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Chemical Priming with β-aminobutyric acid (BABA) for Seedling Vigor in Wheat (*Triticum aestivum* L.)

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ABSTRACT: To achieve efficiency in seedling development, the seed must germinate quickly and homogeneously. Pre-sowing applications such as priming are practiced to eliminate or reduce the negative effects of environmental factors through germination and seedling development. This allows to reach a higher germination rate, yield, and desired plant density, especially by protecting against biotic and abiotic stresses. This study was carried out to examine the effects of pre-sowing seed applications on seedling growth and vigor of bread wheat (*Triticum aestivum L.*). Three different doses of β-amino-n-butyric acid (BABA) were used as seed priming agents and Dimethyl sulfoxide (DMSO) as control groups. The study was carried out according to the completely randomized factorial design with three replications and ten plants per replication. As a result of priming applications, significantly higher values in the number of roots, longest root length, total root length, average root length and shoot fresh weight were obtained compared to control. Our results, for the first time, showed that seed priming with BABA had a promoting effect on many aspects of early seedling growth in bread wheat. This is a preliminary study to understand the mechanism of seed priming and its effects on germination and root growth in wheat. Further studies may shed light on the molecular mechanisms of BABA or other compounds as seed priming agents, benefits for abiotic and biotic stress tolerance, and good stand establishment at the seedling or later stages.

Keywords: β-amino-n-butyric acid, root growth, seed priming

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INTRODUCTION

Bread wheat is one of the most produced and consumed food sources from past to present (Hawkesford et al., 2013). With the global production of more than 760 million tons (FAOSTAT, 2019), wheat is one of three main staple crops with maize and rice as carbohydrate and protein sources for the global population, which is estimated to reach 9 billion by 2050. Therefore, wheat yield should be increased continuously to meet the nutritional and calorie needs (Ray et al., 2013).

New varieties that are adapted to environmental fluctuations, have high yield and quality, and show rapid development, are aimed to be developed for continuously increased consumer demand (Godfray et al., 2010). Increased production also depends on crop stand establishment and homogeneous growth. One of the pre-requisites for homogeneity is healthy germination and seedling vigor. Once seeds are sowed, water absorption from the soil takes a significant time. In this period, seeds may face some abiotic and/or biotic stresses such as high temperature, light intensity, salinity, oxygen, water, soil quality, heavy metal toxicity, and pathogens. All of these stresses can have a great effect on seed germination and stand establishment (Gökçöl et al., 2018).

The application called “seed priming, seed applications, or pre-application” is an old, simple but effective method to improve germination ratio and speed, stand establishment, yield, and resistance to biotic and abiotic stress conditions. This application is based on the progress of germination phases; (I) imbibition, (II) transition, (III) growth, and ends at phase II before the radicle protrudes from the seed coat (Bose et al., 2018). Initially, priming allows the seed to absorb water rapidly in phase I, and that results in the resume of some activities such as respiration and protein synthesis. The following phase, transition, is associated with a slow increase in seed water content and physiological activities related to germination. Various cellular and biochemical events such as the translation of new RNA, and changes in soluble sugars contents, begin in phase II (Bose et al., 2018). Since seed priming allows controlled water uptake to the seed and prevents germination and radicle emergence, it is called a pre-sowing application (Heydecker et al., 1978; Karakurt et al., 2010). Hydropriming, chemical priming, osmopriming, hormone priming, halopriming, and nutrient priming are some of the seed priming techniques that have been used (Ashraf et al., 2018).

Priming applications have various physiological effects on the seed and different results may occur at different moisture and application levels (Taylor, 1997; Elkoca, 2007). Priming applications contribute to the optimum use of storage assimilates, enzyme activities involved in germination and help seeds to quickly adapt/respond to unfavorable environmental conditions (Demir et al., 1994; Elkoca, 2007). Therefore, it has been used successfully in agriculture to accelerate the germination process (Nouman et al., 2012; Aghbolaghi et al., 2014; Bagheri, 2014; Lara et al., 2014; Bose et al., 2018).

Germination and seedling emergence are strongly linked to plant genetics and the development of roots. The seeds of some plant species show different developmental curves in response to different environmental factors. Genotypes with a rapid rooting potential become advantageous to overcome stress conditions from seedling through anthesis (Kaya, 2008). Therefore, it is important to understand the detailed rooting characteristics of priming applied seeds for further stress research such as drought, salt, heat, etc. Understanding seedling growth characteristics of primed seeds can provide novel information to researchers to build up a hypothesis in response to environmental challenges as well as deep down to the molecular mechanism of priming in plants.

Seed priming agents such as nutrients, hormones, chemicals, and microorganisms are reported to increase plant growth, productivity, and stress tolerance (Banerjee and Roychoudhury, 2018; Lal et al., 2018). Moreover, various organic or synthetic compounds such as; sodium, potassium, magnesium, β-aminobutyric acid (BABA), high molecular weight polyethylene glycol (PEG) 6000 or PEG 8000, inorganic salts, glycerol, or sucrose were used for seed priming applications (Karakurt et al., 2010).

β-aminobutyric acid is a non-protein amino acid and one of the potent plant defense inducers. Previous studies reported that the application of BABA in Arabidopsis gives a fast and strong response to stress (Zimmerli et al., 2008; Jisha et al., 2016). Also, other studies showed that foliar or root drenching application of BABA induces resistance against various pathogens, insects, and nematodes as well as resistance to abiotic stresses on various plant species. Some research also demonstrated that BABA can be used as a seed-priming agent and it can improve plant development as well as response to biotic and abiotic stress conditions (Cohen et al., 2016; Dawood, 2018). Exogenous application of BABA to wheat seedlings induced resistance against *S. avenae* (Cao et al., 2014) and nematodes (Oka and Cohen, 2001; Cohen et al., 2016). Moreover, 100 uM BABA was applied to soil to test its effect under soil drying conditions on two spring wheat cultivars. BABA application increased drought-induced abscisic acid accumulation and water use efficiency on mild soil drying, and at the severe stress level, BABA increased ROS production and antioxidant defense enzyme production (Du et al., 2012). BABA was used as a seed priming agent to understand its effects on physiological and proteomic changes under salt-stressed conditions in two barley lines. According to results, BABA application increased relative water content and exhibited different proteomic patterns including root proteins compared to control. Upregulation of antioxidant enzymes, PR proteins, and chaperons demonstrated the induction and effect of BABA on salt stress tolerance (Mostek et al., 2016). Yin et al. (2021) combined Chitooligosaccharide with BABA and showed that seed priming with this compound induces drought tolerance activity on wheat and regulates changes in drought tolerance-related metabolites. We could not find any other related research that tested the activity of BABA as a seed priming agent on wheat. And since BABA is a promising agent for seed priming and abiotic stress responses, especially for drought tolerance, our study aimed; 1) to examine the effects of BABA as a seed priming agent on wheat seedlings, 2) to track root growth parameters deeply with image analysis, and 3) to reveal the effects on root and shoot biomass allocation, and seedling vigor in bread wheat (*Triticum aestivum* L.). This study provides a detailed dissection of seedling root development on bread wheat under seed-priming conditions and provides preliminary data for further in-depth molecular studies.

MATERIALS AND METHODS

Materials

In this study, bread wheat (*Triticum aestivum* L.) cultivar Bezostaja 1 was used as the plant material. To determine the effects of priming applications on the seedling development of bread wheat, BABA (kindly provided by Dr. Ahmet Akköprü Van Yüzüncü yıl University, Department of Plant Protection) and Dimethyl sulfoxide (DMSO; Sigma Aldrich) was used as the solvent. Seeds were surface sterilized with 5% NaOCl for 5 minutes and with 70% ethanol for 1 minute. Sterilized seeds were washed five times with sterile water. Three different doses of BABA (11 mM, 18 mM, and 25 mM) and DMSO (0.2%) as the control group were applied to the seeds in test tubes for 24 hours. After 24 hours of priming agent or control applications, seeds were washed under tap water and dried gently. Then primed seeds were put into Petri dishes for germination.

Experimental design

The modified cigar roll method was used for the evaluation of seedling traits (Zhu et al., 2006; Acikbas et al., 2021). Germinated seeds were transplanted into germination papers (60 x 40 cm) as ten seeds per germination paper. Another layer of germination paper of the same size was used to cover the seeds. Each set was rolled and transferred into beakers filled with sterile water. Seedlings were grown for 15 days under laboratory conditions (25-27°C average temperature, and 14 hours/10 hours (light/dark)) in Siirt University, Faculty of Agriculture. At the end of the fifteenth day, each seedling was scanned with a handheld scanner (Iscan portable mini scanner) at 300 DPI image quality. Image analysis was performed in a PC environment using ImageJ image processing software (Rueden et al., 2017). For seedling root characteristics; the number of roots (NOR), the longest root length (LonRL), total root length (TRL), average root length (aRL), number of lateral roots (NOLatR), plant height (PH), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) were examined. Ratios of SFW/RFW and SDW/RDW were calculated to observe biomass allocation in primed seeds.

Statistical analysis

Statistix 10 software (Analytical Software, Tallahassee, FL) was used for statistical analysis. The results were analyzed using analysis of variance (ANOVA) and multiple comparison was done with the Least Significant Difference (LSD) test. The correlation was analyzed by simple correlation (Pearson) analysis (Steel et al., 1997). The study was carried out according to the completely randomized factorial design with three replications and ten plants per replication.

RESULTS AND DISCUSSION

To achieve earliness and good stand establishment, seeds of a given genotype must germinate in a short time and be as homogeneous as possible. Soil structure and abiotic stresses cause heterogeneity in germination. Climate change also has a significant impact on germination and seedling development in crops. Seed priming aims to shorten the time between sowing and emergence to eliminate the problems that occur during sowing and seedling emergence such as low or high temperature, salinity, drought, and pathogens (Özkaynak et al., 2020). This eventually contributes to a better germination rate, yield, and improves tolerance against biotic and abiotic stresses.

The effect of three different doses of BABA on wheat seedling growth and root development was evaluated in this study. Wheat seeds were immersed into 11-, 18-, and 25-mM BABA or 0.2% DMSO solutions for 24 hours and germinated in Petri dishes immediately. According to results, the effects of priming with BABA on root development were found statistically significant ($p<0.05$) (Figure 1), and different doses had different effects on growth parameters. As a result of BABA applications, significantly higher values were observed in NOR, LonRL, aRL, PH, SFW, SDW, RDW, SFW/RFW, and SDW/RDW parameters compared to control. On the other hand, the effects of priming on NOLatR and RFW were contradictory compared to control (Table 1).

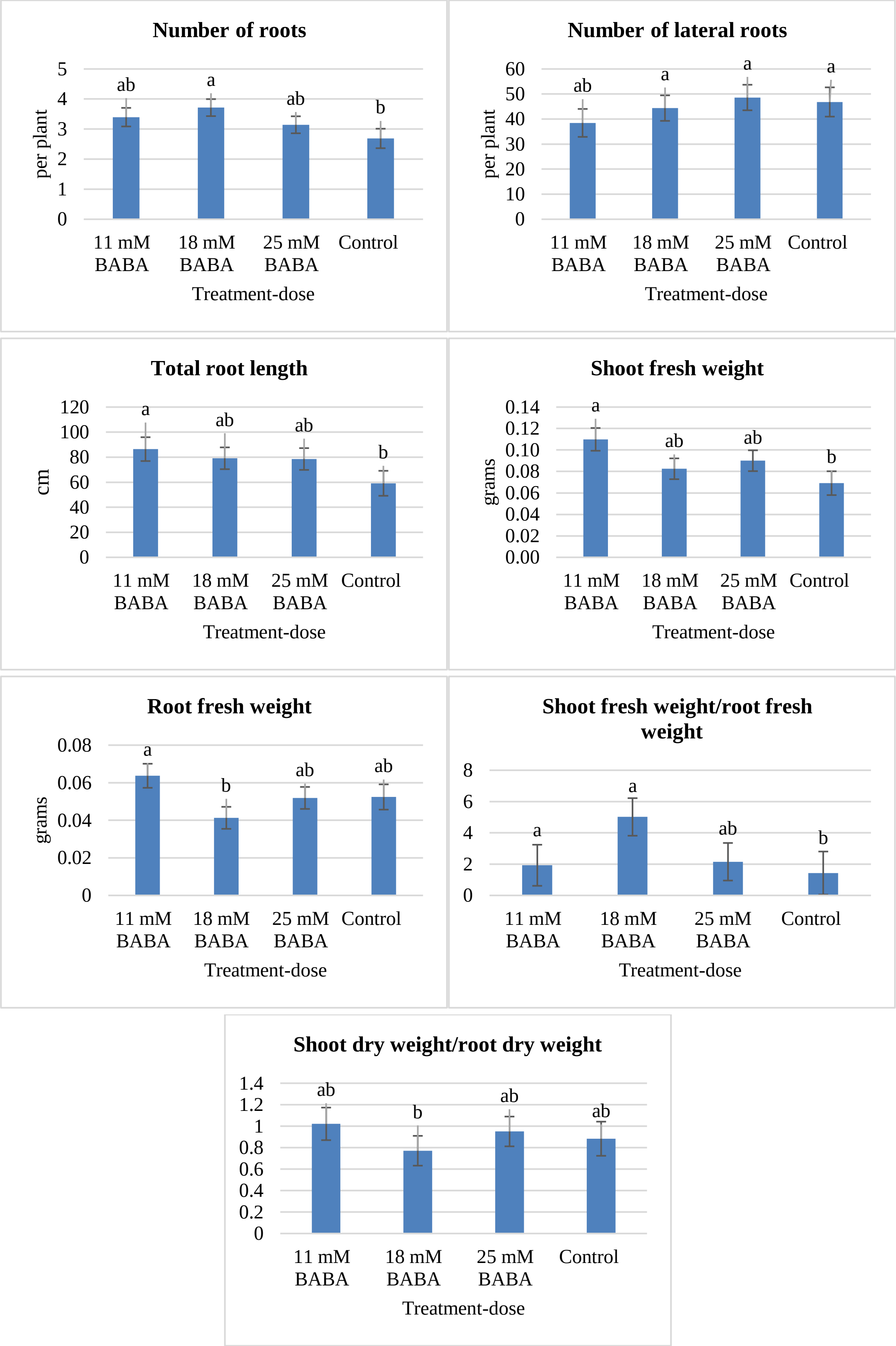

Figure 1. The effects of priming applications (11 mM, 18 mM, and 25 mM BABA) and control on the number of roots (NOR), number of lateral roots (NOLatR), total root length (TRL), shoot fresh weight (SFW), root fresh weight (RFW), SFW/RFW ratio, and shoot dry weight (SDW)/root dry weight (RDW) ratio. The difference between the means in the same group with the same letter is not statistically significant at $p < 0.05$.

As a result of the priming with 11-, 18-, or 25-mM BABA, it was seen that 18 mM BABA caused the highest increase in NOR (3.71 roots plant^{-1}) by 37.92% compared to the control (2.69 roots plant^{-1}). In terms of the LonRL, 25 mM BABA showed an increase of 22.15% compared to the control. Considering the TRL, all priming applications showed an increase compared to control. 11 mM BABA developed 46.16% longer roots compared to control. There was a 22.54% increase in the aRL in 25 mM BABA compared to control. When the effects of priming applications on plant height were examined, it was seen that 25 mM BABA provided 16.68% longer plants compared to control (Table 1).

Table 1: Means and percent differences of each trait compared to control

Treatment	**NOR plant^{-1*}**	**%**	**NOLatR plant^{-1*}**	**%**	**LonRL (cm)**	**%**	**TRL (cm)***	**%**	**aRL (cm)**	**%**	**PH (cm)**	**%**
11mM BABA	3.40	26.39	38.46	-17.86	28.26	19.89	86.41	46.16	24.06	18.93	12.05	3.08
18 mM BABA	3.71	37.92	44.38	-5.21	27.77	17.82	79.10	33.79	20.99	3.76	11.82	1.11
25 mM BABA	3.14	16.73	48.61	3.82	28.79	22,15	78.51	32.80	24.79	22.54	13.64	16.68
Control	2.69	0.00	46.82	0.00	23.57	0,00	59.12	0.00	20.23	0.00	11.69	0.00

Treatment	**SFW (g)***	**%**	**RFW (g)***	**%**	**SDW (g)**	**%**	**RDW (g)**	**%**	**SFW/RFW**	**%**	**SDW/ RDW***	**%**
11mM BABA	0.1099	59.04	0.0637	21.56	0.0119	30.77	0.0126	10.53	1.9268	34.93	1.0215	15.69
18mM BABA	0.0825	19.39	0.0413	-21,18	0.0094	3.30	0.0126	10.53	5.0154	251.22	0.7711	-12.67
25 mM BABA	0.09	30.25	0.0519	-0.95	0.0105	15.38	0.012	5.26	2.1522	50.71	0.9508	7.68
Control	0.0691	0.00	0.0524	0.00	0.0091	0.00	0.0114	0.00	1.428	0.00	0.883	0.00

NOR: Number of roots; LonRL: Longest root length; TRL: Total root length; aRL: Average root length; PH: Plant height; PH/RL: Plant height/root length ratio; SFW: Shoot fresh weight; RFW: Root fresh weight; SDW: Shoot dry weight; RDW: Root dry weight; SFW/RFW: Shoot fresh weight/root fresh weight ratio; SDW/RDW: Shoot dry weight/root dry weight ratio. %: difference compared to control. *: significant at $p<0.05$

When similar priming practices were examined in the literature, Jisha et al. (2016) investigated the effect of seed priming with BABA on the growth of rice seedlings grown under stress-free and stressed (NaCl/PEG-6000) conditions. Under stressed conditions, primed rice seedlings had better stress tolerance compared to control. Priming with BABA provided a significant increase in seedling growth and development compared to the control group under both stressed and normal growth conditions. Our observations with BABA priming were similar to Jisha et al. (2016), except, we did not apply stress. Martínez-Aguilar et al. (2021), on the other hand, tested the effect of treatment with 2,6 dichloro-isonicotinic acid (INA) on resistance against *Pseudomonas syringae* pv in bean (*Phaseolus vulgaris* L.). They reported that the treatment of seeds with INA contributed to its growth and was effective in defense and had an intergenerational effect. Li et al. (2019) used a chitosan application to investigate the effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). They found that chitosan nanoparticles had a growth-promoting effect at 5 µg/mL application dose. They found that the effects of chitosan nanoparticles on wheat germination and seedling growth were positive. These results highlight the promising role of seed priming agents in agricultural practices. Several priming agents, such as salicylic acid (SA) have some commercial use, while most of the rest are still at the experimental stage. Priming has the potential to become a common pre-sowing practice in agriculture.

When we compared priming doses for above and below-ground biomass allocation, 11 mM BABA reached the highest shoot biomass compared to the control with an increase of 59.04% in SFW. The SFW values ranged between 0.0691 g (control) and 0.1099 g (11 mM BABA) applications. (Table 1). On the other hand, when RFW were compared, only 11 mM BABA showed a 21.56% increase compared to control, and the other two doses had lower RFW values compared to control. The lowest values for RFW were observed in the 18 mM BABA application. Shoot dry weight followed the SFW and 11 mM

BABA increased SDW by 30.77% compared to control. At RDW, 11- and 18-mM BABA formed 10.53% more biomass than the control. Goswami et al. (2013) examined the effect of BABA treatment of rice seeds to withstand stress conditions. They reported a significant increase in seedling growth and development at three different concentrations of BABA. In a similar study, Kulak et al. (2021), investigated the seed priming application with SA on plant growth and essential oil composition in basil (*Ocimum basilicum* L.) grown under drought-stressed conditions. Seeds were treated with 0.05- and 0.1-mM SA concentrations. They reported increased shoot length, diameter, and dry weight, the number of branches and leaves in plants treated with SA. Jelali et al. (2021) studied the growth, root acidification, and photosynthetic performance of *Sulla carnosa* plants when treated with SA and hydrogen peroxide. Under non-primed conditions, plant growth and chlorophyll concentrations decreased due to Fe deficiency. They reported that an advantageous effect on the growth of the plant was observed in SA + H_2O_2 application under Fe deficiency. Anosheh et al. (2011) examined the effect of chemical priming on seed viability of hybrid maize (*Zea mays* L.) with Urea and KNO_3 under abiotic stress conditions. They observed that the growth and development of maize plants under stressful conditions were enhanced by priming. Even though mostly preliminary, the above reports and our results clearly show the positive effects of seed priming on plant growth and stress tolerance. There is a need for follow-up studies, under various soil and environmental conditions.

To evaluate the relationships between the traits evaluated, a simple correlation analysis was performed (Table 2). Significant positive correlations were obtained between NOlatR and LonRL (0.35), TRL (0.45), and aRL (0.41). Similarly, a strong positive correlation was observed between LonRL and TRL (0.70) and aRL (0.80). On the other hand, the PH/RL ratio was in a negative correlation with NOR (-0.33), LonRL (-0.84), TRL (-0.46), and aRL (-0.57). The NOR was positively correlated with LonRL (0.42) and TRL (0.70). The results suggest a close association between root system traits. Root systems tend to behave as a system not trait by trait. Therefore, these correlations are expected outcomes of such studies (Bektas et al., 2016).

Table 2: Correlation (Pearson) coefficients between length, and biomass traits

	NOR ($plant^{-1}$)	**NOlatR ($plant^{-1}$)**	**LonRL (cm)**	**TRL (cm)**	**aRL (cm)**	**PH (cm)**
NOlatR	0.19					
LonRL	**0.42**	**0.35**				
TRL	**0.70**	**0.45**	**0.70**			
aRL	0.11	**0.41**	**0.80**	**0.66**		
PH	0.02	0.02	-0.05	-0.03	0.01	
PH/RL	**-0.33**	-0.15	**-0.84**	**-0.46**	**-0.57**	**0.31**
	SFW (g)	**RFW (g)**	**SDW (g)**	**RDW (g)**	**SFW/RFW**	
RFW	**0.59**					
SDW	0.07	0.11				
RDW	0.07	0.14	0.23			
SFW/RFW	-0.13	**-0.36**	-0.06	0.04		
SDW/RDW	0.07	0.02	**0.64**	**-0.51**	-0.07	

NOR: Number of roots; LonRL: Longest root length; TRL: Total root length; aRL: Average root length; PH: Plant height; PH/RL: Plant height/root length ratio; SFW: Shoot fresh weight; RFW: Root fresh weight; SDW: Shoot dry weight; RDW: Root dry weight; SFW/RFW: Shoot fresh weight/root fresh weight ratio; SDW/RDW: Shoot dry weight/root dry weight ratio. Significant at $p<0.05$

While seed priming has a promising aspect for improving seedling emergence, germination uniformity, and resistance to different stress conditions, its detailed molecular mechanisms and/or gene expressions are still not fully understood. Some research provides a clue about its mechanism though. A summary of current findings related to the molecular mechanism of seed priming is shown in Table 3.

Accordingly, the protein analysis and transcriptome profiling were mostly tested with Osmopriming activity with PEG on some plants such as Arabidopsis, Brassica, rice, and wheat. The results suggest that seed priming affects the molecular downstream of the plant and induces development and stress-related mechanisms. Drought stress is one of the most important abiotic stress that plants face. Root characteristics and architecture are one of the most important factors that plants can use to improve drought resistance. Therefore understanding the role of seed priming on root development gave us preliminary results to shed light on its downstream mechanisms and relation to the drought stress response.

Table 3: Molecular finding of seed priming research

Plant material	Application	Molecular findings	Conclusion	Reference
Arabidopsis thaliana	Hydropriming with water Osmopriming with PEG 6000 solution	74 proteins were identified on different phases of seed germination and priming such as cytosolic glyceraldehyde 3-phosphate dehydrogenase protein	For the seed priming and germination process, some protein might be used to characterize priming treatments and seed vigor.	(Gallardo et al., 2001)
Brassica oleracea	Osmopriming with PEG 6000 solution	By priming application; many genes as well as ribosomal proteins, heat shock proteins, etc. were up/down-regulated.	Many genes differentially expressed in different phases of germination, osmopriming, and different drying regimes	(Soeda et al., 2005)
Triticum aestivum	Seeds treated with H_2O_2 under salinity stress	Two heat-stable (stress) proteins with 32 and 52 kDa molecular masses were expressed	H_2O_2 treated induced expression of stress proteins and improve seedling growth under salt stress.	(Wahid et al., 2007)
Oryza sativa	seed priming with polyethylene glycol under nano-ZnO stress	*APXa*, *APXb*, *CATa*, *CATb*, *CATc*, *SOD1*, *SOD2*, and *SOD3* genes were down-regulated	Seed priming with PEG reduced the toxic effects of stress, it also enhanced the cell structure	(Salah et al., 2015)
Brassica napus	Osmopriming with PEG 6000 solution	952 genes and 75 proteins were identified with roles in the regulation of metabolism, cell cycle, protein synthesis, and storage proteins, etc.	All phases of osmopriming rely on complex biochemical processes and express different genes and proteins.	(Kubala et al., 2015)
Oryza sativa	Selenium priming salicylic acid priming on submergence stress	2371 transcripts for Se and 2405 for SA were identified with roles in cellular and metabolic activities such as carbohydrate and nitrogen compound metabolism	Seed priming may induce submergence tolerance.	(Hussain et al., 2016)
Brassica napus	Osmopriming with PEG 6000 solution Salt Priming	One miRNA family (miR172) was upregulated and six microRNA families (miR156, miR169, miR860, miR399, miR171, and miR395) were significantly downregulated.	miRNA plays important role in seed germination and regulation of hormone synthesis under stress conditions.	(Jian et al., 2016)
Oryza sativa	Seeds treated with spermine/spermidine under salinity stress	Spermine/spermidine priming up-regulated stress-response genes and membrane Na+ efflux pumps.	Seed priming can improve plant on salt stress conditions.	(Paul and Roychoudhury , 2017)
Triticum aestivum	Priming with β-aminobutyric acid-modified chitooligosaccharide derivative	Traumatic acid and indol-3-lactic acid pathways were activated. Also photosynthesis as well as stabilization of cell membrane induced and demonstrated promising results for drought stress.	These inducers may be used for an effective drought resistance induction.	(Yin et al., 2021)

CONCLUSION

In this study, we evaluated plant seedling development and vigor, using three different concentrations (11, 18, and 25 mM) of BABA. Significantly higher values in NOR (16.73-37.92%), LonRL (17.82-22.15%), TRL (32.80-46.16%), aRL (3.76-22.54%), and in SFW (19.39-59.04%) were obtained compared to control. Although BABA has been studied very little in priming and seedling root-shoot development studies, it may have an important place in the future. According to significant increases obtained in seedling growth with priming application, BABA may have a commercially important place and high seedling growth efficiency can be achieved with a small amount of chemical application.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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