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# Effect of seed storage and seed coat pigmentation on susceptibility of cowpeas to pre-emergence damping-off

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## Summary

Seeds of four varieties of cowpea (*Vigna unguiculata*) were subjected to prolonged storage conditions of 30°C, 75% RH for three and six months and accelerated ageing for 2 and 4 d at 45°C and 100% RH. Aged seed was sown in soil inoculated with *Fusarium solani*, *Pythium ultimum* or *Rhizoctonia solani*. Varieties with black and tan coloured seeds retained germination best after 4 d accelerated ageing or storage for six months. There was an increase in the leachate conductivity of all the varieties during ageing, with the exception of black seed aged for 2 d, indicating an increase in solute leakage from the seeds. This could be attributed to a decrease in the extent of living tissue within the seeds as indicated by vital staining. Accelerated ageing had little effect on the relative emergence of the black and tan varieties or of the control seeds for the two white varieties. However, all varieties showed reduced emergence after storage at 30°C, 75% RH, with the exception of the black variety, which also retained highest emergence in the presence of all three fungi after ageing. The percentage pre-emergence damping-off of black and tan seeds planted in soil inoculated with *P. ultimum* or *F. solani* was significantly lower than that of the two white varieties. However, in *R. solani*-inoculated soil, reduced damping-off was observed in the coloured varieties only following 4 d accelerated ageing. The significance of seed coat pigmentation to seed quality and susceptibility to infection by soil-borne fungi is discussed.

## Introduction

In tropical countries such as Kenya and Tanzania, cowpea seeds (*Vigna unguiculata* L. Walp.) are stored at high relative humidities combined with high ambient temperatures. These unfavourable storage conditions, combined with high seed moisture contents, result in rapid deterioration of the seed during storage (Roberts, 1972) leading to poor seed quality. However, differences in response to high humidity have been observed in grain legumes differing in testa pigmentation (Abdullah *et al.*, 1992; Asiedu and Powell, 1998). Asiedu and Powell (1998) found that cowpea cultivars having unpigmented seed coats had poor storage potential in comparison to pigmented cultivars in both prolonged storage (30°C, 75.5% RH for six months) and accelerated ageing (40°C, 100% RH for 6 d) conditions.

A second cause of differences in seed quality in grain legumes is the incidence of imbibition damage to the cotyledons arising from rapid water uptake (Powell *et al.*, 1984a). Differences in the rate of water uptake and incidence of imbibition damage have been associated with testa colour in cowpea (Legesse and Powell, 1992), dwarf French bean

(Powell *et al.*, 1986a; 1986b) and long bean (Abdullah, 1988). Cream/beige cultivars showed more evidence of imbibition damage than coloured cultivars and hence had reduced seed quality. The incidence of imbibition damage is however influenced by the extent of seed ageing. Thus in cowpeas, imbibition damage did not occur in unstored seeds whereas the reduced vital staining of the cotyledons after rapid imbibition of stored, i.e. aged seeds, revealed the incidence of imbibition damage (Asiedu and Powell, 1998).

Imbibition damage was also found to enhance the predisposition of pea (Powell and Matthews, 1980) and cowpea (Legesse and Powell, 1992) seed to pre-emergence mortality in unsterilised soil, with coloured seeds showing lower levels of imbibition damage and incidence of infection than seeds with unpigmented seed coats. Stasz *et al.* (1980) reported that pea seeds with seed coats coloured by anthocyanins were resistant to *Pythium ultimum* Trow. seed and seedling diseases whereas seeds with unpigmented seed coats were susceptible. Furthermore, removing seed coats from coloured seeds greatly reduced resistance to *P. ultimum*, whereas removing seed coats from unpigmented seeds had little or no effect on resistance. Similarly, Ginoux and Messiaen (1993) found that black, red buff-coloured and mottled beans were more resistant to *P. ultimum* than white ones.

Cowpea provides an inexpensive source of protein for the rural and urban poor populations in many parts of the world. Unfortunately, it is susceptible to many diseases (Singh *et al.*, 1997). Apart from *Pythium* spp. (Onuorah, 1973; Singh and Allen, 1979; Kataria and Dharam Singh, 1981; Singh and Rachie, 1985; Aveling and Adandonon, 2000), important soil-borne pathogens of cowpea include *Fusarium solani* (Mart.) Sacc. (Singh and Rachie, 1985; Monga and Grover, 1993), *Phytophthora* spp. (Singh *et al.*, 1997), *Rhizoctonia solani* Kühn. (Onuorah, 1973; Singh and Rachie, 1985) and *Sclerotium rolfsii* Sacc. (Singh and Rachie, 1985). Williams (1973) reported that seedling mortality induced by *Pythium* spp. resulted in as low as 25% seedling stand in cowpeas sown during cool, wet weather in Ibadan, Nigeria. *R. solani* seedling mortality of cowpea also occurs during cool, wet weather (Singh and Rachie, 1985).

The aim of this study was to evaluate the effect of accelerated ageing and long-term storage, in relation to seed coat pigmentation, on susceptibility of cowpeas to pre-emergence damping-off caused by *P. ultimum. F. solani* and *R. solani*.

## Materials and methods

### *Seed*

Seeds of four varieties of cowpea differing in seed coat pigmentation (black, tan and two white varieties, called white-3 and white-8) were obtained from an open market in Nairobi, Kenya. Seeds were held in ambient conditions in the laboratory (approximately 20°C for 7 d, then stored in sealed aluminium foil packets and held at 4°C. Seed moisture content was not determined in this work. However, previously, the moisture contents of cowpea seeds that had equilibrated in the laboratory had ranged from 11.5 to 12.5%.

### *Storage*

Prolonged storage under simulated tropical conditions of 30°C, 75% RH was carried out for three and six months. The relative humidity was achieved over a saturated solution

of NaCl in a sealed plastic container (Winston and Bates, 1960). Seeds were weighed and placed in muslin bags on a grid above the salt solution. After three and six months seeds were allowed to dry back to approximately their initial moisture content (mc) by placing in the open in the laboratory (approx. 20°C) overnight. Attainment of this mc was determined by weighing. Seeds were then sealed in aluminium foil packets and stored at 4°C until required for experiments. Accelerated ageing followed a procedure similar to that of Delouche and Baskin (1973). Seeds were placed in muslin bags on a grid in a desiccator held at 45°C and 100% RH. The high relative humidity was achieved by placing water in the well of the desiccator, which was placed at 45°C for 24 h before adding the seeds. Seeds were removed after 2 and 4 d, allowed to dry back to their initial mc as described above and stored in aluminium foil packets at 4°C.

*Seed germination*

Seed germination was tested by placing four replicates of 25 seeds each between moist paper towels, which were rolled up and held upright in plastic beakers. These were placed in trays, in polythene bags and maintained at 20 ± 2°C. Percentage germination was determined after 4 and 8 d according to the International Seed Testing Association rules (International Seed Testing Association, 1999).

*Vital staining*

The testae were removed from 25 seeds after 24 h imbibition in water (fast) or between damp cloths (slow). The cotyledons were separated and placed in a 1% solution of 2, 3, 5 triphenyl tetrazolium chloride (TTC) in the dark for 3 h at 20°C. The degree of red staining (indicative of living tissue) was assessed. The staining of each cotyledon was assessed as either completely stained i.e. completely living, or incompletely stained i.e. including various degrees of dead tissue.

*Conductivity*

The conductivity of 25 seeds was measured for individual weighed seeds, each placed in 4 ml deionised water at 20°C for 24 h, using an automatic seed analyser (G2000, Wavefront Inc, Ann Arbor, Michigan).

*Pathogens*

Cultures of *Pythium ultimum* isolate 279 and *Rhizoctonia solani* were obtained from K. Sutherland, Scottish Agricultural College, Aberdeen. An isolate of *Fusarium solani* (IMI 334478) was obtained from CABI Bioscience, UK. Cultures were maintained on potato dextrose agar (PDA) at 20°C. Discs (6 mm diam.) cut from the actively growing region of 4 d old *P. ultimum* and *R. solani* and 7 d old *F. solani* cultures using a cork borer were used as inoculum.

*Infection studies*

Sterile loam-based compost (John Innes No. 1 compost; sterilised, screened loam containing grit-sand, adjusted to pH 7.0 by addition of $Na_2CO_3$) was used to fill 104-cell plastic seedling trays (40 cm × 28 cm × 3.5 cm). A single seed along with a mycelial

disc was planted at a depth of 2 cm in each cell. Appropriate controls in infested and uninfested compost were also set up. There were four replications of 25 seeds per treatment. Seedling trays were placed in a randomised block design in a controlled environment room at 25°C with a 12 h-light/dark regime. The position of the seedling trays was rotated daily in the room. Seedling trays were watered daily to maintain field capacity (35% mc). Percentage relative emergence (actual emergence in greenhouse expressed as a percentage of laboratory germination) was determined.

*Disease incidence*

Pre-emergence damping-off was expressed as a percentage and determined as follows: 100 – (percentage actual emergence of treatment in greenhouse/percentage actual emergence of control in greenhouse × 100)

*Statistical methods*

Two-way analysis of variance (ANOVA) was performed on the data and least significant differences ($P = 0.05$) were determined.

## Results

The percentage germination of unaged seed of all the varieties was initially more than 85% (table 1). Only the white-3 and white-8 varieties showed a significant reduction in percentage germination (table 1) after 2 d accelerated ageing while the germination of all varieties decreased significantly after 4 d. After 4 d ageing, the black and tan varieties had significantly higher germination percentages than the two white varieties (table 1). Seeds stored for up to six months at 30°C, 75% RH also showed a significant decrease in the percentage germination compared with unaged seed (table 2). However, black and tan seeds retained germination in the first three months whilst after six months, the tan and white-8 varieties retained germination best (table 2).

The seed leachate conductivity of unaged seed of all the varieties ranged between 1696 and 3160 $\mu Scm^{-1}g^{-1}$. The conductivity of all the varieties increased indicating an increase in solute leakage from the seeds during both accelerated ageing and long-term storage, with the exception of black seed aged for 2 d (tables 1 and 2). The largest increases in conductivity occurred for white-3, which also showed the greatest decrease in germination, followed by black seed in both accelerated aged and stored seed (tables 1 and 2).

The differences in the rates of deterioration of the varieties were reflected in their vital staining with tetrazolium chloride following slow imbibition in damp cloths (tables 1 and 2). In both storage conditions (tables 1 and 2) there was a decrease in living tissue in all the varieties, which explained the observed increase in leachate conductivity with ageing. The two white varieties showed a greater reduction in living tissue than did the black and tan seeds (tables 1 and 2). The difference between the percentage of completely living cotyledons when seeds were imbibed slowly compared with that when seeds imbibed rapidly illustrated the extent to which the varieties were susceptible to imbibition damage. Thus the reduced staining in all the seeds after rapid imbibition (unaged seeds, seeds after accelerated ageing and stored seeds) revealed imbibition

Table 1. Seed quality assessments before and after the accelerated ageing of four varieties of cowpea at 100% relative humidity and 45°C.

| Quality assessment | Variety | Unaged seeds | 2d ageing | 4d ageing |
|---|---|---|---|---|
| Germination (%) | Black | 94*ab**y | 95 ay | 83 az |
| | Tan | 97 ay | 93 ayz | 92 az |
| | White 3 | 85 cy | 59 bz | 45 cz |
| | White 8 | 97 ax | 88 ay | 59 bz |
| Conductivity ($\mu Scm^{-1}g^{-1}$) | Black | 3160*** | 2870 | 4121 |
| | Tan | 2264 | 2729 | 2741 |
| | White 3 | 3160 | 3785 | 5257 |
| | White 8 | 1696 | 5257 | 3880 |
| TTC[1]: Slow imbibition | Black | 88*** | 88 | 62 |
| | Tan | 82 | 76 | 68 |
| | White 3 | 76 | 44 | 20 |
| | White 8 | 78 | 62 | 42 |
| TTC[1]: Fast imbibition | Black | 66*** | 70 | 48 |
| | Tan | 76 | 74 | 48 |
| | White 3 | 52 | 40 | 20 |
| | White 8 | 70 | 50 | 36 |

* Each value is a mean percentage of four replicates of 25 seeds. Means within a COLUMN not followed by the same letter are significantly different ($P = 0.05$).
** Means within a ROW not followed by the same letter are significantly different ($P = 0.05$).
*** Each value is a mean of 25 seeds.
[1] TTC = triphenyl tetrazolium chloride test, a mean of 25 seeds expressed as percentage cotyledons with living tissue.

damage in all varieties. Even though all seeds showed evidence of imbibition damage, the black and tan varieties still retained more living tissue following rapid imbibition than did the white varieties (tables 1 and 2). Rapid imbibition appeared to have a greater impact on all varieties stored for three and six months when compared to seed aged for 2 and 4 d.

There was little effect of accelerated ageing on the relative emergence of the black and tan varieties or of the control seeds for the two white varieties (table 3). However, emergence of white-3 variety decreased with age. In the presence of *Pythium*, even the unaged seeds showed reduced emergence with a further fall in emergence after ageing to 0%. Emergence of the two white varieties was significantly less than that of the coloured varieties in the presence of all the fungi after 4 d ageing and after 2 d ageing in the presence of *Pythium* (table 3).

Storage at 30°C and at 75% RH had a greater effect on the emergence of the control seed (table 4). Thus with the exception of the black variety, all varieties showed reduced emergence after six months storage and in the case of white-3, emergence of the control was reduced to 0%. The black variety retained high emergence in the presence of all three fungi after storage, with the exception of an emergence of only 61% in the presence of *Pythium* after six months (table 4). All other varieties again showed reduced emergence as seeds aged during storage. As in accelerated ageing, the emergence of the two white varieties tended to be significantly less than that of the pigmented varieties (table 4).

The effect of the three pathogens on seedling emergence was even more evident when the extent of pre-emergence damping-off was considered (tables 5 and 6). The percentage

Table 2. Seed quality assessments before and after storage of four varieties of cowpea seeds at 75% relative humidity and 30°C for 3 and 6 months.

| Quality assessment | Variety | Unaged seeds | 3 months | 6 months |
|---|---|---|---|---|
| Germination (%) | Black | 94*ab**x | 72 by | 24 bz |
| | Tan | 97 ax | 81 ay | 59 az |
| | White 3 | 85 cy | 20 dz | 7 cz |
| | White 8 | 97 ax | 66 cy | 46 az |
| Conductivity ($\mu Scm^{-1}g^{-1}$) | Black | 3160*** | 4748 | 5311 |
| | Tan | 2264 | 3853 | 3557 |
| | White 3 | 3160 | 5821 | 5446 |
| | White 8 | 1696 | 3948 | 3312 |
| TTC[1]: Slow imbibition | Black | 88*** | 69 | 23 |
| | Tan | 82 | 72 | 21 |
| | White 3 | 76 | 18 | 5 |
| | White 8 | 78 | 60 | 8 |
| TTC[1]: Fast imbibition | Black | 66*** | 42 | 2 |
| | Tan | 76 | 40 | 0 |
| | White 3 | 52 | 18 | 0 |
| | White 8 | 70 | 40 | 0 |

* Each value is a mean percentage of four replicates of 25 seeds. Means within a COLUMN not followed by the same letter are significantly different ($P = 0.05$).
** Means within a ROW not followed by the same letter are significantly different ($P = 0.05$).
*** Each value is a mean of 25 seeds.
[1] TTC = triphenyl tetrazolium chloride test, a mean of 25 seeds expressed as percentage cotyledons with living tissue.

Table 3. Relative emergence (%) of four varieties of cowpea following accelerated ageing and exposure to soil-borne fungi during germination and emergence.

| Soil-borne fungus | Ageing treatment | Variety | | | |
|---|---|---|---|---|---|
| | | Black | Tan | White 3 | White 8 |
| Control | Initial | 103*b**z | 99bz | 80az | 100bz |
| | 2d | 99az | 100az | 124by | 105abz |
| | 4d | 110az | 98az | 109ay | 92az |
| *Rhizoctonia solani* | Initial | 103az | 98az | 90az | 98ay |
| | 2d | 98abz | 102abz | 119bz | 81azy |
| | 4d | 111bz | 97bz | 76az | 76az |
| *Pythium ultimum* | Initial | 100cy | 93cz | 21ay | 65by |
| | 2d | 99by | 96bz | 0az | 3az |
| | 4d | 78bz | 88bz | 0az | 0az |
| *Fusarium solani* | Initial | 102cz | 97cz | 57az | 84by |
| | 2d | 101az | 99az | 86az | 89ay |
| | 4d | 108cz | 104cz | 62bz | 38az |

*Each value is a mean of four replicates of 25 seeds (relative emergence expressed as a percentage of laboratory germination). Means within a ROW not followed by the same letter are significantly different ($P = 0.05$).
**Means within a COLUMN of three values not followed by the same letter are significantly different ($P = 0.05$).

Table 4. Relative emergence (%) of four varieties of cowpea following three and six months storage at 75% relative humidity and 30°C and exposure to soil-borne fungi during germination and emergence.

| Soil-borne fungus | Ageing treatment | Variety | | | |
|---|---|---|---|---|---|
| | | Black | Tan | White 3 | White 8 |
| Control | Initial | 103*b**z | 99by | 80ay | 100by |
| | 3 months | 98az | 116ay | 119ay | 103ay |
| | 6 months | 90bz | 49abz | 0az | 71abz |
| *Rhizoctonia solani* | Initial | 103az | 98ay | 90ay | 98ay |
| | 3 months | 97bz | 107by | 29az | 23az |
| | 6 months | 141bz | 45bz | 30az | 42az |
| *Pythium ultimum* | Initial | 100cy | 93cy | 21az | 65by |
| | 3 months | 95bzy | 97by | 04az | 10az |
| | 6 months | 61bz | 16az | 0az | 4az |
| *Fusarium solani* | Initial | 102cz | 97cx | 57az | 84by |
| | 3 months | 72cz | 69cy | 41bz | 16az |
| | 6 months | 152bz | 46az | 25az | 26az |

*Each value is a mean of four replicates of 25 seeds (relative emergence expressed as a percentage of laboratory germination). Means within a ROW not followed by the same letter are significantly different ($P = 0.05$).
**Means within a COLUMN of three values not followed by the same letter are significantly different ($P = 0.05$).

pre-emergence damping-off of black and tan seeds (unaged, aged for 2 and 4 d or stored for three and six months) planted in soil inoculated with *P. ultimum* or *F. solani* was significantly lower than that of the two white varieties. The only exception was seen in the tan seed after six months storage, which did not differ from the white-8 variety stored for the same period. There was no difference in the pre-emergence damping-off of unaged seeds of all the varieties grown in *R. solani*-inoculated soil. The disease was more severe in white-3 and white-8 seeds aged for 4 d (table 5) or stored for three months (table 6) than in the black and tan seeds. Surprisingly, the white-8 seeds showed a low percentage of pre-emergence damping-off after six months storage. The general trend for all the varieties was that as ageing or storage period increased, disease incidence increased and this was more pronounced in the white varieties.

## Discussion

Seeds tested following accelerated ageing and simulated tropical storage showed more rapid deterioration, with increased solute leakage as a result of a decrease in living tissue, preceding the loss of germination. However, white varieties had poor storage potential compared with the pigmented varieties as previously observed by Asiedu and Powell (1998). All seeds also revealed evidence of imbibition damage, reflected in reduced vital staining after rapid imbibition and seen previously in a number of grain legumes (Powell *et al.*, 1984b). The reduction in seed quality of all varieties as a result of both ageing during storage and imbibition damage was reflected in the reduced emergence of aged seeds and the poorer storage potential of the white varieties was evident in the generally lower emergence than that seen in the pigmented seeds.

Table 5. Pre-emergence damping-off (%) of four varieties of cowpea following accelerated ageing and exposure to soil-borne fungi in the greenhouse.

| Soil-borne fungus | Ageing treatment | Variety | | | |
|---|---|---|---|---|---|
| | | Black | Tan | White 3 | White 8 |
| *Rhizoctonia solani* | Initial | 2.0*a**z | 2.0az | 6.5az | 2.0az |
| | 2d | 2.0az | 1.1az | 9.7ay | 22.6by |
| | 4d | 2.0az | 3.1az | 33cy | 17.9by |
| *Pythium ultimum* | Initial | 4.0az | 6.2az | 69.7cz | 35bz |
| | 2d | 3.0az | 4.3az | 100by | 96.8by |
| | 4d | 28.5by | 9.9az | 100cy | 100cy |
| *Fusarium solani* | Initial | 2.0az | 3.0ay | 25.6bz | 15.2abz |
| | 2d | 2.0az | 3.3ay | 32.3bz | 15az |
| | 4d | 4.1az | 0.0az | 55.5by | 58.6by |

*Each value is a mean percentage of four replicates of 25 seeds. Means within a ROW not followed by the same letter are significantly different ($P = 0.05$).
**Means within a COLUMN of three values not followed by the same letter are significantly different ($P = 0.05$).

Table 6. Pre-emergence damping-off (%) of four varieties of cowpea following three and six months storage at 75% relative humidity and 30°C and exposure to soil-borne fungi in the greenhouse.

| Soil-borne fungus | Ageing treatment | Variety | | | |
|---|---|---|---|---|---|
| | | Black | Tan | White 3 | White 8 |
| *Rhizoctonia solani* | Initial | 2.0*a**z | 2.0az | 6.5az | 2.0az |
| | 3 months | 27.5az | 7.1az | 72.8by | 76.5bx |
| | 6 months | 0az | 24.1by | 100cx | 36.1by |
| *Pythium ultimum* | Initial | 4.0az | 6.2az | 69.7cz | 35bz |
| | 3 months | 6.5az | 15.8az | 95by | 89.7bcy |
| | 6 months | 12.5az | 50.2aby | 100cy | 89.9bcy |
| *Fusarium solani* | Initial | 2.0az | 3.0az | 25.6bz | 15.2abz |
| | 3 months | 25.7ay | 39.9ay | 66.2by | 84.5by |
| | 6 months | 0az | 18.6ay | 100cx | 58.7by |

*Each value is a mean percentage of four replicates of 25 seeds. Means within a ROW not followed by the same letter are significantly different ($P = 0.05$).
**Means within a COLUMN of three values not followed by the same letter are significantly different ($P = 0.05$).

Ageing cowpea seeds prior to planting also increased the susceptibility of the seed to pre-emergence damping-off, an observation previously made in peas by Stasz and Harman (1980). In this study, damping-off was far more severe in the unpigmented, than in the pigmented seeds, often reaching levels of 100% infection. These results are in agreement with those of Legesse and Powell (1992) who reported that cream/beige seeds of cowpea were more susceptible to infection by *Pythium* sp. when sown in soil at 25% and 35% mc and 10°C and 20°C than were coloured seed.

The increased predisposition of the white seeds to the three damping-off pathogens and in particular *Pythium*, after ageing may result from the dead tissue on the cotyledons as a result of acting as an initial site of fungal infection, as suggested by Matthews (1971), or leakage of solutes into the soil (Powell and Matthews, 1980). On the other hand, the resistance expressed by the pigmented cowpea seeds may be attributable to the presence of phenols, tannins and/or lignins in the seed coat. Darkly pigmented cowpea seeds contain more tannins and lignins than unpigmented seeds (Morrison *et al.*, 1995) and Clauss (1961) and Statler (1970) concluded that phenols, present in seed coats and cotyledons of dark-seeded peas and bean selections, were responsible for root rot resistance to *P. ultimum* and *Fusarium solani* f. sp. *solani*, respectively. However, Kraft (1974) found that peas with pigmented seeds, both resistant and susceptible to root rot caused by *P. ultimum*, produced similar amounts of phenols and reducing sugars in exudates from germinating seeds and seedlings, but he noted that resistance in peas to pre-emergence damping-off caused by *P. ultimum* and *F. solani* f. sp. *pisi* was almost always associated with accessions possessing the *A* gene for anthocyanin production. Furthermore, Stasz *et al.* (1980) reported that pea seed, coloured by anthocyanins were resistant to *P. ultimum* seed and seedling diseases, whereas seeds with uncoloured seed coats were susceptible. Muehlbauer and Kraft (1978) found that pea lines homozygous for *A* were more resistant to pre-emergence damping-off presumably due to the fungistatic effects of phenols associated with anthocyanin pigmentation. Lokaprakash (1981) found that the gene Pda 1 which controls pedicel pigmentation, is also involved in the control of seed colour and immature pod pigmentation in cowpea but did not discuss its possible role in resistance to diseases. The gene controlling pigmentation in grain legumes may therefore have effects on seed quality through regulating the rate of imbibition (Powell *et al.*, 1984b), seed storage potential (Asiedu and Powell, 1998) and also the susceptibility of seeds to infection by soil-borne fungi.

Results from this study indicate that farmers should consider rather planting the darker pigmented cowpea varieties or protecting the seeds of light coloured varieties against attack by soilborne pathogens using a fungicide seed treatment such as thiram.

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