International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 11(1): 209-216, February 2018
DOI: 10.30954/0974-1712.2018.00178.27

PLANT PHYSIOLOGY

Accelerated Aging Affects the Germination Physiology of Wheat Seeds

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Paper No. 670 Received: 23-09-2017 Accepted: 19-01-2018

ABSTRACT

In the present piece of work, the caryopses of two wheat varieties (HUR-468 and HUW-234) were taken to study the effects of aging during germination. Accelerated aging (artificial aging) treatments were created by keeping the seeds (with initial moisture content of 12%) at 40°C and relative humidity of 90-95% for 24, 42, 72, 96, 120, 144, 168 and 192h periods. Seeds without any ageing treatment were considered as control (T_1). The parameters like germination percentage, speed of germination (N), mean germination time (MGT), shoot and root lengths, root numbers, fresh and dry weights, α-amylase activity and soluble-insoluble sugar contents were studied upto 10 days after sowing (DAS) in artificially aged and non aged seeds during germination. Result suggested that aging affects the whole process of germination of seeds. The maximum values of the studied parameters were obtained from the 24h aged seeds followed by control and 42h aged seeds in both the varieties. Among the two varieties HUW-234 showed better performance for the above studied parameters.

Highlights

- Accelerated aging inhibits the germination of wheat seeds

Keywords: Aging, wheat, germination physiology

Wheat is the dominant cereal crop in temperate areas, used for food and feed purpose and it is the third among three largest cereal crops, with over 600 million tonnes harvested annually in the world (Shewry and Darwin 2009). Among the factors that affect wheat yield is the quality of seeds used, in respect to the germination and seedling establishment.

Seeds, like other organisms, age and die, although their longevity varies greatly among species and within a species because of differences in genotype and origin (Hong and Ellis 1996). Storage of orthodox seeds for prolonged period induces their deterioration leading ultimately to loss of their viability. The rate of seed deterioration varies among plant species and seed lots, but high moisture content and high temperature accelerate this process (Priestley 1986; Abba and Lovato 1999; Kibinza 2006).

However, little attention has been given to the processes of seed storage, which constitutes a major problem for agriculture (Tekrony 2006). The process is responsible for serious losses worldwide, especially in the tropics, where high temperatures and relative humidity prevail during the maturation and storage of seeds (Bilia *et al*. 1994). It is well established that seed deterioration process is irreversible and inevitable both, but the speed of the process can be controlled with appropriate harvesting, drying and storage techniques. There are several factors that are known to influence the progress of deterioration during seed storage. Both high temperatures and humidity during storage increase the deterioration speed of seeds (McDonald

1999; Pukacka *et al*. 2009), and decreasing either of these factors significantly increases the storage life of seeds (Castellión *et al*. 2010).

Aged seeds show a variety of symptoms ranging from reduced viability to more or less full viability but with abnormal development of the seedling. The reduction in the rate of germination may be an expression of aging of the embryo, but changes in the remainder of the seed could also contribute. Important processes during seed germination, such as the establishment of respiration, ATP production, and protein synthesis, are often perturbed by seed aging (Bewley and Black 1994). However, it is generally accepted that loss of viability with seed aging is mainly connected with the loss of plasma membrane integrity (Senaratna *et al*. 1988). With these points kept in mind our aim in the present study was to test the germination physiology of wheat seeds (HUR-468 and HUW-234) that had been subjected to accelerated aging treatment to evaluate their vigor after planting and food mobilization during storage.

MATERIALS AND METHODS

The present piece of work was carried out in the Seed Physiology Laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, BHU in the year 2013; and the seeds were procured from Department of Genetics and Plant Breeding of the same Institute. For this study, the caryopses of two wheat varieties (HUR-468 and HUW-234) were used for experimental purposes. Accelerated aging treatments were created by keeping the seeds (with initial moisture content of 12%) at 40°C and relative humidity of 90-95% for 24 (T_2), 42 (T_3), 72 (T_4), 96 (T_5), 120 (T_6), 144 (T_7), 168 (T_8) and 192h (T_9) periods. A seed without any accelerated ageing treatment was considered as control (T_1).

For each aging treatment, about 100g of seeds were packed in mosquito net cloth separately within a vacuum desiccator; the floor of the container was covered by distilled water (10% of total container volume). The container was placed in an incubator at a fixed temperature of 40°C with 90-95% relative humidity upto 8 days. After aging treatment, the seeds were used for germination study upto 10 DAS. Germination studies were carried out by using Petri dish, of 3.0 inches diameter. They were washed well, first with water and then with alcohol, autoclaved and oven dried. Filter paper was cut to the required diameter and autoclaved. The lower side of the filter paper was provided with a thin layer of cotton and then placed in Petri dishes. The air dried seeds were then placed equidistantly (50 seeds in each Petri dish) and 5 ml of distilled water was poured in each Petri dish. The parameters taken into consideration were germination percentage, germination index, speed of germination (N), mean germination time (MGT), shoot length (the length of the shoot was taken by using centimeter- scale) and root lengths (the centimeter scale was placed at the base of shoot to the tip of the longest root to measure the root length), root numbers (the number of roots of plants was also counted by placing the root part on a glass plate and by using a needle), fresh and dry weights in which the dry weight of seedlings was obtained by keeping the sample for an hour in an oven pre-set at 100 - 110°C and then these were further placed in another oven, which was set at 60 to 70°C till to get the constant weight. Five number of seedlings were taken into consideration for each treatment and per replication. α-amylase activity and soluble and insoluble sugar contents were determined using the methods introduced by Bernfield(1955) and Dubios *et al*. (1956) respectively.

Germination was calculated by counting and removing the germinated seeds. Germination was observed daily and Germination Index (GI) was observed by counting the number of seedlings emerging daily from the day of planting till the time of germination was completed. Thereafter GI was computed by using the following formulae:

$$G.I. = \Sigma\, n/d$$

Where:

n = Number of seedlings emerging on day 'd', from germinating seeds;

d = Day after planting

Speed of germination (N): Number of germinated seeds was counted every day from the first day and the cumulative index was made to compute the speed of germination by using the formula:

$$\text{Speed of germination} = N1/1 + n2/2 + \ldots\ldots + nx/x = N$$

Where:

N1 = Nx are the no. of seed germinated on day 1 to day x;

1 = X are the no. of days

Mean Germination Time (MGT): Mean Germination Time (MGT) was calculated by using the equation of (Ellis and Roberts 1981).

$$MGT = \Sigma Dn / \Sigma n$$

Where n is the number of seeds that had germinated on day D and D is the number of days counted from the beginning of germination.

The data obtained from various observations were subjected to statistical analysis by adopting appropriate method of Analysis of Variance for Complete Randomized design (CRD). The significance of treatment effect was tested with the help of F-test and the differences between treatments by Critical Difference (C.D) at 1 and 5% level of significance were determined.

RESULTS AND DISCUSSION

Germination of a seed is a most important physiological parameter which emphasizes directly the whole life cycle of a plant. It is also a reliable index in reflecting the variations of the seed vigor. In the present piece of work wheat seeds (var. HUR-468 and HUW-234) were aged at 40°C and relative humidity of 90-95% for 8 days. Table 1 depicts the effect of accelerated aging on germination physiology in which germination percentage was studied at different hours (24h to 120h) in both the varieties and it was observed that treatment T_1 (control seeds) showed the highest germination percentage in both the varieties and in all the studied hours followed by T_2 and T_3. Like germination percentage, in Table 1 GI, N and MGT were also followed the same trend. By comparing the performance of the two varieties it was observed that HUW-234 performed better than the HUR468 in all the studied parameter present in Table 1. The rest of the treatments (T_4 to T_9) were the very poor performer in this regard.

The Table 2 shows the accelerated aging effects of wheat cultivars on shoot and root lengths and root numbers at 6, 8 and 10th days after sowing. In case of shoot and root length T_2 was the best performer followed by either T_1 or T_3 but HUW-234 variety showed better result as compared to HUR-468 in this respect. Unlike shoot and root length T_1 has the more number of roots as compared to other treatments and treatment T_2 showed the at par result with T_1. However, the fresh and dry weights of the seedlings were studied at 10 days after sowing of the aged wheat cultivars (HUR-468 and HUW-234) present in Table 3. In this case T_2 showed better result which was followed by T_3 and T_1. But among the two varieties HUW-234 depicts the best result than the HUR-468.

The figure 1 represents the effect of accelerated aging on α-amylase activity (mg maltose g^{-1} h^{-1} fresh weight of endosperm), soluble and insoluble sugar content (mg g^{-1}) at 48, 72 and 96h after sowing of wheat cultivars in which treatment T_1 has shown higher α-amylase activity at 48h after sowing but at 72 & 96h treatment T_2 obtained maximum α-amylase activity followed by T_3 in HUR-468 but unlikely in HUW-234,T_2 showed maximum α-amylase activity in all the studied hours followed by T_1. In case of soluble and insoluble sugar contents, in both the variety treatment T_2 obtained higher soluble sugar content and lower insoluble sugar content in all the studied 3 days (48, 72 and 96h). However, in between two varieties HUW-234 performed in a better way than HUR-468 in the above studied parameters. Whereas, the other treatments represented very poor performance in this regards.

The agricultural literature suggests that seed storage significantly affects the seed viability (Rao *et al*. 2006; Scalon *et al*. 2012), which may happen due to great loss of their reserves with increasing age of the seed . Seed susceptibility to aging varies among the species, but it is also dependent on the maturity stage and the conditions of development (Priestley 1986, Hay and Probert 1995). These conditions significantly affect seed germination (Bilia *et al*. 1994, Rice and Dyer 2001). Lin (1990) observed a decrease in the germination and vigor of bean seeds subjected to 1, 2, 3 and 4 days of aging that was related to an increase in solute leakage from seed cells, suggesting a close relationship between the deterioration of biological membranes and the loss of vigor and germination. This decay in the viability of aged seeds would normally be attributed to the loss of seed vigor due to ultracellular changes as temperatures increase. Prolonged storage generally

Print ISSN : 1974-1712

Table 1: Effect of accelerated aging on germination percentage, germination index (GI), speed of germination and mean germination time (MGT) of wheat cultivars (HUR468 and HUW234)

Treatments	HUR468								HUW234							
	Germination percentage at different hours					Germination Index (GI)	Speed of Germination (N)	Mean Germination Time (MGT)	Germination percentage at different hours					Germination Index (GI)	Speed of Germination (N)	Mean Germination Time (MGT)
	24 h	48 h	72 h	96 h	120 h				24 h	48 h	72 h	96 h	120 h			
T_1	**86.0**	**86.7**	**97.3**	**97.3**	**98.0**	102.9	45.0	0.32	**74.0**	**89.0**	**97.0**	**98.0**	**98.0**	97.5	42.2	0.32
T_2	38.7	54.7	73.3	78.0	78.0	62.8	27.0	0.44	62.0	67.0	76.0	79.0	79.0	78.2	34.1	0.40
T_3	23.3	41.3	64.7	74.0	74.0	49.4	21.2	0.52	49.0	60.0	73.0	80.0	80.0	69.7	30.3	0.41
T_4	12.0	19.3	29.3	31.3	31.3	22.8	9.8	1.15	7.0	24.0	33.0	33.0	33.0	22.4	9.3	1.18
T_5	4.7	14.7	22.7	24.7	24.7	15.3	6.4	1.70	5.0	15.0	16.0	16.0	16.0	12.5	5.2	2.17
T_6	0.0	6.0	6.7	7.3	6.7	4.3	1.7	4.01	0.0	6.0	6.0	9.0	9.0	4.5	1.9	3.67
T_7	0.0	6.0	6.7	6.7	6.7	4.1	1.6	4.27	0.0	6.0	6.0	6.0	6.0	3.9	1.5	4.67
T_8	0.0	4.0	4.7	4.7	4.7	2.8	1.1	6.14	0.0	3.0	3.0	5.0	5.0	2.4	1.0	6.93
T_9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.32	0.0	2.0	2.0	2.0	2.0	1.3	0.5	14.00
SE	1.1	3.3	1.5	1.3	1.4				1.2	1.1	1.2	0.9	0.9			
CD at 5%	2.3	7.0	3.2	2.8	3.0				2.5	2.3	2.6	2.0	2.0			
CD at 1%	3.1	9.6	4.3	3.8	4.0				3.4	3.1	3.5	2.7	2.7			

Treatment details: T_1=non aged control; T_2=24h, T_3=48h, T_4=72h, T_5=96h, T_6=120h, T_7=144h, T_8=168h and T_9=196h periods of aging (same for other tables(2 and 3) and figure:1)

Online ISSN : 2230-732X

Table 2: Effect of accelerated aging on shoot and root lengths and root numbers of wheat cultivars (HUR468 and HUW234)

Treatments	HUR468								
	Shoot length (cm)			Root length (cm)			Root numbers		
	Days after sowing (DAS)								
	6	8	10	6	8	10	6	8	10
T_1	6.81	10.01	11.99	2.17	2.69	2.84	5.10	6.40	6.90
T_2	7.72	10.87	12.85	2.80	3.45	3.64	5.00	6.40	7.00
T_3	5.66	9.13	11.44	1.97	2.62	3.21	5.00	6.10	6.30
T_4	4.44	8.13	9.88	1.69	2.10	2.89	4.00	4.60	4.90
T_5	2.97	4.78	6.83	1.33	1.64	1.93	3.05	3.60	3.70
T_6	1.58	3.00	4.98	0.87	1.38	1.98	1.70	1.90	1.90
T_7	1.60	2.90	4.74	0.43	0.52	1.56	2.20	2.20	2.20
T_8	0.38	0.85	1.61	0.13	0.21	0.26	0.60	0.60	0.60
T_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE	0.23	0.29	0.41	0.15	0.10	0.15	0.10	0.18	0.17
CD at 5%	0.47	0.62	0.87	0.32	0.21	0.31	0.20	0.39	0.37
CD at 1%	0.65	0.85	1.19	0.43	0.29	0.43	0.27	0.53	0.50
Treatments	**HUW234**								
	Shoot length (cm)			Root length (cm)			Root numbers		
	Days after sowing (DAS)								
	6	8	10	6	8	10	6	8	10
T_1	9.98	12.32	13.34	2.48	3.00	3.52	7.10	7.70	8.30
T_2	10.38	13.03	14.28	3.04	3.33	4.06	6.40	7.10	7.80
T_3	8.57	11.57	13.89	2.84	3.03	3.67	6.40	6.70	7.30
T_4	7.47	10.13	13.56	2.79	2.96	3.39	4.50	6.30	6.90
T_5	5.72	8.42	9.40	2.35	2.59	2.78	4.10	4.90	4.90
T_6	4.38	7.13	7.97	2.15	2.02	2.11	2.20	3.90	4.30
T_7	3.48	5.99	7.59	2.04	1.94	1.97	2.50	3.40	4.10
T_8	3.06	5.11	5.08	1.93	1.82	1.58	2.50	2.90	2.90
T_9	1.75	2.53	2.97	0.74	1.05	1.15	1.30	1.50	1.50
SE	0.37	0.20	0.10	0.06	0.04	0.03	0.25	0.12	0.09
CD at 5%	0.77	0.41	0.21	0.13	0.09	0.07	0.53	0.26	0.20
CD at 1%	1.05	0.56	0.29	0.18	0.12	0.10	0.73	0.35	0.27

Table 3: Effect of accelerated aging on fresh and dry weights at 10 days after sowing (DAS)of wheat cultivars (HUR468 and HUW234)

Treatments	HUR468		HUW234	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
T_1	93.94	14.74	119.89	20.20
T_2	116.44	16.02	150.47	21.35
T_3	107.98	14.89	148.27	20.42
T_4	98.01	13.44	104.25	13.39
T_5	78.69	8.03	85.15	11.49
T_6	54.48	6.90	72.25	9.67
T_7	40.85	6.03	66.21	8.37
T_8	15.41	2.58	63.10	6.88
T_9	0.00	0.00	29.22	4.65
SE	4.84	0.46	5.47	0.89
CD at 5%	10.17	0.96	11.49	1.87
CD at 1%	13.93	1.32	15.75	2.57

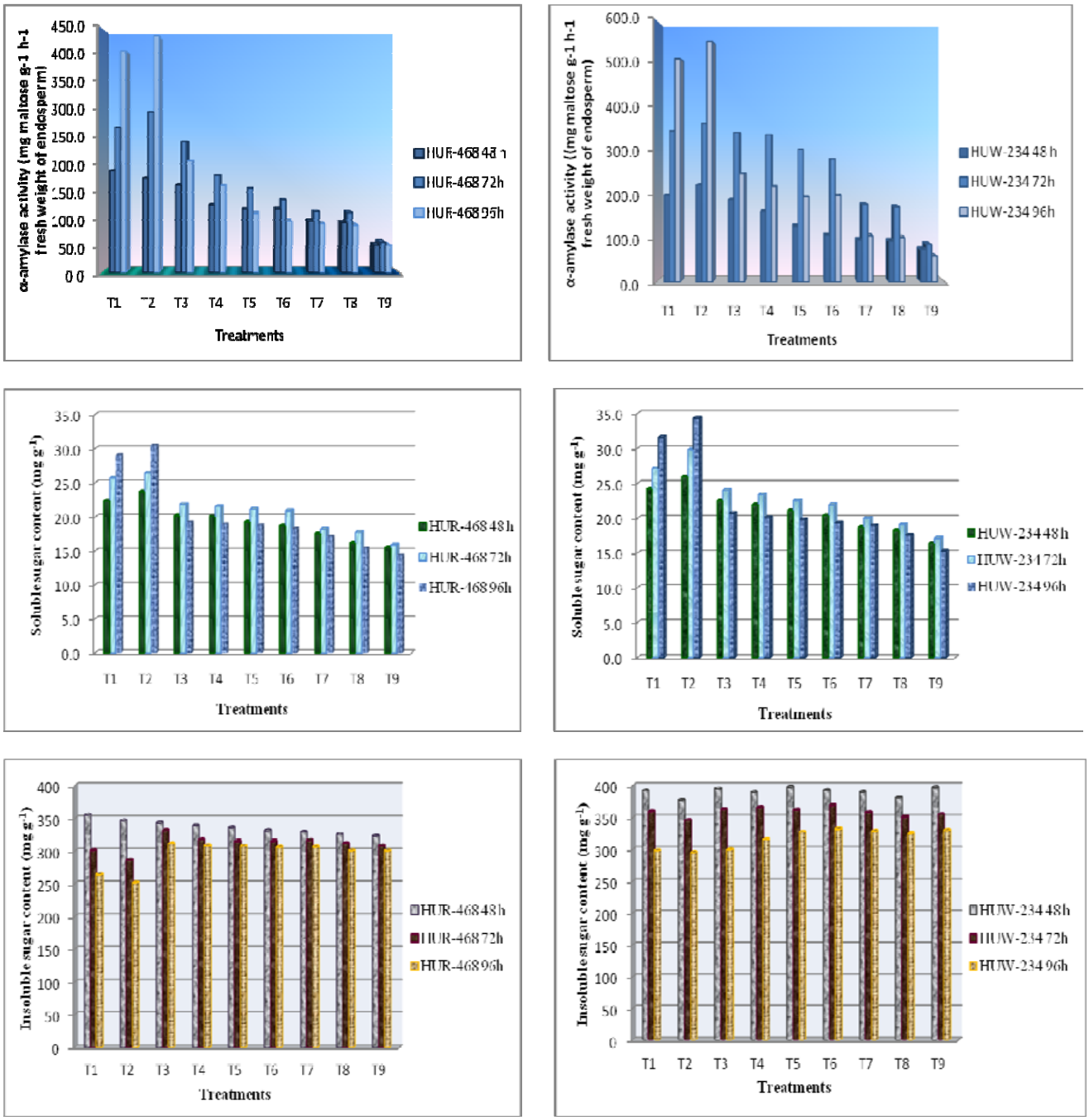

Fig. 1: Effect of accelerated aging on α-amylase activity, soluble and insoluble sugar content at 48, 72 and 96 hour after sowing of wheat cultivars (HUR468 and HUW234)

results in considerable nutrient loss, mainly of sugars and proteins (Sandoval *et al*. 2002; Shah *et al*. 2002). However, in the present case, similar type of results was observed with the wheat cultivars where the germination physiology of wheat cultivars HUW-234 and HUR-468 was shown to be affected by the accelerated aging process. However at the time of accelerated aging high humidity and temperature along with the increasing duration of aging deteriorates the activity of the hydrolyzing enzymes like *α*-amylase was reduced and the stored nutrient elements like soluble and insoluble sugar contents, were degraded and converted into some complex compounds. Sun and Leopold (1995) reported that the hydrolysis of sugars in the seeds would lead to an accumulation of reducing sugars, which would eventually threaten the integrity of proteins due to the formation of Maillard reaction products. In

the present investigation, at the time of accelerated aging due to such type of complexed internal mechanization within the seed system of wheat cultivars the viability of the seeds were lost and the seeds which germinated had lost their vigor. Seed vigor effects the emergence performance of a crop, is well documented by various scientists. Basra *et al*. (2003) stated that percentage emergence decreased with accelerated aging periods in cotton. They also reported that the decline in seed germination during accelerated aging was accompanied with the increase in mean emergence time. Verma *et al*. (2003) indicated that seedling establishment and emergence rate is reduced with increase in seed storage duration. All of these factors can influence finally the dry matter accumulation in the growing seedling. Khah *et al*. (1989) indicated that the poor rate of germination and seedling growth of wheat seed mainly resulted from the deleterious effect of poor seed vigor. So from the present investigation it can be concluded that the least induced aged seeds (T_2) showed higher activities of studied parameters in comparison to more aged one which suggest that humidity (90%) and higher temperature (40°C) for a limited time may improve the germination in wheat; it then may work as seed invigoration treatment or more specifically seed priming treatment. Therefore in case of accelerated aging treatment to seeds upto 24h with 90% relative humidity and higher temperature of 40°C might be worked as an activator for the hydrolyzing enzymes which resultantly improve the germination physiology in wheat.

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