
Effects of Different Storage Duration on Physiological Quality of Maize Inbred Line Seeds

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Abstract: Maize (*Zea mays* L.) seed production is directly related to the quality of seeds obtained from parental inbred lines. Storage duration is an important factor that can determine quality of any crop seed. This research was conducted with the objectives of to assess the effects of storage duration on seed germination, vigor and seedling emergence of different maize inbred lines stored for variant years. The experiment evaluated the effect of five (ML395, CML202, CML165, CML312 and A7033) inbred lines and three seed storage durations of uncontrolled environment (5, 17 and 29 months) infactorial combinations and arranged in laboratory and lath house using a completely randomized design (CRD) with four replications, conducted in 2017/18 main cropping season. The result of analysis of variance was showed significant at ($P < 0.005$) seed physical quality viz seed moisture content and thousand kernel weight was significantly affected by the two main effect and seed physiological quality parameters like germination percentage, speed of germination, field emergence index, seedling vigor index-I and II was significantly affected by two main factors and former combinations of the treatment at $P < 0.005$ respectively. The results of this finding showed that as seed storage duration increased beyond five months, physical and physiological quality parameters of seed become declined. Thus storing seed of maize inbred line for beyond five months are reduce the quality parameters. For that storing seed for short period of time to maintain its quality is preferred.

Keywords: Emergence, Germination Percentage, Inbred line, Maize, Quality Seed, Storage Duration, Vigor Index, Zea Mays

1. Introduction

Maize (*Zea mays* L.) is the third most important cereal grain world wide after wheat and rice. It is the important cereal crop in sub-Saharan Africa and accounts for 15-20% of the total daily calories in the diets of more than 20 developing countries found in Latin America and Africa [2]. The crop is also adaptable across arrange of agro-ecological zones, and every part of the maize plant has economic value. The estimated maize consumption in the African region where it is a staple food ranges from 52g/person/day in Ugandato 328g/person/day in Lesotho [1]. The world maize production area was around 183.2 million hectares in 2014, and that of wheat and paddy rice were 221.60 and 163.25million hectares, respectively [7]. In Ethiopia, maize is produced about two million hectares and it stands first in production (6.49million tons) and productivity (3.254tha^{-1}); and second after tef in area coverage [2]. In addition to strong

demands as common food, there is also the potential for maize to become an increasingly important non-traditional agricultural export crop of Ethiopia [3].

Seed of most crops can be severely damaged and lose vigor when stored under conditions of high humidity and temperature. Maize seed stored while still relatively moist and warm can deteriorate rapidly due to, growth of microorganisms (e.g. fungi and bacteria) and insects [4]. Duration of storage period for maize can also cause adverse effects in growth, vigor and quality of maize. Seed stored for longer period are characterized by delayed germination and slow post germination growth. Quick and uniform germination is very important and decisive for achieving high yields of good quality seed [8] Low quality seeds in many crops such as maize show a decreased rate and percentage of germination and seedling emergence leading to poor stand establishment in

the field and ultimate yield loss [18]. [12] found significant differences in germination and seed vigor among 10 inbredlines with different composition and genetic back grounds. It was reported that the membranes of aged seeds, whose integrity has been reduced by deterioration, are more susceptible to the physical damage such as imbibition damages that result from rapid imbibition. Information is scanty about the effect of storage duration on physiological and physical seed quality of different maize inbred lines in Ethiopia. There for this research was initiated with the objectives of to assess the effect of storage duration on physical and physiological seed quality of maize inbred lines.

2. Material and Methods

2.1. Descriptions of Experimental Site

The experiment was conducted at Bako National Maize Research Center (BNMRC), during 2017 main cropping season. The area is located in East Wollega Zone of the Oromia National Regional State, western Ethiopia. The Center lies between 906'North latitude and 37009' east longitude in the sub-humid agro ecology, at average altitude of 1650 meters above sea level (m.a.s.l). It is 250km far from Addis Ababa, the capital city of

the country. The annual rainfall of the area is 1242mm (BNMRC, 2015). The rainy season covers April to October and maximum rain is received in the months of July and August. The mean minimum, maximum and average air temperature is 13.3, 28.0, and 20.6°C, respectively; and the relative humidity is 63.55%. The soil is reddish brown in color and clay loam in texture. According to USDA soil classification, the soil is Nitosols developed from basalt parent materials, and is deeply weathered and slightly acidic in reaction [18]. The laboratory and pot experiments were also conducted at Bako Agricultural Research Center (BARC) seed testing laboratory.

2.2. Experimental Materials

Stored seed of five maize inbredlines of released hybrids were used in this study. The inbredlines were selected based on availability of their seed in the storage. The seeds of the inbredlines were harvested from Bako National Maize Research Center seed production field in 2014, 2015 and 2016 main rainy seasons and stored under unconditioned ambient storage condition. Therefore, 15 (five inbred lines x three storage periods) treatments in factorial combination were evaluated. List of the inbredlines and year of seed harvest are presented (Table 1).

Table 1. List of maize inbredlines used for the study, year of harvest and storage period.

Inbred lines	Storage duration (month)	Year of harvest
CML395	5, 17 and 29 months	2014, 2015 and 2016 year
CML202	5, 17 and 29 months	2014, 2015 and 2016 year
CML312	5, 17 and 29 months	2014, 2015 and 2016 year
CML165	5, 17 and 29 months	2014, 2015 and 2016 year
A-7033	5, 17 and 29 months	2014, 2015 and 2016 year

CIMMITY= Centro Internacional de Mejoramiento de Maiz y Trigo
BNMRC=Bako National Maize Research Centre.

2.3. Data Collection of Seed Quality Test

The data were collected for every parameter as per the experiment. Percentage data for germination from standard germination test and field emergence index were homogenized using square root transformation as suggested by [11]. The procedures employed for collecting data for each parameter are described as follows:

(1) Physical purity (%)

The 1000 grams of primary sample seeds were taken from each inbredlines and 900 grams of working samples were submitted to laboratory analysis according to [11] then it was divided by mechanical seed divider and weighted in 225 grams for the four replications. The sample seeds were then put on a cleaned illuminated purity working board and the seed components were separated as pure seed, weed seeds, diseased seed, other crops seeds and inert materials, such as soil particles, and particles, broken plant pieces and glumes.

The pure seed component was measured using sensitive balance and finally the purity percentage was calculated as weight of pure seed divide by weight of working sample and the multiplied by hundred.

(2) Seed germination (%)

This parameter was recorded from standard germination test as indicated in 3.3.1.2. Germinated seedlings were counted starting at the 5th day (the first day count of seeds.

Germination) and ends to 10th days (end of seeds germination test) after seed placed on Petridishes. At the end of this test seedlings were categorized as normal, abnormal seedlings, dormant, hard, fresh, dead seeds. The normal seedlings were considered seedlings possessing the essential structures that are indicative of their ability to produce useful mature plants under favorable conditions. Seedling that does not have all essential structures or is damaged, deformed or decayed that prevents normal development were categorized as abnormal seedlings and those which Viable seeds, other than hard seed which fail to germinate when provided prescribed germination conditions was counted as dormant, and seeds which were neither hard nor dormant or have not produced any part of a seedling were counted as dead seeds. Seeds which have imbibed moisture but have failed to germinate and may be dormant were counted as fresh seed, and seeds which remained hard at the end of the test period because their impermeable seed coats prevented the absorption

of water were counted as hard seed.

Seed germination percentage was calculated by considering only the normal seedlings as dividing number of normal seedling by total number of seed sown and then multiplied by hundred.

(3) Speed of Germination

The numbers of germinated seeds were counted daily until there was no further germination and seedlings that were germinated each day were removed after counting. An index was calculated by dividing the number of normal seedlings removed each day by the number of days in which they were placed on the Petridish as per the procedure proposed by [9].

$$\text{Speed of Germination} = \frac{N1}{C1} + \frac{N2}{C2} + \dots + \frac{NF}{CF}$$

Where; N1=number of germinated seed at first count.

N2=number of germinated seed at second count,

NF=number of germinated seed at final count,

C1=days to first count,

C2=days to second count and

CF=days to final count.

(4) Seedling length (cm)

Fifty normal seedlings from standard germination was used to measure seedling length (root and shoot separately) as per [11].

Seed vigor index-I: It was calculated from seedling length and germination percentage as follows:

$$\text{Seed vigor index- I} = \text{GP} \times \text{SL}$$

Where=GP:-is Germination percentage and

SL:-is seedling length (root length +shoot length) were measured at the day of final count.

(5) Field Emergence Index

Emerged seedlings were counted daily until there was no further Seedling emergence conducted in pot experiment. The emergence index was calculated as follows:

$$\text{Field emergence index} = \frac{E1}{D1} + \frac{E2}{D2} + \dots + \frac{EF}{DF}$$

Where=E1=number of seedlings emerged at the first count day,

E2=number of seedlings emerged at the second count day,

EF=number of seedlings emerged at the final count,

D1, D2 and DF are first, second and final days count, respectively.

2.4. Data Analysis of Variance

Analysis of variance (ANOVA) for all data was computed as per the design used in each experiment using ANOVA procedure of SAS 9.1 software computer program. Significant differences were further subjected to Duncan's New Multiple Range Test (DNMRT) and Mean separation was carried out using Least Significant Differences (LSD) at 5% probability level and simple Pearson correlation coefficients were also calculated.

3. Result and Discussion

3.1. Seed Physical Quality

The results of analysis of variances indicated that the two main factors (maize inbredlines and storage durations of seeds) and the interaction of the two factors had significant ($p < 0.05$) effect on moisture content of seed samples and thousand kernels weight. Inbredlines and interaction of the two main effects of seeds had significantly affected the seed purity; however, storage duration of seeds had no significant effect on this parameter.

3.1.1. Seed Moisture Content

The seed samples of CML395 (11.47%), followed by CML202 (11.32%) and CML312 (11.2%) maize inbredlines stored for five months had higher moisture content without significant difference among the mean values but significantly ($p < 0.05$) different from mean values of other treatment combinations. On the other hand, seed sample of CML165 stored for 29 months had significantly lowest (9.25%) moisture content.

3.1.2. Thousand Kernel Weight

Regarding thousand kernel weight, seed samples of CML395 (10.93%) and A7033 (10.97%) stored at 17 and 5 months, (Table 2), had significantly higher mean values while the seed sample of CML165 had significantly lower mean values than other treatment combinations. The seeds of CML395 (30.7g) and CML165 (18g) had significantly highest and lowest thousand kernel weight, respectively. Seeds of inbredlines stored for 5 and 29 months had significantly highest and lowest mean values for thousand kernel weight, respectively. Generally, the seed moisture content and thousand kernel weights reduced as the seeds of inbredlines kept for longer period though it was observed non-significant difference observed between mean moisture content of seeds stored for 5 and 17 months (Table 2).

The seeds moisture content decreased as the storage period was prolonged and there by reduced the seed weight. The inconsistency among and within inbredlines in moisture loss as the storage duration increased might be the differential response to varied seeds storage period. The seed moisture loss and seeds weight reduction as duration of storage was prolonged has been reported by [15]. The seed weight might be also a function of genetic factor related to inherent characteristics of maize inbredlines to produce heavy weight seeds as it was observed in CML395 and A-7033 that had higher thousand kernels weight than other inbredlines at all storage duration. In agreement with this study results, [6] reported that thousand kernel weights of maize seed significantly decreased as storage duration of the seed increased and [17] observed that soybean seeds lost their moisture rapidly after two years of storing period. This finding was also supported by [2, 9] that seed moisture content and thousand kernel weight significantly decreased as storage duration of the seeds extended. However, the authors [13] indicated that, the moisture content of a seed

sample/lot and seed weight of crop varieties may be affected by many factors such as temperature, nature of the seed, relative humidity and a storage duration. [5].

3.1.3. Seed Physical Purity

The overall physical purity of seed samples was 99.85 in the range between 99.68 and 99.95%. All seed samples of inbredlines at all storage durations except the seed samples of A7033 stored for 29 months and seed of CML165 stored for 17 and 5 months had non-significant difference for mean physical purity. The seed samples of CML202 followed by CML395 and CML312 had significantly higher physical purity than seed samples of the two other inbredlines. The seed storage durations had non-significant effect on physical purity of seed but the highest mean values calculated for

seeds stored for 17 months and the seeds of all inbredlines stored for 17 months except CML165 had higher mean values than seeds stored for 5 and 29 months. The result suggested that the seeds physical purity is the function of genetic factor and the seed preparation differences at different periods as the observation is in lined with outhur [10]. The seeds stored for 17 months having higher moisture content without non-significant difference with seeds stored for five months may support the suggestion that the seeds were stored at higher moisture and prepared well in the season where seeds harvested and stored for 17 months. The inbredlines at different storage durations had high physical purity indicated the production and storage of the seeds were carefully done by the research center.

Table 2. Effect of storage duration on seed physical quality of maize inbredlines of seed quality.

Inbred line* duration (month)	Moisture content (%)			Inbred line	Thousand kernel weight (g)			Inbred line	Seed physical purity (%)			Inbred line
	5	17	29		5	17	29		5	17	29	
CML395	11.47 ^a	10.93 ^{bc}	10.36 ^{def}	10.92 ^a	30 ^b	32.3 ^a	29.8 ^b	30.7 ^a	99.93 ^a	99.88 ^a	99.82 ^{abc}	99.88 ^{ab}
CML202	11.32 ^{ab}	10.67 ^{cd}	10.12 ^{ef}	10.71 ^{ab}	24.9 ^c	24.5 ^c	19 ^g	22.8 ^d	99.91 ^a	99.92 ^a	99.93 ^a	99.92 ^a
CML312	11.2 ^{ab}	10.88 ^{bcd}	10.82 ^{bcd}	10.97 ^a	30 ^b	27 ^d	15.5 ^h	24.17 ^c	99.86 ^{ab}	99.95 ^a	99.84 ^{ab}	99.88 ^{ab}
A7033	10.5 ^{cde}	10.97 ^{abc}	9.95 ^f	10.47 ^b	32 ^a	29 ^{bc}	28 ^c	29.67 ^b	99.84 ^{ab}	99.92 ^a	99.68 ^d	99.81 ^{bc}
CML165	10.52 ^{cde}	10.57 ^{cde}	9.25 ^g	10.12 ^c	20 ^g	21.5 ^f	12.5 ⁱ	18 ^c	99.74 ^{bcd}	99.7 ^{cd}	99.86 ^{ab}	99.77 ^c
LSD (5%)		0.516		0.298		1.105		0.638		0.136		0.078
Overall mean		10.64				25.07				99.85		
Storage duration	11 ^a	10.8 ^a	10.1 ^b		27.38 ^a	26.86 ^b	20.96 ^c		99.86	99.87	99.83	
LSD (5%)		0.231				0.494				NS		

Mean values followed by the same letter(s) in columns and rows in the interaction of Inbred line*Storage duration and column of mean values of inbred line had non-significant difference at 5% probability level. LSD (5%) =least significant difference at P<0.05.

3.2. Seed Physiological Quality

3.2.1. Percentage of Seed Germination

The seed samples of CML202 stored for five months followed by CML395 stored for 17 months had higher germination percentage with non-significant difference between the two lines but they had significant differences from the mean values of other treatment combinations. Whereas the seed samples of line CML165 stored for 17 and 29 months and A-7033 stored for 29 months had significantly lower mean for germination percentage. The higher means for germination percentage was registered from CML395 and CML202 while the lowest mean for this trait was registered from seed samples of A-7033 and CML165. The highest germination percentage was obtained at seed samples stored for five months while the lowest was at seed samples stored for 29 months. Germination percentage of all treatment combinations showed reduction when storage duration was extended from 5-29 months except the seeds of A-7033 stored for 17 months. This indicated that the seed germination percentage of inbred lines highly influenced by the prolonged storage duration.

3.2.2. Speed of Germination

The seed samples of CML202, CML312 and CML395 stored for five months followed by seeds from CML395 stored for 17 months had higher speed of germination with non-significant difference among each other but significantly

different from the seed samples stored for 29 months. On the other hand, the seed samples of A-7033 stored for five months and CML165 stored for 29 months had lower speed of germination. The seeds of inbredlines stored for five months except the seeds of A-7033 and CML165 had higher speed of germination than that of seeds stored for 29 months (Table 3). Higher means for speed of germination was registered from line CML202 (19.2), CM312 (18.5) and CML395 (17.66) whereas the lower mean for this parameter was registered from line CML165 (4.08) and A-7033 (4.67). Seeds stored for 5 and 17 months had non-significant difference for speed of germination but had significant difference with seeds stored for 29 months. Generally, germination percentage and speed of germination of maize inbredline are reduced as storage duration of seed was extended and showed significant differences among and within treatments due to varietal variation and the interaction of the two factors. This two traits are reduced as storage period was prolonged so that it causes the reduction of quality parameters of seed. This finding also supported by [14] as they found reduction in quality of maize inbredlines as storage condition was unfavorable and storage duration were prolonged.

Germination percentage of seeds and speed of germinations, storage duration for 5 and 17 months had non-significant difference except for seeds of some inbredlines, but the extended storage duration from 5-29 had a significant effects on germination percentage of seeds and speed of

germinations of all inbred lines. This indicated storage duration beyond 17 months had significant effects on germination percentages and speed of germination of maize inbred lines. As it has been documented by [16], germination decreased over storage time in a sigmoid fashion where by the parameters of these curves seemed to be species specific.

[17] observed the lower germination percentages in seeds of soybean stored for three years as compared to one year storage. However, the current study results are in contrast with finding so [12] that germination percentage of maize was over 80% even after stored for 12 years with increased seeds viability.

Table 3. Interaction effect of storage duration, inbred line and variation of maize inbred line from pre-sowing seed germination.

Inbred line*storage duration (month)	Germination percentage (%)			Speed of germination				
	5	17	29	Inbred lines	5	17	29	Inbred lines
CML395	6.86 ^{bc}	7.37 ^{ab}	5.84 ^{def}	6.69 ^a	17.66 ^{ab}	18.93 ^{ab}	8.52 ^c	14.93 ^a
CML202	7.68 ^a	6.9 ^{abc}	5.6 ^{ef}	6.72 ^a	19.2 ^a	15.24 ^{bc}	14.32 ^{cd}	16.25 ^a
CML312	6.96 ^{abc}	5.98 ^{def}	5.4 ^{fg}	6.1 ^b	18.5 ^{ab}	13.7 ^{cd}	11.12 ^{de}	14.46 ^a
A7033	6.32 ^{cde}	5.9 ^{def}	4.6 ^h	5.6 ^c	4.67 ^f	16.64 ^{abc}	11.1 ^{de}	10.8 ^b
CML165	6.5 ^{cd}	4.6 ^h	4.47 ^h	5.2 ^c	16.5 ^{abc}	8.75 ^e	4.08 ^f	9.78 ^b
LSD (5%)		0.449				1.779		
Overall mean		6.07				13.24		
Storage duration	6.868 ^a	6.158 ^b	5.175 ^c		15.42 ^a	14.44 ^a	9.83 ^b	
LSD (5%)		0.348				1.379		

Mean values followed by the same letter(s) in columns and rows in the interaction of Inbred line*Storage duration and column of mean values of inbred line had non-significant difference at 5% probability level. LSD (5%) =least significant difference at P<0.05.

4. Summary and Conclusion

Maize (*Zea mays* L.) is the third most important cereal grain world wide after wheat and rice. It is produced on an area of two million hectares, and it stands first in terms of production (6.49 million tons) and productivity (3.254 t ha⁻¹), and second after tef in terms of area coverage. Large proportion of maize seed is harvested and stored under hot and humid conditions. This condition aggravates the decline in quality of the product especially under long storage duration. Longevity of storage period for maize can cause adverse effect in quality of maize seed. However, information is scantily that deals the effects of storage duration on maize inbred lines. Therefore, objective of current studies were to evaluate the effect of seed storage duration on physiological quality of maize inbred lines. The experiment was conducted to deal how physical and physiological quality of maize inbred lines can be affected at different duration level in storage.

Five different maize inbred lines and three storage durations were the factors in the experiment. In the current studies, the storage duration, treatment and the interaction effects of the two main factors were significantly affected seed quality attributes: seed moisture content, thousand kernel weight, standard germination percentage, and speed of germination. However, genotype and interaction of the two main factors were significantly affected seed physical purity. In conclusion, current research was found that storage duration, genotype and the interaction of the two main factors were significantly affected seed quality of maize inbred lines. Mainly, storage duration of seed had highly significant effects on the seed quality of seed kept in storage for beyond five months. Therefore, the following recommendation was given. For pre-sowing experiments at beyond five months of storage duration, retardation of

quality traits on maize seed were totally occurred. So storing the seed under ambient temperature for more than five months is not recommended.

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