

## Effect of the storage at low temperatures on the germination and antioxidant activity of *Geum urbanum* seeds

Received for publication, December, 26,2016

Accepted, November, 9, 2017

**RODICA CATANĂ<sup>1</sup>, LARISA FLORESCU<sup>1\*</sup>, ALEXANDRA SIMON-GRUIȚĂ<sup>2</sup>,  
MONICA MITOI<sup>1</sup>**

<sup>1</sup> Institute of Biology Bucharest of the Romanian Academy, Splaiul Independentei No. 296,  
Bucharest 060031, Romania

<sup>2</sup> Department of Genetics, Faculty of Biology, University of Bucharest, 1-3 Intrarea  
Portocalelor, 060101 Bucharest, Romania

\*Address for correspondence to: [aqua.bios@yahoo.com](mailto:aqua.bios@yahoo.com)

### Abstract

The effect of low temperatures (chilling 4° and freezing -75°C) on germination and antioxidant activity of medicinal *Geum urbanum* seeds was investigated. In the recent years, the interest for the conservation of medicinal plants was increased. Thus, the maintenance of seed viability during *ex situ* conservation is essential for the preservation of the medicinal plants and their genetic diversity. In our case, storage at chilling and freezing temperatures improved the germination percentage from 46% (seeds stored at room temperature) to 100% (seeds stored at -75°C). Also, the amylase activity was higher (1.06 and 1.10 U/ml) compared to the seeds stored at room temperature (0.63 U/ml). The antioxidant activity increased after chilling storage from 23.29893 mM Trolox/g to 25.0544 mM Trolox/g and decreased to 19.39789 mM Trolox/g after freezing storage. These values were not correlated with the germination percentage. The study indicated that the exposure at low temperature improved the germination capacity and biochemical particularities of seeds initially stored at room temperature, so cold temperatures could be an efficient storage method for *G. urbanum* seeds.

**Key words:** medicinal plant, germination, conservation, *Geum* sp.

### 1. Introduction

In Romania, the *Geum* genus is represented by 6 taxa, two of them (*G. reptans* and *G. aleppicum*) being sporadic spread. *Geum urbanum* L. is an herbaceous plant belonging to the Rose family (Rosaceae) (CIOCÂRLAN 2009 [1]). This species is a medicinal plant which occurs naturally in shady habitats in Europe and is mentioned as endemic for Europe in the European Red List of Medicinal Plants (ALLEN & al. 2014 [2]). Also, *G. urbanum* is an indicator species for nitrogen soil supply (CIOCÂRLAN 2009 [1]).

*G. urbanum* roots are used as astringent agents in treating diarrhea, indigestion, gingivitis and hemorrhoids based on their main compounds like tannins, phenolic acids, eugenol (main compound of the essential oil) (OWCZAREK & al. 2013 [3]). In the recent years, the importance of secondary metabolites from *G. urbanum* and their effects was recognized (PIWOWARSKI & al. 2014 [4]; PAUN & al. 2015 [5]; OWCZAREK & al. 2015 [6]; GARNICA & al. 2016 [7]). In our country, the underground parts of *G. urbanum* are picked up in moderate quantities from natural habitats (DIHORU & BORUZ 2014 [8]).

*G. urbanum* species is completely self-compatible and its outcrossing rate is low (5- 20%) (RUHSAM & al. 2010 [9]); also, this plant species has a low capacity for vegetative spread (GRIME & al. 1988 [10]), seeds reproduction being more important for their regeneration. Seeds are characterized by a physiological dormancy (GRAVES & TAYLOR 1988 [11]),

which prevents germination until an endogenous chemical change takes place (FENNER & MICHAEL 2005 [12]). The germination rate is ~ 70% after stratification and no persistent seed bank was reported (ROBERTS 1986 [13]). MCDONALD 2005 [14]) described that *Geum* has short (less than 1 year) storage life, which indicates that seeds are likely to lose their viability and vigor over one season. From this reason, a storage method for improving the seed germination is required.

Interest for the conservation of medicinal plants is increasing all over the world. Medicinal plant species are poorly represented in the seedbanks, due to the lack of knowledge on reproduction biology and seed behavior (LIZA & al. 2010 [15]). Storage of the seeds is a technique accessible to a large part of higher plants, serving as a safe and relatively inexpensive method of conservation. Several factors (temperature, seed moisture content, relative humidity etc.) influence the seed longevity during storage (PRADHAN & BADOLA 2008 [16]) and the speed of the biochemical processes (TONIN & PEREZ 2006 [17]). The choice of storage temperature varies considerably according to the plant species and the period for which the seed is to be stored. Maintaining the seed viability during *ex situ* storage is essential for biodiversity conservation programs.

The aim of our research was to evaluate the effect of the storage at low temperatures (chilling at 4° and freezing at -75°C) on the germination process,  $\alpha$ -amylase activity and the antioxidant activity of *Geum urbanum* seeds.

From our knowledge, no data were recorded about the antioxidant activity of *Geum urbanum* seeds after storage at low temperature.

## 2. Material and methods

The biological material was represented by mature seeds (Figure 1) maintained at room temperature in paper bags for one year. Considering that *Geum urbanum* seeds were included in short storage life category, we used one year old seeds to check the germination capacity after the seeds have lost their vigor. The experiments were carried out in laboratory conditions during the years 2015 - 2016.



**Figure 1.** Mature seeds of *Geum urbanum*

### Determination of the seed germination percentage

The seeds were washed 2 hours in tap water and placed in Petri dishes on double layered filter paper, moistened with distilled water. The Petri dishes were covered with aluminum foil and maintained in the refrigerator at 4°C, respectively in the freezer at -75°C for 4 weeks. No seed sterilization protocol was applied. A control, represented by seeds stored at room temperature (~25 °C) was used. After storage at low temperatures, the dishes were placed in a chamber room Weiss Gallekamp Fitotron for germination, at 25°C, with a photoperiod of

16/8 hrs. (using two fluorescent lamps of 36W with maximum intensity of  $\sim 90\mu\text{mol m}^{-2} \text{s}^{-1}$ ). After germination, the seedlings were transferred in a ground-perlite mix at room temperature.

Germinated seeds were counted every day. A seed was considered germinated when the tip of the radicle had grown free of the seed coat.

The estimated parameter was the final germination percentage (%) expressed as total number of germinated seeds/total number of seeds X 100.

Other fifty healthy non-germinated seeds ( $\sim 100\text{mg}$ ) were grounded and extracted in a ratio 1:10 in 0.1 M Na phosphate buffer, pH 7. The homogenates were centrifuged at 10000G, for 20 minutes and the supernatant was used for the subsequent analysis.

#### **Determination of $\alpha$ -amylase activity**

$\alpha$ -Amylase hydrolyzes a starch solution and the substrate remained unhydrolyzed is assayed spectrophotometrically at 620 nm after reaction with iodine solution according to the Fuwa method (Fuwa 1954 [18]). Thus, the enzyme is incubated with 0.5 ml of the starch solution (20 mg /ml in 0.1 M phosphate buffer pH 8) at 37°C for 30 min. The reaction was stopped with 1 ml of 1N HCl, then diluted with 7 ml of distilled water supplemented with 1 mL of iodine reagent (0.2% iodine and 2% KI). The volume was adjusted to 100ml with distilled water.

For estimation of  $\alpha$ -amylase activity was used the difference between the absorbance at 620 nm of starch without adding enzyme and starch remained after enzymatic digestion. To express amylase activity in U/ml, this difference in absorbance relate to aborbance of 1 mg of starch derived from the standard curve and the amount of enzyme used.

#### **Determination of antioxidant capacity by DPPH method**

Diluted 100 $\mu\text{l}$  seeds extract (1:10) was mixed with 2.25 ml of methanol and 150  $\mu\text{l}$  methanol solution of 1.27 mM DPPH according to the method proposed by MARXEN & al. 2007 [19]. The control was represented by the extraction solvent without seed extract. After 30 minutes of incubation at room temperature and darkness, the absorbance at 515 nm was read. Antioxidant capacity represents the difference between the samples and control against a standard curve that uses Trolox (synthetic antioxidant  $\alpha$ -tocopherol analogue) as standard antioxidant. % DPPH inhibition rate was calculated after the formula: % inhibition =  $(1 - \text{OD}_{515\text{nm}} \text{ sample} / \text{OD}_{515\text{nm}} \text{ martor}) \times 100$ , where  $\text{OD}_{515\text{nm}}$  represents optical density at a wavelength of 515 nm.

#### **Statistical interpretation**

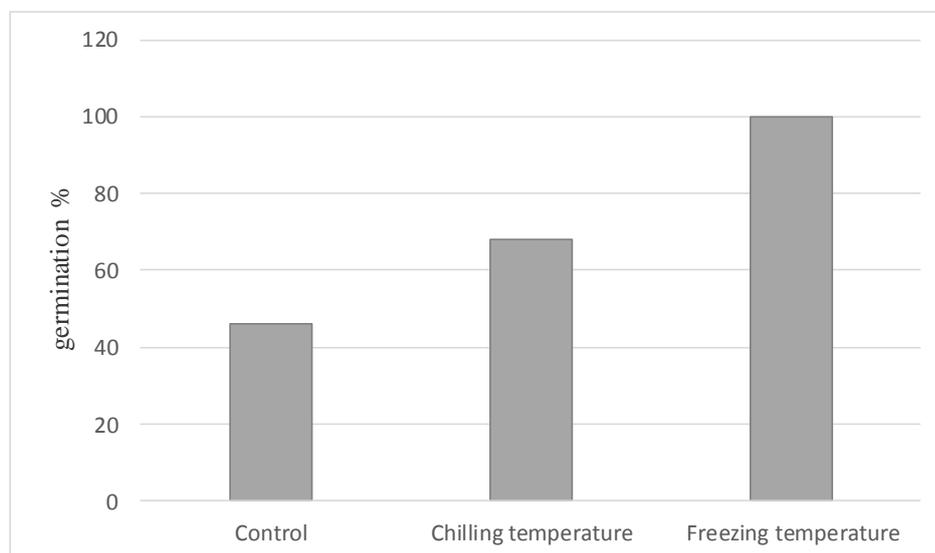
The germination was statistically analyzed using Past program (Hammer & al. 2001 [20]). Considering that the data are ordinal variables, nonparametric tests were used. In order to determine the differences between seeds germination after storage at the tested low temperatures, the Kruskal-Wallis test was applied.

Enzyme activity data are expressed as means ( $\pm$ SD) of three replicates.

### **3. Results and discussion**

Data presented in Figure 2 revealed that under different storage temperatures the *Geum urbanum* seeds germination percentage varied from 46 to 100%. Seeds stored at 4°C germinated in a higher percentage than seeds stored at room temperature. It is known that storage of seeds at room temperature causes low germination level, deterioration and loss of vigor and viability (MÜLLER & al. 2011 [21]). In our case, storage of seeds at room temperature ensured a relatively good germination percentage (46%), but lower than chilling

(4°C) and freezing (temperatures -75°C) where seed germination was increased over 60% (Figure 2).



**Figure 2.** Effect of storage temperatures on the germination percentage of *G. urbanum* seeds

The Kruskal-Wallis test confirmed the significant differences regarding germination percentage between the seeds stored at different temperatures ( $H(\chi^2) = 24.57$ ;  $H_c$  (tie corrected) = 38.91;  $P < 0.0001$ ). In addition, Mann-Whitney post-hoc test revealed that the greatest differences were noted between freezing and room temperature storage (Table 1).

**Table 1.** Mann Whitney pairwise comparisons with Bonferroni correction between groups

	<b>Room temperature</b>	<b>Chilling temperature</b>	<b>Freezing temperature</b>
<b>Room temperature</b>		<b>0.03131</b>	<b>2.85E-07</b>
<b>Chilling temperature</b>	0.09393		<b>3.52E-03</b>
<b>Freezing temperature</b>	<b>8.55E-07</b>	<b>1.06E-02</b>	

The bold values are statistical significant ( $p < 0.0001$ ).

There are few studies concerning the germination of the medicinal plant seeds after storage at low temperatures. CHAUHAN & NAUTIYAL 2007 [22] reported a less viability under room temperature than at low temperature in the case of *Nardostachys jatamansi*, an endangered medicinal herb of high-altitude from Himalaya. In 2012, KHOLINA & VORONKOVA 2012 [23] showed that seeds of a medicinal legume species collected from natural habitats germinated better after storage at low temperatures, surviving after cryostorage better than controls stored at 25°C. Our data are in accordance with those reported by DE SOUSA AGUIAR & al. 2015 [24] regarding two rice cultivar seeds stored at -50°C and 8°C which had higher germination rates compared with seeds stored at 25°C.

The minimum days necessary for initiation of germination, respectively 7 days, were registered at temperatures of 4°C and -75°C. At room temperature, seeds started to germinate after 44 days. The minimum number of days for the initiation of germination reduced with temperature decreasing, indicating that physiological dormancy of *G. urbanum* seeds could be suppressed by low temperatures (BASKIN & BASKIN 1998 [25]).

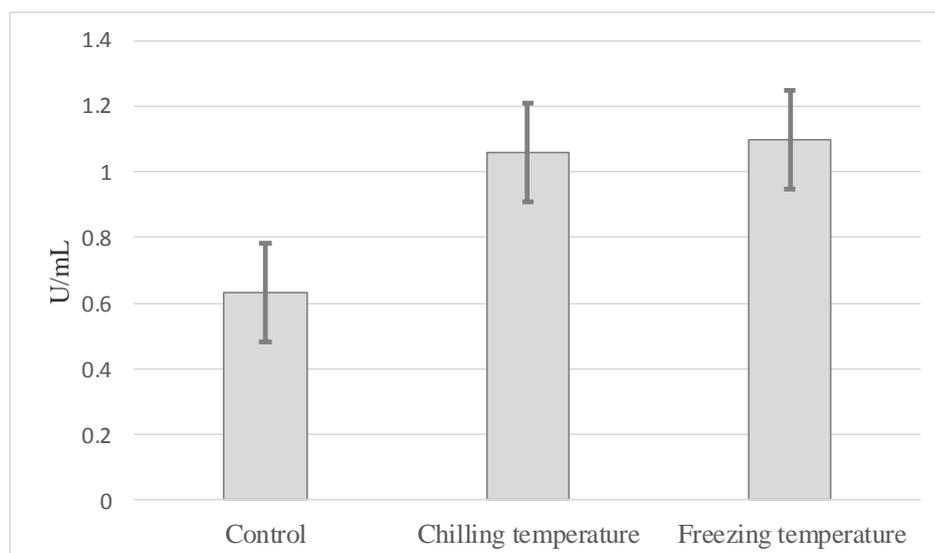
It is known that cold temperatures allow the reduction of seed metabolism, contributing to a longer life; storage at low temperatures favors the maintenance of biochemical processes in the embryo, subsequently enabling normal seedling development and uniform germination (DE SOUSA AGUIAR & al. 2015 [24]).

The  $\alpha$ -amylase and antioxidant activities of *Geum* seeds were measured to determine the influence of low temperatures on the biochemical properties of the seeds, and whether the assay may be used to identify the optimum storage conditions.

$\alpha$ -amylase plays an important role in hydrolyzing the starch into metabolizable sugars, which provide the energy for the growth of roots and shoots (BECK & ZIEGLER 1989 [26]). The  $\alpha$ -amylase test may be of fundamental importance to evaluate the seeds physiology after storage period (PANOBIANCO & al. 2007 [27]).

Amylase activity of *Geum* seeds varied, but not significantly, in response to temperatures. The chilling (4°C) and freezing (-75°C) temperatures had a stimulating effect, amylase activity being increased (Figure 3) in both situations (from 0.63 to 1.06U/ml at 4°C and respectively 1.10 U/ml at -75°C). In the case of seeds stored under uncontrolled conditions like room temperature, the reserve tissues may have been deteriorated as resulted from the measurement of the  $\alpha$  amylase activity.

STRIBUL & al. 1996 [28] investigated the influence of various cold temperatures (-20°C, -75°C, -196°C) on the seeds of corn and observed the stimulating effects of -75°C for the amylase activity. Temperature represents an important factor for seed conservation, directly affecting the speed of the biochemical processes and interfering with the increased amylase enzyme activity. This may be linked to the fact that low temperatures induce the biosynthesis of gibberellin - the precursor of amylase biosynthesis (NEVES & MORAES 2005 [29]).



**Figure 3.** Effect of storage temperatures on amylase activity of *G. urbanum* seeds

Data concerning the antioxidant capacity of the aerial and underground parts of *G. rivale* and *G. urbanum* were reported by OWCZAREK & al. 2015 [6] which using various *in vitro* methods (FC, DPPH, FRAP and linoleic acid peroxidation test) identified *G. rivale* rhizomes as the plant material with the highest antioxidant potential.

From our knowledge, no data were registered concerning the antioxidant capacity of *Geum urbanum* seeds. In this study, seeds of *G. urbanum* were evaluated as potential new sources of antioxidant capacity. There are many researches regarding the utility of the phytochemicals from leaves, roots, flowers, or whole plants. Few reports refer to seeds as sources for pharmaceutical products. Numerous compounds (alkaloids, lectins, phenolic compounds - lactones, tannins and flavonoids) with antioxidant activity are present in seeds or seed coats, protecting the seeds against the microbial degradation until germination occurs (CAI & al. 2004 [30]). In our case, the antioxidant capacity of the seeds stored at positive temperatures (4°C and 25°C) was almost the same, a low decrease being registered in the case of seeds kept at negative temperature, represented by -75°C (table 2).

**Table 2.** The antioxidant capacity and inhibition rate of *G. urbanum* seeds stored at different low temperatures

Temperature variants	Antioxidant capacity mM Trolox/g	% DPPH inhibition rate at 10 mg/mL
Room temperature (25°C)	23.29893	33.98
Chilling temperature (4 °C)	25.0544	36.69
Freezing temperature (-75°C)	19.39789	24.66

Variation of antioxidant activity between storage treatments (-196, -18, 5, 23 and 50°C), which was not related to seed viability was noted by MERRITT & al. 2003 [31] in the case of some Australian species (*Acacia bivenosa*, *Anigozanthos manglesii*, *Banksia ashbyi* and *Mesomelaena tetragona*).

According to PAUN & al. 2015 [5], *G. urbanum* extracts had a remarkable inhibition rate of DPPH (92 ± 3.7% in aqueous extract at 3 mg/ml). Our seed extracts had a lower inhibition rate between 24.66 to 33.98% (table 2) at a concentration of 10 mg seeds/ml phosphate buffer.

Variation of the antioxidant activity between the aerial parts and seeds was observed by ARRAR & al. 2013 [32] in *Capparis spinosa*, the arial parts (flowers and leaves) showing higher levels than the seeds.

Comparing with literature data, the antioxidant activity of *G. urbanum* seeds maintained at 25°C (23.298 mM/g, respectively 2329.8 mM Trolox /100g) was higher than the antioxidant activity of *Geum triflorum* Pursh seeds collected from natural habitats (55.723 µM Trolox /100 g (BORCHARDT & al. 2008 [33])).

Developing vigorous seedlings and enhancing seed germination are crucial for *ex situ* conservation and cultivation. The seedlings obtained from *Geum* seeds stored at 4°C and -75°C had a good development and became vigorous plants, as shown in Figure 3.



**Figure 3.** Vigorous plants obtained from seeds stored at  $-75^{\circ}\text{C}$

#### 4. Conclusion

Seeds stored at  $-75^{\circ}\text{C}$  and  $4^{\circ}\text{C}$  temperatures had higher germination percentage compared with those stored at  $25^{\circ}\text{C}$ . Amylase activity is increased in the seeds stored at low temperatures, proving a good preservation of the seed viability. In terms of antioxidant activity it was observed an increase at chilling temperature, but a decrease at freezing temperature, values which were not correlated with the seed germination percentage. Thus, *G. urbanum* seeds may be used as potential new source of antioxidants. Low temperatures have had a beneficial effect on *G. urbanum* seeds representing a promising method for *ex situ* conservation.

#### 5. Acknowledgements

The study was funded by the Project no. RO1567-IBB06/2016 from the Institute of Biology Bucharest of Romanian Academy.

#### References

1. V. CIORCĂRLAN. Flora ilustrata a Romaniei Pteridophyts & Spermatophyta. Edit. CERES, București, : 320 (2009).
2. D. ALLEN, M. BILZ, D.J. LEAMAN, R.M. MILLER, A. TIMOSHYNA, J. WINDOW. European Red List of Medicinal plants, Publications Office of the European Union, Luxembourg, pp. 51 (2014).
3. A. OWCZAREK, G. JAN, A. KICEL. Composition of essential oil from aerial and underground parts of *Geum rivale* and *G. urbanum* growing in Poland. *Natural Product Communications*, 8(4): 505 (2013).
4. J.P. PIWOWARSKI, S. GRANICA, M. KOSIŃSKI, A.K. KISS. Secondary metabolites from roots of *Geum urbanum* L. *Biochem Syst and Ecol*, 53: 46 (2014).
5. G. PAUN, E. NEAGU, C. ALBU, G.L. RADU. Inhibitory potential of some Romanian medicinal plants against enzymes linked to neurodegenerative diseases and their antioxidant activity. *Pharmacogn. Mag*, 11(1): S110 (2015).
6. A. OWCZAREK, G. JAN, M.A. OLSZEWSKA. Antioxidant activity of *Geum rivale* L. and *Geum urbanum* L., *Acta Pol. Pharm*, 72(6): 1239 (2015).
7. S. GRANICA, A. KLEBOWSKA, M. KOSIŃSKI, J.P. PIWOWARSKI, M.K. DUDEK, S. KAŹMIERSKI, A.K. KISS. Effects of *Geum urbanum* L. root extracts and its constituents on polymorphonuclear leucocytes functions, significance in periodontal diseases, *J. Ethnopharmacol*, 188: 1 (2016).
8. GH. DIHORU, V. BORUZ. The list of main spontaneous medicinal plants from Romania. *Annals of the University of Craiova -Agriculture, Montanology, Cadastrre Series*, 1(XLIV): 328 (2014).
9. M. RUHSAM, P.M. HOLLINGSWORTH, J. SQUIRRELL, R.A. ENNOS. Significant differences in outcrossing rate, self-incompatibility and inbreeding depression between two widely hybridizing species of *Geum*. *Biol. J. Linn. Soc.*, 101: 977 (2010).
10. J.P. GRIME, J.G. HODGSON, R. HUNT. Comparative plant ecology - A functional approach to common British species. *Unwin Hyman*, 1988.
11. J.D. GRAVES, K. TAYLOR. A comparative study of *Geum rivale* L. and *Geum urbanum* L., to determine those factors controlling their altitudinal distribution. III. The response to temperature. *New Phytol*, 110: 391 (1988).

12. M. FENNER, K. THOMPSON. The ecology of seeds. Publisher Cambridge University Press. p. 98 (2005) ISBN 978-0-521-65368-8.
13. H.A. ROBERTS. Seed persistence in soil and seasonal emergence in plant species from different habitats. *J. Appl. Ecol*, 23: 639 (1986).
14. M.B. MCDONALD. Flower seed longevity and deterioration. Chapter 10. In: CAB International Flower seeds: Biology and Technology eds. M.B. McDonald and F.Y. Kwong, pp.188, (2005).
15. S.A. LIZA, M.O. RAHMAN, M.Z. UDDIN, M.A. HASSAN, M. BEGUM. Reproductive biology of three medicinal plants, *BJPT*, 17(1): 69 (2010).
16. B.K. PRADHAN, H.K. BADOLA. Seed germination response of populations of *Swertia chirayita* following periodical storage. *Seed Technology*, 30(1): 63 (2008).
17. G.A. TONIN, S.C.J.G.A. PEREZ. Qualidade fisiológica de sementes de *Ocotea porosa* (Nees & Martius ex. Nees) após diferentes condições de armazenamento e semeadura. *Rev. Bras. Sementes*, 28(2): 26 (2006).
18. H. FUWA. A new method of microdetermination of amylase activity by the use of amylase as the substrate. *J. Biochem*, 41: 583 (1954).
19. K. MARXEN, K.H. VANSELOW, S. LIPPEMEIER, R. HINTZE, A. RUSER, U.P. HANSEN. Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sensors*, 7: 2080 (2007).
20. O. HAMMER, D.A.T HARPER, DAVID, P.D. RYAN. Past: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol Electronica*, 4(1): 9, 178kb (2001).
21. E. MÜLLER, E.J. COOPER, I.G. ALSOS. Germinability of arctic plants is high in perceived optimal conditions but low in the field. *Botany*, 89(5): 337 (2011).
22. R.S. CHAUHAN, M.C. NAUTIYAL. Seed germination and seed storage behaviour of *Nardostachys jatamansi* DC, an endangered medicinal herb of high-altitude Himalaya. *Curr. Sci*, 92(11): 1620 (2007).
23. A.B. KHOLINA, N.M. VORONKOVA. Seed cryopreservation of some medicinal legumes. *J. Bot*, 1: 7 (2012).
24. R.W. DE SOUSA AGUIAR, D.R. BRITO, M. DE MENDONCA LOPES, G.R. DOS SANTOS, C.M. SOUSA, E.M. DA SILVA, J. DIDONET. Physiological and enzymatic changes in rice seeds stored at low temperatures. *Afr. J. Biotechnol.*, 14(31): 2434 (2015).
25. C.C. BASKIN, J.M. BASKIN. Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, London, (1998).
26. E. BECK, P. ZIEGLER. Biosynthesis and degradation of starch in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*, 40: 95 (1989).
27. M. PANOBIANCO, R.D. VIEIRA, D. PERECIN. Electrical conductivity as an indicator of pea seed aging of stored at different temperatures. *Sci. Agric*, 64: 119 (2007).
28. T.F. STRIBUL, S.M. TIMCHUK, O.G. SUPRUN. Induction of amilolytic enzymes in corn seed endosperm rowing after low temperature treatment, In: Problems of cryobiology, 2, Eds. Grishchenko, Institute for problems of cryobiology and cryomedicine National Academy of Science of the Ukraine, pp. 17-22, (1996).
29. L.A.S. NEVES, D.M. MORAES. Análise do vigor e da atividade da amilase em sementes de cultivares de arroz submetidas a diferentes tratamentos com ácido acético. *Rev. Ciênc. Agrovet*, 4(1): 35 (2005).
30. Y. CAI, Q. LUO, M. SUN, H. CORKE. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.*, 74: 2157 (2004).
31. D.J. MERRIT, T. SENARATNA, D.H. TOUCHELL, K.W. DIXON, K. SIVASITHAMPARAM. Seed ageing of four Western Australian species in relation to storage environment and seed antioxidant activity. *Seed Sci. Res*, 13(2): 155 (2003).
32. L. ARRAR, N. BENZIDANE, I. KRACHE, N. CHAREF, S. KHENNOUF, A. BAGHIANI. Comparison between polyphenol contents and antioxidant activities of different parts of *Capparis spinosa* L. *Phcog. Commn*. 3(2): 70 (2013).
33. J.R. BORCHARDT, D.L. WYSE, C.C. SHEAFFER, K.L. KAUPPI, R. G. FULCHER, N.J. EHLKE, D.D. BIESBOER AND R.F. BEY. Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. *J. Med. Plants Res*, 2(4): 081 (2008).