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Research Article

Impact of priming and storage containers on enzyme activities of naturally aged seeds of Barley (*Hordeum vulgare* L.)

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Abstract

The study was conducted on six barley varieties *viz.* BH 885, BH 946, BH 393, BH 902, DWRB 92 and DWRB 101 at CCS HAU, Hisar during 2020-21. The results revealed that maximum catalase activity (243.168 and 157.981umoles/g FW), peroxidase activity (22.33 and 35.03 units/g FW), superoxide activity (22.03 and 35.03 nmoles/g FW) and dehydrogenase activity (0.213 and 0.245 OD/g/ml) were estimated in the seeds primed with ZnO NPs @ 100ppm in cloth and polythene bag, respectively in variety DWRB 92. Among the varieties, DWRB 92 recorded highest catalase activity (217.168 umoles/g FW) in cloth bag while BH 393 recorded maximum catalase activity (232.961 umoles/g FW) in polythene bag. Minimum catalase activity was observed in DWRB 101(60.351 umoles/g FW) in cloth and BH 885 recorded least (121.418 umoles/g FW) in polythene bags. Maximum peroxidase activity (21.22 and 31.56 units/g FW) was estimated in BH 885 while minimum (12.71 and 22.54 units/g FW) in BH 946 in cloth and polythene bag, respectively. Maximum SOD activity (18.033 and 25.033 nmoles/g FW) was measured in DWRB 92 and minimum (12.712 and 22.537 nmoles/g FW) in BH 946 in cloth and polythene bag, respectively. Maximum DHA activity (0.170 and 0.206 OD/g/ml) was recorded in DWRB 101 and least in BH 946 (0.234 and 0.281 OD/g/ml) in cloth and polythene bag. It is concluded that among the various seed priming treatments, priming with ZnO NPs@100ppm at 25°C for 24 hours maintained higher enzyme activity. Barley seed quality can be maintained by storing the seeds at optimum moisture content (<8%) in polythene bags (>700gauge) with germination upto 94.61% as compare to cloth bags (85.89%).

Keywords: Nano-particles, priming, *Hordeum vulgare*, storage container, Catalase, Peroxidase

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the main cereal crop and ranks fourth among grains with production of 156.12 million tonnes after maize, rice and wheat in India as well as world (Anonymous, 2019). Russia ranks first in barley production which contributes about 14 per cent of the world production while India contributes 1.12 per cent in global barley production to the tune of 1.75 million tonnes (Anonymous, 2019). Barley is an important source of carbohydrates (77.7%), protein (9.9%), fat (1.2g), vitamins *viz.,* niacin and pyridoxine and minerals *viz.,* calcium, iron and manganese. The crop is also used as animal fodder, as a source of fermentable material for beer and certain

distilled beverages and as a component of various health foods. Barley grown for malt purpose is called malting barley as opposed to feed barley. Seed is an important component and plays a crucial role in agricultural production as well as in the national economy. Seed deterioration starts once the seed attains physiological maturity in the field. Seed deterioration will lead to some of physiological changes like loss of germination potential, decrease in mean germination time and loss of vigour (Helmer *et al.* 1962). Storage containers or packaging materials mostly influences the seed longevity during storage condition (Oyekale *et al.,* 2012). The use of proper storage containers during storage is one of the most important aspects during storage and maintaining seed quality until the next cropping season. The container properties greatly influence the interaction of seed with the surrounding environment. The rate of entry and exit of moisture content from the storage container will influence the seed longevity (Walters, 2007). Since the seed is hygroscopic in nature, absorbs moisture under ambient storage conditions until seed attains the equilibrium moisture content with surrounding environment. High temperature along with more moisture content enhances the rate of seed deterioration (Roberts, 1972). To overcome all these factors, it is essential to store the seeds in moisture-proof containers such as polythene bags with or without desiccating agents to maintain the seed quality (Vijayalakshm and Malabasari, 2018). The better moisture barrier properties of the storage container are an essential to maintain the germination of seed for longer durations (Fu, 2018). Since seed is a living entity, deterioration is inevitable. Rate of deterioration will be higher under stored seed, however, can be slow down by application of seed quality enhancement techniques *i.e.* priming, coating, pelleting and hardening. Seed priming is one of the scientific techniques used for enhancing the quality of seed at post-harvest season. It is the process of controlled hydration of seeds to a level that allows pre-germination metabolic activity to continue while preventing actual radicle emergence (Vanangamudi, 2014). Seeds are treated with different kinds of chemicals such as inorganic salts (halo-priming), sugars (osmo-priming), plant hormones (hormonal priming), nano-particles (nano-priming) and bioagents (bio-priming). Most of the priming treatments involve imbibing seed with a restricted amount of water to allow sufficient hydration and advancement of metabolic processes but preventing actual seed germination. Seed priming has also been investigated as a pre-sowing or mid-storage treatment for seed batches that have lost vigour due to insufficient storage conditions (Pan and Basu, 1985; Singh *et al.,* 2001).

Superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) are main enzymes which are involved in cell detoxification (Bailly, 2004; Mittler, 2002). The behaviour of seed with different priming treatments depends on various physiological and biochemical factors. There is ample scope for investigating mechanism involved behind the beneficial and adverse effects of seed priming on seed quality. Nanotechnology is a branch of science which deals with the synthesis and application of nano particles having size 1–100 nm (Jasrotia *et al.*, 2018; Roco 2003). Now a days, nanotechnology is emerging as a promising approach to be incorporated in agriculture to improve productivity of different crops through seed treatment with nano particles, their foliar spray on plants, nano-fertilizers for balanced crop nutrition, nano-herbicides for effective weed control, nano-insecticides for plant protection, early detection of plant diseases and nutrient deficiencies using diagnostics kits and nano-pheromones for effective monitoring of pests (Kashyap *et al.*, 2022; Singh *et al.*, 2021). Zinc is essential for plant's enzyme system as it acts as cofactors, metal components and other regulatory factors of many enzymes (Kushwaha *et al.*, 2021; Prasad *et al.,* 2012) which comes in the fourth position after nitrogen, phosphorous and potassium. Nano-priming can be applied to seeds through priming in order to improve seed quality parameters by enhancing various enzyme activities of seed. Less antioxidant enzymes and more lipid peroxidation activities were observed in primed seeds as compared to unprimed seeds in sweet corn (Chang and Sung, 1998). Since enzyme activities in seeds are directly related with the seed quality and a very little information is available on priming effects on enzyme activities naturally aged seeds in barley. Therefore, the present study was planned to assess the effect of different storage containers and priming on nine months naturally aged seeds of barley.

2. Materials and Methods

The study was conducted on seeds of six barley varieties *viz.* BH 885, BH 946, BH 393, BH 902, DWRB 92 and DWRB 101 procured from the Department of Genetics

and Plant Breeding, CCS HAU, Hisar during June, 2020. The seeds (2kg seed of each variety) were stored in cloth bags and polythene bags having 700gauge thickness under ambient conditions (Figure 2) at optimum moisture content (<8%) for nine months. After nine months of storage the seeds were primed with GA_3 (50, 100 and 150ppm, Ethanol (1, 3 and 5%) and ZnO nano-particles (50, 100 and 150ppm) at 25°C for 24 hours and then dried to original moisture content (<8%) under ambient conditions. Enzyme activities *viz*., Dehydrogenase activity, Catalase Assay, Superoxide dismutase Assay (SOD), Peroxidase Assay (POX) were estimated in primed seeds.

Dehydrogenase activity test (OD $g^{-1}ml^{-1}$): One gram of seed sample was taken from each variety and treatment which is replicated three times. The grounded powder of 200mg from each sample was added to a centrifuge tube. Thereafter, 5ml of 0.5% tetrazolium solution was also added it. The mixture was incubated at 38°C for 3 to 4 hours. Tetrazolium chloride (Tz) is reduced to red coloured compound formazan in the seed embryo in the presence of dehydrogenase enzyme. After 4 hours, the mixture was centrifuged for 10mins at 10,000rpm and supernatant was discarded. Then, 10ml of acetone was added to centrifuge tube in order to extract formazon. The tubes were kept at room temperature for 16 hours after that centrifuged at 10,000 rpm for 10mins. Spectrophotometer absorbance was estimated at 520nm taking acetone as blank solution. These observations were indicated as optical density (O.D.) as per the procedure given by Kittock and Law (1968).

Extraction of antioxidant enzymes: One-gram sample of seed from each treatment was placed in a pre-chilled pestle and 5 ml of cold extraction solution containing 0.1 M phosphate buffer (pH 7.0), 2.5 mM DDT, and 1 mM EDTA was added to it. The sample was pulverized thoroughly using a mortar by adding few glass abrasives. After that, the homogenate was placed in a centrifuge tube and was centrifuged at 10,000 rpm for 10 minutes. This whole process of enzyme extraction was performed at temperature of 0-4°C. The resulting supernatant was utilized in various anti-oxidant enzyme tests to determine the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD).

Catalase Assay: In a test-tube, a mixture of chemicals containing 0.55ml of 0.1M potassium phosphate buffer (pH 7.0), 0.4ml of 0.2 M H O, was prepared and 50 µl of enzyme extract was added to it. The whole reaction mixture was incubated at 37°C for a minute. The reaction was stopped by adding 3 ml of 5% (w/v) potassium dichromate and glacial acetic mixture in 1:3 v/v ratio. After that, test tubes were kept in a hot water bath for 10 minutes then gradually cooled. Dichromate acetate solution was used as the blank. Finally, the absorbance was recorded at 570 nm. The amount of H_2O_2 reacted in the mixture was calculated by subtracting the absorbance of other samples from the control. One unit of enzyme activity was given as the amount of enzyme that catalyzes the oxidation of 1 mole H_2O_2 per min. Catalase activity was determined according to the procedure suggested by Sinha (1972).

Superoxide dismutase Assay (SOD): In a test tube, the chemicals 2.5 ml of 60 mM Tris-HCl (pH 7.8), 0.1 ml of 420 mM L-methionine, 0.1 ml of 1.8mM NBT, 0.1 ml of 3.0 mM EDTA were mixed in the serially. The reaction mixture was then made up of 3ml by mixing 0.1 ml of enzyme extract with 0.1 ml of 90uM riboflavin in the test tube. The solution was thoroughly mixed and placed 30 cm below the light source, which consisted three 20 W fluorescent lights. A blank solution containing only the buffer without any enzyme extract was prepared. The reaction was begun by turning on the light and ended by turning it off after 40 minutes of incubation. Once the reaction was completed, the tubes were covered with black material in order to prevent further reaction. The blank is a non-irradiated reaction mixture which hadn't developed any colour. Only the reaction mixture without any enzyme extract developed maximum colour, and its absorbance reduced with increase in volume of enzyme extractin the mixture. At 560 nm, the absorbance was measured. The ability of superoxide dismutase to prevent the photochemical reduction of nitro blue tetrazolium (NBT) was measured using the Beauchamp and Fridovich (1971).

The enzyme activity was estimated in units of g FW, and % inhibition was estimated using the formula given by Asada *et al.,* (1974):

Per cent inhibition = $\frac{V-v}{v} \times 100$

Where,

V-Rate of reaction in absence of SOD

v-Rate of reaction in presence of SOD

One enzyme unit is defined as the amount of enzyme that inhibits the NBT photo reduction by 50%.

Peroxidase Assay: It was initiated by mixing 2.75 ml of 50 mM phosphate buffer (pH 6.5), 0.1 ml of 0.5% hydrogen peroxide, 0.1 ml of 0.2% O-dianisidine dye. Then, the mixture was added with 0.05 ml of enzyme extract. The same mixture without H_2O_2, was taken as a blank. Change in the absorbance was estimated at 430 nm for 3 min and one unit of POD was defined as the quantity of enzyme required to cause O.D. change per minute according to the procedures given by Shannon *et al.* (1966).

Synthesis of Zinc oxide nano-particles: ZnO nano-particles were prepared as the procedure of Moghaddam *et al.* (2009), in the laboratory of Department of Seed Science and Technology, CCS HAU Hisar. The procedure involves, preparation of 0.45 M aqueous solution of Zinc nitrate Zn $(NO_3)_2 2H_2O$ and 0.9M aqueous NaOH in distilled water. After that, the beaker containing NaOH solution was heated at 55^0C temperature. The Zn $(NO_3)_2 2H_2O$ solution was added dropwise slowly up to 40 mins to the above solution. After this the beaker was sealed and kept for 2 hours. Then precipitated ZnO NPs was cleaned with deionized water and ethanol then dried in the air atmosphere at about 60^0C.

Characterization of Zinc oxide nano-particles: The characterization of synthesized ZnO NPs was done by FESEM (Field Emmision Scanning Electron Microscope) and HRTEM (High Resolution Transmission Electron Microscope). As per the results of SEM and TEM, synthesized ZnO NPs had the characteristics with average particle size 35.25nm with purity of 99.9% The particles were white in colour having spheroidal and ellipsoidal shape with inter-planar spacing of 0.85nm (Figure 1).

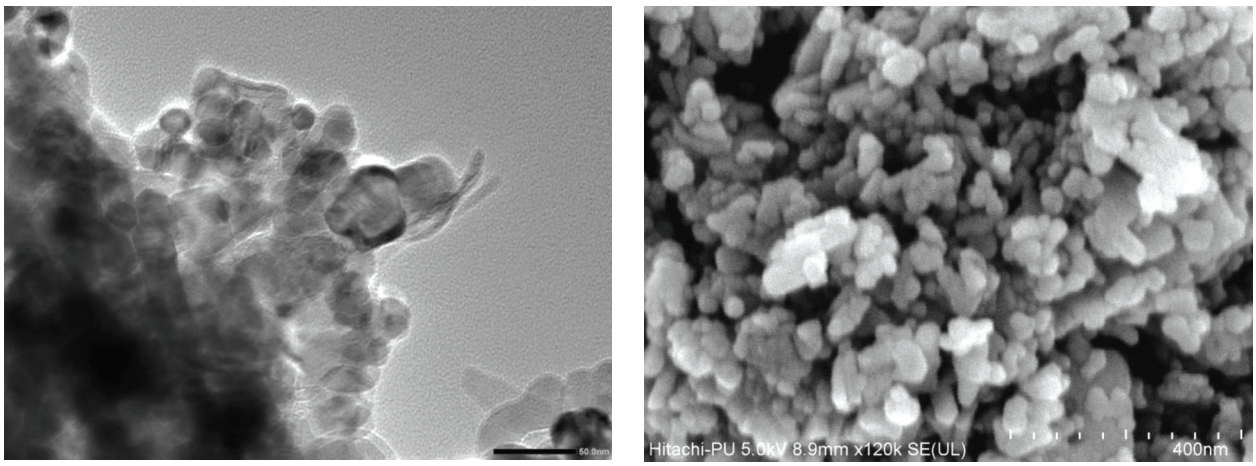

Figure: 1 High resolution transmission electron microscope (HRTEM) and Filed emission scanning electron microscope (FESEM) image of ZnO NPs

The experiment was conducted in completely randomized design (CRD) and data recorded from study were analyzed according to standard method of Panse and Sukhatme (1985) and by using the online statistical tool developed by Sheoran (2010).

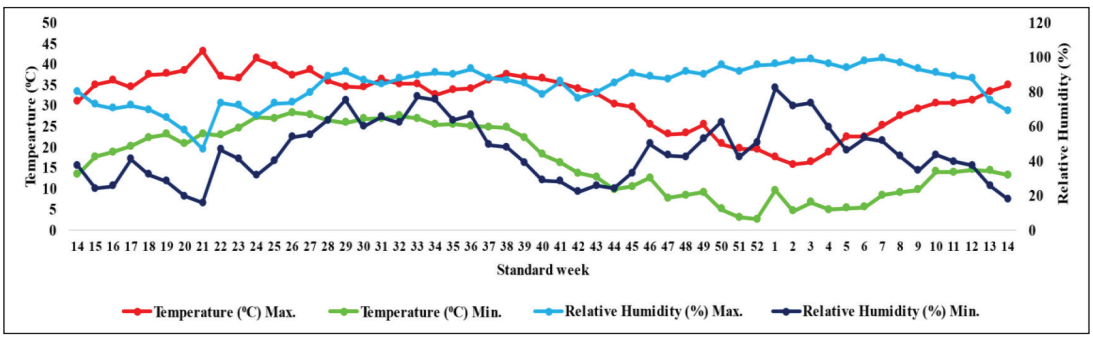

Figure 2: Average weather data of Hisar during storage period (2020-21)

3. Results and Discussion

The data revealed that the priming treatments significantly enhanced catalase, peroxidase superoxide and catalase activities of primed seeds except 5% ethanol. Maximum catalase activity (243.168 and 157.981umoles/g FW), peroxidase activity (22.33 and 35.03 units/g FW), superoxide activity (22.03 and 35.03 nmoles/g FW) and dehydrogenase activity (0.213 and 0.245 OD/g/ml) were estimated in the seeds primed with ZnO 100ppm while minimum catalase activity (114.105 and 141.980 umoles/g FW), peroxidase activity (13.00 and 20.15units/g FW), superoxide activity (15.361 and 20.147 moles/g FW) and dehydrogenase activity (0.140 and 0.197 OD/g/ml) were recorded in seeds primed with 5% ethanol in cloth and polythene bag, respectively (Table 1-4). Although, ethanol at lower concentration (1&3%) showed positive effect on enzyme activities but at higher concentration (5%), it resulted in decrease in enzyme activities. Among the varieties, DWRB 92 recorded the highest catalase activity (217.168 umoles/g FW) in cloth bag while BH 393 recorded maximum catalase activity (232.961 umoles/g FW) in polythene bag. Minimum catalase activity was observed in DWRB 101(60.351 umoles/g FW) in cloth and BH 885 recorded least (121.418 umoles/g FW) in polythene bags. Maximum peroxidase activity (21.22 and 31.56 units/g FW) was estimated in BH 885 while minimum (12.71 and 22.54 units/g FW) in BH 946 in cloth and polythene, bag respectively. Maximum SOD activity (18.033 and 25.033 nmoles/g FW) was measured in DWRB 92 and minimum (12.712 and 22.537 nmoles/g FW) in BH 946 in cloth and polythene bag, respectively. Maximum DHA activity (0.170 and 0.206 OD/g/ml) was recorded in DWRB 101 and least in BH 946 (0.234 and 0.281 OD/g/ml) in cloth and polythene bag stored seeds. The antioxidant enzyme activity such as catalase, peroxidase, superoxide and dehydrogenase activity in stored seeds significantly increased after priming treatments. Only ethanol 5% priming showed the detrimental effect apart from that all other priming treatments increased the enzyme activity. All these four enzymes showed highest activity in ZnO 100ppm priming followed by GA_3 100ppm as compared to other treatments. Ethanol priming also increased the enzymatic activity significantly at lower concentrations (1% and 3%) but higher concentration is toxic which resulted in lower enzyme activities. The significant difference was also observed between cloth and polythene bag which may be due to higher initial activity of enzymes as a result of less deterioration in polythene bag. These results are in accordance with the reports of Rawat *et al.*, (2018) who reported that wheat (variety UP2526) seed treated with four different nano-particles (TiO_2, ZnO, nickel and Chitosan) @ 50ppm and 300 ppm concentration for 4, 6 and 8 hours durations showed better root length, shoot length, seedling length, shoot dry weight, seedling dry weight, seedling vigour index-I and seedling vigour index-II as compared to control. Vijayalaxmi *et al.*, (2013) also conducted an experiment to elucidate the effect of TiO_2 nanoparticles on naturally aged seeds of maize at various concentrations *viz.*, 200, 400, 600, 800 and 1000 mg/kg and reported that seeds treated with 200 mg/ kg recorded significantly higher germination (88%), shoot length (20.52 cm), root length (11.91 cm), dry weight (1.34 g), dehydrogenase activity (0.784 OD value) over the control. Itroutwar *et al.,* (2020) reported that seeds primed with ZnO nano-particles @ 100 mg/L showed maximum improvement in seed quality parameters *viz.*, shoot length (13.0 cm), shoot width (3.4 mm), root length (20.7 cm), root width (1.0 mm), leaf length (60 mm), leaf width (16.0 mm), vigour index (2931.9) and dry matter production (5.33 gm) as compared to ionic control (zinc acetate) and control (hydro-priming). The seeds stored in the polythene bag showed significantly higher enzyme activity as compared to cloth bag. Polythene bag (>700gauge thickness) maintained seed moisture content constant throughout the storage period which resulted in higher enzyme activities. Pavani *et al.,* (2020) reported that ZnO nano-particles have been found to induce the activities of Guaiacol peroxidase, Glutathione Reductase, Catalase and increase in the ascorbic acid and hydrogen peroxide contents in mungbean crop. Nano-particles increases enzymes activities *viz.,* catalases, superoxidase dismutase and guaiacol-peroxidase due to reduction in Reactive oxygen species (ROS) levels in seeds and hence reduces cell damage (Guha *et al.,* 2018). Nano-particles increase water uptake by the seeds which activate germination and increase enzymes activities in phases I and II of germination process (Joshi *et al.*, 2018). Major ROS-scavenging enzymes include superoxide dismutase, glutathione reductatse and catalase. Superoxide dismutase is a key enzyme in the regulation of the quantity of superoxide radicals and peroxides. Hydrogen peroxide

Table 1: Effect of storage container and priming treatments on catalase activity (umoles/g FW) of barley varieties

Treatments(T)	Cloth bag							Polythene bag						
	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean
Control	164.28	112.56	90.75	100.98	39.86	190.61	116.51	194.63	157.64	154.99	104.07	152.05	115.92	146.55
GA_3 50ppm	210.25	179.53	115.97	133.19	64.79	211.54	152.55	213.36	166.57	181.05	131.00	174.98	123.98	165.16
GA_3 100ppm	182.34	133.62	120.47	124.91	78.57	246.24	147.69	219.89	176.69	167.92	120.13	192.11	144.52	170.21
$GA_3$150ppm	170.21	120.56	99.68	111.04	51.92	203.67	126.18	202.69	164.69	161.05	113.00	161.98	122.85	154.38
Ethanol 1%	205.42	206.42	107.42	124.81	53.08	218.47	152.61	211.56	171.57	170.92	117.13	167.11	132.98	161.88
Ethanol 3%	215.30	222.30	218.30	129.27	65.21	231.60	180.33	219.56	178.57	176.92	125.13	172.11	138.98	168.54
Ethanol 5%	157.42	200.42	86.09	98.14	36.75	183.80	114.11	189.56	151.57	150.92	99.00	148.98	111.85	141.98
ZnO 50ppm	219.45	178.16	117.31	121.01	61.49	215.30	152.12	215.69	171.50	175.05	123.00	173.98	139.85	166.51
ZnO100ppm	239.58	204.29	129.44	134.88	83.36	243.17	172.45	220.29	181.69	229.74	144.13	196.11	157.98	288.32
ZnO200ppm	227.45	185.16	124.65	112.68	68.49	227.30	157.62	202.69	164.69	161.05	137.59	152.58	125.54	157.36
Mean	199.17	174.30	121.01	119.09	60.35	217.17	148.52	208.99	168.52	232.96	121.42	169.20	131.45	172.09
C.D. (P=0.05)	Container=0.876,Variety=1.518,Treatment=1.959,ContainerxVariety =2.146, ContainerxTreatment=2.771, VarietyxTreatment= 4.779, ContainerxVarietyxTreatment= 6.787													

Table 2: Effect of storage container and priming treatments on peroxidase activity (Units/g FW) of barley varieties

Treatments (T)	Cloth bag							Polythene bag						
	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean
Control	8.68	14.76	12.52	16.30	13.44	16.10	13.63	17.22	21.92	21.08	27.00	26.02	23.11	22.72
GA_3 50ppm	13.85	21.30	19.26	22.39	17.92	21.11	19.31	23.14	25.84	26.00	33.92	29.94	28.03	27.81
$GA_3$100ppm	16.00	24.61	20.38	27.42	21.01	23.26	22.11	26.14	29.84	28.34	37.92	32.94	34.70	31.65
$GA_3$150ppm	11.60	16.68	14.44	19.22	15.36	20.03	16.22	19.14	24.84	24.00	28.92	28.94	25.03	25.15
Ethanol 1%	12.45	18.91	16.87	19.82	15.43	19.82	17.22	22.14	26.84	29.00	29.59	27.28	27.03	26.98
Ethanol 3%	14.45	21.91	18.87	22.49	18.43	21.82	19.66	25.14	27.84	31.00	32.92	28.28	29.03	29.04
Ethanol 5%	8.27	13.68	12.44	15.56	12.70	15.36	13.00	15.14	18.84	18.00	24.92	22.94	21.03	20.15
ZnO 50ppm	14.60	18.68	17.44	23.22	18.36	20.03	18.72	23.14	25.84	26.00	33.92	30.94	31.03	28.48
ZnO100ppm	16.60	24.68	20.44	27.56	21.36	22.33	22.11	35.03	30.84	29.00	37.59	33.94	35.03	33.57
ZnO200ppm	10.60	15.68	15.44	18.22	16.36	18.03	15.72	19.14	22.84	24.00	28.92	28.94	25.03	24.81
Mean	12.71	19.09	16.81	21.22	17.04	19.76	17.77	22.54	25.55	25.64	31.56	29.02	27.91	27.04
C.D. (P=0.05)	C=0.415, V=0.720, CxV=1.018, T =0.929, CxT=1.314, VxT= 2.275, CxVxT= NS													

Table 3: Effect of storage container and priming treatments on superoxide dismutase (nmoles/g FW) of barley varieties

Treatments	Cloth bag							Polythene bag						
	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean
Control	8.68	14.76	12.52	16.30	13.44	16.10	13.63	17.22	21.92	21.08	27.00	26.02	23.11	22.72
GA_3 50ppm	13.85	76.56	19.26	22.39	17.92	21.11	28.52	23.14	25.84	26.00	33.92	29.94	28.03	27.81
GA_3 100ppm	16.00	24.61	20.38	27.42	20.67	23.26	22.06	26.14	29.84	28.00	37.92	32.94	32.03	31.15
GA_3 150ppm	11.60	16.68	14.44	19.22	15.36	20.03	16.22	19.14	24.84	24.00	28.92	28.94	25.03	25.15
Ethanol 1%	12.45	18.91	16.87	19.82	15.43	19.82	17.22	22.14	26.84	29.00	35.92	27.28	29.03	28.37
Ethanol 3%	14.45	21.91	18.87	22.49	18.43	21.82	19.66	25.14	27.84	31.00	39.92	28.28	27.03	29.87
Ethanol 5%	8.27	17.68	12.44	15.56	12.70	15.36	13.67	15.14	18.84	18.00	24.92	22.94	21.03	20.15
ZnO 50ppm	14.60	18.68	17.44	23.22	18.36	20.03	18.72	23.14	25.84	26.00	33.92	30.94	31.03	28.48
ZnO 100ppm	16.60	19.68	20.44	25.22	21.36	22.03	20.89	35.03	30.84	29.00	35.92	33.94	35.03	33.30
ZnO 200ppm	10.60	15.68	15.44	18.22	16.36	18.03	15.72	19.14	22.84	24.00	28.92	28.94	25.03	24.81
Mean	12.71	24.51	16.81	20.99	17.00	19.76	18.63	22.54	25.55	25.61	32.73	29.02	27.64	27.18
C.D(P=0.05)	C=0.992, V=1.719, T =2.219, CxV=2.431, CxT=3.138, VxT= 5.436, CxVxT=7.689													

Table 4: Effect of storage container and priming treatments on Dehydrogenase activity (OD/g/ml) of barley varieties

Treatments	Cloth bag							Polythene bag						
	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean	BH 946	BH 902	BH 393	BH 885	DWRB101	DWRB92	Mean
Control	0.210	0.204	0.186	0.164	0.142	0.151	0.176	0.256	0.243	0.210	0.196	0.178	0.183	0.211
GA_3 50ppm	0.230	0.234	0.211	0.194	0.163	0.174	0.201	0.276	0.273	0.235	0.226	0.199	0.206	0.236
GA_3 100ppm	0.280	0.276	0.281	0.239	0.211	0.212	0.250	0.326	0.315	0.265	0.271	0.247	0.244	0.278
GA_3 150ppm	0.220	0.209	0.201	0.184	0.157	0.167	0.190	0.266	0.248	0.225	0.216	0.193	0.199	0.225
Ethanol 1%	0.225	0.222	0.205	0.188	0.166	0.168	0.196	0.281	0.261	0.229	0.221	0.205	0.210	0.235
Ethanol 3%	0.246	0.248	0.236	0.212	0.195	0.195	0.222	0.302	0.287	0.260	0.245	0.227	0.237	0.260
Ethanol 5%	0.194	0.187	0.165	0.156	0.133	0.140	0.163	0.240	0.226	0.189	0.188	0.169	0.172	0.197
ZnO 50ppm	0.235	0.230	0.221	0.198	0.171	0.174	0.205	0.281	0.269	0.245	0.230	0.207	0.206	0.240
ZnO 100ppm	0.274	0.273	0.267	0.240	0.207	0.213	0.246	0.314	0.308	0.279	0.264	0.242	0.245	0.275
ZnO 200ppm	0.224	0.220	0.200	0.175	0.151	0.164	0.189	0.270	0.259	0.224	0.213	0.197	0.199	0.227
mean	0.234	0.230	0.217	0.195	0.170	0.176	0.204	0.281	0.269	0.236	0.227	0.206	0.210	0.238
C.D.(P=0.05)	C=0.003, V=0.006, CxV=0.008, T =0.007, CxT=0.01, VxT= NS, CxVxT= NS													

(H_2O_2) can react in the Haber-Weiss reaction forming hydroxyl radicals which cause lipid peroxidation (Mittler *et al.,* 2004). Catalase is implicated in the removal of H_2O_2. Removal of H_2O_2 through a series of reactions is known as an ascorbate- glutathione cycle in which ascorbate and glutathione participate in a cyclic transfer of reducing equivalents resulting in the reduction of H_2O_2 to water (H_2O) using electrons derived form nicotinamide adenine dinucleotide phosphate (Goel and Sheoran, 2003). Seed deterioration is mainly caused by lipid peroxidation which can be is prevented through enhancement in dehydrogenase activity and lower peroxide formation by priming (McDonald, 1999). It is concluded from the study that seed priming with nano-particles at optimum concentration has a potential to enhance the seed quality particularly in poor storer seeds. As Dehydrogenase, Catalase, Superoxide dismutase and Peroxidase activity in seeds are directly related with the seed quality. Hence, seed quality of barley may be enhanced through seed priming with ZnO @100ppm and GA_3 @100ppm at 25°C for 24 hours. Further selection of storage containers also plays a crucial role in storage, storage of barley seeds found to be beneficial in maintaining seed quality in polythene bags (>700gauge) with moisture content (<8%).

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Author's Contribution

Siddu Appasaheb Kurubar: Execution of field/lab experiments and data collection

Axay Bhuker: Designing of the experiments, Analysis of data and interpretation

Sukham Madaan: Preparation of the manuscript

Declaration of interest statement

The authors declare no conflict of interest.

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