Nigerian Journal of Ecology (2003) 5:21-27

ISSN: 1116-753X

Effect of Moisture Content, Genotype and Storage Period on Seed Quality of Thiram-Treated Maize Seeds

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(Accepted 14 July 2003)

ABSTRACT: Changes in seed quality of five genotypes of maize seeds treated with Apron plus (Thiram) were monitored under three moisture levels (I= 9.3 -10.0%, II= 11.3 – 12.0%, and III= 15.7 – 19.3%) and short - term storage period in laboratory experiment for 16 weeks (112 days). Moisture content, genotype and storage period interacted to induce considerable variation in seed viability and seedling vigour. Viability of TZLCOMPICLIN was consistently higher than other genotypes during storage period, followed by TZLCOMP$_3$ C$_2$ and ACR97TZLCOMP$_4$ C$_2$. These three genotypes had viability above 80% at the end of storage which was 13% higher than the two other genotypes. Seedling vigour of TZLCOMPICLIN and TZLCOMP$_3$P$_2$ were consistent and similar during storage periods and followed the same pattern as in viability. Though the difference in seed quality at moisture levels I and II was not significant, seed subjected to moisture level III was significantly different from the other two, and recorded the lowest viability and seedling vigour which was less than 80% and 15.0 respectively; indicating adverse effect of high moisture content on seed storability and quality. Viability and seedling vigour of TZLCOMPICLIN and TZLCOMP$_3$C$_2$ were best at moisture levels I and II, followed by ACR97TZLCOMP$_4$C$_2$. Genotypes like TZLCOMPICLIN, TZLCOMP$_3$C$_2$ and ACR97TZL COMP$_4$C$_2$ can be used in crossing to enable breeders to develop improved genotypes with good storage

Key words: Moisture content, Seed quality, seed viability, seedling vigour, storage period, *Zea mays* L.

Introduction

Maize (*Zea mays* L.) is classified as orthodox seeds along with other cereals and grain legumes which can be safely dried to a low moisture content without any damage to the seed embryo (Ellis *et al.* 1990.). It retains viability if it is thoroughly dried as soon as it matures and viability is highly maintained under proper conditions of temperature and relative humidity.

Moreover, seed lots may react differently to similar conditions and stresses, reflecting differences in seed vigour. Seed quality may be defined as a standard of excellence in certain characters or attributes that will determine the performance of seed when sown or stored (Hampton, 2002). Seed vigour is defined by the International Seed Testing Association (ISTA, 1993) as " the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence" (Hampton and Tekrony, 1995).

Ageing during storage has been recognized as a major cause of declining seed vigour and viability (Tesmer, 2002). Ageing is accomplished by physiological and physical damage to cell membranes (Powell, 1984,1988) as well as by respiratory and hormonal changes, impaired RNA and protein synthesis and accumulation of toxic metabolites (Priestley, 1986). Such deterioration results in reduced speed of emergence and uniformity of germination, decreased tolerance to environmental stresses and arrested growth (Powel *et al.*, 1984 Hampton and Hill, 1990).

In previous reports, it has been observed that fungi play a crucial role in the loss of maize seed viability (Moreno and Christensen, 1970). One way to control these fungi is to keep the seeds under favourable conditions of moisture and temperature to prevent the growth of the fungi (Christensen and Sayer, 1982). Such an approach is not always possible, particularly in tropical areas, where the post-harvest technology is limited and weather conditions encourage the growth of fungi. Under such situation, fungicide application constitutes an alternative way to minimize the damage these fungi can cause to the stored seeds (Moreno and Ramivez, 1985, Moreno-Martinez *et al.*, 1994 Adebisi, 1999, Adebisi and Ajala, 2001). Since seeds are biological entities, the efficacy of these fungicides depends on the moisture content of the seeds after processing and storage. Besides, it is known that maize genotypes differ with respect to degree of seed viability loss during storage (Moreno *et al.*, 1992).

Based on the problem of varying seed moisture content relative to storability after seed treatment, the study will seek to discover what happens to crop seeds at various moisture contents as well as determine actual moisture content that will support its viability when treated seeds are unavoidably stored. Therefore, the following studies were carried out to obtain information pertaining to moisture level at which seed quality of treated maize seeds becomes threatened as well as determine storage period and moisture level suitable for treated and stored seeds of five maize genotypes.

Materials and Methods

Seed Source

Seeds of five maize genotypes (Viz. ACR97TZL COMP$_1$C$_2$, AK96 DMR-LSRW, AMA TZBR-WC TZLCOMPCL1-W and TZLCOMP$_3$C$_2$) were used for the study. The seeds were sourced from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state. The seeds were collected from 5°C seed store. The initial germination and moisture content tests were carried out according to ISTA (1993) and Justice and Bass (1978) respectively (Table 1).

Moisture Adjustment

A 2kg seed of each of the genotypes was divided into three, giving a total of 15 samples. The first part of the seed lot (5 samples) was subjected to 'decreased' moisture content by placing in an oven for 12 hours at temperature of 40°C. The next seed lot (5 samples) were subjected to an 'increased' moisture content by soaking between moisten paper towels in plastic containers for five hours (Herath, 1979). The last part of the samples was left as collected from the source and named 'Normal' moisture content seeds.

Determination of Moisture Content

A 5g seed sample from each genotype was ground and placed in an oven for 2 hours at 130°C. The percentage moisture content (Wet weight basis) was determined using the procedure outlined by Justice and Bass, (1978) (Table 1). The moisture content determination was replicated three times.

Chemical Treatment and Storage

Seeds with 'decreased', 'normal' and 'increased' moisture content of each genotype was treated with Thiram (Apron plus) at the recommended rate of 10g/kg maize seed. Seed and the chemical were agitated for thorough mixing and then kept in paper envelopes and replicated three times. The seeds were kept in the laboratory under ambient environmental conditions at 30-31°C for 16 weeks.

Seed Viability

Every fortnight, samples were drawn for seed viability using standard germination tests according to ISTA (1993). Three replicates of 100 seeds were wrapped

TABLE 1: Initial Quality of the Maize Seed Samples

	Germination	Moisture Content (%)		
		D	N	I
ACR97TZL COMP$_1$C$_2$	100	9.9	12.0	19.3
AK96DMR - LSRW	100	9.3	11.9	18.3
AMA TZBR - WC	95	9.7	11.3	17.5
TZL COMP$_1$CLIN	95	9.3	11.3	17.3
TZL COMP$_3$C$_2$	95	10.0	10.7	15.7

D = Decreased Moisture Content
N = Normal Moisture Content
I = Increased moisture Content

in moist paper towels and placed under ambient laboratory conditions. The first count was made on the fourth day and the final count on the seventh day. The result was expressed in percentage seed germination.

Seedling Vigour

Speed of germination index (SGI) was used to determine the seedling vigour (FAO, 1999). This was calculated according to Maguire (1962) thus:

$$SGI = \frac{a_1}{b_1} + \frac{a_2}{b_2}$$

Where a_1 and b_1 were number of seedlings germinated during the 1st count (4th day) and the second count (7th day) respectively while a_2 and b_2 were number of days before the 1st and 2nd counts respectively.
Seeds were considered to have germinated when a coleoptile was more than 2cm long and radicles were vividly visible.

Data Analysis

The experiment was a factorial in a completely randomized design with three replications. There were three factors: moisture, genotype and period of storage. Seed viability and vigour values were subjected to analysis of variance and their interactions were determined with the use of PROC GLM (SAS Institute Inc., 1990). The detection of differences between treatment means was calculated using Least Significant Difference (LSD) methods of multiple pair wise comparisons according to Wahua (1999).

Results

Analysis of Variance

Results in Table 2 shows that genotypes, storage period and moisture level were highly significant ($P < 0.01$) for the seed viability and seedling vigour evaluated.

TABLE 2: Summary of ANOVA of Seed Viability and Vigour of Treated Maize Genotypes

Sources of Variation	Mean Squares	
	Viability	Seedling Vigour
Genotype (G)	33.89**	32.82**
Storage period (S)	23.32**	26.10**
Moisture Level (M)	648.67**	598.07**
G X S	2.17*	1.76*
G X M	19.29**	17.57**
S X M	5.12**	5.16**
G X S X M	2.44*	2.25*

* Significant at 5% level of probability
** Significant at 1% level of probability

Similarly: the interaction effect between genotype and moisture level and that between moisture level and storage period were highly significant (P< 0.01).

Main effects of genotypes, moisture levels and storage periods.
The differences among the genotypes were statistically significant at P< 0.05 for the two traits (Table 3). AMATZBRWC$_1$ and AK96-DMR-LSRW had reduced viability of 84% and 83% respectively, corresponding to the least seedling vigour values of 16.0 and 15.7. From Table 3, seed viability also varied with the storage periods, with values ranging between 90 to 89% at 6 weeks of storage and thereafter significantly declined to between 84% and 81% at 16WAS. Similarly, an anomalous increase in seedling vigour was recorded from 2 WAS to 6WAS and thereafter declined progressively to 15.8 at 16 WAS.
Data in Table 3 also shows that statistical differences were observed among the moisture level with respect to the two traits evaluated. Seeds subjected to lower moisture levels (I and II) had the highest viability of 94 and 93% respectively whereas at the higher moisture level III, the viability was markedly reduced to 75%. The highest seedling vigour value of 18.0 occurred in the lowest moisture (level I) while the least seedling vigour of 14.0 was recorded in moisture level III.

Interaction effect of genotypes by moisture contents.
A perusal of result in Table 4 shows that seed viability and seedling vigour differed widely among the moisture levels irrespective of the genotypes. Seed viability was drastically reduced at moisture III, irrespective of genotypes. However, TZLCOMP$_1$CLIN and AK 96 DMR-LSRW were superior under moisture level II while TZCOMP$_1$CLIN recorded the lowest

Table 3: Effect of varieties, storage periods and moisture levels on seed viability and seedling vigour of treated maize seeds.

	Seed Viability (%)	Seedling Vigour
Varieties (n = 72)		
ACR97TZLCOMP$_4$C$_2$	88 b	16.8 b
AK96DMR – LSRW	84 c	16.0 c
AMATZBR – WC$_1$	83 c	15.7 c
TZLCOMP$_1$CLIN-W	90 a	17.1 ab
TZLCOMP3C	90 a	17.2 a
Storage Period (n=45)		
2 WAS	90 a	15.7 cd
4 WAS	91 a	17.1 a
6 WAS	90 a	17.5 a
8 WAS	89 b	17.1 a
10 WAS	88 b	17.2 a
12 WAS	84 c	16.1 b
14 WAS	84 c	15.9 c
16 WAS	81 d	15.8 cd
Moisture levels (n=120)		
I	94 a	18.0 a
II	93 a	17.7 b
III	75 b	14.0 c

Values with same letter within the column and parameter are not significantly different at 5% level of probability as determined by Duncan's Multiple Range Tests.
WAS = Weeks After Storage
I = Seeds with 'decreased' Moisture Content (9.3% - 10.0%)
II = Seeds with 'Normal' Moisture Content (10.7 - 12.0%)
III = Seeds with 'Increased' Moisture Content (15.7 – 19.3%)

viability of 76% at moisture level III. Across the moisture levels, the result showed that TZLCOPMP$_1$CLIN consistently maintained the highest seed viability of 97%. However, the poorest seedling vigour was observed at moisture level III in all the genotypes.

Interaction effect of moisture content by storage period.
From the data in Table 5, the general performance of the stored maize seed reveals viability at both moisture levels I and II to be appreciably higher than moisture

Table 4: Effect of Variety and Moisture level on viability and Seedling Vigour of Treated Maize Seed.

Varieties	Seed Viability (%)			Seedling vigour		
	Moisture Level			Moisture Level		
	I	II	III	I	II	III
ACR97TZLCOMP$_1$C$_2$	95 ab	90 b	80 b	18.0ab	17.0c	15.2 a
AK96D DMR – LSRW	93 b	95 a	65 d	17.8 b	17.9 b	12.3d
AMA TZBR –WC	91 b	90 b	68 c	17.3 c	17.1 c	12.9 c
TZL COMP$_1$CLIN	97 a	97 a	76 b	18.5 a	18.5 a	14.4 b
TZL COMP$_3$C$_2$	96 a	92 b	80 a	18.3 a	17.7 a	15.6 a

Values with the same letter within a column and parameter are not significantly different at 5 % level of probability as determined by Duncan's MRT.

I = Seeds with 'decreased' Moisture Content (9.3 – 10.0%)
II = Seeds with 'Normal' Moisture Content (10.7 – 12.0%)
III = Seeds with 'Increased' Moisture Content (15.7 – 19.3%)

Table 5: Effect of Moisture Content and Storage period on Seed viability and Seedling vigour of Maize Genotype

Storage Period	Seed Viability (%)			Seedling vigour		
	Moisture Level			Moisture Level		
	I	II	III	I	II	III
2 WAS	92 d	96 ab	81 a	16.3 c	16.7b	14.2 a
4 WAS	98 a	97 a	76 b	18.6 a	18.4 a	14.1a
6 WAS	98 a	98 a	75 b	19.1 a	19.1 a	14.3 a
8 WAS	97 ab	97 ab	73 bc	18.6 a	18.6 a	14.1 a
10 WAS	95 bc	94 b	76 b	18.5a	18.3 a	14.9 a
12 WAS	92 d	90 c	70 c	17.5 b	17.2 b	13.4 b
14 WAS	93 bc	89 c	71 c	71c	17.7 b	13. 4 b
16 WAS	90 cd	82 d	72 c	17.3 b	16.7 b	13.4 b

Values with the same letter within a column are not significantly different according to Duncan's MRT. (Duncan. 1955)
WAS = Weeks After Storage I = Decreased Moisture Content (9.3 – 10.0%)
II = Normal Moisture Content (10.7 – 12.0%) III = Increased Moisture Content (15.7 – 19.3%)

Table 6: Effect of Variety and storage period on viability and seed vigour

Varieties	2WAS	4WAS	6WAS	8WAS	10WAS	12WAS	14WAS	16WAS
Seed Viability								
ACR 97TZLCOMP$_1$C$_2$	87 bc	88b	94a	90a	91ab	86 ab	87 a	84 a
AK 96 DMR- LSRW	86 bc	89a	85 b	85b	87 b	82 b	82 b	78 b
AMA TZBR – WC	88 b	90 a	86 b	88 a	84 b	76 c	78 b	76 b
TZL COMP$_1$CLIN	96 a	93 a	92 a	90 a	88 ab	87 a	87 a	87 a
TZL COMP$_3$C$_2$	92 ab	92 a	94 a	92 a	92 a	88 a	88 a	82 b
Seed Vigour								
ACR97TZL COMP$_1$C$_2$	15.3 b	16.5 b	18.1 a	17.2 ab	17.6 a	16.6 a	16.4 a	16.2 a
AK96 DMR-LSRW	15.0 b	17.0 ab	16.5 b	16.5 b	17.0 b	15.5 b	15.4 b	15.0 b
AMA TZBR- WC	15.3 b	17.0 ab	16.6 b	16.8 b	16.4 b	14.7 b	14.6 b	14. 6 b
TZL COMP$_1$CLIN	16.9 a	17.5 a	17.8 a	17.4 a	17.2 a	16.6 a	16.6 a	16.8 a
TZL COMP$_3$C$_2$	16.2 a	17.3 ab	18.4 a	17.6a	18.1 a	16.9 a	16.7 a	16.3 a

Values with the same letter within a column are not significantly different according to Duncan's MRT. (Duncan. 1955)

level III, the highest moisture level. Seedling vigour follows exactly the same pattern.
Apart from significant increases in viability and vigour from 2WAS to 4WAS with respect to level I moisture, a gradual decline in these two traits was recorded in all the moisture levels. At moisture level III for instance, no significant reduction in vigour occurred until 12 WAS.

Interaction effect of genotype by storage period.
Result in Table 6 shows the interaction effect of genotype by storage period. There was no particular storage period in which all the genotypes behaved similarly. The observed significant differences were expected as each genotype behaved differently. At 2 WAS and 16 WAS, cultivar TZL COMP1CL1N had the highest seed viability. The viability of cultivar ACR 97 TZLCOMP4C2 was not drastically affected at the end of storage at 16WAS as there was a 3% decline .The seedling vigour of TZLCOMP1CL1N and TZLCOMP3.C2 as well as their seed viabilities were highest at the beginning and end of storage period.

Discussion
Seed viability is a major component of any assessment of quality and germination (Odiemah, 1991). The results indicate that differences among maize genotypes, moisture levels and storage periods were significant, varying from one to another for viability and seedling vigour traits. The interaction effects also indicated that genotype, moisture level and storage period interacted to induce considerable variations in seed viability and seedling vigour.
Differences among the genotypes observed for seed viability and seedling vigour were due to variation in the genetic constitution of the genotypes. This view was supported by Ojo (2000) who studied the inheritance of seed longevity in tropical soybeans. Among all the genotypes, $TZLCOMP_1CLIN$ and $TZLCOMP_3$ C showed an outstanding superiority over other genotypes This was closely followed by $ACR97TZLCOMP_4C_2$. The viability of the two other genotypes were 83 and 84%, which was still above the recommended seed certification value for maize in Nigeria but these two genotypes had low speed of germination.
Maize is a living organism and it undergoes various modification during development, maturity and storage. The behaviour of seed in storage is largely determined by seed moisture and temperature conditions in the immediate surroundings (Ajala,1988).Pooling the genotypes together, our study indicated that there was 9% decline in viability between 2WAS and 16WAS. Even though, storage maintained or improved the seedling vigour of the genotypes evaluated, viability only declined slightly as a result of short duration of storage.
Though there was no significant difference in the viability at moisture levels I and II, the seeds subjected to moisture level III showed the least physiological quality, indicating the lowest seed viability and seedling vigour which was less than 80% and 15.0 respectively. This indicates the adverse effect of high moisture content on seed storability and germinability. Physiological processes in seeds are closely controlled by certain ranges of moisture content. This is not surprising as the range of moisture in level III permits heating to occur sufficiently to kill the seed especially where there is insufficient aeration (Justice and Bass,1978 ; Barton, 1961) .This will result in high rate of seed respiration and increased activity of microorganisms Our findings also confirm the report of FAO (1981) which stated that the keeping quality of seed is largely determined by its moisture content. Genotypes like $TZLCOMP_1CL1N$ and $TZLCOMP_3C_2$ had the best viability and seedling vigour at both moisture levels I and II, closely followed by $ACR97TZLCOMP_4C_2$. Remarkably, the viability and seedling vigour of $TZLCOMP_1CL1N$ were consistently superior at moisture levels I and II compared to other genotypes.
Seedling vigour and viability are usually not equally rated. The extent of seed deterioration can be measured experimentally by determining its vigour (Saxena and Pakeeraiah, 1986). Vigour is more highly correlated with seed deterioration than germination percentage since the latter only measures the last consequence of deterioration.
Seed viability and vigour were variable during storage irrespective of genotypes. Viability of TZLCOMP1CL1N seeds was consistently higher than the others during the storage periods followed by $TZLCOMP_3C_2$ and $ACR97TZLCOMP_4C_2$. These three genotypes had viability above 80% at the end of storage. Our result has proved that the longer the storage period of maize seeds, the lower the seedling vigour and viability, irrespective of genotype and moisture content. We also observed that a demarcation between the seed viability and seedling vigour cannot be drawn as both have the same performance criteria. This can be related to similar report of Justice and Bass (1978) which gave the fact that viability and vigour of seed cannot always be differentiated in storage experiments especially in seed lots that are rapidly deteriorating.
Although seedling vigour of $TZLCOP_1CL1N$ and $TZLCOMP_3C_2$ were consistently superior and similar during the duration of storage and followed the same pattern as in seed viability, the behaviour of treated maize seed in storage was cultivar dependent.

Viability and vigour of seeds subjected to 'low' and moderate moisture i.e. levels I and II and stored for 16WAS was better. Genotypes like TZLCOMP1CL1N, $TZLCOP_3C_2$ and $ACR97TZLCOMP_1C_2$ showed viability in the range of 82 to 87 % at the end of storage, which was 13% higher than the two other genotypes. Similar result was obtained for seedling vigour.

This revelation is very essential for resource poor farmers because they store their seeds under ambient conditions from one season to the next.

Seed deterioration studies like this clearly indicate which categories of seeds (moisture content) could be carried over from one generation to the next. Aim in seed dressing was seen in the light of protecting seed from pathogenic attack or pest infestation but this investigation attests to the fact that seeds with high levels of moisture suffer severe loss of phytotoxicity from seed treatment chemical.

It is noteworthy to state that treatment of some vegetable seed with fungicides increased germination of old seeds to greater extent than in freshly harvested seeds (Wallen et al. 1965). Quite often, the age of the seeds is not usually considered when studying the effect of chemical substances on seeds, even though it is known that fresh seeds react differently to a treatment than old seeds.

CONCLUSIONS

A large percentage of the seed stored by rural and urban farmers in Nigeria are not kept in appropriate moisture content. This reduces germinability in future planting.

Our results have indicated that maize genotypes lose their ability to maintain viability and seedling vigour slowly when treated with Thiram and stored with low moisture content (9.3 -12.0%). Seed subjected to higher moisture content perform poorly in maintaining viability and had the lowest seedling vigour that was not encouraging.

Seed viability and seedling vigour of $TZLCOMP_1CL1N$ and $TZLCOMP_3C_3$ were best at moisture levels I and II during storage followed by $ACR97TZLCOMP_1C_2$. These three genotypes can be used in crossing to enable breeders to develop improved genotypes with the potential of good seed longevity.

For excellent quality seeds needed for improved agricultural practices, seed moisture level before and during storage must be closely monitored. This therefore indicates the importance of using the appropriate moisture content for seed storage. The drying machines that can be used in adjusting the moisture level should be centrally made available to the farmers at an affordable cost.

It is noteworthy to state that in the present study, the seed treatment was carried out on fresh seeds of maize. The result may be different on older seeds. For those farmers who desire information regarding the storage behaviour of new maize genotypes, the information provided by this study should suffice, as there was no appreciable decline in viability at 16 weeks. The two TZL cultivars could be appreciably stored for 4 months after seed treatment without a drastic reduction in viability and vigour.

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