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Seed Priming with $Mg(NO_3)_2$ and $ZnSO_4$ Salts Triggers the Germination and Growth Attributes Synergistically in Wheat Varieties

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Abstract: An experiment was conducted in both laboratory (germinative attributes) and field conditions (growth attributes) with completely randomized design (CRD) and randomized block design, respectively, to view the responses of different priming treatments in two wheat varieties: HUW-234 (V1) and BHU-3(V2). In the present study, seeds were primed with water (hydro; T2), Mg $(NO_3)_2$ (T3), $ZnSO_4$ (T4), and a combination of both salts (T5). Their carry over effects were observed on the germinative and vegetative phases of growth. All treatments were compared with the performance of nonprimed control seeds (T1). Maximum germination percentage (98.33, 100%) was noted with T3, whereas length of shoot (8.83, 10.23 cm) and root (9.47, 10.73 cm) and their fresh (0.34, 0.45 g) and dry weights (0.05, 0.07 g) were recorded maximum in T5 for both varieties; however, the vigor index I and II showed varietal difference, but primed sets were found always superior with respect to nonprimed control. Study of plant height, leaf number and area, fresh and dry weights of total leaves and stem showed the best performance under combined use of both salts, i.e., Mg $(NO_3)_2$ and $ZnSO_4$ as priming agents, followed by $ZnSO_4$, Mg $(NO_3)_2$, hydro, and the nonprimed one. The study of biochemical parameters such as protein content and nitrate reductase activity of leaves showed the highest increment in combined priming treatment and increased 63.77, 90.37, 37.44% and 12.81, 5.61, 7.75%, respectively, after 35, 45, and 60 days after sowing. It is likely that chlorophyll, nitrogen, iron, and zinc content also followed a similar pattern and were enhanced in combined priming treatments as compared to nonpriming treatment. Therefore, the result suggests that priming seeds with Mg $(NO_3)_2$ and $ZnSO_4$ worked synergistically at varietal level and improved growth attributes at field conditions.

Keywords: seed priming; seedling vigor; wheat; stand establishment

1. Introduction

Nutritive food for all, now becomes a global challenge due to increasing population and erratic changes in climate. In the future, the human population will increase continuously and the area for cultivated land reduced proportionally, for shelter or to fulfill other basic needs. Therefore, we need to enhance food production and retain the quality attributes to secure global food security.

Wheat (*Triticum aestivum* L.) is the most important cereal crop for the majority of the world's population and it has the highest position in world trade. World trade in wheat is greater than all other crops combined due to its unlatching viscoelastic property to wheat dough, helping in the preparation of processed food [1,2]. Hence, increasing production potentiality of wheat will always be a demanding issue for crop growers.

Seed germination, representing the first dynamic phase of a plant's life, faces numerous stresses of soil and surrounding environmental conditions. From these stresses a relief can be achieved by using innovative and widely used seed priming technology [3], helping fast and uniform field emergence, combined with the successful establishment of seedlings following improved seedling growth, resulting in enhanced yield of various crops [4,5]. Among frequently used various seed priming technologies, it has been observed that $Mg(NO_3)_2$ primed seeds responded better as compared to KNO_3 and $Ca(NO_3)_2$ [6,7] for crops; the former is also able to induce nitrogen-using efficiency in rice [8]. This might happen due to the presence of Mg, representing the most abundant divalent cation in plant cells and performing critical roles in various physiological processes by serving as the cofactor with ATP in a number of enzymatic reactions [9]. In addition, it is a part of chlorophyll molecules in higher plants. Deficiency of Mg in plants leads to yellowing of leaves or interveinal chlorosis. In various studies, it has been observed that the application of magnesium salt enhanced the nitrogen uptake, which in turn improved protein and chlorophyll contents, nitrate reductase, and glutamate kinase activities in various crops. Use of seeds primed with $Mg(NO_3)_2$ showed an increase in root number, length, and weight, as well as stress improving characteristics in crops cultivated in field conditions [10,11]. Micronutrients, including zinc, also likely play pivotal roles in plant growth and development. Zn acts as a cofactor for various enzymes such as alcohol dehydrogenase (ADH), lactic dehydrogenase (LDH), carbonic anhydrase, and are also involved in the activation of phosphate-transferring enzymes such as hexose kinase of triosephosphate dehydrogenase. Zn is required for the production of tryptophan, which is precursor of plant hormone auxin [12,13]. Zn also participates in several functions of plants such as amino acid, protein, and chlorophyll biosynthesis, and pollen formation. Seed priming with $ZnSO_4$ improves germination related traits in wheat [14,15]; however, the present study highlights the combined role of two seed priming agents, i.e., $Mg(NO_3)_2$ and $ZnSO_4$, in influencing germination and post-germinative traits, including nitrogen enhancing capacity of two wheat varieties, viz., HUW-234 (V1) and BHU-3 (V2). The objective of this study is to visualize the effect of sole and combined salt priming agents on wheat germinative and growth attributes.

2. Material and Methods

2.1. Experimental Site

The present experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, (U.P.) India. The biochemical analysis was performed in Seed Priming Laboratory at the same institute. Geographically, the university is located in the southeastern part of Varanasi city, which is at 25°18′ N latitude, 83°3′ E longitude and at an altitude of 75.7 m above mean sea level.

2.2. Plant Materials and Soil Conditions

Wheat (*Triticum aestivum* L.) varieties HUW-234 (V1) and BHU-3 (V2) were taken as the plant material from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. For experiment purposes only bold, healthy, and disease-free seed was selected. The soil used for the present experiment was Indo–Gangetic alluvial soil with sandy loam texture (fine sand 55.26%, silt sand 27.09%, clay 15.27%), deep, well-drained, and moderately fertile, being low in available phosphorus (17 kg ha^{-1}) and potassium (180 kg ha^{-1}). The pH (7.30) of the soil ranged between neutral to slightly alkaline.

2.3. Seed Priming Treatment

Wheat seeds were surface sterilized by keeping them in 0.01% $HgCl_2$ (mercuric chloride) solution for five minutes and then gently washed with distilled water for 5–6 times. These sterilized seeds were used for further research purposes. The seeds of wheat (*Triticum aestivum* L.) var. HUW-234 and BHU-3 were soaked in different salt solutions and distilled water for 12 h. The experiment contained 5 treatments of each variety (HUW-234 and BHU-3) of combination with three replications containing control (nonprimed, T1), hydro primed (T2), 7.5 mM $Mg(NO_3)_2$ (T3), 50 ppm $ZnSO_4$ (T4), and 7.5 mM $Mg(NO_3)_2$ + 50 ppm $ZnSO_4$ (T5) primed. The best concentration of $Mg(NO_3)_2$ and $ZnSO_4$ used in wheat were standardized earlier in the same laboratory by Singhal and Bose [15]. After 12 h, these seeds were washed smoothly with distilled water and those seeds were further dried back to their original weight at room temperature by placing them under a fan. After that, these seeds were packed in paper bags and then used as per experimental requirements.

2.4. Experimental Layout

The experiment was conducted in a randomized block design (RBD) with three replications in field conditions. In this experiment, each treatment was repeated thrice for each replication. Differences between the means were calculated at 5% level of significance. Additionally, the germinative studies were conducted in completely randomized design (CRD) under laboratory conditions.

2.5. Germination-Related Studies

For germination studies, the air-dried seeds of salt primed (7.5 mM $Mg(NO_3)_2$, 50 ppm $ZnSO_4$, 7.5 mM $Mg(NO_3)_2$ + 50 ppm $ZnSO_4$, and hydro primed) and nonprimed (control) seeds were placed equal-distant (20 seeds/Petri dish) in each Petri dish and 5 mL DW was poured into each Petri dish containing primed and nonprimed seeds. Petri dishes were kept at room temperature in the laboratory.

2.6. Germination Percent (%)

The number of germinated seeds was counted every 12, 18, 24, 30, 36, 48, 72, and 96 h after sowing the seeds into Petri plates. Germination percentage was calculated by the formula given by Singhal and Bose [15]. Germinated seed denotes the seed viability capacity of plants.

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

2.7. Seedling Vigor Index

The seedling vigor index measured in 7-day-old seedlings was calculated by using the formula given by Goodi and Sharifzadeh [16]:

Seedling vigor I: (Shoot length + Root length) × Germination%.

Seedling vigor II: Germination% × Seedling dry weight.

2.8. Dry Weight of Seedling

To take the seedling dry weight, samples of three seedlings in three replications were kept for an hour in an oven preset at 100–110 °C. Thereafter, it was placed in an oven set at 60 ± 2 °C to obtain a constant weight. Later on, the average dry weight of three seedlings was calculated in three replications.

2.9. Root and Shoot Length Measurement

Root and shoot length of three seedlings were determined with the help of a scale.

2.10. Plant Height (cm)

Plant height of the three-tagged tillers was determined from ground to the tip of the longest leaf. The average plant height was analyzed by taking the mean of three observations and expressed in centimeters. Data were observed in wheat at 35, 45, and 60 DAS (days after sowing).

2.11. Leaf Number per Plant

The total number of leaves per plant was counted at different growth stages, i.e., 35, 45, and 60 days after sowing (DAS). At the early stages of the growth, only green leaves were counted and then both green and senescing leaves were counted at the latter stages.

2.12. Total Leaf Area (cm^2)

The total leaf area of the wheat plants was measured using graph paper by measuring the length and width and multiplying with the correction factor. The unit of measurement was cm^2 $plant^{-1}$.

2.13. Determinations of Fresh and Dry Weight

After washing the plants in the tap water and softly wiping them with blotting paper, the fresh weight of three plants was determined by using an electronic balance (Sartorius BT-224S) and the values expressed in grams. After taking fresh weight, the plants were placed in a 100 °C preheated hot air oven for one hour. Then they were placed in an oven, maintained at 60 $\pm$ 2 °C for drying purposes. The weight, expressed in grams, was measured regularly until a constant value was reached.

2.14. Chlorophyll Content

Chlorophyll content of plants was measured to the third leaf from the top of the main tiller by using a SPAD meter.

2.15. Nitrate Reductase Activity (NRA) (n Moles NO_2-Formed g^{-1} F.W. h^{-1})

For estimation of nitrate reductase activity, leaves from uniformly grown seedlings in a homogeneous population were selected. The enzyme activity was assayed in vivo by the method of Srivastava [17] in the first fully expanded mature leaf from the lower side of the plant.

2.16. Estimation of Total Soluble Protein

The method was developed by Bradford [18] and sufficiently sensitive to give a moderately constant value and hence largely followed.

2.17. Nitrogen, Fe, and Zn Content

Nutrient content and portioning in parts of the plant were determined at harvesting. Total nitrogen content in plant samples was determined by nitrogen analyzer (Pelicon, Model KEL 20L), while Zn and Fe concentrations were analyzed by the method used by Paltridge et al. [19]. Ten gram grains were taken from each sample and were stored in new, clean paper envelopes. To avoid any contamination of the grains with dust particles and any other extraneous matter, extra care was taken at each step. Zn and Fe (ppm) analysis were performed by X-ray fluorescence (EDXRF spectrometer X-Supreme8000) installed at the Seed Molecular Laboratory, Department of Genetics and Plant Breeding, B.H.U., Varanasi.

2.18. Statistical Analysis

The data were reported as mean ± standard deviation (SD), critical difference (CD), and standard error of mean for triplet replication, performed by OPSTAT, developed by O.P. Sheoran, CCS, HAU, Hisar, India. Tukey's honestly significant difference (HSD) test with a confidence of 95% was done by using SPSS 19.0 statistical analysis software (IBM, New York, NY, USA). Different alphabetical letters are used in figures and tables for showing significant differences.

3. Results

3.1. Germination Percentage (%)

Table 1 shows the data of germination percentage (G%) of two the wheat varieties, V1 (HUW-234) and V2 (BHU-3), grown in the year 2017–2018. From the data, it was been found that the G% increased with time and observed highest at 96 h (93.67%) and lowest at 12 h (14.33%) in V1, whereas, in V2 variety, the highest G% was recorded at 72 h and 96 h (100%) and lowest at 12 h (40.33%). Moreover, T3 primed sets showed the highest G% (81.67%) followed by T5, T4, and minimum in T1 or nonprimed (51.46%) sets in V1. However, in V2 the highest G% was observed in T5 primed (91.67%) sets followed by T4, T3, and lowest in T1 nonprimed (80.62%) sets. Additionally, it was found that the nonpriming treatment takes more time to fully germinate as compared to the primed one. By comparing the data of both varieties, it can be suggested that the germination percent of V2 was better than V1 at different hours and also in different treatments. Germination percent was found statistically significant at treatments as well as varietal levels, and in V × T interaction for all time periods. Except at 96 h, germination percent was found nonsignificant at treatment levels and V × T interaction (Table 1).

3.2. Shoot and Root Length (cm)

Figure 1 depicts the shoot length (cm) and root length (cm) of both varieties, grown in the year 2017–2018. Shoot length was observed highest in the T5 treatment (8.83 and 10.23 cm) and lowest was in T1, nonprimed sets (7.6 and 8.07 cm) in V1 and V2, respectively. Hence, the result suggested that in the present case, combined treatment of two salts showed superior performance in V1 and V2. Shoot length was found statistically significant in treatment and varietal levels. The interaction between V × T was nonsignificant.

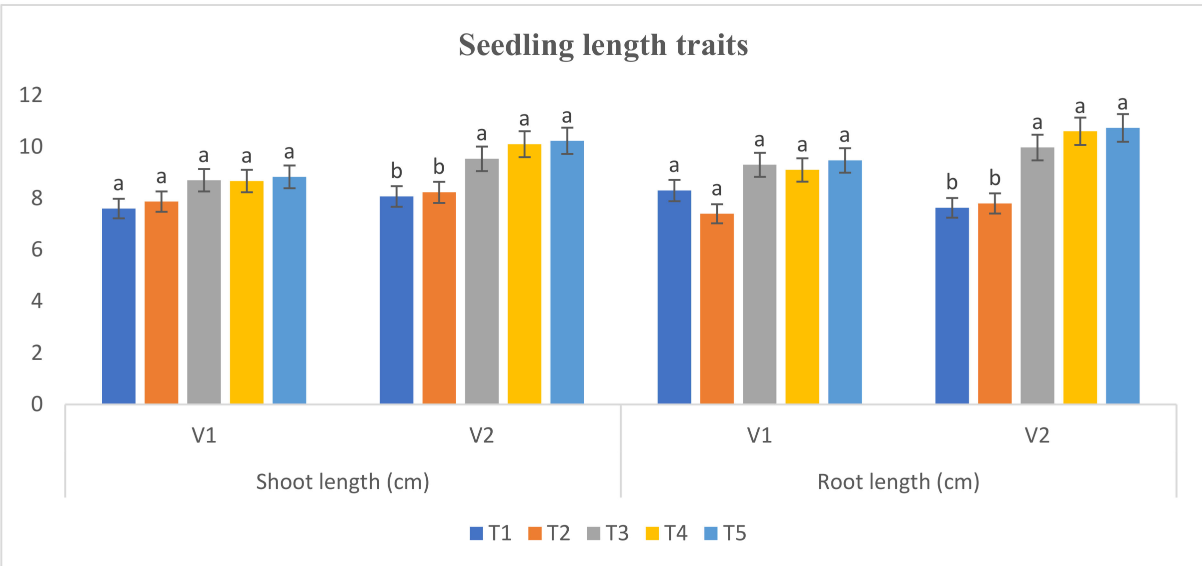

Figure 1. Shoot length (cm) and root length (cm) of wheat varieties under different priming treatments. The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$).

Table 1. Germination of wheat varieties under different priming treatments.

Germination % V1																
Treatments	**12 h**		**18 h**		**24 h**		**30 h**		**36 h**		**48 h**		**72 h**		**96 h**	
T1	1.67 ± 2.36c		13.33 ± 6.24c		33.33 ± 6.24b		53.33 ± 8.50b		63.33 ± 10.27b		75.00 ± 7.07c		85.00 ± 4.08a		86.67 ± 4.71a	
T2	3.33 ± 2.36c		30.00 ± 4.08b		50 ± 4.08b		66.67 ± 2.36b		81.67 ± 8.50a		86.67 ± 6.24b		93.33 ± 2.36a		93.33 ± 2.36a	
T3	28.33 ± 2.36a		53.33 ± 2.36a		88.33 ± 4.71a		93.33 ± 2.36a		95.00 ± 0.00a		98.33 ± 2.36a		98.33 ± 2.36a		98.33 ± 2.36a	
T4	20.00 ± 4.08b		60.00 ± 4.08a		75.00 ± 4.08a		83.33 ± 8.50a		85.00 ± 7.07a		91.67 ± 4.71ab		93.33 ± 6.24a		95.00 ± 4.08a	
T5	18.33 ± 4.71b		48.33 ± 13.12a		76.67 ± 15.46a		88.33 ± 9.43a		90.00 ± 7.07a		95.00 ± 4.08ab		95.00 ± 4.08a		95.00 ± 4.08a	
Mean	**14.33**		**41.00**		**64.67**		**77.00**		**83.00**		**89.33**		**93.00**		**93.67**	
V2																
T1	11.67 ± 6.24c		63.33 ± 8.50b		85 ± 4.08a		90.00 ± 4.08c		96.67 ± 2.36a		98.33 ± 0.00a		100.00 ± 0.00a		100 ± 0.00a	
T2	33.33 ± 6.24b		75.00 ± 4.08ab		83.33 ± 6.24a		91.67 ± 2.36bc		98.33 ± 2.36a		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a	
T3	45.00 ± 8.16ab		80.00 ± 4.08a		95.00 ± 4.08a		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a	
T4	53.33 ± 6.24a		83.33 ± 6.24a		91.67 ± 4.71a		96.67 ± 4.71abc		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a	
T5	58.33 ± 6.24a		81.67 ± 4.71a		95.00 ± 4.08a		98.33 ± 2.36ab		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a		100.00 ± 0.00a	
Mean	**40.33**		**76.67**		**90.00**		**95.33**		**99.00**		**99.67**		**100**		**100**	
	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm
Factor A	4.95	1.67	6.06	2.04	6.26	2.11	5.09	1.72	5.05	1.70	3.50	1.18	2.71	0.91	2.43	1.15
Factor B	7.83	2.63	9.59	3.23	9.90	3.33	8.06	2.71	7.98	2.69	5.54	1.86	4.29	1.44	NS	1.83
Factor A × B	11.00	3.73	13.56	4.56	14.00	4.71	11.39	3.84	11.29	3.80	7.83	2.63	6.06	2.04	NS	2.58

T1; nonprimed (control), T2; hydro primed, T3; $Mg(NO_3)_2$ primed with 7.5 mM solution, T4; $ZnSO_4$ primed with 50 ppm solution, T5; $Mg(NO_3)_2$ + $ZnSO_4$ primed with 7.5 mM + 50 ppm solution, V1; HUW-234, V2; BHU-3, NS; non-significant, CD; critical difference, ± SEm: standard error of mean. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$).

Maximum root length was observed in T5 primed (9.47 and 10.73 cm) sets and minimum was in T1 nonprimed sets (8.3 and 7.63 cm) in both V1 and V2, respectively. Hence, the result recommended that in the present case combined treatment of two salts showed superior performance in V1 and V2. Root length was found statistically significant in treatment level and nonsignificant in varietal level.

3.3. Seedling Fresh Weight (g) and Seedling Dry Weight (g)

Figure 2 represents the data of seedling (root + shoot) fresh and dry weight (g) of both varieties and the maximum seedling fresh weight observed in T5 primed (0.34 and 0.45 g) sets followed by T4, T3 primed, and minimum in nonprimed sets (0.18 and 0.27 g) in V1 and V2, respectively. Hence, T5 primed set is observed to have better performance in V1 and V2. Seedling fresh weight was found statistically significant in both treatment and varietal levels. The interaction between V × T was found to be nonsignificant.

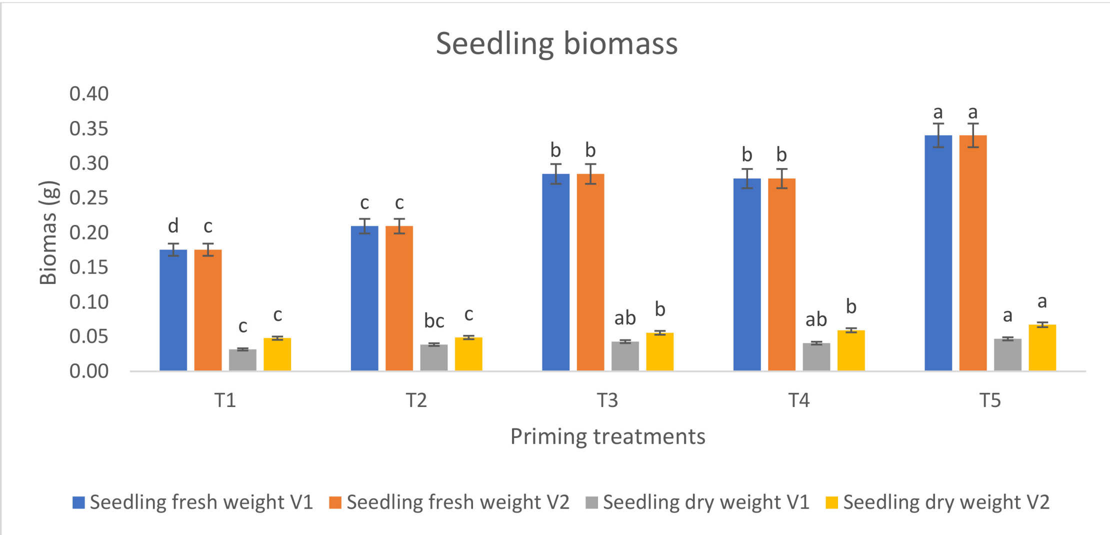

Figure 2. Seedling fresh weight (g) and seedling dry weight (g) of wheat varieties under different priming treatments. The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$).

Highest seedling dry weight was observed in T5 primed (0.05 and 0.07 g) sets followed by T4 and T3 primed sets, and lowest was in nonprimed (control) (0.03 and 0.05 g) in V1 and V2, respectively. Hence, T5 primed set is observed to have better performance in V1 and V2. Seedling dry weight was found statistically significant in both treatment and varietal levels.

3.4. Vigor Index-I

The data showed that vigor index-I (VI-I) increased with time, i.e., increase in hours of germination or day-by-day increase in germination rate. Vigor index-I was observed highest at 96 h (1601.77) and lowest at 12 h (255.20) in V1, but in V2, vigor index was maximum at 72 and 96 h (1858.00) and minimum at 12 h (784.17). In the case of priming treatment, vigor index was highest in the T3 primed (1471.25) set followed by T5, T4 and lowest in T1 or nonprimed (767.27) set in V1. However, in V2 the highest VI-I was observed in the T5 primed (1923.44) sets followed by T4, T3 and lowest in T1 nonprimed (1264.62) sets. By comparing the data, it was observed that V2 was better than V1 at different hours of germination and in different treatments. Vigor index-I was found statistically significant at treatment and varietal levels for all time periods. The interaction between V × T was found nonsignificant for all time periods, while except at 12 h vigor index was found statistically significant at V × T interaction (Table 2).

Table 2. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed, and nonprimed (control) seed treatments on vigor index-I, grown in the year 2017–2018.

				Vigor Index-I (V1)					
Treatments	**12 h**	**18 h**	**24 h**	**30 h**	**36 h**	**48 h**	**72 h**	**96 h**	**Mean**
T1	24.67 ± 34.88c	195.83 ± 86.31b	496.67 ± 79.45b	797.5 ± 116.32b	944.83 ± 129.05c	1120.50 ± 63.65c	1257.50 ± 69.90c	1300.67 ± 101.30b	767.27
T2	51.83 ± 36.86c	480.33 ± 32.71b	806.83 ± 74.40b	1068.17 ± 53.16b	1316.00 ± 132.28b	1395.33 ± 65.18b	1491.67 ± 129.09b	1508.33 ± 109.84ab	1014.81
T3	513.00 ± 82.77a	956.33 ± 49.28a	1588.67 ± 150.79a	1683.00 ± 188.36a	1710.00 ± 157.44a	1773.00 ± 196.58a	1773.00 ± 196.58a	1773.00 ± 196.58a	1471.25
T4	353.67 ± 67.98b	1069.50 ± 123.91a	1334.17 ± 120.12a	1485.67 ± 212.31a	1515.33 ± 194.73ab	1628.50 ± 115.34ab	1659.83 ± 158.55ab	1689.50 ± 134.85a	1342.02
T5	332.83 ± 74.26b	884.00 ± 239.01a	1405.50 ± 302.41a	1617.17 ± 190.11a	1647.50 ± 150.61ab	1737.33 ± 76.76a	1757.33 ± 100.56a	1737.33 ± 76.46a	1389.87
Mean	**255.20**	**717.20**	**1126.37**	**1330.30**	**1426.73**	**1530.93**	**1587.87**	**1601.77**	
					V2				
T1	177.67 ± 89.38d	991.00 ± 116.61b	1335.33 ± 102.79b	1413.83 ± 106.08b	1517.17 ± 75.44b	1542.00 ± 43.30b	1570.00 ± 80.42b	1570.00 ± 80.42b	1264.62
T2	533.00 ± 92.96c	1200.17 ± 23.04b	1332.67 ± 56.79b	1468.50 ± 24.46b	1575.50 ± 33.57b	1603.33 ± 57.35b	1603.33 ± 57.35b	1603.33 ± 57.35b	1364.98
T3	872.5 ± 142.40b	1564.50 ± 168.23a	1855.00 ± 166.31a	1950.00 ± 110.45a	1950.00 ± 110.45a	1950.00 ± 110.45a	1950.00 ± 110.45a	1950.00 ± 110.45a	1755.25
T4	1109.00 ± 171.76ab	1730.00 ± 199.92a	1902.17 ± 189.05a	2005.67 ± 194.31a	2070.00 ± 107.08a	2070.00 ± 107.08a	2070.00 ± 107.08a	2070.00 ± 107.08a	1878.35
T5	1228.67 ± 181.70a	1717.17 ± 181.28a	1990.83 ± 117.62a	2064.17 ± 151.66a	2096.67 ± 106.56a	2096.67 ± 106.56a	2096.67 ± 106.56a	2096.67 ± 106.56a	1923.44
Mean	**784.17**	**1440.57**	**1683.20**	**1780.43**	**1841.87**	**1852.40**	**1858.00**	**1858.00**	

	12 h CD	12 h ± SEm	18 h CD	18 h ± SEm	24 h CD	24 h ± SEm	30 h CD	30 h ± SEm	36 h CD	36 h ± SEm	48 h CD	48 h ± SEm	72 h CD	72 h ± SEm	96 h CD	96 h ± SEm
Factor A	102.3	34.46	132.4	44.57	142.9	48.11	138.6	46.68	119.3	40.17	96.85	32.60	111.2	37.45	107.0	36.02
Factor B	161.8	54.48	209.3	70.48	225.9	76.07	219.2	73.80	188.7	63.52	153.1	51.55	175.8	59.21	169.2	56.96
Factor A × B	228.8	77.05	NS	99.67	NS	107.5	NS	104.3	NS	89.83	NS	72.89	NS	83.73	NS	80.55

The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$). NS; non-significant, CD; critical difference, ± SEm: standard error of mean.

3.5. Vigor Index-II

Table 3 indicates the data of vigor index-II, according to data, it was observed that vigor index-II increases daily. Vigor index-II was observed highest at 96 h (3.78) and lowest at 12 h (0.62) in V1, while in V2 vigor index was highest at 72 and 96 h (5.59) and lowest at 12 h (2.36). Vigor index-II recorded highest in the T5 primed (3.54) sets, followed by T3, T4 and minimum in T1 or nonprimed (1.62) sets in V1. However, in the V2 variety, the highest value was recorded in the T5 primed (6.17) sets followed by T4, T3 and lowest in T1 nonprimed (3.87) sets. By comparing the data, it can be suggested that the V2 variety was better than the V1 variety at different hours and in different treatments. Vigor index-II was found statistically significant at treatment and varietal levels in all the time periods, i.e., 12, 18, 24, 30, 36, 48, 72, and 96 h. The interaction between V $\times$ T was found nonsignificant at all time periods, while except at 12, 36, and 72 h, vigor indexes were found statistically significant for V $\times$ T interaction.

3.6. Plant Height (cm)

Plant height is the most important trait used to measure the vertical growth of a plant. Table 4 represents the data of plant height observed at 35, 45, and 60 days after sowing (DAS) in the year 2017–2018; plant height increased with increasing duration of the wheat crop. Plant heights were highest in T5 primed (30.80 cm) and lowest in T1 primed (29.76 cm) in variety V1; in variety V2, the highest plant height was observed in T4 primed (30.64 cm) and lowest was in the T1 primed (29.44 cm) set at 35 DAS. However, at 45 DAS, the T5 set showed the highest plant height (43.53 and 39.80 cm) in V1 and V2, respectively, and T2 primed showed lowest height in V1, but in the V2 variety T1 showed minimum height. At 60 DAS, it was observed that the T5 primed set had a higher plant height (65.36 and 57.49 cm) and T1 primed showed lower plant height (62.55 and 52.69 cm) in the V1 and V2 varieties, respectively. Thus, it can be determined, based on the result, that T5 primed set's treatment was found to be more effective in both the V1 and V2 varieties at all time periods, except in the V2 variety at 35 DAS. The data were validated again by growing the crop in the year 2018–2019 (Table S1). Although the data showed the same difference from the abovementioned year, i.e., 2017–2018, the trend was found to be the same regarding plant height. The plant height was found statistically significant at 35, 45, and 60 DAS in treatment and varietal level in both experimental years. However, plant height was found nonsignificant at 35 DAS in varietal level in experimental year 2018–2019. The interaction between V $\times$ T was found nonsignificant at all time periods in both experimental years (2017–2018 and 2018–2019).

3.7. Leaf Number (hill^{-1})

Figure 3 signifies the data of leaf number of both varieties that were collected from 35, 45, and 60 DAS-old plants, grown in the year 2017–2018. Generally, leaf numbers increased with increasing age of the wheat crop. Maximum and minimum number of leaf numbers were obtained from T5 primed (18.70 and 19.78) and T1 primed (13.94 and 15.85) seeds in V1 and V2 varieties of wheat, respectively. In addition, at the time of 45 DAS leaf number increased in both varieties. During this time maximum (32.55 and 33.22) leaf numbers in T5 primed and minimum (24.44 and 28.03) in T1 nonprimed sets were found in V1 as well as in V2 varieties. However, at 60 DAS maximum (32.11 and 46.11) and minimum (24.44 and 27.22) leaf numbers were found with the same sets, i.e., T5 and T1 as was observed at 45 DAS. Therefore, the result showed that T5 treatment was more effective in improving the leaf numbers of the V1 and V2 varieties in all the studied periods followed by T4 and T3 primed seed. The data were validated again by growing the crop in the year 2018–2019 (Figure S1). Although the data shows the difference from the abovementioned year, i.e., 2017–2018, the trend was found the same regarding leaf numbers.

Table 3. Vigor index-II of wheat varieties under $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2 + ZnSO_4$ primed, and nonprimed (control) seed treatments.

Vigor Index-II																	
Treatment	**V1**																
	12 h		**18 h**		**24 h**		**30 h**		**36 h**		**48 h**		**72 h**		**96 h**		**Mean**
T1	0.05 ± 0.08c		0.40 ± 0.17c		1.04 ± 0.17d		1.68 ± 0.29d		1.99 ± 0.29c		2.36 ± 0.14d		2.68 ± 0.20d		2.74 ± 0.29d		1.62
T2	0.13 ± 0.09c		1.16 ± 0.20b		1.93 ± 0.16c		2.58 ± 0.21c		3.17 ± 0.45b		3.36 ± 0.39c		3.61 ± 0.23c		3.61 ± 0.23c		2.44
T3	1.22 ± 0.15a		2.29 ± 0.24a		3.81 ± 0.46a		4.01 ± 0.32a		4.08 ± 0.28a		4.23 ± 0.33ab		4.23 ± 0.33ab		4.23 ± 0.33ab		3.51
T4	0.82 ± 0.21b		2.42 ± 0.06a		2.96 ± 0.30b		3.36 ± 0.22b		3.43 ± 0.13b		3.72 ± 0.39bc		3.78 ± 0.35bc		3.85 ± 0.30bc		3.05
T5	0.85 ± 0.20b		2.23 ± 0.49a		3.55 ± 0.49ab		4.12 ± 0.16a		4.20 ± 0.05a		4.45 ± 0.21a		4.45 ± 0.21a		4.45 ± 0.21a		3.54
Mean	0.62		1.70		2.66		3.15		3.38		3.62		3.75		3.78		
	V2																
T1	0.57 ± 0.32d		3.03 ± 0.33d		4.07 ± 0.06b		4.31 ± 0.05c		4.64 ± 0.08c		4.72 ± 0.20c		4.8 ± 0.16c		4.8 ± 0.16c		3.87
T2	1.64 ± 0.34c		3.67 ± 0.18cd		4.07 ± 0.25b		4.49 ± 0.19c		4.81 ± 0.08c		4.9 ± 0.16c		4.9 ± 0.16c		4.9 ± 0.16c		4.17
T3	2.47 ± 0.30bc		4.46 ± 0.51bc		5.30 ± 0.59ab		5.57 ± 0.39b		5.57 ± 0.39b		5.57 ± 0.39b		5.57 ± 0.39b		5.57 ± 0.39b		5.01
T4	3.18 ± 0.47ab		4.96 ± 0.54ab		5.77 ± 0.85a		5.75 ± 0.51b		5.93 ± 0.25b		5.93 ± 0.25b		5.93 ± 0.25b		5.93 ± 0.25b		5.42
T5	3.94 ± 0.55a		5.49 ± 0.46a		6.41 ± 0.63a		6.62 ± 0.41a		6.73 ± 0.37a		6.73 ± 0.37a		6.73 ± 0.37a		6.73 ± 0.37a		6.17
Mean	2.36		4.32		5.12		5.35		5.54		5.57		5.59		5.59		
Interactions	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	CD	± SEm	
Factor A	0.29	0.10	0.33	0.11	0.43	0.15	0.28	0.09	0.26	0.09	0.28	0.09	0.26	0.09	0.26	0.09	
Factor B	0.46	0.15	0.53	0.18	0.69	0.23	0.45	0.15	0.41	0.14	0.44	0.15	0.41	0.14	0.41	0.14	
Factor A × B	0.65	0.22	N/S	0.25	N/S	0.33	N/S	0.21	0.57	0.19	N/S	0.21	0.58	0.19	N/S	0.19	

The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$). NS; non-significant, CD; critical difference, ± SEm: standard error of mean.

Table 4. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed, and nonprimed (control) seed treatments on plant height (cm) of wheat varieties, grown in the year 2017–2018.

Treatments	Plant Height (cm)								
	35 DAS			45 DAS			60 DAS		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T1	27.91 ± 1.40c	26.84 ± 0.38c	27.37	40.26 ± 0.87a	36.78 ± 3.42a	38.52	61.81 ± 0.49d	52.69 ± 3.00a	57.25
T2	28.79 ± 0.83bc	27.97 ± 0.57c	28.38	40.71 ± 0.90a	37.27 ± 2.59a	38.99	62.78 ± 0.85cd	54.35 ± 1.17a	58.56
T3	30.71 ± 1.20ab	29.93 ± 0.33b	30.32	43.28 ± 3.40a	39.54 ± 1.71a	41.41	64.05 ± 1.13bc	55.83 ± 2.95a	59.94
T4	31.15 ± 0.94a	30.64 ± 0.63ab	30.89	43.75 ± 0.66a	40.56 ± 1.51a	42.16	65.14 ± 1.29ab	56.77 ± 1.57a	60.96
T5	32.55 ± 0.71a	31.57 ± 0.61a	32.06	45.95 ± 0.87a	41.52 ± 0.76a	43.73	66.56 ± 0.76a	57.49 ± 1.74a	62.02
Mean	30.22	29.39		42.79	39.13		64.07	55.43	
Factors	35 DAS			45 DAS			60 DAS		
	CD	± SEm		CD	± SEm		CD	± SEm	
Factor A	0.78	0.26		1.77	0.59		1.68	0.56	
Factor B	1.24	0.41		2.79	0.93		2.65	0.89	
Factor A × B	NS	0.59		NS	1.32		NS	1.25	

The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$). NS; non-significant, CD; critical difference, ± SEm: standard error of mean.

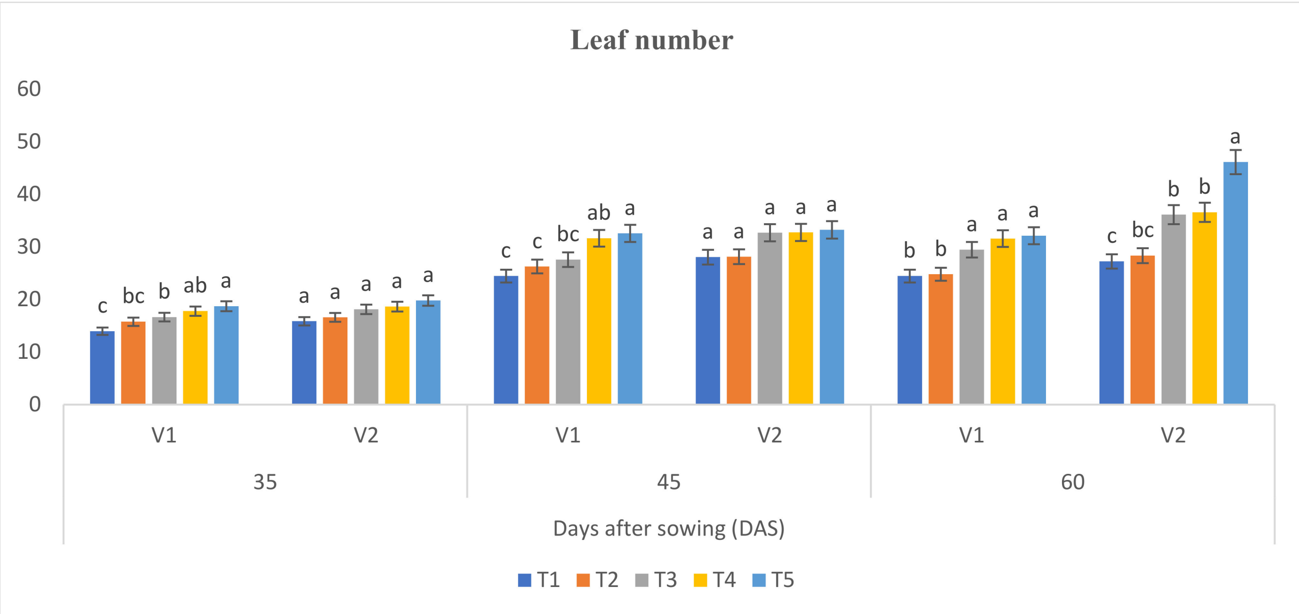

Figure 3. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed, and nonprimed (control) seed treatments on leaf number of wheat varieties, grown in the year 2017–2018. The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$).

3.8. Leaf Area ($cm^2/hill^{-1}$)

Table 5 shows the data of leaf area (cm^2) collected from the two varieties of wheat (V1 and V2), grown in the year 2017–2018. The leaf is the main photosynthetic organ in a plant. When plant leaf area increases, then photosynthetic activity is also increased. Leaf area at 35 DAS is reported to improve the maximum in the T5 primed one (278.57 and 282.41 cm^2) and the minimum is in the T1 nonprimed control set (258.52 and 139.85 cm^2) in the V1 and V2 varieties, respectively. At 45 DAS, maximum leaf area was observed in T5 (415.63 and 356.44 cm^2), followed by T4 and T3 primed sets and the minimum was obtained from the T1 nonprimed (297.24 and 275.14 cm^2) one in both the V1 and V2 varieties, respectively. At 60 DAS, maximum leaf area was reported in the T5 primed (1356.88 and 1270.95 cm^2) one and the minimum was found in T1 treatment (997.55 and 785.04 cm^2) in V1 and V2, respectively. Hence, it can be suggested that in all stages, i.e., the T5 primed one proved to be better at improving leaf area, followed by the T4 and T3 primed ones in V1 and V2. The data were validated again by growing the crop in the year 2018–2019 (Table S2). Although the data showed the difference from the abovementioned year, i.e., 2017–2018, the trend was found to be the same regarding leaf area (cm^2). The leaf area (cm2) was statistically significant at treatment and varietal levels at 35 and 45 as well as at 60 DAS in both experimental years. Moreover, the interaction between V × T was found nonsignificant at 35, 45, and 60 DAS in both experimental years.

3.9. Total Plant Fresh Weight (g)

Figure 4 shows the values of total plant fresh weight at different time periods, i.e., 35, 45, and 60 DAS, obtained from two varieties of wheat (V1 and V2), grown in the year 2017–2018. When the plant's leaf area increased, then plant fresh weight also increased. Total leaf fresh weight at 35 DAS was reported as the maximum in the T5 primed one (12.75 and 13.12 g) and the minimum was found in the T1 nonprimed set (8.63 and 7.88 g) in V1 and V2 varieties, respectively. At 45 DAS, the highest plant fresh weight was observed in T5 primed (20.77 and 18.26 g) and lowest in T1 nonprimed (10.74 and 14.22 g) sets in both V1 and V2, respectively. The plant fresh weight at 60 DAS was again maximum in the T5 primed one (51.89 and 47.94 g) and minimum in the T1 set (41.92 and 29.84 g) in V1 and V2, respectively.

The data were validated again by growing the crop in the year 2018–2019 (Figure S2). Although the data shows the difference from the abovementioned year, i.e., 2017–2018, but the trend was found to be the same regarding total plant fresh weight (g). The total leaf fresh weight was found statistically significant at treatment level at 35, 45, and 60 DAS in both experimental years, except in the experimental year 2018–2019, when at 60 DAS the leaf fresh weight was found statistically nonsignificant in treatment level.

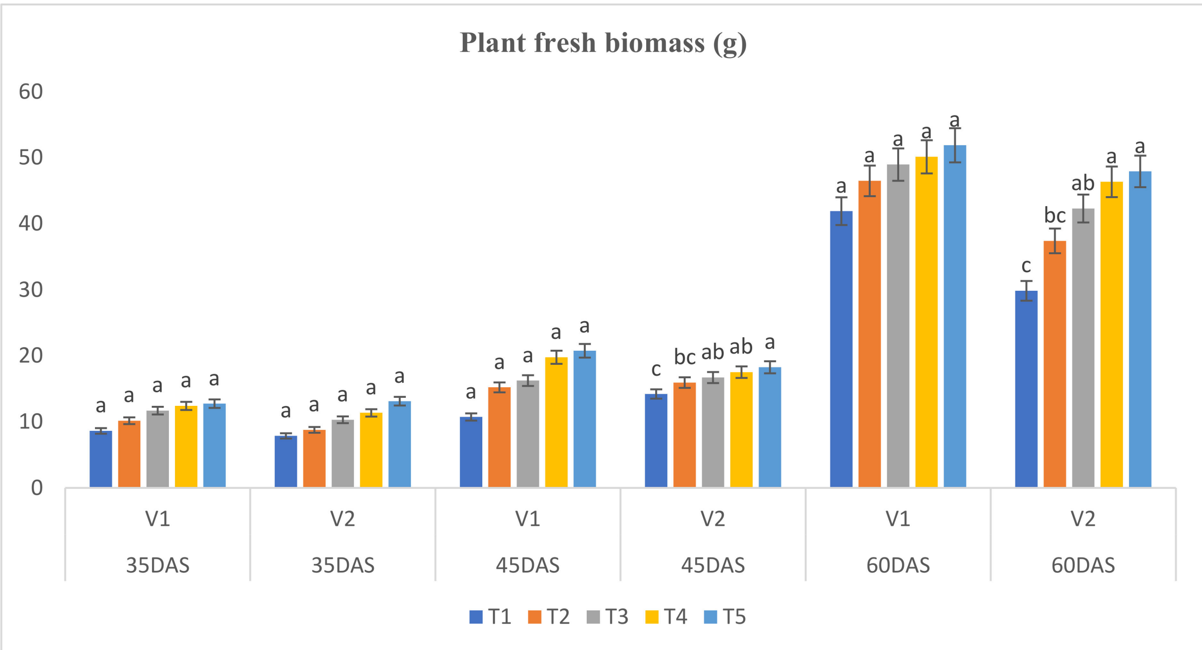

Figure 4. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed, and nonprimed (control) seed treatments on total plant fresh biomass (g) of wheat varieties, grown in the year 2017–2018. The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$).

Table 5. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed, and nonprimed (control) seed treatments on leaf Area (cm^2) of wheat varieties, grown in the year 2017–2018.

	Leaf Area (cm^2)								
Treatments	**35 DAS**			**45 DAS**			**60 DAs**		
	V1	**V2**	**Mean**	**V1**	**V2**	**Mean**	**V1**	**V2**	**Mean**
T1	258.52 ± 18.76a	139.85 ± 32.55a	199.19	297.24 ± 9.20b	275.14 ± 18.85a	286.19	997.55 ± 37.64a	785.04 ± 58.16a	891.29
T2	262.84 ± 45.07a	207.82 ± 71.57a	235.33	300.54 ± 16.70b	287.93 ± 38.83a	294.24	1086.44 ± 109.25a	852.75 ± 18.55a	969.59
T3	274.24 ± 48.48a	222.12 ± 43.21a	248.18	341.62 ± 5.42b	306.51 ± 66.70a	324.06	1148.46 ± 310.96a	1018.21 ± 64.32a	1083.34
T4	276.84 ± 25.81a	255.89 ± 36.02a	266.36	346.67 ± 55.68b	309.13 ± 22.37a	327.90	1264.11 ± 89.33a	1119.45 ± 50.37a	1191.78
T5	278.57 ± 44.03a	282.41 ± 25.84a	280.49	415.63 ± 12.85a	356.44 ± 11.78a	386.03	1356.88 ± 48.22a	1270.95 ± 373.02a	1313.91
Mean	270.20	221.62		340.34	307.03		1170.69	1009.28	
Factors	35 DAS			45 DAS			60 DAS		
	CD	± SEm		CD	± SEm		CD	± SEm	
Factor A	33.72	11.26		27.02	9.02		132.21	44.15	
Factor B	53.32	17.81		42.73	14.27		209.04	69.81	
Factor A × B	NS	25.18		NS	20.18		NS	98.73	

The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$). NS; non-significant, CD; critical difference, ± SEm: standard error of mean.

3.10. Total Plant Dry Weight (g)

Figure 5 depicts the value of total leaf dry weight of the wheat crop, grown in the year 2017–2018. Maximum total plant dry weight at 35 DAS reported in T5 treatment (1.71 and 1.86 g) and minimum was introduced in T1 treatment (1.21 and 1.05 g) in the V1 and V2 varieties, respectively, whereas at 45 DAS highest leaf dry weight was observed in the T5 primed one (2.94 and 2.67 g) and lowest was reported in the T1 nonprimed set (1.47 and 1.95 g) in both V1 and V2 varieties, respectively. However, leaf fresh weight at 60 DAS was highest again in the T5 primed one (12.37 g and 10.57 g) and lowest was in T1 seed (8.32 and 6.17 g) in V1 and V2, respectively. Thus, the report suggested that T5 was the better performer in all stages, i.e., 35, 45, and 60 DAS, among all treatments and the nonprimed (control) was always inferior to all the primed sets.

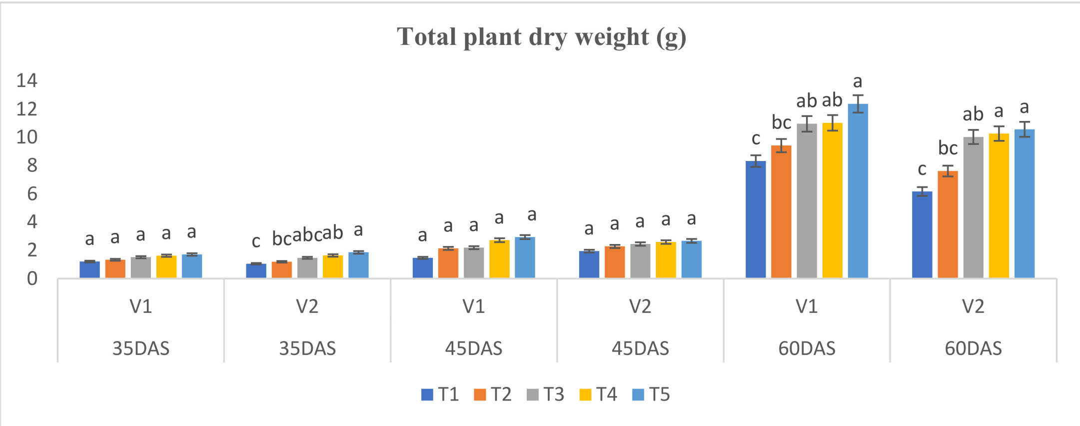

Figure 5. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed and nonprimed (control) seed treatments on total plant dry biomass (g) of wheat varieties, grown in the year 2017–2018. The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$).

The data were validated again by growing the crop in the year 2018–2019 (Figure S3). Although the data shows the difference from the abovementioned year, i.e., 2017–2018, the trend was found to be the same regarding total plant dry weight (g). The total plant dry weight was statistically significant at treatment and varietal level at 35 and 45 days, and also at 60 days after sowing in both experimental years, except at 35 DAS, when leaf dry weight was found statistically nonsignificant in varietal level in experimental year 2017–2018.

3.11. Chlorophyll Content

Figure 6 represents the value of chlorophyll content of both varieties of wheat plant, grown in the year 2017–2018. Maximum chlorophyll content was obtained at 35 DAS in T5 primed (39.73 and 40.48) and followed by T4 and T3, and minimum in T1 nonprimed seed (36.77 and 36.76) in V1 and V2 varieties, respectively. Chlorophyll content slightly increased in both varieties at 45 DAS; this time the maximum value was observed in T3 primed (43.34) seed in the V1 and in V2 varieties, T5 primed (48.10) gave maximum chlorophyll content. While at the stage of 60 DAS, T5 primed (45.90 and 47.68) seed presented higher chlorophyll content in both V1 and V2 varieties and the T1 primed one showed lower (41.63 and 42.41) chlorophyll content in both V1 and V2 varieties.

Therefore, it can be suggested that T5 primed has shown better performance, followed by T4 and T3 treatments in V1 and V2 varieties at all the studied periods except in V1 variety at 45 DAS, when the T3 primed one gave better performance, followed by T5 and T4 treatments. The data were validated again by growing the crop in the year 2018–2019 (Figure S4). Although the data show the difference from the abovementioned year, i.e., 2017–2018, the trend was found same regarding chlorophyll content.

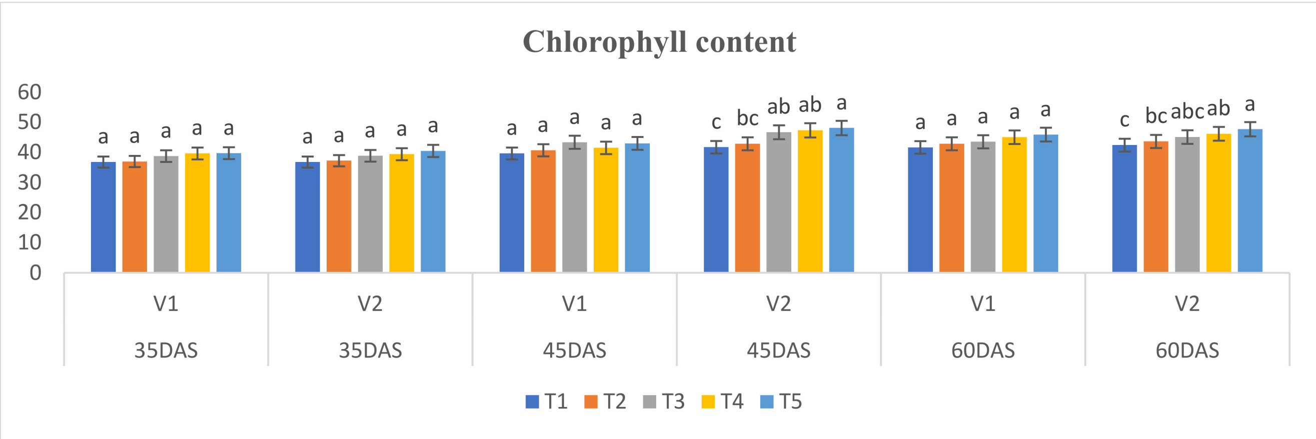

Figure 6. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed and nonprimed (control) seed treatments on chlorophyll content of wheat varieties, grown in the year 2017–2018. The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$).

3.12. Nitrate Reductase Activity of Leaves (n mol g^{-1} h^{-1} Fresh Weight)

Table 6 indicates the value of nitrate reductase activity of leaves, of both varieties of wheat, grown in the year 2017–2018. The values of nitrate reductase activity were measured at 35 DAS and found to be a maximum in T5 primed (339.53 and 412.99), followed by T4 and T3 primed, and the minimum value was observed in the T1 nonprimed (214.78 and 244.71) set in both of V1 and V2 varieties, respectively, while at 45 DAS the value of nitrate reductase was maximum in T5 primed (586.96 and 908.47) and minimum in the T1 nonprimed (338.02 and 447.49) control set in both V1 and V2 varieties, respectively. Again, at the age of 60 DAS, nitrate reductase value was observed to be the highest in the T5 treated one (1127.90 and 1236.10) and lowest was in the T1 nonprimed (792.22 and 927.68) control set in both of V1 and V2, respectively. The percentage of activity that has been calculated also showed the same status for nitrate reductase activity. Thus, the observed results suggest that the T5 primed one showed better performance, which was followed by T4 and T3 primed in V1 and V2 varieties in all the periods studied.

The data were validated again by growing the crop in the year 2018–2019 (Table S3). Although the data showed the difference from the abovementioned year, i.e., 2017–2018, the trend was found to be the same regarding nitrate reductase activity of leaves. The nitrate reductase activity was found statistically significant at treatment and varietal levels at 35, 45, and 60 DAS in both experimental years. Moreover, the interaction between V $\times$ T was found nonsignificant at all stages of study in both experimental years.

3.13. Protein Content in Leaves (mg g^{-1})

Table 7 shows the value of protein content of both varieties of wheat, grown in the year 2017–2018; the maximum amount was reported at 35 DAS in the T5 (146.35) primed one followed by the T4, T3 treated sets, and minimum in the T1 (131.02) nonprimed (control) set in the V1 variety. However, in the V2 variety, maximum protein content reported in the T4 (162.64) primed set was followed by T5, T3, and the minimum was obtained in the T1 (141.26) nonprimed (control) set, whereas at 45 DAS, highest protein content was observed in the T5 (172.70 and 179.88) primed set followed by T3 and T4 treatments and lowest was observed in the T1 (control) nonprimed (162.79 and 171.07) one in both varieties V1 and V2, respectively. Moreover, at the stage of 60 DAS, the higher protein content was reported in the T5 (177.12 and 184.33) primed one and lower content was reported in T1 (162.79 and 172.65) in both varieties V1 and V2, respectively.

Table 6. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed and nonprimed (control) seed treatments on nitrate reductase activity of leaves (n mol g^{-1} h^{-1} fresh weight) of wheat varieties, grown in the year 2017–2018.

Nitrate Reductase (n mol g^{-1} h^{-1} Fresh Weight)												
Treatments	**35 DAS**				**45 DAS**				**60 DAS**			
	V1	**V2**	**Mean**	**% Change**	**V1**	**V2**	**Mean**	**% Increase**	**V1**	**V2**	**Mean**	**% Increase**
T1	214.78 ± 32.79c	244.71 ± 36.48c	229.74	0.0	338.02 ± 42.08d	447.49 ± 33.07c	392.75	0.0	792.22 ± 18.35d	927.68 ± 93.58d	859.95	0.0
T2	241.09 ± 34.84bc	268.19 ± 27.95c	254.64	10.83	396.95 ± 15.63cd	521.85 ± 22.82bc	459.40	16.97	899.40 ± 100.94cd	988.58 ± 25.40cd	943.99	9.77
T3	272.71 ± 46.73abc	317.41 ± 62.38bc	295.06	28.43	442.86 ± 38.43bc	620.32 ± 66.28b	531.59	35.35	989.59 ± 22.63bc	1081.20 ± 49.33bc	1035.39	20.40
T4	301.72 ± 17.81ab	376.52 ± 12.72ab	339.12	47.61	517.48 ± 25.95ab	793.51 ± 133.21a	655.49	66.89	1060.03 ± 70.55ab	1168.83 ± 73.04ab	1114.43	29.59
T5	339.53 ± 34.10a	412.99 ± 13.19a	376.26	63.77	586.96 ± 44.90a	908.47 ± 42.71a	747.71	90.37	1127.90 ± 37.95d	1236.10 ± 64.79a	1182.00	37.44
Mean	273.97	323.96			456.45	658.33			973.83	1080.48		
Factors	**35 DAS**				**45 DAS**				**60 DAS**			
	CD		± SEm		CD		± SEm		CD		± SEm	
Factor A	34.14		11.40		54.46		18.19		59.65		19.92	
Factor B	53.98		18.03		86.11		28.76		94.31		31.49	
Factor A × B	NS		25.49		NS		40.67		NS		44.54	

The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$). NS; non-significant, CD; critical difference, ± SEm: standard error of mean.

Table 7. Effect of hydro, $Mg(NO_3)_2$, $ZnSO_4$, $Mg(NO_3)_2$ + $ZnSO_4$ primed and nonprimed (control) seed treatments on protein content (mg g^{-1}) of leaves of wheat varieties, grown in the year 2017–2018.

Protein Content (mg g^{-1})												
Treatments	**35 DAS**				**45 DAS**				**60 DAS**			
	V1	**V2**	**Mean**	**% Increase**	**V1**	**V2**	**Mean**	**% Increase**	**V1**	**V2**	**Mean**	**% Increase**
T1	131.02 ± 2.90a	141.26 ± 3.77a	136.14	0.0	162.79 ± 20.9d	171.07 ± 4.10a	166.93	0.0	162.79 ± 1.30b	172.65 ± 4.52a	167.72	0.0
T2	129.63 ± 3.33a	148.45 ± 5.00a	139.04	2.13	163.65 ± 2.46cd	173.84 ± 4.85a	168.74	1.08	164.32 ± 0.79b	174.02 ± 7.32a	169.17	0.86
T3	140.21 ± 6.90a	152.99 ± 11.84a	146.60	7.68	170.22 ± 159ab	175.33 ± 2.07a	172.78	3.50	172.02 ± 6.25a	178.57 ± 3.72a	175.29	4.51
T4	143.73 ± 9.36a	162.64 ± 2.67a	153.18	12.45	167.52 ± 0.82bc	177.86 ± 7.07a	172.69	3.45	176.77 ± 2.18a	181.62 ± 12.25a	179.19	6.84
T5	146.35 ± 8.89a	160.80 ± 6.86a	153.58	12.81	172.70 ± 2.78a	179.88 ± 7.70a	176.29	5.61	177.12 ± 2.51a	184.33 ± 1.06a	180.72	7.75
Mean	138.19	153.23			167.38	175.59			170.60	178.24		
Factors	**35 DAS**				**45 DAS**				**60 DAS**			
	CD		± SEm		CD		± SEm		CD		± SEm	
Factor A	5.71		1.91		3.82		1.28		5.25		1.75	
Factor B	9.03		3.02		6.04		2.02		8.31		2.77	
Factor A × B	N/A		4.26		NS		2.85		NS		3.92	

The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$). NS; non-significant, CD; critical difference, ± SEm: standard error of mean.

The percentage data of protein was also calculated and presented in the table to show the effect of priming treatments. Hence, it can be suggested that T5 priming combination predicted to be a better performer for V1 and V2 varieties at all the time periods in both experimental years, except in the V2 variety at 35 DAS in experimental year 2017–2018 when the T4 primed set gave better response. The data were validated again by growing the crop in the year 2018–2019 (Table S4). Although the data shows a difference from the abovementioned year, i.e., 2017–2018, the trend was found to be the same regarding protein content. The protein content was found statistically significant at treatment and varietal levels at 35, 45, and 60 days after sowing in both experimental years. Moreover, the interaction between V × T was found nonsignificant at all the periods of study in both experimental years.

3.14. Nitrogen (mg g^{-1} DW), Zinc, and Iron (ppm) Content of Grains

Table 8 shows the data of nitrogen, zinc, and iron content of wheat grains for the year 2017–2018. V2 showed higher values when compared to V1. Highest N content was measured in the case of combined priming treatment (7.74 and 8.56 mg g^{-1} DW) and the lowest in T1 (4.83 and 5.19 mg g^{-1} DW) treatment. Likewise, in the case of zinc, the highest value was measured in T5 treatment (24.3 and 35.57 ppm) and lowest in the T2 primed set (21.9 and 33.03 ppm). Similarly, in the case of iron, the highest values were recorded in T5 treatment (35.73 and 38.5 ppm) while the lowest were in T1 (33.5 and 38.27 ppm), respectively, in both the varieties. Statistical analysis showed significant differences in nitrogen content but not in case of Zn and iron content, although there are differences in the factor for variety.

Table 8. Effect of different priming treatments on nitrogen, zinc, and iron content.

	Nitrogen Content (mg g^{-1} Dry Weight)			Zinc Content (ppm)			Fe Content (ppm)		
Treatment	**V1**	**V2**	**Mean**	**V1**	**V2**	**Mean**	**V1**	**V2**	**Mean**
T1	4.83 ± 0.18c	5.19 ± 0.42d	**5.01**	24.47 ± 1.37a	33.53 ± 2.96a	**29**	33.5 ± 3.27a	38.27 ± 1.78a	**35.88**
T2	5.63 ± 0.53c	6.78 ± 0.60c	**6.21**	21.9 ± 2.55a	33.03 ± 0.90a	**27.47**	34.03 ± 3.87a	38.47 ± 1.98a	**36.25**
T3	6.38 ± 0.32b	7.23 ± 0.70b	**6.81**	22.87 ± 1.72a	34.47 ± 0.93a	**28.67**	35.23 ± 3.07a	36.8 ± 0.51a	**36.02**
T4	7.47 ± 0.34ab	8.20 ± 0.22a	**7.84**	21.8 ± 2.11a	31.63 ± 2.29a	**26.72**	33.37 ± 1.09a	35.53 ± 2.11a	**34.45**
T5	7.74 ± 0.72a	8.56 ± 0.33a	**8.15**	24.03 ± 2.21a	35.57 ± 2.61a	**29.8**	35.73 ± 2.92a	38.5 ± 1.97a	**37.12**
Mean	**6.41**	**7.19**		**23.01**	**33.65**		**34.37**	**37.51**	
Factors	CD	± SEm		CD	± SEm		CD	± SEm	
Factor A	0.62	0.21		1.81	0.60		2.32	0.77	
Factor B	0.98	0.33		N/S	0.95		NS	1.22	
Factor A × B	NS	0.46		NS	1.35		NS	1.72	

The full description of abbreviations T1, T2, T3, T4, T5, V1 and V2 are represented in material and methods section. Lowercase alphabetical letters (a,b,c) represents the significant differences between the different seed priming treatments (Tukey test; $p < 0.05$). NS; non-significant, CD; critical difference, ± SEm: standard error of mean.

4. Discussion

The main aim of the experiment was to determine and highlight the effects of seed priming treatment with $Mg(NO_3)_2$, $ZnSO_4$, and their combination on germination and growth behavior, and biochemical traits of wheat. Magnesium and zinc are essential plant nutrients required for cellular development, enzyme activation and various functions of plants. The deficiency of Mg and Zn may impair germination and seedling establishment, thus affecting plant growth and production. The study shows that wheat seed priming with $Mg(NO_3)_2$, $ZnSO_4$, and their combination has tremendous capability to improve the crop stand establishment, plant height, leaf expansion, fresh and dry weight of leaf and stem, chlorophyll content, NR activity, and protein content.

Seed priming treatment with $Mg(NO_3)_2$, $ZnSO_4$, and their combination were found to improve the germination-related traits such as germination percent (Table 1), shoot and root length (Figure 1) and seedling weight (Figure 2), and vigor index I and II (Tables 2 and 3), indicating that seed priming plays a positive role in membrane permeability, which leads to enzyme activation such as alpha amylase and metabolism-relative enzymes [11,20].

It was also observed that germination percentage improved in response to seed priming in mustard and maize crops with the use of $Mg(NO_3)_2$ and $ZnSO_4$, respectively [12–22]. However, for the first time it has been observed in these popular varieties of wheat that the combination of these two nutrients worked better after application in a combined form when using as a seed priming treatment (Table 1). Increment in shoot and root length (Figure 2) and seedling weight (Figure 3) indicates that priming accelerates cell elongation and division. Previously, it was observed that $Mg(NO_3)_2$ seed priming increases shoot and root length and fresh and dry weight of seedlings in rice crops [23] and in wheat crops [10,11]. Further, it was observed that seed priming of wheat with ZnSO4 and $ZnCl_2$ improved the rate of germination, final germination, and seedling size [14]. Likewise, improvement in vigor index in response to seed priming in rice and carrot crops with the use of $Mg(NO_3)_2$ and $ZnSO_4$, respectively, were also recorded [24,25]. Moreover, previous studies suggested that seed priming treatment enhanced the relative water content in seed, which hastened the metabolic activity and early emergence of seedlings [26]. Recent studies on seed priming treatments suggested that it enhanced the synthesis of defense metabolites in response to stress conditions, which hastened the seedling growth under adverse conditions [27]. Moreover, seed priming treatment enhanced the solublization of stored food material and transports more towards the growing seedling for its early support [28] Nevertheless, in the extant case, the combination of both the nutrients used in the form of seed priming treatment showed better results relative to other treatments.

Increment in plant height (Table 4 and Table S1), leaf number (Figure 3 and Figure S1), leaf area (Table 5 and Table S2), and fresh and dry weight of plant (Figures 4 and 5, Figures S2 and S3) shows that seed priming with magnesium and zinc salts benefit cell growth and metabolic activities of crops [29]. Previously, it was observed that seed priming with $Mg(NO_3)_2$ increased plant height in wheat [29], number of leaves in maize crops [30], leaf area in rice [7], fresh and dry weights of leaves in wheat, and fresh and dry weights of shoots [31]. In case of zinc, Kaya et al. [32] reported that zinc increases the plant height in tomato crops by increasing internode distances. Various researchers observed an improvement in the number of leaves in maize crops [33], leaf area in plantain crops [34], fresh and dry weight of leaves in wheat [35], and shoot fresh and dry weight [36], by $ZnSO_4$ priming treatment. Seed priming not only enhanced the growth attributes under normal conditions, but it showed the potential under multiple stress conditions [37,38]. Likewise, recently Singhal et al. [39] used the same priming combination in a wheat crop and found that combination of seed priming agents work synergistically and enhanced the growth attributes in wheat under stress condition. Further, they also suggested that the combined treatment enhanced the activity of antioxidant enzymes such as superoxide dimutase (SOD), catalase (CAT), polyphenol oxidase (POX), and peroxidase (POX), and reduced the malonaldehyde (MDA) and H_2O_2 production, which ultimately releases oxidative stress and enhances growth attributes. However, in the present case, the combination of both the nutrients, used in form of seed priming treatment, presented superior in all these traits, relative to other treatments.

Leaf chlorophyll content of both wheat cultivars increased with priming treatment, and higher chlorophyll content was observed in combination treatment (Figure 6 and Figure S4). Likewise, an improvement was found in the total chlorophyll content with use of seed priming in wheat crops regarding $Mg(NO_3)_2$ and $ZnSO_4$ [29,40]. Several other reports also affirm the involvement of Mg and zinc in synthesis of chlorophyll [41,42]. Nitrate reductase activity, protein content, and their percentages were noticed to improve significantly by seed priming treatment, and the maximum was observed in combination with $Mg(NO_3)_2$ and $ZnSO_4$ (Table 6, Table 7, Tables S3 and S4). Improvement in both traits indicates that Mg and Zn are involved in the activation of enzymatic activity and protein synthesis [43–45]. Similarly, it is noticed that seed priming of *Brassica* species with $Mg(NO_3)_2$ improves nitrate reductase (NR) activity and protein content. It was reported that application of $ZnSO_4$ improves protein content in rice [46]. As other researchers also stated, seed priming with $Mg(NO_3)_2$ and $ZnSO_4$ benefits protein content in various crops [47].

In the present analysis, it was found that the priming treatment enhanced the activity of N, Zn, and Fe; although there is significant enhancement in the case of N, it is nonsignificant in the case of Zn and Fe. The enhancement in N content is directly correlated with the chlorophyll content and nitrate reductase activity. Similar results were obtained in the case of Singhal et al. [39], where priming treatment enhanced N content synergistically. In the present case, although the combined salt showed higher Zn and Fe content, it was nonsignificant. It may be because micronutrients are required in very small quantities and a little enhancement, even 1 to 2 ppm, can make large differences. Earlier reports, however, suggest the role of $ZnSO_4$ and other micronutrients in antioxidant defense and in biofortification of crops [13,48]. Overall, seed enhancement and transcriptional regulatory mechanism behind the overall improvement of stand establishment and vigor attributes is also important for sustainability perspective [49–54]. In summary, combining priming salt is effective if the salts act synergistically and enhance growth attributes positively.

5. Conclusions

Our result suggests that priming with a combination of both the nutrients, used in the form of seed priming treatment, exhibited better results relative to other treatments; it indicates that both salts have a synergetic effect when apply as seed priming agents, improving the nitrogen-utilizing capacity of wheat crops. Accordingly, the result suggests that priming of seeds with $Mg(NO_3)_2$ and $ZnSO_4$ worked synergistically at varietal level and improved nitrogen metabolism. The combination of both salts enhanced the growth attributes in the wheat crop and combination of these salt concentrations can be used for wheat-growing areas. More depth molecular understanding, however, is needed to understand the effect at gene levels and how this approach can be suitable for biofortification of crops.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112110/s1, Figure S1: Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on Leaf number per hill of wheat varieties, grown in the year 2018–2019, Figure S2 Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on total plant fresh biomass (g) of wheat varieties, grown in the year 2018–2019. Figure S3 Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on total plant dry biomass (g) of wheat varieties, grown in the year 2018–2019.Figure S4 Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on Chlorophyll content of wheat varieties, grown in the year 2018–2019. Table S1 Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on plant height (cm) of wheat varieties, grown in the year 2018–2019. Table S2 Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on Leaf Area (cm2) of wheat varieties, grown in the year 2018–2019. Table S3 Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on Nitrate reductase activity of leaves (n mol g-1 h-1 fresh weight) of wheat varieties, grown in the year 2018–2019. Table S4 Effect of hydro, Mg(NO3)2, ZnSO4, Mg(NO3)2 + ZnSO4 primed and non-primed (control) seed treatments on Protein content (mg g-1) of leaves of wheat varieties, grown in the year 2018–2019.

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