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Full Length Research Paper

Effect of gibberellic acid and potassium nitrate on seed germination of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae*

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***Ramonda serbica* and *Ramonda nathaliae* are rare resurrection plants, endemic and relict species from Balkan Peninsula. The effect of gibberellic acid (GA_3) and potassium nitrate (KNO_3) were conducted to determine the seed germination response for these two species. An experiment was conducted with four replications and three treatments including: different concentrations of GA_3 (0, 250, 500 and 1000 part per million (ppm)) and KNO_3 (0, 0.1, 0.2 and 0.3% v/v) and their combination. Final germination percentage (FGP), mean germination time (MGT), germination rate index (GRI) and corrected germination rate index (CGRI) were measured. The highest FGP, GRI and CGRI of *R. serbica* were recorded in seeds treated with 1000 ppm GA_3 + 0.3% KNO_3, while MGT in seeds control. The highest FGP and GRI of *R. nathaliae* were recorded in the seeds treated with 500 ppm GA_3, while CGRI in seeds treated with 500 ppm GA_3 + 0.2% KNO_3. The seeds of *R. nathaliae* treated with different concentration of KNO_3 had significantly higher germination compared to the control seeds, while the seeds of *R. serbica* did not have an effect on seed germination. The GA_3 and KNO_3 treatments of *Ramondas* seeds are suitable for the higher percentage of germinations.**

Key words: *Ramonda serbica*, *Ramonda nathaliae*, seed germination, GA_3, KNO_3.

INTRODUCTION

Ramonda serbica and *Ramonda nathaliae* (Gesneriaceae) belongs to a small group of rare resurrection plants of the Northern hemisphere, which originate from the Balkan Peninsula as an endemic and relict species of the Tertiary period. The distribution of *R. serbica* extends in these countries of Balkan Peninsula: Albania, Kosovo, Montenegro, Serbia, FYR Macedonia, Bulgaria and Greece, whereas, *R. nathaliae* is sited only in FYR Macedonia, Serbia and Greece area. Their current distribution is restricted to the northern rocky slopes of gorges and canyons, mainly on foothills, reaching sometimes the alpine belts (Kosanin, 1921; Meyer, 1970). They all prefer limestone rocks, while *R.nathaliae* also settles on ophiolitic bedrock (Kosanin, 1921).

The ability of these *Ramonda* plants to live on harsh environmental conditions up till now have been researched on different aspects of morphological, physiological and biochemical as well as the propagation through *in vitro* cultivation. These researches has conveyed data to generate the cell membrane (Quartacci et al., 2002), antioxidative capacity (Sgherri et al., 2004; Jovanovic et al., 2011), photosynthetic activity (Augusti et al., 2001), CO_2 fixation and chlorophyll *a* fluorescence (Degl'Innocenti et al., 2008), genome size variation and polyploidy (Siljak-Yakovlev et al., 2008), osmotic

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Abbreviations: GA_3, Gibberellic acid; **KNO_3,** potassium nitrate; **PPM,** part per million; **FGP,** final germination percentage; **MGT,** mean germination time; **GRI,** germination rate index; **CGRI,** corrected germination rate index.

adjustment (Zivkovic et al., 2005) and *in vitro* cultivation from seeds of *Ramonda* plants (Kongjika et al., 2002; Dontcheva et al., 2009; Gashi et al, 2011).

Nevertheless, up to now there is only a few data from previous studies on seeds germination of *R. serbica* and *R. nathaliae*. Till date, study on *Ramonda* species on seeds has not been found in literature. Our current study indicates an increase on the percentage of seeds germination for these species.

Gibberellins and potassium nitrate (KNO_3) were used for breaking seed dormancy and promoting seed germination. Gibberellins is most 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). 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). Nitrate (such as KNO_3) clearly stimulates the germination of dormant seeds (Alboresi et al., 2005). The effect of KNO_3 was discovered when it was proven that Knop's solution encourages germination of some plant species.

These species belong to the group of "resurrection plants" and need to be preserved as ornamental plants by cultivation. The propagation of European Gesneriad species from natural seeds is very slow and difficult. Therefore, the objective of this study was to determine the effect of different concentration of GA_3 and KNO_3 treatments on seed germination and devise an effective method for improving seed germination of *R. serbica* and *R. nathaliae*.

MATERIALS AND METHODS

This research was conducted during 2011 in the Department of Biology, University of Pristina, Republic of Kosovo and Department of Biotechnology, University of Tirana, Albania. The study was carried out with *R. serbica* seeds collected from native populations in the gorge of Matos, Sharri Mountains (Kosovo) at an altitude of 910 m, and with *R. nathaliae* seeds collected from gorge of River Vardar near village Radusha (FYR Macedonia) at an altitude of 405 m.

Seed treatments

For the two Ramonda plants, all the seeds were disinfected with ethanol 70% for three minutes and rinsed three times with distilled and sterilized water, before treatments. After the disinfection, the seeds were divided into four treatment groups: (1) seeds of the first group were soaked on H_2O-distilled water (control); 2) seeds of the second group were treated with 0.1, 0.2 and 0.3% (v/v) potassium nitrate (KNO_3) for 24 h; 3) likewise, similar to the previous treatment, the seeds of the third group were put into flasks containing 250, 500 and 1000 ppm gibberellic acid (GA_3) for 24 h; and 4) seeds of the fourth group were soaked in aqueous solutions supplemented with 250 ppm GA_3 + 0.1% KNO_3, 250 ppm GA_3 + 0.2% KNO_3, 250 ppm GA_3 + 0.3% KNO3, 500 ppm GA3 + 0.1% KNO_3, 500 ppm GA_3 + 0.2% KNO_3, 500 ppm GA_3 + 0.3% KNO_3, 1000 ppm GA_3 + 0.1% KNO3, 1000 ppm GA_3 + 0.2% KNO_3, 1000 ppm GA_3 + 0.3% KNO_3, for 24 h in GA_3 and 24 h in KNO_3, respectively. For the all seeds groups, the experiment was conducted with four replicates of 100 seeds which were germinated on top of double layered papers (ISTA, 1996) with 5 ml of water in 10 cm Petri dishes. These Petri dishes were placed in sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate at 24 ± 1°C and at light regime 16 h light (day)/8 h darkness (night).

Germination tests

The germination percentage is an estimate of the viability of seeds. Germinated seeds were counted every 48 h for 29 days. According to Sharma and Sharma (2010), seeds were considered germinated upon emergence of radicles (≥ 2 mm). The following germination parameters were recorded:

1) FGP is: FGP = (number of germinated seeds/number of total seeds) × 100.

2) 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 were germinated on day D, and D is the number of days counted from the beginning of germination.

3) GRI = [(G1/1) + (G2/2) + (Gx/X)]

Where, G is the germination on each alternate day after placement 1, 2 and x is the corresponding day of germination (Esechie, 1994)

4) CGRI = (GRI/FGP) × 100

Where, FGP is the final germination percentage.

Statistical analysis

The statistical design was a completely randomized design. Four replications and 100 seeds per replicate were used. The mean and one-way ANOVA were calculated using SPSS software. The means were compared using Duncan's multiple range tests at 5% level of probability and least significant difference (LSD) test with the statistical significance set at P 0.01 and P 0.05 levels.

RESULTS AND DISCUSSION

The results of the analysis of variance of mean square for the germination parameters of *R. serbica* and *R. nathaliae* are shown in Table 1. The highly significant differences of the double interaction effects on traits (Gibberellin + KNO_3) of FGP, MGT, GRI and CGRI were

Table 1. Analysis of variance of gibberellic acid and potassium nitrate effects on seed germination properties for *R. serbica* and *R. nathaliae*.

SOV	df	Mean Square			
		FGP	MGT	GRI	CGRI
R. serbica					
Gibberellin	3	2527.838**	0.609*	309.129**	15.205*
KNO_3	3	1.198^{NS}	0.399^{NS}	0.118^{NS}	5.527^{NS}
Gibberellin * KNO_3	9	1821.001**	2.080**	275.549**	21.321**
R. nathaliae					
Gibberellin	3	363.888**	15.457**	262.930**	270.130**
KNO_3	3	656.178**	11.852**	99.772*	191.172**
Gibberellin * KNO_3	9	145.870**	7.072**	133.862*	127.556**

NS,** and * are respectively non significant and significant at the P 0.01 and 0.05 levels. FGP, Final germination percentage; MGT, mean germination time; GRI, germination rate index; CGRI, corrected germination rate index.

Table 2. Mean of FGP, MGT, GRI and CGRI for seeds of *R. serbica* under various levels of GA_3 (in ppm) and KNO_3 (in %).

Treatment	FGP	MGT	GRI	CGRI
Control (H_2O)	9.26^{g}	17.14^{h}	2.79^{h}	30.24^{i}
KNO_3 (0.1)	10.37^{g}	16.78^{fg}	3.25^{h}	31.54^{gh}
KNO_3 (0.2)	9.63^{g}	16.59^{fg}	3.12^{h}	32.37^{fgh}
KNO_3 (0.3)	8.89^{g}	16.27^{ef}	2.96^{h}	33.46^{efg}
GA_3 (250)	58.15^{f}	16.48^{fg}	18.36^{g}	31.58^{gh}
GA_3 (500)	68.40^{e}	16.15^{def}	23.90^{f}	34.99^{c-f}
GA_3 (1000)	$71.85d_e$	16.23^{ef}	24.64^{f}	34.33^{c-g}
GA_3 (250) + KNO_3 (0.1)	75.93^{cd}	15.60^{cde}	25.90^{f}	34.11^{d-g}
GA_3 (250) + KNO_3 (0.2)	82.15^{bc}	15.18^{c}	30.10^{de}	36.85^{a-d}
GA_3 (250) + KNO_3 (0.3)	85.93^{ab}	14.79^{abc}	31.48^{cd}	36.65^{a-e}
GA_3 (500) + KNO_3 (0.1)	75.70^{cd}	14.99^{bc}	28.44^{e}	37.58^{abc}
GA_3 (500) + KNO_3 (0.2)	91.81^{a}	15.26^{c}	33.74^{bc}	36.76^{a-d}
GA_3 (500) + KNO_3 (0.3)	89.26^{ab}	14.17^{a}	34.71^{ab}	38.90^{ab}
GA_3 (1000) + KNO_3 (0.1)	87.37^{fg}	15.39^{cd}	31.53^{cd}	36.14^{b-e}
GA_3 (1000) + KNO_3 (0.2)	82.22^{bc}	15.01^{bc}	30.79^{de}	37.32^{a-d}
GA_3 (1000) + KNO_3 (0.3)	92.26^{a}	14.22^{ab}	36.70^{a}	39.82^{a}

Mean in each column followed by same letters are not significantly different at the P 0.05 level using Duncan test. FGP, Final germination percentage; MGT, mean germination time; GRI, germination rate index; CGRI, corrected germination rate index.

ascertained for *R. serbica* and single effect (Gibberellin) for *R. nathaliae*.

Final germination percentage

Based on the results (Table 2) for FGP, significant difference was ascertained for R. serbica between seeds treated with a different gibberellic acid concentration (250, 500 and 1000 ppm GA3) and with combination of different concentration of potassium nitrate (0.1, 0.2 and 0.3% KNO_3) and untreated seeds (control). The highest germination percentage was detected in the seeds treated with 1000 ppm GA_3 + 0.3% KNO_3 (92.26%) and 500 ppm GA_3 + 0.2% KNO_3 (91.81%), compared to the control seeds (9.26%). Our results is in agreement with the results of Shanmugavalli et al. (2007), who treated seeds of sorghum with GA_3 (100 ppm) in combination with 0.5, 1, and 1.5% KNO_3 and obtained a germination percentage of 94%. Additionaly, Amri, (2011) treated seeds of *Terminalia sericea* with GA_3 (400 ppm) and confirmed a higher percentage of germination (67%) compared with the control. In our experiment, the seeds of *R. serbica* treated with different concentration of KNO_3 (0.2 and 0.3%) did not show significant results for FGP, and on the contrary, treatment with 0.3% KNO_3 inhibited the seeds germination (Table 2).

Similar results were reported from other authors, such

Table 3. Mean of FGP, MGT, GRI and CGRI for seeds of *R. nathaliae* under various levels of GA_3 (in ppm) and KNO_3 (in %).

Treatment	FGP	MGT	GRI	CGRI
Control (H_2O)	27.66^{e}	15.30^{c}	11.13^{e}	40.80^{d}
KNO_3 (0.1)	40.00^{bc}	12.80^{b}	20.86^{cd}	51.80^{c}
KNO_3 (0.2)	43.66^{ab}	11.34^{a}	24.85^{bc}	57.10^{abc}
KNO_3 (0.3)	31.66^{cd}	10.89^{a}	18.52^{d}	58.30^{ab}
GA_3 (250)	48.33^{ab}	11.14^{a}	27.59^{ab}	57.13^{abc}
GA_3 (500)	52.33^{a}	10.18^{a}	32.83^{a}	62.85^{ab}
GA_3 (1000)	47.00^{ab}	11.30^{a}	26.77^{abc}	57.14^{abc}
GA_3 (250) + KNO_3 (0.1)	45.66^{ab}	11.08^{a}	26.82^{abc}	58.67^{ab}
GA_3 (250) + KNO_3 (0.2)	48.00^{ab}	10.34^{a}	29.74^{ab}	61.91^{ab}
GA_3 (250) + KNO_3 (0.3)	47.33^{ab}	10.35^{a}	29.10^{ab}	61.45^{ab}
GA_3 (500) + KNO_3 (0.1)	49.33^{ab}	10.02^{a}	30.87^{ab}	62.88^{ab}
GA_3 (500) + KNO_3 (0.2)	50.66^{a}	10.20^{a}	32.05^{a}	63.39^{a}
GA_3 (500) + KNO_3 (0.3)	51.66^{a}	10.68^{a}	30.92^{ab}	59.80^{ab}
GA_3 (1000) + KNO_3 (0.1)	51.00^{a}	11.17^{a}	29.65^{ab}	58.12^{ab}
GA_3 (1000) + KNO_3 (0.2)	48.66^{ab}	11.47^{a}	27.45^{ab}	56.35^{bc}
GA_3 (1000) + KNO_3 (0.3)	45.00^{ab}	10.73^{a}	26.65^{abc}	59.11^{ab}

Mean in each column followed by same letters are not significantly different at the P 0.05 level using Duncan test. FGP, Final germination percentage; MGT, mean germination time; GRI, germination rate index; CGRI, corrected germination rate index.

as Rouhi et al. (2010) for *Tulipa kaufmanniana* seeds treated with 0.1, 0.2 and 0.3% KNO_3 and ascertained that there was no stimulation of the germination. Moreover, the effect of pre-treatment with solutions of potassium nitrate (KNO_3) on seed germination *Terminalia sericea* was not significant (Amri, 2011). 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. Ali et al. (2010) showed that the using 0.1 and 0.2% KNO_3 did not have a huge effect on germination of *Plantago ovata.* Similar results were also reported in *Panicum maximum* by Previero et al. (1996) and Usberti et al. (2000) in many plant species.

Concerning the FGP at *R. nathaliae* seeds for all treatments, significant difference was detected compared with the control (Table 3). Seeds treated with 500 ppm GA_3 had the highest percentage of germination compared to the control seeds (52.33 and 27.66% respectively). According to Liopa-Tsakalidi and Barouchas (2011), the seeds germination of chervil treated with GA_3 concentrations 200, 500 and 1000 ppm is significantly higher than the corresponding one in H_2O treatment. In addition, the best germination percentage for the seeds of *Sabal palmetto* is in treatments with 1% KNO_3 and 500 ppm GA_3 respectively (Dewir et al., 2011). Kabar and Baltepe (1990), showed that seeds of barley and lettuce treated with GA_3 recorded higher values of germination seeds. Gashi et al. (2011) confirmed that the seeds germination of *R. nathaliae* in concentration of higher gibberellic acid did not exceed 50%. At the seeds of *R. nathaliae* (Table 3), higher FGP was confirmed with 0.1 and 0.3% KNO_3, especially with 0.2% KNO_3 compared with the control seeds (40.00, 31.66, 43.66 and 27.66% respectively). Similar results were obtained in the studies carried out on 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 (Shanmugavalli et al., 2007).

R. serbica seeds treated with different concentration of GA_3 and with combination of KNO_3 had higher FGP compared to *R. nathaliae* seeds (Figure 1), whilst at the untreated seeds (control), the highest FGP was gotten from *R. nathaliae* (9.26 and 27.66% respectively). For the seeds of *R. serbica* treated with 1000 ppm GA_3 + 0.3% KNO_3, the germination started on the day 9^{th} of incubation, while for the control seeds on the day 10^{th}. At the *R. nathaliae*, the germination start was the same for treated as well as for the control seeds, the germination started on the day 7^{th} (Figure 1). Regarding the first day of seeds germination of *R. serbica*, our results are consistent with the results obtained by Stefanovic et al. (1986), who in seeds of *R. serbica* treated with 100 µg/ml GA_3, had germination starting after day 9^{th} of incubation and the germination percentage reached around 90%.

Mean germination time

At the *R. serbica* seeds, the best treatment for this trait with the lowest values (14.17) was detected in seeds treatment with 500 ppm GA_3 + 0.3% KNO_3, whilst for *R. nathaliae* (10.02) in treatment with 500 ppm GA_3 + 0.1% KNO_3 (Tables 1 and 2). MGT between different concentration treatments of GA_3 and KNO_3 and their

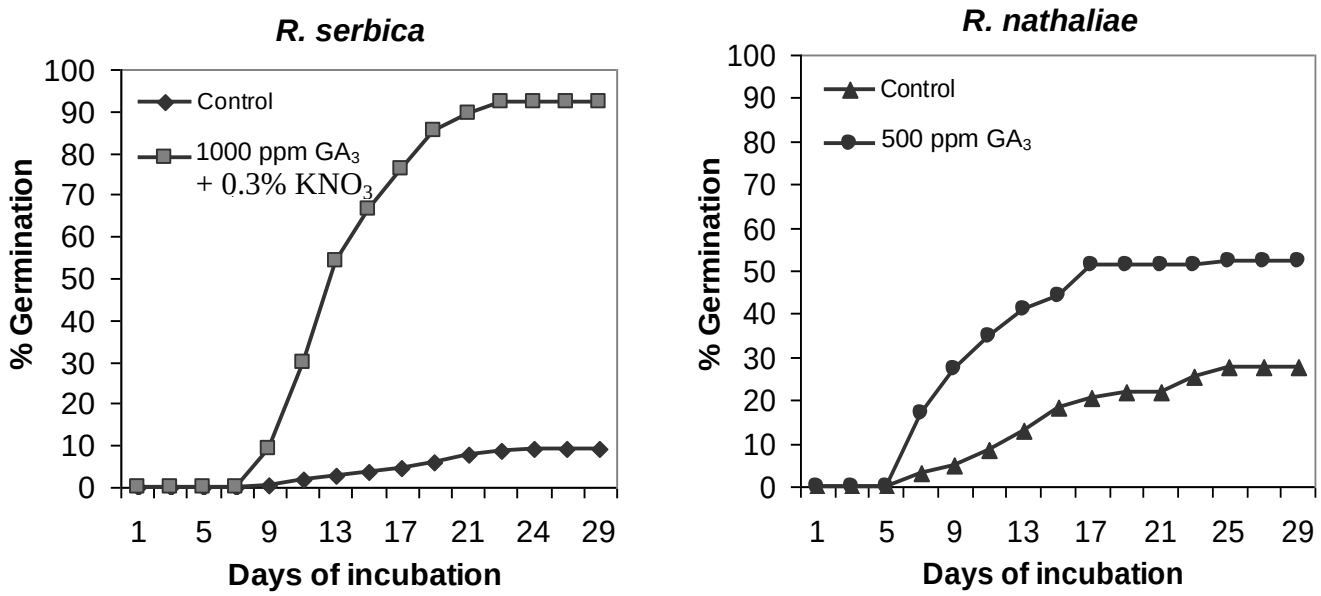

Figure 1. Minimum and maximum germination percentage at *R. serbica* and *R. nathaliae*, counted 29 days after seeds incubation.

combination in comparison with the control seeds, there was a significant difference for *R. nathaliae,* while for *R. serbica* only between different concentration of GA_3 and combination with KNO_3 compared with the control. Our results for this parameter are consistent with the results obtained by Ganaie et al. (2011) on *Arnebia benthamii* seeds, where the low values of MGT was confirmed at treated seeds with different concentration of KNO_3 and 25 ppm GA_3 in comparison with the control seeds. Similar results were reported in *Descurainia sophia* and *Plantago ovata* treated seeds with prechiling 0.3% KNO_3 which have shown significant differences for MGT in comparison with the control seeds (Ali et al., 2010).

Germination rate index and corrected germination index

The highest value of GRI and CGRI in R. serbica (36.70 and 39.82 respectively) was at the treatment with 1000 ppm GA_3 + 0.3% KNO_3. Comparing control and treatments with only KNO_3, the treated seeds with different concentration of GA_3 and with combination of KNO_3 differed significantly for GRI and CGRI at R. serbica.

The application of treatments with different concentration of GA_3 and KNO_3 as well as their combination for R. nathaliae seeds had significant differences for GRI and CGRI between controls and treatments. Treatment in the seeds of these species with 500 ppm GA_3 had highest value (32.83) in GRI, while the treatment with 500 ppm GA_3 + 0.2% KNO3 (63.39) was in CGRI. Dewir et al. (2011) reported the same results for S. palmetto seeds treated with 1, 2 and 3% KNO_3 and 100, 250 and 500 ppm GA_3, significant differences for germination rate index and corrective germination rate index between the treated seeds and those controlled ones was obtained. Zare et al. (2011) confirmed that the *Ferula assa foetida* seeds treated with higher concen-tration of GA_3 (2000 ppm) had higher germination rate values compared to the lower concentrations. Appro-ximate results were ascertained as well in *Lupinus diffusus* seeds (Dehgan et al., 2003) and Anthriscus cerefolium (Liopa-Tsakalidi and Barouchas, 2011).

Conclusions

Based on the analysis of the obtained results, we can conclude that the best way to increase percentage germination of *R. nathaliae* is the seeds treatment during the 24 h with 500 or 1000 ppm GA_3 and thus there will be growth in seeds germination percentage.

For the *R. serbica* seeds, the 24 h treatment with 1000 ppm GA_3 in combination with different concentration of KNO_3 is the treatment which increases the seeds germination up to 90% and more.

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REFERENCES

Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C, Truong HN (2005). Nitrate, a signal relieving seed dormancy in Arabidopsis. Plant Cell. Environ. 28(4): 500-512.

Ali T, Hossein P, Asghar F, Salman Z, Chahkooi (2010). The effect of

different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata*. Afr. J. Biotechnol. 9(39): 6588-6593.
Amri E (2011). Germination of *Terminalia sericea* Buch. Ex DC seeds: Effects of temperature regime, photoperiod, gebberellic acid and potassium nitrate. Int. J. Appl. Biol. Pharm. Technol. 2(2): 104-110.
Augusti A, Scartazza A, Navari-Izzo F, Sgherri CLM, Stevanovic B, Brugnoli E (2001). Photosystem II photochemical efficiency, zeaxanthin and antioxidant contents in the poikilohydric *Ramonda serbica* during dehydration and rehydration. Photosynth. Res. 67: 79-88.
Basra AS (1994). Seed quality basic mechanism and agricultural implications. Food Products Press. Binghamton, New York.
Copeland LO, McDonald MB (1995). Principles of seed science and technology. Chapman and Hall. 3rd edn. New York.
Dehgan B, Norcini GJ, Kabat MS, Perez EH (2003). Effect of seed scarification and gibberellic acid treatment on seedling emergence of Sky-Blue Lupine (*Lupinus diffusus).* J. Environ. Hort. 21(2): 64-67.
Degl'Innocenti E, Guidi L, Stevanovic B, Navari F (2008). CO_2 fixation and chlorophyll a fluorescence in leaves of *Ramonda serbica* during a dehydration-rehydration cycle. J. Plant Physiol. 165: 723-733.
Dewir YH, Mahrouk ME, Naido Y (2011). Effect of some mechanical and chemical treatments on seed germination of *Sabal palmetto* and *Thrinax morrisii* palms. Aust. J. Crop Sci. 5(3): 248-253.
Dontcheva S, Daskalova E, Yahubyan G, Denev I, Minkov I, Toneva V (2009). Conservation of the protected resurrection species *Ramonda serbica* Panc.-Habitat Montana district, Bulgaria as *in vitro* plants through a modified micropropagation system. Biotechnol. Biotechnol. Eq. 23(SE): 369-372.
Esechie H (1994). Interaction of salinity and temperature on the germination of sorghum. J. Agron. Crop. Sci. 172: 194-199.
Finch-Savage WE, Leubner-Metzger G (2006). Seed dormancy and the control of germination. New Phytol. 171(3): 501-523.
Ganaie AK, Aslam S, Nawchoo AI (2011). No chilling obligation for germination in seeds of *Arnebia benthamii*: A critically endangered alpine medicinal plant of north-west Himalayas. Int. J. Biod. Conserv. 3(5): 155-159.
Gashi B, Abdullai K, Mata V, Misimi S, Osmani M, Kongjika E (2011). *In vitro* culture-A tool to overcome the poor *in vivo* development of genus *Ramonda* plants. International conference Biotechnological developments, Acad. Sci. Albania. p. 92.
ISTA (1996).Rules for seed Testing.International Seed Testing Association.Seed sci.Technol.24 suppl.Zurich,Switzerland.
Jovanovic Z, Rakic T, Stevanovic B, Radovic S (2011). Characterization of oxidative and antioxidative events during dehydration and rehydration of resurrection plant *Ramonda nathaliae*. J. Plant Growth Regul. 64: 231-240.
Kabar K, Baltepe S (1990). Effect of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stresses on the germination of Barley and Lettuce seeds. Phyton Horn Austria, 30(1): 65-74.
Kolumbina M, Blesing M, Mares DJ (2006). α-Amylase and programmed cell death in aleurone of ripening wheat grains. J. Exp. Bot. 57(4): 877-885.
Kongjika E, Zekaj Zh, Çaushi E, Stamo I (2002). Bioteknologjia e bimëve-kulturat "*in vitro*". Akademia e Shkencave. Instituti i Kërkimeve Biologjike. Tiranë, Albania.
Kosanin N (1921). La distribution geographique des deux especes de *Ramondia* du Balkan. Academie des Sciences et Arts de Belgrade, pp. 34-49.
Liopa-Tsakalidi A, Barouchas PE (2011). Salinity, chitin and GA_3 effects on seed germination of chervil (*Anthriscus cerefolium*). Aust. J. Crop Sci. 5(8): 973-978.
Meyer KF (1970). *Gesneriaceae* als Glieder der Flora des Tertiar in Europa. Wiss. Ztschr. Friedrich-Schiller Univ. Jena. Math. Naturwiss. Reiche, 19(3): 401-411.
Moradi DP, Sharif-zadeh F, Janmohammadi M (2008). Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). J. Agr. Biol. Sci. 3(3): 22-25.
Previero CA, Martins L, Fonseca RHA, Groth D (1996). Effect of storage of guinea grass *(Panicum maximum* Jacq.) on treatment to break dormancy. Revista brasileira de semetes, 18(1): 143-148.
Quartacci MF, Glisic O, Stevanovic B, Navari-Izzo F (2002). Plasma membrane lipids in the resurrection plant *Ramonda serbica* following dehydration and rehydration. J. Exp. Bot. 53(378): 2159-2166.
Rouhi HR, Shakarami K, Tavakkol Afshari R (2010). Seed treatments to overcome dormancy of waterlily tulip (*Tulipa kaufmanniana* Regel.) Aust. J. Crop. Sci. 4(9): 718-721.
Sgherri C, Stevanovic B, Navari-Izzo F (2004). Role of phenolics in the antioxidative status of the resurrection plant *Ramonda serbica* during dehydration and rehydration. Physiol. Plant. 122: 478-485.
Stefanovic M, Grubisic D, Neskovic M (1986). Effect of light and growth substances on seed germination in *Ramonda serbica* Panc. (Gesneriaceae). Bulletin de l'institut et du jardin botaniques de l'universite de Beograd. 20: 1-6.
Sharma RK, Sharma S (2010). Effect of storage and cold-stratification on seed physiological aspects of *Bunium persicum*: A threatened medicinal herb of Trans-Himalaya. Int. J. Bot. 6(2): 151-156.
Shanmugavalli M, Renganayaki PR, Menaka C (2007). Seed dormancy and germination improvement treatments in fodder sorghum. Int. Crops Res. Inst. Semi-Arid Tropics, 3: 1-3.
Usberti R, Usberti JA, Pateniani RS (2000). Flowering cycle related seed quality characteristics of 15 guinea grass hybrids. Seed Sci. Technol. 28(2): 437-443.
Yakovlev-Siljak S, Stevanovic V, Tomasevic M, Brown CS, Stevanovic B (2008). Genome size variation and polyploidy in the resurrection plant genus *Ramonda*: Cytogeography of living fossils. Environ. Exp. Bot. 62: 101-112.
Yücel E, Yilmaz G (2009). Effect of different alkaline metal salts (NaCl, KNO_3), acid concentrations (H_2SO_4) and growth regulator (GA_3) on germination of *Salvia cyanescens* Boiss. & Bal. seeds. G.U. J. Sci. 22(3): 123-127.
Zivkovic T, Quartacci MF, Stevanovic B, Marinone F, Navari-Izzo F (2005). Low molecular weight substances in the poikilohydric plant *Ramonda serbica* during dehydrationand rehydration. Plant Sci. 168: 105-111.
Zare AR, Solouki M, Omidi M, Irvani N, Abasabadi AO, Nezad MN (2011). Effect of various treatments on seed germination and dormancy breaking *Ferula assa foetida* L. (Asafetida), A threatened Medicinal herb. Trakia J. Sci. 9(2): 57-61.