, 1992). Apart from differences in physiological ageing that occur between pigmented and unpigmented seeds of legumes during storage, storability is also influenced by seed-borne pathogens and damage to seed parts.

The objectives of the experiment were to determine the differences in germination and seed vigour between pigmented and unpigmented cultivars of cowpea stored at an elevated moisture content in a warm environment; the differences in the incidences of disease pathogens and mechanical damage between pigmented and unpigmented seeds of cowpea; and the differences in chemical composition between pigmented and unpigmented seed coats of cowpea.

Materials and methods

Seeds of three cowpea cultivars, namely IT83S-818 (white black-eye), TVX 2724-01F (brown) and IT82D-32 (dark brown) were subjected to controlled deterioration in a similar way as described by Matthews (1980) and then tested for germination. The seed moisture contents were adjusted to 20 per cent by imbibing slowly in damp jay cloths to the desired weight equivalent to this moisture content. Four sets of each cultivar, corresponding to four ageing periods, each containing 100 seeds, were counted into laminated aluminium foil packets. The packets were heat sealed, equilibrated at 10 °C for 24 h, and three packets incubated at 40 °C for 1, 2 and 4 days. After each period of incubation, seeds were dried back to their original moisture contents by placing them in the open at laboratory temperature (about 20 °C) overnight before testing for germination (5 mm radicle emergence) at 24 h intervals for 8 days, and the results compared to the control (unaged) treatment. Seeds of the control treatment had the moisture content raised to 20 per cent, were equilibrated at 10 °C for 24 h, and then dried back for testing for germination without being held at 40 °C.

Four replicates of 25 seeds were germinated between moist paper towels (50 cm × 20 cm) with two paper towels beneath and one above. Seeds were arranged linearly midway between the two long edges of the paper towels. The paper towels were then rolled and placed upright in small plastic containers, a third filled with water. These were packed in plastic trays and each tray covered with a polyethylene bag to reduce loss of moisture from the paper towels, and the trays placed in an incubator at 25-32 °C. Daily counts of radicle

emergence were taken up to 8 days. Seeds with 5 mm radicle protrusion were considered germinated. A graph of germination percentage (y-axis) was plotted against germination time (x-axis), and t_{50} determined as time for 50 per cent of the final germination to occur.

Subsequent to this experiment, the three cowpea cultivars, together with two other unpigmented cultivars, namely IT81D-1137 (white) and TVX 3236 (cream and brown), were evaluated for the incidence of seed-borne pathogens at the Seed Pathology Laboratory of the Crops Research Institute, Kumasi, Ghana. Seeds were produced in the minor season of 1995 for seed health testing, which was conducted in 1996. One hundred seeds per each variety were taken from a working sample and plated on moist blotter paper in Petri dishes (10 seeds per Petri dish). The plates were incubated at 26 °C for 7 days under an alternating cycle of 12 h of near ultraviolet light and 12 h of darkness. The seeds were then examined under low power of a compound microscope to identify the fungal pathogens present, and the percentage incidence of micro-flora was recorded.

The susceptibility of cowpea seeds, IT83S-818 (white), IT81D-1137 (white), TVX 2724-01F (brown) and IT82D-32 (dark brown) to mechanical damage was also evaluated. These cowpea seeds were planted in the minor season of 1996 and harvested in pods for the experiment. Seeds were dried down to 16.5 per cent moisture content and threshed with machine, hand beating of pods in sacs and hand splitting of pods. These were replicated four times and mechanically damaged seeds were counted from 300-g samples and also germination percentages determined.

Chemical pigments were extracted from the seed coats of five cowpea samples, IT835-818 (white), IT81D-1137 (white), TVX3236 (cream and brown), TVX2724-01F (brown) and IT882D-32 (dark brown) using the modification of the procedure described by Carmona, Seidl & Jaffe (1991). The extraction was done after the seed coats had been manually removed from the dry seeds and ground to less than 1 mm using an electric mill. Ground seed coat (2.5 g) of each cowpea sample was extracted in 25 ml of 1 per cent HCl in methanol by shaking at 5 min intervals for 30 min at room temperature (20-22 °C). The supernatants of the extracts were filtered through Whatman No. 1 filter paper and rotary-evaporated to dryness at 35 °C. The dried concentrate was redissolved in 3-ml 95 per cent ethanol and centrifuged at 5000 × g for 10 min. The resulting supernatant (2.1 ml) was pipetted and loaded onto a Sephadex LH-20 column (2 cm × 27 cm) equilibrated in 95 per cent ethanol. The non-tannin polyphenolic compounds were first eluted by 95 per cent ethanol followed by the elution of the tannins by 50 per cent aqueous acetone as described by Strumayer & Malin (1975). Fifty ethanol fractions and 30 acetone fractions were collected. The elution profiles were constructed after measuring the absorbances of the ethanol and the aqueous acetone eluants at 280 and 400 nm, respectively.

Another set of seed coats (100 mg) each in four replicates were weighed into centrifuge tubes and 1 ml of solvent (70% aqueous acetone) added. The tubes were shaken at 5 min intervals whilst being held at 30 °C for 30 min. The extracts in the tubes were centrifuged at 10000 × g and the supernatants (5 ml) decanted into 5-ml volumetric flasks. These were filtered through Whatman No. 1 filter paper and concentrated to dryness before being redissolved in 2.5 ml of methanol. The assay was carried out in water bath at 30 °C following the vanillin-HC1 procedure (Burns, 1970; Price, Scoyoc & Butler, 1978; Morrison *et al.*, 1995) to determine the concentration of tannins (in catechin equivalent). The seed coat lignin content of the six samples of cowpea was determined after using aqueous acetone to remove the tannins. Lignin was then determined by the modified acetyl bromide procedure of Iyama & Wallis (1988) and Morrison *et al.* (1995).

Results and discussion

Results showed high initial germination of 97.01, 100.0 and 100.0 per cent for IT83S-818, TVX 3236

and IT82D-32, respectively (Table 1.) These percentages were statistically comparable; however, during ageing, the white cultivar showed progressive and significant decreases in germination (Table 1, Fig. 1). One of the pigmented cultivars (IT82D-32) showed a slight decrease in percentage germination with ageing, which at the end of 4 days' ageing, had fallen significantly from 100.0 to 93.0. The brown cultivar (TVX2724-01F), however, retained 100 per cent germination throughout the ageing period.

Among the unaged seeds, the initial rate of germination of the white cultivar was more rapid than the two pigmented cultivars. The time to 50 per cent germination (t_{50}) of the white, brown and dark brown cultivars were 19.43, 32.00 and 33.14 h, respectively (Fig. la and 1b; Table 2). Germination rate, however, became progressively slower in the white cultivar and faster in the pigmented cultivars during ageing. At the end of the 4 days' ageing, time for 50 per cent germination, t_{50}, for the white cultivar had increased from 19.43 to 28.00 h; whereas t_{50} for the brown and the dark brown cultivars had decreased from 32.00 to 19.43 h and from 33.14 to 20.00 h, respectively. The time for 50 per cent germination in the pigmented cultivars, therefore, showed trends which were contrary to that of the unpigmented cultivar. The initial rapid germination in the white cultivar may be due to the fact that this cultivar absorbs moisture more rapidly (Asiedu & Powell, 1998), which allows rapid hydration of the embryonic cells to enhance early initiation of the germination processes. However, as the ageing period progressed, the cells of this cultivar became weaker and leaky (Asiedu & Powell, 1998), resulting in the slower rate of germination and low final percentage germination (Fig. 1b and 1c, Tables 1 and 2).

TABLE 1

Effect of Controlled Deterioration at 20% m.c. and 40 °C on Percentage Germination of Cowpea Seeds

Cultivar	*Ageing period (days)*			
	0	*1*	*2*	*4*
Unpigmented				
IT83S-818	97.0ab	94.0bc	85.0d	72.0e
Pigmented				
TVX 2724-01F	100.0a	100.0a	100.0a	100.0a
IT82D-32	100.0a	98.0ab	97.0abc	93.0bc

Means of columns and rows followed by different letters differ significantly (DMRT, $P \leq 0.05$).

In the pigmented cultivars, the initial rate of water uptake before controlled deterioration was slower; thus, it took a longer time for the embryonic cells to become adequately hydrated for germination to take place, resulting in the high t_{50} value. As ageing progressed from 1 to 4 days, the impermeable seed coat of the two pigmented cultivars became progressively softened, leading to increasing rate of water uptake and decreased germination time. The observation of Sousa & Marcos-Filho (1993) that in *Calopogonium mucunoides* deteriorated seeds imbibed water more quickly suports the present finding. Though the dark brown cultivar showed a slight reduction in percentage germination during ageing, high germination above 90 per cent were retained in the two pigmented cultivars throughout the ageing period. These observations show that there are cultivar differences in the response of cowpea seeds to harsh storage conditions and water uptake.

Seed-borne fungal pathogens contribute significantly to loss of germinability in seeds. To find out whether the differences in seed vigour between pigmented and unpigmented cultivars of cowpea could also be related to the differences in the incidence of seed-borne fungal pathogens, the presence of seed-borne fungi was assessed in the three cultivars together with two more unpigmented cultivars in the laboratory. Thus, three unpigmented and two pigmented cultivars were assessed. It was observed that the three white cultivars had higher incidence of seed-borne diseases such as *Fusarium* and *Aspergillus* species (Table 3) when compared to the two

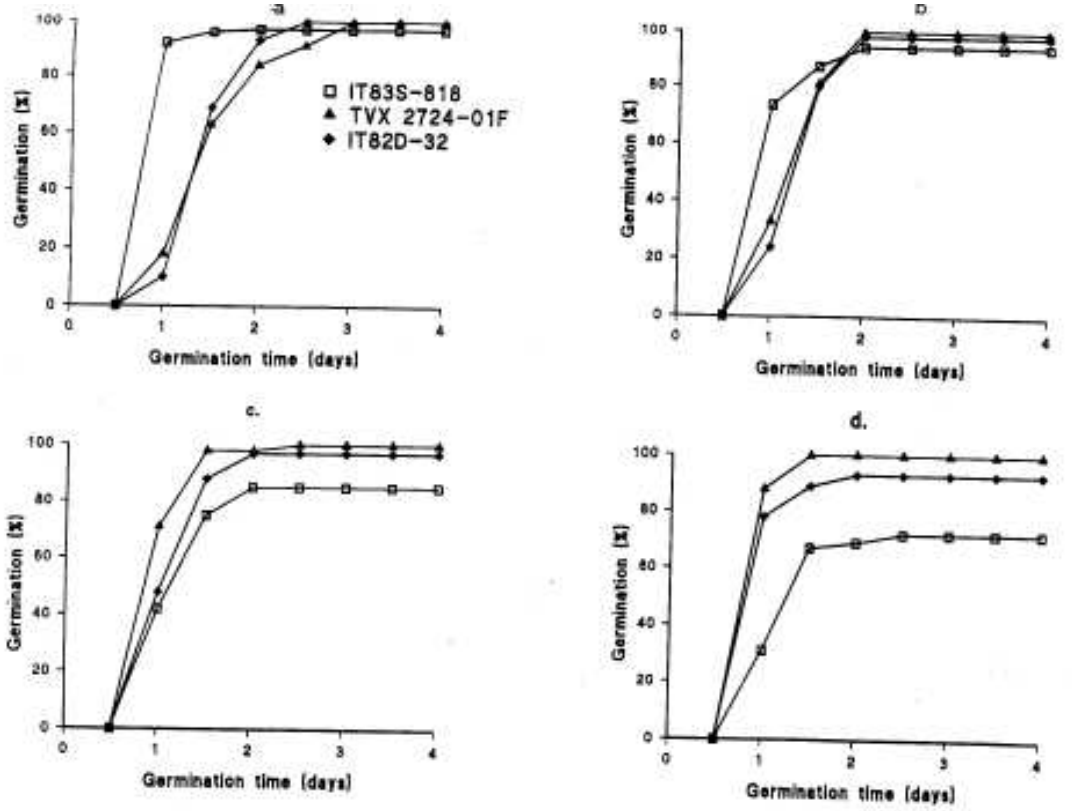

Fig. 1. The effect of controlled deterioration at 40 °C and 20% moisture content on the cummulative germination of seeds of three cowpea cultivars IT83S-818, white (□) TVX 2724-01F, brown (▲) and IT82D-32, dark brown (◆) subjected to a) no ageing, b) one-day ageing, c) two days' ageing and d) four days' ageing.

TABLE 2

Effect of Controlled Deterioration at 20% m.c. and 40 °C on Time (hours) to 50% Radicle Emergence (t_{50})

Cultivar	*Ageing period (days)*			
	0	*1*	*2*	*4*
Unpigmented				
IT83S-818	19.43	19.43	26.29	28.00
Pigmented				
TVX 2724-01F	32.00	27.43	20.57	19.43
IT82D-32	33.14	28.00	25.14	20.00

pigmented cultivars. The earlier observations indicated that the unpigmented varieties were more susceptible to warm and humid storage conditions (Tables 1 and 2). These results also showed that the unpigmented cultivars were more susceptible to the important disease pathogens (Table 3) which could lead to reduction in germination. Among the four varieties (IT83S-818, IT81D-1137, TVX 2724-01F and IT82D-32) subjected to mechanical impact, the white variety, Bengpla, was particularly susceptible to mechanical damage as indicated by reduced germination (Table 4) and high percentage seed damage (Table 5). The controlled deterioration method was, therefore, found to be a useful scientific tool for the prediction of vigour differences among cowpea cultivars, as well as differences in the incidence of seed-borne fungal pathogens and mechanical damage.

The total tannin content determined in the

TABLE 3

Percentage Incidence of Important Seed-borne Fungal Pathogens in Five Cultivars of Cowpea

Seed pathogen	*Non-pigmented cultivar*		*Pigmented cultivar*		
	IT81D-1137	*IT83S-818*	*TVX 3236*	*TVX 2724-01F*	*IT82D-32*
Fusarium semitectum	44.5	33.3	4.7	14.3	15.5
Fusarium oxysporium	16.75	2.0	37.3	0.0	10.3
Fusarium moniliforme	3.25	0.0	0.0	4.5	0.0
Fusarium solani	1.25	2.5	8.0	0.0	0.0
Aspergillus flavus	0.00	52.5	75.5	0.0	6.0

eluants from the Sephadex column and in the 70 per cent aqueous acetone extracts were very low in the white cultivars. For example, the total tannin content (mg catechin equivalent g^{-1}) of the cowpea cultivars determined in the 70 per cent aqueous acetone extracts was below 1.0 mg in the seed coats of the white cultivars (Table 6); the cream and brown cultivar showed slightly higher tannin contents than the white cultivars. However, the pigmented cultivars were very high in tannin content. The ability of tannins (in the seed coats) to complex strongly with carbohydrates and proteins (in the embryo) and the recent use of tannins as industrial adhesives (Porter, 1989) may provide tight adherence between the seed coat and the embryo. This explanation and the water-proofing property of tannins were responsible for the slow rates of water uptake in the pigmented seeds and their ability to combat disease organisms (because all disease-causing organisms have protein components which could be destroyed by tannins).

The pigmented cultivars contained higher amounts of lignin (Table 6) which agrees with the observation by Kannenberg & Allard (1960) in lima bean. The function of lignin in woody plants and tissues, which could also apply to cowpea seed coats, is to provide mechanical strength and also protect them from chemical, physical and biological attacks (Friend, 1981; Schubert, 1973). This contributed to the enhanced ability of the pigmented cultivars of cowpea to withstand disease organisms (Table 3) and mechanical

TABLE 4

Effect of Threshing Methods at 16.5% Moisture Content on Percentage Germination

Variety	*Threshing method*		
	Hand-split	*Machine*	*Beating in sac*
IT81D-1137	86.0	84.0	84.0
IT83S-818	88.0	85.2	65.6
TVX 2724-01F	94.0	93.6	94.4
IT82D-32	89.4	92.0	90.8
Mean	89.3	88.7	83.7
S.E.±	1.7	2.4	6.4

TABLE 5

Effect of Threshing Methods at 16.5% Moisture Content on Percentage Damaged Seed

Variety	*Threshing method*		
	Hand-split	*Machine*	*Beating in sac*
IT83S-818	0.00	0.00	5.20
IT81D-1137	0.00	0.01	1.33
TVX 2724-01F	0.00	0.12	0.l0
IT82D-32	0.00	0.15	0.18
Mean	0.00	0.7	1.7
S.E.±	0.00	0.04	1.0

TABLE 6

Total Tannin and Lignin Contents (mg g^{-1}) of Cowpea Seed Coat. Tannins were Determined from Eluants of Sephadex L-H 20 Column and from Extracts of 70% Aqueous Acetone. Lignin was Determined after 70% Aqueous Acetone Extraction of Tannins

Cowpea variety	*Testa colour*	*Tannin content (mg g^{-1})*		
		Sephadex L-H column × 10^{-3}	*70% acqueous acetone extract*	*Total lignin content (mg g^{-1})*
IT83S-818	White	3.0	0.91 ± 0.16	4.90 ± 0.02
IT81D-1137	White	3.0	0.61 ± 0.08	4.20 ± 0.02
TVX 3236	Cream and brown	49.0	5.22 ± 0.09	5.10 ± 0
TVX 2724-01F	Brown	2880.0	33.36 ± 2.47	10.40 ± 0.07
IT82D-32	Dark brown	1050.0	23.09 ± 0.28	7.80 ± 0.10

damage (Tables 4 and 5). The hydrophobic nature of lignin may also make pigmented seed coats impermeable to water during storage, thereby, reducing respiratory processes which contribute to ageing and deterioration. Thus, differences in seed coat chemical analysis between pigmented and unpigmented seeds of cowpea explain the differences in their physiological behaviour.

REFERENCES

Abdullah, W. D., Powell, A. A. & **Matthews, S.** (1992) Prediction of the storage potential of long bean (*Vigna sesquipedalis* L. Fruhw) seed in the tropics. *Seed Sci. Technol.* **20**, 141-147.

Asiedu, E. A. & **Powell, A. A.** (1998) Comparison of the storage potential of cultivars of cowpea (*Vigna unguiculata*) differing in seed coat pigmentation. *Seed Sci. Technol.* **26.**

Burns, R. E. (1970) Method of estimation of tannin in grain sorghum. *Agron. J.* **63,** 511-513.

Carmona, A., Siedl, S. D. & **Jaffe, W. G.** (1991) Comparison of extraction methods and assay procedures for the determination of the apparent tannin content of common bean. *J. Sci. Fd Agric.* **56,** 291-301.

Friend, J. (1981) Plant phenolics, lignification and plant disease. In *Progress in Phytochemistry* (L. Reinhold, J. B. Harbonne and T. Swain, ed.). Pergamon Press, Oxford, New York, Toronto, Sydney, Paris and Frankfurt.

Hartwig, E. E. & **Potts, H. C.** (1987) Development and evaluation of impermeable seed coat for preserving soybean seed quality. *Crop Sci.* **27,** 506-508.

Iyama, K. & **Wallis, A. F. A.** (1988) An improved acetyl bromide procedure for determining lignin in woods and wood pulps. *Wood Sci. Technol.* **22,** 271-280.

Juliano, B. O., Perez, C. M. & **Chang, T. T.** (1990) Varietal differences in longevity of tropical rough rice stored under ambient conditions. *Seed Sci. Technol.* **18,** 361-369.

Kannenberg, L. W. & **Allard, R. W.** (1960) An association between pigment and lignin formation in the seed coat of the Lima bean. *Crop Sci*. **4,** 621-622.

Krishnasamy, V. & **Seshu, D. V.** (1990) Germination after accelerated ageing and associated characters in rice varieties. *Seed Sci. Technol.* **18,** 147-156.

Kueneman, E. A. (1983) Genetic control of seed longevity in soybeans. *Crop Sci.* **22,** 5-8.

Legesse, N. & **Powell, A. A.** (1992) Comparisons of water uptake and imbibition damage in eleven cowpea cultivars. *Seed Sci. Technol.* **20,** 173-180.

Matthews, S. (1980) Controlled deterioration: A new vigour test for crop seeds. In *Seed Production* (P. S. Hebblethwaite, ed.), pp. 647-660. Butterworths, London.

Morrison, I. M., Asiedu, E. A., Stuchbury, T. & **Powell, A. A.** (1995) Determination of lignin and tannin contents of cowpea seed coats. *Ann. Bot.* **76,** 287-290.

Porter, L. J. (1989) Tannins. In *Methods in Plant Biochemistry* (J. B. Harborne, ed.), pp. 389-419. Academic Press, London, San Diego, New York,

Berkely, Boston, Sydney, Tokyo and Toronto.

Price, M. L., Scoyoc, S. V. & **Butler, L. G.** (1978) A critical evaluation of the Vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Fd Chem.* **26,** 214-1219.

Schubert W. J. (1973) Lignin. In *Phytochemistry, Inoganic Elements and Special Groups of Chemicals* (L. P. Miller, ed.). Van Noltrand Reinhold Company, New York, Cincinnati, Toronto, London and Melborne.

Sousa, F. H. & **Marcos-Filho** (1993) Physiological characteristics associated with water imbibition by *Calopogomium mucunoides* Desv. *Seed Sci. Technol.* **22,** 561-572.

Strumayer, D. H. & **Malin, M. J.** (1975) Condensed tannins in grain sorghum: Isolation, fractionation and characterisation. *J. Agric. Fd Chem.* **23** (5), 909-914.