Trakia Journal of Sciences, No 1, pp 38-46, 2014

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

IMPROVING GERMINATION AND DORMANCY BREAKING IN *ALSTROMERIA LIGTU* HYBRID SEEDS

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ABSTRACT

In vitro and *in vivo A. ligtu* hybrid seed germination tests were conducted at different treatments: soaking in gibberellic acid (GA_3) or salicylic acid (SA) (0, 100, 200 and 400 $mg.l^{-1}$) and Potassium nitrate (KNO_3) (0.1, 0.2, 0.4 and 0.8%) with and without stratification in 5±1 °C. Stratification had a significant effect on seed germination ($p < 0.05$). Maximum germination (86.67%, 96.50% after 16.44, 15.05 days under *in vivo* and *in vitro* conditions, respectively) was found in 100 $mg.l^{-1}$ GA_3 + 21 days of stratification and was followed by 0.8% KNO_3 and 100 $mg.l^{-1}$ SA along with 21 days of stratification. Mean germination time (MGT) decreased with duration of stratification and GA_3, SA and KNO3 application, respectively. The present results indicated that, GA_3, SA and KNO3 stimulated various growth aspects of Alstroemeria seedlings growth in both under *in vitro* and *in vivo*, but seeds treated in 100 $mg.l^{-1}$ GA_3 soulation was more effective. Seeds treated with SA and KNO_3 without stratification and control had no germination. *A.ligtu* hybrid species probably exhibits a combination of physiological dormancy. Stratification at 5±1 °C for 21 days or GA_3 + 21 days of stratification overcame seed dormancy and increased the germination percentage of *A.ligtu* hybrid seeds. Seedlings growth *in vivo* conditions was higher as compared with *in vitro* conditions.

Key words: Alstroemeria, Germination, Dormancy, Gibberellic acid, Salicylic acid, Potassium nitrate

INTRODUCTION

Alstroemeria (Alstroemeraceae) is one of the important commercial cut flowers throughout the world (1). This plant is cultured in greenhouse for cut flower production and is propagated vegetatively by rhizome division. Seed propagation is uncommon due to variability in the germination percent and the time required for germination variability, may be caused by inviable seeds or improper techniques (2). Propagation by seed has the advantage of producing a substantial number of seedlings from one plant (3), but germination is slow in Alstroemeria (*A.ligtu* hybrid). The erratic and unpredictable nature of Alstroemeria germination is undesirable for commercial growers who tend to higher and more synchronous germination. Poor Alstroemeria germination is attributed to its seed dormancy (2). 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 germination is necessary for recovery of seed germination in this plant.

Seed dormancy is a block to the completion of germination of an intact viable seed under favorable conditions (4). Two major forms of physiological seed dormancy have been described, namely embryo and coat dormancy (5). Physiological dormancy can be divided in to three levels: deep, intermediate and slight dormancy (6). 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 (2). In physiological dormant seed, it is thought that temperature and GAs can both release dormancy and promote germination

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(5,6). GAs plays a key role in dormancy release and promotion of germination (5). 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 (7). In case Alstroemeria increased germination of the warm-cold treatment suggests that there are physiological factors in the seed coat that are responsive to cold stratification or that time is required for softening of the seed coat (2). 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 (8). 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 (4).

Potassium nitrate (KNO_3) is most widely used chemical for promoting seed germination of many species (9). According to Bewley and Black (10), KNO_3 raises the ambient oxygen levels by making less oxygen available for citric acid cycle. Functioning of nirates in seed germination through reduction to ammonium ions as occurs in the nutrition of plants (11).

One of the new plant growth regulators is Salicylic acid and its derivates like Acetylsalicylic acid. They could be raised to the status of the above phytohormones because they have a significant impact on the various aspects of the plant life (12). The inclusion of SA at 0.5 mM in the germination medium was associated with increase germination percentage of tomato (13). Basra et al. (14) investigated the possibility of seed invigoration by seed treatments with salicylicate in rice. Pretreatment with SA significantly improved germination potential and growth criteria of maize seedlings at both 25 and 15 ◦C (15). The germination might be attributed to increased metabolic activities in the treated seeds (14,16). Morever, SA treatments maintain the IAA and cytokinin levels in the plant tissues, which enhanced the cell division (17).

The aim of the present study was to find a practical method to promote *A.ligtu* hybrid seed germination and dormancy breaking by mean of stratification and GA_3 SA and KNO_3 application. Therefore, we examine the effect of some treatments on *A.ligtu* hybrid seed germination.

MATERIALS AND METHODS

Seeds of *Alstromeria ligtu* hybrid were immediately washed with tap water and then divided to eight groups (each group was divided to four replicates) and subjected to one of the following treatment: soaking in tap water only for 24 h (control), soaking in water for 24 h and then stratified at 5±1^{o} C up to three weeks, soaking in GA_3 or SA (0, 100, 200 and 400 mg.l^{-1}) and KNO_3 (0, 0.1, 0.2, 0.4 and 0.8%) solutions for 24 h supplemented with and without stratification at 5±1^{o} C up to three weeks. 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 ^{o} C, 16 h light) after sowing in either soil or MS (18) media. Seeds held at growth chamber (21 ^{o} C, 16 h light) for one week before apply chilling.

The seeds were sowing directly in the soil mixture (peat/sand/perlite 1:1:1 V/V) at a depth of approximately 0.5-0.7 cm in pot. 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 ^{0}C and 16h light.

For *in vitro* study the seeds were incubated in 250 ml jars containing 1/2 MS medium, 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 (19). Mean Germination Time (MGT) was calculated to assess the rate of Germination (20). 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^{0}C to reach constant weight.

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

Culture type	Treatments		Germination (%)	Germination of Rate (in day)	Mean of germination time	Number of leaf	Number of root
	GA_3×Chilling						
In vivo	0	+	36.67^{h}	0.17^{r}	23.50^{f}	2.71^{cdefgh}	1.61^{hi}
In vivo	0	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	0	+	40^{h}	0.17^{r}	21.73^{g}	1.55^{klmn}	2.11^{efgh}
In vitro	0	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{J}
In vivo	100	+	86.67^{b}	0.39^{b}	16.44^{o}	5.04^{a}	3.77^{a}
In vivo	100	-	13.33^{i}	0.026^{v}	49.83^{a}	1.97^{ijkl}	1.40^{i}
In vitro	100	+	96.50^{a}	0.41^{a}	15.05^{p}	4.28^{b}	3^{bc}
In vitro	100	-	16.55^{i}	0.033^{u}	47.50^{c}	1.22^{mn}	1.45^{i}
In vivo	200	+	76.70^{cde}	0.34^{g}	18.66^{kl}	3.17^{c}	2.14^{efgh}
In vivo	200	-	10^{i}	0.019^{w}	48.92^{b}	1.82^{ijklm}	1.40^{i}
In vitro	200	+	85.70^{b}	0.36^{d}	16.75^{no}	2.03^{hijk}	2.66^{bcde}
In vitro	200	-	15^{i}	0.025^{v}	43.17^{e}	0.97^{n}	1.75^{hi}
In vivo	400	+	70^{defg}	0.30^{n}	19.84^{hi}	2.8^{cde}	1.77^{hi}
In vivo	400	-	15.50^{i}	0.033^{u}	44.10^{d}	2.1^{fghijk}	1.57^{hi}
In vitro	400	+	80^{bcd}	0.34^{h}	17.41^{mn}	1.91^{ijkl}	2.50^{cdef}
In vitro	400	-	17^{i}	0.044^{t}	38.50^{f}	1.33^{lmn}	1.55^{hi}
	SA×Chilling						
In vivo	100	+	70.50^{defg}	0.31^{l}	18.56^{kl}	4.31^{b}	2.66^{bcde}
In vivo	100	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	100	+	76.33^{cde}	0.37^{c}	18.17^{lm}	3.33^{c}	3.13^{b}
In vitro	100	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vivo	200	+	67^{efg}	0.32^{k}	18.93^{ijkl}	2.47^{defghi}	1.65^{hi}
In vivo	200	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	200	+	$73^{\cdot}33^{cdef}$	0.36^{e}	18.79^{jkl}	2.30^{efghij}	2^{fghi}
In vitro	200	-	0j	0^{x}	0^{q}	0^{o}	0^{j}
In vivo	400	+	66.70^{efg}	0.30^{n}	19.96^{h}	3^{cd}	1.97^{fghi}
In vivo	400	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	400	+	70^{defg}	0.32^{k}	19.34^{hijk}	$2.41^{defghij}$	1.86^{ghi}
In vitro	400	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
	KNO_3×Chilling						
In vivo	0.1	+	63.33^{fg}	0.25^{p}	21.27^{g}	2.75^{cdefg}	1.80^{ghi}
In vivo	0.1	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	0.1	+	73.33^{cdef}	0.32^{k}	19.68^{hij}	1.75^{jklm}	2.37^{defg}
In vitro	0.1	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vivo	0.2	+	60.22^{g}	0.24^{q}	21.17^{g}	3.25^{c}	2.58^{bcde}
In vivo	0.2	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	0.2	+	70.00^{defg}	0.30^{m}	19.72^{hij}	$2.41^{defghij}$	2.85^{bcd}
In vitro	0.2	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vivo	0.4	+	73.50^{cdef}	0.29^{o}	21.20^{g}	2.77^{cdef}	1.70^{hi}
In vivo	0.4	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	0.4	+	76.81^{cde}	0.32^{j}	19.75^{hij}	2.08^{ghijk}	2^{fghi}
In vitro	0.4	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vivo	0.8	+	80^{bcd}	0.33^{i}	19.12^{hijkl}	4.08^{b}	2.99^{bc}
In vivo	0.8	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}
In vitro	0.8	+	83.44^{bc}	0.37^{d}	19.04^{hijkl}	2.47^{defghi}	3.66^{a}
In vitro	0.8	-	0^{j}	0^{x}	0^{q}	0^{o}	0^{j}

†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.

STATISTICAL ANALYSIS

Experiment was randomized complete design with 4 replications. The statistical analysis was made using the GLM 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 ($p < 0.05$). There were significan difference on germination percentage affected by GA_3, SA and KNO_3 along with 21days of stratification ($P<0.05$) **(Table 1).** Results showed that the germination percentage was higher in the seeds treated with GA_3 than in those treated with SA or KNO_3 **(Table 1).** Among the difference GA_3 consentrations and compared with other treatments, soaking seeds in 100 $mg.l^{-1}$ GA_3 +21days of stratification resulted in higher germination (86.67%, 96.50%, respectively *in vivo* and vitro conditions) and was followed by 0.8% KNO_3 +21days of stratification (80%, 83.44% respectively *in vivo* and vitro conditions) and 100 $mg.l^{-1}$ SA +21days of stratification (70.50%, 76.33% respectively *in vivo* and *in vitro* conditions) **(Table 1).** Seeds treated with only stratification gave40% respectively *in vivo* and *in vitro* conditions. Seeds treated with 36.67%,400 $mg.l^{-1}$ GA_3 without stratification gave15.50%, 17% germination, respectively *in vivo* and *in vitro* conditions, whereas seeds treated with SA and KNO_3 without stratification and control had no germination **(Table 1).** These results showed that stratification treatment was more benefit for *A.ligtu* hybrid seed germination. MGT decreased with duration of stratification and concentrations of SA, KNO_3 and GA_3. The seeds treated with 100 $mg.l^{-1}GA_3$ + stratification, MGT reached 16.44, 15.05 day respectively *in vivo* and *in vitro* conditions, But the seeds just treated with GA_3, germinated after 47.50, 43.17 and 38.50 days, respectively, at 100, 200 and 400 $mg.l^{-1}$ *in vitro* conditions **(Table 1).**

In case of SA (100 $mg.l^{-1}$) and KNO_3 along with stratification (0.8%) maximum seed germination obtained after 18.56, 18.17 for SA, 19.12, 19.04 days for KNO3, in *in vivo* and *in vitro* conditions, respectively. In contrast, only stratification treatment of seeds in this expeirment observed on the 23.50 and 21.73 days in *in vivo* and *in vitro* conditions, respectively **(Table 1).** In the current study, seeds germination rate was depended to GA_3 and SA concentrations. In higher concentrations SA and GA_3 (200 and 400 $mg.l^{-1}$) germination and growth of seedlings decreased, showing that 100 $mg.l^{-1}$ was better than other concentrations. Further, MGT decreased in stratified compared to non-chilled treatments. The germination percentage in half strength MS medium was higher than *in vivo* rates **(Table 1).**

Stratification alone or in combination with plant growth substances improved the germination rate and uniformity, and early seedling growth in both under *in vitro* and *in vivo*. Maximum seedlings growth was recorded from seed treatment with 100 $mg.l^{-1}$ GA_3 + stratification treatment under invivo conditions. Therefore, Seeds of treated with 100 $mg.l^{-1}$ GA_3 produced the seedlings with yielded higher leaf number (5.04), root number (3.77), shoot length (11.30 cm), root length (5.50 cm), shoot (396.11 mg) and root (76.50 mg) fresh weight, shoot (25.13 mg) and root (8.83 mg) dry wieght as compared with other treatments under *in vivo* conditions (Table 1, 2). In case of KNO_3 and SA, Seeds of treated with 8% KNO_3 and 100 $mg.l^{-1}$ SA produced the seedlings with yielded higher as compared with other concentrations under *in vivo* conditions. Minimum growth and yield was noted in only GA_3 treatment. Although all GA_3 concentrations had positive effects on seedlings length either *in vivo* or *in vitro* condition, but 100 $mg.l^{-1}$ was better than other concentrations **(Table 2).**

DISCUSSION

Stratification at 5±1°C for 21 days or 100 $mg.l^{-1}$ GA_3 + 21days of stratification overcame seed dormancy and increased the germination percentage of *A.ligtu hybrid* seeds. This showed that these treatments were effective in inducing metabolic activity in the embryo required for the initiation of germination process (21). In current study, seeds able to absorb water, thus, seeds had no physical dormancy as postulated by Willan, (22) and Schmidt, (23) in other plants seed. Results showed that stratification was successful in breaking seed dormancy. Raisi, et al. (24) investigated dormancy break of *Ferula assa-foetida* seed and domenstrated that one period of stratification treatment could increase germination of the seed.

Table 2. *Effect of Gibberellic acid (GA_3) and stratification treatments on germination parameters in Alstroemeria ligtu hybrid seeds.*

Culture type	Treatments		Shoot length (cm)	Root length (cm)	F.W.S (mg)	F.W.R (mg)	D.W.S (mg)	D.W.R (mg)
	GA_3×Chilling							
In vivo	0	+	3.81^{g}	2.91^{h}	82^{f}	34^{hi}	6.43^{ef}	3.4^{fg}
In vivo	0	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vitro	0	+	2.31^{hi}	0.65^{kl}	27^{hij}	14.67^{kl}	3.39^{ghi}	1.6^{ij}
In vitro	0	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vivo	100	+	11.30^{a}	5.50^{a}	396.11^{a}	76.50^{a}	25.13^{a}	8.83^{a}
In vivo	100	-	1.97^{i}	0.95^{jkl}	28^{hij}	9.1^{lm}	1.40^{jk}	1.1^{0ijk}
In vitro	100	+	5.07^{f}	4.39^{defg}	80^{f}	51.67^{cd}	10.10^{d}	5.16^{cd}
In vitro	100	-	1.30^{jk}	0.57^{l}	9.2^{ij}	5^{mn}	0.44^{k}	0.51^{kl}
In vivo	200	+	9.38^{b}	4.54^{def}	340.70^{b}	39.10^{fgh}	17.77^{b}	6.53^{b}
In vivo	200	-	1.85^{ij}	1.20^{jk}	27^{hij}	11^{klm}	1.90^{ijk}	1.4^{ijk}
In vitro	200	+	3.59^{g}	2.78^{hi}	60^{fgh}	36^{hi}	6.20^{ef}	3.20^{fg}
In vitro	200	-	0.91^{k}	0.91^{jkl}	7.1^{ij}	6.9^{m}	0.41^{k}	0.57^{jkl}
In vivo	400	+	9.24^{b}	4.82^{bcd}	250^{c}	47.10^{de}	10.40^{d}	5.63^{bc}
In vivo	400	-	2.10^{i}	1.40^{j}	32^{hij}	14^{kl}	2.50^{hij}	1.6^{ij}
In vitro	400	+	4.21^{g}	2.23^{i}	67^{fg}	33^{hi}	5^{fg}	3.10^{fg}
In vitro	400	-	1.10^{k}	0.86^{jkl}	11^{ij}	5.30^{mn}	0.65^{jk}	0.62^{jkl}
	SA×Chilling							
In vivo	100	+	8.89^{b}	5.17^{abc}	339.3^{b}	63.50^{b}	14^{c}	6.71^{b}
In vivo	100	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vitro	100	+	5.5^{ef}	4.03^{efg}	71^{f}	31.67^{i}	6.30^{ef}	3.40^{fg}
In vitro	100	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vivo	200	+	7.96^{c}	4.8^{bcd}	237^{c}	42.67^{efg}	13.80^{c}	4.56^{de}
In vivo	200	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vitro	200	+	4.96^{f}	4^{fg}	58.67^{fgh}	38^{fghi}	4.86^{fg}	2.96^{gh}
In vitro	200	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vivo	400	+	5.59^{ef}	4.60^{cde}	152^{e}	33^{hi}	8.03^{e}	4^{efg}
In vivo	400	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vitro	400	+	3.97^{g}	3.86^{g}	51^{fgh}	21^{je}	3.90^{gh}	2.10^{hi}
In vitro	400	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
	KNO_3×Chilling							
In vivo	0.1	+	7.82^{c}	4.20^{efg}	168^{de}	34.33^{hi}	7.93^{e}	5.33^{cd}
In vivo	0.1	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vitro	0.1	+	2.50^{hi}	0.95^{jkl}	30.67^{hij}	15^{kl}	3.52^{ghi}	1.70^{l}
In vitro	0.1	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0l
In vivo	0.2	+	7.48^{c}	5.22^{ab}	189^{d}	43.07^{ef}	10.40^{d}	6.10^{bc}
In vivo	0.2	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0l
In vitro	0.2	+	3.96^{g}	2.69^{hi}	55^{fgh}	36.67^{ghi}	6.30^{ef}	3.03^{gh}
In vitro	0.2	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0l
In vivo	0.4	+	6.56^{d}	5.30^{ab}	180^{de}	34.10^{hi}	6.50^{ef}	5.10^{cd}
In vivo	0.4	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0l
In vitro	0.4	+	2.80^{h}	0.93^{jkl}	36^{ghi}	16^{jk}	3.7^{ghi}	1.93^{i}
In vitro	0.4	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vivo	0.8	+	8.84^{b}	5.62^{a}	257.30^{c}	68.20^{b}	12.77^{c}	8.80^{a}
In vivo	0.8	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}
In vitro	0.8	+	5.80^{e}	3.14^{h}	85.33^{f}	57^{c}	7.50^{e}	4.10^{ef}
In vitro	0.80	-	0^{l}	0^{m}	0^{j}	0^{n}	0^{k}	0^{l}

†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.

F.W.S: Fresh weight shoot, F.W.R: Fresh weight root, D.W.S: Dry weight shoot, D.W.R: Dry weight root

Stratification has been reported to induce an increase in GA_3 concentration (25). Control treatment had no germination which indicating a high level of dormancy. It has been reported that germination and dormancy brecking can be induced by GA_3 in many plant species, e.g., *Trichocereus terscheckii* (26), *Rubia tinctorum L* (27) *Pedicularis olympica* (28), *Amaranthus retroflexus* L. (29) and *Acalypha indica* L. (30). According to the results found in this study, *A.ligtu* hybrid species probably exhibits a combination of physiological dormancy. GA_3 improved germination percentage which could indicate the presence of chemical dormancy, as application of GA_3 has shown effect on overcoming dormancy caused by inhibitors (10). Physiological dormancy in seeds is dependent on the ratio of the levels of abscisic acid (a growth inhibitor) and GA (a growth stimulator) (31). 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 (10).

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 (32).

GA_3 is effective in breaking the slight physiological dormancy, but it does not overcome the deep physiological dormancy (33). 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 (6). Further, physiological barriers to germination in embryos have been overcome by cold stratification in a number of rose species (34). Results obtained in this study present strong evidence that the pericarp, the testa, and the embryo play important roles in regulating seed dormancy. King and Bridgen (2) reported that may be physiological inhibitors in the seed coats appear to cause a combined dormancy in Alstroemeria seeds. 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 (35). El-Refaey and El-Dengawy (36), 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, generally in the range of 1-10 °C, can break seed dormancy for a number of species (10), and the data presented here showed that this includes *A.ligtu* hybrid. 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 (4).

Seeds of treated with 100 $mg.l^{-1}$ GA_3 + 21 day of stratification produced the seedlings with yielded higher number of leaf, length of shoot, shoot and root dry weight as compared with other treatments. Application of GA_3 on seeds *Acacia nilotica*, *Albizzia lebbeck* and *Prosopis cineraria*, increased the dry weight of seedlings (37).

Pretreatment with 100 $mg.l^{-1}$ SA supplemented with chilling, significantly improved germination potential and growth *Alstroemeria ligtu* hybrid. This showed that these treatments were effective in inducing metabolic activity in the embryo erquired for the initiation of germination process. These results are in consistent with those of (16) and (38), which showed a promotion in seed germination with SA appliaction. Bean seed treatment with SA improved germination percentage, germination rate and content of Indolacetic acid (IAA), GA_3 increased in response to seed soaking in SA at 15°C (39). In higher concentration SA (200 and 400 $mg.l^{-1}$) germination and growth of seedlings decreased. The might be explained by the fact that lower concentration enhances the activity of hydrolases, which increased the reserve breakdown and earlir start of germination (16). These results are in consistent with reports of Sushma-Negi *et al.*, (40). They found that percentage of seed germination was decreased with the increasing level used of SA. Hayat et al. (41), the leaf number, fresh and dry mass per plant of wheat seedlings raised from the grains soaked in lower concentration of SA, increased significantly. Higher levels of SA may inhibit nitrate uptake system and cause retardation in growth and development (42). Improved seedling fresh and dry weights might be due to

increased cell division within the apical meristem of seedling shoots and roots, which caused an increase in plant growth (16). internal nitrate may provide an inductive concentration to nitrate reductase activity at lower concentrations of SA and/or SA induced modulation of nitrogen use efficiency in cucumber cotyledons (43).

Many nitrogen-containing compounds, promote dormancy release and seed germination in many species (11). 8% KNO_3 significantly enhanced seed germination and seedling growth is presented in Table 1 and 2. Positive effects of KNO3 on seed germination were reported by Osman et al. (44), Kaya *et al*. (19), and Ahmadvand et al. (45) and Jankjo et al. (46), as well. KNO_3 may be helpful for reactivation of metabolic process of seeds. The analysis of the cause for this control of germination is by Roberts, who concludes that nitrate acts an oxidization substrate in a metabolic regulatory process involving NADH-NADP in the pentose phatway of glucose metabolism (47). Morever, it plays role in formation of protoplasm and new cells, as well as encourage plant elongation. The higher root number was observed in 0.8% KNO_3. It is plausible that its positive effect might be due to its role KNO_3 in influencing the permeability of the membranes which ultimately lead to activation of enzymes involved in protein synthesis and carbohydrate metabolism (48).

The results showed that stratification was an effective factor in the breaking of seed dormancy of this plant and KNO_3 had resonance effect. Because KNO_3 concentrations alone and in control treatment (without stratification) had not positive effect. Reports of Cetinbas and Koyuncu (49) on *Prunus avium* seeds and Rouhi et al. (50) on *Tulipa kaufmanniana* Regel is consistent with our results.

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 has a positive effect on *A.ligtu* hybrid seeds germination. In general, *in vivo* germination rates were lower than *in vitro* rates, but seedlings growth under vivo conditions was higher as compared with *in vitro* conditions **(Table 1 and 2)**.

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 $mg.l^{-1}$ GA_3 + 21 days of stratification overcame seed dormancy and increased the germination percentage of *A.ligtu* hybrid seeds. The study suggests KNO_3 treatment is economic and easily applicable by nursery workers and poor farmes in developing mass planting stock, over costly plant growth regulators and associated technicalities. In case of KNO_3 and SA, Seeds of treated with 8% KNO_3 and 100 $mg.l^{-1}$ SA produced the seedlings with yielded higher as compared with other concentrations in *in vivo* and vitro conditions.

ACKNOWLEDGMENT

We thank M.Sc. Mansour Mehri for collecting seeds. We are grateful to Dr. Jaafar Mohammadi for his suggestions and helps.

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