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Investigations on Seed Viability and Vigor during Seed Ageing in Onion (*Allium cepa* L.) cv Bellary Red

Umesh Hiremath, S. N. Vasudevan, K. Bhanuprakash

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Abstract Natural ageing and accelerated ageing studies were conducted in onion cv Bellary red seed to investigate the vigor and viability changes during seed ageing. The seeds were stored up to 9 months under ambient conditions to carry out studies on ageing induced changes at evenly 3 months interval. Accelerated ageing was performed as per ISTA procedure by exposing the fresh seeds to a high temperature 45°C and 75% RH for a period of 9 days. Aged seeds were collected at 3 days interval for seed quality studies. The results revealed that onion seeds lost their vigor and viability progressively with ageing treatment. Germination percentage reduced up to 69% and 55%, field emergence percentage up to 61% and 44% in natural ageing and accelerated ageing respectively. Number of abnormal seedlings, dead seeds and mean germination time (days) increased significantly with the ageing period. The loss of viability in seeds after ageing appeared related to increased membrane destruction (loss of membrane integrity) as evident from higher per cent membrane injury index. This membrane integrity loss may be responsible for the decreased vigor and ultimately viability in onion seeds.

Keywords Accelerated ageing, Membrane injury index, Onion, MGT.

Introduction

It is a known fact that seed is a basic and a very crucial input in agriculture and in fact it is the quality of seed that decides the commercial success of a crop/ variety. Obviously the bumper harvest could be possible only when the planting seed possess high quality standards viz., genetic purity, germination, vigor, uniformity in weight and size apart from freedom from pest and diseases. These quality traits are known to be influenced largely by interaction of environment, cultural practices, harvest and post-harvest management practices at both field and storage levels.

Seed deterioration, a natural process is expressed as the loss of quality, viability and vigor during age-

Umesh Hiremath[1]*, S. N. Vasudevan[2], K. Bhanuprakash[3]
[1]Assistant Professor,
Department of Seed Science and Technology,
College of Agriculture, Kalaburagi,
University of Agricultural Sciences,
Raichur, Karnataka, India
[2]Professor and Head,
ABI Unit, College of Agriculture,
University of Agricultural Sciences,
Raichur 584102, Karnataka, India
[3]Principal Scientist,
Division of Plant Physiology and Biochemistry,
ICAR-Central Plantation Crops Research Institute Kudlu,
Kasaragod, Kerala, India
e-mail : umesh3980@gmail.com
*Correspondence

ing or adverse environmental conditions. It is an irreversible degenerative process that occurs during storage. Three general observations can be made about seed deterioration. First, seed deterioration is an undesirable attribute of agriculture. An understanding of seed deterioration, therefore, provides a template for improved crop production as well as increasing agricultural profits. Second, the physiology of seed deterioration is a separate event from seed development and/or germination. Thus, the knowledge gained from understanding these events likely does not apply to what occurs during deterioration. Third, seed deterioration is cumulative. As seed aging increases, seed performance is increasingly compromised. Thus, it is important that a fundamental understanding of the process of seed deterioration be gained. With these intends in mind, what causes seeds to die? The physiological processes governing seed deterioration vary. For example, short term deterioration in the field is likely a different physiological event than long-term deterioration in storage.

Seed researchers use different methods to study seed deterioration. The most widely accepted single criterion of seed deterioration is reduced germinability. However, many tests for measuring the loss in vigor have been developed based on the physiological effect of ageing [1—4]. Among them the most important method is accelerated ageing which is done by subjecting seeds to elevated temperature, high relative humidity and moisture content. It provides a simple and good method for studying sequence and relationship of process of deterioration with respect to storage atmosphere (temperature, relative humidity and moisture) over short periods. The rate of ageing mainly depends on genotype, moisture and temperature. The pattern of deterioration preceding to death is the same whether seed survives for few hours or decades. Very little is known about changes in seed vigor and viability during storage in onion seeds. Hence, an investigation in this direction was chosen in order to understand what the causes of seed deterioration during ageing.

Materials and Methods

A laboratory experiment to study various physiological and biochemical changes during seed ageing (natural and accelerated ageing) in onion seeds was under taken in the Department of Seed Science and Technology, College of Agriculture, UAS, Raichur. The experimental details are furnished below.

Natural ageing

Freshly harvested seeds of onion cv Bellary red were collected from Department of Seed Science and Technology, College of Agriculture, Raichur and stored up to nine months to carry out studies on ageing induced changes at evenly 3 months interval (T_2- 3 months natural ageing), (T_3- 6 months natural ageing, T_4- 9 months natural ageing). Fresh seeds were treated as control (T_1- Control).

Accelerated ageing

Fresh seeds (untreated) were subjected to artificial ageing as per ISTA procedure for a period of 9 days at 45°C + 75% RH. Samples were collected at 3 days interval for seed quality studies [T_5- 3 days accelerated ageing, T_6- 6 days accelerated ageing, T_7- 9 days accelerated ageing]. All the treatments were replicated thrice. Under natural ageing and accelerated ageing following observations were recorded. The laboratory germination test was done as per the ISTA rules [5] using between paper method. Hundred seeds in three replications were allowed to germinate at temperature of 25 ± 1°C up to 14 days. The germination counts were recorded on 14th day and per cent germination was expressed on normal seedling basis. Mean germination time (MGT) was calculated according to the equation of [6] and expressed in days. The equation is as follows :

$$\text{Mean germination time (MGT)} = \sum D\, n / \sum n$$

Where, D is the number of days counted from the beginning of the test and n is the number of seeds that germinate on day D.

Field emergence (%) was calculated by sowing 100 seeds in four lines of 25 seeds each with three replications in a raised bed and optimum irrigation was provided. Final count was taken on 15th day. The number of normal seedlings emerged was counted

Table 1. Effect of seed ageing on percentage of germination, abnormal seedlings and dead seeds in onion cv Bellary red.*Figures in parentheses indicate are sine transformed values. T_1 - Fresh seeds, T_2 - Natural ageing 3 months, T_3 - Natural ageing 6 months T_4 - Natural ageing 9 months, T_5 - 3 days accelerated ageing, T_6 - 6 days accelerated ageing, T_7 - 9 days accelerated ageing.

Treat-ments	Germination (%)*	Abnormal seedlings (%)*	Dead seeds (%)*
T_1 (Fresh)	93	7.00	0.00
	(74.76)	(15.24)	(0.00)
T_2 (NA3)	88	12.00	0.00
	(69.77)	(20.23)	(0.00)
T_3 (NA6)	77	17.00	6.00
	(61.35)	(24.31)	(14.15)
T_4 (NA9)	69	19.67	11.33
	(56.17)	(26.32)	(19.62)
T_5 (AA3)	81	19.00	0.00
	(64.18)	(25.82)	(0.00)
T_6 (AA6)	55	21.00	24.00
	(47.88)	(27.24)	(29.27)
T_7 (AA9)	0	0.00	100.00
	(0.00)	(0.00)	(90.00)
SEm±	1.29	1.12	1.08
CD @ 5%	3.92	3.40	3.27

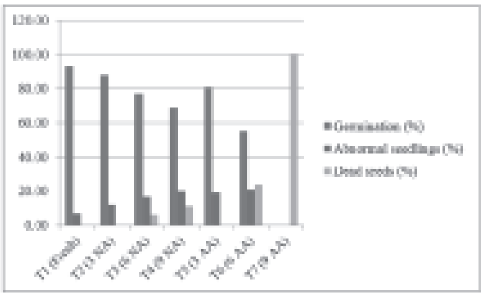

Fig. 1. Germination, abnormal seedlings (%) and dead seeds (%) as influenced by seed ageing in onion cv Bellary red.

and expressed as per cent. Membrane injury index was calculated by the formula :

$$\text{Membrane injury index} = C_1/C_2$$

Where, C_1 = Electric conductivity at 40°C for 30 min and C_2 = Electric conductivity at 100°C for 10 min.

The experimental data were statistically analyzed as per the methods outlined.

Results and Discussion

Seed ageing affected seed germination, abnormal seedlings, mean germination time, field emergence and membrane injury index. The data on seed ageing on germination per cent, abnormal seedlings, dead seeds, mean germination time, field emergence per cent and membrane injury index recorded in different naturally aged (NA) and accelerated ageing (AA) conditions of onion cv Bellary red are presented in Tables 1 and 2.

Ageing showed significant differences in terms of germination percentage, abnormal seedlings and dead seeds in both accelerated ageing and natural ageing (Fig. 1). Significant reduction in per cent germination was noticed starting from 3 months (88%) under NA and 3 days (81%) under AA test when compared to fresh seeds (93%). The fresh seed lot recorded significantly higher (93%) germination per cent followed by NA-3 months (885), NA-6 months (77%) and NA-9 months (69%) and significantly differed compared to the fresh seeds. Accelerated ageing may considerably affect the seed viability and vigor [7]. Thus storage of seeds under such adverse conditions (100 RH and 45°C) results in the production of aged seeds. These aged seeds exhibit a variety of symptoms ranging from reduced germinability (sometimes to zero germination) to more or less full viability (no obvious decline in germinability) but with abnormal development of the seedling (i.e., poor vigor).

In case of accelerated ageing condition, there was significant difference observed in fresh seed lot (93%) and accelerated aged (3 days) seed lot (81%). From AA 6 days (55%) to AA 9 days (no germination was observed) onwards there was reduction in germination per cent significantly compared to fresh and natural aged (9 months) seeds. These data reinforce the concept that the ageing seeds loose the ability to germinate. Similarly, reduction in field emergence was

Table 2. Effect of seed ageing on mean germination time and percent field emergence in onion cv Bellary red.*Figures in parentheses indicate are sine transformed values. T_1 - Fresh seeds, T_2 - Natural ageing 3 months, T_3 - Natural ageing 6 months T_4 - Natural ageing 9 months, T_5 - 3 days accelerated ageing, T_6 - 6 days accelerated ageing, T_7 - 9 days accelerated ageing.

Treat-ments	Germination time (Days)	Field emergence*	% Membrane injury index
T_1 (Fresh)	2.79	88.33 (70.05)	36.0
T_2 (NA3)	3.15	84.00 (66.48)	36.0
T_3 (NA6)	4.00	70.67 (57.23)	37.0
T_4 (NA9)	4.55	61.00 (51.37)	40.0
T_5 (AA3)	2.87	74.67 (59.82)	37.0
T_6 (AA6)	3.85	44.00 (41.55)	60.0
T_7 (AA9)	0.00	0.00 (0.00)	75.0
SEm±	0.03	1.59	0.85
CD @ 5%	0.08	4.82	2.56

noticed in aged (6 DAA and 9 DAA) seeds. There was a large reduction in field emergence per cent from 6 days AA onwards compared to the natural aged (9 months) seeds.

The fresh seed lot recorded significantly higher (88%) field emergence which was on par with NA-3 months (84%). The least field emergence percentage (61%) recorded in NA-9 months. In case of accelerated ageing condition, there was reduction in field emergence per cent significantly from 88.33 to 74.67 in 3 days AA, 44.0 in 6 days AA and 0 in 9 days AA. Loss in onion seed viability and vigor related parameters upon increasing accelerated ageing duration was reported and this might be due to cellular damage under prolonged ageing leading to failure germination [8]. Similar observations in relation to artificial ageing in various crops were noticed.

The fresh seed lot recorded significantly lower (7.0) number of abnormal seedlings followed by NA-3 months (12.0), NA-6 months (17.0) and NA-9 months (19.67). Significant difference was observed between

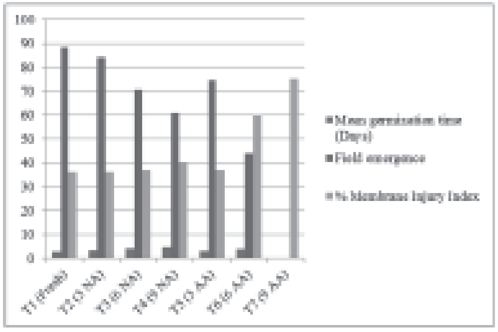

Fig. 2. Mean germination time (Days), field emergence (%) and per cent membrane injury index as influenced by seed ageing in onion cv Bellary red.

fresh (7.0) and accelerated aged (3 days) seed lot (19.0). The AA 6 days (21.0), AA 9 days (no germination was observed) onwards there was increase in abnormal seedlings significantly compared to fresh seed lot. There was a large increase in abnormal seedlings from 6 days AA (21.0) onwards compared to the natural aged 9 months (19.67). The highest number of abnormal seedlings observed in 6 days accelerated ageing treatment (21.0) and lowest was recorded in fresh treatment (7.0) and no germination was observed in 9 days accelerated aged treatment. The damage or deterioration of seed due to seed ageing which develops in the penultimate phase preceding death is irreversible and leads to reduced vigor and the production of abnormal seedlings.

Seed ageing results in deaths of a variable number of seeds, and dead seed provides valuable information on the maintenance of seed viability and the effects of ageing [9]. There were no dead seeds observed in 3 months naturally aged and 3 days AA seed lot. Accelerated ageing conditions for more days resulted in increase in the number of dead seeds. In case of accelerated aged seeds for different days viz., AA 6 days (24.0) and AA 9 days (100.00) dead seeds were more compared to fresh seed lot.

Delayed seedling emergence is among the first noticeable symptoms, followed by a slower rate of seedling growth, development and decreased germination (Fig. 2). Further in our findings, increase in mean germination time (MGT) was noticed due to seed ageing. The fresh seed lot recorded significantly lower mean germination time (2.79) compared to NA - 3 months (3.15), NA - 6 months (4.0) and NA - 9 months (4.55). This increased mean germination time might be due to stronger inhibitory effects during seed ageing [10].

In case of accelerated ageing conditions, there was no significant difference observed between fresh seed lot (2.79) and accelerated aged 3 days seed lot (2.87). Whereas, from AA 6 days (3.85) to AA 9 days (no seed germinated) there was drastic increase in mean germination time significantly compared to fresh seed lot. Further, ageing up to 9 days showed nil values as there was no germination. The findings of the study were in agreement with [11] who also observed, as seed ages, the propensity for genetic mutations increases since, free radicals have effect on chromosomal DNA. These mutations can be detected as chromosomal aberrations. These chromosomal aberration delays the onset of mitosis necessary for germination [12]. Dissociation of polyribosome's must occur before attachment of preformed m-RNA occurs, leading to protein synthesis in germinating seedlings. In non-viable seeds, ribosomes fall to dissociate and protein synthesis is retarded. Ageing depresses the synthesis of newly formed m-RNA. DNA degraded leading to impaired transcription causing faulty translation of enzymes. Possible degradation of longlived m-RNA programmed for enzymes responsible for first stages of germination. Harrington [15] made a strong case for the idea that the breakdown of various germination triggering mechanisms also causes seeds deterioration. It has been noted that the concentrations of various growth hormones are adversely affected by ageing; for example application of gibberelic acid improved germination and vigor in partially aged celery seeds [13].

The membrane injury index significantly differed among the fresh, NA and AA seeds. The lowest membrane injury index (36.0%) was recorded in fresh seed and NA-3 months (36.0%) followed by NA-6 months (37.0%) and AA-3 days (37.0%). Whereas, highest membrane injury index (75%) was observed in AA-9 days treatment followed by AA-6 days (60.0%) and NA-9 months (40.0%). The electrical conductivity of seeds increased with increased ageing, indicating that seed constituents were leaking. The ion leakage is increased by each increment of the accelerated ageing treatment. Present findings indicate that the electrical conductivity of seed is related to its quality. Membrane injury index of seeds is the measure of membrane functions, the results suggest that membrane function is less damaged when the seeds are aged for short time. Increase in the membrane injury index with the ageing of seeds indicates lesions in membrane system. Thus membrane system seems to be among the major events in ageing as proposed by [14]. Membranes are likely to be responsible for the slower growth and germination in aged seeds.

Osborne [15] summarized the transition from viable to non-viable state in dry seeds. In dry (orthodox) seeds, central feature were the damage to membrane systems in all parts of the cell and differential stability of the different enzyme proteins. Cooperative enzymes of a metabolic pathway are not only linked together within organelles (e.g. respiratory enzymes in mitochondria) but often they are intimately associated with membrane structures. Thus disruption of membranes because of ageing could lead to diverse metabolic changes, all of which contribute to different extents to seed deterioration and loss of viability and vigor. Loss of membrane integrity in deteriorated seeds is suggested by increased membrane injury index from aged seeds than from fresh seeds.

Hence, it is concluded that onion seeds aged rapidly showed speedy and significant reductions in the rate of germination and field emergence, which abnormal seedlings, dead seeds and mean germination time were increased significantly with ageing time. Loss in seed viability and vigor was associated with the increased per cent membrane injury index. The observed changes in germination and membrane injury index support the concept that deterioration of cellular membranes is implicated in ageing and loss of viability in seeds. Accelerated ageing test can be used as a successful technique to obtain seed lots of different quality levels thereby reasons associated with loss in seed quality could be explored. The reason for loss in vigor both under natural and accelerated ageing appears unique but distinct in the rate at which they affect various parameters contributing to seed vigor.

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