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Research Article

Seed germination prediction of osmotic-stressed safflower (*Carthamus tinctorius* L.) at different temperatures using hydrotime analysis

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DOI: 10.22099/IAR.2021.39039.1417

ARTICLE INFO

Article history:

Received 19 November 2020

Accepted 8 July 2021

Available online 11 October 2021

Keywords:

Carthamus tinctorius L.

Germination rate

Hydrotime constant

Water potential

ABSTRACT- The hydrotime analysis can predict the germination of plants at reduced water potential (ψ). The effects of reduced ψ on seed germination properties in three safflower cultivars including Padideh, Faraman and Isfahan cultivars at 10, 20 and 30 °C were analyzed using the hydrotime model. Five ψ values (0, -0.3, -0.6, -0.9 and -1.2 MPa) prepared in polyethylene glycol 6000 (PEG) solutions were used in the experiment. The results indicated that all three tested cultivars had the highest germination rate at 30° C and better tolerance to osmotic stress at 20° C. At 20° C, Faraman cultivar had the lowest medium base water potential [$\psi_{b(50)}$]. The standard deviation of ψ_b (σ_b) among cultivars and temperatures could be as high as 0.52 MPa for the Faraman cultivar at 20°. Three tested cultivars had statistically different hydrotime constant (H_t) at 10° C. The lowest $\psi_{b(50)}$ for achieving 50% germination at 10° C was seen in the Isfahan cultivar, and at either 20° C or 30° C was seen in Faraman and Padideh cultivars. The suppressing effect of drought stress on seed germination was greater in Padideh and Faraman cultivars than that in the Isfahan cultivar. Overall, applying of hydrotime model allowed identifying osmotic stress-tolerant cultivars such as Isfahan during germination; and which might be helpful to seed germination prediction and providing more accurate information for sowing time of safflower.

Abbreviations: ψ (water potential); PEG (polyethylene glycol); $\psi_{b(50)}$ (medium base water potential); H_t (hydrotime constant); σ_b (standard deviation of ψ_b); MGT (mean germination time); FGP (final germination percentage).

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated industrial crops, usually grown to produce flowers and oil which is used for coloring, flavoring foods, medicinal properties, dyes, and livestock feed. Despite being underutilized, safflower is gaining attention due to its desired characteristics including its tolerance to drought, salt and heat (Hussain *et al.*, 2015). It has been reported that increasing environmental limitations such as abiotic stresses adversely influence crop productivity (Mittler, 2006; Wu *et al.*, 2011). Incidence, severity and duration of drought could be very unpredictable among abiotic stresses (Chinnusamy *et al.*, 2005). Therefore, in dryland agroecosystems safflower might be a promising

alternate crop due to its insignificant reduction of oil and seed yield under drought stress (Kar *et al.*, 2007).

Safflower is sensitive to water deficit during germination, flowering and seed filling stages. Unfavorable soil moisture at sowing in semi-arid environments where safflower is widespread, often severely affects seed germination and the following growth stages (Mwale *et al.*, 2003; Okcu *et al.*, 2005). Tabatabaei and Ansari (2017) declared that safflower germination was severely decreased under water deficit conditions. Seed germination, a crucial stage in plant development which considered as crop productivity determinant, is the result of a sequence of events governed by water uptake from the soil (Hodge *et al.*,

2009). Thus, the successful crop establishment in semiarid areas depends on quick and uniform germination, which is strictly correlated to the ability of seeds to germinate at low water conditions (Arjenaki *et al.*, 2011). Several external factors determine both the speed and rate of the germination of plant seeds (Krichen *et al.*, 2014). Among these, the temperature is the environmental factor that influences water uptake and the physiological and biochemical process that leading to the onset of germination (Taiz and Zeiger, 2010). There is a positive linear trend between the number of germinated seeds and increasing temperature up to an optimum level, reaching a peak and after that a negative linear reaction (Steinmaus *et al.*, 2000; Bradford, 2002). In addition, the interaction of water and temperature often regulate seed germination, so that drought stress along with high temperature could constrain crop establishment in the field. Therefore, understanding the specific interaction between temperature and drought stress is fundamental to specify the factors controlling seed germination rate and germination time under field conditions. Despite the detrimental effect of drought stress on seed germination, it might generally be reduced at optimum temperature while sub and supra-optimal temperatures decreased germination rate in various species (Khan *et al.*, 2002; Patane *et al.*, 2016).

On the other hand, the effects of water stress strictly depend on the kind of plant species and cultivar. Thus, the selection of cultivars with a better drought tolerance is crucial in dry regions (Tuberosa and Salvi, 2006). However, simulation of uniform and controlled drought in the field is a critical issue that cannot be easily achieved (Shaheen and Hood-Nowotny, 2005). Moreover, a suitable method for selecting a considerable number of genotypes is required to accelerate the development of drought-resistant-cultivars. Using natural field conditions is difficult to gain relevant results because rainfall can eliminate or interfere with water deficits. Therefore, *in vitro* drought screening methods could facilitate the selection of the drought-resistance genotypes. (Pataneh *et al.*, 2006 and 2016). Khakwani *et al.*, (2011) investigated the effect of drought stress on six wheat varieties. The results demonstrated that screening of drought-tolerant genotypes *in vitro* and in field conditions was similar.

To evaluate the influence of drought stress during the germination phase, exposure to polyethylene glycol (PEG-6000) solutions is an effective method to mimic drought stress without metabolic interferences taken up by the plant. PEG is an inert, and non-ionic organic compound extensively used to simulate drought stress during seed germination (Muscolo *et al.*, 2014). It has been shown that PEG maintains uniform low water potential throughout the experiment (Tabatabaei and Ansari, 2017). As PEG (6000) has a high molecular weight, it cannot enter the plant through cell walls. It has been noted that PEG-based *in vitro* screening is an appropriate and accurate method for screening large sets of plant genotypes (Kulkarni and Deshpande, 2007). Khodarahmpour (2011) demonstrated that increasing PEG concentration in the imbibition solution could

induce a decline in seed vigor, germination rate, and root and shoot growth in crop plants.

In drought stress environments, the water needed for germination is available for only a short time and, consequently, successful crop establishment depends not only on rapid and uniform germination of the seeds but also on its ability to germinate under low water availability. All of these attributes can be analyzed through the hydrotime model. The main aim of the present study was to evaluate the influence of PEG-induced drought stress on seed germination of the three safflower cultivars at three different temperatures and prediction of seed germination using hydrotime model.

MATERIALS AND METHODS

Three safflower cultivars including Padideh, Faraman and Isfahan were provided from the Agriculture and Natural Resources Research Center of Darab, Fars Province, Iran. Padideh is a spiny winter cultivar with tolerance to low temperature, while Faraman is a spineless winter-grown cultivar. Also, the Isfahan is a spineless spring cultivar. Seeds were surface-sterilized in a 1% sodium hypochlorite solution, rinsed in distilled water and dried before the experiment.

Seeds were germinated under five levels of water potential of the imbibition solution (): 0.0 (control), -0.3, -0.6, -0.9 and -1.2 MPa. A range of osmotic potential solutions was produced using polyethylene glycol (PEG 6000) according to Michel and Kaufmann (1973). The water used in solutions was deionized and then autoclaved before use. Filter paper and seeds were sterilized as described in Kebreab and Murdoch (1999). Samples of 200 seeds (four replicates of 50 seeds each) set on top of two layers of filter paper (Whatman No. 1, 9 cm circles) that placed in 9 mm diameter Petri dishes. The dishes were moistened with 7 ml of the appropriate solution of PEG to reach to conditions for the seed germination at different levels of osmotic stress. The dishes were then sealed with Parafilm and incubated at 10°, 20° and 30° C. The experiment was conducted once in a completely randomized design (CRD) with four replicates. Germination counting was performed periodically and was stopped 24 hours after germination percentage remained steady. The dishes were sealed again and immediately put back to their original places. At the end of the experiment, the following indices were calculated:

$$\text{Germination index (\%)} = (100G_i/nt_i)$$

where n is the number of seeds in each treatment (50 each replicate) and G_i is the number of seeds germinated on t_i (day) (Rozema, 1975)

$$\text{Mean germination time (MGT)} = \sum ni \times di / \sum ni$$

where n is the number of seeds germinated at day i, di is the incubation period in days and n is the total number of germinated seeds (Orchard, 1977)

$$\text{Final germination percentage (FGP) (\%)} = (n/n_i) \times 100$$

where n marks the number of germinated seeds and n_i is the total number of seeds in each treatment (50 seeds) (Ren and Tao, 2004).

Bradford (1990) formulated a hydrotime model to illustrate the pattern of seed germination in response to . The hydrotime model can be defined as follows:

$$H = [- b_{(g)}] t_g \tag{1}$$

where H is the hydrotime constant (MPa h) that should be accumulated above base water potential to reach 50 % germination, ψ is the actual water potential of the germination medium (MPa), $b_{(g)}$ is threshold or base (MPa) that permits germination of a fraction g of seed lot, t_g is the time (h) to germination of fraction g .

If H is a constant, then t_g will increase as ψ is declined, i.e., t_g is inversely proportional to the difference between actual and $b_{(g)}$ (Bradford, 2002). Change in time to germination within a seed lot is strictly associated with a variation in base water potential among seeds. Therefore, a g fraction of the seed population germinates when the seeds have accumulated H units above their base water potential $b_{(g)}$. The germination time course at any ψ may be described as follows:

$$\text{Probit}(g) = [- (H/t_g - b_{(50)}) / b] \tag{2}$$

where H is the hydrotime constant (MPa h), ψ is the actual water potential of the germination medium (MPa), $b_{(g)}$ is the base (MPa) permitting germination of a fraction g of seed lot, t_g is the time (h) to germination of fraction g and b is the standard deviation of b . To estimate the values of H , $b_{(50)}$, and b probit regression analysis was applied to fit the time courses to the hydrotime model (Bradford, 1990) via a SAS protocol and a nonlinear program. Mean comparison of H , $b_{(50)}$, and b of the three safflower cultivars at three different temperatures was done by t-test, at 5% probability level using Minitab 16.1.0.0 program.

Germination percentage and FGP, arcsin transformed previously, MGT and germination index (GI) were analyzed statistically by a three-way analysis of variance (ANOVA) using SAS package (SAS9.1.3, SAS Institute Inc.). Temperature, water potential, and cultivar were considered as fixed factors. When F ratios were significant, the differences between means were compared using the Duncan test ($P < 0.05$). The relationship of germination rate ($1/t_g$) and water potential and cumulative germination time-courses of three safflower cultivars at different water potentials and temperatures of 10, 20 and 30° C were drawn using SIGMAPLOT 9.0 (software Systat Software Inc., San Jose, CA, USA).

RESULTS

Final Germination Percentage (FGP)

The cultivar, temperature, water potential and their interactions had significant effects on seed germination of safflower (Table 1). The highest germination percentage over different water potentials and temperature regimes was obtained in the Isfahan cultivar (Table 2), whereas the germination percentage of Padideh and Faraman cultivars was significantly lower than that of the Isfahan cultivar. Mean comparison showed that the percentage of germination increased significantly from 10° C to 20° C and then reached to the highest level at 30° C. Seed germination progressively declined with decreasing water potential and there was a significant reduction from -0.6 MPa to -1.2 MPa.

Seed germination which was exceeding 91.77% in all cultivars (Table 2) was not statistically affected by decreasing water potential from 0 MPa down to -0.6 MPa, but mean germination to reach a specific percentage was delayed (Fig. 1). The decreasing water potential to -1.2 MPa negatively affected the final cumulative germination percentage. The adverse effect of increasing level of drought stress was less evident in the Isfahan cultivar with final seed germination of 96.3 and 90.3% at -0.9, and -1.2 MPa, respectively (data not shown). The three-way interaction effect of cultivar, temperature and water potential on FGP was significant, indicating that the supremacy of the Isfahan cultivar over Faraman and Padideh cultivars was not proper at all combinations of temperature and water potential. The highest FGP was observed for the Isfahan cultivar in control condition at 20° C. The lowest FGP was related to Faraman and Padideh cultivars at -1.2 MPa at 10° C (Table 2).

Germination Index

The cultivar, temperature, water potential and the interaction of main factors had significant effects on the germination index of safflower seed (Table 1). The germination index was highest in the Isfahan cultivar, followed by Padideh and Faraman cultivars, respectively. Overall, the germination index increased with rising temperatures, reaching the highest at 30° C. The germination index decreased with decreasing water potentials, reaching the lowest level at -1.2 Mpa (Table 3).

Table 1. Three-way ANOVA of effects of cultivar, temperature and water potential on germination percentage, germination index and MGT of safflower

Source	df	Mean square		
		Germination percentage	Germination index	MGT
Cultivar	2	2855.381****	10153.783****	17.824****
temperature	2	781.678****	29850.218****	100.005****
Water potential	4	5362.542****	25512.945****	71.387****
cultivar × temperature	4	234.869****	758.175****	2.235****
cultivar × water potential	8	561.855****	2116.377****	0.849****
temperature×water potential	8	684.270****	226.251****	4.706****
cultivar×temperature×water potential	16	288.752****	101.648****	0.410****

, * Significant at $P < 0.001$ and < 0.0001 , respectively

Table 2. Water potential, temperature, cultivar and three-way interaction effects on cumulative germination percentage.

cultivar	temperature	Final cumulative germination (%)					Final cumulative germination (%)		
		(MPa)							
		0	-0.3	-0.6	-0.9	-1.2			
Faraman	10° C	97.5 ^{a-f}	96.5 ^{a-f}	96.0 ^{a-g}	90.5 ^{d-j}	15.0 ^l	84.9 ^b	10° C	Temperature
Faraman	20° C	94.0 ^{a-h}	86.0 ^{g-j}	88.5 ^{f-j}	90.0 ^{e-j}	82.5 ^{h-j}	93.43 ^a	20° C	
Faraman	30° C	95.0 ^{a-h}	91.0 ^{c-j}	94.0 ^{a-i}	79.5 ^j	21.0 ^l	85.53 ^b	30° C	
Isfahan	10° C	99.0 ^a	96.5 ^{a-f}	95.5 ^{a-g}	95.0 ^{a-h}	96.5 ^{a-f}			Faraman
Isfahan	20° C	99.0 ^a	99.0 ^a	98.0 ^{a-d}	97.5 ^{a-f}	93.0 ^{b-j}			
Isfahan	30° C	99.0 ^a	94.5 ^{a-h}	98.0 ^{a-c}	96.5 ^{a-f}	81.5 ^{ij}	81.13 ^c		
Padideh	10° C	97.5 ^{a-f}	96.5 ^{a-f}	96.0 ^{a-g}	90.5 ^{d-j}	15.0 ^l	86.83 ^b	Padideh	cultivar
Padideh	20° C	93.5 ^{a-i}	99.0 ^a	98.0 ^{ab}	96.0 ^{a-f}	87.5 ^{d-j}	95.9 ^a	Isfahan	
Padideh	30° C	99.0 ^a	94.5 ^{a-h}	98.0 ^{ab}	90.5 ^{d-j}	51.0 ^k			

Means by the same letter in each column do not differ by Duncan test at 5% probability performed among different temperatures, cultivars, water potentials and three-way interaction effects.

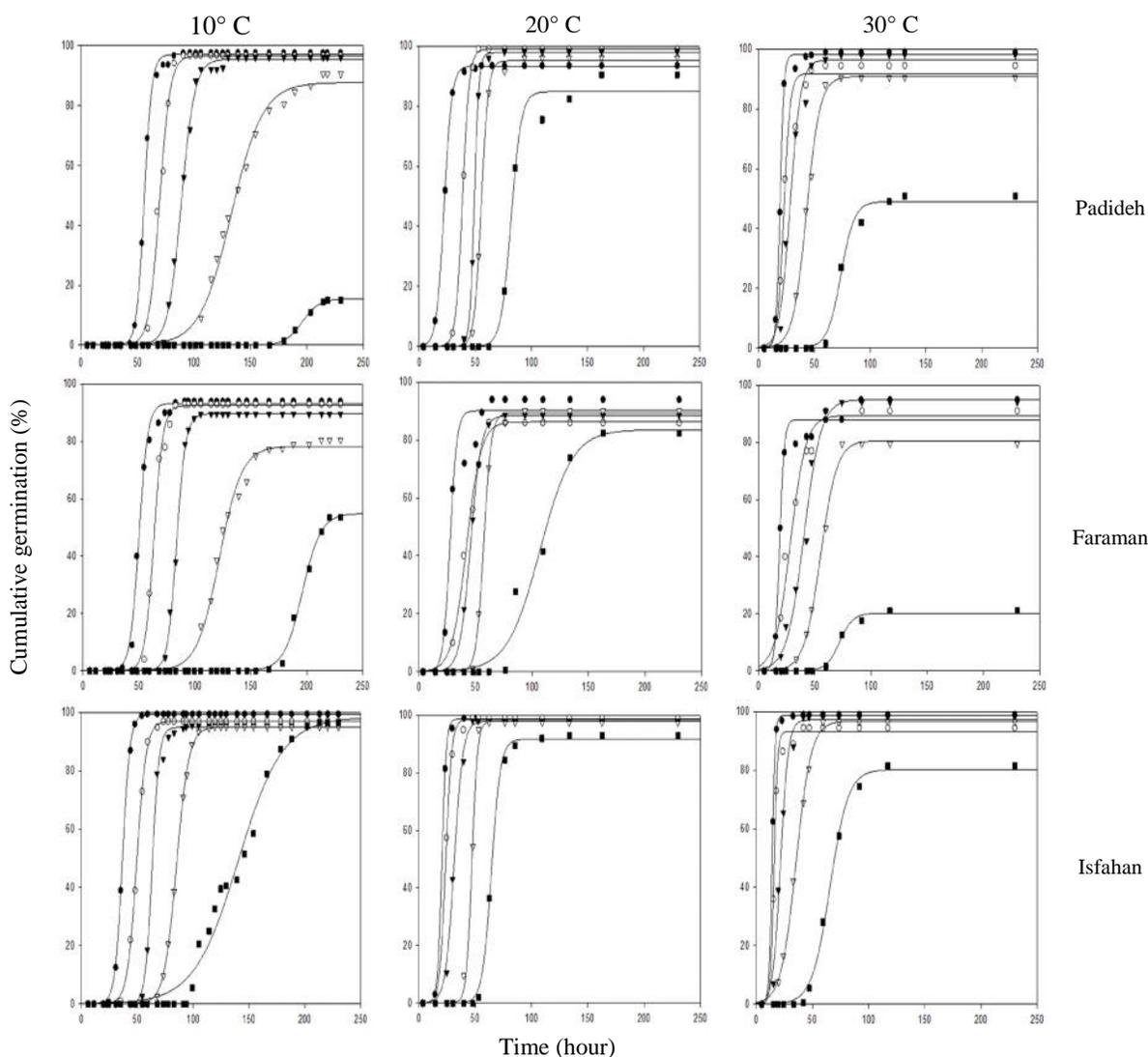


Fig. 1. Cumulative germination time-courses of three safflower cultivars at different water potentials of 0 (○), -0.3 (□), -0.6 (△), -0.9 (◇) and -1.2 (■) MPa and temperatures at 10, 20 and 30° C.

Table 3. Effect of water potential, temperature and cultivar on germination index

	Germination index				
	(MPa)	Temperature		cultivar	
0.0	84.917 ^a	10° C	27.44 ^c	Faraman	40.15 ^c
-0.3	65.24 ^b	20° C	53.40 ^b	Padideh	47.17 ^b
-0.6	52.72 ^c	30° C	71.83 ^a	Isfahan	65.36 ^a
-0.9	35.99 ^d				
-1.2	15.59 ^e				

Means by the same letter in column do not differ by Duncan test at 5% probability.

Mean Germination Time (MGT)

The cultivar, temperature, water potential and the interaction of main factors had significant effects on the MGT of safflower seed (Table 1). The MGT was highest in the Faraman cultivar, followed by Padideh and Isfahan cultivars, respectively. Overall, the MGT decreased with rising temperature, reaching the lowest at 30° C, while the MGT increased with decreasing water potentials, reaching the highest at -1.2 Mpa (Table 4).

Hydrotime Model Parameters

With increasing temperature, the value of θ_H decreased in the three tested cultivars. In other words, the germination rate was the highest at 30° C, followed by 20° C and 10° C, respectively. Moreover, three tested cultivars had statistically different θ_H values at 30, 20, and 10° C. At 10° C and 30° C, the Isfahan cultivar had the highest germination rate, while at 20° C the Padideh cultivar had the highest germination rate, followed by Isfahan and Faraman cultivars (Table 5). At 20° C the Faraman cultivar had the lowest $\theta_{b(50)}$ value followed by Isfahan and Padideh cultivars. On the other hand, at 10 and 30° C, the lowest $\theta_{b(50)}$ value was observed in the Isfahan cultivar (Table 5). Indeed, in the Padideh cultivar, $\theta_{b(50)}$ was not significantly different at the three temperatures tested in this study; however, it was significantly different in Faraman cultivar at the same temperatures. To be more specific, it was significantly lower at 20° C than the corresponding values calculated at 10 and 30° C in the Faraman cultivar. As to Isfahan cultivar, no significant difference was obtained between 10° C and 20° C; however, it was significantly higher at 30° C (Table 5).

The standard deviation of the base water potential (θ_b) was lowest at 10° C followed by 30° C and 20° C for all three tested cultivars. At 10° C the Padideh cultivar, had the lowest θ_b value. At 20° C the Isfahan cultivar had the lowest θ_b value. However, no significant difference was found between Isfahan and

Padideh cultivars at 20° C. At 30° C the Isfahan cultivar had the lowest θ_b value (Table 5). Comparison of the standard deviation of the base water potential revealed that the lowest amount at 10° C was related to cultivar Padideh, and at 20 and 30° C was in seeds of cultivar Isfahan. The lowest quantity of hydrotime coefficient at 10 and 30° C was related to the Isfahan cultivar, and at 20° C related to the Padideh cultivar (Table 5). Variations of $\theta_{b(50)}$ values within cultivars were generally higher at intermediate temperature (20° C) than those at lower (10° C) or higher temperature (30° C) (Table 5). θ_b among cultivars and temperatures can be as high as 0.52 MPa for the Faraman cultivar at 20° C to the lowest quantity of 0.18 MPa for the Padideh cultivar at 10° C. The value of $\theta_{b(50)}$ was highest at 20° C compared to either 10° C or 30° C (Table 5).

The Relationship of Water Potential with Germination Rate

The germination rate of each cultivar at each temperature was linear functions of water potentials, which well fit data of theoretical germination rate versus (Equ. 2). Fig. 2 illustrates the relationship of water potential vs. germination rate for the three tested cultivars of safflower. Cultivars, temperatures and their interactions had a significant effect on germination rate ($P < 0.001$; data not shown). Overall, the germination rate increased with rising temperatures, reaching the highest at 30° C. Among cultivars, the Isfahan cultivar gave the highest germination rate values at all three tested temperatures, with the highest germination rate at 30° C. In contrast, Padideh and Faraman cultivars had no significant difference in germination rate performance. The slope of the linear functions generally increased with increasing temperature in all three cultivars, although the germination rates in the Faraman cultivar at 10 and 20° C increased steadily from the onset of the experiment (Fig. 2).

Table 4. Effect of water potential (θ), temperature and cultivar on MGT.

	MGT				
	(MPa)	Temperature		cultivar	
0.0	1.46 ^c	10° C	4.22 ^a	Faraman	3.15 ^a
-0.3	1.83 ^d	20° C	2.24 ^b	Padideh	2.97 ^b
-0.6	2.28 ^c	30° C	1.80 ^c	Isfahan	2.13 ^c
-0.9	3.21 ^b				
-1.2	4.98 ^a				

Means by the same letter in column do not differ by Duncan test at 5% probability.

Table 5. Mean comparison (mean±SE) of H , $b(50)$, and b of the three tested safflower cultivars at three different temperatures.

		H (MPa h)	$b(50)$ (MPa h)	b (MPa h)
Padideh	10° C	83.66 ^a ±1.86	-1.495 ^b ±0.021	0.181 ^c ±0.008
	20° C	36.56 ^e ±3.86	-1.538 ^{bc} ±0.108	0.468 ^{ab} ±0.069
	30° C	28.64 ^e ±1.59	-1.527 ^b ±0.055	0.380 ^b ±0.028
Faraman	10° C	75.97 ^b ±2.26	-1.475 ^b ±0.028	0.243 ^d ±0.014
	20° C	62.23 ^c ±5.39	-1.908 ^d ±0.123	0.519 ^a ±0.062
	30° C	23.91 ^f ±1.62	-1.167 ^a ±0.051	0.409 ^b ±0.026
Isfahan	10° C	64.58 ^c ±1.78	-1.683 ^{cd} ±0.029	0.218 ^d ±0.013
	20° C	37.79 ^d ±2.55	-1.856 ^d ±0.86	0.337 ^{bc} ±0.037
	30° C	19.93 ^g ±0.92	-1.486 ^b ±0.038	0.301 ^c ±0.017

Means by the same letter in column do not differ by Duncan test at 5% probability.

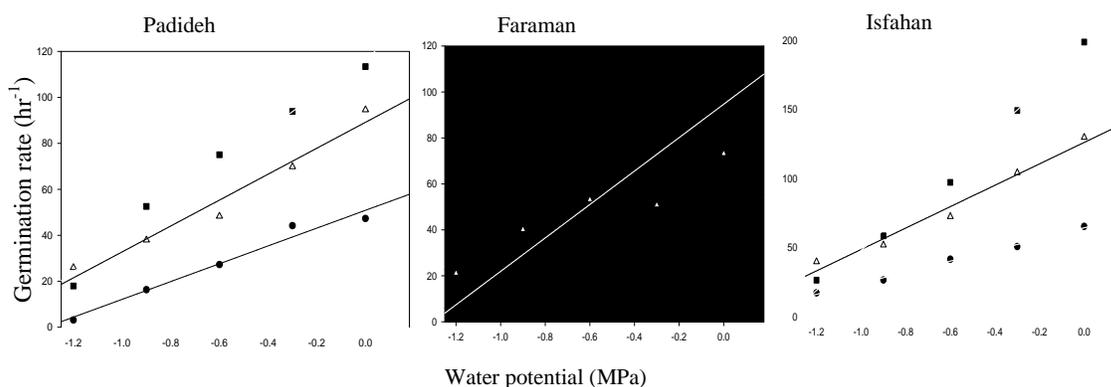


Fig. 2. Relationship between water potential () of seed imbibition solution prepared in polyethylene glycol (PEG) and germination rate of three safflower cultivars including Padideh, Faraman and Isfahan at 10° C (), 20° C () and 30° C (■). Each point represents the average of days and replicates.

Cumulative Germination Time-Courses

The time course function and characteristics of cumulative seed germination curves at different water potentials and temperatures are shown in Fig. 1. The curve trend revealed low germination in the initial phase, followed by a sharp rise up to a maximum germination rate, and finally germination stopped. The time required to achieve the maximum cumulative germination became longer as the water potential decreased. Prolongation of the initial phase of slow germination increased at lower and it was more noticeable in seeds germination (g) at lower water potentials.

DISCUSSION

This study aimed to recognize the tolerance of three safflower cultivars subjected to PEG-induced osmotic stress at three different temperatures as well as to determine their seed germination rate prediction using hydrotime model. The results of this study highlighted significant differences in seed germination rate among tested cultivars exposed to different s (Tables 1 and 2).

The Isfahan cultivar germinated in higher percentages of different s. In other words, the suppressing effect of drought stress on seed germination was more significant in Padideh and Faraman cultivars than in the Isfahan cultivar. It has been reported that germination, vegetative, flowering and seed filling stages of the sweet sorghum are severely affected under water deficit conditions (Patane *et al.*, 2012). It has been previously stated that safflower seed germination severely decreased under water deficit conditions (Hussain *et al.*, 2015). It could be concluded that genetic variability in seed germination under drought stress offers a useful tool to study drought tolerance mechanisms (Muscolo *et al.*, 2014).

The supremacy of the Isfahan cultivar could be attributed to greater capacity for osmotic adjustment allowing seed germination under stress conditions. It has been observed that seeds that remain at very low water potential, although they do not absorb the minimum amount of water, the germination process begins (Delachave and De Pinho, 2003). It has been approved that PEG solutions simulated drought stress conditions for seed germination (Smok *et al.*, 1993; Hu

and Jones, 2004; Toscano *et al.*, 2017). In the present study, the number of final germinated seeds fell gradually with decreasing water potential for each cultivar, as a result of reduced water uptake (Table 2). These results are in agreement with those of other studies reporting that high concentrations of PEG reduce the final seed germination percentage of safflower (Ostadian Bidgoli *et al.*, 2018; Pahlevani *et al.*, 2012).

Unpredictable rainfall and high evaporation rate in arid and semi-arid regions create the opportunity for seeds to germinate at different times during the growing season (Khan and Ungar, 1996). Using hydrottime model, the base, optimum and ceiling emergence temperatures of safflower were estimated to be 3.9, 39.3 and 45 °C, respectively (Ostadian Bidgoli *et al.*, 2018). Delayed germination had adverse fitness consequences, producing smaller plants with shorter life spans, fewer seeds, and lower total reproductive biomass. In this study, it was observed that the temperature of 20° C was the optimum temperature germination over the three cultivars, with no significant differences between 10° C and 30° C (Table 2). In a similar study on safflower, Torabi *et al.*, (2016) declared that the base water potential decreased with increasing temperature from 5 to 20 °C but increasing the temperature from 20 to 40 °C led to a reversed outcome. In addition, hydrottime value decreased with increasing temperature.

Careful and more close and different temperatures are needed to be investigated the effect of temperature on seed germination. In this study, it was found that the relationship between drought stress and the germination rate was well fitted by linear regression, indicating that osmotic limitation is important in determining the germination rate in safflower. Although germination percentage remained statistically uniform from 0 to -0.6 MPa (Table 2), a reduction in germination rate was observed (Fig. 2). This could be described by decreasing water potential in the mentioned range that delayed water uptake. However, at this condition the water content required for germination was adequate.

Germination rate at lower water potentials decreased rapidly, which might be a result of osmotic stress or reduced enzyme activities. Patane *et al.*, (2006) demonstrated that seed metabolic activity and consequently respiration rate at the early stages of seed imbibition are associated with seed water uptake. It has been suggested that adaptive strategies to prevent seed germination under drought stress conditions, e.g. accumulation of solute compounds, might be a reason for delayed or lower seed germination at higher drought stress during seed imbibition in PEG solution (Patane *et al.*, 2012).

Generally, the Isfahan cultivar showed the highest germination rate at all temperatures tested in this study, indicating a more tolerance of this safflower cultivar in terms of germination rate at the different levels of drought stress and higher temperature requirements during the germination phase (Fig. 2).

Variation in seed germination of different cultivars of safflower using the hydrottime model has also been reported by Tabatabaei and Ansari (2017). Genetic

differences in germination behavior under drought stress have also been reported in lentil genotypes (Muscolo *et al.*, 2014), sunflower (Toscano *et al.*, 2017), wheat (Alom *et al.*, 2016), and cowpea (Murillo *et al.*, 2002). The results of this study indicated changes in H_0 , $b_{(50)}$ and b_0 of safflower tested cultivars at different temperatures (Table 5).

The findings of this study are consistent with Bakhshandeh and Gholamhossieni (2019) findings who found variation in hydrottime parameters of radish and cantaloupe at different temperatures. In general, H_0 value was the lowest at 30° C and increased at both 10° C and 20° C in all three tested cultivars. This indicated that the three tested safflower cultivars had the highest germination rate at 30° C.

The value of $b_{(50)}$ indicates the stress tolerance during germination. The lowest base for achieving 50% germination at 10° C was found in seeds of the Isfahan cultivar, and at either 20° C or 30° C was found in seeds of Faraman and Padideh cultivars. So that at 10° C the Isfahan cultivar was the most tolerant cultivar and at 20° C and 30° C Faraman and Padideh cultivars were the most tolerant cultivars, respectively. Moreover, $b_{(50)}$ was lower at 20° C than the corresponding value calculated at 10 and 30° C in all three cultivars. This indicates a better tolerance of seeds of these cultivars to drought stress under 20° C.

Soltani and Farzaneh (2014) reported that, in cotton, the hydrottime constant for H_0 , ranged from 14.7 to 105.5 MPa h, $b_{(50)}$ varied from -1.15 to -0.48 MPa and standard deviation (b_0) ranged from 0.23 to 0.72 MPa. In a study on sweet sorghum, Patane *et al.*, (2016) declared that the predicted H_0 constant was increased when the temperature was declined to 15° C and at this temperature median base water potential for germination [$b_{(50)}$] was more (less negative) than that at 25° C. They concluded that the Keller cultivar exhibited a $b_{(50)}$ more negative but needed a greater H_0 for germination, and demonstrated a greater water-stress tolerance but lower germination rate compared to those of Makueni cultivar. Also, b_0 showed the germination synchrony within the seed population was lower at 15° C than that at 25° C and therefore demonstrated a more regular distribution of base water potential within the seed population at a lower temperature. Bakhshandeh *et al.* (2011), showed that in Velvetleaf plant (*Abutilion theophrasti* med.) and two soybean cultivars (DPX and Williams) seeds, the base water potential ranged from -0.9 to -0.6 MPa and hydrottime for 50% germination ranged from 1.4 to 10 MPa day (MPa d) and in all three plants, they noticeably raised in correspondence to a reduction of temperatures from 25 to 7 °C. Tabatabaei and Ansari (2017) reported that cumulative germination of safflower seed was higher in the Kouseh cultivar in comparison to Sina, Faraman, and Talaei cultivars. Also, hydrottime constant (H_0) in Sina, Faraman, Talaei, and Kouseh cultivars were 0.93, 0.84, 0.78 and 0.72 MPa d, and $b_{(50)}$ in Sina, Faraman, Talaei and Kouseh safflower cultivars were -0.56, -0.67, -0.64 and -0.77 MPa, respectively. Farzaneh and Soltani (2011) showed that the estimated values of $b_{(50)}$, b_0 , and H_0 differed in different sugar beet cultivars. The $b_{(50)}$ ranged from

1.30 (cv. H5505) to -1.67 (cv. 7233) MPa, and reduced from about 85 MPa d for cv.H5505 and cv. PP22 and to 100 MPa d for cv.7233. In the present study, the highest uniformity in germination at 10° C was observed in seeds of Padideh, while at 20° and 30° C, the Isfahan cultivar had the highest uniformity. In total, σ_b was the lowest at 10° C and increased at both 20° C and 30° C in all three tested cultivars. This indicated that the three tested cultivars had the highest uniformity in germination at 10° C (Table 5).

CONCLUSIONS

In the current study, it was demonstrated that safflower germination was affected by cultivar, temperature, and water potential parameters. The highest germination percentage occurred in the Isfahan cultivar and at 20° C. The differences

observed in germination behavior of the three tested cultivars might indicate the necessity of screening of more safflower genotypes for adaptation to sowings in the semi-arid areas. As well, the interaction effect between the investigated factors (cultivar, temperature, and water potential) was significant showing the differences in germination capacity of the cultivars in response to water availability and temperature. The results of this study also indicated that the hydrotime model might be helpful to predict seed germination and provide accurate information for the sowing time of safflower in specified regions.

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پیش‌بینی جوانه‌زنی بذر گلرنگ در شرایط تنش اسمزی و درجه حرارت‌های مختلف با استفاده از تحلیل هیدروتایم

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اطلاعات مقاله

تاریخچه مقاله:

تاریخ دریافت: ۱۳۹۹/۸/۲۸

تاریخ پذیرش: ۱۴۰۰/۴/۱۳

تاریخ دسترسی: ۱۴۰۰/۷/۱۹

واژه‌های کلیدی:

گلرنگ

سرعت جوانه‌زنی

ثابت هیدروتایم

پتانسیل آب

چکیده - تحلیل هیدروتایم، قادر به پیش‌بینی روند جوانه‌زنی با کاهش پتانسیل آب () می‌باشد. اثرات کاهش پتانسیل آب روی جوانه‌زنی بذر در سه رقم گلرنگ شامل رقم های پدیده، فرامان و اصفهان در دمای ۱۰، ۲۰ و ۳۰ درجه سانتی‌گراد از طریق مدل هیدروتایم واکاوی شد. در این آزمایش پنج پتانسیل آب () (۰، ۰/۳، -۰/۶، -۰/۹، -۱/۲- مگاپاسکال) که در محلول‌های پلی‌اتیلن گلیکول (PEG) ۶۰۰۰ تهیه شدند استفاده شد. نتایج نشان داد که هر سه رقم مورد آزمایش، دارای بیشترین سرعت جوانه‌زنی در دمای ۳۰ درجه سانتی‌گراد و تحمل بهتر تنش خشکی در دمای ۲۰ درجه سانتی‌گراد بودند. در دمای ۲۰ درجه سانتی‌گراد، رقم فرامان دارای کم‌ترین پتانسیل آب پایه متوسط (b(50) بود. در میان ارقام و دماهای مختلف، رقم فرامان دارای بیشترین انحراف معیار b (b) به میزان ۰/۵۲ مگاپاسکال در دمای ۲۰ درجه سانتی‌گراد بود. ثابت هیدروتایم (H) در دمای ۱۰ درجه بین سه رقم مورد آزمایش به طور معنی‌داری متفاوت بود. پایین‌ترین b(50) برای دستیابی به جوانه‌زنی ۵۰ درصد در ۱۰ درجه سانتی‌گراد، مربوط به رقم اصفهان بود و در دمای ۲۰ یا ۳۰ درجه سانتی‌گراد، پایین‌ترین مقدار مربوط به رقم فرامان و پدیده بود. اثر مهارکنندگی تنش خشکی روی جوانه‌زنی بذر در ارقام پدیده و فرامان بیشتر از رقم اصفهان بود. به‌طورکلی، کاربرد مدل هیدروتایم، امکان شناسایی ارقام متحمل به تنش اسمزی مانند رقم اصفهان را در طی جوانه‌زنی فراهم کرده و می‌تواند در پیش‌بینی جوانه‌زنی بذر و ارائه اطلاعات دقیق‌تر در خصوص زمان کاشت گلرنگ سودمند باشد.