Australian Journal of Crop Science AJCS
AJCS 4(9):718-721 (2010) ISSN:1835-2707

Seed treatments to overcome dormancy of waterlily tulip (*Tulipa kaufmanniana* Regel.)

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Abstract

Dormancy and germination requirements were investigated in seeds of *Tulipa kaufmanniana* Regel (Liliaceae). The present study was conducted to study the dormancy breaking treatment in Tulip seed. An experiment was conducted with four replications and three treatments including: 3 different stratification periods (0, 5 and 7 weeks), varying concentrations of GA_3 (0, 250 and 500ppm) and 4 levels of KNO_3 (0, 0.1, 0.2 and 0.3% v/v). Germination percentage and mean germination time were significantly enhanced by treating seeds with mentioned treatments compared with the untreated control seeds. It was concluded that stratification for 7 weeks was more effective treatment on studied traits than 5 weeks. Moreover, cold stratification was a better treatment on breaking seed dormancy of waterlily seeds than GA_3 and KNO_3 treatments. Applying 500ppm concentration of GA_3 and 0.1 of KNO_3 after stratification resulted in higher germination in waterlily dormant seeds.

Keywords: Germination, Dormancy, Seed, *Tulipa kaufmanniana.*
Abbreviations: GA_3_gibberellic acid; KNO_3_potassium nitrate.

Introduction

A tulip is a flower in the genus *Tulipa*, comprising about 150 bulbous species, and in the family Liliaceae (Jaap et al., 2007). The native range of the species includes southern Europe, North Africa, and Asia from Anatolia and Iran in the west to northeast of China. The center of diversity of the genus is in the Pamir and Hindu Kush mountains and the steppes of Kazakhstan (Jaap et al., 2007). A number of species and many hybrid cultivars are grown in gardens, used as pot plants or as fresh cut flowers. Most cultivars of tulip are derived from *Tulipa gesneriana*. Waerlily tulip is a hybrid made by man (PlantCare.com). Seed dormancy has been described as "one of the least understood phenomena in seed biology" (Finch-Savage and Leubner, 2006) and remains confusing despite much recent progress. This confusion reflects the likelihood that dormancy is not a single phenomenon but a condition with many contributing causes (Finkelstein et al., 2008). Traditionally this condition has been primarily negatively defined as a developmental state in which a viable seed fails to germinate under superficially favorable environmental conditions (e.g., adequate moisture). All types of dormancy impose a delay between seed shedding and germination, but the underlying causes may vary. This variety has been classified in terms of whether germination is inhibited owing to embryonic immaturity or physical or physiological constraints, and whether the controlling structure or substances are embryonic or in the surrounding tissues of the seed, i.e., coat imposed (Finch-Savage and Leubner, 2006). Hard seededness, which is prevalent in many species of a number of families like Leguminousae, Malvaceae and Liliaceae, is one form of dormancy and is caused by both genetic and environmental factors (Copeland and McDonald, 2001). Seed dormancy has been further negatively categorized in terms of the requirements for release from this block, such as disruption of the seed coat (scarification), a period of dry storage (after-ripening) or moist chilling (stratification), or exposure to light (Finkelstein et al., 2008). The situation is further complicated by the fact that, although germination is an all or nothing event for each seed, populations display variable degrees of dormancy that are reflected in the rate or percentage of germination under specific conditions. Presumably, each seed is in a state somewhere along the continuum from deeply dormant to nondormant, but it remains unclear how the tipping point between nonpermissive and permissive for germination is sensed for each seed (Finkelstein et al., 2008). However, this point is of critical agronomic and ecological significance because it determines both the degree of synchronous germination in a given season and the reservoir of ungerminated viable seeds remaining in the soil until a later season, i.e., the seed bank. Gibberellins [e.g., gibberellic acid (GA)] are a family of 136 tetracyclic diterpenes, a small subset of which are active as plant hormones and known to stimulate seed germination in a wide range of plant species; the predominant active GA depends on the species (Thomas et al., 2005). Gibberellins stimulate germination by inducing hydrolytic enzymes that weaken the barrier tissues such as the endosperm or seed coat, inducing mobilization of seed storage reserves, and stimulating expansion of the embryo (Bewley and Black, 1994). GA may also stimulate germination via the transition from embryonic to vegetative development, in part mediated by the chromatin remodeling factor PICKLE (PKL) (Henderson et al., 2004). Incubation of seeds in moist conditions to break dormancy, usually in cold to simulate overwintering is known as stratification (Finkelstein et al,2008).

Table 1. Analysis of evaluation variance of germination traits of studied via dormancy breaking treatment in waterlily tulip

	Mean of squares		
SOV	df	FGP	MGT
Stratification	2	152908**	27884.33**
Gibberellin	2	2.33 ns	17.69 ns
KNO_3	3	6.66 ns	136.95**
Gibberellin* Stratification	4	1.33 ns	10.26 ns
KNO_3* Stratification	6	12.44*	123.62**
KNO_3*Gibberellin	6	10.77*	196.53**
KNO3* Gibberellin* Stratification	12	8.22*	172.19**

ns,**,* Respectively non significant and significant at the P 0.01 and 0.05 levels

The effect of GA_3 as a germination promoter is hypothesized to increase with stratification treatment (Yamauchi et al., 2004). Stratification also plays an important role in providing the stimulus required to overcome dormancy. Stratification has been reported to induce an increase in GA_3 concentration (Bretzloff and Pellett, 1979; Yamauchi et al., 2004). Many nitrogen-containing compounds, including NO gas, nitrite (NO_2^-), nitrate (NO_3^-), nitrogen dioxide, ammonium, azide, and cyanide, promote dormancy release and seed germination in many species, possibly as a means of sensing soil N availability (Bethke et al., 2007). Potassium nitrate is well documented as a compound, which increases the germination of photo-dormant seeds (Shanmugavalli et al., 2007). Many gardeners choose potassium nitrate to break seed dormancy and increase the health of plants. According to Bewley and Black (1994), KNO_3 raises the ambient oxygen levels by making less oxygen available for citric acid cycle.
The aim of this research was to determine the effects of different seed dormancy breaking treatments which are able to stimulate and enhance germination of this important ornamental plant.

Materials and methods

This study was carried out in January 2008 at the Department of Horticulture, University of Tehran, Iran. Waterlily is commonly grown in Iran and seeds were received from Mahde Laleha, Institute of Gachsar. Germination Percentage and Mean Germination Time of the cultivar were studied using following treatments: Gibberellin (GA_3) including: 0, 250 and 500 ppm, KNO_3 levels including: 0, 0.1, 0.2 and 0.3% (v/v). Stratification treatments for 0, 5 and 7 weeks were done before using Gibberellin and KNO_3 treatments.

Seed treatments

For stratification treatment, Waterlily seeds were mixed in perlite medium and distilled water in vessels, then transferred to arefrigerator for 5 and 7 weeks at 5±1◦C. These vessels put into sealed plastic bags to avoid moisture loss. After this time the seeds were rinsed with distilled water three times. The treated seeds were put into flasks contained 250 and 500ppm Gibberellin for 24 hours (flasks were shaken on orbital shaker under dark condition). Likewise, similar to previous treatment, the stratificated seeds were treated by 0, 0.1, 0.2 and 0.3% (v/v) KNO_3 for 24 hours. In this case, flasks were shaken on orbital shaker in light condition.

Germination tests

Four replicates of 25 seeds were germinated on top of double layered papers (ISTA, 1996) with 5 ml of water in 10cm Petri dishes. These Petri dishes were put into sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate at 13±1◦C in the dark condition for 24 days (the reason of choice this temperature was lack of germination in 20-30◦C as an optimum temperature). Germination was considered to have occurred when the radicles were 2mm long. Germination percentage was recorded every 24h for 24 days. Mean germination time (MGT) was calculated by following equation (Schilin *et al.,* 2003).

Mean Germination Time (MGT): $\dfrac{\sum fini}{N}$

fi: Day during germination period (between 0 and 24 day).
ni: Number of germinated seeds per day
N: Sum of germinated seeds

Statistical analysis

The statistical design was a completely randomized design in a factorial arrangement with three factors. Four replications and 25 seeds per replicate were used. Data for germination and abnormal germination percentage were subjected to arcsine transformation before analysis of variance. Statistical analysis was carried out using SAS program. Mean comparison was performed with Duncan's test at the 5% level of significance.

Results and discussion

Analysis of variance showed that (Table 1), triple interaction effects on traits of final germination percentage and mean germination time was significant. Therefore, it was decided to avoid explaining and discussing about main or double interaction effects.

Final Germination Percentage (FGP)

Based on the results, the highest germination percentage was detected for the stratification of 7 weeks in concentration of 500 ppm GA_3 and 0.1% KNO_3. However it was not different significantly to some treatments as shown with the same letter (Table 2).

Table 2. Effect off seed dormancy breaking treatments on germination traits of waterlily tulip, In each column means followed by the same letter are not significantly different at the $P < 0.05$ level

Treatment	Final Germination Percentage (%)	Mean Germination Time (seed/day)
Control	-	-
Stratification(0)+GA3(0)+KNO3(0.1)	-	-
Stratification(0)+GA3(0)+KNO3(0.2)	-	-
Stratification(0)+GA3(0)+KNO3(0.3)	-	-
Stratification(0)+GA3(250)+KNO3(0)	-	-
Stratification(0)+GA3(250)+KNO3(0.1)	-	-
Stratification(0)+GA3(250)+KNO3(0.2)	-	-
Stratification(0)+GA3(250)+KNO3(0.3)	-	-
Stratification(0)+GA3(500)+KNO3(0)	-	-
Stratification(0)+GA3(500)+KNO3(0.1)	-	-
Stratification(0)+GA3(500)+KNO3(0.2)	-	-
Stratification(0)+GA3(500)+KNO3(0.3)	-	-
Stratification(5)+GA3(0)+KNO3(0)	97 abc	45.63 hi
Stratification(5)+GA3(0)+KNO3(0.1)	91 d	48.53 ijk
Stratification(5)+GA3(0)+KNO3(0.2)	99 ab	40.38 gh
Stratification(5)+GA3(0)+KNO3(0.3)	99 ab	47.09 hijk
Stratification(5)+GA3(250)+KNO3(0)	97 abc	45.21 jk
Stratification(5)+GA3(250)+KNO3(0.1)	97 abc	46.7 hij
Stratification(5)+GA3(250)+KNO3(0.2)	96 bc	40 gh
Stratification(5)+GA3(250)+KNO3(0.3)	98 abc	48.09 ijk
Stratification(5)+GA3(500)+KNO3(0)	99 ab	36.6 g
Stratification(5)+GA3(500)+KNO3(0.1)	97 abc	43.36 hi
Stratification(5)+GA3(500)+KNO3(0.2)	95 c	73.22 l
Stratification(5)+GA3(500)+KNO3(0.3)	99 ab	46.92 hij
Stratification(7)+GA3(0)+KNO3(0)	99 ab	18.92 c
Stratification(7)+GA3(0)+KNO3(0.1)	98 abc	18.25 c
Stratification(7)+GA3(0)+KNO3(0.2)	99 ab	17.54 b
Stratification(7)+GA3(0)+KNO3(0.3)	98 abc	24.12 ef
Stratification(7)+GA3(250)+KNO3(0)	97 abc	16.44 b
Stratification(7)+GA3(250)+KNO3(0.1)	98 abc	19.27 c
Stratification(7)+GA3(250)+KNO3(0.2)	99 ab	23.29 ef
Stratification(7)+GA3(250)+KNO3(0.3)	99 ab	18.6 c
Stratification(7)+GA3(500)+KNO3(0)	99 ab	21.54 de
Stratification(7)+GA3(500)+KNO3(0.1)	100 a	12.39 a
Stratification(7)+GA3(500)+KNO3(0.2)	99 ab	26.03 f
Stratification(7)+GA3(500)+KNO3(0.3)	97 abc	21.37 d

Stratification treatment including three levels: 0, 5 and 7 weeks, Gibberellin(GA3) treatment including three levels: 0, 250 and 500 ppm

KNO3 treatment including four levels: 0, 0.1, 0.2 and 0.3% (v/v), Dash in front of each combined treatment means zero germination

Mean Germination Time (MGT)

The best treatment for this trait was detected in the stratification treatment for 7 weeks in concentration of 500 ppm GA_3 and 0.1% KNO_3 (Table 2). In our experiment, application of GA_3 not stimulated the germination of waterlily tulip. Researchers mentioned that the role of gibberellins in dormancy release is controversial. Although GA accumulation is associated with dormancy release and/or germination, GA treatment alone does not stimulate germination in all species (Bewley, 1997; Ali-Rachedi et al., 2004).

At lower concentrations of GA, germination was zero. Similarly, levels of KNO_3, also did not stimulate the germination of waterlily tulip (Table 2). Shanmugavalli et al (2007) showed that seeds of sorghum soaked in 0.5% and 1% potassium nitrate (KNO_3) improved germination up to 44%, but again it was not a complete success. Potassium nitrate has been used for many years, with positive studies beginning in the 1980's but it often increased the germination of photo-dormant seeds (Shanmugavalli et al., 2007). Similar results were reported in *Panicum maximum* by Previero et al (1996) and Usberti et al (2000) in many plant species.Despite the single treatments (GA_3 and KNO_3), treatment with stratification for 5 and 7 weeks were most effective and germination of waterlily seeds improved by this treatment. Stratification plays an important role in improving sensitivity to other treatment to overcome dormancy (Bretzloff and Pellett, 1979;Yamauchi et al., 2004). It seems combining treatments of 7 weeks stratification in the concentration of 500ppm of GA_3 and 0.1% of KNO_3(v/v) resulted in the best treatment. The effect of GA_3 as a germination promoter is hypothesized to increase with stratification treatment (Bretzloff and Pellett, 1979;Yamauchi et al., 2004). Stratification has been reported to induce an increase in sensitivity to GA_3 concentration (Oh et al., 2006). The lack of a positive response of stratified waterlily seeds to only GA_3 may be due to this fact that seed may possess physiological dormancy. Moreover, there are various reports about the different physiological effects of gibberellings. For example,

GA_7 was more effective than GA_3 in promoting germination of *Sanguinaria candensis* L. (bloodroot) (Deno, 1996). Because of GA did not promote the germination of *Tulipa tarda* seeds, Nikolaeva (1977) concluded that they had deep physiological dormancy (PD), in addition to morphological dormancy (MD). Razumova and Pozdova (1997) reported the same results as Nikolaeva in *Tulipa greigii*. Subsequent studies have shown that GA by itself does not stimulate the germination of seeds *Delphinium tricorne* (Baskin and Baskin, 1994). Finkelstein et al (2008) mentioned that GA may not trigger the onset of after-ripening, but it may be necessary though not sufficient for seed dormancy release and germination. Dormant seeds which require chilling, dry storage, after ripening, and light as a germination stimulator, are often treated with GA_3 to overcome their dormancy (Gupta, 2003). In this study 7 weeks of stratification along with GA_3 and KNO_3 were the most effective treatment. Similar to our results, Nadjafi et al. (2006) reported that, in natural habitats of *Ferula gummosa*, higher seed germination occurred in colder regions with higher precipitation. Oh et al (2006) suggest that stratification promotes germination by increasing the potential for bioactive GA accumulation. Stratification led to increased expression of the GA biosynthesis genes *GA20ox1* (*GIBBERELLIN 20 OXIDASE*), *GA20ox2*, and *GA3ox1* and decreased expression of the GA catabolic gene *GA2ox2* (Yamauchi et al., 2004). In some reports stratification was introduced as the best method for dormancy breaking. Bubel (1988) reported that seeds of tulip tree (*Liriodendron tulipifera*) need stratification at 1◦C to 4◦C for two to three months to break dormancy.

Conclusions

The dormancy of waterlily tulip seed was broken by stratification treatment, but GA_3 and KNO_3 had no effect. Our results showed that applying 500ppm concentration of GA_3 and 0.1% of KNO_3 after stratification could be the best treatment for waterlily dormant seeds.

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