



World Scientific News

An International Scientific Journal

WSN 144 (2020) 13-29

EISSN 2392-2192

Wheat seedlings as affected by $Mg(NO_3)_2$ and $ZnSO_4$ priming treatments

Rajesh Kumar Singhal*, **Bandana Bose**

Seed Priming Laboratory, Department of Plant Physiology, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi 221005, India

*E-mail address: rajasinghal151@gmail.com

ABSTRACT

Seed is the most crucial and prime input in agriculture, and the seed must be of high quality. Seed priming is one of the reliable and cost-effective strategies to enhance seed germination and seedling establishment in crops. This study was conducted to determine the effects of seed priming with $Mg(NO_3)_2$ (S_1) and $ZnSO_4$ (S_2) in two wheat (*Triticum aestivum* L.) varieties (HUW-468 and HUW-510) on germination and seedling growth. Varied levels of $Mg(NO_3)_2$ and $ZnSO_4$ from C_0 to C_{11} were used for the present study and number of seed germinated, seedling establishment and biochemical parameters were recorded. From the study of germination %, mean germination time (MGT), mean germination rate (MGR), coefficient of velocity of germination (CVG), germination rate index (GRI) (% / day), germination index (GI), seedling vigor (SV), shoot, root and seedling length, α -amylase activity, soluble and insoluble sugar content and protein content, it was revealed that 7.5 mM of $Mg(NO_3)_2$ and 0.05 mM of $ZnSO_4$ were the best treatments in improving seedling establishment, performance and biochemical status of wheat seed. Further, this analysis also reveals a strong correlation between the seedling establishment and the activity of α -amylase and protein content.

Keywords: α -amylase activity, Mean germination time, Seed priming, Soluble and insoluble sugar content, Seedling vigour, *Triticum aestivum*

1. INTRODUCTION

Wheat is the prime global staple crop and cultivated over an extensive latitudinal range of areas mainly in the rain-fed climatic conditions. Food security has become the global challenge to given the projected needed to increase world food supply by about 70% by the next four decades. Considering the limitations of expanding cultivated areas, a remarkable rise in crop productivity, will be needed to achieve this target (Reynolds et al. 2011). Moreover, adverse environmental conditions diminishing in germination potential and induce the several changes in physiological, biochemical and molecular levels (Sadeghian and Yavari 2004; Soltani et al. 2006; Vibhuti et al. 2015). Time to time agriculturist find new and advance seed enhancement strategies to resolve the problem of seed germination and seedling establishments (Taylor et al. 1998). Among them seed priming is a best pre-sowing strategy known for reliable, cost-effective, easy to use and eco-friendly (Paparella et al. 2015; Singhal et al. 2019). Seed priming is hydration and time-based approach, which allows the controlled seed rehydration, to accelerate the specific metabolic processes but avoid the seed transition before the seedling emergence (Bose et al. 2018).

Priming of seed with $Mg(NO_3)_2$ showed an increment in root number, length, weight as well as stress ameliorating characters in crops cultivated under field condition (Sharma and Bose 2006; Anaytullah and Bose 2007). Likewise, Bose et al. (2007) used nitrate seed priming/hardening technology, where the seeds of various field crops hardened with some nitrate salts before sowing and they found an enhancement in germination physiology, vegetative growth, nitrogen metabolism antioxidant processes and yield traits. Although a wide range of soils in the world deficient in zinc, to combat this problem seed priming combined with zinc element can be useful in these types of land. Zinc sulphate superiority is probably due to role of zinc in protein synthesis, cell membrane and cell elongations. To considering above facts this research experiment conduct to find the best concentrations of $Mg(NO_3)_2$ and $ZnSO_4$ in two wheat varieties and the change in the physiological and biochemical status of wheat seedling during germination and seedling establishment as compared to non-primed seed.

2. MATERIAL AND METHOD

Experimental site: This experiment conducted in the Seed Priming and Seed Physiology Laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

Choice of plant material: Wheat (*Triticum aestivum* L. var. HUW-468 and HUW-510) varieties chosen as the plant material for the present experiment. The varieties HUW-468 and HUW-510 were developed at the Department of Genetics and Plant Breeding, of same institute.

The layout of the experiment: The experiment laid down in a Completely Randomised Design (CRD) with three replications.

Hardening of the seeds: The sterilized seeds (with 0.1% $HgCl_2$) were primed by immersing the seeds in different concentrations of (0, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 40 and 50 mM represented $C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}$ and C_{11}) $Mg(NO_3)_2$ (S_1) and 0.03, 0.05, 0.1,

0.3, 0.5, 0.75, 1, 1.25, 1.5 and 1.75 mM represented C₁ to C₁₁ of ZnSO₄ (S₂). Whereas C₀ represents the non-primed seed set. After 11 hr. of treatment, seeds were dried under the shade using forced air by fan to bring back to its initial dried stage. Further, the seeds were kept in packets and used immediately for germination purpose.

Germination studies: Final germination percentage, germination index, germination rate index, mean germination time, coefficient velocity of germination and vigour index were calculated using the following formula

Final germination percentage: Final germination percentage calculated by the formula given by Al-Mudaris (1998).

$$\text{Germination} = \frac{\text{No.of seeds germinated}}{\text{No.of seeds present in Petri dish}} \times 100\%$$

Mean germination time: Mean germination time calculated by the formula given by Al-Mudaris (1998).

MTG (in days) = $\Sigma F \cdot x / \Sigma F$ Where F is the number of seed germinated on day x

Germination index (GI): Germination index of wheat seed calculated by the formula given by Ranal et al. (2009). $GI = (4 \times N_1) + (3 \times N_2) + \dots + (1 \times N_4)$

Where N₁, N₂, ... N₄ is the number of germinated seeds on the first, second and subsequent days until 4th day.

Germination Rate Index (GRI): GRI calculated by the formula given by the Al-Mudaris (1998). $GRI = G_1/1 + G_2/2 + \dots + G_x/x$

Where G₁ is the germination percentage on day 1, G₂ is the germination parentage on day 2; and so on.

Coefficient of Velocity of Germination (CVG): CVG calculated by the formula given by Al-Mudaris (1998). $CVG = N_1 + N_2 + \dots + N_x / 100 \times N_1 T_1 + \dots + N_x T_x$

N = No. of seeds germinated each day, T = No. of days from seeding corresponding to N

Mean germination rate (MGR): MGR calculated by the formula given by the Ranal et al. (2009). $CV/100 = 1/T$; where T is mean germination time and CV: coefficient of velocity.

Seedling vigour index (SVI): Seedling vigour index measured in 7-day old seedling by the formula given by Goodi and Sharifzadeh (2006).

SVI = germination % × seedling length

α-amylase activity: For the estimation of the α-amylase (EC3.2.1.1) enzyme activity in the endosperm of germinating wheat seeds, method of Bernfeld (1955) followed.

Soluble and insoluble sugar content: For the estimation of the soluble and insoluble sugar content in the endosperm of germinating wheat seeds, method of Dubois et al. (1956) followed.

3. RESULTS

Germination percentage (G%) is the most crucial trait reveals the germination capacity of represented in table 1. Presented data depicted that the germination % increased with time hours from 24 h to 96 h. and variety V_2 showed a higher germination % as compared to V_1 . Likewise, treatment (T) S_1C_0 always showed the lower germination % in all the studied conditions. Whereas, the highest G% observed in case of S_1C_1 , at 24h and 48 h and at 72 h 7.5mM Mg (NO₃)₂ primed seed. The final result showed that in starting hours, hydro primed gives best results, whereas overall 7.5 mM Mg(NO₃)₂ priming set represents the best treatment in both the varieties. Similarly, Table 2 describes the data of germination percent under different concentrations of ZnSO₄ (S_2) and from the data, it was depicted that treatment S_2C_0 always showed the lowest germination per cent. Whereas, the treatment 0.05 mM ZnSO₄ priming exhibited the highest percentage of germination.

Table 3 represents the data of mean germination rate (MGR), mean germination time (MGT), coefficient of velocity of germination (CVG) and germination rate index (GRI) (%/day) of wheat varieties under different concentration of Mg(NO₃)₂. Usually, variety V_2 showed higher mean MGR values as compared to variety V_1 . Further, data revealed the lowest MGR, CVG and GRI value found in S_1C_0 and the highest in treatment S_1C_1 . In case of MGT, lowest value of MGT was recorded in case of S_1C_1 , whereas the highest values in treatment S_1C_0 .

Table 4 represents the data of MGR, MGT, CVG and GRI of wheat varieties under different concentration of ZnSO₄ (S_2). In case of MGR lowest value was found in case of C_0 condition, whereas, highest in treatment S_2C_1 in V_1 and S_2C_3 in V_2 . Similarly, the lowest MGT value found in case of S_2C_1 in the V_1 (1.60 days) and S_2C_3 in the V_2 (1.54 days). Whereas, the highest MGT values were recorded in case of treatment S_2C_0 . Likely, the lowest CVG value found in case of S_2C_0 , whereas, the highest value of CVG found in concentration S_2C_1 (62.51) and S_2C_3 (64.93) respectively. In case of GRI data lowest value was found in case of S_2C_1 in the varieties V_1 (47.00%/day) and V_2 (46.17%/day), whereas, the highest value of CVG measured in the S_2C_3 .

The values of GI indicate both the percentage of germination and germination speed. Fig. 1 a & b represents the GI data of wheat varieties V_1 and V_2 under different concentrations of Mg (NO₃)₂ and ZnSO₄. Data on Mg (NO₃)₂ priming showed that variety V_2 showed the higher mean GI values as compared to V_1 . Consequently, the lowest GI value recorded in S_1C_0 and the highest in treatment S_1C_1 . While in case of ZnSO₄ priming, the lowest value of GI was found in the case of S_2C_0 and the highest value in S_2C_4 treatment.

Seedling vigour (SV) is the crucial parameter indicates the seedling establishment and growth. Fig. 1 a & b represents the SV data of wheat varieties V_1 and V_2 under different concentrations of Mg(NO₃)₂ and ZnSO₄ from C_1 to C_{11} after seven days of sowing. In Mg (NO₃)₂ priming set variety V_1 showed higher SV values as compared to V_2 . Whereas, the lowest SV value recorded in S_1C_0 and the highest SV value recorded in treatment S_1C_3 . Further, the data on ZnSO₄ primed sets showed that the lowest values of SV found in the case of S_2C_0 and the highest in S_2C_3 treatment.

Shoot length represents the vertical growth of the plant and root length is critical for the seedling parameter, indicates the establishment capacity of seedling. Fig. 2 a describes the shoot length (SL) (cm), root length (RL) (cm) and seedling length (cm) data of wheat varieties after seven days of sowing under Mg (NO₃)₂ priming. Data revealed the lowest SL value in S_1C_0 , while the highest found in treatment S_1C_3 . Likewise, lower RL values were recorded in

treatment S₁C₁₀, while the higher in the treatment S₁C₃. In case of seedling length lowest value was recorded in treatment S₁C₈, while the highest in case of treatment S₁C₃ in both the varieties. Fig. 2 b represents the similar traits data under ZnSO₄ priming and data revealed the lowest SL value was in treatment S₂C₀ and highest in treatment S₂C₃ in both the varieties. In case of RL lowest value was found in the case of S₂C₈ in the varieties V₁ (4.07 cm) and S₂C₆ in V₂ (4.10 cm), whereas the highest in S₂C₄ in V₁ (4.73 cm) and S₂C₂ in V₂ (5.33 cm) respectively. In case of seedling length lowest value was found in the case of S₂C₂, while the highest in S₂C₃ in both varieties.

α- amylase activity is the crucial enzyme represents the metabolic activity of seed. It hydrolyses the stored carbohydrate in the seed. Fig 3 a & b represents the data of α- amylase activity at 24 and 72 h in the endosperm of primed wheat seed. The α-amylase activity was higher at 72 h as compared to 24 h. Similarly, at 24 and 72 h the lowest activity of α- amylase was observed in treatment C₀, and higher in case of treatment C₃ in both varieties.

Soluble and insoluble content of the seed represents the metabolism of stored sugar and mobilisation into seedling. Table 5 describes the soluble and insoluble sugar content in mg g⁻¹ DW of wheat endosperm at 24h and 72h in Mg(NO₃)₂ primed seed. In the presented data lower soluble sugar content at 24 h was recorded in treatment S₁C₀, whereas the highest in treatment S₁C₃ in both the varieties. The similar trend was also observed at 72 h also. Analysis of insoluble sugar content of wheat endosperm revealed that treatment S₁C₀ showed the highest value whereas, the lowest value in treatment S₁C₃.

Table 6 represents the soluble and insoluble sugar content in mg g⁻¹ DW of wheat endosperm at 24h and 72h in wheat varieties under different concentration of ZnSO₄. In the presented data lowest soluble sugar content at 24 h was observed in case of treatment S₂C₀, whereas the highest in treatment S₂C₃. The similar trend recorded at 72 h in both the varieties. Analysis of insoluble sugar content of wheat endosperm revealed that treatment S₂C₀ shows the highest value and S₁C₃ lowest value in both the varieties.

Fig 4a&b represents the protein content of wheat endosperm after 48 h germination in both the varieties under different concentrations of Mg (NO₃)₂ and ZnSO₄. Presented data depicted that treatment C₀ having lowest protein content, whereas the highest protein content in case of treatment C₃ in both the varieties.

Table 1. Effect of seed priming with different concentrations of Mg(NO₃)₂ salts on the germination percentage of two selected wheat varieties at different study periods of germination.

Treatments (T/S)	Germination %											
	24h			48h			72h			96h		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
S ₁ C ₀	17.0	18.0	17.5	57.0	54.0	55.5	84.0	83.0	83.5	88.0	85.0	86.5
S ₁ C ₁	56.0	58.0	57.0	78.0	80.0	79.0	94.0	93.0	93.5	95.0	95.0	95.0
S ₁ C ₂	49.0	45.0	47.0	71.0	76.0	73.5	93.0	93.0	93.0	98.0	98.0	98.0
S ₁ C ₃	49.0	50.0	49.5	76.0	78.0	77.0	97.0	95.0	96.0	98.0	99.0	98.5

S ₁ C ₄	43.0	49.0	46.0	67.0	76.0	71.5	92.0	94.0	93.0	97.0	98.0	97.5
S ₁ C ₅	44.0	47.0	45.5	78.0	73.0	75.5	95.0	91.0	93.0	97.0	97.0	97.0
S ₁ C ₆	40.0	50.0	45.0	75.0	75.0	75.0	90.0	96.0	93.0	97.0	97.0	97.0
S ₁ C ₇	39.0	49.0	44.0	72.0	73.0	72.5	88.0	94.0	91.0	95.0	95.0	95.0
S ₁ C ₈	44.0	44.0	44.0	74.0	75.0	74.5	92.0	92.0	92.0	95.0	95.0	95.0
S ₁ C ₉	45.0	50.0	47.5	75.0	75.0	75.0	91.0	95.0	93.0	93.0	96.0	94.5
S ₁ C ₁₀	42.0	48.0	45.0	72.0	77.0	74.5	91.0	93.0	92.0	96.0	97.0	96.5
S ₁ C ₁₁	45.0	45.0	45.0	77.0	72.0	74.5	93.0	92.0	92.5	96.0	97.0	96.5
Mean	42.7	46.1		72.67	73.67		91.67	92.58		95.42	95.75	
Factors	24 h			48 h			72h			96h		
	CD	±SEm		CD	±SEm		CD	±SEm		CD	±SEm	
Factor A	0.67	0.24		0.65	0.23		0.65	0.23		N.A	0.18	
Factor B	1.65	0.58		1.6	0.56		1.6	0.56		1.24	0.43	
AXB	2.33	0.82		2.3	0.8		2.3	0.8		1.75	0.61	

Table 2. Effect of seed priming with different concentrations of ZnSO₄ salts on the germination percentage of two selected wheat varieties at different study periods of germination.

Treatments (T/S)	Germination %											
	24h			48h			72 h			96 h		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
S ₂ C ₀	17.0	18.0	17.5	57.0	54.0	55.5	84.0	83.0	83.5	88.0	85.0	86.5
S ₂ C ₁	56.0	58.0	57.0	78.0	80.0	79.0	94.0	93.0	93.5	95.0	95.0	95.0
S ₂ C ₂	53.0	55.0	54.0	71.0	74.0	72.5	88.0	94.0	91.0	92.0	95.0	93.5
S ₂ C ₃	57.0	63.0	60.0	81.7	82.0	81.8	97.7	97.7	97.7	98.7	98.7	98.7
S ₂ C ₄	56.0	62.0	59.0	81.0	81.0	81.0	97.0	97.0	97.0	98.0	98.0	98.0
S ₂ C ₅	53.0	55.0	54.0	73.0	75.0	74.0	94.0	94.0	94.0	95.0	95.0	95.0
S ₂ C ₆	51.0	47.0	49.0	72.0	70.0	71.0	93.0	92.0	92.5	95.0	93.7	94.3
S ₂ C ₇	48.0	55.0	51.5	75.0	69.0	72.0	92.0	88.0	90.0	94.0	91.0	92.5
S ₂ C ₈	52.0	48.0	50.0	72.0	70.0	71.0	92.0	92.0	92.0	94.0	93.7	93.8
S ₂ C ₉	44.0	61.0	52.5	77.0	80.0	78.5	93.0	94.0	93.5	95.0	96.0	95.5

S ₂ C ₁₀	36.0	46.0	41.0	68.0	69.0	68.5	90.0	84.0	87.0	92.0	89.0	90.5
S ₂ C ₁₁	37.3	47.0	42.2	77.0	71.0	74.0	92.0	87.0	89.5	94.0	91.0	92.5
Mean	46.7	51.3		73.56	72.91		92.22	91.31		94.22	93.42	
Factors	24 h		48 h			72h			96h			
	CD	±SEm	CD	±SEm	CD	±SEm	CD	±SEm	CD	±SEm		
Factor A	0.8	0.28	N/A	0.25	0.52	0.18	0.47	0.16				
Factor B	1.95	0.68	1.75	0.61	1.27	0.45	1.15	0.4				
AXB	2.76	0.97	2.48	0.87	1.8	0.63	1.62	0.57				

Table 3. Effect of seed priming with different concentrations of Mg(NO₃)₂ salts on the mean germination rate (MGR), coefficient of velocity of germination, mean germination time (MGT) and germination rate index of two selected wheat varieties.

Treatments (T/S)	Germination parameters											
	MGR			CVG			MGT (days)			GRI (%/day)		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
S ₁ C ₀	0.5	0.5	0.5	45.4	46.0	45.7	2.2	2.2	2.2	47.0	46.2	46.6
S ₁ C ₁	0.6	0.6	0.6	62.5	63.8	63.2	1.6	1.6	1.6	72.6	73.8	73.2
S ₁ C ₂	0.6	0.6	0.6	54.8	55.1	54.9	1.8	1.8	1.8	68.6	67.4	68.0
S ₁ C ₃	0.6	0.6	0.6	57.7	57.3	57.5	1.7	1.8	1.7	69.8	70.7	70.2
S ₁ C ₄	0.5	0.6	0.6	52.2	56.7	54.4	1.9	1.8	1.8	64.6	69.5	67.0
S ₁ C ₅	0.6	0.6	0.6	56.8	54.8	55.8	1.8	1.8	1.8	67.2	67.5	67.3
S ₁ C ₆	0.5	0.6	0.6	53.0	58.1	55.6	1.9	1.7	1.8	64.3	69.8	67.0
S ₁ C ₇	0.5	0.6	0.6	52.5	57.9	55.2	1.9	1.7	1.8	62.6	68.3	65.4
S ₁ C ₈	0.6	0.6	0.6	55.9	56.2	56.1	1.8	1.8	1.8	65.8	65.9	65.8
S ₁ C ₉	0.6	0.6	0.6	57.8	58.6	58.2	1.7	1.7	1.7	65.8	69.4	67.6
S ₁ C ₁₀	0.5	0.6	0.6	53.7	57.1	55.4	1.9	1.8	1.8	64.6	68.8	66.7
S ₁ C ₁₁	0.6	0.5	0.6	56.8	54.2	55.5	1.8	1.9	1.8	67.1	66.4	66.8
Mean	0.55	0.56		54.91	56.31		1.83	1.79		64.98	66.98	
Factors	MGR			CVG			MGT (days)			GRI		
	CD	±SEm		CD	±SEm		CD	±SEm		CD	±SEm	
Factor A	0.01	0.002		0.5	0.2		0.02	0.005		0.37	0.13	

Factor B	0.01	0.004	1.2	0.4	0.04	0.013	0.9	0.32
AXB	0.01	0.006	1.63	0.6	0.05	0.018	1.28	0.45

Table 4. Effect of seed priming with different concentrations of ZnSO₄ salts on the mean germination rate (MGR), coefficient of velocity of germination, mean germination time (MGT) and germination rate index of two selected wheat varieties.

Treatments (T/S)	Germination parameters											
	MGR			CVG			MGT (days)			GRI (%/day)		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
S₂C₀	0.5	0.5	0.5	45.4	46.0	45.7	2.2	2.2	2.2	47.0	46.2	46.6
S₂C₁	0.6	0.6	0.6	62.5	63.8	63.2	1.6	1.6	1.6	72.6	73.8	73.2
S₂C₂	0.6	0.6	0.6	59.0	60.5	59.8	1.7	1.7	1.7	68.7	71.4	70.0
S₂C₃	0.6	0.7	0.6	62.3	64.9	63.6	1.6	1.5	1.6	74.9	78.0	76.5
S₂C₄	0.6	0.7	0.6	62.0	64.5	63.3	1.6	1.6	1.6	74.1	77.1	75.6
S₂C₅	0.6	0.6	0.6	59.4	60.9	60.1	1.7	1.6	1.7	70.3	71.6	70.9
S₂C₆	0.6	0.6	0.6	57.9	56.6	57.2	1.7	1.8	1.8	69.0	66.3	67.6
S₂C₇	0.6	0.6	0.6	58.4	59.9	59.1	1.7	1.7	1.7	67.7	69.1	68.4
S₂C₈	0.6	0.6	0.6	58.8	56.9	57.8	1.7	1.8	1.7	69.2	66.8	68.0
S₂C₉	0.6	0.7	0.6	57.2	64.5	60.9	1.8	1.6	1.7	66.3	75.7	71.0
S₂C₁₀	0.5	0.6	0.6	52.9	56.7	54.8	1.9	1.8	1.8	59.8	63.8	61.8
S₂C₁₁	0.6	0.6	0.6	55.4	57.3	56.3	1.8	1.8	1.8	62.7	65.3	64.0
Mean	0.58	0.6		57.61	59.36		1.75	1.7		66.85	68.74	
Factors	MGR			CVG			MGT (days)			GRI		
	CD	±SEm		CD	±SEm		CD	±SEm		CD	±SEm	
Factor A	0.01	0.002		0.5	0.2		0.01	0.01		0.45	0.16	
Factor B	0.01	0.004		1.2	0.4		0.03	0.01		1.1	0.39	
AXB	0.02	0.006		1.7	0.6		0.05	0.02		1.56	0.55	

Table 5. Effect of seed priming with different concentrations of Mg(NO₃)₂ salt on the soluble and insoluble sugar content (mg g⁻¹DW) of two selected wheat varieties.

Treatments (T/S)	Soluble sugar						Insoluble sugar					
	24h			72h			24h			72h		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
S ₁ C ₀	9.3	8.3	8.8	17.7	15.7	16.7	30.7	31.7	24.5	22.3	24.7	23.5
S ₁ C ₁	15.0	12.7	13.8	20.0	17.7	18.8	25.0	26.3	23.3	19.3	22.7	21.0
S ₁ C ₂	15.7	13.3	14.5	21.3	21.3	21.3	24.3	23.7	24.0	16.3	19.0	17.7
S ₁ C ₃	18.7	17.0	17.8	26.7	26.7	26.7	21.3	28.3	24.8	12.0	13.7	12.8
S ₁ C ₄	16.3	13.3	14.8	23.7	23.7	23.7	17.0	26.0	21.5	14.0	16.7	15.3
S ₁ C ₅	15.0	12.7	13.8	21.7	21.7	21.7	25.0	24.0	24.5	16.0	18.7	17.3
S ₁ C ₆	16.0	12.3	14.2	20.7	20.7	20.7	24.0	23.3	23.7	16.7	19.7	18.2
S ₁ C ₇	15.0	13.0	14.0	20.0	20.0	20.0	25.0	24.0	24.5	16.0	20.3	18.2
S ₁ C ₈	13.3	11.7	12.5	19.3	19.3	19.3	26.7	21.3	24.0	19.0	21.0	20.0
S ₁ C ₉	13.0	12.0	12.5	23.0	23.0	23.0	27.0	23.0	25.0	17.0	17.3	17.2
S ₁ C ₁₀	12.7	11.0	11.8	23.0	23.0	23.0	27.3	23.0	25.2	17.0	17.3	17.2
S ₁ C ₁₁	12.3	10.7	11.5	18.7	18.7	18.7	27.7	20.3	24.0	19.7	21.7	20.7
Mean	14.36	12.33		20.94	20.94		25.28	22.89		17.11	19.39	
Factors	24 h			72h			24h			72h		
	CD	±SEm		CD	±SEm		CD	±SEm		CD	±SEm	
Factor A	0.75	0.26		N/A	0.33		0.85	0.36		0.79	0.28	
Factor B	1.84	0.65		2.28	0.8		N/A	0.88		1.95	0.68	
AXB	N/A	0.91		3.22	1.13		3.55	1.24		N/A	0.97	

Table 6. Effect of seed priming with different concentration of ZnSO₄ salt on the soluble and insoluble sugar content (mg g⁻¹DW) of two selected wheat varieties

Treatments (T/S)	Soluble sugar						Insoluble sugar					
	24h			72h			24h			72h		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
S₂C₀	9.3	8.3	8.8	17.7	15.7	16.7	30.7	31.7	31.2	22.3	24.7	23.5
S₂C₁	15.0	13.7	14.3	20.0	17.7	18.8	25.0	26.3	25.7	19.3	22.7	21.0
S₂C₂	17.0	15.7	16.3	24.0	19.7	21.8	23.0	24.3	23.7	16.0	20.7	18.3
S₂C₃	18.7	17.3	18.0	26.0	23.0	24.5	21.3	22.7	22.0	14.0	17.3	15.7
S₂C₄	16.7	13.3	15.0	22.0	19.0	20.5	23.3	26.7	25.0	18.0	21.3	19.7
S₂C₅	16.0	14.0	15.0	19.7	18.0	18.8	24.0	26.0	25.0	20.3	22.3	21.3
S₂C₆	13.3	12.0	12.7	22.0	17.0	19.5	26.7	28.0	27.3	18.3	23.3	20.8
S₂C₇	15.7	12.7	14.2	23.0	19.3	21.2	24.3	27.3	25.8	17.0	21.0	19.0
S₂C₈	15.3	12.7	14.0	19.0	17.7	18.3	24.7	27.3	26.0	21.0	22.7	21.8
S₂C₉	13.7	12.7	13.2	20.0	17.7	18.8	26.3	27.3	26.8	20.0	22.7	21.3
S₂C₁₀	14.3	12.7	13.5	18.0	17.0	17.5	25.7	27.3	26.5	22.0	23.3	22.7
S₂C₁₁	14.7	12.3	13.5	18.0	16.7	17.3	25.3	27.7	26.5	18.7	23.7	21.2
Mean	14.97	13.11		20.78	18.19		25.03	26.89		18.92	22.14	
Factors	24 h			72h			24h			72h		
	CD	±SEm		CD	±SEm		CD	±SEm		CD	±SEm	
Factor A	0.83	0.29		0.78	0.27		0.83	0.29		0.83	0.29	
Factor B	2.02	0.71		1.9	0.67		2.02	0.71		2.04	0.71	
AXB	N/A	1		N/A	0.94		N/A	1		N/A	1.01	

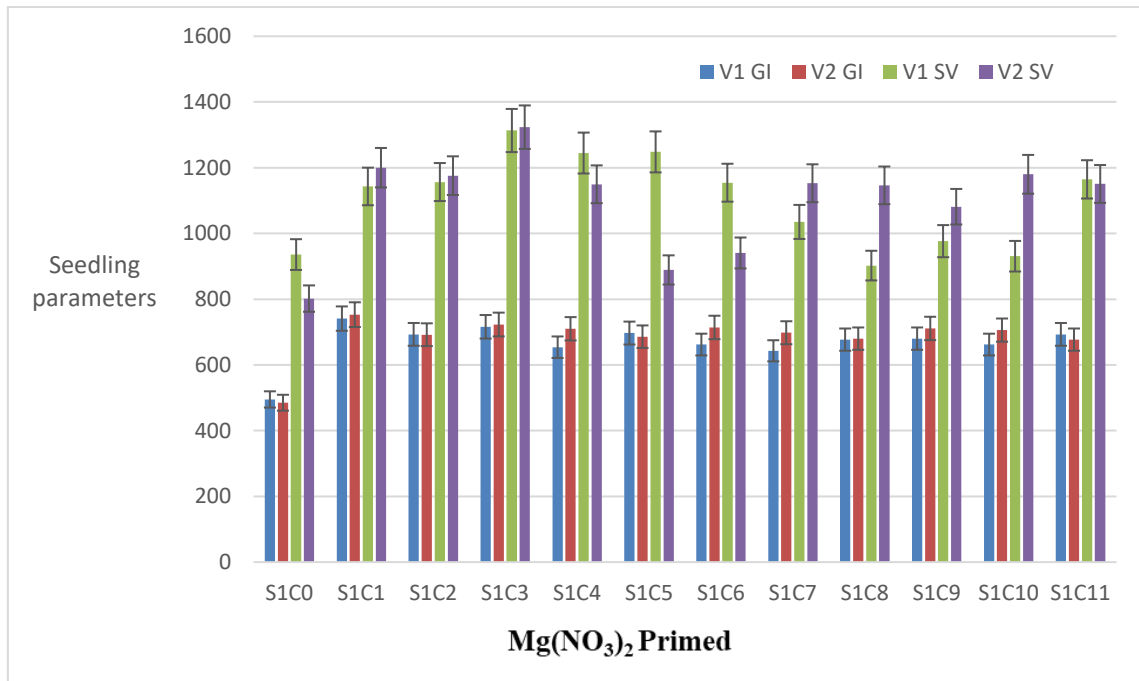


Fig. 1(a). Effect of seed priming with different concentrations of $Mg(NO_3)_2$ on the germination index and seedling vigour of two wheat varieties.

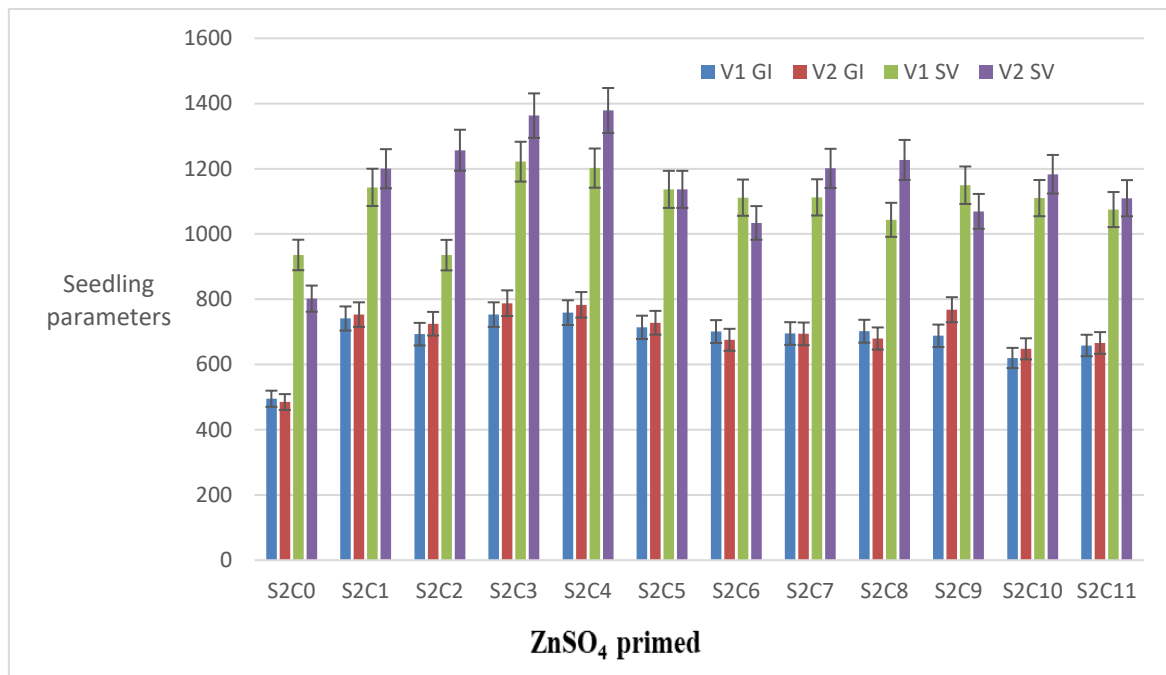


Fig. 1(b). Effect of seed priming with different concentrations of $ZnSO_4$ on the germination index and seedling vigour of two wheat varieties

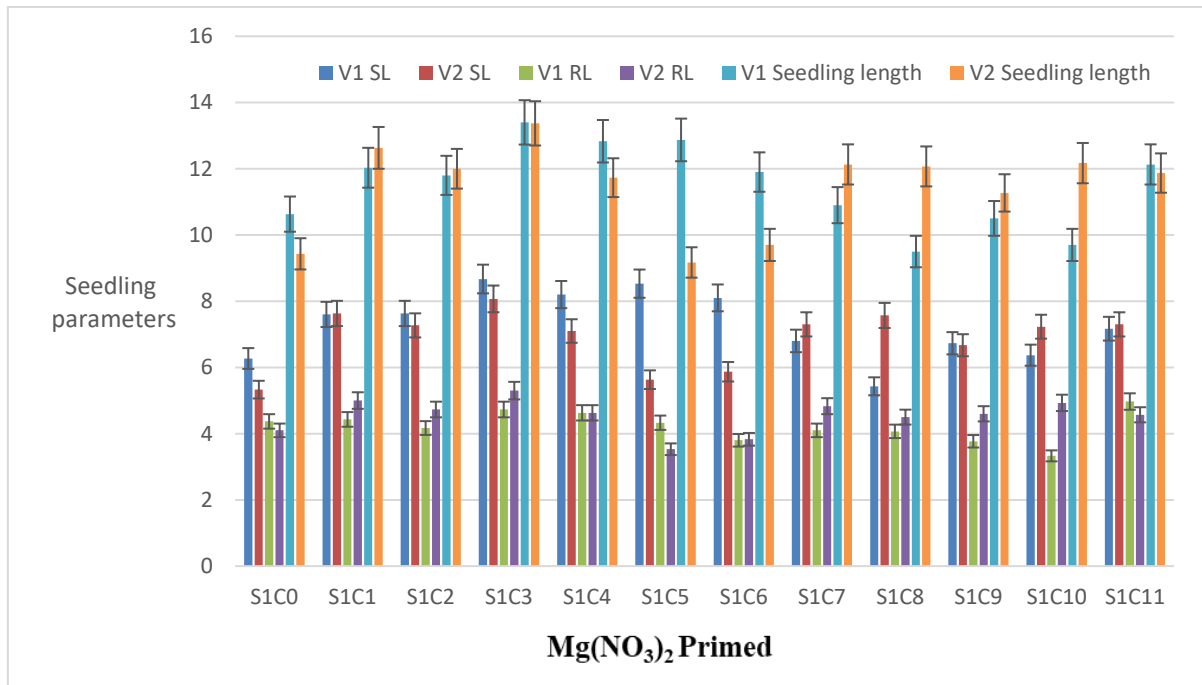


Fig. 2(a). Effect of seed priming with different concentrations of $Mg(NO_3)_2$ on the shoot length, root length and seedling length (cm) of two wheat varieties.

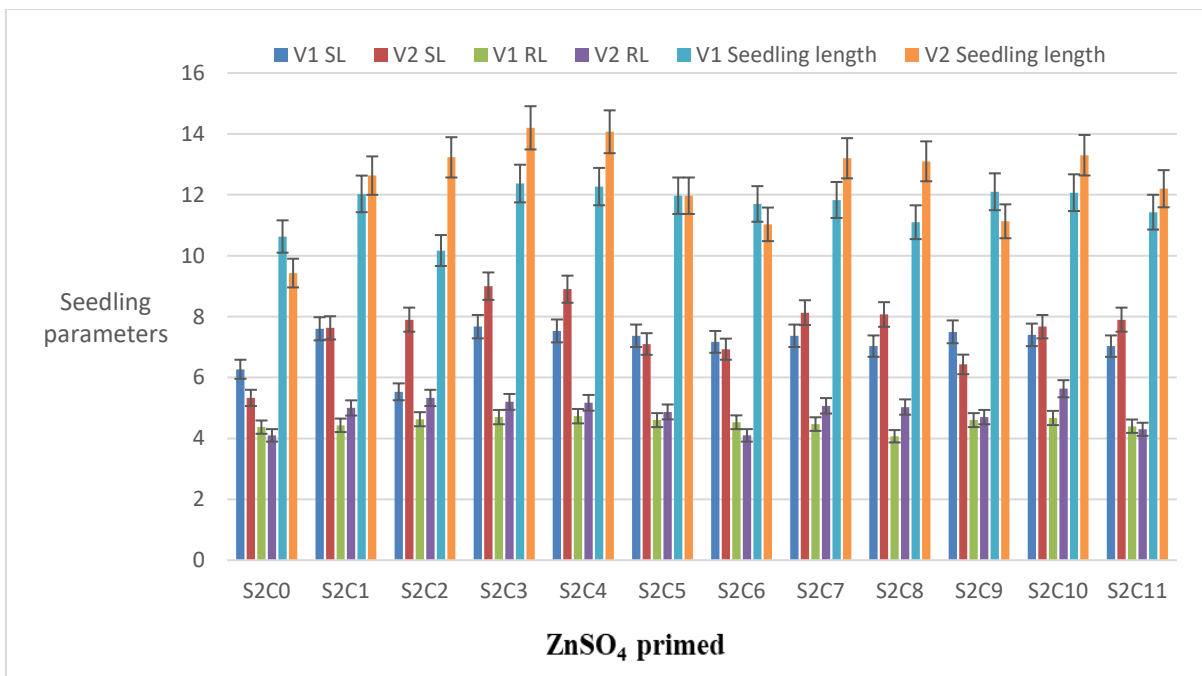


Fig. 2(b). Effect of seed priming with different concentrations of and $ZnSO_4$ on the shoot length, root length and seedling length (cm) of two wheat varieties.

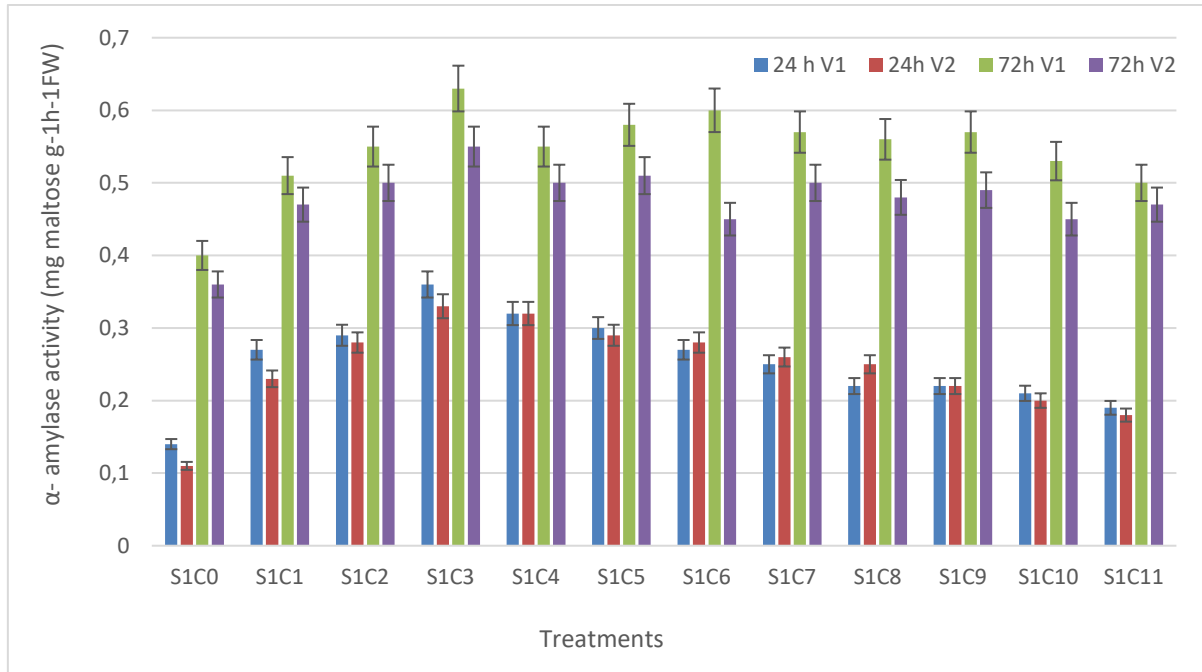


Fig. 3(a). Effect of seed priming with different concentrations of $Mg(NO_3)_2$ on the α -amylase activity (mg maltose $g^{-1}h^{-1}$ FW) of two wheat varieties.

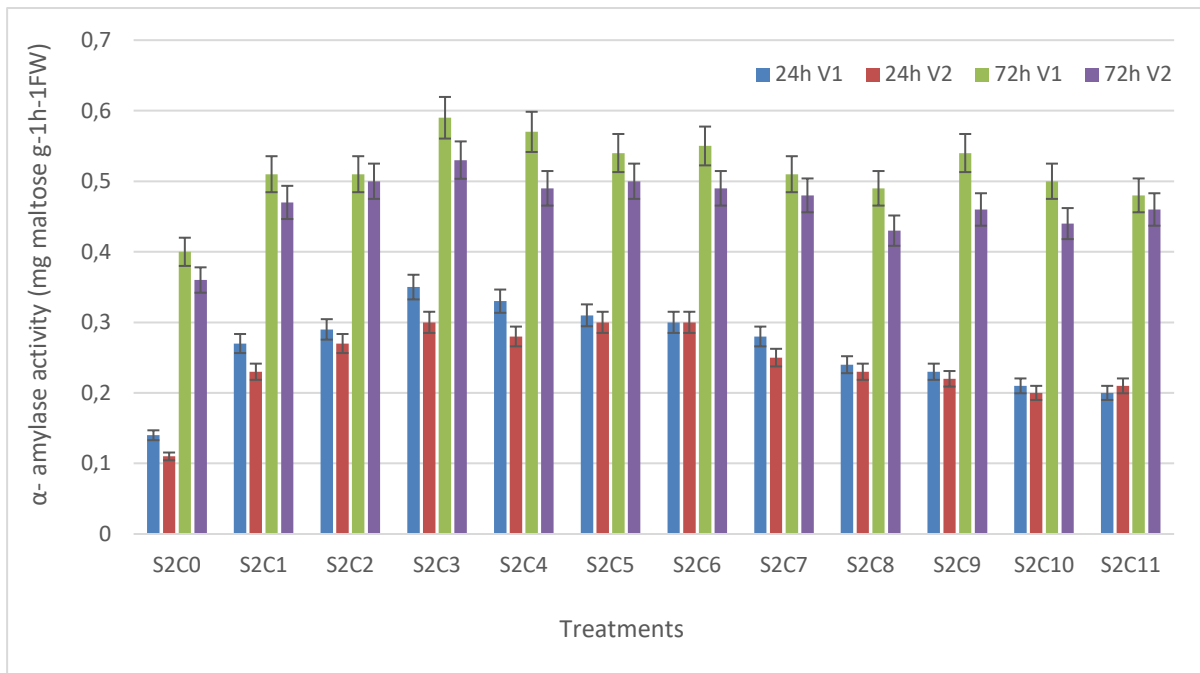


Fig. 3 (b). Effect of seed priming with different concentrations of $ZnSO_4$ on the α -amylase activity (mg maltose $g^{-1}h^{-1}$ FW) of two wheat varieties.

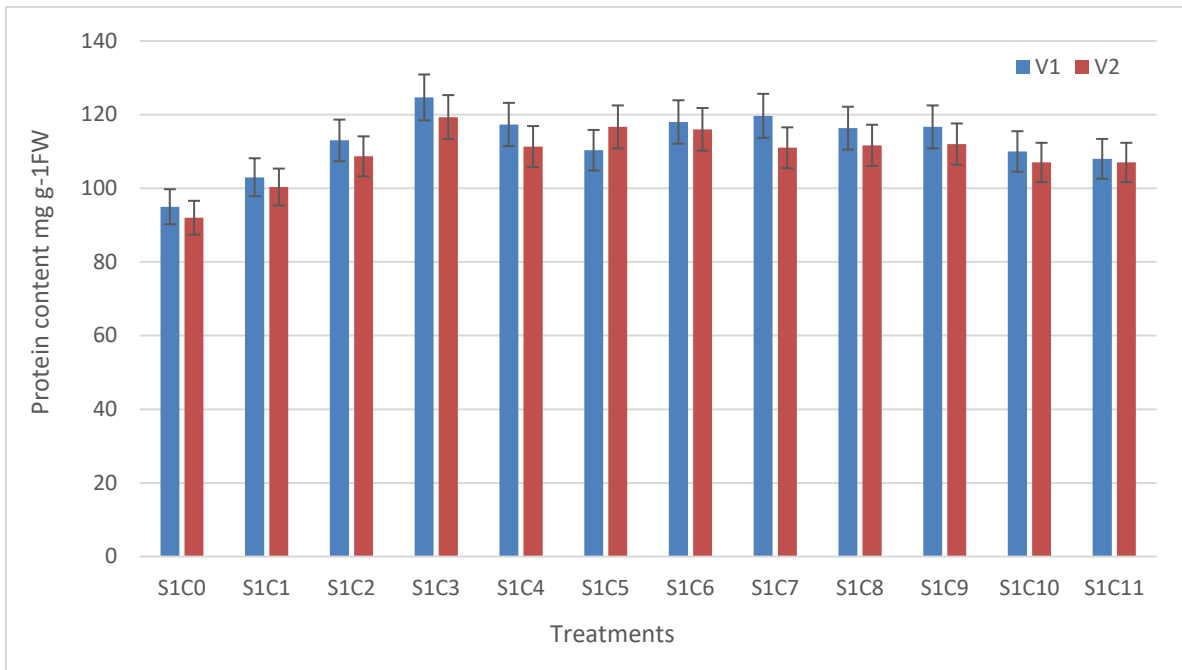


Fig. 4(a). Effect of seed priming with different concentrations of $Mg(NO_3)_2$ on the protein content ($mg\ g^{-1}\ FW$) of two wheat varieties

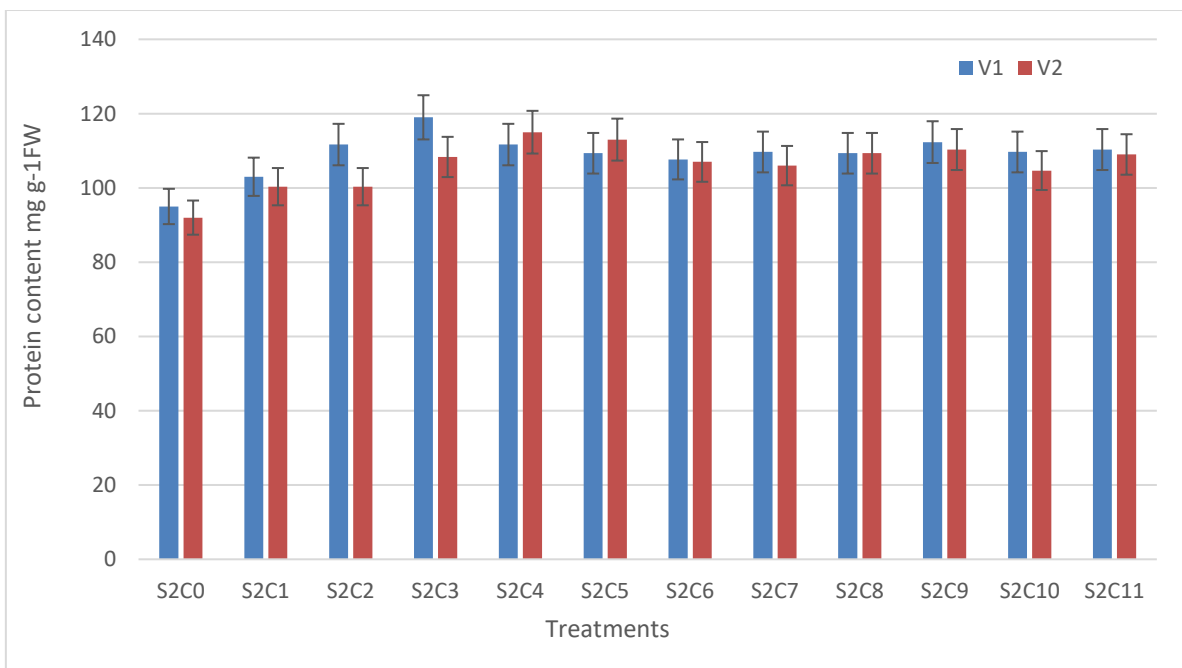


Fig. 4 (b). Effect of seed priming with different concentrations of $ZnSO_4$ on the protein content ($mg\ g^{-1}\ FW$) of two wheat varieties

4. CONCLUSIONS

Seed invigoration techniques are pragmatic approaches to achieve the proper seedling stand establishment and improving multiple abiotic stresses. Seed priming has been commonly used to reduce the time between the sowing of seed and the appearance of seedling (Bose et al. 2018). In the present study priming with 7.5 mM $Mg(NO_3)_2$ and 0.05 mM $ZnSO_4$ showed the best results in both the varieties. The data of presented investigation (Table 1 & 2) reflects an improvement in germination in primed seeds is associated with the work done by (Kumar et al. 2016). However, Germination percentage represents only the number of seed germinated on a particular day. Real germination potential of seed evaluated by the help of number of parameters, which discussed in the following paragraphs.

MGT is precise estimation of the time taken for a seed lot to germinate. It is attentive on the day when the most germination events occurred. As Table 3 and 4 showing that non-primed set having lower germination percentage, reflecting the more time to take germination, as compared to a primed seed. Moreover, MGT demonstrates the comparison of relative emergence rate of seed lots among the different treatments. Resultantly the former may germinate first as compared to later one. Second GRI shows the percentage of germination per day, so this parameter mash-up with germination % and MGT values. The higher the final germination percent (FGP) and the lower the MGT values, represents the more elevated of GRI. Data reveals that primed set always shows the higher GRI values as compared to non-primed one. Consequently, MGR shows the germination speed of seed. Generally it was predicted that a higher germination percentage, higher CVG and lower MGT seed lots having a higher rate of germination. Similarly, priming increases the progress of seed germination, which accelerates the germination rate. Data presented in Table 3 & 4 matched with this report. Another important germination parameter is CVG the significance of the time required shows to attend maximum germination percentage. From the Table 3 & 4, it reflects that non-primed set shows the lower CVG value as compared to the primed one. It is because of priming improves the velocity of germination and reduce the time of lag phase to start germination.

Germination index (GI) is the most comprehensive measurement evaluates both FGP and speed of germination. It magnifies the variation among the seed treatments with an easily compared numerical analysis. Data on GI in present investigation reflect the higher values in case of primed sets as compared to non-primed one. All these germination parameters indicate the germination potential of seeds. In the present experiment presence of NO_3^- and SO_4^{2-} ions improves the germination process via induction of nitrogen and sulphur transfer, and induction of protease activity, which may induce the solubilisation of endosperm protein and synthesis of new proteins. Seed vigour (SV) is a crucial quality parameter for assessment of supplement germination and viability tests to gain insight into the performance of a seed treatment in the field conditions. Seedling vigour or seedling health or seedling growth rate is associated with several other factors related to genetics and environment. In the presented work primed seed lots showed better germination parameters as compared to non-primed one, also confirmed the healthy seedling growth and SV. Shoot length, root length and seedling data of wheat seed reflected with the data of seedling vigour data. A higher the seedling length and germination percentage indicate the higher SV data, which seen in case of primed seed. In the case of presented treatment it was found that $ZnSO_4$ was more effective in shoot and root length compared to $Mg(NO_3)_2$. This might be possible that $ZnSO_4$ priming improves the root traits by synthesis of auxin.

As already we discussed that germination is a result of several biochemical reactions. During seed germination process first and essential step is the entry of water into the seed, which causes the activation of several enzymes, for the metabolism of seed reserve (carbohydrate, lipid and protein) into more straightforward molecular form and these end products translocate to new emerging seedlings. In this array, α -amylase, β -amylase, protease and lipase are the critical enzyme to break seed reserve into a more straightforward form. Most studies on the connections between α -amylase activity and the seed dormancy focused on the genetic defects of late-maturity, α -amylase activity and pre-harvest sprouting in crop cereals and poor bread-making quality (Kondhare et al. 2015). A positive correlation between the α -amylase and soluble sugars found, where increased α -amylase activity resulted in increased soluble sugar content, whereas the higher the soluble sugar content in primed seed reflects the lower content of insoluble sugar. In case of non-primed seeds, insoluble content was higher which correlates with the reduced α -amylase activity. The similar result obtained in the case of protein content whereas higher protein content found in case of primed seed as compared to non-primed because of protease activity and solubilisation of protein. Moreover, priming with $ZnSO_4$ improves the seed germination and early seedling establishment in wheat. Therefore, this study concluded that seed priming treatments improve the seed germination parameters, seedling vigor, seedling growth and biochemical parameters, which help in early emergence, vigorous growth and better seedling establishment compared to non-primed seeds.

Acknowledgement

Authors are very thankful to Institute of Agricultural Sciences, Banaras Hindu University for providing all the facilities.

References

- [1] M. Reynolds, D. Bonnett, S.C. Chapman, R.T. Furbank, Y. Manès, D. E. Mather, M. A. Parry, Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. *Journal of Experimental Botany* 62(2) (2011) 439-452
- [2] Sadeghian, S.Y., Yavari, N., Effect of water-deficit stress on germination and early seedling growth in sugar beet. *Journal of Agronomy and Crop Science*, 190(2) (2004) 138-144
- [3] Soltani, A., Gholipoor, M., Zeinali E., Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environmental and Experimental Botany* 55 (2006) 195-200
- [4] C. S. Vibhuti, K. Bargali, and S. S. Bargali. Seed germination and seedling growth parameters of rice (*Oryza sativa* L.) varieties as affected by salt and water stress. *Indian Journal of Agricultural Sciences*, 85 (2015)102-108
- [5] A. G. Taylor, P. S. Allen, M. A. Bennett, K. J. Bradford, J. S. Burris, and M. K. Misra. Seed enhancements. *Seed Science Research*, 8(2) (1998) 245-256

- [6] S. Paparella, S. S. Araújo, G. Rossi, M. Wijayasinghe, D. Carbonera, and A. Balestrazzi. Seed priming: state of the art and new perspectives. *Plant Cell Reports*, 34 (2015) 1281-1293
- [7] B. Bose, M. Kumar, R. K. Singhal, and S. Mondal. Impact of Seed Priming on the Modulation of Physico-chemical and Molecular Processes During Germination, Growth, and Development of Crops. In *Advances in Seed Priming*. Springer, Singapore, (2018) pp. 23-40.
- [8] M. K. Sharma, and B. Bose. Effect of seed hardening with nitrate salts on seedling emergence, plant growth and nitrate assimilation of wheat (*Triticum aestivum* L.). *Physiol. Mol. Biol. Plants* 12 (2006) 173-176
- [9] S. A. Anaytullah, and B. Bose. Nitrate hardened seed increase germination, amylase activity and proline content in wheat seedling at low temperature. *Physiol. Mol. Biol. Plants* 13 (2007) 61-163
- [10] B. Bose, R. Kumar, S.K. Kuril, A.K. Shrivastava. Hardening of mustard seeds with magnesium nitrate increases seed germination, vegetative growth, nitrogen assimilation and yield. *Brassica* 9 (2007) 33-38
- [11] M. Al-Mudaris. Notes on various parameters recording the speed of seed germination. *Der Tropenlandwirt*, 99 (1998) 147-154
- [12] M. A. Ranal, D. G. D Santana, W. R. Ferreira, and C. Mendes-Rodrigues. Calculating germination measurements and organizing spreadsheets. *Brazilian Journal of Botany*, 32 (2009) 849-855
- [13] M. Goodi, F. and Sharifzadeh. Evaluation effect of hydropriming in barley difference cultivars. *Mag Biaban*. 11 (2006) 99-109
- [14] P. Bernfeld. Amylases alpha and beta. *Meth Enzymoogy*. 1 (1955) 149
- [15] M. Dubois, K.A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28 (1956) 350-356
- [16] M. Kumar, B. Pant, S. Mondal, and B. Bose. Hydro and halo priming: influenced germination responses in wheat Var-HUW-468 under heavy metal stress. *Acta Physiol Plant* 38, 217 (2016). <https://doi.org/10.1007/s11738-016-2226-3>
- [17] B. Bose, H. S. Srivastava, S. N. Mathur. Effect of some nitrogenous salts on nitrogen transfer and protease activity in germinating *Zea mays* L. seeds. *Biol Plant* 24, 89 (1982). <https://doi.org/10.1007/BF02902849>
- [18] A. Rehman, M. Farooq, R. Ahmad, and S. M. A. Basra. 2015. Seed priming with zinc improves the germination and early seedling growth of wheat. *Seed Science and Technology* 43(2) (2015) 262-268
- [19] R.K. Singhal, V. Kumar, and B. Bose. Improving the yield and yield attributes in wheat crop using seed priming under drought stress. *Journal of Pharmacognosy and Phytochemistry* 8(2) (2019) 214-220