Indian Journal of Agricultural Sciences **91** (1): 123–7, January 2021/Article
https://doi.org/10.56093/ijas.v91i1.110940

Biochemical and physiological studies in ultra-dried seeds of cotton, safflower and groundnut during various storage conditions

DEEPSHIKHA DIXIT*, NEETA SINGH and ANKIT MALIK

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110 012, India

Received: 27 June 2020; Accepted: 17 July 2020

ABSTRACT

An experiment was conducted at National Bureau of Plant Genetic Resources, New Delhi to assess physiological and biochemical parameters to identify factors contributing to the loss of seed viability. Ultra-dried seeds of Cotton, Safflower and Groundnut stored at different MTS and ambient condition, viz. Cotton MTS 3.3%, Cotton Ambient 3.3%; Safflower MTS 6%, Safflower Ambient 3.8%; Girshell MTS, Gir Ambient 6.2% were used for present study. Result showed significant difference for all the physiological and biochemical traits as the seeds that were stored at MTS condition having high germination percentage, high vigour index, less moisture content, and lower Electrical conductivity (E.C.) and higher Dehydrogenase activity in comparison with seeds that were stored at Ambient condition. In SDS-PAGE profile of Cotton, Safflower and Groundnut some of the old bands were found to be missing at Ambient condition while some new bands were found to be appear in case of seeds that were stored at MTS condition. Deterioration was observed in seeds that were stored at Ambient condition. Whereas seeds that were stored at MTS condition were not deteriorated as much when compared to seeds that were stored at Ambient condition.

Key words: Ambient, MTS, Ultra-dried

The preservation of seed viability and proper storage of seed is an important sequel to the proper production of seed. It is of no use to produce good quality seed if the seed becomes worthless before it can be planted. Therefore the purpose of good storage is to preserve the high germination and vigour (healthy physiological status) of the seed from harvest to planting time. The two most important environmental factors which influence the physiological status (quality) are temperature and relative humidity for controlling seed moisture. In crops with orthodox seed storage behavior, seed moisture is high, therefore germination loss is more rapid (Umarani *et al.* 2015). A thumb rule to the effect of moisture content on seed ageing explained by Harrington (1959) by stating that for every decrease of 1% in seed moisture content, seed can be stored without seriously effecting the germination is almost doubled. Proper moisture content during storage ensures good germination and vigour of seeds.

High purity of seeds with respect to infestation and their germination capacity and vigour is also the most important factor contributing to maximum agriculture production per unit of land. Thus, during seed storage to make good quality of seeds, it is require preventing seeds from pests, disease and unfavorable weather conditions (Wimalasekera 2015, Maity and Pramanik 2013).

Ultra dry is one of the methods was first used to describe the seeds dried below 5% moisture content (fresh weight basis) and was originally intended for oily seeds, recognizing that in several cases the optimum moisture content for storage was below the 5% level recommended for seed gene banks. Subsequently ultra dry has been used to describe seeds dried to equilibrium at 10–12% relative humidity (RH) and 20°C (Bewley *et al.* 2013). The application of ultra dry conditions for seed storage was conceived as a means of maximizing the longevity of seeds in circumstances where facilities for low temperature storage were not available. Groundnut, cotton and safflower show large variability among its genotypes for seed germination, seedling vigour and storability during long term storage at ambient temperature. This obviates the need to find out suitable storage condition for these seeds to ensure minimum losses in seed quality and germination during storage due to ageing.

MATERIALS AND METHODS

Experimental material: Present experiment was conducted at National Bureau of Plant Genetic Resources Pusa Campus, New Delhi. During the present study, seeds of the three most important crops, cotton, safflower and groundnut, were obtained from Germplasm conservation unit, NBPGR, New Delhi, stored and managed under

*Corresponding author e-mail: dixit.deepshikha@gmail.com

different ultra dried MTS and ambient condition including Cotton (MTS 3.3%, Ambient 3.3%), Safflower (MTS 6%, Ambient 3.8%) and Groundnut (Girshell MTS, Gir Ambient 6.2%). Various physiological and biochemical parameters were recorded to find out the quality and germination in Ultra dried seeds.

Physiological parameters

Moisture content determination (%): Moisture content of seeds was determined by most commonly used method air–oven (ISTA 1995) based on the principle upon finding out the differences between initial weight and final weight of the seed after complete elimination of water from the seed under precisely controlled time and temperature conditions. The moisture content is expressed as % moisture content on dry weight basis.

$$M = \frac{M2 - M3}{M2 - M1} \times 100$$

$$= \frac{\text{Differences in weight of sample}}{\text{Intitial weight of sample}} \times 100$$

Where, M, Moisture content of sample; M_1, Weight of empty bottle with its lid; M_2, Weight of bottle with its lid and seed sample before drying; M_3, Weight of bottle with its lid and sample after drying.

Germination percentage (Paper towel method): Germination test was conducted by using 25 seeds sown in two replications on wet paper towel as a substratum and adequate moisture and a temperature of 20°C±2°C (ISTA 1995) was provided during germination.

Seedling vigour (cm): Ten seeds were placed with uniform arrangement on germination paper towels with two replications and placed vertically in a germination chamber maintaining adequate humidity and temperature (20°C). Observations for each of 10 seeds were recorded on the seventh day of sowing. The length of roots and shoots were measured in millimeter and average calculated on the basis of total no of seeds placed.

Vigour index: Vigour index on the seventh day of sowing was calculated by adopting method of Abdul Baki and Anderson (1973) as:

Vigour index = Root length + Shoot length (in mm) × Germination percentage

Biochemical parameters

Electrical conductivity (ms/cm/g): To measure electrical conductivity, ten undamaged seeds in three replications from each sample were taken and weighed, followed by soaked overnight in 10 ml distilled water (at 20±2°C). Electrical conductivity of leachate was measured using conductivity meter consisting a sensitive electrode.

Dehydrogenase activity: It is quick method to predict seed viability. Some seeds take weeks to month to germinate for these types of problems, we found out some other methods. Dehydrogenase activity was measured by colorimetric estimation of red colored formazon produced during the reaction of TTC with Dehydrogenase present in the seed (Kittock and Law 1968). In this experiment 10 seeds were taken in the three replications from each seed lot. Seeds were then soaked overnight in 10 ml distilled water at room temperature. Water was then decanted and seeds were dried. After removing the seed coat, the seeds were pierced with the help of a needle to facilitate the entry of TTC. The pierced seeds were then put in a beaker containing 5 ml of 1% TTC solution. The beaker was then covered with aluminium foil and kept in dark for 4 h in germinator at 30°C. After 4 hrs, the beakers were taken out and TTC solution decanted off. Seeds were then washed with distilled water for 2 min. Formazon was then extracted by using 5 ml of 2-methoxy-ethanol solution for each replication. The activity of dehydrogenase was then determined by estimating the intensity of the extracted colored solution at 470 nm (visible range) using spectrophotometer. 2- methoxy-ethanol was used as a blank.

Lipid peroxidation: This involves measuring the amount of a product of lipid peroxidation malondialdehyde (1, 3 - propandial) by the colorimetric (Health and Packer 1968). Ten seeds in two replications were weighed and allowed to imbibe water for 48 hrs by placing over wet filter paper placed in germinator at 20-25ºC. After 48 h, these were homogenized in 5 ml distilled water with a pinch of quartz powder using a pestle and mortar and then mixture was taken in centrifuge tube. Now 5 ml of 0.5% TBA (2-thio barbituric acid) and 20% TCA (Trichloro acetic acid) was added and the sample was incubated at 95ºC for 30 min in incubater. The reaction was stopped by placing the reaction tubes on ice. The samples were then centrifuged at 10,000 rpm for 10 min. The red colored supernatant was separated and recorded absorbance at 575 nm. The value for non- specific absorption at 600 nm was read and subtracted from this.

Soluble protein estimation by SDS-polyacrylamide gel electrophoresis

SDS is most widely used quantitative method for analyzing soluble protein mixture comparing the protein pattern between seeds of cotton, safflower and groundnut that were stored at MTS and ambient condition.

RESULTS AND DISCUSSION

Present investigation was based on to assess seed quality studying the physiological and biochemical parameters of Ultra-dried seeds of Cotton, Safflower and Groundnut at MTS and ambient condition.

Physiological parameters

Moisture content: In all the three crops, significant differences were observed for moisture content (Table 1). In cotton, moisture content of seeds that were stored at MTS condition, at 3.3% was 6.217, while at Ambient at 3.3% condition was 7.641 which was greater than MTS condition. Same trend was found in safflower and Groundnut as on MTS condition moisture content of seeds was 5.434 and 6.519% respectively and on ambient condition it was

Table 1 Physiological parameters at different MTS and Ambient condition of cotton, safflower and groundnut

Ultra dried seeds	Moisture content (%)	Germination percentage in normal seedlings	Seedling vigour		Vigour index
			Root length (nm)	Shoot length (nm)	
Cotton					
MTS 3.3%	6.217	80%	85.4	66.9	12184
Ambient 3.3%	7.641	68%	74.4	66.6	9588
Safflower					
MTS 6%	5.434	64%	171.1	91.9	16832
Ambient 3.8%	7.828	12%	0	0	12
Groundnut					
Girshell MTS	6.519	92%	73.2	31.3	9623
Gir Ambient 6.2%	7.291	36%	57.2	22.2	2858

7.828 and 7.291% respectively (Table 1). In the present study, moisture content in the seeds of all three crops was estimated higher that were stored at ambient condition as compared to storage under MTS condition in which seeds exhibited less moisture content possibly due to reduction in the values of relative humidity in the air of the environment over time (Filho 2016). Estimated higher moisture content in seeds under ambient condition as observed in our results, sometimes leads to seed deterioration because lack of control of temperature situations may increase the susceptibility of the material to latent damage, or even worsen deterioration, thus reducing the storage potential of the product and its physiological quality (Mbofung *et al.* 2013). Therefore, suitable range of the temperatures should be maintained during storage to avoid damage to the seeds, when they are more dried. So that seed quality could be precisely sustained for long storage of seeds.

Germination Percentage: Significant differences were observed for germination percentage between MTS and Ambient condition of Cotton, Safflower and Groundnut. In cotton Germination percentage of seeds at MTS condition, 3.3% was 80% and that were stored at Ambient condition, 3.3% was 68% which was lower when compared to seeds stored at MTS condition having 80% Germination percentage. In safflower and Groundnut also germination percentage was higher in MTS condition 64 and 92% respectively when compared with ambient condition 12 and 36% respectively (Table 1). Seed germination strongly influenced by temperatures in comparison with other environmental factors under ambient condition (Rosbakh and Poschlod 2015). In the present investigation, when seeds were stored at different ultra dried MTS and ambient conditions, germination percentage exhibited lower at Ambient condition as compared to MTS condition may be caused by temperature fluctuation which rapidly deteriorate seed viability. Thus, both the Ambient and MTS conditions indicate that germination of crop seeds have distinct physiological responses to temperature.

Seedling Vigour: Significant effect of MTS and Ambient condition were found on root and shoot length in all the crops. In cotton Root and shoot length of seeds that were stored at MTS condition, 3.3% was 85.4 mm and 66.9 mm respectively which was higher as compared to seeds that were stored at Ambient condition, 3.3% having root and shoot length 74.4 mm and 66.6 mm. Most interestingly in safflower root and shoot length of seeds at MTS condition, 6% was 171.1 mm and 91.1 mm respectively while at Ambient condition, 3.8% was 0 mm and 0 mm, there was no germination at al. In groundnut also root and shoot length of Girshell MTS was 73.2 mm and 31.3 mm respectively and at Ambient condition 6.2% was 57.2 mm and 22.2 mm respectively (Table 1).

Vigour Index: Vigour index was also found higher at MTS condition in all the three crops. Vigour index of cotton seeds that were stored at MTS condition 3.3% was 12184, and at Ambient condition was 9588, which was much less as compared to MTS condition seeds vigour index. In Safflower and Groundnut also vigour index of seeds at MTS condition was 16832 and 9623 respectively and at ambient condition was 12 and 2858 (Table 1). Standard laboratory germination test performed earlier is not indicative of field emergence potential. Therefore, vigour test also carried out to verify germination test result and to find out if the seeds showing high germination percentage in laboratory will also show better emergence in the field as well. Vigour index need to be evaluated to increase performance of a seed lot in storage (Khan *et al.* 2017). Prior to seeds in all three crops that were stored at ambient condition, vigour index and germination was observed to be highest for seeds that were stored at MTS condition. This observation was in conformity with earlier studies of Copeland and McDonald (1995). The results confirm that cotton, safflower, groundnut seeds that were stored at ambient condition have lower germination and vigour and the field emergence will be lesser in case of these seeds as compared to seeds that were stored at MTS condition. This can be attributed to deterioration or faulty synthesis of those protein and enzyme during this ambient condition time which is required to germination (Kapoor *et al.* 2010).

Biochemical Parameter

Electrical conductivity and dehydrogenase activity: Electrical conductivity (E.C.) of seed is an index of vigour status of the seed especially in case of some leguminous seeds. A poor quality seed shows high E.C. due to membrane disruption, which leads to excess leakage of solutes, electrolytes into surrounding medium, thereby increasing its E.C (Nawaz *et al.* 2013). In the present study significant differences were observed for electrical conductivity and dehydrogenase activity between MTS and Ambient condition of cotton, safflower and groundnut (Table 2). The deterioration had certainly occurred due to ageing at membrane level, due to which electrical conductivity was higher in seeds that were stored at ambient condition, when compared to seeds that were stored at MTS condition

Table 2 Estimation of Electrical conductivity and Dehydrogenase activity at both MTS and Ambient condition in seeds of Cotton, Safflower and Groundnut

Ultra dried seeds	Electrical conductivity (ms/cm/gm)	Dehydrogenase activity (Absorbance at 470 nm)	Lipid peroxidation		
			Absorbance at 575 nm	Absorbance at 600 nm	Differences
Cotton					
MTS 3.3%	0.493	1.275	3.448	2.729	0.719
Ambient 3.3%	0.569	1.213	4.500	3.927	0.573
Safflower					
MTS 6%	1.081	0.845	0.056	0.032	0.023
Ambient 3.8%	1.129	0.302	0.042	0.030	0.012
Groundnut					
Girshell MTS	0.171	1.586	2.564	2.351	0.213
Gir Ambient 6.2%	0.296	1.317	1.685	1.497	0.187

exhibiting lower EC which reduces seed viability during storage (Fotouo *et al.* 2015). Seed ageing and bed storage condition is characterized by oxidative degradation of unsaturated lipid fractions rendering the cell membrane more permeable upon imbibition. Therefore, deteriorated seeds would leach more than the vigorous seed into the surrounding medium due to localized cellular autolysis (Dawidowicz-Grzegorzewska and Podstolski 1992). This was in agreement with current findings of E.C in the seeds (cotton, safflower, groundnut) stored at ambient condition was higher due to more leakage and lower in seeds that were stored at MTS condition.

Lipid Peroxidation: In the present experiment, Lipid peroxidation was lower in all the three crops at 600 nm than at 575 nm (Table 2). Lipid peroxidation is major causes of seed ageing under accelerated ageing conditions (Priestley 1986, Wilson and McDonald 1986, McDonald 1999). Lipid peroxidation of seeds stored at MTS condition was higher and the seeds that were stored at ambient condition shown lower because Ambient condition caused more deterioration of seeds and their enzyme activity also decreased as compared to seeds that were stored at MTS condition and also disturb the normal biochemical process (Murthy *et al*. 2003).

Qualitative Protein estimation (SDS-PAGE): In SDS-PAGE profile of Cotton, Safflower and Groundnut some new bands were observed and some bands were considered missing when compared to ambient condition. In SDS-PAGE profile Of Cotton, At Rm value 0.2654 presence of new band was observed at MTS condition. At Rm values 0.4247 and 0.7345, band was not present in Cotton Ambient. One band was also missing in Cotton Ambient at Rm value 0.8672. At Rm values 0.3362, 0.8318, 0.8938 and 0.9292 band was present in both MTS and Ambient, while, when observed SDS-PAGE profile of Safflower, at Rm value 0.2831 presence of new band was observed in

Table 3 Protein (SDS-PAGE) scoring pattern of groundnut, cotton and safflower

Band No.	Rm value	Groundnut girshell MTS	Groundnut Gir Ambient 6%	Cotton MTS 3.3%	Cotton Ambient 3.3%	Safflower MTS 6%	Safflower Ambient 3.8%
1	0.0619	+	-	-	-	-	-
2	0.2035	-	-	-	-	+	+
3	0.2654	+	+	+	-	+	+
4	0.2831	-	-	-	-	+	-
5	0.3362	-	-	+	+	+	+
6	0.4247	+	+	+	-	+	+
7	0.4955	+	+	-	-	+	+
8	0.5309	+	+	-	-	+	+
9	0.7345	-	-	+	-	+	+
10	0.8141	+	+	-	-	+	+
11	0.8318	-	-	+	+	-	-
12	0.8672	+	+	+	-	-	-
13	0.8938	+	-	+	+	+	+
14	0.9292	+	+	+	+	-	-

MTS condition. In SDS-PAGE profile of Groundnut at Rm value 0.0619 presence of new band was observed in MTS. At Rm value 0.8938 band was not present condition in Groundnut Ambient (Table 3). In SDS-PAGE profile, differences in banding patterns of seeds that were stored at MTS and Ambient condition were significantly differ, which help to detect the extent of deterioration of a seed. In SDS-PAGE profile of Cotton, Safflower and Groundnut some of the old bands were found to be missing while some new bands were found to be appear in case of seeds that were stored at MTS condition. This could be attributed to increased activity of proteolytic enzymes or development of lesions in the component of RNA and protein synthesis system of cells during storage of seed in ambient condition (Raz *et al.* 2001, Locascio *et al.* 2014).

The main external factors causing seed deterioration during storage are high temperature, R.H. and O_2/CO_2 concentration which increase the respiration rate causing seeds to deteriorate more rapidly (Goel *et al.* 2003, Kapoor *et al.* 2010) and therefore, possibility to regulate these factors makes the basis for longer seed storage (Mohammadi *et al*. 2011). In the present study Cotton, Safflower and Groundnut seeds that were stored at MTS condition were viable with high vigour. However deterioration was observed in seeds that were stored at Ambient condition. Whereas seeds that were stored at MTS condition were not deteriorated as much when compared to seeds that were stored at Ambient condition.

REFERENCES

Abdul-Baki A and Anderson J D. 1973. Vigor determination in Soybean seed by multiple criteria. *Crop Science* **13**: 630–33.

Antonella Locascio, Irma Roig-Villanova, Jamila Bernardi and Serena Varotto. 2014. Current perspectives on the hormonal control of seed development in Arabidopsis and maize: a focus on auxin. *Frontiers in Plant Science* **5**: 412.

Aniruddha Maity and Pragati Pramanik. 2013. Climate change and seed quality: an alarming issue in crop husbandry. *Current Science* **105** (10): 1336–38.

Bewley J D, Bradford K J, Hilhorst H W M and Nonogaki H. 2013. *Seeds, Physiology of Development, Germination and Dormancy*. 3rd Edn. New York, NY: Springer.

Copeland L O and M B McDonald. 1995. *Principles of Seed science and Technology*. 3rd Edn. Chapman an Hall, New York, NY. 409pp.

Dawidowicz-Grzegorzewska A and Podstolski A. 1992. Age-related changes in the ultrastructure and membrane properties of *Brassica napus* L. seeds. Annals of Botany **69**: 39–46.

F A Khan, S A Bhat, Sumati Narayan, Rifat Maqbool, Imtiyaz Murtuza and F U Khan. 2017. Seed deterioration and priming - An Overview. *SKUAST Journal of Research* **19**(1): 12–21.

Filho C P H, Goneli A L D, Masetto T E, Martins E A S, Oba G C and Siqueira V C. 2016. Quality of second crop soybean submitted to drying and storage. *Pesquisa Agropecuária Tropical* vol) 46, p. 267–75.

Fotouo-M H, du Toit E S and Robbertse P J. 2015. Germination and ultrastructural studies of seeds produced by a fast-growing, drought-resistant tree: implications for its domestication and seed storage. AoB PLANTS 7: plv016.

Goel A, Goel A K and Sheoran I S. 2003. Change in oxidative stress enzymes during artificial ageing in cotton (Gossypium hirsutum L.) seeds. *Journal of Plant Physiology* **160**(9): 1093–1100.

Harrington J F. 1959. The value of moisture resistant containers in vegetable seed packaging. *California Agricultural Experiment Station Bulletin* **792**: 1–23.

Health R L and Packer L. 1968. Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* **125**: 189–98.

ISTA.1995. *Handbook of Vigour Test Methods.* J Hampton and D M Tekrony (Eds). International Seed Testing Association, Ziirich. Switzerland.

Kapoor N, Arya A, Siddiqui M A, Amir A and Kumar H. 2010. Seed deterioration in chickpea (Cicer arietinum L.) under accelerated ageing. *Asian Journal of Plant Science* **9**(3): 158–62.

KIttock D L and Law A G. 1968. Relationship of seeding vigor and TTC reduction by germination wheat seeds. *Argon Journal* **60**: 286.

Mbofung G C Y, Goggi A S, Leandro L F S and Mullen R E. 2013. Effects of storage temperature and relative humidity on viability and vigor of treated soybean seeds. *Crop Science* **53**(3): 1086–95.

McDonald M B.1999. Seed deterioration: physiology, repair and assessment. *Seed Science and Technology* **27**: 177–237.

Mohammadi H, Soltani A, Sadeghipour H R and Zeinali E. 2011. "Effect of seed aging on subsequent seed reserve utilization and seedling growth in soybean". *International Journal of Plant Production* **5**(1): 65–70.

Nawaz A, M Amjad, S M Khan, I Afzal, T Ahmed, Q Iqbal and J Iqbal. 2013. Tomato seed invigoration with cytokinins. *The Journal of Animal & Plant Sciences* **23**(1): 121–28.

Priestley D A. 1986. *Seed Ageing*. Ithaca, New York: Cornell University Press.

Raz V, Bergervoet J H, Koornneef M. 2001. Sequential steps for developmental arrest in Arabidopsis seeds. *Development* **128**: 243–52.

R Umarani, E Kanthaiya Aadhavan and M Mohamed Faisal. 2015. Understanding poor storage potential of recalcitrant seeds. *Current Science* **108**(11): 2023–34.

Sergey Rosbakh and Peter Poschlod. 2015. Initial temperature of seed germination as related to

species occurrence along a temperature gradient. *Functional Ecology* **29**: 5–14.

U M Narayana Murthy, Prakash P Kumar and Wendell Q Sun. 2003. Mechanisms of seed ageing under different storage conditions forVigna radiata(L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. *Journal of Experimental Botany* **54**(384): 1057–67.

Wilson D O and McDonald M B.1986. The lipid peroxidation model of seed ageing. *Seed Science and Technology* **14**: 269–300.

Wimalasekera R. 2015. *Role of Seed Quality in Improving Crop Yields*, pp 153-168. Hakeem K (Eds). Crop Production and Global Environmental Issues. *Springer Cham*.