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INFLUENCE OF SEED INITIAL MOISTURE CONTENT, STORAGE CONDITION AND TIME OF STORAGE ON SEEDLING GROWTH STAGES OF COFFEE (COFFEA ARABICA L.)

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ABSTRACT

Coffee is one of the most important agricultural products in the international market and many countries are involved in its production, trade or consumption. Arabica coffee is self-pollinated, homozygous, and normally propagated by seeds. Seeds have been considered intermediate storage behavior with varying results. It is highly desirable that seeds are stored safely to optimize coffee seedling production at the appropriate time and season. The objective of this study was to evaluate the effect of storage temperature, time of storage and initial seed moisture contents on early seedling performances of coffee seeds and to determine the appropriate seed handling method. In this experiment, the influence of initial seed moisture content with four levels (12, 17, 22 & 27%) with storage two temperature (15°C & ambient), time of storage with six levels (sowing after each month and upto 6 months) and on coffee seedling growth stages were studied in a split-split-plot factorial design. The data collected were subjected to ANOVA. The storage environment with cold temperature (15°C) accelerated seedling growth stages parameters much better performances than did ambient temperature condition. All tested seedling growth stages were faster at initial time of storage. After third month seed quality drastically reduced especially under ambient storage condition. Seeds dried to 12% moisture content showed delayed performance throughout the trial period. Seeds with 27% initial moisture content took shorter days to reach at different growth stages at initial storage time but when aged took much days. Storage temperature, time of storage and initial seed moisture contents showed highly significant main and interaction effects and seeds dried to intermediate moisture level (17 and 22%), stored under cold temperature and sown at early times resulted in enhanced seedling growth. It was concluded that drying coffee seeds to 17% to 22% moisture contents and kept under storage with relatively lower temperatures (15°C) for not more than six months of storage showed better performance.

Keywords: Arabica coffee, Coffee Seedling, Seedling growth stage.

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1. INTRODUCTION

Coffee has enormous economic, social and environmental significance in Ethiopia. But despite its enormous genetic variability, favorable ecology for production and its importance in the national economy of the country, productivity of the crop remained much lower than released varieties yielding potential which primarily associated to use of poor seed that result in poor seedlings causing poor establishment in the field (Alemayehu et al. 2008; Mojo et al. 2017; Nasiro et al. 2017; Hirons et al. 2018).

As most commercially grown cultivars of Arabica coffee are largely self-pollinated and homozygous, they are normally propagated by seeds. Vegetative propagation is used for multiplication of clones and hybrids at research stations (Dussert et al. 2018). The Coffee seed normally presents high germination potential, just after appropriate harvest and desiccation. However, it loses its physiological quality very rapidly under usual storage conditions. Ellis et al. (1990) have grouped coffee under intermediate category for seed behavior, because seed viability lasts for only short periods and must be planted soon after collection (Trujillo et al. 2019). But one may need to store seeds for up to six months or more because of the gap between harvesting (during October and November) and sowing seeds (between April and August). Hence, storage and preservation of quality seed lots until sowing is as important as producing quality seeds.

Jimma agricultural research center (JARC), which is the sole mandated coffee seed producer in the country, is conventionally using seed moisture contents ranging from 12 to 15% for seed storage. But feedback from various

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users repeatedly indicted that there is a vast problem on seed germination and need further investigation in order to come-up with the best solution (Steinbrecher and Leubner-Metzger 2017; Ahmed et al. 2010). Therefore, due to the damage that seeds have during the storage and limited information in our country about the storability of the seeds justified necessity of doing the experiment with the aim of giving practical information about storing of coffee seeds. Thus, objectives were to evaluate the effect of storage temperature, time of storage and initial seed moisture content on seedling growth performances of coffee seeds and to determine the appropriate seed handling method.

2. MATERIALS AND METHODS

2.1. Description of the Study Site

The experiment was executed in a nursery site at Jimma Agricultural Research Center (JARC), of the Ethiopian Institute of Agricultural Research. It is the National Coffee Research Coordinating Center in the country and is located 365 km away from Addis Ababa, 12 km from the Jimma town in the south west direction. The center is found within tepid to cool humid highland agro-ecological zone of the country at an altitude of 1750 meters above sea level, 7046" N, latitude, and 36047" E longitude in the sub humid tropical belt of south western Ethiopia. Average annual rain fall of the area is 1594 mm with 67% mean relative humidity. The mean minimum and maximum temperatures are about 11.6°C and 26.3°C, respectively. The soil of the center has a characteristic of reddish to reddish brown clay nature, where erutic nitosols and chronic cambisols are dominant types with pH range of 5 to 6.

2.2. Experimental materials

Following the conventional procedures widely applied in coffee seed preparation (Van der Vossen 1979; Goodman 1980; Rothofs 1980), fully ripe red cherries were harvested from selected mother trees in the seed orchard of cultivar 74110 (which was selected as it is widely adapted, highly demanded and much produced) at, JARC, on 21st November 2011 growing season. This cultivar was selected for the present study, because it is highyielding CBD resistant, widely adaptable, highly demanded and produced much. The cherries were sorted out and pulped in a hand pulp separator. After pulping the selected cherries, the wet parchment beans were again sorted out, thoroughly washed, and taken to drying room, hut made of grass roof.

Then the wet parchment coffee was laid on wire mesh for drying under shade and when its skin dried dressed in fine wood ash following the JARC conventional practice and kept till it attained the desired four levels of moisture contents (27, 22, 17 and 12% [fresh weight base]). One kg of parchment coffee seeds was taken on 5th January 2012 till 2nd July 2012 from each of the four batches and kept separately in each of the two storage conditions. The storage conditions used in this study were cold store with 15°C (SC1) and a room at ambient temperature (SC2). All the seed lots with four levels of MCs were kept under both storage conditions.

Representative samples of seeds were taken every month from each treatment combinations and were subjected to a series of tests in the laboratory and nursery trials to evaluate the potential viability (germination) and early seedling growth potential status of each seed lot.

2.3. Treatments and experimental design

A split-split plot factorial design was used with three replications.

2.4. Factors

A. Storage condition (cold 15°C and ambient temperature)

B. Time of storage (sowing after 1, 2, 3, 4, 5 and 6 months of storage)

C. Initial seed moisture content (12, 17, 22 and 27%)

As presented in Table 1, in this experiment, storage conditions (SC) were assigned to main plot, time of storage (ST) was assigned to sub-plots while seed initial moisture level was assigned to sub-sub-plots. Total number of treatment combinations were 48 (2*6*4) replicated three times and the total number of experimental plots were 144. The treatments were randomly and independently assigned to main plots, sub plots and the sub-sub plots. Every routine nursery activity was practiced uniformly to all experimental units as per the recommendation of the JARC (Institute of Agricultural Research, 1996).

2.5. The model

Three factor analysis of variance model was used with General Linear Model (GLM) Procedures of SAS Version 9.2. The linear statistical model for the split-split-plot design would be:

$$Yijk = \mu + \alpha i + \beta j + \gamma k + (\alpha x \beta)ij + (\alpha x \gamma)ik + (\beta x \gamma)jk + (\alpha x \beta x \gamma)ijk + \pounds \mu ijk Where: -$$

Yijk = the response measurement for the ijkth observation

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 μ = is the overall mean effect.

 $\alpha i = is$ the effect of the ith level of sub-sub-plot

 $\beta j = is$ the effect of the jth of main plot

 $\gamma k = is$ the effect of the kth of sub-plot

 $(\alpha x \beta)ij = is$ the effect of the interaction b/n actor A&B

 $(\alpha x y)ik = is$ the effect of the interaction b/n factor A&C

 $(\beta x y)$ jk = is the effect of the interaction b/n factor C&B

 $(\alpha x \beta x y)$ ijk = the effect of interaction b/n the three factors

£ μ ijk = is a random error component for all factors

2.6. Sampling and data collection

The observations were recorded on seed germination percent, shoot length, seedling root length, girth diameter, leaf area, seedling dry weight and seedling vigor Index and they were explained below.

2.7. Determination of percentage of seedling growth stages

The plots were checked consistently every five days starting from the 65th day after sowing till all the potentially capable seedlings in each plot developed their cotyledonary leaves, total percentage of seedlings at the butterfly growth stage (%BFS) was determined for each plot. Using the number of seedlings that produced their cotyledonary leaves, or that attained the "butterfly" stage of growth, the mean days required to attain this stage of growth (MDBFS) was also calculated following the same procedure used for determination of MDE above.

Similarly, the number of seedlings that developed their pair of true leaves were further counted from each plot and recorded at five-days interval starting from the 80th day after sowing and the percentage of seedlings that produced their first true leaf (%TLS) was determined. The mean number of days required by each plot to attain this growth stage (MDTLS) was also computed using the method described above.

2.8. Statistical analysis

The seedling growth data collected were subjected to Analysis of variance (ANOVA) for split-split-plot design and treatment mean separation was carried out using least significant difference (LSD) at 0.01 and 0.05 probability levels using General Linear Model (GLM) procedure of SAS statistical version 9.2 software (SAS, 2009).

3. RESULTS AND DISCUSSION

3.1. Butterfly Growth Stage (BFS %)

The data of the experiment showed that the number of seedlings attained butterfly growth stage was significantly (P<0.01) influenced by the main and interaction effects of storage condition, storage time and seed moisture content (Table 1). Treatment combinations of seeds stored in cold condition dried to 22% moisture contents and sown at first month maintained significantly higher butterfly growth stage (88.00%). Similarly, seeds stored in cold condition dried to 12, 17 and 27% moisture contents and sown at first month and seeds with 17, 22 & 27% moisture contents that sown at second month also maintained higher percentage of butterfly growth stage (Table 2). This indicated that cold condition better maintained coffee seeds quality and it is consistent with the findings of Barboza and Herrera (1990) and Ellis et al. (1990; 1991) who have proposed the use of low temperatures (around 15°C) to preserve coffee seed viability and vigor for a considerable period of time.

The least butterfly growth stage was recorded in the seeds stored in ambient condition dried to 12 and 17% moisture contents and sown at sixth month of storage (31.00 and 32.67%, respectively) (Table 2). This could be attributed to the general decline in viability of seed lots as a result of prolonged storage.

Table I: Analysis of Variance for butterfly stage (%BFS), true leaf stage (%TLS), mean days to butterfly (MDBFS) and true leaf stage (MDTLS)

Source of Variation	DF	BFS	TLS	MDBFS	MDTLS
Rep	2	73.42**	85.33**	5.76**	6.30*
SC	I	9136.17**	10885.44**	680.34**	1778.03**
Error (a)	2	26.76**	21.36*	11.17**	22.13**
ST	6	3732.66**	4021.23**	523.62**	1390.04**
SC*ST	6	870.99**	830.33**	24.72**	79.06**
IMC	3	179.58**	156.75**	26.43**	54.43**
SC*IMC	3	15.90*	30.35**	5.78**	10.94**
ST *IMC	18	14.23**	14.44**	3.64**	2.09 ns
SC*ST*IMC	18	13.33**	17.59**	0.90*	6.06**
Error (b)	12	22.68**	25.00**	7.62**	9.13**
CV%		2.89	3.51	0.81	0.89

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*,*** = Indicate significant differences at the 5% and 1% probability levels, respectively. ns = non-significant at 5%. probability levels.

Beside failure in germination, which is a sign of seed death, the general symptoms of deterioration exhibited by seeds include: reduced germination rate and vigor, and development of abnormal seedlings (Decouche and Caldwell 1960; Anderson 1970; Negussu 1986; Vivas et al. 2017; Fătu et al. 2017). Initial seed moisture contents with 22% maintained better performance for percent butterfly stage with prolonged storage time. These results are in accordance with the reports of (IAR 1996; Wondyifraw 1994).

Table 2: The interaction effects of storage condition (SC), storage time (ST) and initial seed moisture content (IMC) on

percentage of seedlings attained butterfly stage (%)

SC	IMC	Storage Time (in month)					
		I	2	3	4	5	6
Cold (I5°C)	12%	83.67 ^{a-e}	80.33 ^{d-i}	77.33g-l	72.33 ^m -p	71.33 ^{nop}	68.67P9
	17%	86.67 ^{ab}	83.33 ^{a-f}	80.00 ^{d-i}	75.00j-n	72.33 ^m -p	66.33 9
	22%	88.00 ^a	84.33 ^{a-d}	82.00 ^{b-g}	80.33 ^{d-i}	76.67 ^{i-m}	71.33 ^{nop}
	27%	87.67 ^a	86.00 ^{abc}	81.00 ^{d-i}	77.33g-l	73.00 ^{l-} P	69.00P9
Ambient	12%	81.67 ^{c-h}	78.00g-k	72.67 ^{l-} P	55.33 ^r	41.33 ^u	31.00W
	17%	80.67 ^{d-i}	78.67 ^{f-k}	74.00 ^{k-o}	54.33 ^{rs}	49.00 ^t	32.67VW
	22%	82. 00 ^{b-g}	79.33 ^{e-j}	77.00 ^{h-m}	65.33 q	49.67 st	41.67 ^u
	27%	82.00 ^b -g	74.67 ^{j-0}	70.00 ^o Pq	56.33 ^r	47.00 ^t	37.33 ^{uv}
CV%		4.12					
LSD (5%)		4.6806					

Means followed by the same letter(s) are not significantly different at 5% level of probability.

3.2. True Leaf Growth Stage (TLS %)

Interaction effects of storage condition, storage time and seed moisture content on true leaf growth stage was significant (P<0.01) similarly with the results of emergence percentage butterfly stage (Table 1). Treatment combinations of seeds stored in cold condition dried to 17% moisture content and sown after a month maintained significantly higher true leaf growth stage (85.67%). However, not significantly differed from seeds stored under cold condition that dried to 22 and 27% seed moisture content and sown after a month and with the same three levels of moisture contents that sown after second month. While, the least percentage of true leaf growth stage was recorded in the seeds stored in ambient condition dried to 12 and 17% moisture contents and sown after sixth month of storage (27.67%). Similarly, seeds stored in ambient condition dried to 22 and 27% moisture contents and sown after sixth month of storage also showed lower percentage of seedlings attained true leaf stage (Table 3). This could be attributed to the general decline in viability of seed lots as a result of prolonged storage.

In the same manner to that of butterfly stage seeds with 22% moisture content maintained better performance for percent true leaf stage with prolonged storage time. These results are in accordance with the reports of (IAR 1996; Wondyifraw 1994). The general symptoms of deterioration exhibited by seeds include: reduced germination rate and vigour, and development of abnormal seedlings (Decouche and Caldwell 1960; Anderson 1970; Negussu 1986).

Table 3: The interaction effects of storage condition (SC), storage time (ST) and initial seed moisture content (IMC) on

percentage of seedlings attained at true leaf stage (%)

SC	IMC	Storage Time (in month)					
		I	2	3	4	5	6
Cold (15°C)	12%	79.33 ^{b-e}	75.00 ^e -j	73.67 ^{f-l}	69.67 ^{k-0}	69.33 ^k -P	64.33P-s
	17%	85.67 ^a	80.67 ^{a-d}	76.67 ^{c-g}	70.00j-o	65.67 ^{o-r}	61.00 ^{rs}
	22%	85.33 ^a	81.33 ^{abc}	78.67 ^{b-f}	75.00 ^{e-j}	71.00 ⁱ⁻ⁿ	67.67 ^m -q
	27%	85.00 ^a	83.33 ^{ab}	79.00 ^{b-e}	73.67 ^{f-l}	68.67 ^l -q	66.00 ^{n-r}
Ambient	12%	78.00 ^{c-f}	71.678 ^{-m}	64.009-s	48.67 ^{tu}	36.33 ^W	27.67×
	17%	76.67 ^{c-g}	75.67 ^{d-i}	71.33 ^{h-m}	51.00 ^t	43.67 ^{uv}	27.67 [×]
	22%	75.67 ^{d-i}	76.33 ^{c-h}	71.00 ⁱ⁻ⁿ	60.00 ^s	44.00 ^{uv}	32.67 ^{wx}
	27%	74.33 ^{e-k}	71.00 ⁱ⁻ⁿ	65.00 ^{o-s}	52.67 ^t	43.00 ^v	30.33×
CV%		4.76					
LSD (5%)		5.0679					

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Means followed by the same letter(s) are not significantly different at 5% level of probability.

3.3. Mean Days to Butterfly Stage (MDBFS)

The data of the experiment showed interaction between storage condition, time of storage and initial seed moisture content was highly significant (P<0.01) for mean days to butterfly stage (Table 1). The treatment combinations of seeds dried to 27%, stored under cold condition and sown after a month showed significantly short MDBFS (87.67 days). While, extended time was recorded for treatment combination of seeds stored in ambient condition, dried to 12% moisture content and sown after the end of the sixth month of storage (107 days). This could be attributed to the general decline in viability and vigor of seed lots as a result of prolonged storage, fluctuation of temperature and high respiration under ambient condition. Present study results revealed that seeds dried to 22% moisture and stored in cold condition better maintained rapidity and uniformity of seedlings than did the other treatment combinations (Table 4). These results agree with various reports (Van der Vossen 1979; Bradford et al. 1990; Pammenter and Berjak 1999; Rosa et al. 2005) who worked on various crops including coffee.

Mean days to butterfly stage progressively declined with prolonged storage time irrespective of moisture content, storage environment and their interaction. These results are in accordance with the reports of (IAR 1996; Wondyifraw 1994). There was a positive relationship between the mean number of days required to reach a given growth stage and time in storage. Vigorous seeds rapidly germinate, metabolize and establish in the field. Therefore, any method used to determine the rapidity of growth of the seedling will give an indication of seed vigour level (Perry 1984).

Table 4: The interaction effects of storage condition (SC), storage time (ST) and initial seed moisture content (IMC) on mean

days taken to seedlings to attain at butterfly stage (no of days)

SC	IMC	Storage Time (in month)					
		I	2	3	4	5	6
Cold	12%	90.00bcd	91.00 ^{b-f}	93.00 ^{f-i}	95.67j-m	98.33 ^{n-q}	101.33 ^{stu}
(15°C)	17%	91.33c-f	93.00 ^{f-i}	94.33h-k	95.00 ^{i-l}	97.67 ^m -p	99.33P-s
	22%	89.00 ^{ab}	91.00 ^{b-f}	92.00 ^d -g	93.678 ^{-j}	95.67 ^{j-m}	97.67 ^{m-p}
	27%	87.67 ^a	90.67 ^{b-e}	92.67 ^{e-h}	94.67 ^{h-k}	96.00 ^{klm}	100.009 ^{-t}
Ambient	12%	93.00 ^{f-i}	95.00 ^{i-l}	97.67 ^m -p	101.00 ^{r-u}	104.33 ^{wx}	107.00 ^y
	17%	92.00 ^d -g	95.00 ^{i-l}	96.33 ^{k-n}	99.00 ^{o-r}	102.67 ^{uvw}	105.33 ^{×y}
	22%	90.00 ^{bcd}	93.00 ^{f-i}	95.33jkl	99.33P-s	102.00 ^{tuv}	104.67 ^{wx}
	27%	89.67 ^{abc}	93.00 ^{f-i}	97.00 ^{l-o}	101.33 ^{stu}	104.00 ^{vwx}	107.33У
CV%		1.40					
LSD (5%)		2.1862					

Means followed by the same letter(s) are not significantly different at 5% level of probability.

3.4. Mean Days to True Leaf Stage (MDTLS)

The result the present study showed that interaction between storage condition, time of storage and initial seed moisture content was highly significant (P<0.01) for mean days to true leaf stage with the same manner to MDBFS (Table 1). The treatment combinations of seeds dried to 27% moisture content, stored under cold condition and sown after a month showed significantly short MDTLS (116.33 days). While, extended time was recorded for treatment combination of seeds stored under ambient condition, dried to 12 and 27% moisture and sown at the end of the sixth month of storage (145.33 days). This could be attributed to the general decline in viability and vigor of seed lots as a result of prolonged storage, fluctuation of temperature and high respiration under ambient condition. The result of this experiment revealed that seeds dried to 27% moisture and stored in cold condition better maintained rapidity and uniformity of seedlings than did the other treatment combinations (Table 5). This evidenced that the advantage of having optimum initial seed moisture content in maintaining seed viability and vigor for a longer time (Agrawal 1992). Moreover, Bradford et al. (1990) also stressed that such rapid early growth of seedlings was associated with high vigor, a measure of the inherent potential of seed lots to produce strong plants, which in turn is affected by moisture content of the seed to be sown.

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Mean days to true leaf stage progressively declined with prolonged storage time irrespective of moisture content, storage environment and their interaction. These results are in accordance with the reports of (Wondyifraw 1994). This could be attributed to the general decline in viability of seed lots as a result of prolonged storage. As reported by Perry (1984), vigorous seeds rapidly germinate, metabolize and establish in the field and therefore, any method used to determine the rapidity of growth of the seedling will give an indication of seed vigour level.

Table 5: The interaction effects of storage condition (SC), storage time (ST) and initial seed moisture content (IMC) on mean

days taken to seedlings to attain at true	leaf stage (No. of days)
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SC	IMC	Storage Time (in month)					
		I	2	3	4	5	6
Cold	12%	119.67 ^{b-e}	120.67 ^{b-e}	123.67 ^{f-h}	127.67 ^{jmn}	131.67 ^{op}	135.00 ^{qr}
(15°C)	17%	119.00 ^{a-d}	120.00 ^{b-e}	122.00 ^{e-h}	124.67 ^{h-k}	128.00 ^{lmn}	135.00 ^{qr}
	22%	118.67 ^{a-d}	120.00 ^{b-e}	122.33 ^{e-h}	125.33 ^{i-l}	130.00 ^{no}	136.33 ^{rs}
	27%	116.33 ^a	118.33 ^{abc}	120.67 ^{b-e}	124.00g-j	126.67 ^{j-m}	129.33 ^{mno}
Ambient	12%	122.33 ^{e-h}	126.00 ^{i-l}	130.33 ^{no}	135.33 ^{qr}	140.33 ^{tuv}	145.33 ^W
	17%	121.00 ^{c-f}	123.67 ^{f-i}	127.67 ^{lmn}	133.67Pqr	138.67 st	143.00VW
	22%	120.00 ^{b-e}	124.33 ^{h-k}	128.00 ^{lmn}	134.00Pqr	140.00 ^{tu}	143.00 ^{vw}
	27%	118.00 ^{ab}	121.33 ^d -g	127.00 ^{klm}	133.33P9	142.00 ^{uv}	145.33 ^W
CV%		1.31					
LSD (5%)		2.7214	1 1	F0/ I			

Means followed by the same letter(s) are not significantly different at 5% level of probability.

Conclusion

According to the results obtained in this investigation, it was observed that early seedling growth parameters tested resulted to be statistically influenced by the main and interaction effects of storage conditions, storage time and initial seed moisture contents. After four more months of storage all the factors resulted in highly significant (P<0.01) difference for almost all the variables tested. Storage environment with cold condition (15°C) maintained seed germination and early seedling growth parameters much better performances than did ambient condition. All tested seed and seedling growth parameters were highest at initial time of storage and declined progressively with storage time. After third month seed quality drastically reduced especially under ambient storage condition. Seeds dried to 12% moisture content showed inferior performance throughout the trial period. Storage condition, time of storage and initial seed moisture contents showed highly significant interaction effects and seeds dried to intermediate moisture level (17 and 22%), stored under cold condition and sown at early times resulted in enhanced early seedling growth and uniformity in seedling. Hence, for storing coffee seeds, it is advisable drying the seeds to about 17% to 22% moisture contents and keep under storage with relatively lower temperatures (at about 15°C) for not more than five or six months of storage is advisable.

Recommendations and Future line of work

- 1) For immediate sowing of coffee seeds higher seed moisture content of 27% may be used for better seed germination and early growth potentials that result in vigorous seedling.
- 2) But if it is to be used after storage for more than two months initial seed moisture content needs to be reduced to around 17% for maintaining better seed quality for about six months.
- 3) For prolonged coffee seed storage up to six months storage temperatures nearly 15°C advised to be used and seeds kept under ambient condition may be sown immediately or within two to three months otherwise preferable not to be used as seed material.

The experiments conducted so far in this area are not sufficient to draw a reliable conclusion. Since, the present study was done for a single cultivar (74110) and under Jimma conditions further experiment needs to be conducted for varieties at different environmental conditions with narrower ranges of seed initial moisture content and storage temperatures.

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