Research Journal of Biology Sciences
Biyoloji Bilimleri Araştırma Dergisi
E-ISSN: 1308-0261,11(1): 38-41, 2018

Effects of Different Pre-Treatments on Wild Safflower (*Carthamus* spp.) Seed Germination

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Geliş Tarihi: 22 Mayıs 2018
Kabul Tarihi: 30 Temmuz 2018

Abstract
Within the framework of this study, we collected seeds from 40 locations for *C. dentatus* Vahl., 38 locations for *C. lanatus* L., 3 locations for *C. persicus* Willd., 11 locations for *C. glaucus* Bieb., 1 location for *C. tenuis* (Bois and Balansa) Bornm. However, this study was performed to determine differences between species. In this study, we aimed to determine the effects of gibberellic acid doses (0, 100, 300 and 500 ppm) and potassium nitrate doses (0, 100, 300 and 500 ppm) on germination and seedling growth of five safflower species. Germination ratio, root and shoot length, dry weight ratio were investigated.In this study, the best results in terms of germination ratios, which is the primary objective of the research, are attained from 300 ppm concentration of gibberellic acid for *C. dentatus*, 500 ppm gibberellic acid for *C. tenuis*, 500 ppm potassium nitrat for *C. glaucus* and *C. lanatus*, and 500 ppm gibberellic acid for *C. persicus*.
Keywords: Emergence percentage, gibberellic acid, Wild *Carthamus* spp., potassium nitrate.

INTRODUCTION

The Asteraceae family comprises about 20,000 species of herbaceous plants in more than 800 genera of oil seed (e.g. *Guizotia abyssinica* (L. f.), Cass), leafy vegetables (e.g. *Lactuca sativa* L., lettuce), edible tubers (e.g. *Helianthus tuberosus* L., Jerusalem artichoke), safflower (*Carthamus*), and other products (e.g. *Chrysanthemum cinerariaefolium* (Trev.), Bocc, pyrethrum).Traditionally the crop was grown for its seeds but results indicate that it has phytostabilization plants in remediation of Cd contaminated areas [1]. Seeds are achene and show orthodox storage behavior. In most genera, seeds normally exhibit dormancy, i.e. the failure of a seed to germinate even when environmental conditions permit germination. This can cause various difficulties in promoting seed germination, depending on both the species and accession.

There are two types of dormancy: embryo dormancy and impermeable testa dormancy. The latter occurs when water and oxygen are prevented from permeating the seed, while embryo dormancy occurs when the seed does not reach a sufficient morphologic and physiologic maturity level, or because of complicated biochemical determinants [2]. Cultivated safflower (*C. tinctorius L.*) are non-dormant. However, wild species e.g. *C. alexandrius, C. flavescens, C. glaucus, C. lanatus, C. oxyacantha*, and *C. palaestinus* may show considerable dormancy [3, 4, 5, 6, 7, 8].

Plant growth regulators stimulate seeds for germination or dormancy, with gibberellic acid (GA_3) playing a significant role in initiating germination by removing the effects of abscisic acid and expediting the hydrolysis of starch and storage proteins [9, 10]. When externally applied to seeds, GA_3 encourages germination by increasing the α-Amylase activity [11]. Furthermore, applying GA_3 stimulates polyploidy in the germination case, thus removing the necessity of pre-chilling the seeds [12]. Finally, GA_3 initiates germination by taking on the task of the environmental stimulants such as light or temperature and directly effects embryo growth by providing hydrolysis in the endosperm [13].

Partly-successful dormancy-breaking treatments exist for *C. lanatus*, such as pre-soak: 2-24hours, continuous light, 10^{-5} mol m^{-2} s^{-1}[14] for *C. oxyacantha*, such as constant temperatures: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, optimum at 15°-20°C, in dark [5]. Pre-chill: 0°C, 1 month [5]; 3°-5°C, 2-8weeks, then cut off tip of seed coat [6]. Polyethylene glycol: co-applied, -1, -2 bar [15]. However, successful dormancy-breaking treatments for *C. lanatus*: Pre-soak: 24hours, continuous light, 650 nm, 7×10^{-6} mol m^{-2} s^{-1}, germinate at 25°C in dark (Wright 1980). For *C. oxyacantha*: Pre-soak: 1-4 days, germinate at 18°C in dark [8]. Additionally, there are successful dormancy-breaking treatments for *C. lanatus* such aspre-soak: 24 hours, continuous light, 650 nm, 7×10^{-6} mol m^{-2} s^{-1}, germinate at 25°C in dark [14] and for *C. oxyacantha* such aspre-soak: 1-4 days, germinate at 18°C in dark [8]. Demir [16] reported that yield and oil rates of safflower cultivars varied significantly in various ecologies in connection with yield and oil rate parameters.

This study aimed to record the effects of GA_3 and potassium nitrate (KNO_3) on germination and seedling growth of five safflower species, which represent all wild safflower species cultivated in Turkey.

MATERIAL and METHODS

Trials were conducted in germination cabinets of laboratories at the Central Research Institute for Field Crops, Ankara, Turkey using seeds from five different species of wild safflower collected from natural habitat in Turkey. The analyses were built on coincidence parcels which were split separately for each species with three replicates, according to methods of [17]. Main parcels comprised applications of GA_3 and KNO_3 and lower parcels were composed of application dosages (0, 100, 300 and 500 ppm). Germination papers were used as the germination environment. In every germination paper 50 seeds were used and for the germination tests of each species 1200 seeds were used, for a total of 6000 seeds. Counting was done on days 4 and 14 to determine germination ratios. Seedling height, root length, and dry weight measurements were taken on the 14^{th} day. Germination ratios were converted into angle values but the real values of germination ratio were given in the charts and; discussions and comments were done through these values. All statistical analyses were made using MSTATC.

RESULTS

Germination Ratios: There was no change in germination ratio due to applications on day 4. On day 14, statistically significant importance doses of 0.05 for *C. dentatus*, importance doses of 0.01 on both days for *C. tenuis*, 0.05 on both days for *C. glaucus*, and insignificant effects on both days for *C. lanatus* and *C. persicus*. Moreover it is also recorded that with the effect of applications x dose interaction, changes such as 0.01 on the 4th and 0.05 on the 14th days in terms of importance doses for *C. tenuis*, on the other side an importance doses of change of 0.05 on both days for *C. glaucus* (Table 1).

Table 1. Variance analysis of the effects of different Gibberellic Acid and Potassium Nitrate doses on germination rate (%) at 4th and 14th day, seedling dry weight ratio(%), seedling root length(cm), and seedling length (cm) at 14th day in wild safflower species.

Source of Variation	DF	Mean square				
		4th day germination ratio (%)				
		C. dentatus	*C. tenuis*	*C. glaucus*	*C. lanatus*	*C. persicus*
Replication	2	132.7	8.7	155.2	268.7	2.2
Application	1	181.5	2904.0**	726.0*	32.7	28.2
Dose	3	4778*	2021.3**	1753.6**	3436.4**	5225.5**
Application *Dose	3	25.9	984.0**	306.4*	6	9.5
Error	14	60.7	43.7	45.1	66	71.9
Total	23	5178.7	5961.7	2986.2	3809.8	5337.2
CV		15.40	19.1	19.3	13.4	12.8
		14th day germination ratio (%)				
Replication	2	33.5	18.7	128.7	131.2	0.9
Application	1	522.7*	3360.7**	160.2*	24	0.3
Dose	3	4799.6*	1016.2**	2418.8**	2573.8**	5.7**
Application *Dose	3	82.2	449.1*	587.3*	86.7	0.2
Error	14	26.1	49.5	32.3	67	0.3
Total	23	5464	4894.2	3327.2	2882.6	7.3
CV			16.3	11.7	10	7.1
		14th day seedling dry weight ratio (%)				
Replication	2	10.6	6.7	0.5	1.1	21.5
Application	1	0.5	73.9	1611.4**	4.9*	65.0*
Dose	3	264.2*	163.2*	295.0**	2.9*	472.0*
Application *Dose	3	10.5	339.9*	303.1**	1.1	76.6**
Error	14	4.9	25	4	0.5	14.9
Total	23	290.6	608.7	2214	10.5	650.1
CV		17.0	50.5	16.8	16.8	26.4
		14th day seedling root length (cm)				
Replication	2	0.1	0.8	1.4	0.8	0.2
Application	1	3.3*	5.2*	15.6*	2.7	4.7*
Dose	3	0.05	4.9**	5.4*	1.6	0.5
Application *Dose	3	1.1	1.6*	2	1.4	1.7
Error	14	0.5	0.2	0.6	0.7	0.7
Total	23	5	12.6	25.1	7.1	7.7
CV		41719	15.7	19.2	13.1	15.2
		14th day seedling length (cm)				
Replication	2	0.1	4.2	2	1	1.5
Application	1	24.5*	3.9*	36.3**	13.1*	19.7*
Dose	3	7.6*	12.6**	7.1*	0.5	3.4*
Application *Dose	3	9.3	2.2*	6.0*	4.1	3.1*
Error	14	1.1	0.6	0.5	1.3	0.9
Total	23	42.6	23.5	52	20	28.6
CV		22.0	24.1	20.4	19	16.5

*:significant at P ≤ 0.05, **:significant at P ≤ 0.01

On day 4, the lowest germination ratios was observed in *C. dentatus* and *C. tenuis* (9.3%),followed by *C. glaucus* (10.7%), *C. persicus* (22.7%), and *C. lanatus* (25.3%) in the control group seeds.When 500 ppm GA_3 was applied, the highest germination ratios were observed in *C. persicus* (88.7%), *C. lanatus* (78.7%), *C. dentatus* (75.3%), and *C. tenuis* (73.3%). When 300 ppm GA_3 was applied to *C. glaucus*, the germination ratio was 54.0% (Fig. 1).

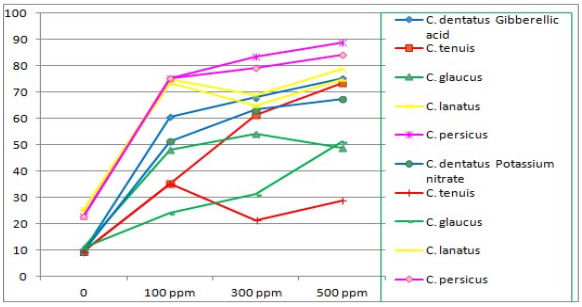


Figure 1. Effects of different gibberellic acid doses and potassium nitrate doses on germination ratio of wild safflower species at 4th day

On day 14, the lowest germination ratios were observed in *C. dentatus* (18.7%), *C. tenuis* and *C. glaucus* (24%), compared to 50.7% in *C. lanatus* and 48% in *C.persicus* in the control group seeds. The highest germination ratios were observed in *C. dentatus* (83.3% with the application of 300 ppm GA_3), *C. tenuis* (76.7% with the application of 500 ppm GA_3), and *C. lanatus, C. persicus,* and *C. glaucus*, which achieved germination ratios of 96.7%, 84%, and 82%, respectively, when 500 ppm KNO_3 was applied (Fig. 2).

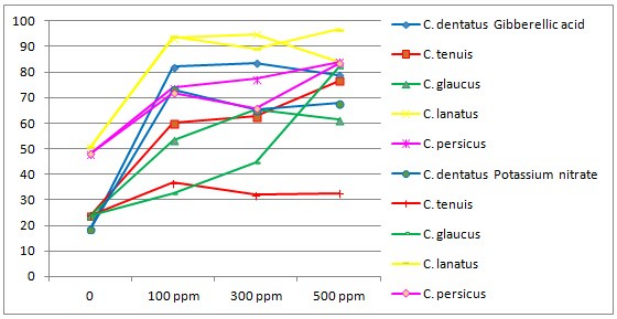


Figure 2. Effects of different gibberellic acid doses and potassium nitrate doses on germination ratio (%) of wild safflower species at 14th day

Seedling Dry Weight Ratios: Analysis of variance was perfomed to test the significance of difference among doses and different applications for seedling dry weight ratios (%). Analysis of variance revealed that were found importance owing to application of doses as significant at 0.05 in C. dentatus, *C. tenuis*, *C. lanatus*, *C. persicus* and was found 0.01 in *C. glaucus*. Applications was found significant at 0.01 in *C. glaucus*, was found significant at 0.05 in *C. lanatus* and *C. persicus*. Applications x doses interaction was found significant at 0.01 in *C. glaucus* and *C. persicus*, and significant at 0.05 in *C. tenuis* (Table 1).

On day 14, the lowest dry weight ratios were observed in *C. lanatus* (3.2%), *C. dentatus* (3.7%), and *C. persicus*(5.1%) in the control group seeds; *C. tenuis* (3.4%) when 100 ppm KNO_3 was applied, and *C. glaucus* (3.1%) when300 ppm KNO_3 was applied.The highest dry weight ratios were observed in *C. dentatus* (18.6%) when 500 ppm GA_3 was applied, *C. tenuis* (7.6%) when 300 ppm GA_3 was applied, *C. glaucus* (37.3%) when 100 ppm GA_3 was applied, *C. lanatus* (5.6%) when 300 ppm KNO_3 was applied, and *C. persicus* (32.6%) when 300 ppm KNO_3 was applied (Fig.3).

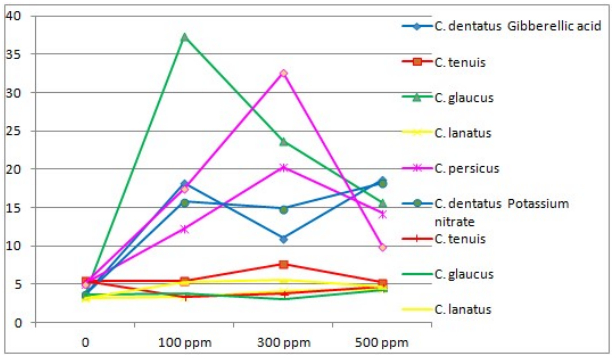


Figure 3. Effects of different gibberellic acid doses and potassium nitrate doses on dry weight ratio of wild safflower species at 14th day

Seedling Root Length: Analysis of variance was used to test the effect of different applications on root length(cm). Analysis of variance revealed that the importance owing to of the applications was significiant at 0.05 in *C. dentatus*, *C. tenuis*, *C. glaucus*, *C. persicus*. Doses was found significant at 0.05 in *C. glaucus*, was found significant at 0.01in and *C.*

tenuis. Applications x doses interaction was found significant at 0.05 in 0.05 in *C. tenuis* (Table 1).

Onday 14, the lowest seedling root lengths were observed in *C. dentatus*(3.4 cm) and *C. lanatus* (5.3 cm) when 500 ppm KNO_3 was applied; *C. tenuis* (1.8 cm)and *C. glaucus* (2.8 cm) in the control group;and *C. persicus* (3.8 cm) when 300 ppm GA_3 was applied.The highest seedling root lengths were obtained from *C. dentatus* (4.2 cm) and *C. tenuis* (4.7 cm) when 500 ppm KNO_3 was applied; *C. glaucus* (5.8 cm) with applications of 100 and 500 ppm KNO_3;*C. lanatus* (7.7 cm) when 300 ppm KNO_3 was applied; and *C. persicus* (6.2 cm) when 300 ppm KNO_3 was applied (Fig.4).

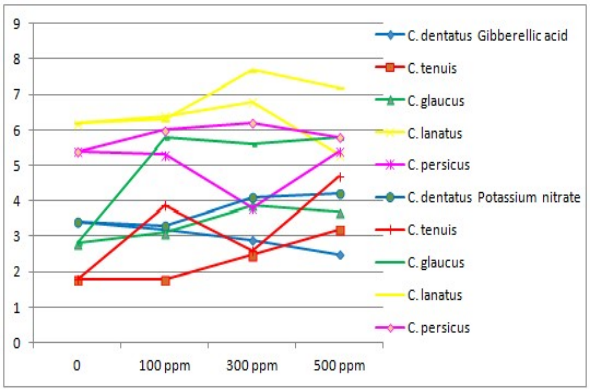


Figure 4. Effects of different gibberellic acid and potassium nitrate doses on seeding root length of wild safflower species at 14th day

Seedling Length: The distinctions due to the applications over seedling lengths were occurred at importance dose of 0.05 in *C. dentatus*, *C. tenuis*, *C. lanatus* and *C. persicus* and 0.01 in *C. glaucus*. The changes due to the doses were recorded at the importance dose of 0.05 for *C. dentatus*, *C. glaucus* and *C. persicus* and 0.01 in *C. tenuis* and as insignificant for *C. lanatus*. In addition to this, it is recorded that the effect of application x dose interaction has occurred at the importance dose of 0.05 in *C. tenuis*, *C. glaucus* and *C. persicus* (Table 1).

On day 14, the lowest seedling lengths were observed in *C. dentatus* (2.8 cm) when 500 ppm KNO_3 was applied; *C. tenuis* (2.0 cm) when 100 ppm KNO_3 was applied;*C. glaucus* (1.9 cm) in the control group; *C. lanatus* (4.5 cm) when 300 ppm KNO_3 was applied; and *C. persicus* (3.5 cm) when 300 ppm KNO_3 was applied. The highest seedling lengths were obtained from *C. dentatus* (8.6 cm) with 100 ppm KNO_3, *C. glaucus* (6.6 cm) and *C. lanatus* (8.0 cm) with 300 ppm KNO_3, and *C. tenuis* (6.6 cm) and *C. persicus* (7.8 cm) with 500 ppm KNO_3, (Fig.5).

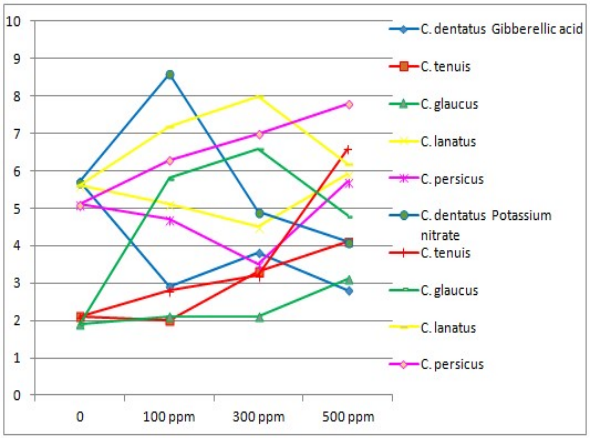


Figure 5. Effects of different gibberellic acid and potassium nitrate doses on seedling length of wild safflower species at 14th day

DISCUSSION

The results show that applications of 500 ppm GA_3 and KNO_3 give high germination ratios in *C. tenuis*, *C. glaucus*, *C. lanatus,* and *C. persicus*. The preferred germination ratio for *C. dentatus* is attained using100 ppm concentrations of both applications. Thus GA_3 and KNO_3 can be used in order to break dormancy. While the effects of each application vary across species, they show increasing effects on dry weight ratio, seedling root length and seedling length.

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