PLANTAGO MEDIA L. GERMINATION RESPONSE

Luminiţa COJOCARIU, N. M. HORABLAGA , Adina HORABLAGA, A. COJOCARIU[2], Despina- Maria BORDEAN, Aurica BOROZAN, C. BOSTAN, C. RUJAN

[1]Banat's University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania;
[2]"Tibiscus" University of Timisoara , Faculty of Economic Science Timisoara, Romania.
E-mail: luminitacojocariu@yahoo.com

Abstract: *The aim of the paper is to study the germination response of Plantago media seeds, collected from their natural growth environment in order to identify the weaknesses and threats regarding the conservation of this species in grassland habitats from the piedmont areas near Zărand Mountains. The biologic material was represented by Plantago media L. seeds, collected in September from grasslands found at 617 m altitude.We assessed the potential of Plantago media seeds to produce normal plants in a wide range of field conditions. For that, we used the test "top of paper" (TP) in two variants: ITP – temperature 20°C, humidity 90%, with light, without special treatments and IITP at an alternating temperature 20-30°C, humidity 90%, with light, with precooling at 10 C° for five days. Our studies revealed that the highest percentage of normal Plantago media seedlings appeared 21 days after the seeds had been placed for germination (ITP and IITP). The maximum germination (65%) was recorded in variant IITP, when Plantago media seeds were kept at 10° C for 5 days, and then subjected to high humidity (90%) and alternating temperature (20-30°C), with light. The mathematical model used suggests that variant IITP (top of paper) indicates the potential of Plantago media seeds to germinate in early spring on cold and humid soils in Zărand Mountains, where this species was identified and from where we took the seeds for analysis. In the area under analysis, species Plantago media L. is not under any survival risk.*

Key words: *germination, top of paper test, Plantago media L., grassland habitat, Zărand Mountains.*

INTRODUCTION

Plantago media L. is a plant that grows on wet grasslands. It has average fodder value, but it is recognized as a valuable medical herb, due to its therapeutic effects.

The study on the germination of *Plantago media* seeds can offer unique perspectives on the processes of range expansion and adaptation to the possible threats on grasslands in piedmont areas and mountain areas in Zărand Mountains, the place where the seeds were taken from.

Unscientific exploitation of semi-natural grasslands (abandon or excessive grazing), calamities, road constructions and climate change have all led to the decrease or even disappearance of some species from grassland vegetation (KUMAR et al., 2011). A change in the size and density of population over a certain period of time can indicate that the plant species are rare, endangered or on the verge of extinction (MAIKHURI et al.,1998 quoted by VINAY et al., 2011).

Taking into account the increasing demand for plant-based medication, it is extremely important to preserve species that have a pharmaceutical value (AIRI et al, 2000).

There are several internal and external factors which prevent seeds from germination. Among the internal factors, some important ones are: the presence of a seed coat, which is a barrier to the penetration of water and oxygen; the presence of a biochemical inhibitor in the seed; and immature embryos. Among the external factors, the most common are soil water content and temperature (FERNÁNDEZ-QUINTANILLA et al., 1991 quoted by Christoffoleti and R.S.X. CAETANO, 1998).

According to ELIAS et al. (2006), other factors that can affect seed germination in plants, after harvest, refer to seed maturation, harvest methods, drying, cleaning and storage.

Therefore, the information on germination characteristics of *Plantago media* offers valuable knowledge on the factors that might contribute to the successful expansion of the habitat of this species, as well as to its adaptation to the new threats that may affect piedmont and mountain grasslands in the Romanian Carpathians. Germination tests can assess the effect of these factors on seed quality and establish the real value of *Plantago media* seeds.

MATERIAL AND METHODS

The biological material investigated was represented by *Plantago media* L. seeds. The seeds belonging to this species were collected from their natural habitat (617 m altitude), in September 2012 and then kept in suitable storage conditions.

In order to determine the germination of *Plantago media* L. seeds, method TP-top of paper was used (International Seed Testing Association ISTA-2002, Order SR 1634/1999, AOSA 2002, 2004, http://www.icrisat.org/what-we-do/genebank/genebank-manual/seed-processing-4.pdf).

Research methodology

In conformity with the **TP** -top of paper method, the *Plantago media* seeds were placed on the surface of the filter paper *CLASS 601- medium filtration*, in Petri dishes. The paper layer was moistened at the beginning to the maximum absorption capacity. After setting up each experiment, the dishes were covered with a glass lid in order to reduce evaporation.
The variants used for this method were ITP and IITP.

In the ITP variant (temperature 20°C, humidity 90%, with light), no special treatments were applied. The temperature was constant: 20°C.

In the IITP variant (alternating temperature 20-30°C, humidity 90%, with light), before the germination test, pre-cooling was performed. Thus, the seeds were kept at 10ºC for five days. This period is not included in the 21 days necessary for the germination of *Plantago media* seeds. After that, the samples were introduced in the germination chamber at alternating temperature (20-30°C), where the higher temperature alternated with the lower temperature. In this technique, the seeds are kept 16 hours at the lower temperature, 20°C, and the rest of 8 hours they are kept at the higher temperature of 30°C. The change in temperature is gradual, taking place over 3 hours.

For every variant under study, the determination was made in four repetitions of 100 seeds each.

In order to find the initial quality of the samples under study, the following determinations were made: normal seedlings (%), abnormal seedlings (%); dead seedlings (%). Assessment of the normal seedlings was performed seven days (E7= evaluation of normal seedlings after 7 days %) and 21 days after the seeds were placed for germination (E21 = evaluation of normal seedlings after 21 days %), respectively.

In the end, the germination value (%) of *Plantago media* seeds was calculated.

Statistical analysis

The statistical evaluation of the experimental data was made using PAST 2.14.

RESULTS AND DISCUSSIONS

For the identification of the survival risks in piedmont and mountain grasslands, the studies on the germination of *Plantago media* seeds play a key role in the maintenance of the measures for the conservation of this species. Therefore, the focus of this study was on finding the germination capacity of *Plantago media* seeds, collected from their natural growth environment (Zărand Mountains).

The germination capacity of seeds in cold and moist soils is influenced by plant heredity, physiology, seed particularities and mechanic damage at harvest (AOSA 2002). Seed exposure to temperature variations (low and high temperature) is different from one species to another, as well as the period of exposure (VINAY et all., 2011).

The research methods on seed germination can be based on any characteristic that can be used for distinguishing the most obvious particularities of the species under study (RUJAN et all., 2012).

Plantago media seeds were assessed by the TP - top of paper test, in compliance with the ISTA rules in force (International Seed Testing Association ISTA, 2002) and Order SR 1634/1999. The TP (top of paper) test was performed in two variants: ITP = variant 1 code (temperature 20°C, humidity 90%, with light, without special treatments); IITP = Variant 2 code (temperature 20-30°C, humidity 90%, with light, with precooling at 10 C° for five days)**.** In addition, we determined the number of germinated plants 7 and 21 days after starting the experiment, respectively, in order to get a clear image of the biological material under study and the period necessary for the germination of *Plantago media* seeds (Figure 1. and 2.).

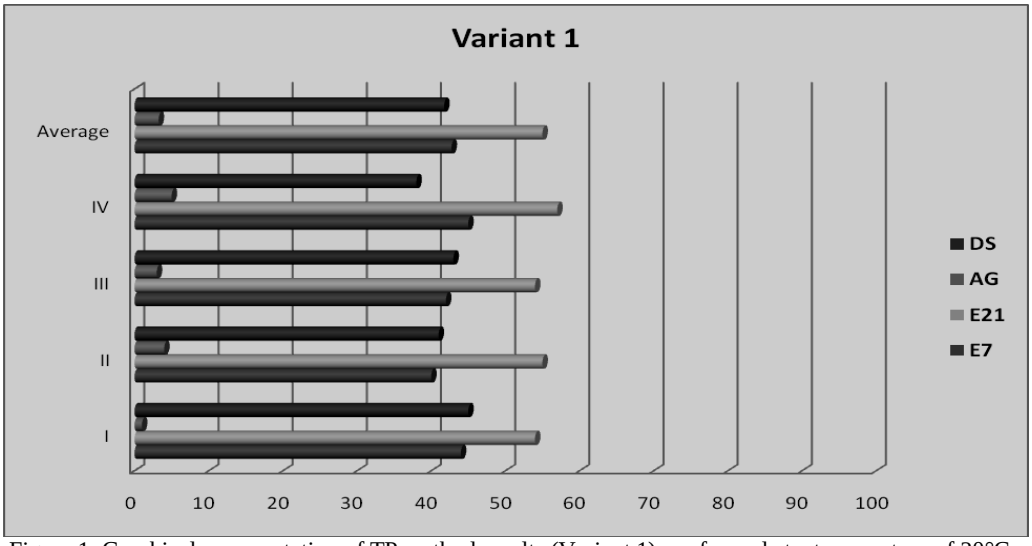

Figure 1. Graphical representation of TP method results (Variant 1), performed at a temperature of 20°C, with light, without special treatments

Legend: I, II, III, IV = 4 repetitions / 100 pc; E7= evaluation of normal seedlings after 7 days (%); E21 = evaluation of normal seedlings after 21 days (%); AG = abnormal seedlings (%); DS = dead seedlings (%).

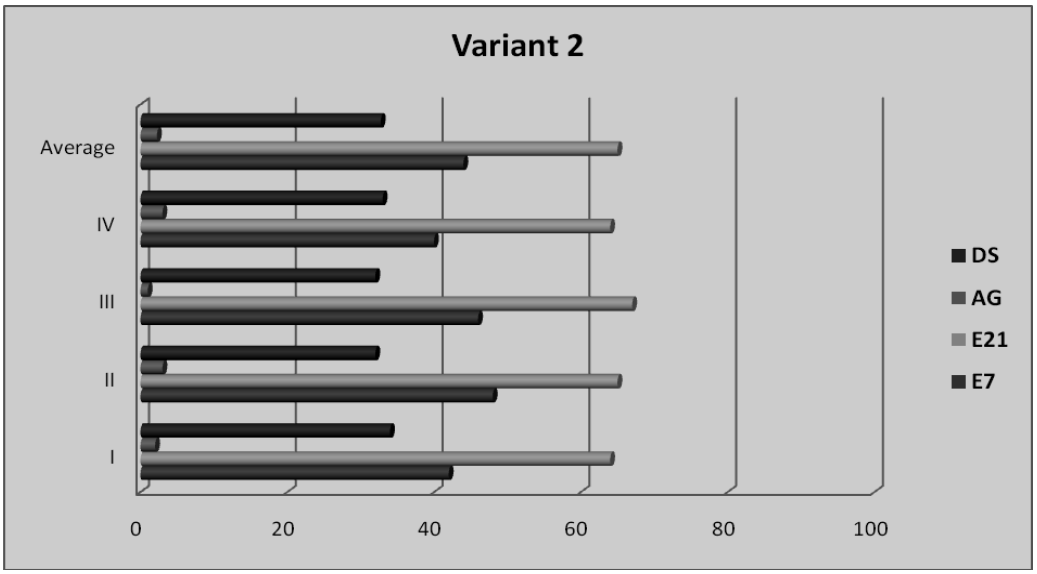

Figure 2. Graphical representation of TP method results (Variant 2), performed at a temperature of 20-30°C, with light, with special treatments, chilling at 10°C for 5 days

Legend: I, II, III, IV = 4 repetitions / 100 pc; E7= evaluation of normal seedlings after 7 days (%); E21 = evaluation of normal seedlings after 21 days (%); AG = abnormal seedlings (%); DS = dead seedlings (%)

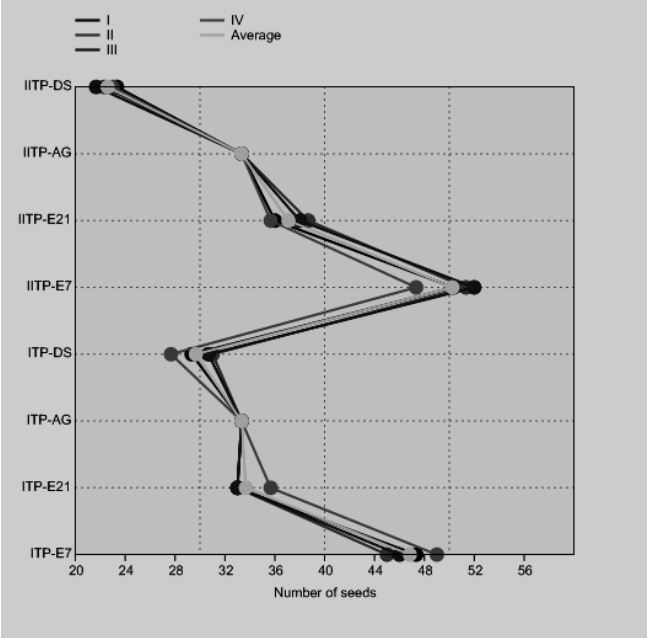

Figure 3. Comparative representation of the variants *ITP and IITP* seed germination

Legend: I, II, III, IV = 4 repetitions / 100 pc; ITP = variant 1 code; IITP = Variant 2code; E7= evaluation of normal seedlings after 7 days (%); E21 = evaluation of normal seedlings after 21 days (%); AG = abnormal seedlings (%); DS = dead seedlings (%)

Figures 1, 2 and 3 show that a small part of the normal seedlings appeared after seven days; the highest percentage of normal *Plantago media* seedlings appeared 21 days after the seeds were placed for germination (ITP and IITP). The best results on the germination of *Plantago media* seeds were recorded in variant IITP, where the germination percentage was 65%, whereas in variant ITP the germination process was 55% after 21 days (Figure 3.).

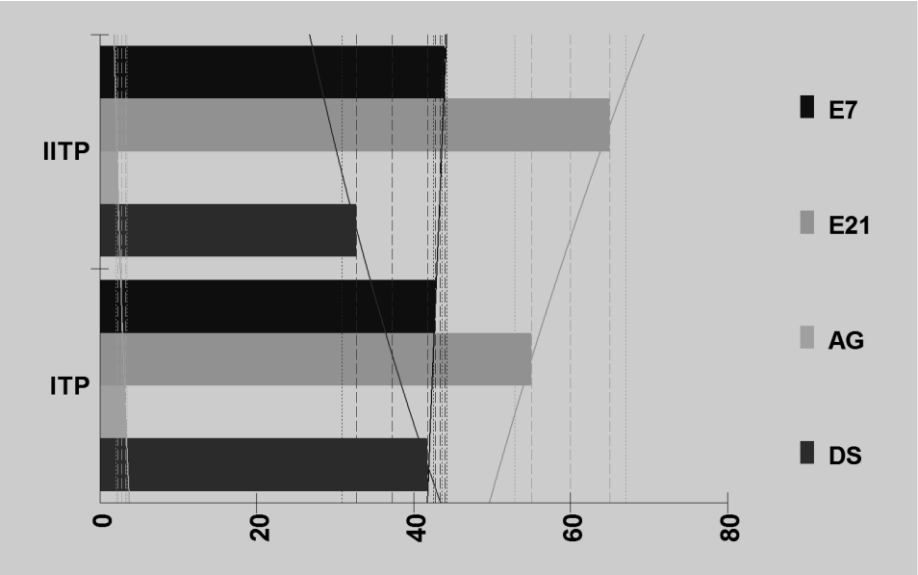

Figure 4. Graphical representation of methods developmental variations

Legend: ITP = variant 1 code; IITP = Variant 2code; E7= evaluation of normal seedlings after 7 days (%); E21 = evaluation of normal seedlings after 21 days (%); AG = abnormal seedlings (%); DS = dead seedlings (%)

The highest germination appears in variant 2 (IITP = Variant 2code), which presents the highest germination tendency, described by an exponential 1 curve fit (Figure 4.). According to this, we may conclude that a reduced number of abnormal seeds will generate higher germination, which could rich 70% under specific developmental circumstances. The applied germination model, Variant 2, is a TP (top of paper) method, performed at variable temperature of 20-30°C, in presence of light, with chilling at 10°C for 5 days, which suggests that IITP is a more adequate germination model to compare with realistic field conditions.

CONCLUSIONS

Based on the results of our study, we drew the conclusion that maximum germination (65%) was obtained when *Plantago media* seeds were kept at a temperature of 10° C for five days and then in high humidity (90%) at alternating temperature (20-30°C), with light.

Variant IITP (top of paper) indicates the potential of *Plantago media* seeds to germinate in early spring on cold and humid soils in the grassland in Zărand Mountains, where this species was identified and from where we collected the seeds for research. The optimal temperature

and humidity that appear in the germination test may not appear also under the conditions of the area under study.

Taking into account the large number of seeds produced by the species *Plantago media*, this low seed germination of 55-65% does not endanger the life of the species in the piedmont and mountain grasslands in Zărand Mountains.

BIBLIOGRAPHY

1. AIRI S., R. S. RAWAL, U. DHAR, O. PUROHIT, 2000. Assessment of availability and habitat preference of Jatamansi–a criticallyendan gered medicinal plant of west Himalaya, Current Science, Vol. 79, No. 10.http://www.iisc.ernet.in/currsci/nov252000/1467.pdf.
2. CHRISTOFFOLETI P.J., CAETANO R.S.X. Soil seed banks, Sci. agric. vol.55 special issue Piracicaba, p.74-78.1998. http://www.scielo.br/pdf/sa/v55nspe/3152.
3. ELIAS SABRY, GARAY ADRIEL, SCHWEITZER LEE, HANNING SHERRY, 2006. Sees quality testing of native species, Native Plants J. 7(1): 1-19. http://www.nativeplantnetwork.org/Journal/ArticleAbstract.
4. HIERRO JOSÉ L., ÖZKAN EREN, LIANA KHETSURIANI, ALECU DIACONU, KATALIN TÖRÖK, DANIEL MONTESINOS, KRIKOR ANDONIAN, DAVID KIKODZE, LEVAN JANOIAN, DIEGO VILLARREAL, MARÍA E. ESTANGA-MOLLICA, RAGAN M. CALLAWAY, 2009. Germination responses of an invasive species in native and non-native ranges. Ecology & Organismal Biology , Oikos, Vol 118 Issue 4. Abstract. http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0706.2009.17283.x/full
5. KUMAR, G.P., R. KUMAR AND O.P. CHAURASIA, 2011. Conservation status of medicinal plants in Ladakh: Cold arid zone of Trans-Himalayas. Res. J. Med. Plant, 5: 685-694.CrossRef | Direct Link.
6. MANAGING AND ENHANCING THE USE OF GERMPLASM - Strategies and Methodologies, Tehnical Manual No 10, Section 4: Seed Processing, ICRISAT, p. 77-79. http://www.icrisat.org/what-we-do/genebank/genebank-manual/seed-processing-4.pdf
7. RUJAN CRISTIAN, LUMINIȚA COJOCARIU, HORABLAGA NICOLAE MARINEL, DESPINA - MARIA BORDEAN, CARMEN DURĂU, CICIO JAMES, 2012. Quality Assessment of *Festuca Arundinacea (Schreb.)* Seeds through Laboratory, Bulletin UASVM Cluj-Napoca Agriculture 69(1)/2012, p.126-132. http://journals.usamvcluj.ro/index.php/agriculture/article/download/8667/7341.
8. RUJAN CRISTIAN, LUMINIȚA COJOCARIU, DESPINA - MARIA BORDEAN, CAMELIA GIUCHICI, BOSTAN CRISTIAN, AURICA BOROZAN, MANUELA PĂLCUȚ, CARMEN DURĂU, HORABLAGA NICOLAE MARINEL, 2012. Testing *Festuca arundinaceae* seed during storage, Research Journal of Agricultural Science vol.44 (1) 1 -299 (2012), U.S.A.M..V.B. Timisoara, Faculty of Agriculture, p. 216- 222, Agroprint Timisoara, Romania. http://www.rjas.ro/index.php/rjas/article/view/1651/1394.
9. VINAY PRAKASH, HEMLATA BISHT and M.C. NAUTIYAL, 2011. Seed Germination Enhancement in High Altitude Medicinal Plants of Garhwal Himalaya by Some Pre-sowing Treatments. Research Journal of Seed Science, 4: 199-205.
10. *** ASSOCIATION OF OFFICIAL SEED ANALYSTS [AOSA], 2002. Seed vigor testing handbook. Las Cruces (NM): Contribution No. 32.
11. *** ASSOCIATION OF OFFICIAL SEED ANALYSTS [AOSA], 2004. Rules for testing seeds. Las Cruces, NM.
12. *** INTERNATIONAL SEED TESTING ASSOCIATION [ISTA], 2002. International rules for seed testing. *Seed Sci. Technol*., 37(Suppl.): 54-59.
13.*** SR (STANDARD ROMÂN) 1634, iun.1999. Semințe pentru însămânțare. Determinarea germinației.