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Hydropriming influences physio-chemical responses of fresh and naturally aged seeds of bottle gourd (*Lagenaria siceraria* L.)

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ABSTRACT

Hydropriming is a simple and fundamental technique where seeds are soaked in distilled water to improve germination rate, accelerate germination speed, and promote uniform crop establishment, especially in challenging conditions. An investigation was carried out to study the effect of hydropriming on various enzyme activities in fresh harvested, one year old, and two year old seeds of bottle gourd (Pusa Naveen variety and HBGH-35 hybrid). The study was conducted with Completely Randomized Design (CRD) consisting four replications. Seeds of variety as well as hybrid were soaked in distilled water for 48 hours, using half the volume of water relative to seed weight (w) maintaining a constant temperature of 25°C. Subsequently, various enzyme assays and physiological parameters were evaluated to assess the impact of hydropriming on antioxidant enzyme activities and seed quality. The results indicated that hydropriming significantly improved the quality of the different seed lots. The highest levels of dehydrogenase activity, catalase activity, peroxidase activity, superoxide dismutase activity, speed of germination, and field emergence index were observed in fresh seeds, followed by one year old and then two year old seeds. However, the greatest percentage enhancement was noted in the one year old seed lot, followed by the two year old and fresh seed lots. Therefore, the study concluded that the one year old seed lot responded more favourably to process of hydropriming, followed by the two year old and fresh seed lots.

KEYWORDS

Antioxidant; Dehydrogenase; Enzyme; Genotypes; Hydropriming

ARTICLE HISTORY

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Introduction

Lagenaria siceraria (Molina) Stand commonly known as bottle gourd is a key vegetable crop in the Cucurbitaceae family cultivated for its fleshy fruits in the regions of tropics and subtropics. Believed to have originated in Africa, the species exhibits significant diversity, which suggests its broad environmental adaptability [1]. In the western part of the country, it is cultivated during both the Kharif and summer seasons, while in tropical regions, it is grown year-round under mild temperature conditions [2]. The edible portion of bottle gourd is rich in nutrients such as carbohydrate, protein, fat and minerals including calcium and phosphorus [3]. It is considered one of nature's most beneficial fruits for human health, as it contains essential nutrients. Additionally, bottle gourd is useful in managing various ailments such as cardiac disorders, hepatic disorders, and ulcers [4]. It is also reported to contain high levels of choline, a compound known to help treat mental disorders [5,6]. In India, bottle gourd has traditionally been used to alleviate stomach complications.

However, the popularity and cultivation of bottle gourd landraces have declined due to the growing preference for exotic members of the Cucurbitaceae family, such as pumpkins, watermelons, butternut squash, and squashes. There is an urgent need for research to document the agronomy of bottle gourd landraces and their potential to enhance food security in marginal areas. Seed priming could help in boosting antioxidative systems, lowering lipid peroxidation rates and contributing to seed vigor [7,8]. Hu et al. found that priming enhanced the activity of antioxidant systems [9]. One consequence of seed deterioration or exposure to environmental stress is the increased production of reactive oxygen species (ROS), including free radicals [10,11]. Antioxidant enzymes, such as superoxide dismutase and catalase, play a crucial role in eliminating free radicals and reactive oxygen species [10]. Seed priming repairs the damage in aged seeds or those exposed to abiotic stress, thereby improving germination performance [12]. While the positive effects of seed priming on germination and antioxidant activity are well-documented in other plant species, there is limited research on the application of these techniques specifically to bottle gourd landraces. This study aims to fill this gap by investigating the effectiveness of seed priming in enhancing the germination and resilience of bottle gourd landraces.

Methodology

Seed materials

The experiment was conducted in the laboratories of the Department of Seed Science and Technology at Chaudhary Charan Singh Haryana Agricultural University, Hisar

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(29°9'14.18"N, 75°43'22.58"E). Fresh seeds, along with one year old and two year old seeds (stored under ambient conditions) of the Pusa Naveen variety and the HBGH-35 hybrid of bottle gourd, were sourced from the Department of Vegetable Science at the same university. To evaluate the effects of hydropriming on various enzyme activities in the two bottle gourd genotypes, seeds were fully immersed in petri dishes containing distilled water at a ratio of half the seed weight (w). The petri dishes were maintained at a constant temperature of 25°C for 48 hours under controlled conditions. This hydropriming protocol had been standardized in a previous experiment (data not shown). All seeds were removed from the petri dishes simultaneously. The experimental units (petri dishes) were arranged in a completely randomized design with four replications. Dry or non-primed seeds were used as the control.

Seed physiological parameters

The primed seeds were evaluated for various seed physiological parameters, including speed of germination, field emergence index, and electrical conductivity (μS $cm^{-1}g^{-1}$). Speed of germination was assessed by observing the seeds daily for germination (radicle emergence of up to 2 mm) and calculated using the following formula.

Speed of germination= n1/d1+n2/d2+n3/d3+---------- Where, n= number of germinated seeds, d= number of days. The speed of germination and electrical conductivity was evaluated as per the standard procedure in International Rules for Seed Testing (ISTA, 2017; https://www.seedtest.org/).

Enzyme assay

Hydroprimed seeds and dry seeds were evaluated for enzyme activities. Observations were recorded for various enzyme activities, including dehydrogenase activity, catalase activity, peroxidase activity, and superoxide dismutase activity. The recorded data for each parameter were subjected to a three-way analysis of variance using OP-STAT software to find out the significance of variance ($p<0.05$).

Results and Discussion

Speed of germination

The speed of germination was remarkably improved by hydropriming treatment in fresh seed, one year old seed and two year old seed of bottle gourd variety Pusa Naveen and hybrid HBGH-35 (Table1). Maximum values of speed of germination were obtained in fresh seeds (66.99) followed by one year old seeds (39.71) and two year old seeds (21.65). However, in terms of percent improvement, aged seeds showed better performance as compared to fresh seeds. Additionally, the HBGH-35 hybrid seeds exhibited a higher germination speed (44.14) compared to the Pusa Naveen variety seeds (41.43). The enhancement in speed of germination in hydroprimed seeds can be attributed to the fact that, during hydropriming, seeds have already completed phases I and II of germination. This enables them to complete the imbibition process more quickly when they come into contact with water, reducing the time required for cellular activities to commence, thus accelerating germination. Sanchez et al. reported that a single cycle of hydropriming was sufficient to enhance and speed up germination in cucumber cultivars [13]. Similarly, Aragao et al. observed that hydropriming promoted faster and more favorable germination in bean seeds [14]. Our findings are consistent with the results of Das et al. in bottle gourd and Shakuntala et al. in cucumber [15,16].

Table 1. Effect of hydropriming treatment, genotypes and different aged seed lots on speed of germination of bottle gourd genotypes.

G/L/T	Pusa Naveen variety (G1)				HBGH-35 hybrid (G2)				
	L1	L2	L3	Mean (G1)	L1	L2	L3	Mean (G2)	Mean (T)
T0	64.15	24.66	19.74	36.18	68.51	26.82	21.38	38.90	37.54
T1	65.46	52.41	22.16	46.68	69.84	54.96	23.30	49.37	48.02
Mean (L)	64.81	38.54	20.95	41.43	69.17	40.89	22.34	44.14	
Mean	L1- 66.99		L2- 39.71		L3- 21.65				
CD (P=0.05%)	G (main effect)	L (main effect)	G x L (Interaction effect)	T (main effect)	G x T (Interaction effect)	L x T (Interaction effect)	G x L x T (Interaction effect: 3 factors)		
	1.26	1.55	NS	1.26	NS	2.19	NS		

G: Genotype; L: Seed lot (L1: Fresh, L2: one year old, L3; two year old); T: Priming duration (T0: Control; T1: 48 hour)

Field emergence index

The highest emergence index was observed in fresh seeds (33.86), whereas one year old seeds (17.81) and two year old seeds (12.83) exhibited lower values as shown in Table 2. However, the percentage of improvement was greatest in one year old seeds (62.16%), compared to two year old seeds (18.91%) and fresh seeds (2.32%). Among the genotypes, the HBGH-35 hybrid had a higher emergence index (22.75) than the Pusa Naveen variety (20.25). Hydroprimed seeds exhibited a significantly higher field emergence index (23.40) compared to the control or dry seeds (19.59). Similar findings were reported by Amooaghaie et al. in tomato; Catiempo et al. in sunflower and Adhikari et al. in bitter gourd. [17-19].

Electrical conductivity (μS $cm^{-1}g^{-1}$)

The data in Table 3 show that the electrical conductivity of seed leachates was higher in the control (699.83) compared to hydroprimed seeds (664.00). Among the different seed lots, fresh seeds had the lowest electrical conductivity (354.50), while one year old seeds (730.75) and two year old seeds (960.50) exhibited higher values. Electrical conductivity generally reflects the integrity of cell membranes and the overall quality of seeds, with lower values indicating stronger membrane integrity and higher seed quality. The higher electrical conductivity observed in control seeds may be due to rapid electrolyte leakage and membrane damage during imbibition, which compromises the selective permeability of the cell membrane, leading to increased seed leachates. Hydropriming enhanced

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seed quality, as demonstrated by the reduced electrical conductivity in primed seeds, likely due to the gradual hydration process that helped maintain the structure of the plasma membrane. Similar results were observed by Basra et al. in wheat, Abdulrahmani et al. in barley, Rouhi et al. in coriander and Shakuntala et al. in cucumber [16,20-22].

Table 2. Effect of hydropriming treatment, genotypes and different aged seed lots on speed of germination of bottle gourd genotypes.

G/L/T	Pusa Naveen variety (G1)				HBGH-35 hybrid (G2)				
	L1	L2	L3	Mean (G1)	L1	L2	L3	Mean (G2)	Mean (T)
T0	31.14	12.78	10.66	18.19	35.81	14.39	12.77	20.99	19.59
T1	32.21	21.49	13.20	22.30	36.29	22.57	14.66	24.51	23.40
Mean (L)	31.68	17.14	11.93	20.25	36.05	18.48	13.72	22.75	
Mean	L1- 33.86		L2- 17.81		L3- 12.83				
CD (P=0.05%)	G (main effect)	L (main effect)	G x L (Interaction effect)	T (main effect)	G x T (Interaction effect)	L x T (Interaction effect)	G x L x T (Interaction effect: 3 factors)		
	0.61	0.75	1.06	0.61	N/A	1.06	N/A		

G: Genotype; L: Seed lot (L_1: Fresh, L_2: one year old, L_3; two year old); T: Priming duration (T_0: Control; T_1: 48 hour)

Table 3. Effect of hydropriming treatment, genotypes and different aged seed lots on electrical conductivity (µS $cm^{-1}g^{-1}$) of bottle gourd genotypes.

G/L/T	Pusa Naveen variety (G1)				HBGH-35 hybrid (G2)				
	L1	L2	L3	Mean (G1)	L1	L2	L3	Mean (G2)	Mean (T)
T0	382.00	775.00	985.00	714.00	336.00	748.00	973.00	685.67	699.83
T1	371.00	728.00	950.00	683.00	329.00	672.00	934.00	645.00	664.00
Mean (L)	376.50	751.50	967.50	698.50	332.50	710.00	953.50	665.33	
Mean	L1- 354.50		L2- 730.75		L3- 960.50				
CD (P=0.05%)	G (main effect)	L (main effect)	G x L (Interaction effect)	T (main effect)	G x T (Interaction effect)	L x T (Interaction effect)	G x L x T (Interaction effect: 3 factors)		
	1.64	2.01	2.84	1.64	2.32	2.84	4.01		

G: Genotype; L: Seed lot (L_1: Fresh, L_2: one year old, L_3; two year old); T: Priming duration (T_0: Control; T_1: 48 hour)

Dehydrogenase activity (OD g^{-1} ml^{-1})

The results of the current study showed that hydropriming significantly increased dehydrogenase activity in fresh, one year old, and two year old seeds of both the Pusa Naveen variety and the HBGH-35 hybrid of bottle gourd (Table 4). Among the seed groups, fresh seeds exhibited the highest dehydrogenase activity (0.640) compared to one year old seeds (0.526) and two year old seeds (0.423). However, aged seeds demonstrated a more pronounced improvement in dehydrogenase activity than fresh seeds. As illustrated in Figure 1A, the one year old seeds showed the largest increase (10.72%), followed by two year old seeds (8.63%) and fresh seeds (3.02%). HBGH-35 hybrid seeds recorded the highest dehydrogenase activity (0.600), surpassing the Pusa Naveen variety seeds (0.459).

Dehydrogenase activity is a key marker for assessing seed viability, as it is produced by all living, respiring seeds. In this investigation, less viable seeds exhibited reduced dehydrogenase activity due to the death of viable tissues. Priming significantly increased dehydrogenase activity in both highly viable and less viable seeds. The enhanced dehydrogenase activity in hydroprimed seeds can be attributed to the initiation of enzyme activity and increased cell cycle activity during the priming process. These findings are consistent with observations in cucumber, sunflower, barley and stevia [23-26].

Table 4. Effect of hydropriming treatment, genotypes and different aged seeds lots on dehydrogenase activity (OD g-1 ml-1) of bottle gourd genotypes.

G/L/T	Pusa Naveen variety (G1)				HBGH-35 hybrid (G2)				
	L1	L2	L3	Mean (G1)	L1	L2	L3	Mean (G2)	Mean (T)
T0	0.558	0.442	0.329	0.443	0.702	0.556	0.482	0.580	0.512
T1	0.577	0.484	0.363	0.475	0.721	0.621	0.518	0.620	0.547
Mean (L)	0.568	0.463	0.346	0.459	0.712	0.588	0.500	0.600	
Mean	L1- 0.640		L2- 0.526		L3- 0.423				
CD (p<0.05)	G (main effect)	L (main effect)	G x L (Interaction effect)	T (main effect)	G x T (Interaction effect)	L x T (Interaction effect)	G x L x T (Interaction effect: 3 factors)		
	0.004	0.004	0.006	0.004	0.005	0.006	0.009		

G: Genotype; L: Seed lot (L_1: Fresh, L_2: one year old, L_3; two year old); T: Priming duration (T_0: Control; T_1: 48 hour)

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Catalase activity (µmoles g^{-1} dry weight)

Hydropriming treatment led to significantly higher catalase activity (µmoles g-1 dry weight) in HBGH-35 hybrid seeds (427.74) compared to the Pusa Naveen variety, which exhibited lower catalase activity (410.81). Among the different seed lots, fresh seeds showed the highest catalase activity (475.88), whereas one year old seeds (430.05) and two year old seeds (351.89) showed comparatively low activity (Table 5). However, the greatest percentage increase in catalase activity was observed in the one year old seed lot (5.74%), compared to two year old lot (5.23%) and the fresh seed lot (2.97%), as shown in Figure 1B. The mean catalase activity was higher in hydroprimed seeds (428.58) compared to the control (409.97).

Table 5. Effect of hydropriming treatment, genotypes and different aged seed lots on catalase activity (µmoles g^{-1} dry weight) of bottle gourd genotypes

G/L/T	Pusa Naveen variety (G1)				HBGH-35 hybrid (G2)				
	L1	L2	L3	Mean (G1)	L1	L2	L3	Mean (G2)	Mean (T)
T0	460.33	407.56	337.19	401.69	477.52	428.54	348.66	418.24	409.97
T1	474.29	430.18	355.28	419.92	491.38	453.91	366.43	437.24	428.58
Mean (L)	467.31	418.87	346.24	410.81	484.45	441.22	357.55	427.74	
Mean	L1- 475.88		L2- 430.05			L3- 351.89			
CD (P=0.05%)	G (main effect)	L (main effect)	G x L (Interaction effect)	T (main effect)	G x T (Interaction effect)	L x T (Interaction effect)	G x L x T (Interaction effect: 3 factors)		
	1.33	1.63	2.30	1.33	1.88	2.30	N/A		

G: Genotype; L: Seed lot (L_1: Fresh, L_2: one year old, L3; two year old); T: Priming duration (T_0: Control;T_1: 48 hour)

The enhancement in catalase activity due to hydropriming may be attributed to the restoration and improvement of gene expression related to catalase, which helps mitigate the effects of aging on enzyme activity [27]. Catalase plays a crucial role in scavenging free radicals during lipid peroxidation and aid in protecting mitochondrial components from oxidative damage [28]. It also protects cells from ROS [29,30]. Catalase accumulates in the cytosol along with hydrogen peroxide during seed priming [31]. Decreased catalase activity has been associated with aging, leading to increased lipid peroxidation, loss of vigor, and reduced viability in maize and sunflower [32,33]. Our results are consistent with studies by Parmoon et al., Huang et al. in cucumber, Wang et al. in bitter gourd, Hsu et al. in bitter gourd, Kulsumbi et al. in spinach, and Sepehri and Rouhi in groundnut, Garcia-Caparros et al., Lee et al. in Brassica oleracea [8,34-38].

Peroxidase activity (nmoles g^{-1} dry weight)

Hydropriming treatment had a significant impact on peroxidase activity (nmoles g $^{-1}$ dry weight), as shown by the higher mean peroxidase activity in hydroprimed seeds (40.31) compared to the control (37.93) (Table 6). Seeds of the HBGH-35 hybrid exhibited greater peroxidase activity (47.96) com-pared to the Pusa Naveen variety (30.28). Among the different seed lots, fresh seeds recorded the highest peroxidase activity (51.63), comparing the one year old seeds (39.61) and two year old seeds (26.12). However, the greatest percentage increase in peroxidase activity was observed in the one year old seed lot (14.78%), followed by fresh seed lot (2.40%) and two year old lot (1.85%), as shown in Figure 1C.

Table 6. Effect of hydropriming treatment, genotypes and different aged seed lots on peroxidas eactivity (nmoles g $^{-1}$ dry weight) of bottle gourd genotypes

G/L/T	Pusa Naveen variety (G1)				HBGH-35 hybrid (G2)				
	L1	L2	L3	Mean (G1)	L1	L2	L3	Mean (G2)	Mean (T)
T0	41.20	27.45	19.02	29.22	60.84	46.32	32.75	46.64	37.93
T1	42.36	32.17	19.50	31.34	62.13	52.50	33.23	49.29	40.31
Mean (L)	41.78	29.81	19.26	30.28	61.49	49.41	32.99	47.96	
Mean	L1- 51.63		L2- 39.61		L3- 26.12				
CD (P=0.05%)	G (main effect)	L (main effect)	G x L (Interaction effect)	T (main effect)	G x T (Interaction effect)	L x T (Interaction effect)	G x L x T (Interaction effect: 3 factors)		
	0.34	0.42	0.60	0.34	N/A	0.60	N/A		

G: Genotype; L: Seed lot (L1: Fresh, L2: one year old, L3; two year old); T: Priming duration (T0: Control;T1: 48 hour)

The increase in peroxidase activity in hydro primed seeds may be attributed to the role of peroxidase in catalyzing the oxidation of various electron donors with the help of hydrogen per-oxide, aiding in the scavenging of endogenous hydrogen peroxide [39]. Germination percentage in maize is positively associated with peroxidase activity [32]. Enhanced peroxidase activity may help mitigate the effects of lipid peroxidation, which produces highly reactive free radical intermediates

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capable of damaging membranes, proteins, and nucleic acids. Lipid peroxidation has been noted to occur before the decline in viability in maize [40]. Therefore, antioxidant enzymes like peroxidase play a role in slowing down damage caused by lipid peroxidation. Our results are consistent with studies reported in spinach and in rice [37,41].

Superoxide dismutase activity (Units g^{-1} dry weight)

Significant differences were noted in superoxide dismutase (SOD) activity (Units g^{-1} dry weight) among seeds subjected to hydropriming (Table 7). The highest SOD activity was recorded in seeds of the HBGH-35 hybrid (12.13), while seeds of the Pusa Naveen variety had lower activity (11.27). Fresh seeds exhibited the highest SOD activity overall (15.77), as compared to one year old seeds (11.01) and two year old seeds (8.32). However, the greatest percentage increase in SOD activity was observed in one year old seed lot (23.23%), followed by two year old lot (15.96%) and the fresh lot (9.56%), as illustrated in Figure 1D. Overall, the mean SOD activity was higher in hydroprimed seeds (12.53) compared to the control (10.87). The SOD enzyme, located in the mitochondria, plays a crucial role in scavenging ROS and preventing oxidative damage to cells. Therefore, priming enhances the antioxidant defense system of these cells. Parvar et al. found that wheat seeds primed with 100 µM Sodium Nitroprusside under oxidative stress exhibited increased SOD activity, helping to reduce cold-induced oxidative stress [42]. Dong et al. also reported improved SOD activity in aged Welsh onion seeds as a result of priming. Similar findings have been reported by Huang et al. in cucumber, Wang et al. in bitter gourd, Hsu et al. in bitter gourd, Sepehri and Rouhi in groundnut, Rouhi et al. in coriander and Monajjem et al. in rice [8,10,22,35,36,38,41].

Table 6. Effect of hydropriming treatment, genotypes and different aged seed lots on peroxidas eactivity (nmoles g^{-1} dry weight) of bottle gourd genotypes

G/L/T	Pusa Naveen variety (G1)				HBGH-35 hybrid (G2)				
	L1	L2	L3	Mean (G1)	L1	L2	L3	Mean (G2)	Mean (T)
T0	14.41	9.49	7.16	10.35	15.70	10.23	8.25	11.39	10.87
T1	16.18	11.76	8.64	12.19	16.81	12.54	9.23	12.86	12.53
Mean (L)	15.30	10.63	7.90	11.27	16.25	11.39	8.74	12.13	
Mean	L1- 15.77		L2- 11.01		L3- 8.32				
CD (P=0.05%)	G (main effect)	L (main effect)	G x L (Interaction effect)	T (main effect)	G x T (Interaction effect)	L x T (Interaction effect)	G x L x T (Interaction effect: 3 factors)		
	0.21	0.26	N/A	0.21	N/A	0.37	N/A		

G: Genotype; L: Seed lot (L_1: Fresh, L_2: one year old, L_3; two year old); T: Priming duration (T_0: Control; T_1: 48 hour)

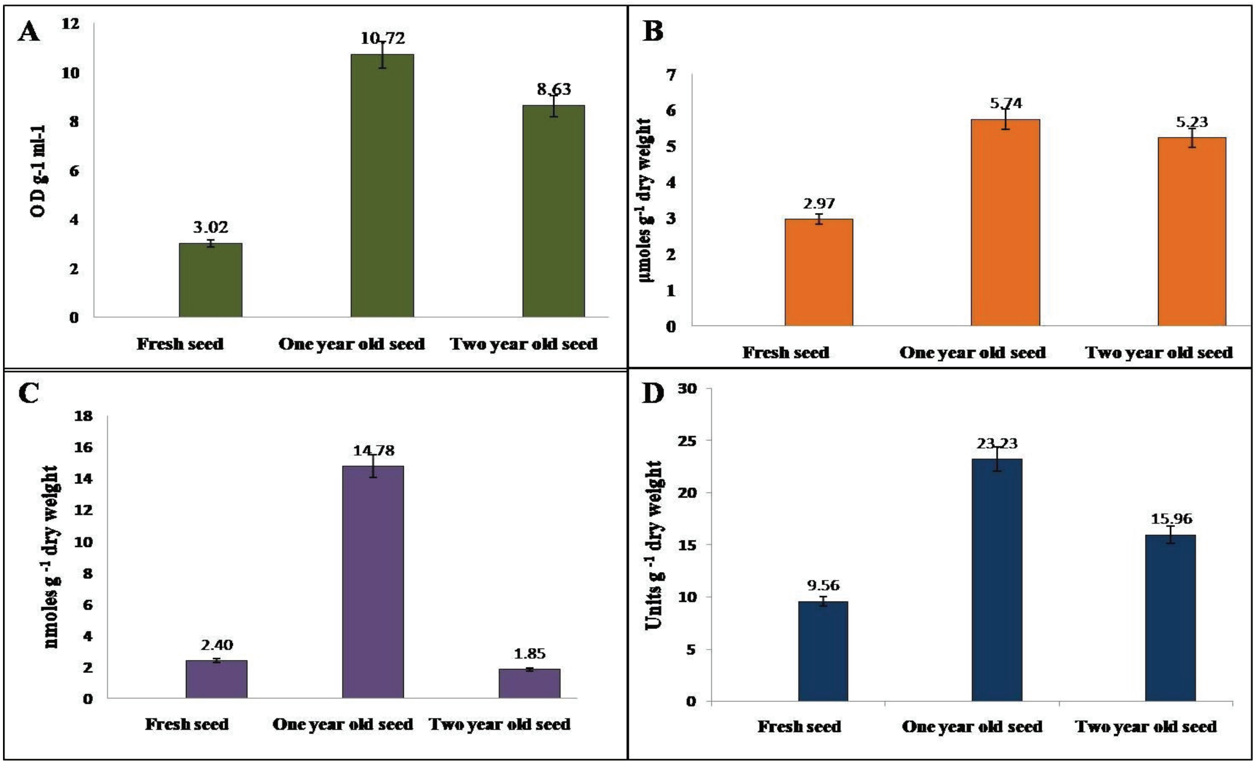

Figure 1. Percent enhancement in enzyme activities of different aged seed lots of bottle gourd by hydropriming. A: Dehydrogenase activity, B: Catalase activity (µmoles g^{-1} dry weight), C: Peroxidase activity (nmoles g^{-1} dry weight), D: Superoxide Dismutase activity (Units g^{-1} dry weight).

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Conclusions

Hydropriming is a cost-effective technique that comprises seed soaking in water to increase germination rates and to promote uniformity in plant establishment. In bottle gourd, hydropriming led to enhancement in the activity of various enzymes across differently aged seed lots of variety as well as hybrid. Soaking seeds for 48 hours using half the water volume relative to seed weight (w) at a consistent temperature of 25°C produced markedly better outcomes compared to the control. Hydroprimed fresh seeds showed the highest enzyme activity, followed by one year old seeds, with two year old seeds having the lowest activity. Interestingly, hydropriming had a more pronounced positive effect on the physiological processes of aged seeds than on fresh seeds. Consequently, the one year old seed batch exhibited the greatest improvement, followed by the two year old batch, and lastly, the fresh seeds. Among the genotypes, HBGH-35 hybrid seeds responded more effectively to hydropriming than the Pusa Naveen variety.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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