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Effect of production sites and seed age on seed quality parameters of malt barley (*Hordium vulgare* L.) varieties

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Quality malt barley production, processing and marketing had paid a greater degree of attention among farmers, traders, malting and brewing factories in Ethiopia. *there is no quantified research information available on effect of production sites, seed ageing and potential varietal differences in meet up basic seed and grain quality standards for malt*. Hence, this study was designed to evaluate the effect of production sites and seed age on seed quality of malt barley varieties. The experiment was conducted under laboratory condition using 12 treatments consisting 3 barley sample collections obtained from DARC, ESE and OES,2 seed ages (year 1and year 2) and 2 malt barley varieties (Beka and Holker). Seed quality analyses were performed using standard laboratory procedures. All measured seed quality parameters were subjected to ANOVA using SAS version 9.1. Highly significant (P < 0.01) variation was observed among the tested quality traits. Two years aged seed samples gave the highest percentage of abnormal seedlings and dead seeds before accelerated ageing under standard germination test. Similarly, artificially accelerated aged seeds of malt barley displayed a marked decline in germination % as compared to newly harvested one. Highly significant differences (P <0.01) were observed between one and two years aged seeds after accelerated ageing. The highest percent of normal seedlings (37.1%) was recorded in one year aged seeds compared to two years aged (19.8%) samples. With increased age of seeds in storage there was proportional increase in seed deterioration rates. This might indicate how natural and artificial seed ageing affect seed and other end use quality in malting barley. In general, natural as well as artificially seed aged under prolonged storage were perceived to be a leading yield and quality reducing factor by decreasing rate of seedling emergence and stress survival ability of malt barley in the field. Therefore, it is necessary to use freshly harvested seeds in order to ensure satisfactory yield attainment in the field and have better quality barley grain for malting industry.

Key words: Production site, seed age, artificial seed ageing, malt barley.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the most important crop often grown in areas with low rainfall where other crops such as wheat fail to grow (Whabi and Gregory, 1989). To ensure food security, standardized living condition and bring general economic development of the country, the production and productivity of the farm should be increased in Ethiopia. Hence, agricultural production and productivity increment certainly lead to the issue of quality seed. The use of improved seeds on cultivated area is very low level which is less than 3% of the total cultivated area (CSA, 2007). In case of malt barley, most of the demand for malt is compensated met through imports, which accounts for 69% of the total annual requirement (ORDA, 2008a).Currently, barley production in Ethiopia covers about 1.04 million ha with annual production of 15.9 million quintals (CSA, 2011). The share of malting barley production is quite low (about 2%) in the country (Birhanu et al., 2005), despite the fact that the country has conducive environment and potential market opportunity which is 2-3 fold of their current market volume (Getachew et al., 2006).

MATERIALS AND METHODS

Laboratory experiment was conducted at Asella Seed Quality Control Centre and Seed Quality Testing Laboratory of the National Seed Industry Agency to determine the effects of production sites and seed age on seed quality traits in malt barley. 3 × 2 × 2 factorial experiment consisting 3 potential malt barley collection sites [Debrebrihan Agricultural Research Center (DARC), Ethiopia Seed Enterprise (ESE) and Oromia Seed Enterprise (OSE)] with varying altitudes and soil types which most likely affect malt barley quality requirements, 2 seed ages (seeds harvested in 2009 and 2010) and 2 malt barley varieties (Beka and Holker) was laid out in CRD with four replications. Seed lots of malt barley varieties Beka and Holker (obtained from Debrebrihan Agricultural Research center, Ethiopia Seed Enterprise and Oromia Seed Enterprise) which were harvested in the years 2009 and 2010 were used for the experiment. Two years aged seed samples were harvested in the years 2009 while one year aged seed samples were harvested in the year 2010. Both one and two years aged seed samples were stored for one year under room temperature condition. For standard germination and other vigor parameter test, 100 seeds randomly drawn from 400 seeds were used as per the standard of laboratory procedures.

RESULTS AND DISCUSSION

Seed moisture content for collected samples

Average percentage of moisture content was determined for each of the treatments in collected and harvested samples. The results of the difference between replications for all the treatments were fallen in the tolerance level for barley (< 0.02 %) stipulated by ISTA (2008). The three ways interaction effect of seed age by site by variety depicted highly significant (P≤ 0.01) difference for collected samples while significant (P≤0.05) differences were recorded for harvested samples (Appendix Table 3). Accordingly, the highest mean value was recorded in two years age Holker (12.12%) collected from DARC which is not statistically different from two years aged Holker (12.07 %) from OSE. The lowest mean value was recorded in one year aged Beka (11.17 %) collected from DARC which is not statistically different from one year aged Beka variety (11.12 %) collected from ESE (Table 1).

Seed moisture content for harvested samples

In the harvested samples, the highest value of moisture content was recorded in one year aged Holker from ESE (12.53%) followed by two years aged Beka from OSE (11.57%). Conversely, the lowest moisture content was recorded in two years aged Beka (9.65%) from ESE (Table 2). Even though there were statistical differences among the tested parameters, each observed values were within tolerable range of moisture content set for barley (Appendix Table 1). This might show us that seeds utilized for this experiment were harvested at appropriate and safe moisture level. Moisture is the most critical factor which determines the storability and longevity of seeds. In orthodox seeds like barley, 1% difference in moisture content shortens the life span of seeds by half (ISTA,1985).

Thousand kernel weight for collected samples

Highly significant (P≤ 0.01) variations for thousand kernel weight were observed among varieties due to main effect of sites and seed age (Appendix Table 3). Accordingly, the highest TKW was recorded in one year aged Holker variety (39.10 g) collected from ESE compared to two years aged Holker under same source (33.90 g) and one year aged Holker collected from DARC (32.4 g). The least value of TKW was recorded in two years aged Beka variety (23.0 g) from ESE (Table 2). This is in agreement with the work of Ihsan et al. (2005), Ajmal et al. (2000) and Grzesiak (2001) who reported decrease of TKW with increased seed age in maize. Similarly, Rukavina et al. (2002) also reported significant difference in TKW among three barley varieties as seed age increases. Hence, difference in maintaining seed quality among cultivars might be due to their various genetic potential while increased storage life is compensated by decrease in thousand seed weight.

Thousand kernel weight for harvested samples

Regardless of seed source, main effect of seed age and variety exhibited highly significant (P≤) difference on

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Table 1. Interaction effect of seed age x site x variety on MC, TW and TKW for collected and harvested samples.

Treatments			Parameters			
Age	**Site**	**Variety**	**MCc**	**MCh**	**TKWc**	**TKWh**
Year 1	DARC	Beka	11.17	11.15	29.35	30.35
	DARC	Holker	11.36	11.27	32.95	37.70
	ESE	Beka	11.12	11.32	22.60	31.80
	ESE	Holker	12.04	12.53	37.50	39.05
	OSE	Beka	11.47	11.33	28.25	29.15
	OSE	Holker	11.54	11.36	32.00	30.45
Year 2	DARC	Beka	11.90	11.05	24.50	28.25
	DARC	Holker	12.12	11.37	29.50	32.35
	ESE	Beka	11.86	9.65	22.60	26.25
	ESE	Holker	11.91	11.08	34.55	31.20
	OSE	Beka	11.67	11.57	33.60	28.65
	OSE	Holker	12.07	11.00	24.85	31.65
LSD (0.05%)			0.13	0.32	5.11	8.66
CV (%)			1.26	2.10	7.75	12.53

DARC= Dabrebrihan Agricultural Research Center ; ESE=Ethiopia Seed Enterprise ; OSE= Oromia Seed Enterprise ; MCc= Moisture content for collected samples ; MCh= Moisture content for harvested samples ; TWc= Test weight for collected samples ; TWh = Test weight for harvested samples ; LSD (0.05%) = Least significant difference at 5% probability level ; CV (%) = Coefficient of Variation.

Table 2. Main effect of seed age, site and variety on SG (%) and vigour parameters for collected samples.

Treatments	Parameters						
	SG	**SPG**	**RL**	**SHL**	**SDW**	**VI**	**VII**
Age							
Year 1	95.25	16.85	13.82	11.15	0.19	2330.08	18.11
Year 2	92.50	15.14	13.94	11.28	0.18	2333.92	16.82
P<0.05	1.22	0.99	ns	ns	ns	ns	ns
Site							
DARC	91.00	16.98	13.82	11.33	0.18	2288.70	16.51
ESE	95.13	15.74	12.94	11.29	0.18	2284.52	17.65
OSE	95.50	15.27	14.08	11.03	0.19	2422.80	18.24
P<0.05	1.49	1.21	ns	ns	ns	118.9	ns
Variety							
Beka	93.25	16.32	13.24	10.67	0.17	2229.80	16.10
Holker	94.50	15.66	13.99	11.75	0.19	2434.20	18.83
P<0.05	1.22	ns	ns	0.46	0.02	97.08	1.78
CV(%)	2.21	10.41	14.04	6.91	0.02	7.60	18.09

SGc= standard germination, SPGc =speed of germination, RL (cm) = Root Length, SHL = Shoot length (cm), SDW =Seedling dry weight (mg), VI = Vigor index one, VII=Vigor index two, LSD (0.05%) = Least significant difference at 5%, CV (%) = Coefficient of Variation.

thousand kernel weight (Appendix Table 1). Hence, the highest TKW was recorded in one year aged Holker variety from ESE (37.80 g) which is not statistically different from one year aged Holker from DARC (37.57 g). The least value of TKW was recorded in two years aged Beka variety (23.0 g) from ESE (Table 2). This result contradicts the standard set for TKW by local maltsters since it does not exceed 40 g (Tadesse, 2003). However, according to the Ethiopian quality standard the acceptable TKW and TW (hectolitre weight) for raw

Table 3. Main effect of seed age, site and varieties on purity components (%) for collected and harvested samples.

Treatments	Collected				Harvested			
	PS	OCS	WS	IM	PS	OCS	WS	IM
Age								
Year 1	99.47	0.18	0.01	0.45	99.08	0.21	0.18	0.39
Year 2	99.35	0.17	0.01	0.33	99.02	0.34	0.31	0.23
P<5%	ns	ns	ns	ns	ns	ns	ns	0.15
Site								
DARC	99.24	0.18	0.01	0.54	98.90	0.16	0.40	0.32
ESE	99.55	0.12	0.01	0.32	99.40	0.14	0.11	0.30
OSE	99.29	0.32	0.01	0.31	98.87	0.53	0.21	0.30
P<%	ns	ns	ns	ns	Ns	0.17	0.20	ns
Variety								
Beka	99.33	0.20	0.01	0.35	99.15	0.33	0.20	0.30
Holker	99.34	0.22	0.01	0.42	98.96	0.23	0.29	0.38
P<5%	ns	ns	ns	ns	Ns	ns	ns	ns
CV(%)	0.14	13.50	4.29	11.89	0.35	13.13	4.29	11.93

Number in parenthesis indicates square root transformed mean value. PS=Pure seeds; OCS=other crop seeds; WS=weed seeds; IM=Inert matter; LSD (0.05%)=least significant difference at 5% ; CV (%)=Coefficient of Variation.

barley are in the range 25–35 g and 48–62, respectively (EQSA, 2006). Therefore, the result exhibited by the present study has never been beyond acceptable levels of TKW for malt except for aged seeds. With increased seed age there was a proportional decrease in TKW. It is related to size which is equally an important malting quality parameter because aged seeds loose aggregates of grain composition leading to less carbohydrate, less grain weight, and low malt extract (Zhao et al., 2006). Reduction in the final TKW due to seed age is linearly related to the reduction in starch content (Savin et al., 1997). If seeds having high TKW are used for planting purpose, it will give good stand establishment which could give witness for high biomass in final harvest while seeds with low TKW will give low biomass. Hence, it is more important than the relative growth rate in determining early plant biomass in wild barley (Van Rijn et al., 2000), good TKW is much more important than the relative growth rate in determining early plant biomass in wild barley. In similar fashion López-Castaneda et al., (1996) reported that, from the major factors responsible for variation in early vigour in barley, wheat, and oat, embryo size was found to be the most important one.

Physical purity for collected samples

Analysis of variance showed that neither the independent nor the interaction effect of seed age, site and variety exhibited any significant effect on purity components for collected samples of malt barley (Appendix Table 1). This might tell us that under each collection sites varieties were well maintained and met minimum physical purity requirements under laboratory condition for barley seed certification set by quality and standards authority of Ethiopia. In agreement to this, National Seed Industry Agency (NSIA, 1997) stated that minimum seed physical purity must meet 97% pure seed, 0.2% other crop seed, 0.1% weed seeds and 2% inert matter respectively for certified seed. Hence, the collected samples used for physical purity determination was not beyond the minimum physical purity standard (Table 3).

Physical purity for harvested samples

Sites posed highly significant (P≤0.01) difference in OCS (%) for harvested samples (Appendix Table 2). Main effect of seed age was found to be significant (P≤0.05) in weed seeds (%) and inert matter (%). Similarly, the main effect of site was significantly (P≤0.05) affected by WS (%). To this effect, the highest value of other crop seeds (%) was recorded in samples from OSE (0.53%) followed by DARC (0.16%) which is not statistically different from ESE (0.146%). The highest mean value of WS (%) was recorded in samples from DARC (0.40%). Statistically the highest mean value of IM (%) was recorded in samples from DARC (0.32%) (Table 3). Seed physical characteristics are influenced by many environmental factors. In general, this variation might be attributed to the surrounding environment in the field especially how mother seed was managed and maintained in storage. Therefore, it gives the impression to develop management practices pertinent to maintain on-farm seed quality control trend that include thoroughly cleaning all seed production field and machinery used for seed cleaning, storage and clearly communicate these practices to all farm employees involved.

Standard germination and vigour parameters for collected samples

The independent effect of seed age and site had showed highly significant (P ≤ 0.01) difference in standard germination (SG %) and speed of germination (SPG). Likewise, site and variety had exerted significant (P ≤ 0.05) effect on SG (%) and SPG respectively (Appendix Table 5). Similarly, variety posed highly significant (P ≤ 0.01) difference in shoot length (SHL), vigour index (VI) and VII except for SDW which was significantly (P ≤ 0.05) affected. Hence, higher value of SG (%) was recorded in one year aged samples (95.25%) compared with two years aged samples (92.50%). This is consistent with the work of Abdalla and Roberts (1969) where slow germination rate from aged seeds resulted in poor stand establishment and delayed flowering in the field and uneven germination during malting. Moreover, consequence of seed physiological aging progressively had reduced germination rate, and did increase abnormal seedlings and dead seed (ISTA, 1981; Wang and Hampton, 1990).

Greater SPG was recorded in one year aged samples (16.85) compared with two years aged seed samples (15.14). In agreement to this result, Turk and Tawaha (2002) had observed increased germination percentage and greater speed of germination in freshly harvested seeds as compared to old seeds in barley. The highest SG (%) was recorded in samples collected from OSE (95.5%) which is not statistically different from ESE (95.13%) and followed by DARC (91 %). Similarly, the highest SPG was recorded in samples collected from DARC (16.98) followed by ESE (15.74) and OSE (15.27), respectively.

Likewise the highest value of vigour index (VI) was recorded in sample collected from OSE (2422.80) followed by DARC (2288.69) and ESE (2284.52) respectively. From the result of analysis of variance, high SG (%), SHL (cm), SDW (g), VI and VII were recorded in Holker as compared to Beka variety (Table 2). Hence, it can be concluded that different varieties of malt barley with different storage periods possess different levels of seed vigour. In line with this, Kotze (2009) reported that choice for vigour seed is an important decision for farmers to have good stand establishment in the field beside factors determining cultivar choice for profitable malting barley production. In similar fashion, Berzy et al. (2013) suggested that as duration of seed storage increases, there is an equivalent decline in the vigour of the seed lots, resulting in retarded germination, slow development and shorter seedlings under stress conditions in maize.

CONCLUSION AND RECOMMENDATION

Highly significant (P < 0.01) variation was observed among the tested seed quality traits. Two years aged seed samples revealed the highest percentage of abnormal seedlings and dead seeds before accelerated ageing under standard germination test. Similarly, artificially aged seeds of malt barley displayed a marked decline in germination percent as compared to newly harvested one. Highly significant differences (P <0.01) were observed between one and two years aged seed samples after accelerated ageing. The highest percent of normal seedlings (37.1%) were recorded in one year aged seeds as compared to two years aged (19.8%) samples. As seed age increased, there was proportional increase in seed deterioration rates. This can best indicate how natural and artificial seed ageing negatively affect seed and other end use quality in malting barley. In general, natural seed aging due to prolonged storage duration were apparent to be a leading yield and end use quality reducing factor by decreasing rate of seedling emergence and stress survival ability of malt barley in the field and grain after harvest. Therefore, it is necessary to use freshly harvested seeds in order to ensure satisfactory yield attainment in the field and have better quality barley for malting and brewery industries.

Conflict of interests

The authors have not declared any conflict of interest.

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Appendix Table 1. Analysis of variance for TKW, MC and TW for collected and harvested samples.

Sources	df	Mean Squares					
		Thousandkernel weight		Seed moisture content		Test weight (kg/hl)	
		TKWc	TKWh	MCc	MCh	HLWc	HLWh
Replication	1	1.900ns	13.253ns	0.280ns	0.0003ns	8.729ns	0.632ns
Seed age	1	98.34**	105.06**	1.325**	1.744**	20.020**	130.020**
Site	2	22.75*	14.96ns	0.017ns	0.058ns	1.583ns	10.937**
Variety	1	206.88**	163.41**	0.564**	1.088**	1.687ns	13.020**
Agex Site	2	19.79*	15.12ns	0.111**	1.573**	1.083ns	10.020**
Age x Variety	1	10.56ns	1.40ns	0.045*	0.005ns	0.187ns	9.187*
Site x Variety	2	84.61**	26.28ns	0.048**	1.32**	7.000*	4.396*
Age x Site x Variety	2	38.13**	1.31ns	0.197**	0.113*	14.250**	14.813**
Error	11	(22) 5.67	(22) 11.4	(11) 0.005	(11) 0.020	(33) 1.65	(33) 1.28

TKWc= Thousand Kernel Weight for Collected Samples; TKWh= Thousand Kernel Weight for harvested samples; MCc= Moisture content for collected samples; MCh = Moisture Content for Harvested Samples; HLWc=Hectolitre Weight for Collected Samples; HLWh=Hectolitre Weight for Harvested Samples; Number in parenthesis indicate error degree of freedoms as same replication was not used across all parameters, (*) single astrix shows significant difference (P≤0.05) while (**) shows highly significant difference at (P≤0.01).

Appendix Table 2. Effects of seed age, sites and variety on physical purity of collected and harvested samples.

Sources	Df	Mean squares							
		Collected samples				Harvested Samples			
		PS	OCS	WS	IM	PS	OCS	WS	IM
Replication	1	0.150 ns	0.266 (0.024) ns	0.002 (0.00 1) ns	0.001 (0.0002) ns	0.162ns	0.150 (0.0001) ns	0.0003 (0.021) ns	0.181(0.001)ns
Seed Age (A)	1	0.062ns	0.010 (0.010) ns	0.001 (0.001) ns	0.085 (0.025) ns	0.021 ns	0.098 (0.001) ns	0.100 (0.012) ns	0.150 (0.001) *
Site (S)	2	0.170 ns	0.030 (0.025) ns	0.003 (0.001) ns	0.135 (0.039) ns	0.735ns	0.370 (0.002) **	0.176 (0.023) *	0.0002 (0.001) ns
Variety (V)	1	0.010 ns	0.002 (0.001) ns	0.002 (0.001) ns	0.035 (0.010) ns	0.218 ns	0.062 (0.001) ns	0.051 (0.002)ns	0.002(0.001) ns
A x S	2	0.110 ns	0.016 (0.015) ns	0.002 (0.002) ns	0.101 (0.029) ns	0.290 ns	0.075 (0.001) ns	0.095 (0.013) ns	0.066 (0.002) ns
A x V	1	0.020ns	0.001 (0.0001) ns	0.003 (0.002) ns	0.055 (0.018) ns	0.015ns	0.056 (0.001) ns	0.263 (0.002) ns	0.082(0.003) ns
S x V	2	0.090 ns	0.044 (0410) ns	0.006 (0.002) ns	0.014 (0.002) ns	0.698 ns	0.309 (0.001) **	0.162 (0.044) *	0.004(0.003) ns
A x S x V	2	0.050 ns	0.029 (0.027) ns	0.001 (0.001) ns	0.021 (0.007) ns	0.546 ns	0.250 (0.0003) **	0.001 (0.027) ns	0.016 0.002 ns
Error	11	0.068	0.012	0.001	0.047	0.444	0.026	0.035	0.028

Numbers in the parenthesis indicate transformed mean square values; h= harvested seed samples from experimental site; c= collected samples from different growing sites; (*) single astrix shows significant difference (P≤0.05) while (**) shows highly significant difference at (P≤0.01).

Appendix Table 3. Effects of seed age, sites and varieties on standard germination for collected samples.

Sources	Df	Mean squares			
		Nsc (%)	**Absc (%)**	**Fsc (%)**	**Dsc (%)**
Replication	3	7.020ns	2.277ns	1.576ns	3.361ns
Seed age	1	266.02ns	4.08**	0.75ns	2.52*
Site	2	21.33ns	0.90ns	1.02ns	3.00**
Variety	1	2.52ns	2.08*	0.75ns	9.18**
Age x Site	2	8.34ns	3.27**	4.94**	2.58*
Age x Variety	1	25.52*	16.33**	0.00ns	0.52ns
Site x Variety	2	10.65ns	2.77**	2.31ns	0.25ns
Age x Site x Variety	2	19.15*	13.40**	0.56ns	0.58ns
Error	33	4.9	0.4	0.6	0.5

Nsc= Normal Seedlings for Collected Samples;Absc= Abnormal Seedlings; Fsc= Fresh Ungerminated Seeds; Dsc=Dead Seeds; (*) Single Astrix Shows Significant Difference (P≤0.05) While (**) Shows Highly Significant Difference at (P≤0.01).