Effect of different storage environment on sorghum (*Sorghum bicolor* (L.) Moench.) seed vigor

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Abstract. The research to evaluate the effect of different storage environment to sorghum (*Sorghum bicolor*) seed vigor was conducted in seed laboratory of Indonesian Cereal Research Institute. The treatments were arranged in completely randomized design with 2 sorghum varieties (Numbu and Super 1) in two storage environment: warehouse storage and cool storage. Observation on seed moisture content, germination percentage, germination rate, shoot and root length, shoot and root dry weight, electric conductivity of seed leakage were carried out in every storage period (0, 4, 8, 12 months). At 12 month storage, Numbu variety showed higher increase of moisture content (34.44%) than that of Super 1 variety (24.18%). Germination percentage has still remained at 99-100% with germination rate of 31.1-32.2%/day until 4 months storage but signficantly decreased at 3% (8 months) and 9% (12 months) on Super 1 variety, decreased on Numbu variety to 4% (8 months) and 12% (12 months) in cool storage. In warehouse storage, at 4, 8 and 12 months storage, germination percentage significantly decreased to 9%, 19% and 25% respectively on Super 1 variety, while for Numbu variety the decrease was 10%, 24%, and 30% respectively. Electrolyte leakage has significantly increased with decrease of germination, germination rate, root length, shoot length, root dry weight, shoot dry weight in every period storage. Storing the sorghum seeds in cool storage showed lower decrease of its germination, germination rate, root length, shoot length and lower electrolyte leakage than in warehouse storage.
Key Words: storage, seed quality, sorghum, aging, vigor.

Introduction. Sorghum (*Sorghum bicolor* (L.) Moench.) grain in Indonesia was used as food in East Nusa Tenggara, South Sulawesi, Central Sulawesi, East and Central Java, and many other areas (ICERI 2018). In East Flores District of East Nusa Tenggara Province, sorghum grain was used as principle staple food. In many sorghum producing areas, productivity is still low, on average of 500-750 kg ha^{-1}.

Poor seed quality is one of the most important factors contributing to this low productivity. Sorghum seed industry is still not well developed in Indonesia. Farmers usually keep part of their produce to be used as seed in the following planting season, or in many cases, farmers may obtain seeds from the local market or from other farmers. They usually store sorghum seed in traditional storage facilities and different type of warehouses. Seeds were usually stored for varying lengths of time after harvest. Seed viability at the end of any storage period is the result of the initial viability at harvest, initial moisture content before storage, temperature and humidity of storage and length of seed storage (Arief et al 2013).

Factors affecting seed quality in the storage are environmental condition during seed production, seed viability at harvest, initial seed moisture content, mechanical damage during seed processing, packaging material, temperature and humidity in storage and biochemical injury of seed tissue (TeKrony et al 1987; Anfinrud 1997; Al-Yahya 2001). According to Copeland & McDonald (2001), for one year seed storage seed moisture content should be less than 11% and the temperature should not exceed 20^{o}C. Seed storage longevity is influenced by seed quality and storage conditions. Low initial seed quality, unfavourable storage condition (storage temperature and relative humidity), contribute to accelerating seed deterioration in storage. The effective storage period is difficult to asses because the storability of the seed is a function of initial seed quality and the storage conditions (Anfinrud 1997; Fabrizius et al 1999). Some tests such as tetrazolium, seedling growth rate and seedling dry weight, accelerated aging test, first count, speed of germination, cool germination test and cold test are used to estimate seed vigour (AOSA 2011). Electrical conductivity test termed as the bulk conductivity test, is now extensively used in Europe, Australia and New Zealand for garden pea (*Pisum sativum* L.) (Matthews & Powell 2006). Seed vigour may be reduced by damage to the embryo or seed coat during harvesting and processing. Discrepancies between germination capacity and field performance are not equal in different species. Discrepancies occur much more commonly than in cereals (Matthews & Powell 2006).

Beside the biochemical, cytological and genetic changes, there are a lot of external factors that can influence the seeds vigour. The integrity of the seed vessel is another factor that can affect the vigour of the seeds. From the injured seeds it is obtained a bigger percentage of abnormal germs (Roberts 1972). If the injuries affects only the pericarp, the decrease of germination is not too high; but in case a part of the endosperm falls off or it has deep rents, the energy and the germinative faculty decrease significantly, and when the embryo seed is affected it becomes totally improper, or even being dead (Gill & Delouche 1973).

The sanitary condition of the seeds can influence their vigour, because the seed may carry pathogens (Oroian 2008) or pest agents that can be found on the surface of the seeds, under the layer or even inside them. The seeds age can influence the capacity of germination and the seeds vigour, because during their ageing there are important changes, starting with the accumulation of delayer substances of raising, the metabolic decay of the provision substances, the denaturizing of the proteins and the lipids. The nutritive substances within the embryo that are necessary to a proper functioning of the respiratory system, which are usually found in small quantities, are fully consumed during the breathing process, and in case of dry seeds they cannot be replaced due to the lack of free water which allows hydrolyze and the transport between the cells (Bucurescu et al 1992). Handling during storage period can do physical damage to the seed quality (Goftishu & Belete 2014). Bad handling of seed during long term storage would make the seed deteriorated and their vigor will decrease during storage (Akter et al 2014).

Variations in humidity and temperature during the development and the maturation and improper conditions of harvesting and storing cause the natural and physical deterioration of the seeds, shortening their longevivity. Storing in good conditions of the seeds depends especially on the water they contain. If the storing humidity is as lower as possible, the storing can last longer, because the water is the deciding factor which determines the resistance while storing (Bucurescu et al 1992). Increased temperature and long storage period cause a decrease in the seed germination percentage; also the number of normal seedling will be drecreased, along woth the enzymes activity (Azadi & Younesi 2013).

Vigorous seeds will produce excellent emergence and stand in proper soil environment. It can improve the chances for satisfactory emergence. Vigor is often implied when discussing seed quality and most seed growers have to use the terms and quality and vigor interchangeably. Seeds vigor comprises those properties, which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions (Bewley 1997). The rapid and synchronous germination rate as well as good field establishment will be characteristic of vigorous seeds. The deterioration of stored seed is a natural phenomenon and the seeds tend to lose viability and vigor even under ideal storage conditions (Burris & Navratil 1979). The rate of seed deterioration varies greatly from one species to another and even among varieties of the same species. The performance capabilities of many seeds deteriorate due to variations in temperature, relative humidity and moisture content in storage (Abdul-Baki 1980).

The aim of this research was to evaluate the effect of storage environment to sorghum seed vigor.

Material and Method. The research was conducted under Laboratory of Indonesian Cereal Research Institute (ICERI), Maros, South Sulawesi, Indonesia, from July 2017 until August 2018. The treatments were arranged in completely randomized design:
A. sorghum variety: (A1) - Numbu, (A2) - Super 1;
B. storage duration: (B1) - without storage (0 month), (B2) - 4 months, (B3) - 8 months, (B4) - 12 months;
C. storage type/environment: (C1) - warehouse storage, (C2) - cool storage.

The treatments were replicated thrice. Statistical analysis according to Gomez & Gomez (1984). Means of studied factors were compared using Duncan's multiple range tests at 5% level of probability (Duncan 1955).

Storage type. Two storage type were used: (1) cool storage - room under controlled temperature with air conditioner and dehumidifier with average temperature between 18 and20^{o}C and relative humidity (RH) 50-55%, (2) warehouse storage - room with uncontroled temperature, with brick walls, alluminium and iron roof and cement floor with average temperature between 26 and 30^{o}C and RH 65-85%.

Seed preparation. Two seed lots of sorghum of Super 1 and Numbu varieties were prepared for storage into two storage environment. Fresh harvest sorghum from the field were dried and processed, seeds were then inserted into an airtight plastic recipient and tightly closed, ready for storing. Seeds weighed of 250 g each were obtained from the storage room for quality analysis at every storage period.

Determinations. All determinations for seed quality analysis were carried out according to International Rules for Seed Testing (ISTA 2006).

Moisture content. A number of 100 seed samples were ground and oven-dried at 130^{o}C for two hours. Calculations were based on the wet basis.

Germination. A number of 100 seeds from each treatment and each replication were germinated in rolled germination towels moistened with water in an incubator at 25^{o}C. The first count was made 5 days later. Normal seedlings were evaluated after 7 days. Germination percentage was expressed by the percentage of seed germinating normally after 7 days (ISTA 2006).

Germination rate. Data were obtained from substrate of seed germination test. Every observation time, total percentage of normal shoot is divided by day (24 hours). Cumulative day value is obtained when seeds are planted until the time of observation. The formula used as follows:

$$KT = \frac{(Xi\text{-}Xi\text{-}1)}{Ti}$$

where: KT = germination rate (%/day);
Xi = the percentage of normal seed day i;
Ti = time of observation (day).

Shoot length. About 10 normal seedlings were taken at random from each replicate at the end of standard germination test to evaluate shoot length (cm).

Root length. The same 10 normal seedlings of shoot length evaluation test were used to evaluate the root length (cm).

Shoot dry weight. Ten normal seedlings were taken and dried in a forced air oven at 110°C for 17 h (Agrawal 1986) to obtain shoot dry weight and expressed as grams.

Root dry weight. Ten normal seedlings were taken and dried in a forced air oven at 110°C for 17 h (Agrawal 1986) to obtain root dry weight and expressed as grams.

Electrical conductivity. Three replications of 50 seeds of each treatment were weighed and moisture content recorded. The seeds of each replication were placed in 200 mL beaker and 50 mL of deionized water was added. Seeds were stirred gently to ensure that all seeds were completely immersed and evenly distributed. The beakers were placed at temperature of 20^oC for 24 hours. The electrical conductivity of the leachates of each replication was measured by using a conductivity meter (sension 5) and conductivity per gram of seed weight was calculated (ISTA 2006):

$$\text{Conductivity } (\mu S\ cm^{-1}\ g^{-1}) = \frac{\text{conductivity reading- blank reading}}{\text{weight (g) of replicate}}$$

Results and Discussion. Initial seed moisture contents before seeds stored, for the two varieties, were almost the same, between 9.0 and 9.1%, there were no statistical differences (Table 1). The highest value of moisture content, 12.1% was recorded in one year storage on Numbu variety (Table 1). It showed that seed moisture content in the cool storage maintained between 8.8 and 9.1%. Conversely, in the warehouse storage the lowest moisture content was recorded at initial observation, and increased with increasing of storage duration for both varieties. Sorghum of Numbu variety showed higher increasing (34.44%) than that of Super 1 variety (24.18%) when stored for 12 months in warehouse (Table 1).

Table 1

Seed moisture content of sorghum of Super 1 and Numbu variety at different storage type and storage duration

Variety	*Storage type*	*Storage duration*			
		0	*4*	*8*	*12*
		Seed moisture content (%)			
Super 1	Cool storage	9.1^{g}	9.0^{h}	8.9^{i}	8.9^{i}
	Warehouse storage	9.1^{gh}	9.4^{f}	10.9^{c}	11.3^{b}
Numbu	Cool storage	9.0^{gh}	8.9^{i}	8.8^{i}	8.9^{i}
	Warehouse storage	9.0^{gh}	9.8^{e}	10.4^{d}	12.1^{a}

*) numbers followed by the same letters are not significantly different by DMRT at 1% level.

Moisture is the most critical factor which determines the storability and longevity of seeds. In orthodox seeds like sorghum, 1% difference in moisture content shortens the life span of seeds by half (ISTA 2006). Storage temperature and RH are two major external factors affecting storage duration and degree of seed deterioration. In this experiment, the average temperature of the cool storage was between 19 and 23^oC, and RH between 39 and 55%. In the warehouse, average room temperature was between 26 and 28^oC and RH between 65 and 89% (Table 2).

The warehouse storage in our study has no special temperature and humidity control. For longer and more reliable seed storage, facilities with specific temperature and RH control should be used. Seed moisture content is the most important determinant of longevity in storage. An important aspect from the perspective of chemical reactions in the seed is water activity, which means the chemical potential of water in the system (Basra 1995). According to Basra (1995), reduced capacity of old seed to absorb water seems to be associated with degradative changes in insoluble carbohydrates and protein macromolecules, which are primary components responsible for water absorption. When the initial water content in seed is low due to damage caused by unfavourable environmental conditions the rate of swelling is low, especially when water viscosity is increased.

Sorghum as orthodox seed, may be stored for several years if the seed moisture is maintained between 5 and 8% (Arief et al 2013). The seed moisture content depends on relative air humidity in the seed storage facility. Higher air humidity increases seed moisture content, leading to more rapid seed deterioration, particularly at the moisture content above 12%. Condition of seed storage and duration can significantly influence the quantity of water imbibed by the seeds (Vertucci & Leopold 1987). Higher storage temperature and higher seed moisture content may cause the appearance of fungal growth in storage inducing decrease of germination. Sometimes, seed deterioration during storage is manifested not as a significant decline in germinability, but as a loss of seed vigour (Timotiwu et al 2017).

Table 2

Average temperature and relative humidity of cool storage and warehouse storage during period of seed storage

Storage period (month)	Cool storage		Warehouse storage	
	Temperature (^oC)	Relative humidity (%)	Temperature (^oC)	Relative humidity (%)
1	20	51	27	85
2	21	44	26	89
3	21	41	27	86
4	19	40	28	81
5	20	40	28	78
6	20	39	27	83
7	20	45	27	77
8	21	48	27	72
9	19	52	28	65
10	20	49	28	71
11	23	46	28	82
12	21	55	27	85
Average	20.4	45.8	27.3	79.5

Germination of these two sorghum varieties in cool storage still remained at 99-100% with germination rate 31.1-32.2%/day until 4 months storage duration, but at 8 months, germination signficantly decrease 3% (8 months) and 9% (12 months) on Super 1 variety, and the decrease of Numbu variety was 4% (8 months) and 12% (12 months). At 4, 8, and 12 months storage, germination percentage significantly decrease 9%, 19% and 25% respectively on Super 1 variety, while in Numbu variety, higher decrease was significantly, 10%, 24%, and 30% at 4, 8, and 12 months storage respectively (Table 3).

Germination rate on the two tested varieties showed no significant decrease until 4 months storage, but at 8 and 12 months storage, germination rate decrease 1.6%, and 6.2% respectively for Super 1 variety in cool storage and 0.9% and 7.5% respectively for Numbu variety.

Table 3

Germination and germination rate of sorghum seed from different storage type and storage duration

Variety	Storage type	Storage duration (month)			
		0	4	8	12
		Germination (%)			
Super 1	Cool storage	99^a	99^a	96^b	90^d
	Warehouse storage	99^a	90^d	80^f	74^h
Numbu	Cool storage	100^a	100^a	94^c	88^e
	Warehouse storage	100^a	90^d	76^g	70^i
		Gemination rate (%/day)			
Super 1	Cool storage	32.1^a	32.1^a	31.6^c	30.1^e
	Warehouse storage	32.2^a	32.1^a	27.3^g	23.3^i
Numbu	Cool storage	32.2^a	32.2^a	31.9^b	29.8^f
	Warehouse storage	32.2^a	31.1^d	25.8^h	23.3^i

*) numbers followed by the same letters are not significantly different by DMRT at 1% level.

Shoot length, root length, shoot dry weight and root dry weight were significantly affected by storage environment and duration of storage. Root length and shoot length of seedlings showed significant decrease with longer seed storage. Stored sorghum seed for 12 months in warehouse storage showed higher decrease of shoot and root length compare to cool storage; Super 1 variety which stored in cool storage decrease its root length 11.5% and in warehouse storage the decrease was 30.9%, while in Numbu variety which stored in the cool storage, the decrease of root length was 18.9% and in warehouse storage, the decrease was 42% (Table 4). Higher percentage decrease of shoot length and root length of the seedlings which were stored in ware house indicated that vigor decreased.

Table 4

Root length, shoot length, root dryweight and shoot dryweight of sorghum of Super 1 and Numbu variety from different storage type and storage period

Variety	*Storage type*	*Storage period*			
		0	*4*	*8*	*12*
		Root length (cm)			
Super 1	Cool storage	11.15^{a}	11.03^{c}	10.20^{g}	9.87^{i}
	Warehouse storage	10.92^{d}	9.72^{k}	8.64^{m}	7.54^{n}
Numbu	Cool storage	10.91^{e}	10.90^{f}	9.80^{j}	8.84^{l}
	Warehouse storage	11.08^{b}	9.88	7.03^{o}	6.43^{p}
		Shoot length (cm)			
Super 1	Cool storage	6.08^{b}	5.90^{e}	5.72^{g}	5.12^{i}
	Warehouse storage	6.09^{a}	5.67^{h}	4.97^{m}	4.63^{o}
Numbu	Cool storage	6.03^{c}	5.86^{f}	5.43^{i}	5.01^{l}
	Warehouse storage	6.01^{d}	5.03^{k}	4.76^{n}	4.41^{p}
		Root dry weight (g)			
Super 1	Cool storage	0.382^{ab}	0.382^{ab}	0.326^{e}	0.295^{i}
	Warehouse storage	0.383^{a}	0.328^{d}	0.297^{h}	0.242^{l}
Numbu	Cool storage	0.382^{ab}	0.376^{c}	0.305^{f}	0.275^{j}
	Warehouse storage	0.381^{b}	0.302^{g}	0.264^{k}	0.225^{m}
		Shoot dry weight (g)			
Super 1	Cool storage	0.301^{b}	0.297^{c}	0.263^{f}	0.258^{g}
	Warehouse storage	0.300^{b}	0.264^{e}	0.237^{i}	0.220^{k}
Numbu	Cool storage	0.302^{a}	0.275^{d}	0.258^{g}	0.249^{h}
	Warehouse storage	0.301^{b}	0.258^{g}	0.224^{j}	0.213^{l}

*) numbers followed by the same letters are not significantly different by DMRT at 1% level.

Electrical conductivity of seed leakage was used to assess the degree of membrant leakage in the seed. Use of conductivity measurement of seed leachates to determine seed viability is based on Osterhout (1922) in McDonald & Nelson (1988) who established the relationship between cell death and release of electrolytes. During harvesting and processing, seed vigour may be reduced by damage to the embryo or seedcoat. In different species, germination capacity and field performance may not be equal, and it occurs much more commonly in cereals (Arief et al 2013).

The results showed that there was no significant difference between varieties at initial observation (before seed storage) of electrical conductivity of seed leakage, but at 4 month storage, electrolyte leakage increased significantly with seed age and different seed storage. Seed in the warehouse had an increase of electrolyte leakage higher than that of seed in cool storage. Super 1 variety has 35% increase electrolyte leakage for seed in cool storage after 12 months, while seed in warehouse storage has 65% increased. Numbu variety has 48% increase of electrolyte leakage for seed in cool storage while in the warehouse has 74% increase. The results also showed that Super 1 variety has lower electrolyte leakage comparing to Numbu variety in every storage period and in two storage environments (cool storage and warehouse storage) (Table 5). As seeds age, the seed membrane becomes more permeable, so many substances in seeds such as sugars, free amino acids, organic acids and various elements leach out in the presence of water. The concentration of seed leakage is measured by the electrical conductivity. The integrity of cell membranes can be considered to be the fundamental cause of the differences in seed vigour which are indirectly measured as electrolyte leakage during the conductivity test (Komalasari & Arief 2019). Seed lots with low electrolyte leakage are considered to be high in vigour under stressful conditions. Results showed that vigour loss symptoms in this research are reductions in germination rate and uniformity, increasing electrolyte leakage of membrane cell and inferior seedling emergence and growth.

Table 5

Electrical conductvity of seed leakage of sorghum of Super 1 and Numbu variety from different storage type and storage period

Variety	*Storage type*	*Storage period*			
		0	*4*	*8*	*12*
		Electric conductivity of seed leakage (uS cm^{-1} g^{-1})			
Super 1	Cool storage	9.45^{m}	11.45^{l}	12.67^{j}	14.46^{i}
	Warehouse storage	9.42^{m}	14.74^{g}	18.97^{d}	27.98^{c}
Numbu	Cool storage	9.44^{m}	12.24^{k}	14.66^{h}	18.22^{f}
	Warehouse storage	9.45^{m}	18.85^{e}	28.98^{b}	34.56^{a}

*) numbers followed by the same letters are not significantly different by DMRT at 1% level.

Conclusions. Sorghum seeds which stored in average temperature 20.4^{o}C and relative humidity 45.8% for 8 and 12 months decrease germination percentage 3 and 9% respectively on Super 1 variety and 4 and 12% on Numbu variety. Sorghum seeds which stored in warehouse with average temperature 27.3^{o}C and relative humidity 79.5% for 4, 8, and 12 months, significantly decrease germination percentage 9, 19 and 25% respectively on Super 1 variety and 10, 24, and 30% respectively in Numbu variety. Electrolyte leakage was signifinatly increased with decrease of germination percentage, germination rate, root length, shoot length, root dry weight, shoot dry weight in every observation period in the storage.

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