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The Effect of Seed Priming Applications on Germination Parameters of Red Clover (*Trifolium pratense* L.)

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ABSTRACT: This study aimed to determine the effects of silicon (Si) and salicylic acid (SA) seed priming applications on the germination parameters of red clover (*Trifolium pratense* L.). The study was carried out in Siirt University, Faculty of Agriculture, Field Crops Laboratory, under controlled conditions at 24±1 °C. The plant material of the study was Rajan red clover (*T. pratense* L.) cultivar. The laboratory study was carried out in Petri dishes according to the randomized plots trial design with 4 replications. The subject of the study consists of hydropriming application with 2 mM, 4 mM, and 6 mM priming doses of Si and 0.5 mM, 1.0 mM, and 1.5 mM priming doses of SA. The study also included the non-priming application as a control subject. The germination percentage, mean germination time, germination index, coefficient of uniformity of germination, and germination energy properties were examined in terms of the effect on germination development in different priming applications applied to red clover. Significant differences were found between the priming applications in terms of all germination parameters examined. It is thought that early germination, more uniform germination, and higher germination rate can be achieved with priming applications in plants with germination problems such as red clover. In this respect, 2 mM Si priming application can be recommended.

Keywords: Red clover, silicon, salicylic acid, seed priming, germination index, germination energy

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INTRODUCTION

Seed priming is accepted as a simple, cost-effective, and efficient approach to increase seed germination, early seedling growth and development under both stress and non-stress conditions (Hameed et al., 2013; Maiti and Pramanik, 2013). To this end, different chemicals, ions, organic compounds, hormones, and some antioxidants are used, and it was observed that positive results were obtained in germination and seedling growth. For example, seed priming with acetylsalicylic acid was reported to provide uniform germination and seedling formation in hot pepper (*Capsicum annuum* L.) under normal and salty conditions (Khan et al., 2009), sodium silicate seed priming was reported to improve tolerance to water deficiency stress of wheat seeds and improve germination and seedling growth of wheat seeds (Abro et al., 2009; Hameed et al., 2013), seed priming with gibberellic acid (GA3) was reported to reduce salinity stress and increase germination and seedling growth parameters in alfalfa (Younesi and Moradi, 2014), and seed priming applications with KNO_3 and KH_2PO_4 positively affected germination and seedling growth parameters of *Citrullus colocynthis* seeds (Ghiyasi et al., 2019). It is also a common practice to use silicon (Si) and salicylic acid (SA) in seed priming.

Silicon is the second most abundant element in the Earth's crust (Zhu and Gong, 2014) and is the only nutrient element that is not harmful when excessively accumulated in plants (Ma et al., 2001). Although not essential for plant growth (Zhang et al., 2017), Si was reported to be effective in reducing stresses related to salinity (Liang et al., 2003, 2005; Parveen and Ashraf, 2010; Malhotra et al., 2016) and drought (Maghsoudi et al., 2019) in plants. Furthermore, it was stated that Si increased the activity of antioxidant enzymes, thereby reducing stress and improving plant growth (Al-Aghabary et al., 2004).

Salicylic acid is a plant hormone that regulates physiological processes in plant growth and development and plays a role in the plant's resistance to biotic stress (Farhangi-Abriz and Ghassemi-Golezani, 2016; Dempsey and Klessig, 2017). Exogenous administration of SA improves growth and physiological processes and increases protection against abiotic stress (He et al., 2002; Kaya et al., 2002; Arfan et al., 2007; Eraslan et al., 2007; Noreen et al., 2012). Additionally, it was reported that SA had a very important function in stomatal regulation (Morris et al., 2000; Barros et al., 2019), mechanism of plant water stress tolerance (Ashraf and Foolad, 2007), seed germination in plants (Shakirova et al., 2003; Simaeia et al., 2011; Kim and Lee, 2013), increasing the dry weight of root and upper parts (Khodary, 2004; Stevens et al., 2006). Furthermore, Chakma et al. (2021) emphasized that it could be used as a seed priming material.

Red clover (*Trifolium pratense* L.) is one of the widely grown forage legumes in temperate regions (Boller et al., 2010). Red clover is a valuable feed source for ruminants as green forage, straw, silage or dried feed and is a nutrient-rich, nutritious plant that provides forage on the farm throughout the year (Przybylska et al., 2021). It is known that certified seed production of red clover, which has high forage yield especially under irrigable conditions, is insufficient (Zuk-Gołaszewska et al., 2019). Varieties of forage legume species usually produce seeds with low seed yield or low viability (Amdahl et al., 2017). Furthermore, hard seededness (Desai et al., 1997), a special type of dormancy found only in legumes, is a common feature in the cultivars of *Trifolium* species (Tomic et al., 2020). Tomic et al. (2020) reported that the important indicators of seed quality in legumes are germination, hard seededness, and seedling growth parameters.

Seed germination and seedling growth are two critical stages for the establishment of crops (Hubbard et al., 2012). Rapid and uniform seed germination and emergence are key factors in better and optimum crop formation (Hameed et al., 2013). To this end, seed priming, which initiates the germination process with some biochemical changes such as the activation of enzymes and dormancy breaking, is important (Ajouri et al., 2004; Hameed et al., 2013). On the other hand, the response of plants to certain priming agents may differ, whether under stress or non-stress conditions. The concentration of the said substances, processing time, plant species, and the plant organ used are effective in this difference. In this sense, there is little information available for a large number of crops, and therefore further research is needed.

In this study, the effects of Si and SA seed priming applications on the germination parameters of red clover (*T. pratense* L.) were investigated.

MATERIALS AND METHODS

The study was carried out in Siirt University, Faculty of Agriculture, Department of Field Crops Laboratory. Rajan red clover (*T. pratense* L.) cultivar was used as a plant material. Sodium metasilicate pentahydrate ($Na_2SiO_3.5H_2O$) was used as the Si source, and distilled water was used for hydropriming.

Experimental Design and Treatment Details

The subject of the study consists of hydropriming application with 2 mM, 4 mM, and 6 mM priming doses of Si and 0.5 mM, 1.0 mM, and 1.5 mM priming doses of SA. The study also included the non-priming application as a control subject.

A laboratory trial was set up according to a randomized plot trial design with 4 replications. Twenty-five seeds were used for each replication. Seeds were sterilized in 70% ethyl alcohol for 1 minute and rinsed 3 times with sterile water. Then, surface sterilization was carried out to cover the seeds with 10% sodium hypochlorite (NaOCl) for 5 minutes to deform the microorganisms on the seed surface. The sterilized seeds were placed between Whatman (Little Chalfont, Buckinghamshire, UK) Grade 2 filter paper in Petri dishes (90 mm x 15 mm). Si, SA and hydropriming solutions prepared according to the doses were applied to each Petri dish as 3 ml, and the seeds were kept in hydropriming for 18 hours (Farooq et al., 2017), in Si for 12 hours (Othmani et al., 2020), and in SA for 12 hours (Sundstrom et al., 1987; Ceritoğlu and Erman, 2020). The seed/solution ratio for each Petri dish was adjusted as 2:1 g ml^{-1} (Johnson et al., 2005). After priming, the seeds were washed with distilled water to clean the entire surface and were first roughly dried in blotting paper, then again placed between dry filter paper and dried to the initial moisture (3% ±) (Jatana et al., 2020). The dried red clover seeds were placed in new Petri dishes, and 3 ml of distilled water was added to each Petri dish. Petri dishes were left to germinate in an oven set at 25±1 °C (BINDER, GmbH, Germany).

Germination controls were made every 24 hours during the test, and the germination test was completed on the 10^{th} day. While germination was detected in seeds, at least 2 mm rootlet emergence was accepted as a germination criterion (Scott et al., 1984; Soleymani and Shahrajabian, 2018).

Germination Measurements and Observations

The germination percentage (GP), mean germination time (MGT), germination index (GI), coefficient of uniformity of germination (CUG), and germination energy (GE) properties were examined in terms of the effect on germination development in different priming applications applied to red clover.

The GP parameter was determined according to Equation 1 used by Scott et al. (1984) by counting the seeds that germinated every 12 hours.

$$GP = (NGS/TS) \times 100 \qquad (1)$$

In the equation, NGS is the number of normally germinated seeds, and TS is the total number of seeds used.

The MGT is generally used to determine the germination day of seeds and was calculated according to Equation 2 (Ellis and Roberts, 1981).

$$MGT = \sum(N_i T_i / N_i) \quad (2)$$

Where N_i is the number of seeds germinated on day T_i, and T_i is the number of days counted from the beginning of germination.

The GI was calculated with the help of Equation 3 (Wang et al., 2004), CUG was calculated with the help of Equation 4 (Bewely and Black, 1994), and GE was calculated with the help of Equation 5 (Li et al., 2020).

$$GI = \sum(G_i / T_t) \quad (3)$$

Where Gi is the germination percentage on the ith day, and Tt is the days of the germination test.

$$CUG = \sum n / \sum[(MGT - t)^2 n] \quad (4)$$

Where t is the time in days, starting from day 0, the day of sowing, and n is the number of seeds completing germination on day t.

$$GE = (T_1 / N) x100 \quad (5)$$

Where T_1 is the number of seeds germinated on the first day, and N is the total number of seeds.

Statistical Analysis

The ArcSin transformation was applied to GP values before analysis of variance (Zar, 1996). The obtained data were subjected to analysis of variance according to the randomized plot trial design, and the differences between the means were checked by Tukey's multiple comparison test (Açıkgöz and Açıkgöz, 2001).

RESULTS AND DISCUSSION

The GP results of the red clover plant in different priming applications are presented in Figure 1. The effect of different priming applications in terms of GP was found to be statistically significant at the $p<0.01$ level.

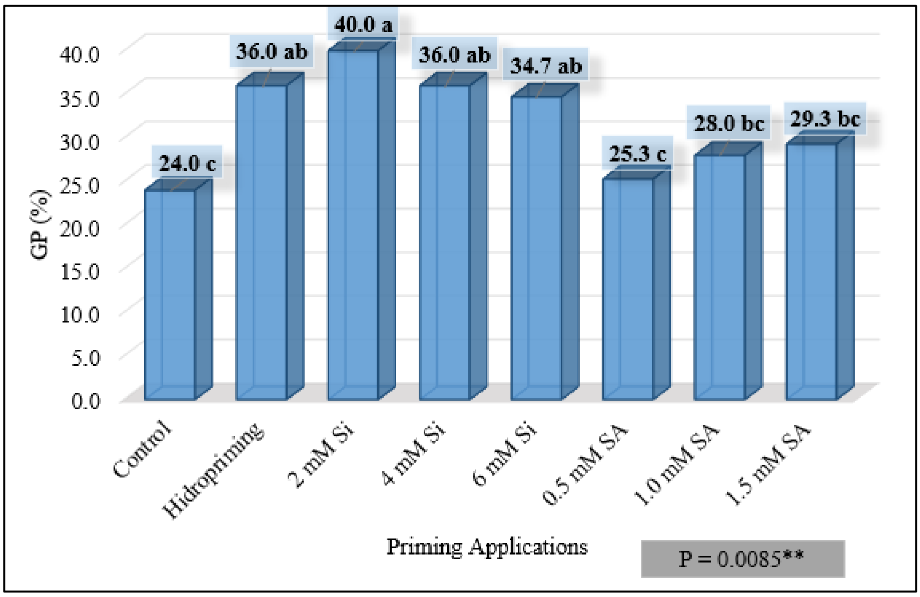

Figure 1. Effect of different priming applications on GP in red clover

In the study, in terms of GP, the highest value was determined in 2 mM Si priming application with 40.0%, and the difference between all Si priming applications and hydropriming application was statistically insignificant. The lowest GP was determined in the control group (24.0%) without priming application and 0.5 mM SA priming application (25.3%). It was determined that Si priming showed more positive effects in terms of GP than SA priming (Figure 1).

When the effects of different priming applications were evaluated together in terms of GP, it was determined that all priming applications, except 0.5 mM SA priming, showed positive effects compared to the control, and especially Si priming applications promoted germination more. In studies conducted on many plant species, it was reported that hydropriming (Ghafoor et al., 2020; Açıkbaş and Özyazıcı, 2021; Ceritoglu and Erman, 2021), silicon priming (Janmohammadi et al., 2015; Khalaki et al., 2016; Biju et al., 2017; Bijanzadeh and Egan, 2018; Ivani et al., 2018; Ayed et al., 2021) and salicylic acid priming (Rajasekaran et al., 2002; Shakirova et al., 2003; Afzal et al., 2006; Farooq et al., 2008; Ceritoğlu and Erman, 2020; Ghafoor et al., 2020) had positive effects on the GP.

The findings of the MGT are shown in Figure 2. The effect of different priming applications in terms of MGT was found to be statistically significant at the $p<0.05$ level. When the MGT were examined, the latest germination was determined as 3.23 days in the control group. This was followed by hydropriming (2.60 days), 0.5 mM SA priming (2.50 days) and 1.5 mM SA priming applications (2.40 days), which were statistically in the same group. With the effect of priming applications, the earliest germination was detected in all Si priming applications (2, 4 and 6 mM) and 1.0 mM SA priming (Figure 2).

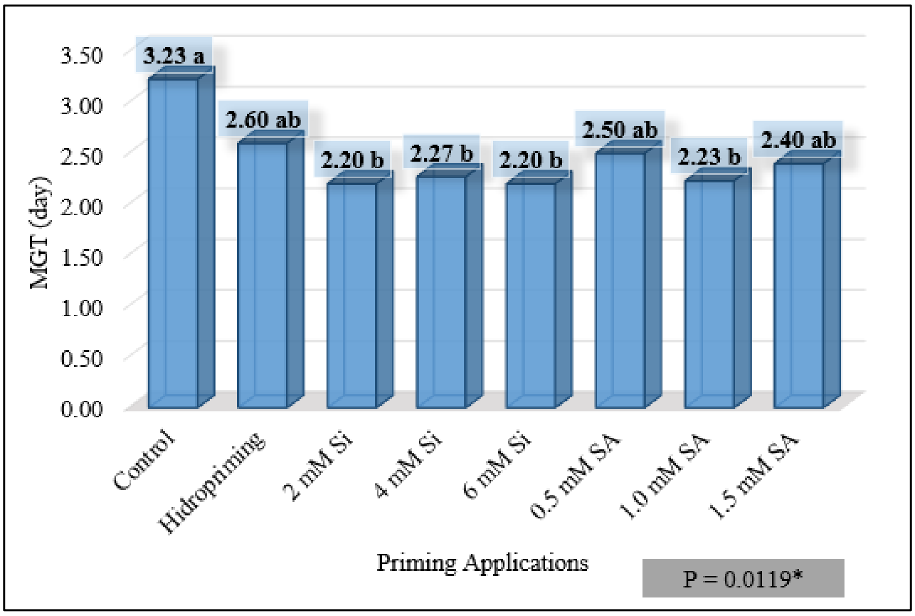

Figure 2. Effect of different priming applications on MGT in red clover

In general, it can be said that the MGT is shortened with priming applications. In this sense, it was observed that Si priming applications and 1.0 mM SA priming were more effective. As in this study, it was reported that hydropriming, Si and SA priming applications significantly affected the MGT and shortened the germination time in some other studies conducted on different plant species (Afzal et al., 2006; Bijanzadeh and Egan, 2018; Ivani et al., 2018; Ceritoğlu and Erman, 2020; Moghaddam et al., 2021).

Different priming applications affected the GI of red clover seeds in a statistically significant manner ($p<0.01$). The highest value in terms of the GI was determined in 2 mM Si priming with 5.67. The difference between this application, hydropriming and 4-6 mM Si priming applications was statistically insignificant. The lowest value was found in the control group with 2.03, in which no priming was applied (Figure 3).

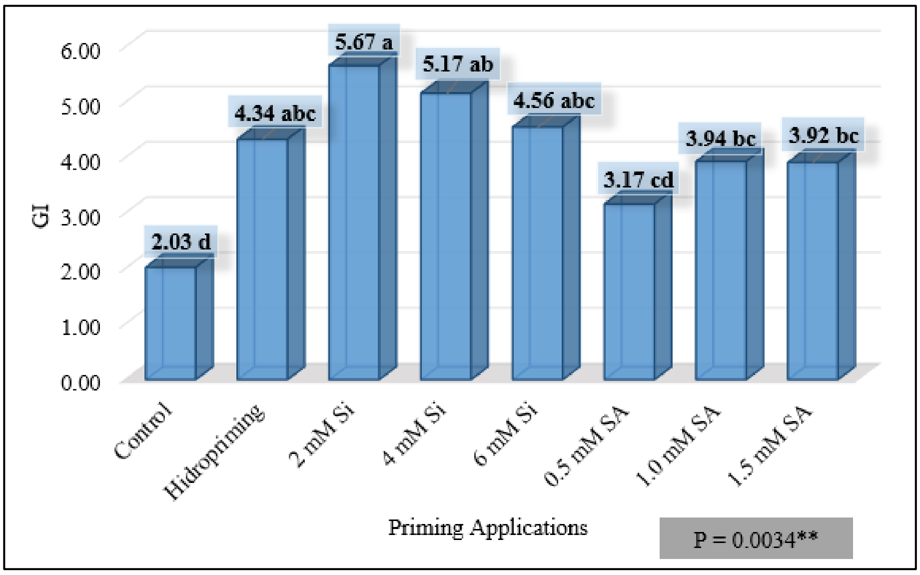

Figure 3. Effect of different priming applications on the mean GI in red clover

Since the GI parameter is affected by the germination ratio and calculated according to the number of germinated seeds on the germination days, the effect of different priming applications was clearly observed. Thus, many studies reported that the GI values of priming applications with Si and SA increased in plant species such as wheat (Hameed et al., 2013; Meena et al., 2014; Ayed et al., 2021), tomato (Shi et al., 2014), lentil (Biju et al., 2017), faba bean (Anaya et al., 2018), and bitter vetch (Moghaddam et al., 2021).

The values related to the CUG determined in red clover in different priming applications are given in Figure 4. There were statistically significant differences at the $p<0.05$ level between different priming applications in terms of the CUG. In terms of the CUG, the most uniform germination was revealed in 2 mM Si priming. In the study, in terms of the CUG, the difference between 0.5 mM SA priming and the other priming applications, excluding the control group, was statistically insignificant (Figure 4).

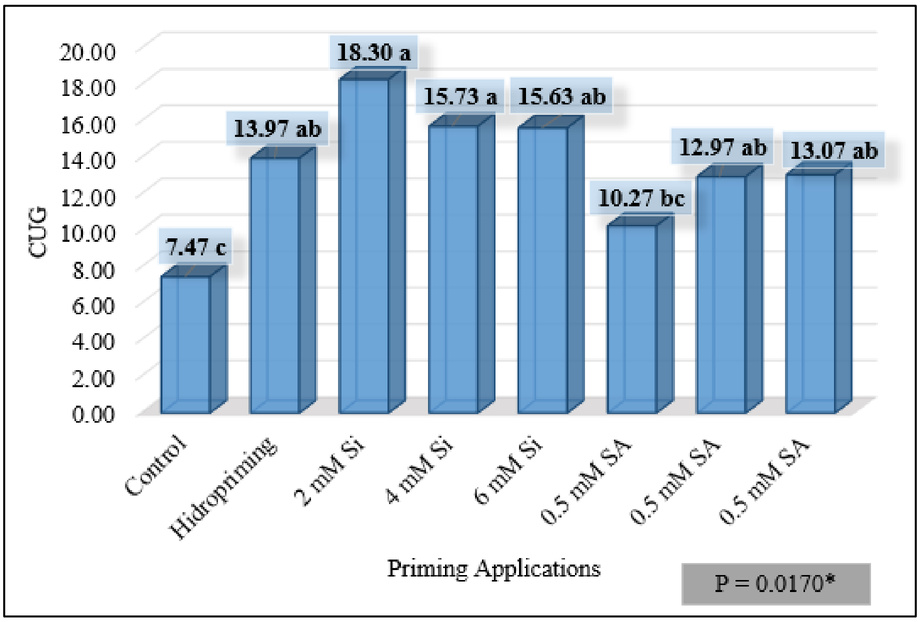

Figure 4. Effect of different priming applications on the CUG in red clover

The CUG is a parameter that expresses fluctuations and stability in germination times between the first and last germinated seeds. Higher values in this feature represent the stability of seeds, while lower values indicate variability in germination under the same conditions (Souhail and Chaabane, 2009). In a study on chickpea regarding germination uniformity, it was reported that hydropriming and SA priming applications (0.1 mM, 0.2 mM, 0.3 mM) increased the CUG compared to the control group (Ceritoğlu and Erman, 2020).

The results of the GE values in the study are shown in Figure 5. Differences between different priming applications in terms of GE were found to be statistically significant at the $p<0.01$ level. The highest values in terms of GE were observed in 2 mM and 4 mM Si priming applications with 12.0. It was determined that no seeds germinated on the first day in the control group in which no priming was applied, and it constituted the lowest group (Figure 5).

Germination energy is generally a germination parameter related to how many seeds germinate on the first day. In terms of GE, 2 mM and 4 mM Si priming applications and 1.0 mM SA priming were found to be effective in seed germination on the first day. It was indicated that the effects of different priming applications on GE were different, and in a study conducted with *Triticum aestivum* L. in terms of GE, priming with sodium silicate had positive effects compared to hydropriming and control (Hameed et al., 2013). In another study, it was reported that different priming applications, including SA, had different effects on the GE of the maize plant (Kumari et al., 2017).

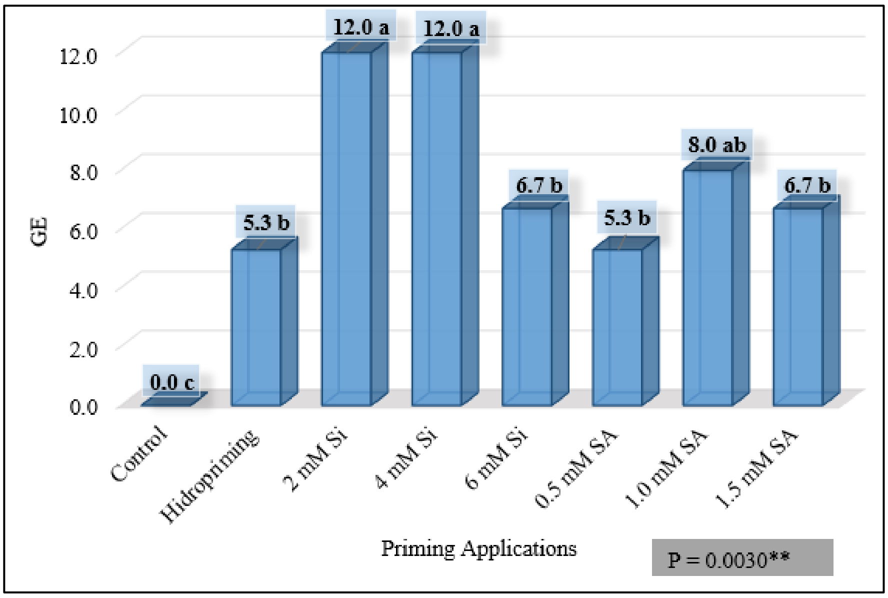

Figure 5. Effect of different priming applications on GE in red clover

CONCLUSION

As a result of the study, it was observed that the effects of hydropriming, Si and SA priming applications on the germination characteristics of red clover showed significant differences. In particular, it was concluded that silicon priming and hydropriming were more effective than salicylic acid priming in terms of germination percentage and germination index, while in the other examined parameters, all priming applications had a positive effect compared to the control group in which no priming was applied. It is thought that early germination, more uniform germination, and higher germination percentage can be achieved with priming applications in plants with germination problems such as red clover. In this respect, 2 mM Si priming can be recommended.

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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