African Journal of Biotechnology Vol. 11(68), pp. 13173-13178, 23 August, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1131
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Effects of cold-stratification, gibberellic acid and potassium nitrate on seed germination of yellow gentian (*Gentiana lutea* L.)

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Accepted 8 August, 2012

The purpose of this study was to show the effect of cold-stratification, gibberellic acid and potassium nitrate on seed germination of yellow gentian (*Gentiana lutea* L.). The seeds of natural yellow gentian populations of the Albanian Alps (Kosovo) were collected in September 2010. Seeds placed in filter paper (plastic bag) and in mixture of sand-soil were cold-stratified (2±1°C) for 72 days. The effect of different concentrations of gibberellic acid (250, 500 and 1000 ppm GA_3) and potassium nitrate (0.1, 0.2 and 0.3% KNO_3) in final germination percentage (FGP), mean germination time (MGT) and germination index (GI) in non-stratified and stratified seeds were examined. Seeds stratified in filter paper and treated with 1000 ppm GA_3 and 0.1% KNO_3 showed significantly higher percentage of germination (FGP and GI), while seeds stratified in mixture of sand-soil showed the highest values of FGP and GI in treatments with 500 ppm GA_3 and different concentrations of KNO_3. The cold-stratification and GA_3 treatments of yellow gentian seeds are suitable for the higher percentage of germinations.

Key words: *Gentiana lutea* L., cold-stratification, seeds, germination, gibberellic acid (GA_3), potassium nitrate (KNO_3).

INTRODUCTION

Gentiana lutea L. is a perennial herb which grows up to 1.5 m, with large, parallel-veined leaves. The yellow flowers are borne in dense clusters along a sturdy flowering stalk. The native range of the species includes the mountains of Central and Southern European and Asia Minor. These species belong to two subspecies: subsp. *lutea* and subsp. *symphyandra* Murb. Occurrence centre of this species is in European Alps (Southern and Central parts) (Tutin, 1972). Gentian root is a traditional bitter tonic that stimulates appetite. It is a roborant and cholagogue, and is used in the case of poor appetite, flatulence and bloating, as well as for dyspepsia and anorexia. It is also widely used in homoeopathy (Ben-Erik and Wink, 2004). Its medicinal traits were known to the ancient people. Illyrian King Gentian was the first to indicate the medicinal traits of this plant in 200 BC (Jedjović and Maletić, 2007). The native populations of this species in Kosovo (Albanian Alps and Sharri Mountains) grow on mountain meadows and pasture, rarely in forest and rocky terrains at altitudes of 1200 to 2500 m (Millaku, 1999, 2005). The temperature

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Abbreviations: FGP, Final germination percentage; **GI,** germination index; **MGT,** mean germination time; **GA_3,** gibberellic acid; **KNO_3,** potassium nitrate.

influences the percentage and rate of germination through its effects on seed deterioration, loss of dormancy and the germination process itself (Roberts, 1988). It was observed that alternating temperatures profoundly enhanced breaking of seed dormancy in barnyard grass, common lambs-quarters and redroot pigweed when soil water content was high enough for germination (Martinez-Ghersa et al., 1997).

Gibberellins (GAs) and potassium nitrate (KNO_3) are used for breaking seed dormancy and promoting seed germination. Mostly, gibberellins are directly implicated in the control and promotion of germination. A biochemical reaction known to be enhanced by GA is the synthesis of hydrolases (especially α amylase) in the endosperm of cereal grains. Its breakdown is generally assumed to be an essential process of germination (Kolumbina et al., 2006). GA stimulates seed germination via amylase synthesis (Finch-Savage and Leubner, 2006). Also, nitrate (such as KNO_3) clearly stimulates the germination of dormant seeds (Alboresi et al., 2005). KNO_3 is the most widely used chemical for promoting germination. Solutions of 0.1 to 0.2% KNO_3 are common in routine germination testing and are recommended by the Association of Official Seed Analysts and the International Seed Testing Association for germination tests of many species (Copeland and Mc Donald, 1995; Basra, 1994). The effect of KNO_3 was discovered when it was proven that Knop's solution encourages germination of some plant species. Recently, it was confirmed that potassium nitrate interacts with light and temperature. A major problem in cultivating gentian is the seed which has extremely low germination rates.

The aim of this research was to determine the effect of cold-stratification, gibberellic acid and potassium nitrate on seed germination rates of yellow gentian.

MATERIALS AND METHODS

This study was carried out during 2010 to 2011 in the Department of Biology and Faculty of Agriculture, University of Pristina, Republic of Kosovo.

Seeds of yellow gentian (*G. lutea* L. subsp. *symphyandra* Murb.) were collected from native population in the Albanian Alps (Kosovo) in the subalpine zone at altitude of 1850 m. The collected seeds were stored in normal conditions. The seeds were divided into three groups (variants): non-stratified seeds (A), stratified seeds with filter paper in plastic bag (B) and stratified seeds with mixture of sand-soil (C). Stratification was carried out for 72 days at the temperature 2±1°C. final germination percentage (FGP), mean germination time (MGT) and germination index (GI) were examined after the treatment of the three seed groups with different concentrations of gibberellic acid (0, 250, 500 and 1000 ppm GA_3) and potassium nitrate (0.1%, 0.2% and 0.3% KNO_3).

Chemical and GA_3 treatments

Before treatment with GA_3 and KNO_3, all seed groups were disinfected with 70% ethanol for three minutes and rinsed three times with distilled and sterilized water. The three seed groups were then put into flasks containing 250, 500 and 1000 ppm GA_3 for 24 h in the dark and 0.1, 0.2 and 0.3% potassium nitrate (KNO_3) for 24 h in light condition.

Seed germination assays

For the three seed groups, the experiment was carried with four replications of 100 seeds each for 15 treatments and the control. Seeds were inoculated on top of double layered papers (ISTA, 1996) with 5 ml water in 10 cm Petri dishes. These Petri dishes were put into sealed plastic bags to avoid moisture loss. Incubation was done in a germinator for 30 days with photoperiod of 16 h with fluorescent light, 40 μmol $m^{-2}s^{-1}$ and in shifting temperature of 25±1°C.

Germinated seeds were counted every 48 h for 30 days. According to Sharma and Sharma (2010), seeds were considered to have germinated upon emergence of radicles (≥ 2 mm). MGT was calculated according to the following equation (Moradi et al., 2008).

$$MGT = \Sigma Dn / \Sigma n$$

Where, n is the number of seeds, which germinated on day D and D is the number of days counted from the beginning of germination.

GI was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following the formula:

$$GI = \frac{\text{No. of ger. seed}}{\text{Day. of first count}} + ... + \frac{\text{No. of ger. seed}}{\text{Day. of final count}}$$

Where, ger. is germinated.

Statistical analysis

Least significant differences (LSD) at *P<0.05* and *P<0.01* level of probability was determined after analysis of variance (ANOVA) for all treatments according to the randomized complete block design (RCBD). For the analysis of variance, statistical analysis software (SAS, SAS institute, 2002) was used. Correlations between variables were examined using the Pearson correlation.

RESULTS AND DISCUSSION

The analysis of the variance with ANOVA (Table 2) showed highly significant differences for the mean square of the treatments of stratified seeds for all investigated parameters (FGP, MGT and GI). Also, the impact of interactions between treatment and stratification (TxS) was with higher influence on the different traits. Significant variation confirmed in our study can be attributed to the hormonal (GA_3) treatments and cold-stratification. Bhan and Sharma (2011) confirmed that the interaction effect of stratification and chemical treatments on *Prunus armeniaca* L. had significant influence on the germination of seeds. From the correlative analysis of treatments according to Pearson, there are ascertained variable values of the phenotypic correlation coefficient. In all parameters measured, coefficients of phenotypic variation were 10.81 (MGT), 39.34% (FGP) and 40.47 (GI). There was positive significant correlation (P<0.01;

Table 1. Effect of seed stratification, gibberellic acid and potassium nitrate on FGP, MGT and GI in *G. lutea* L., the same letters are not significantly different at the $P \leq 0.01$ and $P \leq 0.05$ levels.

Treatment	A			B			C		
	FGP (%)	MGT	GI	FGP (%)	MGT	GI	FGP (%)	MGT	GI
Control (H_2O)	6.33	8.37	4.48	7.67	13.11	4.02	21.67	9.57	14.40
GA_3 (0) + KNO_3 (0.1%)	8.00	10.50	4.67	12.00	14.77	5.30	26.00	11.49	14.71
GA_3 (0) + KNO_3 (0.2%)	7.00	9.51	4.50	12.00	12.66	6.43	34.67	9.86	22.09
GA3 (0) + KNO_3 (0.3%)	8.00	12.53	3.76	11.00	12.46	5.81	34.33	12.24	18.51
GA_3 (250) + KNO_3 (0)	21.00	12.26	11.38	64.33	13.17	30.48	46.00	13.47	22.85
GA_3 (250) + KNO_3 (0.1%)	15.00	12.06	8.13	60.00	13.60	29.59	57.33	12.26	31.60
GA_3 (250) + KNO_3 (0.2%)	16.67	12.77	8.58	59.67	14.31	28.39	52.00	12.57	27.28
GA_3 (250) + KNO_3 (0.3%)	14.00	12.05	7.69	60.00	12.64	31.95	53.00	12.46	28.41
GA_3 (500) + KNO_3 (0)	17.67	14.29	7.97	79.67	14.12	36.81	70.67	12.51	37.86
GA_3 (500) + KNO_3 (0.1%)	14.33	11.43	8.25	78.67	14.30	37.90	72.67	11.58	43.34
GA_3 (500) + KNO_3 (0.2%)	16.00	13.25	7.87	75.67	14.55	34.48	71.33	10.67	45.38
GA_3 (500) + KNO_3 (0.3%)	15.33	12.90	7.74	78.67	13.44	39.78	74.33	11.83	44.69
GA_3 (1000) + KNO_3 (0)	21.33	12.87	10.45	88.67	11.46	50.06	69.67	13.17	37.94
GA_3 (1000) + KNO_3 (0.1%)	18.00	12.56	9.18	93.67	10.39	58.28	71.67	11.43	43.20
GA_3 (1000) + KNO_3 (0.2%)	17.00	12.21	8.85	93.33	10.99	56.30	68.67	12.10	40.22
GA_3 (1000) + KNO_3 (0.3%)	15.00	13.64	7.03	92.00	10.68	56.47	71.00	11.99	41.40
Mean for treatments	14.42	12.08	7.53	60.44	12.92	32.00	55.94	11.83	32.12
LSD P≤0.05	7.008^{NS}	3,885^{NS}	3,259^{NS}	18,205^{NS}	3,495^{NS}	10,752^{NS}	25,928^{NS}	3,127^{NS}	21,328^{NS}
LSD P≤0.01	9.437**	5,232^{NS}	4,389**	24,517**	4,706^{NS}	14,480**	34,918**	4,211^{NS}	28,722**

A, Non-stratified seeds; B, seeds stratification with filter paper; C, seeds stratification with sand-soil; FGP, final germination percentage; MGT, mean germination index; GI, germination index; Gibberellic acid (GA_3) treatment including four levels: 0, 250, 500 and 1000 ppm; KNO_3 treatment including four levels: 0, 0.1, 0.2 and 0.3% (v/v), NS, non-significant; *significant at 0.05 probability level; **significant at 0.01 probability level.

r=0.96) between FGP and GI on non-stratified seeds. In addition, a positive significant correlation (P<0.01; r=0.87) of FGP and FGP between non-stratified and filter paper stratified seeds was seen. Results are presented in Table 3.

The effect of seed stratification on FGP, MGT and GI

The FGP of stratified seeds of yellow gentian with filter paper and sand-soil was higher when compared with non-stratified seeds (7.67, 21.67 and 6.33%) (Table 1). Our results of higher FGP of stratified seeds are in accordance with other authors results. Kouncu and Sesli (2000) showed that seeds of *Juglans regia* stratified for 125 days had a higher germination percentage. In addition, Jedjović and Maletić (2007) using seeds of yellow gentian moistened and cooled at 4°C for 90 days showed best germination percentage. Stratification might act simply to lower the rate of enzymatic reactions taking place in the seed, and might cause differential changes in enzyme concentrations (Bewley and Black, 1994). The mean of FGP of the overall treatments for non-stratified seeds was 14.42%, for stratified seeds in sand-soil, it was 55.94% and for stratified seeds in filter-paper in plastic bags, it was 60.44%. The lower FGP of non-stratified seeds in our study is in accordance with results of Sharma and Sharma (2010), who in fresh seeds of *Bunium persicum* showed deep dormancy, confirmed no

Table 2. Mean square of physiological traits of *G. Lutea* L.

Sources of variation	FGP	MGT	GI
Treatment (T)	516.72**	6.70**	288.54**
Stratification (S)	1121.94**	5.82**	617.405**
T x S	108.67**	6.10**	76.404**
CV%	39.34	10.81	40.47

*Significant at 0.05 probability level; **significant at 0.01.

Table 3. Coefficients of correlation for characteristics studied according to Pearson.

Treatment		A			B			C		
		FGP	MGT	GI	FGP	MGT	GI	FGP	MGT	GI
A	FGP	1								
	MGT	0.72**	1							
	GI	0.96**	0.54*	1						
B	FGP	0.87**	0.73**	0.81**	1					
	MGT	-0.21	-0.13	-0.17	-0.35	1				
	GI	0.80**	0.66*	0.74**	0.97**	-0.54	1			
C	FGP	0.75**	0.75**	0.66*	0.94**	-0.24	0.89**	1		
	MGT	0.71**	0.67*	0.66*	0.48	-0.11	0.43	0.36	1	
	GI	0.64*	0.65*	0.56*	0.91**	-0.24	0.86**	0.97**	0.17	1

A, Non-stratified seeds; B, seeds stratification with filter paper; C, seeds stratification with sand-soil; NS, non-significant; *significant at 0.05 probability level; **significant at 0.01 probability level; FGP, final germination percentage; MGT, mean germination index; GI, germination index.

germination until 120 days of optimum germination conditions. The germination percentage of non-stratified seeds of *P. armeniaca*, was lower when compared with stratified seeds (Bhan and Sharma, 2011). The average of FGP for each treatment of the three seed groups (Figure 1) was highest in the treatment of 1000 ppm of GA_3. The obtained results are in accordance with previous researches (Moussa et al., 1998; Rouhi et al., 2010; Nadjafi et al., 2006; Zare et al., 2011).

The effect of GA_3 and KNO_3 treatments on FGP, MGT and GI of non-stratified seeds

In non-stratified seeds (Table 1) treated with 1000 ppm GA_3 and 0% KNO_3, the value of FGP was significantly higher (P<0.01) in comparison with three treatments without gibberellins (0 GA_3 and 0.1% KNO_3; 0 GA_3 and 0.2% KNO_3; 0 GA_3 and 0.3%) and control (21.33%; 6.33 to 8%, respectively). In the same seed treatments, the highest value of MGT was found in the case of treatment with 500 ppm GA_3 and 0% KNO_3 (14.29). The highest value of GI in all treatments was recorded in treatment with 250 ppm GA_3 and 0% KNO_3 (11.38). The value of GI is significantly higher in comparison (P<0.01) with the control. In addition, Gashi et al. (2011, 2012) confirmed that the seeds of *Ramonda nathaliae* treated with gibberellic acid and potassium nitrate germination did not exceed 53%.

The effect of GA_3 and KNO_3 treatments on FGP, MGT and GI of filter-paper stratified seeds

The values of FGP and GI of seeds stratified in the filter paper (group B) and treated with 1000 ppm GA_3 and 0.1% KNO_3 (Table 1) are significantly higher in comparison with without gibberellins (0 GA_3 and 0.1% KNO_3; 0 GA_3 and 0.2% KNO_3; 0 GA_3 and 0.3%) and control (P<0.01; FGP: 93.67% and 7.67 to 12% and GI: 58.28, 4.02 to 6.43, respectively). The highest value (but not statistically significant) of MGT was recorded on seed treated with 0 GA_3 and 0.1% KNO_3 (14.77). Our results of effect of GA_3 on germination seeds of yellow gentian are in accordance with results of Erken and Kaleci (2010), who in seeds of yellow gentian treated with 600 ppm GA_3 recorded higher values of germination seeds. Our results of effect of GA_3 on germination of seed treated with GA_3 are also consistent with results of Petrova et al. (2006) who in treated seeds of *G. lutea* with GA_3 (50 mg J1)

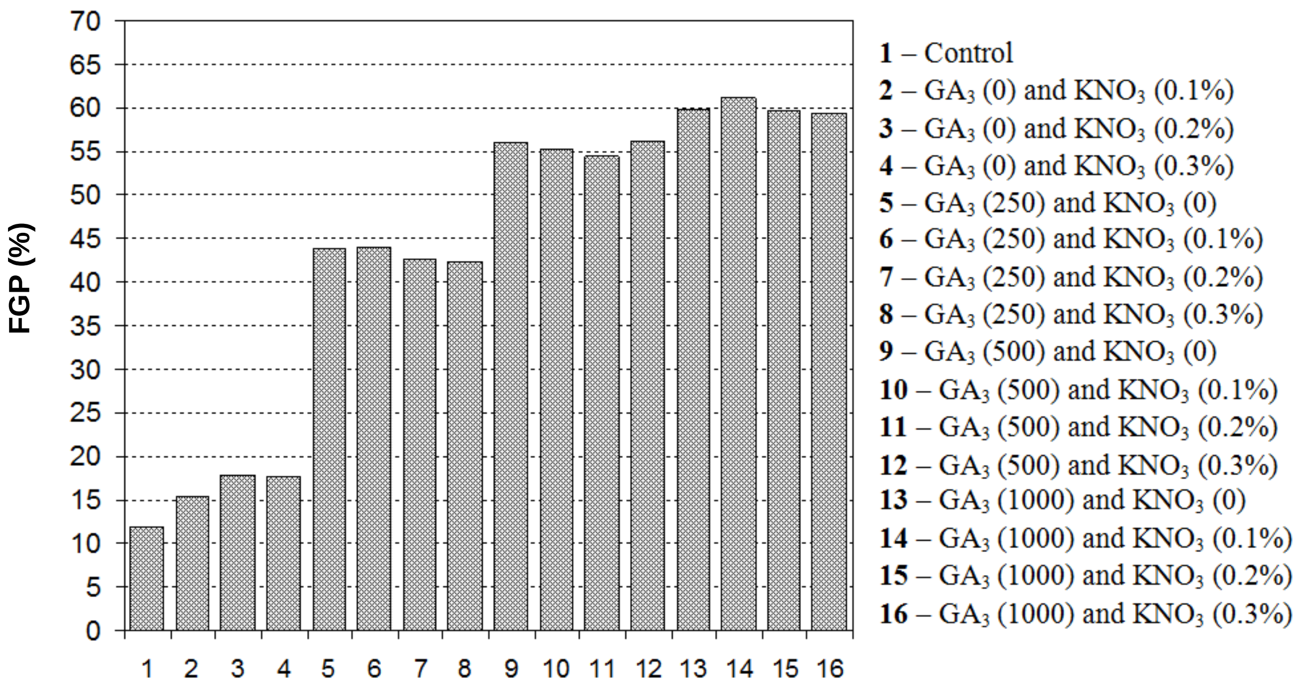

Figure 1. Mean of final germination percentage (FGP) of three seeds groups (non-stratified; stratified seeds with filter paper in plastic bag and stratified seeds with mixture of sand-soil) after their treatment with different concentrations of GA_3 and KNO_3.

confirmed higher percentage of germination (42.50% germination for the unscarified seeds and 60% for the scarified ones). Research carried out in recent years have shown that gibberellin is an effective germination stimulator in many plant species (Giba at al., 1993; Samaan et al., 2000; Çetinbaş and Koyuncu, 2006; Dewir et al., 2011; Bhan and Sharma, 2011)

The effect of GA_3 and KNO_3 treatments on FGP, MGT and GI of mixture of sand-soil stratified seeds

The seeds of yellow gentian stratified in a mixture of sand-soil (group C) and treated with 500 ppm GA_3 and 0.3% KNO_3 showed significantly FGP ($P<0.01$; 74.33%) in comparison with seeds treated with 0 GA_3 and different levels of KNO_3 and control (Table 1). For this seed groups, MGT had higher values in the treatment with 250 ppm GA_3 and 0% KNO_3 (13.47). For GI, the highest value was in the treatment with 500 ppm GA_3 and 0.2% KNO_3 (45.38), whereas the lowest value was in the treatments without gibberellins (0 GA_3 and 0.1% KNO_3; 0 GA_3 and 0.2% KNO_3; 0 GA_3 and 0.3%) and control (14.71, 22.09, 18.51 and 14.40, respectively). The treatments of three seed groups of yellow gentian with different concentration of KNO_3 (0.1, 0.2 and 0.3%) had no significant effect on seed germination. On the other hand, Yücel and Yilmaz (2009) confirmed the inhibiting effect of germination on seeds of *Salvia cyanescens* treated with different concentrations of KNO_3.

Conclusion

Seeds of yellow gentian have to undergo a period of cold stratification in order to obtain better results in seed germination. In order to obtain a value above 93.33% of FGP, the seed stratification of yellow gentian is recommended with the temperature of 2±1°C for 72 days in filter-paper placed in plastic bags hermetically sealed, and thereafter treated with 1000 ppm of GA_3 and 0.1% KNO_3.

ACKNOWLEDGEMENT

We would like to thank Prof. Dr. Isa Elezaj for professional collaboration.

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