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Effect of seed priming on germination of relict beautiful vavilov, *Vavilovia formosa* (Stev.) Al. Fed.

Tükenme eşiğindeki güzel vavilovia (*Vavilovia formosa* (Stev.) Al. Fed.) türünün çimlenmesine tohum priming etkisi

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ABSTRACT

Priming of seeds increases uniformly germination rate in cultivated and wild species as well, it also shortens germination and emergence time. Beautiful vavilovia, *Vavilovia formasa* (Stev.) Al. Fed. is a relict and endangered legume crop which is the closest relatives of the genus *Pisum* L. In beautiful vavilovia, seed priming has ignored due to insufficient seed production of the plant. In the present report, three seed priming including hydro-priming, glycerol (%1) and potassium chloride (KCl) of 5% were compared to control (no-priming application). Findings have shown that seed priming with glycerol significantly accelerated germination time in beautiful vavilovia. Despite better germination time with hydro-priming after glycerol, hydro-priming had almost similar to control. Germination was stalled off at KCl treatment indicating that beautiful vavilovia could be salt susceptible. Priming with glycerol can be suggested as the fast and reliable germination of seeds of relict beautiful vavilovia.

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ÖZ

Tohumlarda ekim öncesi priming uygulamaları kültürü yapılan bitkilerde olduğu kadar yabani türlerde de çimlenmeyi homojen olarak arttırmasının yanında, aynı zamanda çimlenme ve çıkış süresini de kısaltmaktadır. Güzel vavilovia *Vavilovia formasa* (Stev.) Al. Fed., *Pisum* L. cinsinin en yakın akrabası, nesli tükenmekte ve tehlike altında olan bir baklagil bitkisidir. Güzel vavilovia bitkisinde tohum priming uygulamaları günümüze kadar bitkinin yetersiz tohum üretiminden dolayı göz ardı edilmiştir. Bu çalışmada güzel vavilovia bitkisinde hidro-priming, gliserol (%1) ve potasyum klorid (KCl) (%5) priming uygulamaları kontrol ile karşılaştırılmıştır. Sonuçlar gliserol ile yapılan priming uygulamasının vavilovia bitkisinde çimlendirmeyi önemli seviyede arttırdığını göstermiştir. Hidro-priming uygulaması gliserolden sonra çimlenme oranının artırması bakımından ikinci sırada yer almasına rağmen, bu uygulamadan kontrole oldukça yakın sonuçlar elde edilmiştir. KCl uygulaması ise bu bitkide çimlendirmeyi geciktirmiştir, dolayısıyla buradan vavilovia bitkisinin tuza hassas olabileceği sonucu çıkarılabilir. Araştırma sonuçlarına göre vavilovia bitkisinde gliserol ile yapılan priming uygulamasının hızlı ve homojen çimlenme için etkili bir priming yöntemi olarak önerilebilir.

1. Introduction

Vavilovia formosa (Stev.) Al. Fed. is monotypic genus, dwarf, alpine perennial (Davies 1970) and diploid species with 2n= 14 chromosomes (Abramova 1971). The plant was first described by Steven in 1813 and then it was assigned to the extinct genus *Orobus* L. as *O. formosus* Stev. meaning of "*formosus*" was stated as beautiful (Golubev 1990; Kenicer 2009). It was considered as a member of the genus *Pisum* L. as *P. formosum* (Steven) Alefeld (Kenicer et al. 2009). In 1939, the plant was revised by Federov on the base of morphological characteristics and placed in a new genus as *Vavilovia* Fed. named to honour of Nikolai Ivanovich Vavilov (Kenicer et al. 2009; Mikic et al. 2009; Mikic et al. 2013; Smykal et al. 2017). The following synonymous of the plant were reported by Davies (1970) and Mikic et al. (2009): Syn: *Orobus formosus*

Stev., *Pisum aucheri* Jaub. & Spach, *P. formosum* (Stev.) Alef. and *P. frigidum* Alef., *Vavilovia aucheri* (Jaub. &. Spach) A. Fed., *Vicia aucheri* Boiss., *Alophotropis formosa* (Stev.) Grossh. and *Lathyrus frigidus* Scott & Kotschy. In the late of 1970s, the plant was assigned to be a member of the genus *Pisum* L. (Gunn and Kluve 1976) and later it was suggested to transfer from the genus *Vavilovia* Fed. to *Pisum* L. as *P. formosum* according to molecular findings (Oskoueiyan et al., 2010). However, Kenicer et al. (2009) reported that the genus *Vavilovia* Fed. considerably differed from wild and cultivated peas. It is the closest species to the genus *Pisum* L. in the tribe Fabeae (Schaefer et al. 2012).

Beautiful vavilovia was depicted by Davies (1970) as below: Leaves are broadly cuneate-obovate to suborbicular, 4-15 x 4-13 mm, thick, glaucous or purple, usually glabrous, one pair and end with a mucronate, while its stipules are small semi-sagittate form. Flowers are solitaire with carmine in colour and 16-20 mm, legumes are linear-oblong, length of 25-35 mm and consist of 3-5 seeds (Davies 1970; Kenicer et al. 2009; Mikic et al. 2009). Seeds of beautiful vavilovia are globosely smooth, while dormancy in seeds was reported (Cooper and Cadger 1990).

Beautiful vavilovia is distributed from the Central and Eastern Caucasus (Grossheim 1952; Galushko 1980) to Syria, Iran, Iraq, Lebanon and Turkey (Davies 1970; Maxted and Ambrose 2000; Deniz and Sümbül 2004; Eren et al. 2004; Smykal et al. 2017). It is also found in high mountain areas in Azerbaijan (Carjagin 1954), Armenia (Gabrielyan 1962), Dagestan (Murtazaliev 2012; Burlyaeva et al. 2014; Kimeklis et al. 2015; Vishnyakova et al. 2016) and Georgia (Kolakovskiy 1958; Arabuli 1981). Among these countries, Turkey is the first rank on the basis of richness of the beautiful vavilovia (Davies 1970; Deniz and Sümbül 2004; Eren et al. 2004; Kenicer et al. 2009; Mikic et al. 2009, 2014; Smykal et al. 2017). Beautiful vavilovia was categorized as endangered species in many of these countries (Smykal et al. 2017) due to fact that it is in danger in their natural environment owing to the grazing by herds of goat. In addition to these treats, seeding capacity per year was found as low in some populations of the plant, causing them to decrease in size of population (Deniz and Sümbül 2004; Eren et al. 2004; Sarukhanyan et al. 2009). Some population near Antalya, Turkey neither produce flowers nor pods and seeds in 2018 (Personnel observation by C. Toker). Thus, beautiful vavilovia was officially under protection (Popov 1988; Gabrielyan 1990). Because the presence of the plant in mountainous and rocky places and the difficulties of germination it cannot be easily propagated.

To germinate seeds, seed priming, a process of regulating the germination process by managing the temperature and seed moisture content, has been developed to increase the resistance of the seeds to various stress conditions (Heydecker 1973; Khan 1992; Taylor et al. 1998). Seed priming treatments are not only applied to reduce the effect of all these adverse conditions on germination, but they are also useful to shorten the time between sowing and seedling (Basu 1994; Parera and Cantliffe 1994). Also, the treatments uniformly and early germinate seeds (Sadeghian and Yavari 2004; ur Rehman et al. 2011). The essence of the seed priming is based on activating the enzymes that enable the storage material in the seed to be mobilized in the first stage of the water intake (Heydecker 1973; Harris et al. 2007). Various seed priming techniques have been improved such as hydro-priming, halo-priming, osmo-priming and hormonal priming. The most used priming materials are polyethylene glycol (PEG), inorganic salts such as KCl, KNO_3, K_3PO_4, KH_2PO_4, $MgSO_4$, and $CaCl_2$, and low molecular weight organic compounds such as mannitol, glycerol and sucrose (Bodsworth and Bewley 1981; Yacoubi et al. 2013). Belmont et al. (2018) emphasized that the usefulness of seed priming has usually proven in cultivated species but ignored in wild species. Although seed priming has been successfully applied to important legume crops including chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* (L.) Walp.), common bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.), mung bean (*V. radiata* (L.) Wiczek), pea (*Pisum sativum* L.) and soybean (*Glycine max* Merr.) (Bensen et al. 1990; Harris et al. 1999; Hosseini et al. 2002; Sun et al. 2003; Arif et al. 2008; Golezani et al. 2008; Abebe and Modi 2009; Bassi et al. 2011; Ahmadvand et al. 2012; Gupta and Singh 2012; Cokkizgin 2013; Sarika et al. 2013; Chavan et al. 2014; Singh et al. 2014), it has been overlooked in beautiful vavilovia (WOS 2020). The aim of this study is to detect to convenient priming method for fast and reliable germination of relict beautiful vavilovia.

2. Materials and Method

2.1. Plant materials

Seeds of beautiful vavilovia were collected from Kızlarsivrisi location (36°49' N, 30°20' E and 2400 m asl), Antalya, Turkey (Figure 1) at the end of July, 2017. Seeds were maintained at 4°C for six months to get rid of dormancy and vernalization requirement since dormancy and vernalization were reported in wild species of the genus *Pisum* (Highkin 1956; Cechova et al. 2017; Hradilova et al. 2019) the nearest relative (Oskoueiyan et al. 2010; Schaefer et al. 2012). The plant starts flowering from middle June to at the end of June and matures at the end of July (Figure 2).

2.2. Priming treatments

At the initial of the study, all seeds were cleaned from dust and plant debris. In total, one control group (no-priming) and three different priming treatments including potassium chloride (KCl) of 5%, glycerol of 1% and hydro-priming were performed as three replications at temperature of 25°C for 12 hours because glycerol and hydro-priming treatments were accelerated germination in pea plant (Sivritepe and Dourado 1995; Benamar et al. 2003), while potassium chloride was found as an effective treatment under saline conditions in some food legumes (Chavan et al. 2014; Toklu 2015; Farooq et al. 2018). It is not appropriate to collect hundreds of seeds from the valuable wild plant species that are endangered in their natural habitat. Therefore, three seeds were used in each replication in order to be an initiative to the germination issue of beautiful vavilovia and this study was dealt with three priming treatments and control (no-treatment). Prior to germination test, KCl (5%) and glycerol (1%) solutions were prepared and then solutions were poured onto the seeds in sterile glass bottles.

At the end of the 12 h period, the seeds were washed with sterile water and then dried. The dried seeds were placed in petri dishes and immediately irrigated with pure water at 25°C under laboratory conditions. The seeds were daily checked and the records were started with germination event that emerges from the seed of the radicle and plumule as the embryo develops under appropriate conditions. Both of days to radicle emergence and days to plumule emergence were recorded in day for each seed priming treatment.

Figure 1. Location of beautiful vavilovia with red dot in Antalya, Turkey.

Figure 2. Flower and pod of beautiful vavilovia at Antalya location, Turkey.

2.3. Statistical analyses

The data on seed priming were subjected to analysis of variance (ANOVA) with MINITAB 16. Results were statistically expressed ($P<0.05$), Tukey's test was performed for comparison of means using SPSS 22.

3. Results

According to results of ANOVA, differences among means obtained from seed priming treatments were significant for days to radicle emergence ($P<0.01$) (Table 1). The first radicle emergence was determined at glycerol treatment with a mean of 6.3 days (Figure 3 and 4). Glycerol treatment was found to be significantly different from the other treatments for days to radicle emergence. After the treatment of glycerol, the second radicle emergence was determined at hydro-priming treatment with a mean of 13.8 days and followed by the control treatment with a mean of 14.4 days. Hydro-priming and the control treatments were found at the same group since there was not a significant difference between the hydro-priming treatment and the control treatment. The latest radicle emergence occurred at KCl treatment with a mean of 15.8 days (Figure 3).

ANOVA results indicated that there were significant differences among means for days to plumule emergence ($P<0.01$) (Table 1). The first plumule emergence was determined at glycerol treatment with a mean of 10.8 days and glycerol treatment as a single treatment was significantly different from the other treatments' means (Figure 3). Glycerol treatment was followed by hydro-priming and days to plumule emergence was determined to be 21.2 days for hydro-priming

Table 1. ANOVA results for days to radicle and plumule emergences of beautiful vavilovia.

Source	Degrees of Freedom	Sum of Square	Mean of Square	F values	*P*
Treatments (Radicle)	3	329.1	109.7	258.1**	0.000
Error	20	8.5	0.4		
Treatments (Plumule)	3	566.3	188.8	198.7**	0.000
Error	20	19.0	0.95		

** indicate that there were significant differences among means at $P \leq 0.01$.

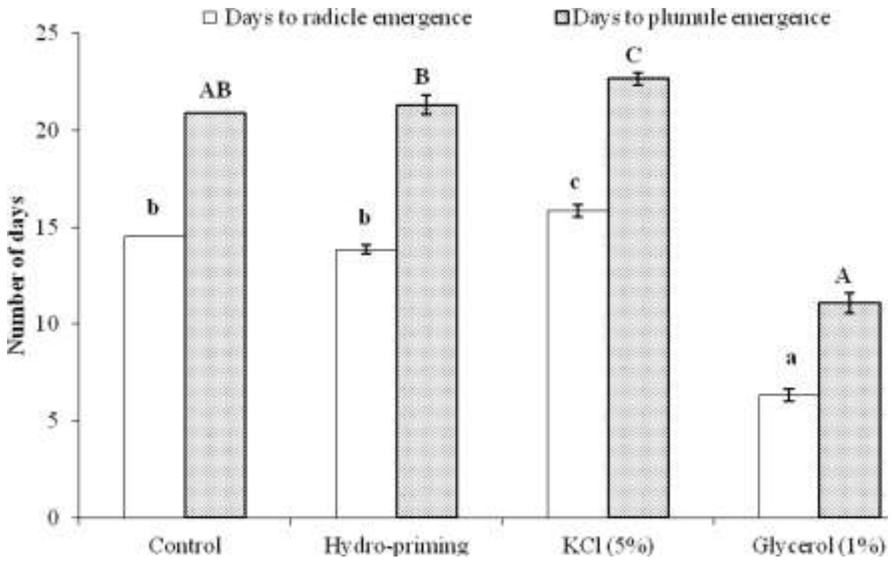

Figure 3. Days to radicle and plumule emergence in different seed priming treatments in beautiful vavilovia (Bars indicate mean±standard errors. Different common and capital letters indicate that there were significant differences among means).

Figure 4. Radicle and plumule emergences in glycerol (1%) treatment in beautiful vavilovia.

Control treatment was the third rank after hydro-priming treatment with 21.7 days. As in the emergence of radicle, plumule emergence was the last rank at KCl treatment with a mean of 23 days (Figure 3). Radicle and plumule emergences were given in Figure 4.

4. Discussion

Differences among means obtained from seed priming treatments were significant for days to radicle and plumule emergences ($P<0.01$) in Table 1, indicating that different priming treatments stimulated or retarded germination of seeds of beautiful vavilovia (Figure 3). Glycerol, which is important metabolite that transports of reducing-equivalents from the cytosol to the mitochondria (Chanda et al. 2011), treatment was markedly grouped at different category from the other treatments (Figure 3) and glycerol obviously stimulated germination in beautiful vavilovia. Glycerol treatment was pointed out to be stimulated growth in diverse plant species including maize (*Zea mays* L.), carrot (*Daucus carota* L.) and spearmint (*Mentha spicata* L.) by Tisserat and Stuff (2011).

After glycerol, hydro-priming was detected as the second stimulator on germination of seeds of beautiful vavilovia (Figure 3). Prior this report, Cooper and Cadger (1990) studied germination of beautiful vavilovia in petri dishes under laboratory condition and they did not use any seed priming treatments but they removed a piece of the seed coats to accelerate water intake. The first radicle was seen in the seeds of beautiful vavilovia after 5 days, which was approximately similar with glycerol treatment in this report (Figure 3). In pea, seed priming treatments such as PEG, abscisic acid and hydro-priming were encouraged to increase germination and decreased in the mean germination time (Sivritepe and Dourado 1995). Hydro-priming treatment was detected as favor of field performance of common bean (*Phaseolus vulgaris* L.) owing to higher yield than that of no-priming seeds (Golezani et al. 2010). In other research carried by Golezani et al. (2008), hydro priming treatment in lentil stimulated seed germination and field emergence rate when hydro priming was compared to osmo-priming treatments. In sorghum (*Sorghum bicolor* L.), Tiryaki and Buyukcingil (2009) reported that germination rate, percentage and spread were significantly improved by all seed priming treatments including PEG, NaCl, KNO_3, glycerol and boric acid.

In *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*, Tsegay and Gebreslassie (2014) found that priming with sodium chloride (NaCl) considerably increased the germination time of *Pisum sativum* var. *abyssinicum*. As a similar finding to this report, KCl extended germination time of seeds of beautiful vavilovia (Figure 3 and 4). Retard of germination with KCl treatment in this report was able to a clue for sensitivity to salinity of beautiful vavilovia. When the habitat of beautiful vavilovia is considered (Figure 1), the species is grown at locations where elevation is more than 2000 m from sea level in Turkey (Eren et al. 2004; Deniz and Sümbül 2004). The species were covered by snow longer than a half of year and then inorganic salts are removed from soil with snow water during melting (Data not shown). In mung bean, osmotic priming of seeds with PEG was detected as decline in longevity of seeds (Sun et al. 2003). In soybean, the following priming treatments; hydro-priming, KCl, $CaCl_2$, KH_2PO_4 and gibberellic acid (GA) were in favored of field performance and they increased seed yield (Chavan et al. 2014).

A field experiment was carried out by Gupta and Singh (2012) to reveal the effects of seed priming on chickpea and plant height, nodule dry weight, seed index, dry matter accumulation, yield and yield characteristics of chickpea were significantly and positively affected by seed priming treatments. Musa et al. (1999) also noted that seed priming treatments help chickpea to escape from terminal drought. The effect of osmo-priming consisting of KNO_3 and hydro-priming on germination and early growth of cowpea was studied by Singh et al. (2014), and their results showed that osmo-priming was superior to no-priming (Singh et al. 2014).

Inter-generic hybrids between *P. sativum* (cultivated pea) × *V. formosa* (beautiful vavilovia) and vice versa were reported by Golubev (1990). In this respect, inter-generic crosses of *Vavilovia* with *Pisum* deserve attention to improve novel traits in pea breeding (Kenicer et al. 2009; Mikic et al. 2009, 2013; Ochatt 2015).

In habitat of beautiful vavilovia at the location, Antalya, Turkey, the plant is continuously exposed to a/biotic stresses (Figure 5). We detected ascochyta blight on leaves of beautiful vavilovia (Figure 5) in its habitat (Figure 1). Yankov and Golubev (1999) reported similar susceptibility to the pea-specialized fungi *Ascochyta pisi* Lib. (Figure 5) and *Uromyces pisi* prior the present report. In addition to a/biotic stresses, the plant is under pressure of grazing by goat and wild animals. In the location (Figure 5), not only beautiful vavilovia but also *Cicer isauricum* P.H. Davies treated by road construction (Tekin et al. 2018) and ski buildings. Due to these reasons, the species was pushed to edges of relict. Therefore, the species was categorized as endangered species in many of these countries (Schoener 1968; Schlueter and Harris 2006; Mikic et al. 2009, 2014; Zoric et al. 2010; Safronova et al. 2015, 2017, 2018; Lam et al. 2016; Provorov and Andronov 2016; Sazanova et al. 2017; Smykal et al. 2017).

Results have shown that seed priming with glycerol significantly triggered germination of seeds of beautiful vavilovia. After glycerol, hydro-priming had almost similar to no-priming (control), while KCl treatment was stalled off germination in beautiful vavilovia that may be susceptibility salt. Priming with glycerol can be recommended because of not only the fastest but also the most reliable germination treatment in relict beautiful vavilovia. The germination and cultivation of beautiful vavilovia was considered to be an important bottleneck. It is essential to cultivate the relict beautiful vavilova in order to get the maximum benefit since inter-generic crosses between *Vavilovia* and *Pisum* deserve attention to improve novel traits in pea breeding.

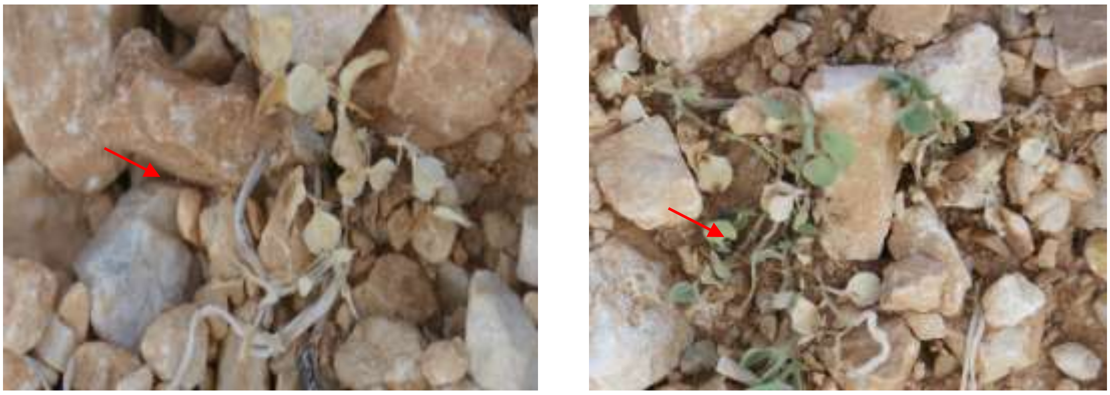

Figure 5. Relict beautiful vavilovia exposed to different biotic and abiotic in its habitat.

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