African Crop Science Journal, Vol. 28, No. 1, pp. 11 - 22
Printed in Uganda.
ISSN 1021-9730/2020 $4.00

DOI: https://dx.doi.org/10.4314/acsj.v28i1.2

BOLL POSITIONING AND SEED AGEING EFFECTS ON SEED QUALITY OF COTTON IN BUSIA COUNTY, KENYA

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(*Received 12 February, 2018; accepted 18 February, 2020*)

ABSTRACT

Cotton (*Gossypium hirsutum*) seed is an agricultural product which is prone to deterioration during storage. The objective of this study was to determine the effect of boll position on seed the plant and storage period on seed quality of cotton seed. Cotton variety KSA 81M was planted at Busia Agricultural Training Centre (ATC) and bolls were harvested from the basal, middle and top branches. Seeds were stored and subsequently tested to determine seed quality. In general, germination percentage of the seeds declined during the storage period; while electrical conductivity and mean germination time increased. Remarkably, germinative capacity of seeds originating from the basal stems dropped below the germinative capacity of seeds from the middle and top branches, after six months despite having a higher initial germination 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. Our results suggest that the quality of cotton seeds from the basal branches at harvest is higher than the quality of seeds from the middle and top branches. However, when subjected to storage, the seeds from the basal branches show higher deteriorative changes than those obtained from the middle and top branches. This might be related to duration of seed development and increased solute leakage following imbibition, which is usually accompanied by inevitable leakage of metabolites necessary for germination and normal seedling growth.

Key Words: Cotton seeds, germination, *Gossypium hirsutum*

RÉSUMÉ

La graine de coton (*Gossypium hirsutum*) est un produit agricole susceptible de se détériorer pendant le stockage. L'objectif de cette étude était de déterminer l'effet de la position de la capsule sur les

graines de la plante et la période de stockage sur la qualité des graines de coton. La variété de coton KSA 81M a été plantée au Centre de Formation Agricole de Busia (ATC) et les capsules ont été récoltées dans les branches basale, centrale et supérieure. Les graines ont été stockées puis testées pour déterminer la qualité des graines. En général, le pourcentage de germination des graines a diminué pendant la période de stockage; tandis que la conductivité électrique et le temps moyen de germination augmentaient. Remarquablement, la capacité germinative des graines provenant des tiges basales est tombée en dessous de la capacité germinative des graines des branches médianes et supérieures, après six mois malgré un pourcentage de germination initial plus élevé. En revanche, la conductivité électrique et le temps moyen de germination des graines des branches basales ont montré une augmentation significative après six mois par rapport aux branches moyennes et supérieures. Nos résultats suggèrent que la qualité des graines de coton des branches basales à la récolte est supérieure à la qualité des graines des branches médianes et supérieures. Cependant, lorsqu'elles sont soumises au stockage, les graines des branches basales présentent des changements de détérioration plus élevés que ceux obtenus des branches moyenne et supérieure. Cela pourrait être lié à la durée du développement des graines et à l'augmentation des fuites de soluté après l'imbibition, qui s'accompagne généralement d'une fuite inévitable des métabolites nécessaires à la germination et à la croissance normale des semis.

Mots Clés: Graines de coton, germination, *Gossypium hirsutum*

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 fiber that is used in the textile industry, and has the potential to generate other micro-enterprise activities. In addition, the crop offers employment opportunities to women and youth in the cotton industries (G.O.K, 1999). Despite the sub-sector's decline in recent years, cotton is still considered one of the few cash crops with real potential for increasing employment opportunities and food security, through income generation in the arid and semi-arid lands (ASALs) of Kenya (CODA, 2011). According to Kenya's Vision 2030 Strategic Plan, and its Medium Term Plan, 2008-2012 (GOK, 2008), revitalising the cotton sector is one of the government's key developmental initiatives to trigger industrial revival in the ASAL regions, and also in areas with high potential for cotton growing.

In the report by the World Bank (2005) and CODA (2008), some of the key factors responsible for the cotton sector's poor performance in the past, and to some extent today, include periodic drought, volatile producer prices, delayed payments to farmers, lack of access to quality seeds, high cost of pesticides, competition with other farm enterprises over scarce resources, collapse of co-operative societies and former state-owned textile firms, and competition from synthetic fiber substitutes and cheap imports of new and second-hand clothes.

Poor seed handling techniques by the farmers and ginneries in Kenya has contributed to the loss of quality of cotton seed. Most farmers and ginneries are not aware of the processing requirements that maintain seed quality, because the seed is considered as a secondary or by-product; and 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 when seeds are stored at high temperatures and/or high relative humidity conditions (Trawatha *et al.,* 1995).

Seed attains maximum germination ability and highest vigour when it is at its maximum dry weight, a stage known as physiological

maturity in most crops (TeKrony and Egli, 1997). However, seeds are seldom planted immediately after harvesting. Indeed, seed is processed and stored for a certain period of time before sowing, and during this period seeds start deteriorating, moving inexorably towards death (Gregg *et al.,* 1994).

Low vigour and germination have been reported when farmers plant seeds which 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 reduction 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 germination capacity during periods 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 integrity; although the exact causal effect of viability loss is still not clear (Sung *et al.* 1995).

Among the factors affecting seed quality during storage; the initial quality of the seed lot; environment for conservation (with its variations on temperature, moisture, oxygen availability; and the packaging), as well as characteristics inherent to the species, should be taken into account. The type of packaging during storage assumes relevant importance on seed quality, since packaging helps in lessening the speed of deterioration by maintaining the initial moisture content of seeds stored, and by lowering their respiration rate (Tonin and Perez, 2006).

The peroxidation of lipids may be the most frequent cause of deterioration and loss of viability of seeds, since it is a factor that leads to reduction in content of lipids in seeds during the storage procedure (Silva *et al*., 2011). Many times, such a factor is activated by the action of oxygen on a given polyunsaturated fatty acid, which is present in the membranes of seeds. In addition, in the process of seed deterioration, the increase in peroxidation of lipids results in damages to the cell membrane, and consequent generation of toxic sub-products (Schwember and Bradford, 2010). Enzymatic changes may seem to also be useful in studies on seed deterioration. Thus, the decrease in antioxidant enzymes is linked to increase on peroxidation of lipids, as well as to accelerated aging process, with a positive correlation between antioxidant capacity of the enzyme and the vigour of seeds (Bailly *et al.,* 2002).

Cotton seeds are rich in oil (25 to 40%), and require special care during storage so that their quality is maintained (Passos, 1977; Medeiros-Filho *et al.,* 1996). Such seeds, which are classified as aleuro-oleaginous, have a lower storage potential than the amylaceous seeds due to the low chemical stability of the lipids in relation to starch, since a moderate elevation of the temperature is sufficient for the decomposition of the lipids and increase on deterioration rate as consequence of the respiration process.

The content of oil in seeds may vary according to the position of the seed on the plant, and the storage conditions, especially temperature and relative humidity (Koutroubas *et al.*, 2000); and such variations directly influence the integrity of the oil during storage.

Cotton seeds in bolls borne on different positions on the plant from the ground to the top part 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 and the 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 (Esclante and Wilcox. 1993). Seed filling, as influenced

by the position of seed on the plant, influences the germination potential of the seed due to variation occurring in nutrients and assimilates supply and other factors at each position (Sharma *et al.*, 2009). The biochemical changes which take place during storage of seed harvested from different positions on the stem axis are expected to account for the loss in viability. The objective of the study was to define the ideal harvest position on the stem axis and evaluate the storage period for the seeds obtained from the different levels on the plant.

MATERIALS AND METHODS

A field study was conducted at the experimental fields of the Agricultural Training Centre (ATC) in Busia County, Western Kenya. The experimental site lies at an altitude of 1212 m above sea level, and at N- 0° 27' 307'' and E- 034° 06' 900''. The soil types are Chromic and Orthic Acrisols and Ferralic Cambisols (Enserink, 1985).

The region experiences a bimodal rainfall pattern, with an annual rainfall of 1200-1800 mm. 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 is increasingly un-predictable. Current weather trends show that the first rains of the long wet season have become unreliable and on average significantly reduced. The first rains are sometimes insufficient to support a harvest, especially in the east of the county. While the average number of rainy days during the short wet season has reduced from 60 to 30, rainfall has become more intense and the season is being prolonged into January and February, leading to higher total rainfall for this season. On a broader scale, the area of west-central Kenya receiving 500 mm of rain or more has shrunk since 1960 and is likely to keep shrinking over the next 30 years (UNEP, GEAS, 2011). Mean annual temperatures have increased by 1 ºC since 1960, equal to an average increase of 0.2 ºC per decade (Parry *et al*., 2012)

Cotton seeds. Cotton seeds of variety KSA 81M used in the study were obtained from Cotton Development Authority (CODA) at the Bungoma substation. The seeds were planted and the crop raised following the recommended agronomic practices for cotton growing in Kenya as per the farm management hand book for Kenya (MOA, 1983). Planting holes spaced 40 cm apart, were dug with a hand hoe, within the rows spaced 70 cm. A minimum of six seeds was applied per hill at a planting depth of 3 -5 cm.

After germination, the plants were thinned to two seedlings per hole. After two months, the plants were top-dressed with calcium ammonium nitrate (CAN) fertiliser. Weeds were controlled through regular hand-hoeing. Insect and disease monitoring took place throughout the growth period, and sprays using Cypermetrin and Mancozeb were applied whenever pests and diseases were noticed.

Mature balls that were fully open were harvested separately from each section of the stem axis (Basal=B(1st 4 branches on the lower stem axis), Middle=M(2nd 4 branches on the middle of stem axis) and Top=T (3rd 4 branches on the top the stem axis), pooled and kept in khaki paper bags for further analysis. The seeds were hand ginned and dried on a bench in a greenhouse to a moisture content of 10% as tested by a moisture meter, then stored in a cold room with temperatures of between 5-10 ºC for further tests.

Seed storage. A seed sample was randomly picked from each of the three bags holding seeds from the three plant positions (basal, middle and top). They were 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 sterilised sand, according to ISTA (1996). The only modification was the use of 50 seeds per germination tray, instead of 100 seeds because the trays could not hold 100 seeds when sparsely spaced. There were 3 replications per treatment. The seeds were then buried with a thin layer of sand and the trays kept in a growth chamber, maintained at a temperature of 25 °C and relative humidity of 70%. The growth medium was moistened with distilled water regularly, until seedling evaluation for germination percentage was done. After 12 days, normal and abnormal seedlings and dead seeds were screened and assessed for % germination according to ISTA (2005).

Electrical conductivity. The electrical conductivity test was carried out to assess seed vigour, was done according to the method of Hampton and Tekrony (1995). Twenty seeds were weighed and placed in 200 ml of distilled water in plastic jars. The jars were covered with aluminum foil and allowed to stand at room temperature for 24 hours. The electrical conductivity of the seed leachates 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 gramme of the seed weight as $\mu S\ cm^{-1}\ g^{-1}$ for each sample.

Speed of germination. Seeds were germinated between moistened Whatman filter papers, according to ISTA Rules (2008). The seeds in trays were then placed in a growth chamber (25°C and 70% RH) for 12 days. On a daily basis, the number of seeds that had germinated was counted. Seeds were considered as having germinated when the radicle length exceeded 3 mm (Tobe *et al.,* 2005). Let g_d be the number of seeds that are considered as having germinated *d* days after sowing. The mean germination time (MGT) is computed as follows:

$$MGT = \frac{1}{n}\sum_{d=1}^{12} d \times g_d$$

Where:

n = the number of seeds that had germinated at the end (day 12) of the experiment (Khan *et al.,* 2003; Babiker *et al.,* 2010).

Statistical analysis. The data collected were analysed using three general linear models at 95% level of significance. The factor position, and the covariate storage time were included as independent variables, as well as the interaction term between position and storage time. The germination rate, MGT and electrical conductivity were tested parameters. Diagnostic plots were made to assess the quality of the model fit, normality and homoscedasticity of the residuals.

RESULTS

Germination rate. Figure. 1 presents values for germination rate as a function of storage time for the different positions on the plants. It is evident that the germination rate linearly decreased with storage time. The effect of storage time on the germination rate became more pronounced for the seeds harvested from the lower branches on the stem axis, and decreases towards the middle and upper branches. The general linear model analysis showed a strongly significant interaction effect ($F(2,78) = 11.690$, $p = 0.000$) between position of seeds on the plant axia and storage time. i.e., the slopes of the regression lines in Figures 1 (a)-(c) differed significantly.

Table 1 shows the coefficients for the individual regression models. From this Table, it can be inferred that germination rate decreased as a function (with statistical significance for each height) of storage time, and that the decrease was strongest in basal

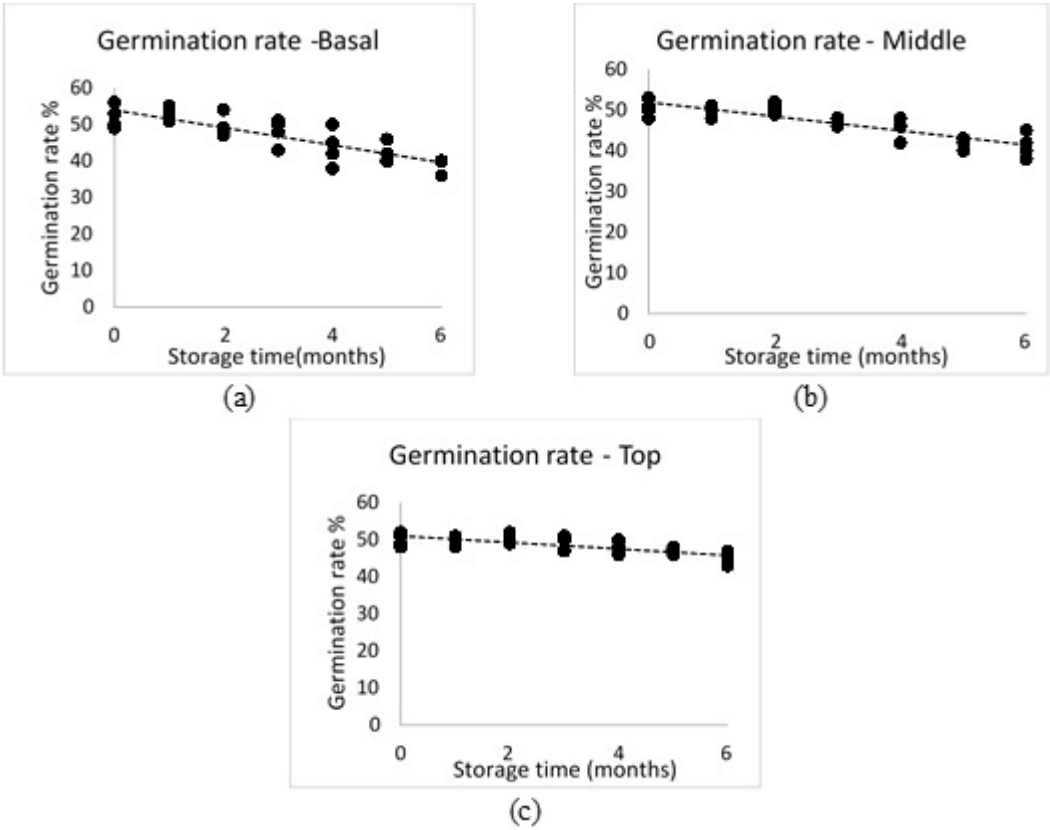

Figure 1. Influence of storage time on germination rate for cotton seeds harvested from basal (a), middle (b) and top (c) plant positions.

TABLE 1. Regression coefficients for the effect of storage time on germination rate of cotton seeds, standard deviations and confidence intervals are computed based on the joint general linear model

Effect	Coeff.	Std.Error	95% C.I.	t-value	P-value
Position: basal					
Intercept	53.902	.803	[52.302,55.501]	67.088	0.001
Effect of storage time	-2.384	.223	[-2.828,-1.940]	-10.698	0.001
Position: middle					
Intercept	51.938	.803	[50.338,53.537]	64.643	0.001
Effect of storage time	-1.741	.223	[-2.185,11.297]	-7.813	0.001
Position: top					
Intercept	51.027	.803	[49.427,52.626]	63.510	0.001
Effect of storage time	-.866	.223	[-1.310,-.422]	-3.887	0.001

seeds and weakest in top seeds; middle seeds; had an intermediate value.

A closer inspection of the data reveals that germination rate directly after harvesting was 52, 50.5 and 50%, for basal, middle and top seeds, respectively. By the end of six months after storage, the germination rate of the basal seeds (39%) had dropped below those from the middle (41.25%) and top (44.5%) seeds, despite having higher initial germination rate.

Germination time. Figure 2 presents the germination time as a function of storage time, for the different seed positions on the plants. For this parameter, a general linear model analysis showed that the slope of the regression lines significantly differed between seed positions ($F(2,57) = 8.417$, $P = 0.000$). Table 2 shows the coefficients for the individual regression models, and it can be deduced that the mean germination time was a statistically significantly increasing function of storage time for positions basal and middle. The influence of storage time was not significant for seeds harvested from the top branches. The effect of storage time was most pronounced for basal seeds, and negligible for top seeds; middle seeds have an intermediate value.

Mean germination time directly after harvesting was 3.985, 4.642 and 4.763 days, respectively for basal, middle and top seeds, respectively. By the end of six months after storage, germination time of the basal seeds (6.903 days) had risen above those from the middle (5.703 days) and top (4.833 days) seeds

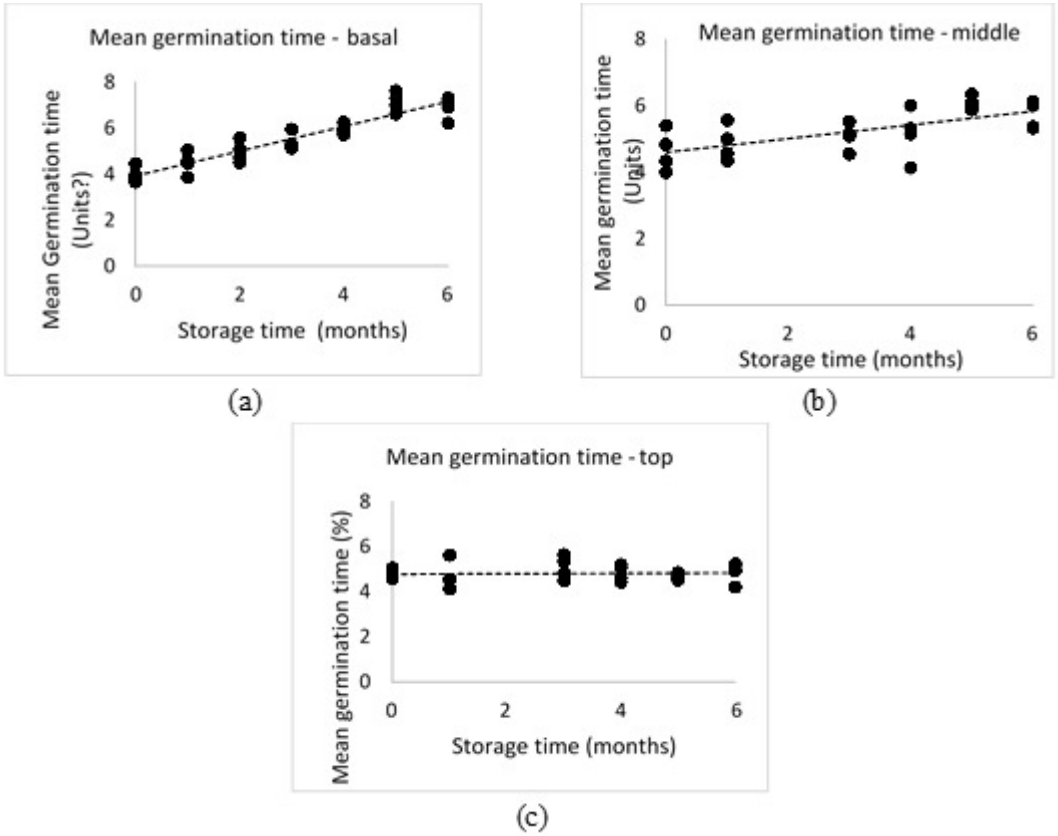

Figure 2. Influence of storage time on mean germination time for cotton seeds harvested from basal (a), middle (b) and top (c) plant positions.

TABLE 2. Regression coefficients for the effect of storage time on mean germination rate of cotton, standard deviations and confidence intervals are computed based on the joint general linear model

Effect	Coeff.	Std.Error	95% C.I.	t-value	P-value
Position: basal					
Intercept	3.935	0.157	[3.623,4.247]	25.136	0.001
Effect of storage time	0.537	0.043	[.450,.624]	12.368	0.001
Position: middle					
Intercept	4.606	0.169	[4.269,4.943]	27.274	0.001
Effect of storage time	0.204	0.044	[.116,.293]	4.605	0.001
Position: top					
Intercept	4.788	0.169	[4.451,5.125]	28.351	0.001
Effect of storage time	0.008	0.044	[0-.081,0.096]	0.178	0.860

despite having a lower initial mean germination time.

Electrical conductivity. Figure 3 presents electrical conductivity values of the seed leachates as a function of storage time for the different seed positions on plants. There was a general increase of electrical conductivity of the seed leaches, with storage time from the seeds harvested from the different points on the plant. The general linear model analysis showed that the slope of the regression lines significantly differed between seed positions, ($F(2,63) = 4.117$, $P = 0.000$). For all positions, the regression lines had a significantly positive slopes (Table 3), which was the highest in basal seeds and the lowest in top seeds.

Correlation of EC and germination rates. Overall, there was a strong, and negative correlation between germination rate with, electrical conductivity r = -0.774, n = 84, P = and mean germination time. Decreases in germination rate were correlated with increases in electrical conductivity and mean germination time.

The correlation between electrical conductivity 0.000 was r = -0.624, n = 84, P = 0.000 germination time was (r = -0.641, n = 84, P = 0.000).

DISCUSSION

Cotton seeds harvested from the basal branches showed a higher initial germination percentage to the seeds from the middle and top branches (Table 2). This differential effect could have resulted from the accumulation of different amounts of seed storage nutritional reserves (protein and lipids), which are essential during germination and initial seedling growth. The seeds from the top branches were probably still synthesizing storage components when compared with the seeds located at the basal branches, as observed in Indian mustard inflorescence (Munshi and Kumari, 1994) and soybean (Guleira *et al.*, 2007). Studies by Sharma *et al.* (2013), on soybean found that lipid content in mature seeds was higher at the basal position than at the apical position; whereas protein content showed the reverse. Collins and Cartter (1956) showed that seeds developing in the upper one-fourth portion of the stem axis plant contained higher concentration of proteins and lower concentrations 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 were attributed

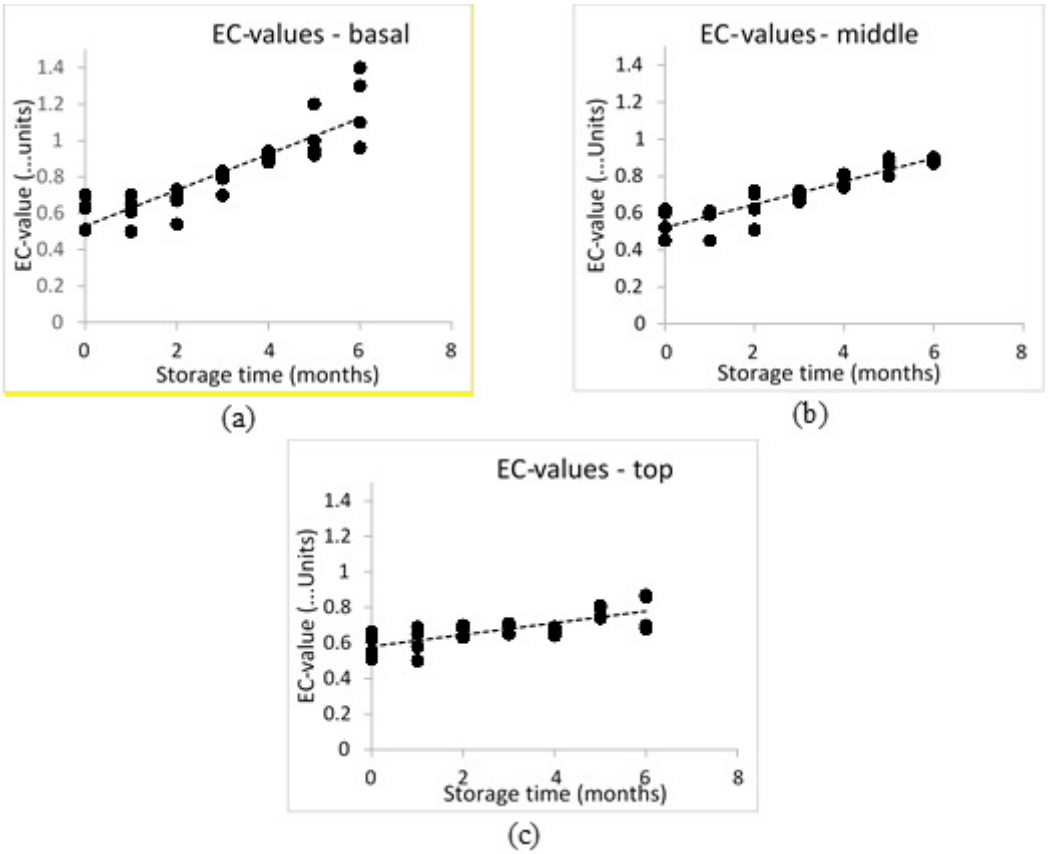

Figure 3. Influence of storage time on electrical conductivity for cotton seeds harvested from basal (a), middle (b) and top (c) plant positions.

TABLE 3. Regression coefficients for the effect of storage time on electrical conductivity of the cotton seed leachates, standard deviations and confidence intervals are computed based on the joint general linear model

Effect	Coeff.	Std.Error	95% C.I.	t-value	P-value
Position: basal					
Intercept	0.531	0.027	[0.477,0.584]	19.617	0.001
Effect of storage time	0.099	0.008	[0.084,0.114]	13.165	0.001
Position: middle					
Intercept	0.522	0.027	[0.468,0.575]	19.287	0.001
Effect of storage time	0.062…	0.008	[0.047,0.077	8.309	0.001
Position: top					
Intercept	0.582	0.027	[0.528,0.635]	21.502	0.001
Effect of storage time	0.033	0.008	[0.018,0.048]	4.392	0.001

to environmental factors such as light, water availability and temperature (Wolf *et al*., 1982; Maestri *et al.,* 1998). This implied that the seeds harvested from the branches located on the upper stem axis were comparatively immature and synthesis of storage compounds hampered due to premature senescence. The seeds on the basal branches were exposed to long durations of seed development and greater photoperiod to synthesize seed storage compounds compared to the seeds located at the top branches.

Reduced seed germination, following seed storage, 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. Figure 3 shows that the electrical conductivity of seed leachates 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 membrane of imbibed seeds (McDonald, 1999). 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).

Difference in vigour of seeds from harvest positions (Fig. 3) after storage for six months, 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 accumulated 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.

ACKNOWLEDGEMENT

The authors thank VLIR-UOS (Flemish Interuniversity's Council- University Development Co-operation) for funding this research work.

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