The Influence of Hydropriming and Osmopriming with KNO_3 on Seed Germination of Dalmatian Pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip.)

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Summary

Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip.) produces pyrethrin which is currently the most important natural insecticide. Due to the growing interest for its use in organic agriculture, the demand for this valuable plant is increasing. However, poor seed germination and inaccessibility of high quality planting material is one of the main constrains for the introduction of Dalmatian pyrethrum into agricultural production. Priming treatments are known to be able to induce rapid and more uniform seed germination, seedling emergence and plant growth and their efficiency was tested on Dalmatian pyrethrum seed. Seed of four natural Dalmatian pyrethrum populations was subjected to hydropriming or osmopriming with 0.2% KNO_3 with the aim of determining their effect on germination parameters (germinability, mean germination time, mean germination rate, coefficient of variation of the germination time, uncertainty of the germination process and synchrony of the germination process). The significantly highest(P<0.05) germinability was recorded for the Biokovo population (27.00%), followed by the population from Šolta (19.67%), Konavle (17.67%) and population from Šibenik (6.67%). Hydropriming for 24h significantly increased the seed germination and germination index (P<0.05). The treatment with 0.2% KNO_3 improved germinability and germination index when compared to control, however, the differences were not significant (P>0.05). As a low-cost, simple and practical, hydropriming can be recommended as an effective method to promote germination performance of Dalmatian pyrethrum seed.

Key words

Dalmatian pyrethrum, germination, natural insecticide, priming, pyrethrin

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Introduction

Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip.) is the most important plant species of the genus *Tanacetum,* the botanical family Asteraceae. It is indigenous to the eastern Adriatic coast and wild populations can be found in Croatia, Bosnia and Herzegovina and Montenegro. Wild populations of Dalmatian pyrethrum in Croatia are distributed on the Eastern sub-Mediterranean dry grasslands in extremely degraded habitats with shallow rocky soils (Grdiša et al., 2014). The reason of interest for this indigenous plant species as well as the increasing demand on the world market stems from the fact that the species synthesizes natural insecticide pyrethrin, comprised of six active ingredients pyrethrin I and II, cinerin I and II and jasmolin I and II (Casida and Quistad, 1995; Hedayat et al., 2009). Pyrethrin is the most important insecticide of plant origin (Hedayat et al., 2009). It rapidly penetrates the insect organisms acting on the central nervous system (Davies et al., 2007). The most particularly important characteristic of pyrethrin is rapid degradation under the influence of light, water and high temperatures (Godin, 1968; Dickinson, 1982) with no negative impact on the environment (Casida and Quistad, 1995).

The cultivation and use of Dalmatian pyrethrum and its products in households and agricultural systems are well documented in Croatian history. However, nowadays in Croatia it is not cultivated on significant agricultural areas. The interest from the farmers exists but the unavailability of high quality planting material is one of the obstacles in the process of re-introducing this plant species into agricultural production. The available scientific literature reports low and poor germination of Dalmatian pyrethrum seed (Wandahwa et al, 1996; Vasisht, 2001; Sladonja, 2014), where generally no reasons are provided for the germination failure. Barton (1966) poor germination attributes to the presence of a high proportion of empty achenes. Due to weak and uneven germination and slow growth of the seedlings cultivation of Dalmatian pyrethrum by direct sowing is often unsuccessful (Vasisht, 2001). Our previous experience with seed of natural populations has also shown low germinability and slow seedling emergence (unpublished data).

Low quality seed requires more time for germination and seedling emergence, which makes them more susceptible to unfavorable environmental conditions resulting in irregular emergence (de Oliveira and Gomes-Filho, 2016). Different seed priming techniques have been found useful for enhancing seed quality, seedling emergence and establishment and crop yields as well as increasing tolerance to adverse environmental stresses in various plant species (Chen and Arora, 2013; Bhargava et al., 2015; de Oliveira and Gomes-Filho, 2016; Ibrahim, 2016). The use of priming treatments stimulates earlier and faster activation of enzymes, mobilization of stored nutrients and transport of hydrolyzed compounds to embryos, DNA replication, etc. These processes combined with initiated physiological processes result in acceleration of germination and seedling emergence (Grzesik and Janas, 2014). Nowadays, numerous seed priming methods have been developed and tested, including hydropriming, osmopriming, hormopriming, solid matrix priming and biopriming (Farooq et al., 2006; Singh et al., 2015). The efficiency of each priming method depends on treated plant species, development stage, concentration of the priming agent and duration time of the treatment. Hydropriming (seed soaking in pure water) is the simplest method of seed priming (Taylor at al., 1998). Kaur et al. (2002) describe hydropriming as an economical and safe technique for seed germination enhancement, which promotes the physiological and biochemical processes in the seed needed for germination process to begin (Barsa et al., 2003). Following the treatment with distilled water, the protoplasm of the seed shows higher capacity for the absorption of water and nutrients and has a higher capacity for water retention in the dry periods (Thomas et al., 2000). Yagmur and Kaydan (2008) state that the increase in cell growth after treatment and higher water seed capacity is the main feature of hydropriming. The effect of hydropriming on Dalmatian pyrethrum seeds in distilled water (dH_2O) was investigated by Li et al. (2011). The authors investigated the influence of light on seed germination and seedling growth as well as possibilities of improving seed germination and tolerance to salt and drought stress by hydropriming. Hydropriming has been used successfully to promote rapid and uniform germination in many other crops (Artola et al., 2003; Demir and Ermis, 2003; Srivastava et al., 2010; Zulueta-Rodríguez et al., 2015; Singh, 2017). Osmopriming involves soaking seeds in aerated, osmotic solution with low water potential instead of pure water. A variety of chemicals are used to create low-water-potential solutions (Thomas et al., 2000). Values of water potential together with duration of priming treatment should be always adjusted to species, cultivar and sometimes seed lot. Different compounds are used in osmopriming procedure including potassium nitrate (KNO_3), hydrogen peroxide (H_20_2), sodium chloride (NaCl), etc. (Yacoubi et al., 2013). In numerous studies KNO_3 proved to be an effective mean of promoting seed germination (Shim et al., 2008; Fariman et al., 2011; Bian et al., 2013; Ruttanaruangboworn et al., 2017).

To diminish the problem of variable and poor seed germination priming techniques could be successfully applied in Dalmatian pyrethrum that in turn would ensure greater profit for potential commercial producers. Therefore, the objective of this study was to determine the effect of hydropriming and osmopriming with 0.2% KNO_3 on germination parameters (germinability, mean germination time, mean germination rate, coefficient of variation of the germination time, uncertainty of the germination process and synchrony of the germination process) of Dalmatian pyrethrum.

Materials and methods

Seed collecting

The experiment was conducted at the Department of Seed Science and Technology at the Faculty of Agriculture, University of Zagreb. The seed of four natural Dalmatian pyrethrum populations were collected in summer 2016 and stored at 4°C in dark conditions. The collected seed is maintained as a part of the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology (Table 1, Figure 1).

Priming treatments and germination tests

Prior to the priming treatments the seeds were surface-sterilized for 1 minute with 2% sodium hypochlorite solution (NaOCl) and rinsed in dH_2O water three times. The experimental design was two-factor factorial arranged in a randomized complete block design with four replications and 25 seeds per replicate. The first factor was the population (P1, P2, P3, P4) and the second seed priming treatment (hydropriming and 0.2% KNO_3,). For priming treatments seed of each population was placed in dH_2O water or 0.2% KNO_3 for 24h. Unprimed seeds were used as the control treatment. The seeds were evenly placed on a filter paper in 9 cm Petri dish and germination was carried out under controlled conditions in a growth chamber at a temperature of 20°C, relative humidity of 70% and under a 16/8 h day/night regime. Distilled water was added as needed to the Petri dishes to assure the sufficient amount of water

Table 1. Sampling sites of natural Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip.) populations

Population	Accession number[a]	Location	Latitude (N)[b]	Longitude (E)[b]	Elevation (m)
P1	MAP02763	Šibenik	43.78	15.91	88
P2	MAP02768	Šolta - Tatinja	43.38	16.29	62
P3	MAP02769	Konavoska brda	42.60	18.25	448
P4	MAP02771	Ravna Vlaška - Biokovo	43.29	17.09	1235

[a] Accession number from the Collection of Medicinal and Aromatic Plants, Zagreb, Croatia; available at Croatian Plant Genetic Resources Database (http://cpgrd.hcphs.hr/); [b] N-North; E-East; Coordinates are in degree decimal format.

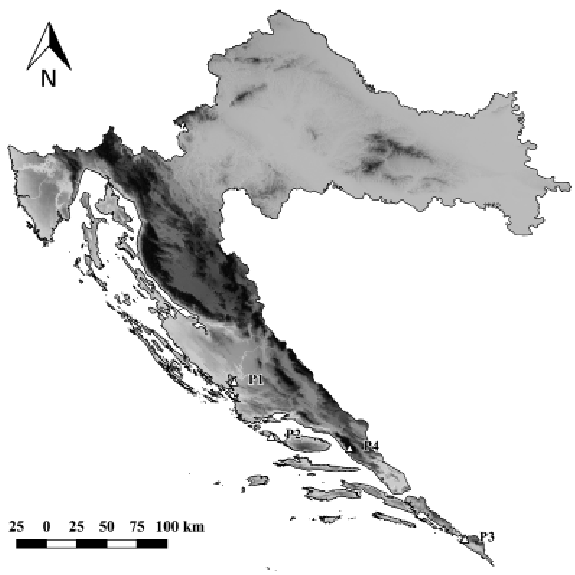

Figure 1. Geographic distribution of analyzed populations of Dalmatian pyrethrum (Populations P1-P4 are presented in Table 1.)

required to germinate the seeds. Seeds were considered germinated when the radicle has extended at least 2 mm. The number of germinated seeds was recorded every 48 h over a period of 21 days.

Germination parameters

Seven germination parameters were calculated to describe the process of germination: germinability (*G*), mean germination time ($\bar{t}$), mean germination rate (*MR*), coefficient of variation of the germination time (CV_t), uncertainty of the germination process (*U*) and synchrony of the germination process (*Z*).

Germinability (*G*; %) was calculated using the following equation (Scott et al., 1984):

$$G = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} \times 100$$

Mean germination time ($\bar{t}$), mean germination rate (*MR*), coefficient of variation of the germination time (CV_t), uncertainty of the germination process (*U*) and synchrony of the germination process (*Z*) were calculated according to formulas given by Ranal et al. (2009):

Mean germination time ($\bar{t}$; days):

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where t_i is time from the start of the experiment to the i^{th} observation (day in this case); n_i is number of seeds germinated in the i^{th} time (not the accumulated number, but the number correspondent to the i^{th} observation), and *k* is last time of germination.

Mean germination rate (*MR*):

$$MR = \frac{1}{\bar{t}}$$

Coefficient of variation of the germination time (CV_t; %):

$$CV_t = \frac{s_t}{\bar{t}} 100$$

where s_t is standard deviation of the germination time and $\bar{t}$ mean germination time.

Uncertainty of the germination process (*U; bits*):

$$U = -\sum_{i=1}^{n} f_i \ log_2 f_i$$

$$f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$$

where n_i is number of seeds germinated on the i^{th} time, and *k* is the last day of observation.

Synchrony of the germination process (*Z*)

$$Z = \frac{\sum_{i=1}^{k} C_{n_i,2}}{C_{\Sigma n_i,2}}$$

being $C_{n_i,2} = n_i(n_i - 1)/2$

where $C_{n_i,2}$ is combination of the seeds germinated in the i^{th} time, two by two, and n_i is the number of seeds germinated in the i^{th} time.

Germination index (*GI*) was calculated as described by the Association of Official Seed Analysis (1983) using the following formula:

$$GI = \frac{No.\ of\ germinated\ seeds}{Days\ of\ first\ count} + \ldots + \frac{No.\ of\ germinated\ seeds}{Days\ of\ final\ count}$$

The obtained data was subjected to statistical analysis using SAS software (SAS Institute, 2004). Analysis of variance was carried out using PROC GLM in SAS followed by post-hoc Tukey's adjustments at 0.05 probability.

Results and discussion

Analysis of variance revealed significant effect of population ($P<0.01$) and priming treatment ($P<0.05$) on germinability (*G*) and germination index (*GI*). Furthermore, the statistical significance ($P<0.01$) of the differences of mean germination time ($\bar{t}$), mean germination rate (*MR*) and uncertainty of the germination process (*U*)

Table 2. Germination characteristics of Dalmatian pyrethrum seed

Population	*G* (%)	$\bar{t}$ (days)	*MR*	CV_t (%)	*U* (bit)	*Z*	*GI*
P1	6.67c	13.44a	0.08b	33.69	0.59b	0.08	0.14b
P2	19.67ab	10.01b	0.11a	31.79	1.05ab	0.32	0.57a
P3	17.67bc	15.16a	0.07b	32.65	1.39a	0.10	0.37ab
P4	27.00a	13.75a	0.08b	37.31	1.70a	0.18	0.60a
P(F)	**	**	**	ns	**	ns	**

G- germinability; $\bar{t}$ -mean germination time; *MR*- mean germination rate; CV_t - coefficient of variation of the germination time; *U* - uncertainty of the germination process; *Z* - synchrony of the germination process; *GI* - germination index; ns, non-significant; *, significant at *P*< 0.05; **, significant at *P*< 0.01; Values followed by the same letter in each column are not significantly different based on the Tukey test at 0.05 probability.

were determined for population. Population by priming treatment interaction was statistically significant (P<0.05) only for the uncertainty of the germination process (*U*) and germination index (*GI*).

In concordance with the findings of other authors (Wandahwa et al., 1996; Vasisht, 2001; Sladonja, 2014) our results have shown low germinability of Dalmatian pyrethrum seed. The highest germinability was found for population P4 (27.00%). This average was significantly higher (*P*<0.05) than those of all the other populations except for the population P2 (19.67%). The lowest germination percentage of 6.67% was recorded for the population P1 (Table 2). Environmental conditions such as amount of precipitation, soil moisture, temperature and nutrient availability to which the mother plant is exposed during seed maturation, are those that strongly affect seed quality (Andersson and Milberg, 1998; Hamilton, 2013). The natural plant populations of Dalmatian pyrethrum inhabit dry, extremely degraded habitats with shallow rocky soils. Therefore, the possible explanation of such low seed germinability might also be the fact that these natural populations thrive on drylands with low nutrient content, high temperatures and low precipitation. This might lead to low seed quality and absence of germination, as the availability of maternal resources affects continued seed growth, its development, seed weight and quality. The influence of drought and high temperatures during the flowering and seed development has been discussed by Fulton (1998). He has reported that drought stress that occurs prior to flowering will impact seed quantity, while drought occurring during the reproductive phase can have considerable negative impact on both seed quantity and quality. Furthermore, higher temperatures during seed development might influence seed weight, i.e., higher temperatures cause smaller seed. Higher temperatures increase the rate of seed ripening reducing the available time for assimilation of nutrients (Fenner, 1992). The greatest differences in habitat environmental conditions are those between population from Mt. Biokovo (P4) that was collected at considerably higher altitudes (1228 m a. s. l.), and other analysed populations. The influence of altitude on the germination of *Chenopodium bonus-henricus* L. was investigated by Dorne (1981). His results have shown that the altitude is one of the important factors that can affect seed germination percentage and speed. The seed collected at higher altitudes is more difficult to germinate than those at lower altitudes because of the thickening of the seed coat. In our study, that was not the case, the Mt Biokovo (P4) population had the highest total germination and mean germination time did not statistically differ from mean germination time of populations P1 and P3. This might be explained by the fact that the seed at higher altitudes is under the influence of somewhat lower temperatures and higher amounts of rainfall during maturation resulting in better seed quality.

Immaturity or different maturity stages of seed at harvest is another possible explanation of the obtained results. The seed harvest at optimum time is of most importance to obtain high quality seed. However, due to different flower maturities that exist on the plant its determination is complicated. The different flower maturities result in seed being immature and other being lost or overmatured, even at most appropriate time of harvest. If harvested too early seed may not yet have developed quality germination characteristics, whereas late harvest may result in seed deterioration (Fulton, 1998). Harvesting seed in optimal maturity stage is particularly difficult in natural populations and it is inevitably to collect seeds at different stages of maturity due to the variability of flowering within and between populations (Hay and Probert, 2013). It is possible that some seed involved in the study were physiologically immature therefore failed to germinate or displayed poor germination characteristics.

Mean germination time ($\bar{t}$) reflects germination speed. The results of mean comparison of mean germination times for populations indicated the significantly lowest (*P*<0.05) values in population P2 (10.01 days) (Table 2). For all the other populations (P1, P3 and P4) mean germination time varied from 13.44 to 15.16 days (*P*>0.05). Analysis of variance revealed significant differences in mean germination rate among populations (*P*<0.01). The highest (*P*<0.01) mean germination rate (0.11) was observed in population P2 (Table 2). All the other populations had similar values in the range from 0.7 to 0.8 (*P*>0.05). On the other hand, seeds of four natural Dalmatian pyrethrum populations did not present significant differences in the coefficient of variation of the germination time (CV_t). Coefficient of variation of the germination time explains the germination uniformity or variability in relation to the mean germination time, whereas higher values indicate more irregular germination. The analysed population showed similar values (*P*>0.05) in the range from 31.79 to 37.31 (Table 2).

The germination index (*GI*) describes the germination percentage/speed relationship (Kader, 2005). A higher germination index value denotes higher germination percentage and germination rate (Bench et al., 1991), whereas higher germination rate values indicate higher and faster germination. Analysis of variance revealed significant differences in the germination index of analysed populations (*P*<0.01). The highest germination index was found in the population P4 and P2, followed by P3 and P1 (Table 2).

Germination uniformity is defined by the measurement of the synchronization index (*Z*) and the uncertainty (*U*) associated to the distribution of the relative frequency of germination. The analysis of variance did not show any significant difference in synchrony of the germination process in populations and priming treatments.

Table 3. Effect of priming treatments on germination characteristics of Dalmatian pyrethrum seed

Priming treatment	*G* (%)	$\bar{t}$ (days)	*MR*	CV_t (%)	*U* (bit)	*Z*	*GI*
KNO_3	17.00ab	14.19	0.08	40.91	1.11	0.07	0.36b
dH_2O	23.50a	12.78	0.08	30.62	1.45	0.21	0.59a
Control	12.75b	12.30	0.09	30.06	0.98	0.23	0.31b
P(F)	*	ns	ns	ns	ns	ns	*

G - germinability; $\bar{t}$ -mean germination time; *MR*- mean germination rate; CV_t - coefficient of variation of the germination time; *U* - uncertainty of the germination process; *Z* - synchrony of the germination process; *GI* - germination index; ns - non-significant; * - significant at $P< 0.05$; ** - significant at $P< 0.01$; Values followed by the same letter in each column are not significantly different based on the Tukey test at 0.05 probability.

Table 4. Interaction effects of populations and priming treatments on germination index (*GI*) and the uncertainty of the germination process (*U*) of Dalmatian pyrethrum seed

Population	*GI*			*U* (bit)		
	dH_2O	KNO_3	Control	dH_2O	KNO_3	Control
P1	0.10 aA	0.11aB	0.22aA	0.25aA	0.50 aA	1.02 aA
P2	0.28 bA	1.10 aA	0.34 bA	0.88 abA	1.89 aA	0.38 bA
P3	0.41 aA	0.50 aAB	0.19 aA	1.66 aA	1.53 aA	0.98 aA
P4	0.65 aA	0.64 aAB	0.51 aA	1.64 aA	1.92 aA	1.55 aA

GI - germination index; *U* - uncertainty of the germination process; Average/mean values followed by the same lowercase letter are not statistically different within the same population; Average/mean values followed by the same uppercase letter are not statistically different within the same treatment.

However, there was a significant variation in the uncertainty of the germination process between populations and interaction population x priming treatment. The low *Z* values recorded for analysed populations (0.08-0.32, much less than 1) indicate a very low degree of synchrony between the seeds during germination. This lack of synchrony was further confirmed by the low *U* values observed, which suggest a low degree of germination spreading over time. Hence, germination of population P1 was the most concentrated in time when compared to other populations (Table 2). Furthermore, significant interaction population x priming treatment indicated that populations have different response to priming treatments (Table 4). Hydroprimed seed of population P2 presented more heterogenous germination, while seed treated with KNO_3 and untreated seed presented greater homogeneity.

Germinability and germination index were improved by priming with H_2O.For both parameters the significantly higher ($P<0.05$) values were observed (23.50% and 0.59, respectively) when compared to control (Table 3). One of the rare investigations dealing with the effect of priming treatments on seed of Dalmatian pyrethrum is that of Li et al. (2011). In their investigation hydropriming improved the germination of pyrethrum seeds and the authors recommend this technique as a practical and low costly method for improving germination in both saline and non-saline agronomic conditions.

Use of potassium nitrate (KNO_3) has long been known as a suitable chemical method for enhancing germination in different plant species and generally as a priming agent or germination media (McDonald, 2000; Shim et al., 2008). It produces a hormonal balance and reduces the concentration of the substances that inhibit germination. The effect of seed priming with KNO_3 on germination, seedling emergence and growth has been well documented. Aghilian et al. (2014) found that treatment with 2% KNO_3 improved the germination of marigold (*Calendula officinalis* L.) and artichoke (*Cynara scolymus* L.) as well as St John's wort (*Hypericum perforatum* L.) in dark conditions. Ocvirk et al. (2012) reported significant increase in the germination of chicory (*Cichorium intybus* L.) seed primed with KNO_3. Olmez et al. (2004) conducted a study investigating the effect of different chemical treatments on seed germination of caper (*Capparis ovata* Desf.). Treatment with 0.2% KNO_3 solution for eight hours after application of sulfuric acid(H_2SO_4) for 20 minutes showed the best efficacy to germinate caper seed. In our study germination parameters of seed primed with 0.2% KNO_3 were not significantly different ($P>0.05$) from those obtained with untreated seeds, although somewhat higher values of germination percentage (17%) and germination index (0.36) were found when compared to control. Among treatments no significant differences in mean germination times were observed ($P>0.05$), therefore the priming of Dalmatian pyrethrum seed did not result in its shortening (Table 3). Decrease in mean germination time of seeds primed with KNO_3 was observed in rapeseed (*Brassica napus* L.) (Ghassemi-Golezani et al., 2010), marigold (*Calendula officinalis* L.) (Karimi and Varyani, 2016) and *Quercus castaneifolia* C. A. Mey. (Hadinezhad et al., 2013). Whereas, hydropriming reduced mean germination time in sage (*Salvia officinalis* L.) (Dastanpoor et al., 2013) and green bell pepper (*Capsicum annum* cv. Goliath) (Uche et al., 2016).

For germination index, interaction of population and priming treatment was significant ($P<0.05$). Population P2 showed higher value of germination index in treatment with H_2Oin comparison to treatment with KNO_3 and control, while among populations P1, P3 and P4 there were no significant differences ($P>0.05$) between the treatments (Table 4).

Conclusions

Hydropriming can be recommended as one of the practical, safe, low-cost and effective method to promote germination, given that in this study it has shown the best results. Even though increase in germination parameters was not significant with 0.2% KNO_3, this treatment has improved germinability and germination index.

In future investigations different concentrations of KNO_3 should be tested as well as the duration time of the treatment. Also, for further research it is recommended to use the uniform seed lot, equalized by size, mass and specific weight. Other priming agents documented in literature and their combinations should also be tested for effectiveness in promoting seed germination.

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