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Araştırma Makalesi - Research Article

Determination of Optimum PGPB-Priming Protocol on Germination and Seedling Growth in Lentil

Mercimekte Çimlenme ve Fide Gelişimi Üzerine Optimum PGPB-Priming Protokolünün Belirlenmesi

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ABSTRACT

Plant growth promoting bacterias (PGPBs) can be described as bacterial strains increasing water and nutrient uptake, gaining nitrogen and phosphorus to plants by biological nitrogen fixation and phosphate mineralization and promoting plant growth. Besides, PGPB increase stress tolerance due to mechanisms as secretion of various phytohormones, vitamins and growth regulators, restriction of ethylene synthesis with ACC deaminase activity, decreasing of pathogen damage by the secret of antibiotic and fungicidal compounds. This study was carried out in laboratory of Field Crops Department in Siirt University under controlled conditions. The two original PGPB strains (KF3B and KF63C) and five different priming times (control, 1, 2, 4 and 6 hours) were applied on the Fırat-87 lentil variety. The study was laid out in a completely randomized design with three replications. It was aimed with this study that investigating effects based on bacterial biodiversity and priming time on germination characteristics and seedling growth in lentils. According to results, biodiversity-induced differences were observed in germination percentage, seedling fresh weight, seedling dry weight, seedling length and seedling vigor index while priming time significantly affected all investigated parameters except for germination percentage. However, the interaction of strains and priming times did not lead to any significant differences on traits except for seedling dry weight. In conclusion, microbial diversity and priming time have a critical role on successful of the priming technique. Optimum priming time for lentils was determined as four hours. Besides, the strain of KF63C had a noteworthy stimulative effect on especially seedling growth in the experiment.

Keywords- ACC Deaminase, Bacterial Inoculation, Microbial Diversity, Lens Culinaris, Seed Priming

ÖZET

Bitki gelişimini teşvik eden bakteriler (PGPB), su ve besin alımını artıran, biyolojik azot fiksasyonu ve fosfat mineralizasyonu ile bitkilere azot ve fosfor kazandıran, bitki büyümesini teşvik eden bakteri ırkları olarak tanımlanabilir. Ek olarak, PGPB çeşitli fitohormonlar, vitaminler ve büyüme düzenleyici salgılar gibi mekanizmalar sayesinde stres faktörlerine karşı toleransın artırılmasını, ACC deaminaz aktivitesi ile etilen sentezinin kısıtlanmasını, antibiyotik ve fungisidal bileşiklerin sentezi ile patojen hasarının azaltılmasını sağlar. Bu çalışma Siirt Üniversitesi Tarla Bitkileri Bölümü Laboratuvarında kontrollü koşullarda yürütülmüştür. Fırat-

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87 mercimek çeşidine iki orijinal PGPB ırkı (KF3B ve KF63C) ve beş farklı priming süresi (kontrol, 1, 2, 4 ve 6 saat) uygulanmıştır. Araştırma tesadüf parsellerinde faktöriyel deneme desenine göre üç tekerrürlü olarak planlanmıştır. Bu çalışma ile mercimeklerde bakteri biyo-çeşitliliğin ve priming süresinin çimlenme özellikleri ve fide gelişimi üzerine etkilerinin araştırılması amaçlanmıştır. Sonuçlara göre, çimlenme yüzdesi, fide yaş ağırlığı, fide kuru ağırlığı, fide uzunluğu ve fide canlılık indeksinde biyo-çeşitlilik kaynaklı farklılıklar gözlenirken, priming süresi çimlenme yüzdesi dışında incelenen tüm parametreleri önemli ölçüde etkilemiştir. Bununla birlikte, bakteri ırkları ve priming sürelerinin interaksiyonu fide kuru ağırlığı dışında özellikler üzerinde önemli bir farklılığa yol açmamıştır. Sonuç olarak, priming tekniğinin başarılı olmasında mikrobiyal çeşitlilik ve priming süresi kritik bir role sahiptir. Mercimek için en uygun priming süresi dört saat olarak belirlenmiştir. Ayrıca KF63C ırkı denemede özellikle fide büyümesi üzerinde kayda değer bir uyarıcı etkide bulunmuştur.

Anahtar Kelimeler- ACC Deaminaz, Bakteri İnokulasyonu, Microbiyal Çeşitlilik, Lens Culinaris, Tohum Priming

I. INTRODUCTION

Lentil is one of the most consumed foods in the world. Turkey is a major lentil producer country with 430,000 tons of production on 292,000 ha area annually [1]. However, lentil cultivation is generally designed as a dry farming system, therefore, high yield losses occur many times due to drought stress. Although many different methods have been tried to mitigate environmental stress, not only for drought and the other stress factors like salinity, extreme temperatures, nutrient deficiency, etc., seed priming and plant growth promoting bacteria (PGPB) applications have started up due to their sustainable, eco-friendly and cost-effective properties for last decades [2,3].

Indeed, the seed priming technique is based on the exposure of seeds to lower external water potential [5]. Seed priming, which is based on soaking seeds to water or a low osmotic potential of solution pre-sowing, has commonly been used to improve both seed germination and seedling growth, and also protect the plants against environmental stress and pathogens [4]. Several researchers stated that seed priming applications provide faster and more homogeneous germination and improve seedling growth by controlled water up take, activating starch disruption and enzyme actions, ATP synthesis and antioxidant defence systems, thereby, causes increase a stress tolerance to negative environmental conditions [6-8]. Common seed priming techniques are hydro-priming, osmo-priming, halo-priming, nano-priming, thermos-priming, solid matrix priming and bio-priming [9]. Out of them, bio-priming with PGPB strains, which have superior properties like nitrogen fixation, phosphate solubilizing, siderophore and IAA production, ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity, has a special position due to its regulative impacts on biotic and abiotic stress [10]. ACC deaminase activity has a critical role for plant sunder stress conditions due to its inhibitory impact on the ethylene hormone. Mechanism of ACC deaminase activity bases on hydrolysis of ACC which is the immediate precursor of ethylene hormone, thereby, reduction of ethylene level [11]. Therefore, PGPB strains can improve stress tolerance in plants exhibiting ACC deaminate activity. Moreover, PGPR shaving ACC deaminase activity promote root elongation and growth by reducing root ethylene level [12]. In the bio-priming technique, the secretions produced by the seed create a valuable food and energy source for the bio-control agents, allowing beneficial microorganisms to multiply and colonize the seed surface. This situation also positively affects the water and nutrient uptake of the seed [13]. The superior feature of the bio-priming technique compared to other priming applications is to has a more intense effect on the germination rate and uniformity and supports the suppression of soil and seed-borne pathogens [2]. Bacteria commonly used in bio-priming technique are species belonging to *Trichoderma*, *Enterobacter*, *Pseudomonas* and *Bacillus* genera [14,15]. Bio-priming with PGPB also includes biological fertilizer (nitrogen binder, phosphate and potassium solvent), bio-agent (suppression of disease factors) and plant growth [16-18]. Many researchers have denoted that PGPB-priming applications have positive results on germination, seedling growth and nutrient uptake in eggplant [19], chickpea [7], maize [20, 21], soybean [22] and tomato [23].

The effectiveness of seed priming applications depends on many external factors such as priming technique, material, seed species, ambient temperature, the concentration of priming solution, aeration, osmotic potential of the solution, age of seed, light and priming duration [24-27]. So, this study aimed to determine the most suitable protocol for bio priming with PGPB on lentils.

II. MATERIAL AND METHODS

The Fırat-87, which is the most grown red lentil variety in South-eastern Anatolia Region, was used as the material in the study. Seed material was obtained from GAP International Agricultural Research and Training Center (GAPUTAEM). Homogeneous seeds were selected to avoid endosperm effects on the results. Two superior bacterial strains (KF3B and KF63C) from a large-scale collection isolated from rural lands of Southern of Siirt province in the spring season of 2020 were used in the experiment. Soil samples were collected from rhizosphere of superior plants, one gram soil was diluted with 9 ml distilled water, gradual dilution was continued, thereby, bacterial strains were isolated, purified, and stored [28]. Nitrogen fixation, phosphate solubilizing, siderophore production and ACC deaminase activity properties of strains were determined [29-31]. Universal primer was used for the amplification of 16S rDNA gene in all bacterial isolates. Samples were sent to a special laboratory to sequence analysis after the clean-up process [32]. Purified bacterial strains were stored at -86 °C to use in experimental studies. The bacterial properties of KF3B and KF63C were given in Table 1.

Table 1. Species information and bacterial properties such as N2-fixation, P-solubilizing, ACC deaminase activity and siderophore production of used PGPG strains

Code of strains	Species information	N2-fixation	P-solubilizing	ACC Deaminase activity	Siderophore production
KF3B	*Paenarthrobacter nitroguajacolicus*	+	-	+++	+
KF63C	*Paenibacillus xylanilyticus*	+	++	++	+

(-: passive, +: active, ++: strong, +++: very strong)

The study was carried out as a petri experiment in Siirt University, Faculty of Agriculture, Field Crops Department Laboratory in 2021. The five different priming duration (control, 1, 2, 4 and 6 hours) were applied with two bacterial strains. Seeds were inoculated with bacterial strains for a few minutes in control groups. Also, there is not a control group for bacterial strains since the study did not aim a comparison between inoculated and non-inoculated seeds. We focused on priming duration with different bacterial strains. The study was laid out in a completely randomized design with three replications. Before starting the experiment, all materials such as petri, forceps were sterilized in the autoclave (HIRAYAMA, HV-110L, Japan) at 121 °C for 20 minutes. Pure cultures of KF3B and KF63C were grown in nutrient agar medium. A single colony from each strain was transferred into a 250 ml Erlenmeyer containing nutrient broth and grown aerobically overnight on a rotating shaker (120 rpm) at 28±2 °C. Strains grown on nutrient broth were diluted with sterile distilled water to a final concentration of 10^8 CFU ml^{-1} by turbidimeter [33]. In order to allow bacteria to adhere to the seed surface, 3% of sugar solution was added to the suspension and mixed in a shaker for 30 minutes. Before priming, seeds were subjected to surface sterilization with 10% sodium hypochlorite (NaOCl) + 0.01% Tween20 solution for 10 minutes. After sterilization, the seeds were washed 3 times with distilled water and dried with filter paper. The homogeneous 25 seeds were placed in petri dishes (90mm x 15mm) and bacterial suspensions were added with a seed: solution ratio of 1:4 g ml^{-}. The petri dishes were placed in an oven set at 24±2 ° C (BINDER GmbH, Germany) and kept in the dark for required durations. After bio-priming process, seeds were transferred into filter paper for re-drying to initial moisture content. After this stage, all seeds were sown between 2 layers of filter papers placed in petri dishes as 25 pieces / petri dish. The 4 ml distilled water was added to each petri dish. Petri dishes were kept at 24±2 °C through experiment. The 2 ml distilled water was added to each petri dish every 48 hours until the end of the study. The study was completed on 7^{th} day.

The germination percentage (GP), mean germination time (MGT), germination index (GI) and uniformity of germination (UG) were determined to investigate germination characteristics depending on applications. So, germinated seeds were counted daily up to the end of the experiment. Besides, seedling vigor index (SVI), seedling length (SL), seedling fresh weight (SFW) and seedling dry weight (SDW) were calculated to observe seedling growth. The observations for seedling growth were taken from 10 plants randomly selected from the germinated seeds in each petri dish. Before taking observations about the weight, the seeds were cleared of excess water on the surface with filter paper and immediately weighted with a sensitive scale. Image analysis software was used to obtain more precise measurements for SL. Randomly selected samples were placed on a platform and scanned in colour at 600 dpi resolution by a portable scanner (ISCAN, handheld scanner). Root images were analysed using ImageJ image analysis software [34]. The germination characteristics were calculated by equations given below:

$$GP\ (\%) = \frac{G7}{N} \text{x}100 \text{ [35]} \quad (1)$$

$$MGT\ (day) = \sum \frac{G*t}{N} x100\ [36] \quad (2)$$

$$GI = \sum \frac{G_t}{t}\ [37] \quad (3)$$

$$UG = \frac{GP}{MGT}\ [38] \quad (4)$$

$$SVI = GP * SL\ [39] \quad (5)$$

G_7 is the number of total germinated seeds on the 7th day, N is the number of total sowing seeds, t is the time from sowing to counting day. Data were calculated with analysis of variance in the JMP (5.0.1) according to the completely randomized design. The obtained results were grouped according to TUKEY multivariate test.

III. RESULTS AND DISCUSSION

The study was conducted to form a protocol for bio-priming with PGPB in lentils so that optimum priming performance can be obtained. The experiment indicated that both germination characteristics and also seedling growth are significantly affected depending on the duration of priming and bacterial strains. According to the analysis of variance, all parameters were affected by bacterial strains and/or priming duration except for the GP. There were statistically significant differences (<0.01 or <0.05) between treatments and interactions of applied factors. In general, priming durations caused more differences in germination characteristics while bacterial strains were more effective on seedling growth. There was not any evidence for the impact of bacterial-priming duration interaction (Table 2).

Table 2. Analysis of variance (ANOVA) for germination characteristics and seedling traits of lentil depending on PGPR-priming and priming durations

		GP		MGT		GI		UG	
	DF	**MS**	**F prob.**	**MS**	**F prob.**	**MS**	**F prob.**	**MS**	**F prob.**
Bacteria (B)	1	83.3	ns	0.00005	ns	0.0004	ns	8.4	ns
Time (T)	4	30.4	ns	0.19425	**	0.0396	**	208.7	**
B x T	4	10.4	ns	0.01097	ns	0.0005	ns	8.5	ns
	DF	**SFW**		**SDW**		**SL**		**SVI**	
Bacteria (B)	1	0.000504	**	0.0000008	*	7.40	**	44313	*
Time (T)	4	0.000288	**	0.0000021	**	4.18	**	44037	**
B x T	4	0.000015	ns	0.0000004	*	0.04	ns	1648	ns

(GP: Germination percentage, MGT: Mean germination time, GI: Germination index, UG: Uniformity of germination, SFW: Seedling fresh weight, SDW: Seedling dry weight, SL: Seedling length, SVI: Seedling vigor index DF: Degree of freedom, MS: Means of square)

The GP, MGT, GI and UG varied depending on priming duration and bacterial strains between 93.3-100.0-93.3, 1.62-2.13 days, 0.49-0.70, 45.9-62.5, respectively. In addition, SVI, SL, SFW and SDW changed between 551-880, 5.70-8.80cm, 0.059-0.083g and 0.0054-0.0075 g, respectively (Table 3). In general, the highest values were obtained from KF63C and 4 hours of priming duration while the lowest ones were observed in control groups. Although bacterial strains did not cause any significant difference in germination traits, seedling growth was affected depending on bacterial strains and their interactions with priming durations. Improving investigated traits increased up to 4 hours priming duration, however, they were adversely affected over 4 hours. Maximum GP (100%) was observed with four hours priming whereas lowest one (94.2%) was obtained by six hours priming. However, there was no significant differences between priming durations in terms of the GP. Although bacterial strains did not significantly affect the GP, KF3B exhibited higher performance. While bacterial strains did not affect the MGT, priming duration significantly reduced (18.4%) germination time. The GI increased with four hours priming from 0.50 to 0.70 compared with control seeds. Similarly, the UG enhanced with four hours priming from 47.2 to 62.2 compared with control seeds. Four hours priming and KF63C improved the SVI. The highest seedling length was obtained from four hours priming duration (8.80cm) in the KF63C bacteria strain. The SFW and SDW were increased with four hours priming as 30.7% and 28.1, respectively. KF63C strain promoted the SDW and dry matter accumulation (Table 3). It was determined that four hours is the threshold value for bio priming applications in lentils. Moreover, the impacts of interaction between bacteria and priming duration were just observed in seedling growth.

Table 3. Means of germination and seedling traits of Fırat-87 lentil depending on bio priming applications with KF3B and KF63C bacterial strains and priming durations

Traits	Priming duration	KF3B	KF63C	Mean	Traits	Priming duration	KF3B	KF63C	Mean
GP (%)	Control	93.3	96.7	95.0	MGT (day)	Control	2.00	2.13	2.07^{A}
	1 hour	93.3	98.3	95.8		1 hour	1.95	1.95	1.95^{AB}
	2 hours	96.7	98.3	97.5		2 hours	1.87	1.86	1.86^{BC}
	4 hours	100.0	100.0	100.0		4 hours	1.62	1.62	1.62^{D}
	6 hours	93.3	95.5	94.2		6 hours	1.77	1.66	1.71^{CD}
	Mean	95.3	97.8			Mean	1.84	1.85	
GI	Control	0.51	0.49	0.50^{D}	UG	Control	48.4	45.9	47.2^{C}
	1 hour	0.53	0.54	0.54^{CD}		1 hour	50.5	48.1	49.3^{BC}
	2 hours	0.59	0.58	0.59^{BC}		2 hours	53.6	50.3	51.9^{BC}
	4 hours	0.69	0.70	0.70^{A}		4 hours	61.9	62.5	62.2^{A}
	6 hours	0.63	0.67	0.65^{AB}		6 hours	54.0	56.3	55.2^{B}
	Mean	0.59	0.60			Mean	53.7	52.6	
SVI	Control	551	673	612^{C}	SL (cm)	Control	5.70	6.95	6.33^{C}
	1 hour	653	696	674^{BC}		1 hour	6.63	7.43	7.03^{BC}
	2 hours	688	736	712^{A-C}		2 hours	6.88	7.88	7.38^{A-C}
	4 hours	785	880	832^{A}		4 hours	7.85	8.80	8.32^{A}
	6 hours	737	812	775^{AB}		6 hours	7.73	8.69	8.21^{AB}
	Mean	683^{B}	760^{A}			Mean	6.96^{B}	7.95^{A}	
SFW (g)	Control	0.059	0.065	0.062^{C}	SDW (g)	Control	0.0060^{CD}	0.0054^{D}	0.0057^{C}
	1 hour	0.064	0.072	0.068^{BC}		1 hour	0.0058^{CD}	0.0062^{B-D}	0.0060^{BC}
	2 hours	0.065	0.079	0.072^{B}		2 hours	0.0059^{CD}	0.0066^{A-C}	0.0063^{BC}
	4 hours	0.078	0.083	0.081^{A}		4 hours	0.0071^{AB}	0.0075^{A}	0.0073^{A}
	6 hours	0.070	0.079	0.074^{AB}		6 hours	0.0061^{B-D}	0.0067^{A-C}	0.0064^{B}
	Mean	0.067^{B}	0.076^{A}			Mean	0.0062^{B}	0.0065^{A}	

(GP: Germination percentage, MGT: Mean germination time, GI: Germination index, UG: Uniformity of germination, SFW: Seedling fresh weight, SDW: Seedling dry weight, SL: Seedling length, SVI: Seedling vigor index)

Although all seed priming techniques provide higher germination traits and seedling growth, improving antioxidant defence systems, bio-priming with PGPB occupies a major position due to superior properties (N fixation, P-solubilizing, ACC deaminase activity, siderophore and IAA production) of used bacterial strains [40,41]. Besides, promotive effects of bio-priming with PGPB are considered to be caused by bio-active substances (gibberellins, auxins, cytokinins, amino acids and vitamins) secreted by used bacterial strains [42]. Many researchers have stated that bio-priming applications with bacterial strains from different species positively affect germination characteristics and seedling growth in wheat [43], maize [44], mung bean [45], soybean [46], common bean [47], pea, lentil, red gram and chickpea [48]. However, information about bio-priming applications in lentils is still limited.

Our results are in agreement with the Meshram and Sharma et al. [48], in which the seeds were soaked in *Tirchoderma* solution for 4 hours and seedling vigour index, germination rate, seedling length and root growth improved depending on bio-priming. Similarly, Darabi et al. [49] stated that plant height, root nodules, the number of branches, the number of leaves per plant and grain yield increased depending on bio priming with *Azospirillum* spp. Also, they point out to improve photosynthesis in primed seeds. Pankaj et al. [50] applied bio priming with various bacterial strains on four lentil cultivars and determined that *Azotobacter* formulation and Pant bio-agent-3 noteworthy improved stand establishment, grain yield, vigour index and seed quality. Yadav et al. [51] bio-primed with *Trichoderma*, *Pseudomonas* and *Rhizobium* spp. the chickpea and common bean seeds so that they investigated individual and synergistic impacts of bacterial strains on germination, growth and yield. The study indicated that co-inoculated bio priming with three strains showed higher promotive effects compared with individual applications and it was considered to be caused by synergistic relationships. Ahmad et al. [52] stated that bio priming with PGPB provided to mitigate the salinity stress in mung bean and improved both shoot and

root growth. They reported that bio-priming with bacterial strains that have high ACC deaminase activity exhibited higher promotive performance than the others. It is considered to be caused by differences between growing conditions, in which salinity stress caused ethylene synthesis and restricted growth, therefore, ACC deaminase activity alleviated the stress and exhibited more helpful performance in the study [52]. Although superior traits of bacterial strains did not affect the germination characteristics, significant differences were observed on seedling growth depending on strains in the study. It is considered that production of phytohormones, enzyme activities, nitrogen fixation ability and synergistic root colonization were effective on seedling growth and vigour index [53,54]. KF63C, which has higher P solubilizing capacity, promoted seedling growth. It denotes that P solubilizing in PGPB is an effective mechanism on seedling growth. Many researchers were in agreement this approach [16,18,22,29]. However, there is needed to carry out long-term pot and field studies in which some physiological, biochemical and molecular traits should be observed. So, impacts of ACC deaminase activity and siderophore production on plant growth, grain yield and quality under stress conditions can be observed.

The priming duration has a vital position on priming success and growth indices. Many studies have denoted that germination and seedling growth show differences depending on priming duration [55-57]. Moreover, the optimum duration for seed priming changes with priming technique, species, seed size, ambient temperature and aeration [25, 26]. So, this experiment aimed the optimization of bio-priming duration for lentil seeds, and ultimately four hours exhibited the maximum promotive effects on lentil seeds regardless of bacterial strains (Table 3). It is considered that a higher or lower duration time than four hours could exhibit lower performance due to differences in solution uptake or secreted bio-active substances. The findings of Kokila and Bhaskaran [58] are in agreement with our considerations.

IV. CONCLUSIONS

The study was conducted to form a protocol for bio-priming with PGPB in lentils so that optimum priming performance can be obtained. Germination characteristics and seedling growth parameters were enhanced by seed priming with 1:1 diluted bacterial solution for four hours at 24±2 °C. Although superior traits of bacterial strains did not affect the germination characteristics, significant differences were observed on seedling growth depending on strains. It is considered that IAA production, promoting germination and antioxidant enzyme systems, nitrogen fixation and better root elongation were effective on better seedling development. However, it is also estimated that ACC deaminase activity and siderophore production had no impact due to any stress threatens in the growth ambient. So, there is needed to carry out long-term pot and field studies to understand which traits are important for bio-priming where some physiological, biochemical and molecular traits can be observed under optimum and stress conditions.

REFERENCES

[1] FAO. (2019). *Lentil production*. Available from: http://www.faostat.fao.org/beta/en/#data/OA > (Accessed at: 10.08.2021).

[2] Reddy, P. P. (2012). *Bio-priming of seeds*. *Recent advances in crop protection 1st ed*. Springer, New Delhi.

[3] Prajapati, R., Kataria, S., & Jain, M. (2020). Seed priming for alleviation of heavy metal toxicity in plants: An overview. *Plant Science Today*, 7(3), 308-313.

[4] Kumar, A., Droby, S., White, J. F., Singh, V. K., Singh, S. K., Zhimo, V. Y., & Biasi, A. (2020). Endophytes and seed priming: agricultural applications and future prospects. *Microbial Endophytes: Functional Biology and Applications*. Elsevier Inc., Switzerland.

[5] Ceritoglu, M., & Erman, M. (2021). Effect of silicon priming treatments on germination and some agronomic traits in lentil. *3. International African Conference on Current Studies*, 27-28 February, Abomey-Calavi, Benin, 436-444.

[6] Sita, K., & Kumar, V. (2020). Role of gamma aminobutyric acid (GABA) against abiotic stress tolerance in legumes: A review. *Plant Physiology Reports*, 25(4), 654-663.

[7] Ceritoglu, M., & Erman, M. (2020). Mitigation of salinity stress on chickpea germination by salicylic acid priming. *International Journal of Agriculture and Wildlife Science*, 6(3), 582-591.

[8] Açıkbaş, S., & Özyazıcı, M. A. (2021). Silisyum tohum ön uygulamasının tuz stresine maruzbırakılan yem bezelyesi [*Pisum sativum* ssp. Arvense L. (Poir.)]'nin çimlenme gelişimine etkisi. *Middle East International Conference on contemporary Scientific Studies*-V, 27-28 March, Ankara, 148-158.

[9] Hasanuzzaman, M., & Fotopoulos, V. (2019). *Priming and Pretreatment of Seeds and Seedlings 1st ed.* https://doi.org/10.1007/978-981-13-8625-1

[10] Ahammed, G. J., Gantait, S., Mitra, M., Yang, Y., & Li, X. (2020). Role of ethylene crosstalk in seed germination and early seedling development: A review. *Plant Physiology and Biochemistry*, 151, 124-131.

[11] Raghuwanshi, R., & Prasad, J. K. (2018). *Perspectives of rhizobacteria with ACC deaminase activity in plant growth under abiotic stress*. *Root Biology 1st ed.* Springer, Cham.

[12] Penrose, D. M., & Glick, B. R. (2001). Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth-promoting bacteria. *Canadian Journal of Microbiology*, 47, 368-372.

[13] Wright, B., Rowse, H. R., & Whipps, J. M. (2003). Application of beneficial microorganisms to seeds during drum priming. *Biocontrol Science and Technology*, 13(6), 599-614.

[14] Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Huckelhoven, R., Neumann, C., & Von-Wettstein, D. (2005). The endophytic fungus Piriformis indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences*, 102, 13386-13391.

[15] Paparella, S., Araujo, S. S., Rossi, G., Wijayasinghe, M., Corbonera, D., & Balestrazzi, A. (2015). Seed priming: State of the art and new perspectives. *Plant Cell Reports*, 34, 1281-1293.

[16] Çakmakçı, R., Erman, M., Kotan, R., Çığ, F., Karagöz, K., & Sezen, M. (2010). Growth promotion and yield enhacement of sugar beet and wheat by application of plant growth-promoting rhizobacteria. In: *Proceedings of the International Conference on Organic Agriculture in Scope of Environmental Problems*, 3-7 February, Famagusta, 204-208.

[17] Erman, M., Kotan, R., Çakmakçı, R., Çığ, F., Karagöz, K. & Sezen, M. (2010). Van Gölü havzasında nizole edilen azot fikseri ve fosfat çözücü bakterilerin buğday ve şeker pancarında büyüme ve verim özelikleri üzerine etkiler. *Türkiye IV. Organik Tarım Sempozyumu*, 28 Haziran-1 Temmuz, Erzurum, 326-330.

[18] Glick, B. R. (2020). *Beneficial Plant-Bacterial Interactions 2nd ed*. Springer Nature Switzerland, Cham. https://doi.org/10.1007/978-3-319-13921-0

[19] Forti, C., Ottobrino, V., Bassolino, L., Toppino, L., Rotino, G. L., Pagano, A., Macovei, A., & Balestrazzi, A. 2020. Molecular dynamics of pre-germinative metabolism in primed eggplant (*Solanum melongena* L.) seeds. *Horticulture Research*, 7, 87.

[20] Nawaz, H., Hussain, N., Jamil, M., Yasmeen, A., Bukhari, S. A. H., Aurangzaib, M., & Usman, M. (2020). Seed biopriming mitigates terminal drought stress at reproductive stage of maize by enhancing gas exchange attributes and nutrient uptake. *Turkish Journal of Agriculture and Forestry*, 44, 250-261.

[21] Singh, S., Singh, U. B., Triverdi, M., Sahu, P. K., Paul, S., Paul, D., & Saxena, A. K. (2020). Seed biopriming with salt-tolerant endophytic *Pseudomonas geniculata*-modulated biochemical responses provide ecological fitness in maize (*Zea mays* L.) grown in saline sodic soil. *International Journal of Environmental Research and Public Health*, 17(1), 253.

[22] Paul, S., & Rakshit, A. (2021). Effect of seed bio-priming with *Trichoderma viride* strain BHU-2953 for enhancing soil phosphorus solubilization and uptake in soybean (*Glycine max*). *Journal of Soil Science and Plant Nutrition*, 21, 1041-1052.

[23] Peixoto da Silva, M. B., Silva, V. N., & Vieira, L. C. (2021). Biopriming of sweet pepper and tomato seeds with *Ascophyllum nodosum*. *RevistaFacultad Nacional de Agronomia*, 74(1), 9423-9430.

[24] Perez-Garcia, F., Pita, J. M., Gonzalez-Benito, M. E., & Iriondo, J. M. (1995). Effects of light, temperature and seed priming on germination of celery seeds (*Apium graveolens* L.). *International Seed Testing Association*, 23(2), 377-383.

[25] Elkoca, E. (2007). Priming: Ekim öncesi tohum uygulamaları. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 38(1), 113-120.

[26] Yari, L., Aghaalikani, M., & Khazaei, F. (2010). Effect of seed priming duration and temperature on seed germination behavior of bread wheat (*Triticum aestivum* L.). *ARPN Journal of Agricultural and Biological Science*, 5(1), 1-6.

[27] Popovic, V., Ljubicic, N., Kostic, M., Radulovic, M., Blagojevic, D., Ugrenovic, V., Popovic, D., & Ivosevic, B. (2020). Genotype × environment interaction for wheat yield traits suitable for selection in different seed priming conditions. *Plants*, 9(12), 1804.

[28] Schmidt, E. L., & Belser, L. W. (1982). *Nitrifying bacteria, in methods of soil analysis part 2*. *Chemical and Microbiological Processes 1st ed*. ASA, Wisconsin.

[29] Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologiya*, 17, 362-370.

[30] Li, Z., Chang, S., Lin, L., Li, Y., & An, Q. (2011). A colorimetric assay of 1-aminocyclopropane-1-carboxylate (ACC) based on ninhydrin reaction for rapid screening of bacteria containing ACC deaminase. *Letters in Applied Microbiology*, 53, 178-185.

[31] Vaikuntapu, P. R., Dutta, S., Samudrala, R. B., & Rao, V. R. V. N. (2014). Preferential promotion of Lycopersicon esculentum (tomato) growth by plant growth promoting bacteria associated with tomato. *Indian Journal of Microbiology*, 54, 403-412.

[32] Miller, C. S., Handley, K. M., Wrighton, K. C., Frischkorn, K. R., Thomas, B. C., & Banfield, J. F. (2013). Short-read assembly of full-length 16S amplicons reveals bacterial diversity in subsurface sediments. *PloS one*, 8(2), e56018.

[33] Sonkurt, M., & Çığ, F. (2019). The effect of plant growth-promoting bacteria on the development, yield and yield components of bread (*Triticum aestivum* L.) and durum (*Triticum durum*) wheats. *Applied Ecology and Environmental Research*, 17(2), 3877-3896.

[34] Ceritoglu, M., Ceritoglu, F., Erman, M., & Bektas, H. (2020). Root system variation of pulse crops at early vegetative stage. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 48(4), 2182-2197.

[35] Al-ansari, F., & Ksiksi, T. (2021). A quantitative assessment of germination parameters: The case of *Crotalaria Persica* and *Tephrosia Apollinea*. *The Open Environmental Research Journal*, 9, 13-21.

[36] Ellis, R. H., & Roberts, E. H. (1981). The quantification of aging and survival in orthodox seeds. *Seed Science and Technology*, 9, 373-409.

[37] AOSA. (1983). Seed Vigor Testing Handbook. Association of Official Seed Analysts, Ithaca, New York, USA.

[38] Noorhosseini, S. A., Jokar, N. K., & Damalas, C. A. (2018). Improving seed germination and early growth of garden cress (*Lepidium sativum*) and Basil (*Ocimum basilicum*) with hydro-priming. *Journal of Plant Growth Regulation*, 37, 323-334.

[39] Abdul-Baki, A. A., & Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria. *Crop Science*, 13, 630-633.

[40] Forti, C., Shankar, A., Singh, A., Valestrazzi, A., Prasad, V., & Macovei, A. (2020). Hydropriming and biopriming improve Medicago truncatula seed germination and upregulate DNA repair and antioxidant genes. *Genes*, 11(3), 242.

[41] Nawaz, H., Hussain, N., Ahmed, N., Rehman, H., & Alam, J. (2021). Efficiency of seed bio-priming technique for healthy mung bean productivity under terminal drought stress. *Journal of Integrative Agriculture*, 20(1), 87-99.

[42] Afifi, M. H., Manal, F. M., & Gomaa, A. M. (2003). Efficiency of applying biofertilizers to maize crop under different levels of mineral fertilizers. Annals of Agricultural Science *Moshtohor Journal*, 41(4), 1411-1420.

[43] Fathollahy, S., & Mozaffari, A. (2020). Investigation the effect of seed biopriming with plant growth promoting rhizobacteria (PGPR) on antioxidant enzymes activity of seedling and germination indices of two wheat cultivar under salt stress conditions. *Iranian Journal of Seed Science and Technology*, 9(1), 27-44.

[44] Panuccio, M. R., Chaabani, S., Roula, R., & Muscolo, A. (2018). Bio-priming mitigates detrimental effects of salinity on maize improving antioxidant defense and preserving photosynthetic efficiency. *Plant Physiology and Biochemistry*, 132, 465-474.

[45] Kumari, P., Meena, M., Gupta, P., Dubey, M. K., Nath, G., & Upadhyay, R. S. (2018). Plant growth promoting rhizobacteria and their biopriming for growth promotion in mung bean (*Vigna radiata* (L.) R. Wilczek). *Biocatalysis and Agricultural Biotechnology*, 16, 163-171.

[46] Arif, M., Jan, M. T., Khan, N. U., Khan, A., Khan, M. J., & Munir, I. (2010). Effect of seed priming on growth parameters of soybean. *Pakistan Journal of Botany*, 42(4), 2803-2812.

[47] Monalisa, S. P., Beura, J. K., Tarai, R. K., & Naik, M. (2017). Seed quality enhancement through biopriming in common bean (*Phaseolus vulgaris*. L). *Journal of Applied and Natural Science*, 9(3), 1740-1743.

[48] Meshram, S., & Sarma, B. (2017). Comparative analysis of effects of seed biopriming on growth and development in different Pulses: Pea, lentil, red gram and chickpea. *International Journal of Current Microbiology and Applied Sciences*, 6(10), 2944-2950.

[49] Darabi, F., Hatami, A., Zarea, M. A., & Naseri, R. (2016). Investigation of important morphological traits and grain yield of lentil under shading and bio-priming. *Iranian Journal of Pulses Research*, 7(1), 145-160.

[50] Pankaj, K., Deepa, K., Birendra, P., Srinivas, P., & Rejendra, P. (2016). Effect of seed bio-priming on seed quality parameters of lentil under mid Himalayas. *Journal of Hill Agriculture*, 7(2), 195-200.

[51] Yadav, S. K., Dave, A., Sarkar, A., Singh, H. B., & Sarma, B. K. (2013). Co-inoculated biopriming with *Trichoderma*, *Pseudomonas* and *Rhizobium* improves crop growth in *Cicer arietinum* and *Phaseolus vulgaris*. *International Journal of Agriculture, Environment & Biotechnology*, 6(2), 255-259.

[52] Ahmad, M. A., Zahir, Z. A., Arghar, H. N., & Arshad, M. (2012). The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. *Annals of Microbiology*, 62, 1321-1330.

[53] Siddiqui, Z. A., Baghel, G., & Akhtar, M. S. (2007). Biocontrol of *Meloidogyne javanica* by *Rhizobium* and plant growth-promoting rhizobacteria on lentil. *World Journal of Microbiology and Biotechnology*, 23, 435-441.

[54] Kumar, R., & Chandra, K. (2008). Influence of PGPR and PSB on *Rhizobium leguminosarum* Bv. *viciae* strain competition and symbiotic performance in lentil. *World Journal of Agricultural Sciences*, 4(3), 297-301.

[55] Soughir, M., Aymen, E. M., & Cherif, H. (2012). Effect of NaCl priming duration and concentration on germination behavior of fenugreek. *Albanian Journal of Agricultural Sciences*, 4(11), 193-198.

[56] Ghasemi-Golezani, K., Jabbarpour-Bonyadi, Z., Shafagh-Kolvanagh, J., & Nikpour-Rasdabad, N. (2013). Effects of water Stress and hydro-priming duration on field performance of lentil. *International Journal of Farming and Allied Sciences*, 2(21), 922-925.

[57] Musa, M., Singh, A., & Lawal, A. A. (2014). Influence of priming duration on the performance of amaranths (*Amaranthus cruentus* L.) in Sokoto semiarid zone of Nigeria. *Hindawi Publishing Corporation International Journal of Agronomy*, Article ID: 475953, 1-4.

[58] Kokila, M., & Bhaskaran, M. (2016). Standardization of *Azospirillum* concentration and duration of biopriming for rice and vigor improvement. *International Journal of Agricultural Sciences*, 12(2), 283-287.