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The Effects of Bio-Priming on Seed Germination and Seedling Growth of Italian Ryegrass (Lolium multiflorum Lam.)

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The Effects of Bio-Priming on Seed Germination and Seedling Growth of Italian Ryegrass (*Lolium multiflorum* Lam.)

Abstract

Seed bio-priming applications with plant growth promoting rhizobacteria (PGPR) have been widely used recently to improve germination and seedling growth. Therefore, the aim of this study was to investigate the effects of bio-priming with different bacterial strains on germination and seedling development of Italian ryegrass seeds. The sterilized seeds of the Elif variety (*Lolium multiflorum* Lam) were inoculated with nine different bacterial strains belonging to Bacillus species (108 cfu/mL bacterial suspension) for 15 min at 120 rpm and then dried at room temperature. The treated seeds were germinated in petri dishes with 25 seeds between 3 filter papers at 22 ±2 ˚C. The study was carried out in a completely randomized design with three replications. As a result of the study, no significant difference was obtained between the treatments in germination percentage and root length, but it was determined that SY2 and SY5 (*Bacillus* isolates) showed superior performance compared to the control in terms of shoot length and seedling fresh and dry weights.

İtalyan Çiminde (Lolium multiflorum Lam.) Biyo-Priming Uygulamalarının Tohum Çimlenmesi ve Fide Büyümesi Üzerine Etkileri

Özet

Bitki büyümesini teşvik eden rizobakteriler (PGPR) ile tohum biyo-priming uygulamaları, çimlenmeyi ve fide gelişimini iyileştirmek amacıyla son zamanlarda yaygın olarak kullanılmaktadır. Bu nedenle, bu çalışmanın amacı, farklı bakteri suşları ile biyo-priming uygulamalarının İtalyan çimi tohumlarının çimlenmesi ve fide gelişimi üzerindeki etkilerini araştırmaktır. Çalışmada Elif çeşidi (*Lolium multiflorum* Lam) İtalyan çimi tohumları kullanılmıştır ilk olarak sterilize edilmiş tohumlar *Bacillus* türlerine ait dokuz farklı bakteri suşu (108 cfu/mL bakteri süspansiyonu) ile 120 rpm'de 15 dakika süreyle inoküle edilmiş ve ardından oda sıcaklığında kurutulmuştur. Uygulama yapılan tohumlar petri kaplarında 3 filtre kağıdı arasında 25 tohum olacak şekilde 22 ±2 ˚C'de çimlendirilmiştir. Çalışma üç tekerrürlü olarak tesadüf parselleri deneme desenine göre yürütülmüştür. Çalışma sonucunda, çimlenme yüzdesi ve kök uzunluğu bakımından uygulamalar arasında önemli bir fark elde edilmemiş, ancak SY2 ve SY5'in (*Bacillus* izolatları) sürgün uzunluğu ve fide taze ve kuru ağırlıkları bakımından kontrole kıyasla üstün performans gösterdiği belirlenmiştir.

1. INTRODUCTION

Italian ryegrass (*Lolium multiflorum* Lam.) is a plant in the genus Lolium belonging to the Poaceae family of the Poales order (Lale and Kökten, 2020). It is best adapted to cool and humid climates. It can be easily grown in places with annual rainfall above 400 mm (Açıkgöz, 2021). The optimum air temperature required for the most efficient development is 18-24˚C (Pişkin, 2007). Due to its high yield and forage quality, sown area and green herbage production have increased over the years in Türkiye and the sown area reached 536256 decares and green herbage production was 2154518 tons in 2023 (Özkan et al., 2022; TUIK, 2024)

Plant growth-promoting rhizobacteria (PGPR) is a group of bacteria that colonized the plant roots (Wu et al., 2005). PGPRs can produce phytohormones (Egamberdiyeva 2007; Shaharoona et al., 2006), fix atmosperic nitrogen (Salantur et al., 2006; Yaman et al., 2023) and activate various enzymes during early growth (Yıldız et al., 2022; Yıldız et al., 2023). PGPRs play an important role in yield and quality in agricultural production (Bashan et al., 2004). Uniform germination and emergence are crucial for plant development. In recent years, various treatments have been applied to seeds during the early development period. Various PGPR strains affect seed germination and contribute to early seedling growth and development (Miljakovic et al., 2022). Bio-priming of seeds with PGPR is one of the inexpensive and eco-friendly solutions to increase the growth in the early or primary stages of its growth (Deshmukh et al., 2020). The use of beneficial PGPRs such as *Pseudomonas spp*. (Chitra and Jijeesh, 2021), *Enterobacter* spp. (Roslan et al., 2020), *Bacillus spp*. (Li et al., 2021), *Azotobacter spp*. (Bidabadi and Mehralian, 2020), and *Azospirillum spp*. (Gowthamy et al., 2017) as a bio-inoculant or seed bio-priming agent has been well documented and utilized to improve stress tolerance, nutrient uptake and seed germination (Mitra et al. 2021).

Bio priming enables microorganisms to penetrate into the seed by soaking the seeds in microbial solutions (Mahmood et al., 2016). In recent years, seed biopriming with plant growth-promoting rhizobacteria (PGPR) has been widely used as it improves germination and seedling development. Therefore, this study aimed to investigate the effects of bio-priming applications with different bacterial strains on germination and seedling development of Italian ryegrass seeds.

2. MATERIALS AND METHOD

Elif, an Italian ryegrass variety registered by Aegean Agricultural Research Institute, was used in the study. The seeds used in the study were sterilized with 10% sodium hypochlorite for 5 minutes and then rinsed 3 times with pure water. In the study, 9 strains belonging to Bacillus species with a density of 108 cfu/mL were used for bio-priming application (Table 1). The sterilized seeds were subjected to bacterial inoculation at 120 rpm for 15 minutes and then allowed to dry at room temperature. Seeds were sown in 25 pieces between 3 filter papers in petri dishes and allowed to germinate at 22 ± 2 ˚C. Seeds were considered germinated when the root (≥2 mm) emerged and germinated seeds were counted for 14 days. At the end of the 14th day, germination percentage (number of germinated seeds/25 x 100) was calculated and shoot and root length, fresh and dry weight of seedling were determined at randomly selected 10 seedlings.

Table 1. Bacteria used and their properties.

Bacteria no	Type of Bacteria	Properties
SY 1	*Bacillus spp. İsolate*	ACC deaminase
SY 2		Siderophores
SY 3		
SY 4		Nitrogen fixation
SY 5		
SY 6		
SY 7		Phosphotase, Nitrogen fixation
SY 8		
SY 9		ACC deaminase

The research was carried out in a completely randomized design with three replications. The data obtained as a result of the research were analyzed on the computer with the 'JMP 13.2.0' program. Treatment means were compared with the Tukey

multiple comparison test (Snedecor and Cochran, 1967).

3. RESULTS AND DISCUSSION

Biopriming can promote rapid and even germination as well as shoot development (Moeinzadeh et al., 2010). This study was designed to investigate the effect of different PGPRs on germination and early growth of Italian ryegrass plants.

As a result of the study, the effects of treatments on shoot length, seedling fresh and dry weights were found statistically significant at $p<0.01$ level, while the effects on germination percentage and root length were found statistically insignificant (Table 2). The highest germination percentage was 100% in SY 4, the lowest was 90.66% in SY 8, and in the control treatment, it was recorded as %96. Bio-priming with bacteria did not cause a statistically significant increase in germination percentage compared to the control, in line with Erman et al. (2022). However, Perez-García et al. (2023) found that different PGPRs increased the germination percentage compared to the control treatment in their study on lettuce plants.

Table 2. Germination percentage, shoot and root length and seedling fresh and dry weight data

Bacteria no	Germination Percentage (%)	Shoot length (cm)	Root length(cm)	Fresh weight (mg/per seedling)	Dry weight (mg/per seedling)
SY 1	96.00	3.82 abc	3.84	9.63 cd	1.27 cde
SY 2	94.66	5.73 a	4.58	13.66 a	1.80 a
SY 3	98.66	4.11 abc	4.82	7.10 e	0.63 e
SY 4	100.00	3.66 bc	3.62	10.73 bc	1.41 bc
SY 5	96.00	4.93 ab	4.04	11.70 b	1.54 ab
SY 6	96.00	3.85 abc	3.04	9.00 d	1.18 de
SY 7	97.33	4.67 ab	4.61	10.40 bcd	1.37 cd
SY 8	90.66	2.28 c	2.64	6.93 e	0.91 e
SY 9	96.00	3.95 abc	3.37	10.66 bcd	1.40 bcd
Control	**96.00**	**3.64 bc**	**3.41**	**9.93 cd**	**1.30 cd**

The highest shoot lengths was 5.73 cm in SY 2, the lowest were 2.28 in SY 8, and shoot length was 3.64 cm in the control treatment. Root length varied between 2.64 and 4.82 cm in the bacteria treatments and was determined as 3.41 cm in the control treatment. Miljakovic et al. (2022) reported that bio-priming with Bacillus megaterium improved shoot and root length in soybean. Hormones such as indole acetic acid and gibberellic acid produced by PGPRs can promote shoot growth and development (Perez-Garcia et al., 2023; Chabbi et al., 2024).

The highest seedling fresh and dry weights were obtained from SY 2 at 13.66 and 1.80 mg, respectively, while the lowest weights were obtained from SY 8 at 6.93 and 0.91 mg. The fresh and dry weights of the control treatment were determined to be 9.93 and 1.30 mg. Houida et al. (2022), demonstrated that all the their tested bacterial strains improved seedling biomass of maize. When other studies were examined, increases in germination, shoot and root length and fresh and dry weights were observed with PGPR applications in the germination stage (Widawati and Suliasih 2018; Houida et al., 2022; Chabbi et al., 2024).

3.1 CONCLUSION

Uniform germination and quality seedling formation significantly affect crop yield. As a result of the study, no significant difference was obtained between treatments in germination percentage and root length. However, in terms of shoot length and seedling fresh and dry weights, SY2 and SY5 (*Bacillus spp*. isolate) showed superior performance compared to the control. It was concluded that two Bacillus strains can be evaluated as biopriming inoculants for Italian ryegrass.

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