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The Effect of Gibberellic Acid and Stratification on Germination of Alstroemeria (*Alstroemeria ligtu* hybrid) Seed Under *In Vitro* and *In Vivo* Conditions

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Abstract

The dormancy characteristics and optimum conditions for seed germination of *Alstroemeria ligtu* had not been explained. *In vitro* and *in vivo* alstroemeria (*A. ligtu* hybrid) seed germination tests were conducted in a Randomized Completely Design at two different treatments (gibberellic acid (GA_3) 0, 100, 200 and 400 mg/l with and without stratification in 5±1°C) in four replications. Seeds were planted in the soil mixture (peat/sand/perlite 1:1:1) or 1/2 MS media (1% sucrose, 0.7% agar and pH to 5.8). After 3-weeks keeping in the stratification conditions, transferred to the growth chamber (21°C and 16h photoperiod). Shoot and root length, number of root and leaf, root and shoot fresh and dry weight, seed germination percentage, germination rate and mean germination time were recorded during experiment. Stratification had a significant effect on seed germination ($p<0.05$). Soaking for 24 h in 100 mg/l GA_3 supplemented with stratification under *in vitro* and *in vivo* conditions increased germination up to 76.67% and 70.00%, respectively. Mean germination time (MGT) decreased with duration of stratification and concentration of GA_3. Seeds treated with 100 $mg.l^{-1}$ GA_3 plus 21 days of stratification produced the seedlings with the higher number of leaf, length of shoot, shoot and root dry weight in both *In vivo* and *in vitro* conditions. Non-stratified seeds without GA_3 application fail to germinate, whereas seeds chilled for 21 days had 36.6%, 40.0% germination under *in vivo* and *in vitro*, respectively. Stratification was successful in breaking seed dormancy; stratification at 5±1°C for 21 days or 100 mg/l GA_3+21 days of stratification overcame seed dormancy and increased the germination percentage of *A.ligtu* hybrid seeds. Thus, seeds of *A.ligtu* hybrid species probably exhibit a combination of physiological dormancy. In general, *In vivo* germination rates were lower than *in vitro* rates.

Keywords: *Alstroemeria*, Physiological dormancy, Pre-germination treatments.

INTRODUCTION

The genus *Alstroemeria* L. (Alstroemeraceae) has been distributed in South America with two main centers; one Chile and the second throughout the eastern of Brazil and contiguous Paraguay and Argentina (Bayer, 1987; Aker and Healy, 1990). *Alstroemeria* is one of the important commercial cut flowers throughout the world (Gonzalez-Benito and Alderson, 1992). All species are herbaceous, perennial and rhizomatous plants with big flowers, living in a wide range of habitats from rainy forest to desert areas and from the mountains to the coast (Munoz and Moreira, 2003). This plant is planted in greenhouse for cut flower production and is propagated vegetative by rhizome division. Seed propagation is uncommon due to variability in the germination percent and the time required for the germination, may be caused by viability of seeds, seed dormancy or improper techniques (King and Bridgen, 1990). The dormancy characteristics and optimum conditions for seed germination of this species had not been explained. Thus, some information about effective factors on dormancy breaking and optimal conditions of seedling growth is necessary for recovery of seed germination in this plant. The erratic and unpredictable nature of *Alstroemeria* germination is undesirable for commercial growers who tend to higher and more synchronous germination. Also, determination of optimum germination conditions could aid breeding efforts and hybrid seed distribution.

Seed dormancy is a block to the completion of germination of an intact viable seed under favorable conditions (Hilhorst, 1995; Bewley, 1997). This block to germination has evolved differently across species through adaptation to the prevailing environment, so that germination occurs when conditions for establishing a new plant generation are likely to be suitable (Hilhorst, 1995; Bewley, 1997; Baskin and Baskin, 2004). Dormancy is an innate seed property that defines the environmental conditions which seed is able to germinate. It is determined by genetics with a substantial environmental influence which is mediated, at least in part, by the plant hormones such as abscisic acid and gibberellins (GAs) (Finch-Savage and Leubner-Metzger, 2006). Two major forms of physiological seed dormancy have been described, namely embryo and coat dormancy (Kucera *et al*., 2005). Physiological dormancy is the most abundant form and found in seeds of gymnosperms and all major angiosperm (Finch-Savage and Leubner-Metzger, 2006). Physiological dormancy can be divided in to three levels: deep, intermediate and slight dormancy (Baskin and Baskin, 2004). Genotypic and physical constraints, morphologically immature embryos, and may be physiological inhibitors in the seed coats appear to cause a combined dormancy in alstroemeria seeds (King and Bridgen, 1990).

In physiological dormant seed, it is thought that temperature and GAs can both release dormancy and promote germination (Kucerna *et al.,* 2005; Baskin and Baskin, 2004). GAs plays a key role in dormancy release and promotion of germination (Kucerna *et al.*, 2005; Cetinbas and Koyuncu, 2006). Gibberellic acid (GA_3) is widely used to break dormancy of seeds of various plant species. Dormant seeds which require stratification, dry storage after ripening and light as a germination stimulator, are often treated with GA_3 to overcome their dormancy (Gupta, 2003). Increased germination of alstroemeria seeds with a warm-cold treatment suggests that there are physiological factors in the seed coat of this species that are responsive to cold stratification or that time is required for softening of the seed coat (King and Bridgen, 1990). The embryo of many seeds fails to germinate because oxygen dose not diffuse through the seed coat. At low temperature more oxygen dissolves in water and therefore more oxygen is prepared for embryo (Young and Young, 1992). Dormancy and germination are complex phenomena that are controlled by both developmental and environmental factors (Bewley, 1997; Koornneff *et al.*, 2002). When seeds released from dormancy, the receptors then initiate a signal transduction cascade, perhaps involving synthesis of or sensitization to germination-promoting GAs that lead to the completion of germination (Bewley, 1997). Imbibition stimulates GA secretion from embryo, secreted GA increases synthesis of hydrolytic enzymes located under aleuron layer. Synthesized enzymes are transported to endosperm

via scutulum and are used for decomposing of stored food to supply the energy required for germination (Cirak *et al*., 2004).

The aim of the present study was to find a practical method to promote *A.ligtu* hybrid seed germination and dormancy breaking by means of stratification and GA_3 application. Therefore, we examine the effect of some treatments on *A.ligtu* hybrid seed germination.

MATERIALS AND METHODS

This investigation was carried out in the Department of Horticultural Science, Agriculture Faculty, university of Zanjan, Iran. Seeds of *Alstromeria ligtu* hybrid were immediately washed with tap water and then divided to four groups (each group was divided to four replicates) and subjected to one of the following treatment:1. soaking in tap water only for 24 h (control), 2. soaking in water for 24 h and then stratified at 5±1°C up to three weeks, 3. soaking in a GA_3 solution at 0, 100, 200 and 400 mg/l for 24 h supplemented with stratification at 5±1°C up to three weeks and 4. soaking in a GA_3 solution at 0, 100, 200 and 400 mg/l for 24 h without stratification. Seeds were sterilized by 70% ethanol (1 min), 3% sodium hypochlorite solution (20 min) either after soaking in water or GA_3 solutions and then rinsed with sterilized water (10 min each). Treatments were held at growth chamber (21°C, 16 h light) after sowing in either soil or MS media. For stratification treatments seed held at growth chamber (21°C, 16 h light) for one week before apply chilling.

The seeds were sow directly in the soil mixture (peat/sand/perlite 1:1:1) at a depth of approximately 0.5-0.7 cm in pot. Irrigation was done every 3 days. Each pot was containing 10 seeds. Germination of seeds was recorded at daily interval. After three-weeks keeping seeds in the stratification conditions, transferred to the growth chamber, and cultures were placed under 21°C and 16h photoperiod.

For in vitro study the seeds were incubated in 250 ml jars containing half strength MS medium (Murashige and Skoog, 1962), supplemented with 1% sucrose and 0.7% agar, and pH was adjusted to 5.8. Each jar was containing 10 seeds.

The progress of seed germination was recorded daily for a period of 30 days after treatment application. Radicle length of 2 mm was scored as germinated seed (Kaya *et al.*, 2006). Mean Germination Time (MGT) was calculated to assess the rate of Germination (Ellis and Roberts, 1981). Shoot and root length, number of root and leaf, root and shoot fresh and dry weight, seed germination percentage, germination rate and mean germination time were recorded during experiment. The oven-dried weight was obtained by drying seedlings at 70°C to reach constant weight.

Experiment was randomized completely design with 4 replications. The statistical analysis was made using the ANOVA procedure of SAS. The difference between the means was compared using the Duncan's multiple test ($p < 0.05$).

RESULTS

Stratification had a significant effect on seed germination of *A. ligtu* hybrid ($p < 0.05$). Seed germination percentage in stratified seeds without GA_3 treatments was 36.67 and 40% *in vivo* and *in vitro* conditions, respectively. Application of GA_3 (supplemented with or without stratification) affected total germination in these experiments (Table 1). Seeds treated with 100 mg/l GA_3 without stratification gave 13.33% and 16.67% germination, whereas seeds treated with 100 mg/l GA_3 + 21 day stratification gave 70% and 76.66% germination *in vivo* and *in vitro* conditions respectively. The germination percentage in half strength MS medium was higher than *in vivo* rates (Table 1). The difference between *in vivo* and *in vitro* seed germination under stratification was 5.02%. Therefore, the application of GA_3 (100 mg/l) resulted in higher germination percentage and rate, shoot and root fresh weight, shoot and root dry weight than those of seeds treated with 200 and 400 mg/l GA_3.

MGT decreased with duration of stratification and concentration of GA_3 (Fig. 1). MGT in

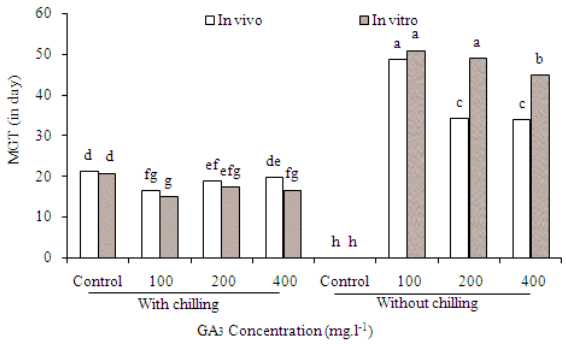

Fig. 1. Effects of gibberellic acid (GA_3) concentrations and stratification during the germination phase on mean of germination time (MGT) in *Alstroemeria ligtu* hybrid seeds.

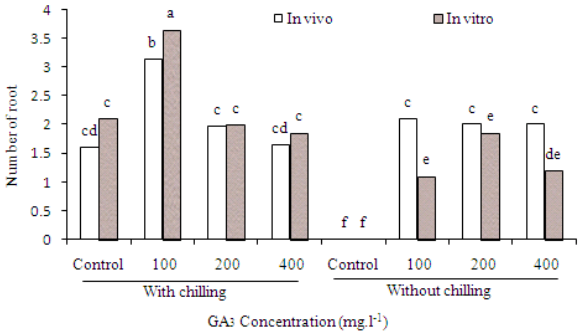

Fig. 2. Eeffect of gibberellic acid (GA_3) concentrations and stratification during the development and growth phase on root number in *Alstroemeria ligtu hybrid* seeds.

in vivo and *in vitro* conditions were reduced to 16.44 and 15.05 days by 100 mg/l GA_3 + stratification treatment. In the other hand seeds treated with GA_3, germinated after 51, 49 and 45 days at 100, 200 and 400 mg/l GA_3 in vitro conditions (Fig. 1). These results showed that stratification treatment was more benefit for A. ligtu hybrid seed germination.

GA_3 treatments increased germination percentage and rate in compare to those did not treated with GA_3. In the current study, seed germination rate was depended to GA_3 concentration. In higher concentration (200 and 400 mg/l) germination rate decreased. Further, MGT decreased in stratified compared to non-chilled treatments.

Stratification alone or in combination with GA_3 improved seedling characteristics including seedling length, root dry weight, shoot dry weight significantly and has a larger effects than the GA_3 treatments applied (Table 1). The highest root number was observed in 100 mg/l GA_3 + stratification treatment (Fig. 2). Although all GA_3 treatments had positive effects on seedlings length either *in vivo* or *in vitro* condition, but 100 mg/l was better than the other concentrations. Therefore, maximum seedling fresh and dry weights (roots and shoots) were recorded from seed treatment with 100 mg/l GA_3 (Table 1).

Table 1. Effect of gibberellic acid (GA_3) and stratification treatments on germination parameters in *Alstroemeria ligtu* hybrid seeds.

Culture Type	Treatments GA_3×Chilling		G.P (%)	G.R (per day)	No. of Leaves	Shoot Length (cm)	Root Length (cm)	F.W.S (mg)	F.W.R (mg)	D.W.S (mg)	D.W.R (mg)
In vivo	0	*	36.66c	0.17g	2.47 cde	3.81d	2.91d	82d	34cd	6.43cd	3.4cd
In vivo	0	-	0.0e	0m	0 J	0i	0h	0h	0i	0i	0i
In vitro	0	*	40c	0.17g	1.55fjhi	2.32e	0.65g	27fg	14.67f	3.39efg	1.6ef
In vitro	0	-	0.0e	0m	0J	0i	0h	0h	0i	0i	0i
In vivo	100	*	70ab	0.31e	5.04a	8.89a	5.17a	339.3a	63.5a	17.77a	8.8a
In vivo	100	-	13.33d	0.025k	1.97defgh	1.85efg	0.95fg	28fg	9.1fgh	1.4hi	1.1fgh
In vitro	100	*	76.67a	0.37a	3.34b	5.5c	4.03c	71de	31.67d	6.3cd	3.4cd
In vitro	100	-	16.67d	0.027k	0.97i	0.91h	0.57g	9.2gh	5hi	0.45i	0.52hi
In vivo	200	*	67b	0.35c	2.71bcd	7.97b	4.81ab	237b	42.67b	13.8b	4.57b
In vivo	200	-	10d	0.019l	1.82efgh	1.97ef	1.2ef	27fg	11fg	1.9ghi	1.4efg
In vitro	200	*	73ab	0.36b	2.30cdef	4.96c	4c	58.67de	38bc	4.87de	2.97d
In vitro	200	-	15d	0.034j	1.22h	1.1gh	0.91fg	7.1gh	6.9gh	0.41i	0.58hi
In vivo	400	*	66.67b	0.30f	3bc	5.6c	4.61b	152c	33cd	8.03c	4bc
In vivo	400	-	16.67d	0.032j	2.1defg	2.1e	1.4e	32fg	14f	2.5fgh	1.6ef
In vitro	400	*	70ab	0.32d	2.41cde	3.97d	3.86c	51ef	21e	3.9ef	2.1e
In vitro	400	-	17 d	0.044i	1.33ghi	1.3fgh	0.86fg	11gh	5.3ghi	0.66hi	0.62jhi

Means in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's.
*: with stratification - : without stratification.
G.P: Germination percentage, G.R: Germination rate, F.W.S: Fresh weight shoot, F.W.R: Fresh weight root, D.W.S: Dry weight shoot, D.W.R: Dry weight root.

DISCUSSION

Stratification at 5±1°C for 21 days or in combination with 100 mg/l GA_3 overcame seed dormancy and increased the germination percentage of *A.ligtu* hybrid seeds. This results showed that these treatments were effective in inducing metabolic activity in the embryo required for the initiation of germination process (Al-Menaie *et al*., 2007). Releasing dormancy can also be associated with an increasing gibberellin biosynthesis during stratification (Yamauchi *et al.*, 2004). The germination percentage higher than *in vivo* rates could have been due to the effect of various elements used in the medium. In vitro germination condition is a nutrient medium containing macro and micro elements and sucrose (1/2MS) that had a positive effect on *A.ligtu* hybrid seeds germination. Control treatment had no germination which indicating a high level of dormancy. In current study, seeds were able to absorb water, which indicates no physical dormancy as postulated by Willan, 1987 and Schmidt, 2000 for other plant seeds. Results showed that stratification was successful in breaking seed dormancy. Raisi *et al.* (2013) investigated dormancy break of Ferula assa-foetida seed and domenstrated that one period of stratification treatment could increase germination of the seed. GA_3 is effective in breaking the slight physiological dormancy, but it does not overcome the deep physiological dormancy (Baskin and Baskin, 1990). Application of GA_3 during and after stratification on Pistachio seed increased the length, shoot diameter, internodes length, leaf area and fresh and dry weight of seedlings (Rahemi and Baninasab, 2000). It has been reported that germination and dormancy breaking can be induced by GA_3 in many plant species, e.g., *Trichocereus terscheckii* (Baes and Rojas-Arechiga, 2007), *Rubia tinctorum* L. (Sadeghi *et al*, 2009), *Pedicularis olympica* (Kirmizi *et al.*, 2010), *Amaranthus retroflexus* L. (Ke˛pczyn´ski and Sznigir, 2013) and *Acalypha indica* L. (Gupta and Bandopadhyay, 2013). According to the results found in this study, *A.ligtu* hybrid species probably exhibits a combination of physiological dormancy. Improvement of germination percentage by GA_3 could indicate the presence of chemical dormancy as well, as application of gibberellic acid has shown effect on overcoming dormancy caused by inhibitors (Bewley and Black, 1994). Physiological dormancy in seeds is dependent on the ratio and the levels of abscisic acid (a growth inhibitor) and GA (a growth stimulator) (Hilhorst and Karssen, 1992). GAs are known to obviate the requirement of seeds for various environmental cues, promote germination, and counteract the inhibitory effects of ABA, frequently in combination with cytokines (Bewley and Black, 1994). Giba *et al*. (1993) reported that the inhibitory effect of retardants was overcome by GA_3. Stratification might act simply to lower the rate of enzymatic reactions taking place in the seed, and might cause differential changes in enzyme concentrations or in enzyme production (Bewley and Black, 1994).

Incidence of abnormal seedling growth observed in seeds treated with only GA_3 treatment. It is suggested that the onset of embryo dormancy is associated with accumulation of growth inhibitors and breaking of dormancy with a shift in the balance of growth regulators towards growth promoters to overcome the effect of inhibitors (Khan, 1971).

Seeds of treated with 100 mg/l GA_3 + 21 days of stratification produced the seedlings with yielded higher number of leaf, length of shoot, shoot and root dry weight as compared with other treatments. Similar results were observed by Mostafa and Abou-Alhamd *et al.* (2011) and Dhupper (2013) where they found that application of GA_3 showed remarkable increase in the number of leaves, length of shoots and dry weight of seedlings. da Silva Vieira *et al*. (2010) reported that the increase in height of the plant can be attributed to auxin, since it can cause the synthesis of gibberellins and thereby induce cell elongations. The increase in the dry weight of seedling due to treatment with GA_3 might be attributed to increase in cell elongation, cell division and accumulation of building units that accompanied by greater sacharids content than those of untread plants (Akhtar *et al.*, 2008; Abdel- Latef *et al.*, 2009;).

These findings, except for the scarce response to GA_3, firmly support the hypothesis that

A.ligtu hybrid seeds fit the characteristics a non-deep physiological dormancy according to the dormancy classification of Baskin and Baskin (2004). Further, physiological barriers to germination in embryos have been overcome by cold stratification in a number of rose species (Zhou *et al.*, 2009). Results obtained in this study present strong evidence that the pericarp, the testa, and the embryo play important roles in regulating seed dormancy. The negative effect of the testa on germination can be attributed to some inhibitory substances in the testa and not to its role as a mechanical barrier or in restricting access to water (Bo *et al.*, 1995). King and Bridgen (1990) reported that may be physiological inhibitors in the seed coats appear to cause a combined dormancy in *Alstroemeria* seeds. El-Refaey and El-Dengawy (2005), shown that stratification of seeds at 4-5°C or treatment of seeds with GA_3 was successfully overcome dormancy in *Eriobotrya japonica* seeds. Cold stratification increased the germination percentage and rate of *A.ligtu* hybrid, as generally is known for a number of other species (Bewley and Black, 1994).

CONCLUSION

The current study demonstrated that the *A.ligtu* hybrid seeds were in a dormant state, which suggests that stratification at 5±1°C for 21 days or 100 mg/l GA_3 plus 21 days of stratification overcame seed dormancy and increased the germination percentage of *A.ligtu* hybrid seeds.

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