Journal of Materials and Environmental Sciences
ISSN : 2028-2508

JMES, 2017 Volume 8, Issue 6, Page 2034-2039

http://www.jmaterenvironsci.com/

In vitro seed germination of some Algerian medicinal plants and the effect of Gibberellic acid (GA_3) on breaking dormancy

S. Mehalaine [1, 2*], T. Menasria [3], S. Bouguessa [1], A. Yahia[4]

[1]*Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Tebessa 12002, Algeria.*
[2]*Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, University of Oum El Bouaghi, Oum El Bouaghi 04000, Algeria.*
[3]*Department of Applied Biology, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Tebessa 12002, Algeria.*
[4]*Institute of Science and Technology, University Centre of Mila, Mila 43000, Algeria.*

Received 11 Dec 2016
Revised 5 March 2017
Accepted 9 March 2017

Keywords

- ✓ *Thymus algeriensis;*
- ✓ *Rosmarinus officinalis;*
- ✓ *Marrubium vulgare*;
- ✓ Seed germination;
- ✓ Seed dormancy;
- ✓ GA_3

S. Mehalaine
souadmehalaine@yahoo.fr
+213773529638

Abstract

In the present study, we investigated the seed germination behaviour of three medicinal plants growing wild in Algeria: *Thymus algeriensis* Boiss & Reut., *Rosmarinus officinalis* L., *Marrubium vulgare* L. and assessed the effect of gibberellic acid (GA_3) on breaking seed dormancy. The seeds were sterilized in 5% sodium hypochlorite solution (NaOCl) and subjected to two experiments. First, an examination of the seed germination ability without pre-treatment by incubating the seeds at ambient temperature (23±2 °C) and continuous darkness. Secondly, the seeds were treated with various doses of GA_3 (125, 250, 500 mg/L) and incubated in thermoperiod (25°C/16h, 15°C/8h) and continuous darkness. From the experiment one, *T. algeriensis* seeds presented the highest germination percentage (94.43 %) followed by *M. vulgare* (57.76 %). However, *R. officinalis* seeds did not germinate. The GA_3 treatment did not exhibit any significant effect ($P > 0.05$) on the germination of the three tested plants. In addition, the highest germination rates were observed in *T. algeriensis* seeds (80 and 100 %) and *M. vulgare* (53.3, 73.3 and 86.7 %). *R. officinalis* seeds presented very low germination rates with (3.3, 6.7 and 16.7 %).

1. Introduction

Medicinal plants have been used, since antiquity, to treat common infectious diseases and to prevent a variety of ailments [1,2]. In the recent decades, novel diseases have been emerged from irrational uses of antibiotics and increasing of industrialization. Therefore, it is necessary to work more extensively on natural sources such as the plants [2].

In Algeria, many species belonging to the Lamiaceae family are intensely used in traditional medicine to treat several pathologies, among them *Thymus algeriensis* Boiss & Reut., *Marrubium vulgare* L. and *Rosmarinus officinalis* L. [3,4]. *Thymus algeriensis* is a North African endemic species. It is known by a vernacular name Djertil [5]. And widely used in folk medicine for its antiseptic, antispasmodic, antibacterial and antifungal properties [6]. *Rosmarinus officinalis* is a common and an endemic species to Algeria. It is locally known as: Klil, Hassalhan, Lazir [5]. Due to its antioxidant properties, Rosemary is largely used as spice in culinary and food condiment [7, 8]. In Algerian folk medicine, *R. officinalis* is used as stimulant, antispasmodic, diuretic and vermifuge [3]. *Marrubium vulgare* is commonly distributed in Algeria and locally known as Marriout [5]. Its aerial parts and extracts are widely used to treat cough and as choleretic in digestive and biliary ailments [9].This plant is used for its beneficial anti-hepatotoxic and anti-inflammatory properties [10,11].

Medicinal plants are under threat due to intensive exploitation by herbalists, traditional healers and excessive grazing. Considering their crucial role in traditional medicine, their conservation is therefore fundamental and strategies of exploitation and preservation should be investigated [12].

Plant regeneration from seeds have been used to produce and maintain various species and a number of researches regarding seed germination and the effect of gibberellic acid on breaking dormancy have been reviewed [13-18]. Seed germination and dormancy are important processes influencing life cycle of plants. These processes are under the effect of various factors, including plant hormones. Gibberellins, abscisic acid, auxins, cytokinins and ethylene can affect seed germination [19]. Moreover, plant hormones imbalance inhibit seed germination [15].
In this context, the objectives of this study were to assess the germination ability and the effect of gibberellic acid (GA_3) on breaking seed dormancy of *Thymus algeriensis*, *Rosmarinus officinalis* and *Marrubium vulgare*, species growing wild in Algeria, in order to develop an efficient protocol for their production.

2. Experimental details

2.1. *Seeds collection*
Seeds of *Thymus algeriensis*, *Rosmarinus officinalis* and *Marrubium vulgare* were harvested at the fruiting stage from their natural environment in Ain Beida (latitude: 35.805° N, longitude: 7.376° E, altitude: 920 m asl), Algeria during 2012-2013. The seeds were stored at room temperature until the experiment started.

2.2. *Seeds sterilization*
The seeds were cleaned under running tap water and surface-sterilized with 5% sodium hypochlorite solution (NaOCl) for 5 min. Thereafter, seeds were rinsed three times in sterile distilled water. The sterilized seeds were subjected to two experiments.

2.3. *In vitro seed germination experiments*
2.3.1. *Preliminary germination test*
The disinfected seeds were impregnated in sterile distilled water for one hour. Then, 30 seeds per species were placed on three layers of sterile filter paper in Petri dishes and incubated at ambient temperature (23±2 °C) and continuous darkness in the laboratory. Seeds were watered daily with sterile distilled water. The experiment was performed with three replications.

2.3.2. *Effect of gibberellic acid (GA_3)*
Decontaminated seed samples of the three species were treated with three doses of gibberellic acid (GA_3) (125, 250, 500 mg/L) for 2 hours, along with a non-treated check. Then, 10 seeds per species were placed on sterile agar (7g/L) in Petri dishes and incubated in darkness at thermoperiod (25°C/16h, 15°C/8h) in a phytotron (Snijders, Model ECP 01E). The experiment was performed with three replications. In both experiments, the germinated seeds were counted daily during 30 days-period. Seeds were considered as germinated when the radicle reached (radicle length ≥ 2 mm). Germination percentage was calculated as:

Number of germinated seeds / Number of tested seeds × 100.

2.3.3. *Data analysis*
The means and standard deviations were calculated from independent experiments. Data were subjected to a one way analysis of variance (ANOVA) to test species and GA_3 effects on seed germination at 5% probability level.

3. Results and Discussion

3.1. Preliminary germination test
The Figure 1 illustrates the dynamic of germination for *M. vulgare* (A) and *T. algeriensis* seeds (B) during thirty days. It describes three important phases. The first one of latency is corresponding to seed imbibitions. This phase was very short and took two days for *T. algeriensis*. However, it seemed long for *M. vulgare* seeds and took six days. The second phase is exponential, corresponding to the acceleration of germination that began on fifteenth day for *M. vulgare* seeds and on sixth day for *T. algeriensis* with significant asymptotes during at least 10 days (*M. vulgare:* $t = 45.09$, $P < 0.001$; *T. algeriensis*: $t = 135.20$, $P < 0.001$) (Table 1). Then, the stationary phase which is corresponding to the stopping of germination and constancy of germinated seed number after obtaining the maximum germination capacity on twenty-seventh day and twenty-third day for *M. vulgare* and *T. algeriensis* respectively. The germination curve of two species shows also a notable phase corresponding to the stopping of germination between the beginning of this process and the ascendant phase. It was very short for *T. algeriensis* (two days) but long for *M. vulgare* (six days). This fluctuation in the dynamic of germination can be

assigned to the seed coat characteristics of target species. During our seed manipulation, we observed that *M. vulgare* seed coat was very hard compared to that of *T. algeriensis* seeds. This may decrease the water entry speed and delay the seed imbibitions for *M. vulgare*.

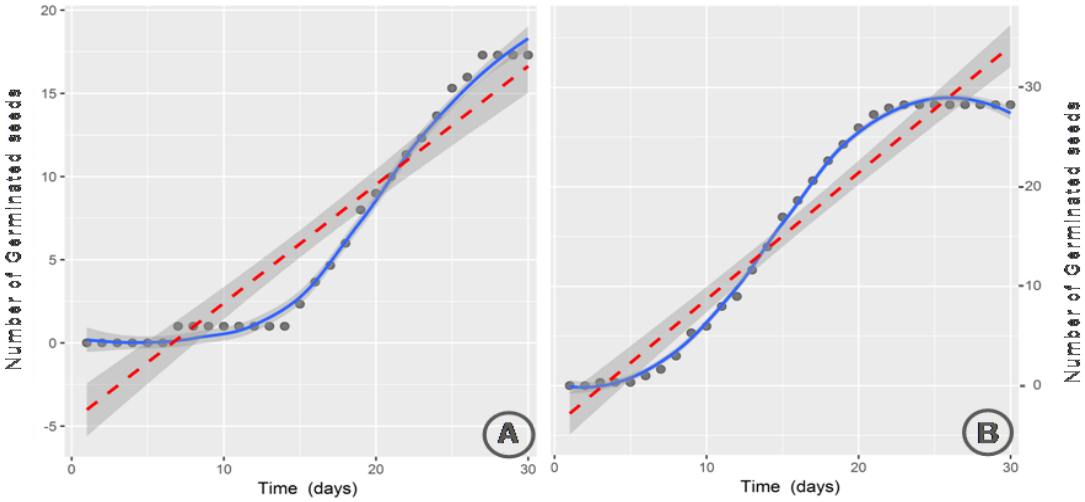

Figure 1. Seed germination of *M. vulgare* (A) and *T. algeriensis* (B). *Dashed red lines* are predicted curves obtained from a non linear regression (four-parameter logistic model). *Continuous blue lines* are LOESS curves (locally weighted polynomial) fitted to the data with a 95 % confidence interval region.

Table 1. Summary of the non linear regression (four-parameter logistic S-shaped functions) analyzing seed germination of *T. algeriensis* and *M. vulgare*.

T. algeriensis				
Parameters	**Estimate**	**SE**	**t-value**	**P**
A	-0.50	0.278	-1.80	0.084
B	28.93	0.214	135.20	<0.001
C	13.98	0.112	124.75	<0.001
D	2.96	0.107	27.63	<0.001
M. vulgare				
Parameters	**Estimate**	**SE**	**t-value**	**P**
A	0.09	0.163	0.58	0.566
B	18.41	0.408	45.09	<0.001
C	20.42	0.208	98.01	<0.001
D	3.02	0.179	16.85	<0.001

In addition, the obtained results showed that *T. algeriensis* presented the highest number of germinated seeds (28.33 ± 2.89) followed by *M. vulgare* (17.33 ± 3.21) corresponding to the germination percentages of (94.43 % and 57.76 %) respectively. However, *R. officinalis* seeds did not germinate (Figure 2).

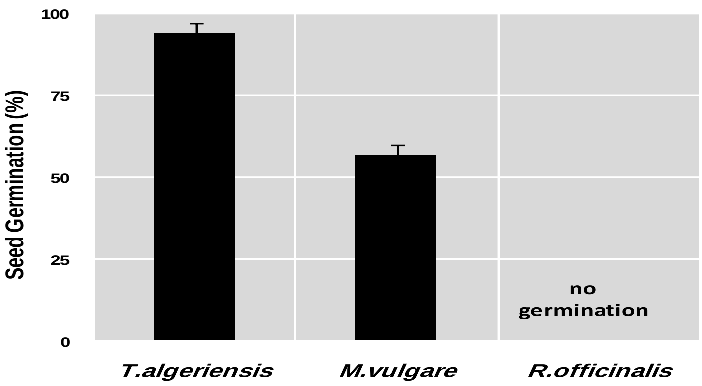

Figure 2. Seed germination rates of *T. algeriensis*, *M. vulgare* and *R. officinalis*.

3.2. Effect of gibberellic acid (GA_3)

Gibberellic acid treatment did not significantly affect the number of germinated seeds for the three tested plants ($P > 0.05$). High number of germinated seeds was observed with *T. algeriensis* for the control (8 ± 1.73) and the three applied GA_3 doses with the same value (10 ± 0.00) corresponding to the percentages of (80 and 100 %) respectively followed by *M. vulgare* with a number of germinated seeds ranging between (5.33 ± 1.53 and 8.67 ± 0.58) corresponding to the rates of (53.3 and 86.7 %). Whereas, *R. officinalis* showed a very low number of germinated seeds between (0.33 ± 0.58 and 1.67 ± 0.58) corresponding to the germination rates of 3.3 and 16.7 % (Table 2).

Table 2. Effect of different GA_3 doses on the number of germinated seeds (NGS). Values are expressed as Mean ± standard deviation (SD), (NS: not significant).

Source	GA_3 doses				$F_{(3, 8)}$	P
	Control	125 mg/L	250 mg/L	500 mg/L		
T. algeriensis **NGS**	8 ± 1.73	10 ± 0.00	10 ± 0.00	10 ± 0.00	4.00	0.052 **NS**
R. officinalis **NGS**	0.33 ± 0.58	0.33 ± 0.58	1.67 ± 0.58	0.67 ± 0.58	3.58	0.066 **NS**
M. vulgare **NGS**	5.33 ± 1.53	7.33± 2.08	7.33± 0.58	8.67 ± 0.58	3.09	0.090 **NS**

The results obtained from the preliminary germination test indicated that *T. algeriensis* and *M. vulgare* seeds were able to germinate under ambient conditions without pre-treatments. According to the results from the experiment two, the GA_3 treatment presented no effect on promoting their germination.

Therefore, considering the obtained high germination percentages from both experiment one and two and according to Baskin and Baskin [20], our tested seeds were non-dormant. This can be attributed to the action of endogenous gibberellic acid contained in the tested seeds and to the experimental conditions which appeared favourable to improve the germination process in *T. algeriensis* and *M. vulgare* seeds.

During the germination, seed absorbs water that induces the expansion and elongation of embryo, causing the radicle growth out of the seed testa [21]. Furthermore, the embryo is a source of gibberellic acid and with the presence of the aleurone layer in periphery of the albumen, the gibberellic acid stimulates the production of α-amylase, a hydrolytic enzyme responsible for the digestion of nutrients necessary for embryo growth and seedling development [22,23].

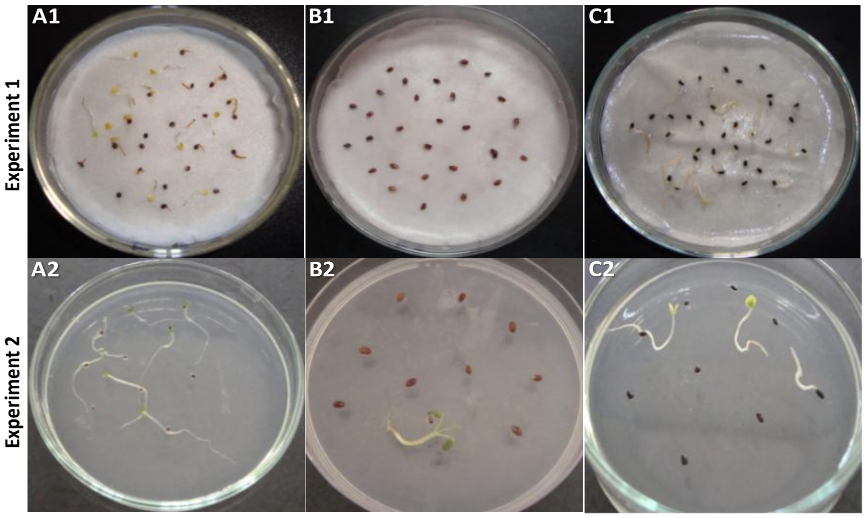

Figure 3. Seed germination experiments
Experiment one (Preliminary test): *T. algeriensis* (A1), *R. officinalis* (B1), *M. vulgare* (C1).
Experiment two (Effect of GA_3): *T. algeriensis* (A2), *R. officinalis* (B2), *M. vulgare* (C2).

The non-germination of *R. officinalis* seeds during the preliminary test and the low germination percentages obtained from the experiment two indicated that these seeds were unable to germinate under the experimental conditions. This can be assigned to the dormancy type and different interactions between endogenous plant hormones and exogenous GA_3.
The dormancy is a mechanism which allows seeds to inhibit their germination in order to synchronise it with favourable environmental conditions (secondary dormancy) [24,25]. While, primary dormancy is caused by the effect of abscisic acid during seed development [26]. According to these data, we suggest that *R. officinalis* seeds may contain a high amount of abscisic acid (ABA). The plant hormones gibberellins are necessary for seed germination through the release of coat dormancy (weakening of endosperm and expansion of embryo cell) [19].
Findings reported by Dissanayake et al. [15] showed that GA_3 induced germination and overcame dormancy in *Parthenium argentatum* seeds but the application of exogenous abscisic acid almost prevented the germination in this plant. In addition, the abscisic acid can totally reverse the effect of GA_3 and the seed dormancy/germination is regulated by the balance of germination promoters (GA_3) and inhibitors (ABA).
According to Graeber et al. [27], the inhibitory effects of abscisic acid on seed germination is through delaying the radicle expansion and weakening of endosperm. Furthermore, the excess of abscisic acid suppresses the embryo expansion which inhibits the effect of gibberellins on radicle growth, and hence it will not germinate through the testa [28].
On the other hand, the growth hormones, cytokinins are able to enhance plant cell division [29]. According to Güleryüz et al. [16], the combination of GA_3 and cytokinin (Kinetin) broke dormancy and increased the germination rates in *Stachys germanica* seeds. Moreover, the high content of abscisic acid in these seeds could only be deceased by a combination of GA_3 and Kinetin. Besides, Khan [30] reported that gibberellins enhance germination but they cannot exhibit their effect in the presence of abscisic acid unless a sufficient quantity of cytokinin is present. In fact, it appears that there is a synergistic relationship between cytokinins and gibberellins in the regulation of dormancy and germination [16].
The temperature is also an important external factor that influences germination and seeds need appropriate temperature for different metabolic processes required for the germination [31]. In this regard, many researchers have elucidated the effect of temperature on the germination. Padilla and Encina [31] observed that a high temperature (30 °C) was suitable for promoting germination rate of *Annona cherimola* seeds. Whereas, many studies demonstrated that temperatures ranging between (15 and 20 °C) broke dormancy and increased the germination rates for several plant species such as (*Myrsine parvifolia* and *Thymus lotocephalus*) [17,32-34]. Moreover, the cold stratification of seeds is an effective means to break dormancy in many species, especially those from temperate regions [24].
The GA_3 levels can also affect the germination. It has been reported that the optimum concentrations of GA_3 to overcome dormancy are 250 and 500 ppm [15-17]. In addition, the concentrations may depend on the level of dormancy [15]. Therefore, the applied GA_3 doses may be insufficient to promote germination in *R. officinalis*.
Another factor may play a role in the non-germination of *R. officinalis* seeds: the seed coat characteristics. Previous researches showed that the hard seed coat prevents the entry of growth regulators into seeds and the chemical or mechanical scarification may reduce the inhibitory action exhibited by the seed coat on the embryo [13,15,34].
Besides, the seeds geographic origin can influence their germination [16,35,36] and the assessing of germination behaviour should comprise several populations from the same species [32].

Conclusions

The preliminary germination test showed that *T. algeriensis* and *M. vulgare* seeds were not dormant and able to germinate under ambient temperature and continuous darkness contrary to *R. officinalis* seeds which seemed dormant. In addition, *T. algeriensis* seeds presented the highest germination percentage followed by *M. vulgare*. The GA_3 treatment had no significant effect on increasing the germination rate in *R. officinalis* seeds. Furthermore, the treated seeds and the control of both *T. algeriensis* and *M. vulgare* did not reveal any significant differences with or without GA_3 treatment. In conclusion, further studies should be achieved in order to overcome dormancy and increase the germination rate in *R. officinalis* seeds.

Acknowledgments-The authors thank the laboratory of Botany at the Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Algeria, for all facilities provided in caring out this study.

References

1. Rios J.L., Recio M.C., *J Ethnopharmacol.* 100 (2005) 80-84.
2. Ahmed M.J., Murtaza G., *J Ethnopharmacol*. 159 (2015) 209-214.
3. Beloued A., *Office des publications universitaires,* Alger. (2005) pp. 184, 206.
4. Boudjelal A., Henchiri C., Sari M., Sarri D., Hendel N., Benkhaled A., Ruberto G., *J Ethnopharmacol*. 148 (2013) 395-402.
5. Quezel P., Santa S., Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome 2 Ed: *C.N.R.S,* Paris. (1963) pp. 793, 801, 806.
6. Hazzit M., Baaliouamer A., Veríssimo A.R., Faleiro M.L., Miguel M.G., *Food Chem*. 116 (2009) 714-721.
7. Harach T., Aprikian O., Monnard I., Moulin J., Membrez M., Beolor J.C., Raab T., Mace K., Darimont C., *Planta Med*. 76 (2010) 566-571.
8. Ben Jemia M., Tundis R., Maggio A., Rosselli S., Senatore F., Menichini F., Bruno M., Kchouk M.E., Loizzo M.R., *J Funct Foods*. 5 (2013) 1873-1882.
9. Wichtl M., Anton R., Plantes Thérapeutiques. *Tec & Doc*, Paris. (1999) pp. 341.
10. Sahpaz S., Garbacki N., Tits M., Bailleul F., *J Ethnopharmacol*. 79 (2002) 389-392.
11. Verma A., Masoodi M., Ahmed B., *Asian Pac J Trop Biomed*. (2012) S1308-S1311.
12. Yetein M.H., Houessou L.G., Lougbégnon T.O., Teka O., Tente B., *J Ethnopharmacol*. 146 (2013) 154-163.
13. Colin R. Norton., *Sci Horticult*. 27 (1985) 163-169.
14. Conversa G., Lazzizera C., Elia A., *Sci Horticult*. 125 (2010) 196-202.
15. Dissanayake P., George D.L., Gupta M.L., *Ind Crops Prod*. 32 (2010) 111-117.
16. Güleryüz G., Kırmızı S., Arslan H., Sakar F.S., *Flora*. 206 (2011) 943-948.
17. Darrudi R., Hassandokht M.R., Nazeri V., *J Appl Res Med Aromat Plants*. 2 (2015) 168-173.
18. Medeiros M.J., Oliveira M.T., Willadino L., Santos M.G., *Plant Physiol Biochem*. 97 (2015) 278-286.
19. Miransari M., Smith D.L., *Environ exp bot*. 99 (2014) 110-121.
20. Baskin J.M., Baskin C.C., *Seed Sci Res*. 14 (2004) 1-16.
21. Hermann K., Meinhard J., Dobrev P., Linkies A., Pesek B., Heß B., Machackova I., Fischer U., Leubner-Metzger G., .*J Exp Bot*. 58 (2007) 3047-3060.
22. Hamman B., Koning G., Him Lok K., *Homeopathy*. 92 (2003) 140-144.
23. Hopkins W.G., Physiologie végétale. Edition *De Boeck.* Bruxelles (2003) pp. 323.
24. Baskin C.C., Baskin J.M., Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination. *Academic Press,* San Diego. (1998).
25. Finkelstein R., Reeves W., Ariizumi T., Steber C., *Ann Rev Plant Biol*. 59 (2008) 387-415.
26. Bewley J.D., *Plant Cell*. 9 (1997) 1055-1066.
27. Graeber K., Linkies A., Muller K., Wunchova A., Rott A., Leubner-Metzger G., *Plant Mol Biol*. 73 (2010) 67-87.
28. Nonogaki H., *Plant Sig Behav*. 1 (2008) 65-67.
29. Miller C., Skoog F., Saltza M.V., Strong M., (1955). In: Miransari, M., Smith, D.L., *Environ exp bot*. 99 (2014) 110-121.
30. Khan A.A., *Science*. 171 (1971) 853-859.
31. Padilla I.M.G., Encina C.L., *Sci Horticult*. 97 (2003) 219-227.
32. Coelho N., Gonçalves S., González-Benito M.E., Romano A., *Sci Horticult*. 145 (2012) 84-86.
33. Emamipoor Y., Maziah M., *Asian Pac J Trop Biomed*. 4 (8) (2014) 642-649.
34. Ribeiro J.N.S., Costa C.S.B., *S Afr J Bot*. 98 (2015) 128-133.
35. Urbanska K.M., Schütz M., *Bot Helvet*. 96 (1986) 43-60.
36. Pérez-García F., Hornero J., González-Benito M.E., *Isr. J Plant Sci*. 51 (2003) 117-124.