Positional and seed ageing effects on seed quality of cotton *(Gossypium hirsutum)* grown in Western Kenya

Rugut Eliud Kiprotich, Kris Audenaert, Lucas Ngode, Linnet Gohole, Godelieve Gheysen, Geert Haesaert.

[1]*Faculty of Bioscience Engineering, Department of Applied Biosciences, Ghent University, Valentine Vaerwyckweg 1, 9000 Ghent, Belgium*

[2]*University of Eldoret, P.O. Box 1125, 30100 Eldoret*

Correspondence: *Email: ruguteliud@yahoo.com*

Abstract

Cotton seed is one of the most sensitive agronomic seeds where significant deterioration occurs after a short period of storage. This study was conducted to determine the effects of boll position and storage period on seed quality of cotton seed. Cotton variety KSA 81 M was planted at Busia ATC and bolls harvested from the Basal, Middle and Top branches for storage and subsequent seed quality tests. Germination of seeds from all the three plant positions declined with storage period while electrical conductivity and mean germination time increased. Germination percentage of seeds from the basal stems dropped below the middle and top branches after six

months despite having a higher initial percentage. On the other hand, electrical conductivity and mean germination time of seeds from the basal branches showed a significant increase after six months compared to the middle and top branches. Results suggest that the quality of cotton seeds collected from the basal branches is high compared to the middle and top branches. However, when subjected to storage, the seeds showed higher deteriorative changes in comparison to the seeds obtained from the middle and top branches. This might be related to duration of seed development and the increased solute leakage following imbibition which is usually accompanied by inevitable exit of materials necessary for germination and normal seedling growth.

Keywords: *Cotton seeds· Boll position· Storage period· Seed quality*

Introduction

Cotton (*Gossypium spp*.) is considered as the 'white gold'and king of fibre crops (Mayee and Rao, 2002). In Kenya, cotton accounts for over 50% of fibre that is used in the textile industries and has the potential to generate other micro-enterprise activities besides offering employment opportunities to women and youth in the cotton industries (G.O.K, 1999). The industry started to decline in the 1990s due to multiple problems among them unavailability of good quality seed.

Poor seed handling techniques by the farmers and ginneries in Western Kenya has contributed to the loss of quality of cotton seed. Seed possesses maximum germination ability and highest vigour when it is at its maximum dry weight, a stage known as physiological maturity in most crops (TeKrony & Egli, 1997),

however, like any other form of life, they cannot retain this identity indefinitely. Seed is seldom planted immediately after harvesting; it is processed and stored for a certain period of time before sowing. After harvest, seeds start deteriorating, moving inexorably towards death (Gregg *et al.,* 1994). Most farmers and ginneries during harvesting and processing do not take into consideration the requirements that maintain seed quality because the seed is considered as a secondary or by-product and the lint as the primary product. Hence the whole process from harvesting, storage of un-ginned cotton, ginning and storage of seed after ginning is geared towards production of quality lint and not quality seed. The losses are exacerbated if seeds are stored at high temperature and/or high relative humidity conditions (Trawatha et al., 1995).

Cotton seed is one of the most sensitive agronomic seeds where significant deterioration occurs after a short period of storage. Complaints of low vigour and germination have been reported when farmers plant seeds that are stored without protection from fluctuations in temperature and relative humidity (Nyongesa and Johnson, 1990). Numerous studies have documented yield reductions when poor quality seed is planted and the reductions in yield is attributed to inadequate stand establishment and is directly connected to seedling germination and emergence (Barradas and Lopez-Bellido, 2007). Seeds usually lose their germinability during period of prolonged storage (Gidrol et al.1989). Seed deterioration involves many biochemical and physiological changes which include loss of enzymatic activities, genetic alterations and membrane intergrity, although the exact causal effect of viability loss is still not clear (Sung et al. 1995).

During deterioration, vigour is the first component of seed quality, which is lost; this is followed by loss of germination capacity and viability (Trawatha *et al.,* 1995). Delayed emergence, increased seedling disease, and reduced stands are documented effects of

low quality of cotton seed (Bourland *et al.,* 1988). The loss in cotton seed quality during storage is related to many factors which include the production conditions in the field, pests, diseases, seed oil content, seed moisture content, processing, packaging and temperature and relative humidity of the storage environment (Simic et al. 2004; Guberic et al. 2003). Longevity of seed in storage is influenced by the initial seed quality and the storage environment which only serves to accelerate the rate of deterioration. Soluble carbohydrates generally decline with seed aging (Petruzelli and Toranto 1989) and this decline may result in reduced respiratory substrates needed during germination (Sharma et al. 2005). The lipid related changes in oil seeds during storage are responsible for the decline in seed vigour (Priestly and Leopold 1983).

Cotton seeds in bolls borne on different positions on the plant from the ground to the top part of the plant are subjected to positional effects (Bennet et al. 2003). Oil content and fatty acid composition vary between positions of bolls on the stem axis (Guleria et al. 2007, 2008). Seeds that develop in the upper one third part of the plant contain a higher concentration of protein and lower oil content and vice versa for the seeds borne on the lower one third of the plant (Esclante and Wilcox 1993) This difference in the protein and oil content at the different positions on the plant has been described to be due to variation occurring in nutrients and assimilates supply and other factors at each position. Seed filling, as influenced by the position of seed on the plant influences the germination potential of the seed (Sharma et al. 2009). The biochemical changes that take place during storage of seed harvested from different positions on the plant are expected to account for the loss in viability. The information obtained will be useful to the cotton farmers in Kenya to plan the harvesting, sorting and storage of cotton for seed purposes.

Materials and Methods

Field experiment

The field trial was conducted at the experimental fields of the Agricultural Training Center (ATC) in Busia county, Western Kenya. The fields for a long time were used for demonstration of good agricultural practices for crops grown within the region. The experimental site, geographically lies at an altitude of 1212m above sea level and is located at N- 0° 27' 307'' and E- 034° 06' 900". The soil types can be classified as chromic and orthic acrisols and ferralic cambisols (Enserink 1985). The county experiences a bimodal rainfall pattern with an annual rainfall of 1200-1800mm. Long rains fall from March to June and the short rains from September to December. With emerging unreliable rainfall patterns due to climate change, this pattern can be un-predictable.

Cotton seeds

Cotton seeds of variety KSA 81 M used in the study were obtained from Cotton Development Authority (CODA) at the Bungoma Sub station. The seeds were planted and the crop raised in the field following the recommended package of practices. Pests and diseases were controlled throughout the growth period of the crop. Mature balls that were fully open were harvested individually from each section of the plant (Basal=B, Middle=M and Top=T), pooled and stored in paper (khaki) bags for further analysis. The seeds were hand ginned and dried to a water content of 10% then seed quality tests were done.

Seed storage

A sample was drawn from the three plant positions and placed in paper bags and stored at room temperature. Samples were drawn at 0, 1, 2, 3, 4, 5 and 6 months of storage, in quadruplicate and subjected to seed quality tests.

Germination

Seeds were germinated in sterilized sand according to ISTA (1996) rules. The only modification was the use of 50 seeds instead of 100 seeds per replication for each treatment. Moistened sand was placed in germination trays and the seed sparsely spaced and then buried with a thin layer of sand. The trays were kept in a growth chamber maintained at a temperature of 25°C and relative humidity of 70%. The growth medium was moistened regularly until seedling evaluation was done. After 12 days, normal and abnormal seedlings and dead seeds were screened and assessed according to ISTA (2005) rules.

Electrical conductivity

The electrical conductivity test was carried out according to the method of Hampton and Tekrony (1995). 20 seeds were weighed and placed in 200ml of distilled water in plastic jars. The jars were then covered with aluminum foil and allowed to stand at room temperature for 24 hours. The electrical conductity of the seed leachetes was then measured using an EC meter, type Fielblab- Lf and LF 513T-electrode dip-type cell (Schott Gerate Glass Company, Mainz, Germany). The electrical conductivity of the seed leachates was expressed per gram of the seed weight as µS cm^{-1} g^{-1} for each sample.

Mean germination time

Seeds were germinated between moistened whatman filter papers, according to ISTA (2008) rules except that 25 seeds per replication were used instead of 100 seeds per replication for each treatment. The seeds in trays were then placed in the growth chamber (25°C and 70% RH) for 12 days. The seeds were considered as germinated when the radicle length was approximately 3 mm. Each day counts were made and the mean

germination time (MGT) was calculated for each lot using the formula cited by Ellis and Roberts (1980) given below:

$$MGT = \frac{\sum(nT)}{\sum n}$$

where,

n = number of seeds newly germinated (3 mm, radicle emergence) at time T

T = days from the beginning of the germination test,

$\sum = n$final germination.

Results

Germination

Cotton seeds harvested from the basal, middle and top branches showed a significant difference in percent germination as revealed by ANOVA, $F(2,63) = 4.896$, $P=0.011$and when the seeds were subjected to storage for six months, ANOVA revealed an effect of $F(6,63) = 30.064$, $P=0.000$. Post hoc LSD test showed that the quality of seeds from the basal and middle branches differed significantly at $p<0.05$ from the seeds obtained from the top branches (Table1). The ANOVA on the effect of harvest position on seed quality during storage also revealed $F(12,63) = 2.401$, $P=0.013$ an indication that seed quality during storage was affected by the position of seed on the plant. Fig. 1 indicates that there is a general reduction in germination of seeds harvested from the three harvest points with storage time. However, the reduction from the seeds from the basal branches was higher and by the end of six months the percent germination had dropped below those of the middle and top branches despite having a higher initial percent germination.

Table 1: Analysis of variance for germination for the seeds harvested from the basal, middle and top branches

Harvest level	Germination
Basal	46.75a
Middle	46.71a
Top	48.43b

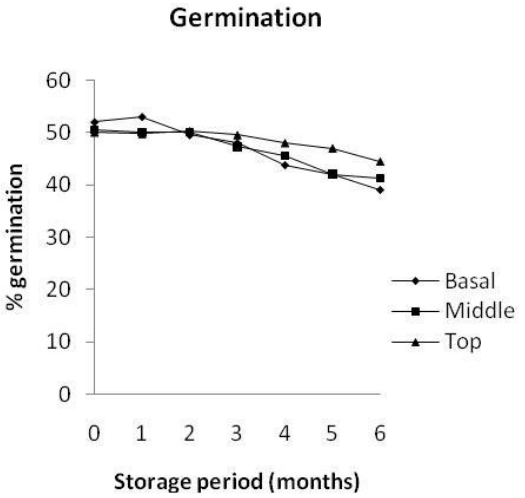

Fig.1: Germination trends of seeds from different branch positions over time

Electrical conductivity

The electrical conductivity of the seeds harvested from the different harvest point differed significantly as revealed by ANOVA of $F(2,63) = 27.443$, $P=0.000$ and during the six month storage period the ANOVA was $F(6,63) = 39.193$, $P=0.000$. Post hoc LSD test showed that there was a significant difference at

P<0.05 between the seeds form the seeds harvested from the basal branches compared those obtained from both the middle and top branches (Table 2).

The interaction effect between the harvest level and the harvest point also showed a significant difference as revealed by ANOVA of $F(12,63) = 4.117$, $P=0.000$.

Table 2: Analysis of variance for electrical conductivity for the seeds harvested from the basal, middle and top branches

Harvest level	Electrical conductivity
Basal	0.827a
Middle	0.709b
Top	0.680b

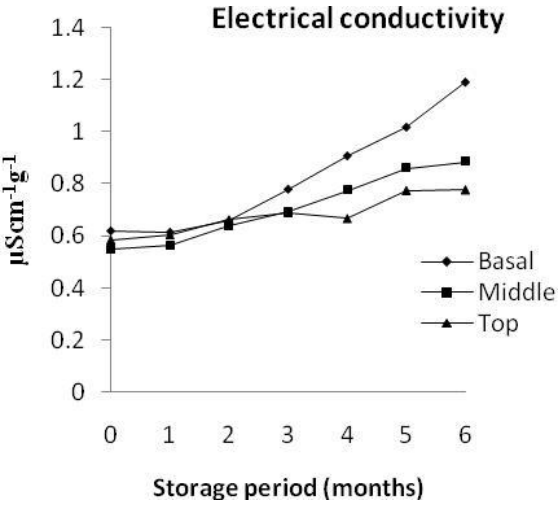

Fig 2: Electrical conductivity of seeds from different branch positions stored over time

There was a general increase of seed leachets with storage time from the seeds harvested from the different points on the plant (Fig. 2). However, the increase from the seeds obtained from the lower branches was higher compared to the middle and top branches whose increase did not differ significantly by the end of the storage period.

Mean germination time

The analysis of variance on mean germination time for the seeds obtained from the different harvest positions revealed $F(2,63) = 13.192$, $P=0.000$ and the storage period as $F(6,63) = 19.400$, $P=0.000$ indicating that both the harvest position and the storage duration had an effect on the quality of seed. The interaction effect of harvest level and storage duration showed a significant difference as revealed by ANOVA test, $F(12,63) = 9.735$, $P=0.000$. When post hoc test was done, results indicated that there was a significant difference at $P<0.05$ between the seeds harvested from both the basal and middle branches compared to the seeds from the top branches (Table 3).

During the storage time, there was a general increase in time taken by the seeds to germinate from the three harvest points; however the increase was higher from the seeds harvested from both the lower and middle branches compared to the top branches (Fig. 3).

Table 3: Analysis of variance for mean germination time for the seeds harvested from the basal, middle and top branches

Harvest level	Mean germination time
Basal	5.55a
Middle	5.35a
Top	4.95b

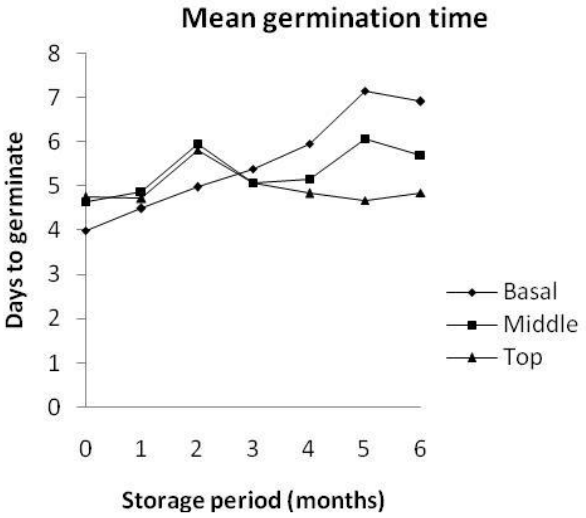

Fig.3: Days to germinate by seeds from different plant positions during storage.

Discussion

Cotton seeds harvested from the basal branches showed a higher initial germination percentage compared to the seeds from the middle and top branches and this might be due to differences in the mobilization of assimilates within the plant. The apical portion of the plant is exposed more to photosynthesis compared to the basal branches (Guleria et al 2007). This differential effect could have resulted in the accumulation of different amounts of seed storage reserves (protein and lipids) which are essential during germination and initial seedling growth. Studies by Sharma et al. 2013, on soybean found that lipid content in mature seeds was higher at the basal position and it decreased towards the apical position, whereas protein content showed the reverse. Collins and Cartter (1956) have shown that seeds developing in the upper one-fourth portion of the plant contained higher concentration of

proteins and lower concentration of oil than from the lower one-fourth of the plant. Variability in protein and oil content existed among the different positions on the plant (Escalante and Wilcox 1993; Bennet et al. 2003; Guleria et al. 2007) and these differences are attributed to environmental factors (Wolf et al. 1982; Maestri et al. 1998). The seeds from the top branches probably were still synthesizing storage components in comparison to the seeds located at the basal branches as observed in Indian mustard inflorescence (Munshi and Kumari 1994) and soybean (Guleira et al. 2007). This implied that the seeds were comparatively immature and synthesis of storage compounds hampered due to premature senescence of seeds in the top branches. The seeds on the basal branches acquired longer duration of seed development and optimum photoperiod to synthesize seed storage compounds compared to the seeds located at the top branches.

Reduced seed germination following seed ageing might have resulted from the increased solute leakage following imbibition which is usually accompanied by inevitable exit of materials necessary for germination and normal seedling growth. Fig 2 shows that the electrical conductivity of seed leachetes increased with time in all the cases indicating that seed vigour reduced with storage time. The increased seed leakage is believed to be associated with ageing induced changes in the cellular membranes of imbibed seeds. Increased electrolyte leakage with ageing confirmed the inferior quality of aged seeds (Slddiqui et al. 2008). Many studies have shown that peroxide changes in fatty acid composition of membranes lipids lead to massive dysfunction of cellular membranes associated with increased viscosity and permeability of bilayers (priestly, 1986; Copland McDonald, 1995). Changes in the composition of membrane lipids therefore could account for the increase in solute leakage (Sung, 1996).

The vigour of seeds from the different harvest positions as shown in fig. 3 differed significantly after storage for six months and this could be explained by the fact that there was more leakage of seed

storage components (Fig 2) from the seeds obtained from the basal branches compared to the top branches. The seeds from the basal branches, because of the longer deposition period could have more storage lipids than the seed from the top branches. Lipids are responsible for degradation of the cellular membranes which in turn leads to more leakage of the seed solutes that are primary compounds during germination.

This study revealed that seeds harvested from the basal branches have high viability and vigour compared to the seeds from the top branches; however they exhibit a rapid decline in quality with storage time. Studies to evaluate biochemical changes that lead to these variations in seed quality loss are ongoing.

References

Barradas, G. and R. J. Lopez-Bellido, 2007. Seed weight, seed vigour index and field emergence of six upland cotton cultivars. *J. Food. Agric. Environ*. 5: 116-121

Bennet J O, Krishman A H, Weidbold W J, Krishman H B (2003). Positiona effect on protein and oil content and composition of soybeans. *J Agric Food Chem* 51:6882-6886

Bourland F M, S M Furbeck and G Kaiser (1988). Effects of seed deterioration on cotton seed development in uniform plant stands. *Proc. Beltwide Cotton Prod. Res. Conf,*. p. 108, National Cotton Council of America, Memphis, TN,

Collins F I and cartter J L (1956). Variability in chemical composition of seeds from different positions of the soybean plant. *J Agron* 4:216-219

Copland L O, M B McDonald (1995). Principles of seed science and technology. Chapman and Hall, New York, USA.

Ellis, R.H. and Roberts, E.H.(1980) Improved equations for the prediction of seed longevity. *Annals of Botany* **45**, 13-30.

Enserink, H.J. (1985). Appendix to soils of Busia and Siaya Districts. In H.J. Enserink (Eds), Sorghum Agronomy Investigations in Kenya, using a Farming Systems Perspective. Wageningen: Netherlands Soil Survey Institute. http://library.wur.nl/ WebQuery/isric/17938. Accessed 15 October 2013

Escalante E E and Wilcox J R (1993). Variation in seed protein among nodes of normal and high protein soybean genotypes. *Crop Sci* 33:1164-1166

G.O.K (Government of Kenya), (1999). Sessional Paper N0.1 of 1999 on 'The Revitalization of the Cotton Industry'

Gidrol, X., Serghini, H., Noubhani, A., Mocoquot, B. and Mazliak, P., 1989. Biochemical changes

Gregg, B., S.A.E. Wanis, Z. Bishaw and A.J.G. van Gastel, 1994. Safe Seed Storage. WANA Seed Network Publication No. 5/94. ICARDA, Aleppo, Syria.

Guberac V, Maric S, Lalic A, Drezner G, Zdunic Z. Hermetically sealed storage of cereal seeds and its influence on vigor and germination. *J Agron Crop Sci.* 2003;189:54–56. doi: 10.1046/j.1439-037X.2003.00596.x.

Guleria S, Sharma S, Gill BS, Munshi SK. Distribution and biochemical composition of large and small seeds of soybean (*Glycine max* L.) *J Sci Food Agric.* 2008; 88:269–272.

Guleria S, Sharma S, Munshi SK. Compositional changes in soybean seeds influenced by their positions on stem axis. *J Food Sci Technol*. 2007; 44:607–610.

Hampton, J.G.; Tekrony, D.M. Handbook of vigour test methods. 3rd ed. Zürich: Ista, 1995. 117p.

Induced by accelerated aging in sunflower seeds. I. Lipid peroxidation and membrane

ISTA (International Seed testing Association) 1996. International rules for seed testing (1996). *Seed Science and Technology 24* (Suppl): 1996.

ISTA (International Seed Testing Association) 2005. International rules for seed testing edition 2005. International Seed testing Association (ISTA), Basserdorf CH-Switzerland.

ISTA. International Rules for Seed Testing. Ed. 2008/1, Pub. The ISTA, CH – 8303, Bassersdorf Switzerland.

Maestri DM, Laburkas DO, Meriles JM, Lamarque AL, Zygadlo JA, Guzman CA. Seed composition of soybean cultivars in different environmental regions. *J Sci Food Agric.* 1998;77:494–498.

Mayee, C.D. and M.R.K. Rao, 2002. Current Cotton Production and Protection Scenario including GM Cotton, Crop report Cotton Agro look. Apr-June: 14-16.

Munshi SK, Kumari A. Physical characteristics of siliqua and lipid composition of seeds located at different positions in mature mustard inflorescence. *J Sci Food Agric*. 1994;64:289–293.

Nyongesa, T. E. and Jonson, S. (1990). The acceptability of new seed varieties: report of a survey to determine farmers response to new varieties. EMI projects, Embu, Kenya.

Petruzelli L, Taranto G. Wheat ageing: the contribution of embryonic and non-embryonic lesions to loss of seed viability. *Physiol Plant.* 1989;76:289–294.

Priestley DA, Leopold AC. Lipid changes during natural ageing of soybean seeds. *Physiol Plant*. 1983;59:467–470.

Priestly D A (1986). Seed ageing. Comstock Publishing Associates Itheca, New York, pp 137-55

Sharma S, Kaur A, Bansal A and Gill B S. (2013). Positional effects on soybean seed composition during storage. *J Food Sci Technol*.50(2): 353-359.

Sharma S, Kaur A, Sital JS. Effect of storage on germinability and composition of seeds from different positions on soybean stem axis. *Ind J Agric Biochem*. 2009;22:94–97

Sharma S, Virdi P, Gambhir S, Munshi SK. Changes in soluble sugar content and antioxidant enzymes in soybean seeds stored under different storage conditions. *Ind J Agric Biochem*. 2005;18:9–12.

Simic B, Popovic S, Tucak M. Influence of corn bred lines seed processing on their damage. *Plant Soil Environ*. 2004;50:157–161.

Slddiqui S U, Ali A, Chaudhary A M (2008). Germination behavior of wheat (Triticum aestivum) varieties to artificial ageing under varying temperatures and humidity. *Pak J Bot* 40(3):1121-1127

Sung J M (1996). Lipid peroxidation and peroxidase scavenging in soybean seedsduring ageing. *Physiol Plant* 97:85-9

Sung JM, Chiu CC. Lipid peroxidation and peroxide scavenging enzymes of naturally aged edible soybean seed. *Plant Sci.* 1995;110:45–52.

Tekrony D M and D B Egli, (1997). Relationship between standard germination, accelerated ageing and field emergence in soybean. *Basic and Applied Aspects of seed Biology*, pp 593-600. Kluwer Academic.

Trawatha S E, D M Tekrony, D F Hildebrand (1995). Relationship of soybean quality to fatty acid and C6-aldehyde levels during storage. *Crop Sci* 35:115-42

Wolf RB, Cavins JF, Kleiman R, Black LT. Effect of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acid and sugars. *J Am Oil Chem Soc*. 1982;59:230–232.