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EFFECT OF SEED PRIMING TECHNIQUES ON GERMINATION PARAMETERS OF SAFFLOWER (*Carthamus tinctorius* L.)

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Summary: A useful approach for improving seed germination and seedling growth is a seed priming technique. Application of the priming technique enhances water absorption, causing activation of metabolic activities in the seed. The objective of this study was to evaluate the effect of seed priming on germination parameters of safflower and to compare different priming techniques: priming by soaking and priming on filter paper. The priming treatments included hydropriming (distilled water) and osmopriming with 0.1% and 0.5% solutions of KNO_3 for 8 and 16 hours. The experiment revealed significant difference between the priming treatments and the control. The highest germination (89.50%) was recorded within the priming treatments by soaking in the solution of 0.1% KNO_3 and priming on filter paper moistened with 0.5% KNO_3 for 8 hours. Considering germination index, mean germination time and time to 50% germination, the best results were obtained within hydropriming on filter paper for 16 hours. This study has shown that the priming techniques significantly improved germination parameters of safflower. Although priming on filter paper showed better results, the soaking technique – due to its simplicity, low cost and easiness of application – can be successfully used to improve germination parameters of safflower and increase the number of plants per unit of area and thus increase the seed yield per acreage.

Key words: germination parameters, priming techniques, safflower, osmopriming

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the oldest oilseed crops commercially grown for edible oil extracted from seeds. Although safflower is grown in around 60 countries around the world, it is one of the most unexploited crops with adaptability to be grown worldwide (Gilbert, 2008). Native to arid regions, it is a minor oilseed crop in terms of production today. Flowers are used for production of natural dyes for coloring cloth and carpets, as well as for medicinal purposes (Yau and Ryan, 2010). It is a highly branched plant with a deep root system adapted to arid and semi-arid regions. Leaves have many, long and sharp spines contributing to good drought and heat tolerance. Safflower seeds are a rich source of oil (35-40%), which is rich in unsaturated fatty acids, either linoleic (C18:2) or oleic (C18:1). Safflower oil has high potential in fuel industry, as biodiesel is becoming more important due to depletion of natural fossil fuel reserves. Because of high linoleic acid content (75-80%), safflower oil has good characteristics in low temperature environments (Meka et al., 2007).

Seed germination is one of the most important properties of the seed that affect the plant productivity, as it has significant effect on the following stages of the plant growth. In order to achieve high yields in annual crops, it is essential to have rapid and uniform field emergence, especially in adverse conditions. Ambika et al. (2014) reported that using good quality seed may increase the seed yield by 15-20% and the extent of this increase is directly proportional to the quality of the seed that is being sown. An important precondition for successful crop production, in terms of seed, is ability to germinate under low water availability. As previously reported by Jajarmi (2008), it is estimated that 25% of the world's agricultural lands are affected by water stress. One of the most important abiotic factors limiting seed germination and plant growth is water stress caused by drought and salinity (Janmohammadi et al., 2008; Khayatnezhad et al., 2010). Higher salt concentration in soil generates lower water potential and results in water deficit condition in plants. As reported by Mahajan and Tuteja (2005), the major ions involved in salt stress

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include Na^+, K^+, H^+ and Ca^{2+}. Being a meteorological term, drought is defined as combined interplay of reduced rainfall, and limited water availability with rise in temperature, resulting in more than 50% yield losses for most of the crops (Singh and Laxmi, 2015; Singh et al., 2015). A useful approach for improving the rate and the uniformity of seed germination and seedling growth is seed priming. Seed priming is a pre-sowing treatment that commonly implies two techniques: osmopriming and hydropriming. Osmopriming involves soaking seeds in osmotic solutions, such as polyethylene glycol (PEG), potassium nitrate (KNO_3) or sodium chloride (NaCl), while hydropriming is simply soaking seeds in water. The basis of osmopriming is reflected in the fact that controlled hydration of seed, by decreasing water uptake, will prevent the completion of germination (radical emergence), which will later result in enhanced seed germination under low water availability. Osmopriming is considered to be an economical and safe technique and can significantly improve seed and plant performance particularly in stressed conditions (Ashrafi and Razmjoo, 2010; Nabizadeh et al., 2012). As stated earlier, an appropriate priming treatment can improve seed germination, seedling quality and drought tolerance (Rahimi, 2013; Čanak et al., 2014). In one of the studies, Bijanzadeh et al. (2010) tested various priming treatments in rapeseed and concluded that seed priming is an enhanced technique for improving the overall germination and seedling emergence. In a comprehensive study by Espanany et al. (2016), it is concluded that seed priming also enhanced tolerance of seedlings to toxic metals such as cadmium. Moreover, several previous studies of priming in safflower were performed by soaking seeds in different solutions, such as KNO_3, PEG, NaCl and KCl, as well as different priming durations (Elouaer and Hannachi, 2012; Elouaer et al., 2014; Kandil et al., 2016). On the other hand, different priming techniques, such as priming on moistened filter paper, were successfully applied in Fendler's bladder pod and cucumber (Windauer et al., 2007; Ghassemi-Golezani and Esmaeilpour, 2008).

This study was conducted in order to evaluate the effect of seed priming on germination parameters of safflower and to compare different seed priming techniques.

MATERIAL AND METHODS

Safflower seed was provided from the collection of oilseed species of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The basic data for the genotype used in this study are: oil content - 21%, protein content - 17%, days to flowering - 53, days to harvest – 114.

Maximum duration time for seed priming was tested in Petri dishes containing 50 seeds in 4 replicates. Seeds were placed on moistened, double layered filter paper (Whatman) at 25 °C. Radicle appearance was checked every hour. After 18 h, radicle appearance was recorded in few seeds and accordingly 16 h was chosen as maximum duration of seed priming.

Laboratory experiment was carried out at the Industrial Crops Department of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Seeds were initially surface sterilized by soaking in 0.1% solution of sodium hypochlorite for 5 minutes, followed by three rinses with distilled water and dried for two days at room temperature. Seed priming was performed by applying two different techniques: (1) by soaking seeds in cups with different priming solution, and (2) by placing seeds on filter paper moistened with priming solution. Priming treatments included hydropriming and osmopriming. Osmopriming included potassium nitrate (KNO_3) solutions of 0.1% and 0.5%. After priming, seeds were thoroughly rinsed with tap water and then dried out at room temperature for three days. Two durations of priming were tested (8 h and 16 h). Priming was conducted in the dark at 25 °C. Unprimed, sterilized seeds were used as the control.

Germination was tested in Petri dishes (15 cm diameter), on double layered filter paper moistened with 10 ml of distilled water, in 4 replicates with 50 seeds. Seeds were left to germinate in the dark at 25 °C for seven days (relative humidity (RH) - 65%). Germination was recorded every 24 h. Seed was considered germinated when the radicle was 2 mm long (ISTA, 2009). Germination (G) was recorded on 7-th day of counting.

Germination index (GI) was calculated according to formula:

GI = No. of germinated seeds / Day of first count + … + No. of germinated seeds / Day of final count

Mean germination time (MGT) was determined with formula:

$MGT = \sum Dn / \sum n$

where *D* is the number of days counted from the beginning of germination and *n* is the number of seeds that germinated on day *D*.

Time to 50% germination (T_{50}) was calculated according to the formula:

$T_{50} = t_i + (N/2 - n_i)(t_j - t_i) / (n_j - n_i)$

where *N* is the final number of germinating seeds, n_j and n_i are the cumulative numbers of seeds germinated by adjacent counts at times t_j and t_i, respectively, when $n_i < N/2 < n_j$.

Data analysis

For statistical analysis software STATISTICA 10 was used. Data were analyzed using one factorial analysis of variance. The differences between the treatments were compared using Duncan's multiple range test ($p<0.05$).

RESULTS

By comparison of the effect of the priming treatments on germination parameters of safflower, analysis of variance showed that there were significant statistical differences between the priming treatments. Regarding the germination parameters, the priming treatments showed statistically higher differences within germination index (GI), mean germination time (MGT) and time to 50% germination (T_{50}) (Table 1).

Table 1. Analysis of variance of germination parameters of safflower

Germination percentage (G)				
Source	**DF**	**SS**	**MS**	**F**
Treatment	12	1308.77	109.06	2.10*
Residual	39	2025.00	51.92	
Total	51	3333.77		
Germination index (GI)				
Treatment	12	6570.2	547.5	5.18**
Residual	39	4121.5	105.7	
Total	51	10691.7		
Mean germination time (MGT)				
Treatment	12	0.173891	0.014491	6.18**
Residual	39	0.091399	0.002344	
Total	51	0.265290		
Time to 50% germination (T_{50})				
Treatment	12	0.334424	0.027869	6.15**
Residual	39	0.176767	0.004532	
Total	51	0.511191		

*=p<0.05; **= p<0.01

Experimental results showed that some of the priming treatments significantly improved the germination parameters of safflower seed compared to the control (Table 2).

Table 2. Germination parameters of safflower seed under different priming treatments

Treatments	**h**	**G (%)**	**GI**	**MGT (days)**	**T_{50} (days)**
Control		72.50**b**	110.70**d**	4.56**a**	1.59**a**
Technique 1 (soaking)					
Hydropriming	8	88.00**a**	143.50**abc**	4.49**abc**	1.49**abc**
	16	80.00**ab**	134.20**c**	4.46**bcd**	1.47**bcd**
0.1% KNO_3	8	89.50**a**	143.50**abc**	4.50**abc**	1.52**abc**
	6	86.50**a**	139.40**bc**	4.50**abc**	1.52**abc**
0.5% KNO_3	8	80.50**ab**	126.60**c**	4.52**ab**	1.54**abc**
	16	87.00**a**	141.30**abc**	4.49**abc**	1.50**abc**
Technique 2 (filter paper)					
Hydropriming	8	85.50**a**	136.90**c**	4.50**abc**	1.52**abc**
	16	83.00**ab**	156.80**a**	4.34**e**	1.27**e**
0.1% KNO_3	8	89.00**a**	138.30**c**	4.54**ab**	1.56**ab**
	16	87.50**a**	155.20**ab**	4.40**de**	1.38**d**
0.5% KNO_3	8	89.50**a**	141.00**abc**	4.52**ab**	1.53**abc**
	16	78.00**ab**	133.00**c**	4.44**cd**	1.43**cd**
$LSD_{0.05}$		11.27	14.70	0.07	0.10

h – priming duration (hours); **G** – germination percentage (%); **GI** – germination index; **MGT** – mean germination time (days); **T_{50}** – time to 50% germination (days); Values with the same letter(s) are not significantly different

For more illustrative presentation of the effects of the priming treatments, experimental results are presented in Figure 1. The highest germination percentage (G) of 89.50% was recorded within the treatments priming by soaking in the solution of 0.1% KNO_3 and priming on filter paper moistened with 0.5% KNO_3 for 8 hours. Both priming treatments showed significant differences compared to the control but did not differ significantly from other priming treatments. Non-primed seeds (the control) had the lowest G (72.50%). Significant improvement in germination index (GI) was present in all priming treatments compared to the control. The seed hydroprimed for 16 hours on filter paper had the highest GI (156.80). In respect of mean germination time (MGT) and time to 50% germination (T_{50}), the most effective priming treatment was also hydropriming on filter paper for 16 hours (4.34 and 1.27 days). In addition to the mentioned parameters, significantly better treatments, compared to the control, were hydropriming by soaking and priming on filter paper with 0.1% and 0.5% KNO_3 solution for 16 hours. Non-primed seeds, i.e. the control, had the least favorable germination parameters. When comparing the priming techniques (soaking vs. filter paper) in terms of G, there were no significant differences between the priming treatments. Regarding GI, significant differences were observed in the treatments hydropriming and priming with 0.1% KNO_3 for 16 hours in favor of filter paper. In terms of MGT and T_{50}, significant difference was also determined between the treatments hydropriming and priming with 0.1% KNO_3 for 16 hours in favor of filter paper.

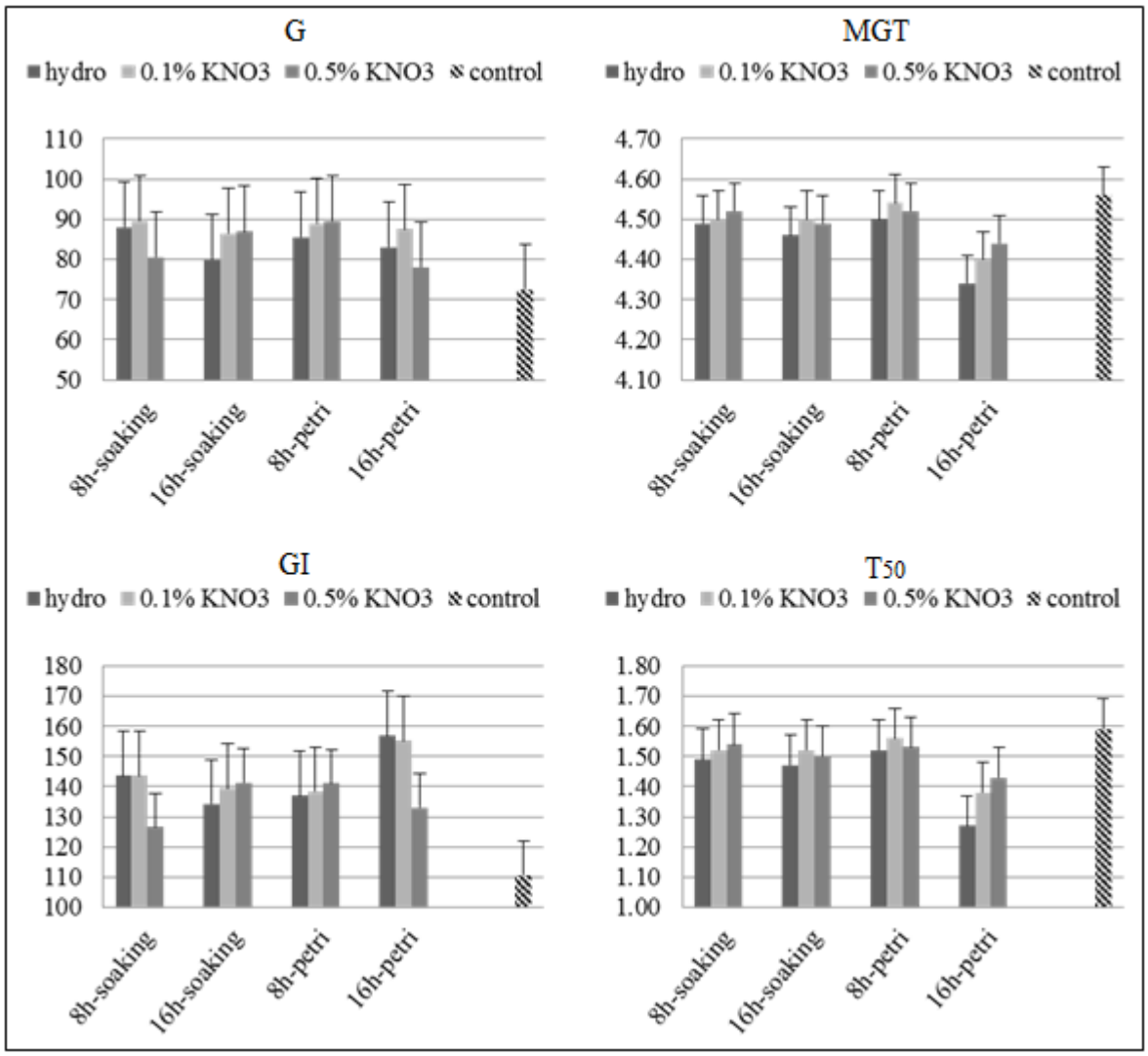

Figure 1. Effect of priming treatments on safflower seed germination parameters (Bars on the columns present $LSD_{0.05}$)

DISCUSSION

Germination (G) is a very important parameter for plant establishment and crop uniformity especially in unfavorable conditions. Unequal germination and emergence, caused by numerous factors, have adverse effect as seedlings are exposed to soil pathogens and unfavorable conditions for a longer period. It is known that osmopriming activates mechanisms related to germination through enhanced activity of enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and increase respiratory capacity which enhances energy (ATP) and assimilates supply (Ghasemi et al., 2014). Confirming our results on beneficial effect of priming, Eskandari et al. (2014) previously reported that hydropriming and osmopriming with KNO_3 improved germination properties of lentil under salt and drought conditions. Recent studies by Miladinov et al. (2015) and Ruttanaruangboworn et al. (2017) highlighted beneficial effects of KNO_3 priming treatments on germination in soybean and rice, respectively. Positive effect of 0.5% KNO_3 solution on MGT and T_{50} in maize was recently reported by Čanak et al. (2016). Significant improvement in MGT is due to increased and synchronized seed germination as a result of osmopriming, which enhanced stimulation of internal regulatory metabolism. In earlier studies by Patane et al. (2009) and Vasconcelos et al. (2017), it is stated that priming treatments are used to improve germination, emergence and seedling growth especially in unfavorable conditions. Although in the saline solution the water uptake is decreased by generated external osmotic potential, our study showed that KNO_3 solutions had stimulative influence on germination (G). Čanak et al. (2014) stated that seed priming is useful for overcoming problems related to poor germination as well as seedling establishment in drought conditions. All priming treatments used in this study significantly improved germination index (GI), which is very important especially in stressed conditions because higher GI means that less time is needed for germination. Seeds having greater GI are considered to be more vigorous. In a previous study on malting barley, Frančáková and Líšková (2009) stated that germination index is by far the best predictor for dormancy depth. Priming treatments improved mean germination time (MGT) due to stimulative influence on initiation of primary metabolic processes. Priming treatments increase antioxidants in seed, such as glutathione and ascorbate, which reduce lipid peroxidation activity and increase germination speed. MGT and T_{50} are especially important on sandy soils which rapidly dry out because faster germination gives chance to seedlings to absorb more water. The stimulatory effects of seed priming are a result of the completion of metabolic activities in a pre-germination stadium, which makes the seeds ready for radicle protrusion. By comparing two priming techniques, this study has shown that the best results for most of the evaluated germination parameters were recorded on filter paper. This can be explained by the fact that seed on filter paper had better aeration. However, the advantage of the soaking method compared to filter paper can be in the fact that it is faster, cheaper and easier because it does not require laboratory equipment such as Petri dishes and filter paper.

CONCLUSION

Bearing in mind the aforementioned results, this research has shown that the seed priming techniques enhanced germination parameters of safflower. Most priming treatments significantly improved germination percentage (G). Considering GI, MGT and $T_{50,}$ the best results were obtained by hydropriming for 16 hours on filter paper. Although priming on filter paper showed better results, the soaking technique – due to its simplicity, low cost and easiness of application – can be successfully used to improve germination parameters of safflower and improve plant establishment and thus increase seed yield.

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EFEKAT TEHNIKA POTAPANJA SEMENA NA PARAMETRE KLIJAVOSTI ŠAFRANJIKE (*Carthamus tinctorius* L.)

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Izvod: Koristan pristup za poboljšanje klijavosti semena i rasta klijanaca jeste tehnika potapanja semena. Primena tehnike potapanja poboljšava apsorpciju vode što dovodi do aktiviranja metaboličkih aktivnosti u semenu. Cilj ovog istraživanja bila je ocena efekta potapanja na parametre klijavosti semena šafranjike, kao i poređenje različitih tehnika potapanja, potapanje kvašenjem i potapanje na filter papiru. Tretmani potapanja uključivali su potapanje vodom (destilovana voda) i potapanje u prajmere sa 0,1% i 0,5% rastvorom KNO_3, tokom 8 i 16 časova. Eksperiment je pokazao značajne razlike između tretmana potapanja i kontrole. Najviša klijavost (89,50%) zabeležena je kod tretmana potapanja kvašenjem u 0,1% rastvoru KNO_3 i potapanje na filter papiru nakvašenom sa 0,5% rastvorom KNO_3, tokom 8 časova. Uzimajući u obzir indeks klijanja, prosečno vreme klijanja i vreme do 50% klijavosti najbolji rezultati su dobijeni potapanjem vodom na filter papiru tokom 16 časova. Ova studija je pokazala da tehnike potapanja značajno poboljšavaju parametre klijavosti semena šafranjike. Iako je potapanje na filter papiru pokazao bolje rezultate, tehnika potapanjem zbog jednostavnosti, niskog troška i lakoće primene može se uspešno koristiti za poboljšanje parametara klijavosti šafranjike kao i da unapredi broj biljaka po jedinici površine a na taj način i poveća prinos semena po površini.

Ključne reči: parametri klijavosti, tehnike potapanja, šafranjika, potapanje u osmotske rastvore

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