# Cotton SA Project Report

# Investigating seed germination and vigour of cotton seeds


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## Aim:

To determine which cotton cultivar would be best suitable for replanting after storage of seed for two different periods to break any possible dormancy the seed has.

## Trial objectives:

- To determine the effect of storage period on cotton seed germination. Cotton seed that has been in storage for at least one season, is presented for planting during the second season, and compared to cotton seed, that has been harvested around May-June, kept in storage and packed for sale for the season directly following. Thus, one seed lot has rested for around 12-15 months, while the other has rested for 3-4 months.
- To evaluate vigour of stored cotton seeds in addition to germination, as described above.

### Materials and Methods:

Table 1 represents the four cultivars of cotton seeds viz. Candia, DP1531, DP1240 and DP1541. Newly harvested seed of the 2018/19 season were compared to seed that were harvested in harvested in the 2018/2019 season. Seed was prepared for sale and planting in the industry for the 2019/20 season. Seed of the previous season (2017/18) will be referred to as "well-rested seed:, while seed harvested in 2018/19, will be referred to as "rested-seed". Seed that originates from the same lots that were planted in the Sweizer-Reneke area was sent to Cotton SA. Seed was kept under cool conditions in storage for the resting period. Seed were

delivered to the Seed Science laboratory (University of Pretoria), by A. Bennett. Seeds were stored at standard room conditions for future evaluations.

**Table 1:** Cotton seed samples tested in this study

| Cultivar | Sample code and year | |
|---|---|---|
| Candia | SP17/18-1-well rested seed | SP18/19-1-rested seed |
| DP1531 | SP17/18-2- well rested seed | SP18/19-2– rested seed |
| DP1240 | SP17/18-3 – well-rested seed | SP18/19-3– rested seed |
| DP1541 | SP17/18-4-well rested seed | SP18/19-4- rested seed |

## Moisture content test

Moisture content of cotton seeds was measured before proceeding with seed germination and vigour tests. The high-constant oven temperature method as stipulated by the International Seed Testing Association (ISTA) (2019) was used to determine moisture content of cotton seeds. Sub-samples of 10 g were randomly taken from cotton seed samples, to form two replicates that were ground to a fine powder and weighed. Powdered samples were dried in an oven at 131 °C for 24 h. At the end of the drying period, powdered samples were placed in a desiccator for 5 min to cool. Thereafter, final weight of powdered samples was measured and changes in moisture determined.

## Standard germination test

Seed germination of cotton seeds was done using the between paper method according to the rules of ISTA (2019). Four replicates of fifty seeds were placed on top of three layers of moistened germination paper before covering them with a fourth layer of moistened germination paper. The layers of germination paper were rolled, sealed in a polythene bag and incubated at 25 °C under an alternating 12 h darkness and normal light regime. Seed

germination was evaluated at the fifth and twelfth day, where counts of germinated seeds and abnormalities were recorded.

## Seed vigour tests

### 1. Accelerated aging (AA) test

Two hundred seeds (four replicates of 50) from each sample were randomly selected. Seeds were placed uniformly on sterilised metal sieves, which were then placed into plastic accelerated aging (AA) boxes on grids above 45 mL of sterile distilled water. The AA boxes were sealed and placed in an incubator and incubated for 48 h at a temperature of 41±1 °C. Following accelerated aging of the seeds, the seeds were subjected to the standard germination test (ISTA, 2019) as described above.

### 2. Cold soil test

Cotton seeds were placed on moistened germination paper as for the standard germination test and each row of seeds was covered with a thin layer of soil collected from a maize field at the Experimental farm of the University of Pretoria, Pretoria, South Africa. Four replicates of 50 seeds were used. Seeds were incubated for 7 days at 18 °C and then at 25±1 °C for a further 7 days. Seeds were visually assessed according to the ISTA rules (ISTA 2019.

### 3. Electrical Conductivity (EC) test

Cotton seeds were randomly chosen from each seed sample making four replicates of 25 seeds. Seeds were weighed and soaked in conical flasks filled with 250 mL sterilised distilled water. Flasks were wrapped with aluminium foil and incubated for 24 h at 20±1 °C. At the end of the incubation period, the electrical conductivity of the leachate in the solution was read on an E215 conductivity meter (Hanna Instruments, Johannesburg, South Africa).

## Statistical analysis

All experiments were conducted under controlled laboratory conditions laid out in a complete randomized design. Collected data (i.e. germination percentage and conductivity values) were statistically analysed using a General Linear Model (GLM) procedure to perform an Analysis of Variance (ANOVA) using Statistical Analysis Software (SAS) version 9.4. Means were separated using Fisher's Least Significant Difference test (P= 0.05).

## Results

### Moisture content

The moisture content of cotton seed lots is presented in Table 2. All cotton seed lots had moisture content below the recommended 12% for *Gossypium* spp. as stipulated by ISTA (2019).

**Table 2:** Moisture content (%) of treated cotton seed from 2017/18 and 2018/19 growing seasons

| Cultivars | Season | |
|---|---|---|
| | **SP17/18 – well rested seed** | **SP18/19-rested seed** |
| Candia | 6.75 | 4.83 |
| DP1531 | 6.60 | 7.64 |
| DP1240 | 8.22 | 8.94 |
| DP1541 | 8.18 | 8.09 |

*No statistical analysis was done. Above values represent overall percentage moisture content of two replicates of each seed sample at each season.

### Standard germination

Results of the seed germination tests are shown in Table 3. For cotton seed harvested in 2018 (Sp17/18), the cultivar DP1240 (well rested seed) had the highest percentage seed germination (83.5%) and differed significantly from cultivar DP1531 (rested seed) which had the lowest germination (67.5%). However both these cultivars did not differ significantly from cultivars Candia and DP1541.

**Table 3:** Germination (%) of treated cotton seed harvested from two seasons, 2017/18 and 2018/19

| Cultivar | Season | | $LSD^{x-y}$ | CV (%) |
|---|---|---|---|---|
| | Sp17/18 – well rested seed | Sp18/19 – rested seed | | |
| | Percentage germination | | | |
| Candia | $70^{ab,x}$ | $83.5^{ab,x}$ | 15.52 | 11.69 |
| DP1531 | $67.5^{b,x}$ | $72.5^{c,x}$ | 19.14 | 15.80 |
| DP1240 | **$83.5^{a,x}$** | **$73^{bc,y}$** | 9.13 | 6.72 |
| DP1541 | $76^{ab,x}$ | $84.5^{a,x}$ | 10.83 | 7.80 |
| $LSD^{a-c}$ | 14.33 | 10.72 | | |
| CV (%) | 12.53 | 8.89 | | |

*Values in the table are mean germination of four replicates of 50 seeds per seed sample. Means with the same letter do not differ statistically according to Fisher's LSD test at *P = 0.05*. **Means with $LSD^{a-c}$ are a comparison of germination percentage of seed samples within each column. *Means values with $LSD^{x-y}$ are a comparison of germination percentage amongst rows.

For cotton seed harvested in 2019 (Sp18/19), cultivars DP1541 (84.5%) and Candia (83.5%) had the highest percentage seed germination although the latter did not differ significantly from cultivar DP1531 ($p>0.05$).

A comparison between the two seasons, i.e. 2017/18 and 2018/19, showed that seed germination was significantly lower only on cotton seeds of cultivar DP1240 with a reduction of 10.5%.

Table 4 summarises abnormalities observed on cotton seedlings in the standard seed germination bioassay.

**Table 4:** Percentage of abnormal cotton seedlings in the standard germination bioassay

| Description of abnormalities | 2017/18 | | | | 2018/19 | | | |
|---|---|---|---|---|---|---|---|---|
| | Cultivar | | | | | | | |
| | Candia | DP1531 | DP1240 | DP1541 | Candia | DP1531 | DP1240 | DP1541 |
| Ungerminated | 12 | 7 | 8.5 | 7 | 11 | 14.5 | 7 | 5.5 |
| No primary roots | | 1 | 0.5 | | | | 9.5 | 1.5 |
| Stubby roots without hypocotyl | | | | | | | | |
| Abnormally spiralled | | 1.5 | | | 1 | | 1 | 0.5 |
| Very tiny hypocotyl | 0.5 | | | 6 | 1 | | | 1 |
| Poor primary roots and very short hypocotyl | | | | | | | | |
| Negative geotropism | | | | | 0.5 | | | |
| Very tiny hypocotyl and no secondary roots | | | | | | 0.5 | | |
| Poor primary roots and no hypocotyl | 1 | 2.5 | | | | | | |
| More than 50% of cotyledon damaged | 9.5 | 5 | 5 | 6 | 1 | 4.5 | 2.5 | 4.5 |
| Diseased/ rotting seed | | 6 | | | | | 1 | |
| Hypocotyl split | 5.5 | 3.5 | 3 | 0.5 | 2 | 2 | 0.5 | 2 |
| Rotten roots | 1 | | | | | | | |
| Primary roots trapped in the seed coat | | 2 | | 0.5 | | | 2.5 | |
| Hypocotyl not intact | | | | 4 | | | | |
| **% Abnormal seedlings** | **29.5** | **28.5** | **17** | **24** | **16.5** | **21.5** | **24** | **15** |

## Seed vigour tests

### Accelerated aging (AA) test

There was no significant difference in the germination of all cultivars harvested in 2018 (Sp17/18) (Table 5). However, for cotton seed harvested in 2019 (Sp18/19), highest seed germination was recorded on both cultivars Candia and DP1541 (69.5%) and the lowest on cultivar DP1531.

**Table 5:** Cotton seed germination following the accelerated aging test

| **Cultivar** | **Season** | | **LSD$^{x-z}$** | **CV (%)** |
|---|---|---|---|---|
| | **Sp17/18 – well rested seed** | **Sp18/19- rested seed** | | |
| | **Percentage germination** | | | |
| Candia | 69$^{a,x}$ | 69.5$^{a,x}$ | 19.05 | 16.06 |
| DP1531 | **80$^{a,x}$** | **47.5$^{c,y}$** | 9.24 | 8.37 |
| DP1240 | 65$^{a,x}$ | 57$^{b,x}$ | 18.53 | 17.55 |
| DP1541 | 69.5$^{a,x}$ | 69.5$^{a,x}$ | 17.62 | 14.65 |
| LSD$^{a-c}$ | 19.58 | 7.29 | | |
| CV (%) | 17.94 | 7.78 | | |

*Values in the table are mean germination of four replicates of 50 seeds per seed sample. Means with the same letter do not differ statistically according to Fisher's LSD test at *P = 0.05*. **Means with LSD$^{a-c}$ are a comparison of germination percentage of seed samples within each column. *Means values with LSD$^{x-y}$ are a comparison of germination percentage amongst rows.

When comparing percentage germination between the two seasons, i.e. 2017/18 and 2018/19, only cultivar DP1531 had a significantly reduction (32.5%) in germination. Table 6 shows abnormalities observed on cotton seedlings in the accelerated aging bioassay.

**Table 6:** Percentage of abnormal cotton seedlings in the accelerated aging bioassay

| Description of abnormalities | 2017/18 | | | | 2018/19 | | | |
|---|---|---|---|---|---|---|---|---|
| | Cultivar | | | | | | | |
| | Candia | DP1531 | DP1240 | DP1541 | Candia | DP1531 | DP1240 | DP1541 |
| Ungerminated | 14 | 10.5 | 15.5 | 9.5 | 10 | 17 | 12.5 | 6 |
| No primary roots | 2 | | | | | | | |
| Stubby roots without hypocotyl | | | | | | | | |
| Abnormally spiralled hypocotyl | | | | | 6 | 2.5 | | |
| Very tiny hypocotyl | | | 5 | 2.5 | 0.5 | | | |
| Poor primary roots and very short hypocotyl | | | 2 | | | | | |
| Very tiny hypocotyl and no secondary roots | | 0.5 | | 2 | | | | |
| Poor primary roots and no hypocotyl | 1 | 1 | | | | | | |
| More than 50% of cotyledon damaged | 2.5 | 2.5 | 5.5 | 5.5 | 5.5 | 12.5 | 8 | 8.5 |
| Diseased/ rotting seed | | | | | | | | |
| Hypocotyl split | 10.5 | 4 | 7 | 9 | 5.5 | 11 | 12 | 5 |
| Primary infection (roots, hypocotyl) | 1 | 1 | | 3 | 3 | 9.5 | 11 | 9 |
| Primary roots trapped in the seed coat | | 0.5 | | | | | 3.5 | 2 |
| **% Abnormal seedlings** | **31** | **20** | **35** | **31.5** | **30.5** | **52.5** | **47** | **30.5** |

**Cold soil test**

The percentage germination of cotton seed harvested in 2018 (Sp17/18) only differed between cultivar DP1240 (68%) and Candia (51%) (Table 7). However, for the seed harvested in 2019, cultivar DP1541 (76%) only differed from cultivar DP1240 (64.5%). When comparing percentage germination in the two seasons, i.e. 2017/18 and 2018/19, the percentage germination was significantly higher in the 2018/19 seed, showing that the seed had higher vigour except for DP1240. Table 8 shows abnormalities observed on cotton seedlings in the cold soil bioassay.

**Table 7**: Cotton seed vigour following cold soil test

| **Cultivar** | **Season** | | **LSD$^{x-y}$** | **CV (%)** |
|---|---|---|---|---|
| | **Sp17/18** | **Sp18/19** | | |
| | **Percentage Germination** | | | |
| Candia | **51$^{b,y}$** | **70.5$^{ab,x}$** | 15.72 | 14.95 |
| DP1531 | **58$^{ab,y}$** | **72$^{ab,x}$** | 9.97 | 8.70 |
| DP1240 | 68$^{a,x}$ | 64.5$^{b,x}$ | 14.60 | 12.73 |
| DP1541 | **59.5$^{ab,y}$** | **76$^{a,x}$** | 7.83 | 6.68 |
| LSD$^{a-c}$ | 11.73 | 10.35 | | |
| CV (%) | 12.87 | 9.50 | | |

*Values in the table are mean germination of four replicates of 50 seeds per seed sample. Means with the same letter do not differ statistically according to Fisher's LSD test at *P = 0.05*. **Means with LSD$^{a-c}$ are a comparison of germination percentage of seed samples within each column. *Means values with LSD$^{x-y}$ are a comparison of germination percentage amongst rows.

**Conductivity**

The values of conductivity of seed leachate are presented in Table 7. There were significant differences in seed leachate values for seeds harvested in 2018 (Sp17/18), where highest leachate value was recorded on cultivar DP1531 (790.42 µS.cm$^{-1}$. g$^{-1}$) and cultivar DP1541 (742.65 µS.cm$^{-1}$. g$^{-1}$). The lowest seed leachate value was recorded on cultivar Candia (487.65 µS.cm$^{-1}$. g$^{-1}$). However, there were no significant differences between seed leachate values of seeds harvested in 2019 (Sp18/19). Comparing the two seasons, seed leachate values for

cultivars Candia and DP1240 were significantly higher for the season 2018/19 than those of 2017/18 season.

**Table 7:** Conductivity leachate values of treated cotton seed harvested from two seasons, 2017/18 and 2018/19

| Cultivar | Season | | $LSD^{x-z}$ | CV (%) |
|---|---|---|---|---|
| | Sp17/18 | Sp18/19 | | |
| | Conductivity $\mu S.cm^{-1}. g^{-1}$ | | | |
| Candia | **487.65$^{c,y}$** | **794.94$^{a,x}$** | 178.23 | 16.06 |
| DP1531 | 790.42$^{a,x}$ | 762.02$^{a,x}$ | 110.9 | 8.26 |
| DP1240 | **667.15$^{b,y}$** | **801.93$^{a,x}$** | 58.55 | 4.61 |
| DP1541 | 742.65$^{a,x}$ | 771.14$^{a,x}$ | 58.91 | 4.50 |
| $LSD^{a-c}$ | 71.3 | 122.97 | | |
| CV (%) | 6.89 | 10.20 | | |

Values in the table are the mean conductivity of four replicates of 25 seeds per seed sample. **Means with $LSD^{a-c}$ are a comparison of the conductivity of seed samples within each column. *Means values with $LSD^{x-y}$ are a comparison across rows. Mean conductivity with similar letters (a-c in columns; x-y in rows) do not differ significantly according to Fisher's LSD test at P = 0.05.

## Discussion and conclusion

According to literature, a good quality cotton seed should have a percentage germination of above 65% (Charlotte *et al*., 2018; http://seednet.gov). Based on the standard germination test results, all the cultivars from both seasons, i.e. 2017/18 and 2018/19, had a percentage germination above the acceptable standard minimum value for cotton. Moreover, an inter-season comparison within each cultivar was done, only cultivar DP1240 had a significant reduction of percentage germination but this was still above the acceptable standard minimum value for cotton.

Based on the AA test results, only cultivar DP1531 from the season 2017/18, had the highest percentage germination (80%). However, seedling vigour of cultivar DP1531 was significantly reduced to a germination of less than 50% after storage ($p<0.05$), which is far below the

acceptable standard minimum value for cotton. Percentage germination after the cold test indicated that, except for DP1240, all the cultivars from the 2018/19 season had a higher germination. Assessing the membrane integrity of cotton seeds using the conductivity test showed higher seed leachate values for cultivars Candia and DP1240 harvested in the 2018/19 season than those of 2017/18 season.

In conclusion, results of moisture content, standard germination, accelerated aging and conductivity tests showed that cultivar DP1541 was consistent throughout the two seasons showing the best results. Therefore, cultivar DP1541 can be recommended for planting in the following season. Some caution should be taken when planting with cultivars DP1240 and DP1531 especially if conditions are unfavourable, as these two seed lots had lower vigour even though only DP1531 showed a reduction in germination from the one season (2017/18) to the next (2018/19). It must be kept in mind that these tests all take place in the laboratory and should be compared with results of emergence in the field. One will then be able to get an indication of which of the vigour tests is/are most suitable for cotton seed and which ones should be repeated for the next storage period.

## References

Charlotte, TD, Clovis KN., Faustin SD ., Djinkin KK., Lacina S., Francis SE., Selastique AD., Justin KY., Severin A. (2018). Study of germination and health quality of cotton seed grown in the West Central Region of Côte d'Ivoire. Scholars Journal of Agriculture and Veterinary Sciences 5, 148-155.

Seed Standards for Foundation and Certified. Available online at http://seednet.gov.in/QualityControl/SeedStandard13.htm. [Accessed on 17 December 2019]

## Supplementary information


**Figure 1:** Seed germination of cotton seed cultivar Candia (Sp17/18) from an accelerated aging bioassay


**Figure 2:** Seed germination of cotton seed cultivar  DP1531 (Sp17/18) from an accelerated aging bioassay


**Figure 3:** Seed germination of cotton seed cultivar DP1240 (Sp17/18) from an accelerated aging bioassay


**Figure 4:** Seed germination of cotton seed cultivar DP1541 (Sp17/18) from an accelerated aging bioassay


**Figure 5:** Seed germination of cotton seed cultivar Candia (Sp18/19) from an accelerated aging bioassay


**Figure 6:** Seed germination of cotton seed cultivar  DP1531 (Sp18/19) from an accelerated aging bioassay


**Figure 7:** Seed germination of cotton seed cultivar  DP1240 (Sp18/19) from an accelerated aging bioassay


**Figure 8:** Seed germination of cotton seed cultivar DP1541 (Sp18/19) from an accelerated aging bioassay