BULG. J. PLANT PHYSIOL., 1997, 23(1–2), 61–66

STUDY ON THE GERMINATION OF *ATROPA BELLA-DONNA* L. SEEDS

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Received 14 July 1997

Summary. The germination of *Atropa bella-donna* L. seeds, a medicinal species with restrictive mode of use, was studied. It was established that variable temperature (6 h at 30 °C and 18 h at 15 °C) significantly stimulates seed germination – 82.5%. A maximum germination was obtained by treatment with gibberellic acid (GA_3) 1 mg/l H_2O – 89.5%

Kew words: *Atropa bella-donna* L., seed germination, scarification, variable temperature, gibberellic acid

Introduction

From ancient times *Atropa bella-donna* L. (banewort) is a well known medicinal plant with roots, leaves and stems used as herbal drugs. The whole plant contains tropane alkaloids mainly atropine and a small quantity of hyosciamine, scopolamine and apoatropine. The herbal drugs possess spasmolytic and mydriatic activity (Modern Phytotherapy, 1982). An interesting fact can be noticed that the atropine inhibits the multiplication of enveloped viruses (Duke, 1986).

Banewort row material has been collected from habitats for the need of the pharmaceutic industry. This leads to significant increase of sizes and numbers of its populations. The necessity of strict control on usage of *A. bella-donna* ressources brought to its inclusion in the group of species with restrictive mode of use according to Order No. 973/1991 of the Bulgarian Ministry of Environment. With a view of protecting this species the formation of plantations in regions with suitable ecological conditions is recommended (Genova et al., 1996).

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Because of the slow and irregular germination of *A. bella-donna* seeds some of its biological peculiarities have been studied – permeability of testa and influence of some mechanical and chemical reagents on seed germination (Dubinskaya, 1949; Jankulov, 1961).

The germination of seeds which have a hard testa can be significantly increased by treatment with the plant growth regulator gibberellic acid. The effect is expressed by change in proportion of inhibitors and growth regulators in favour of the last and the viable seeds germinate rapidly (Ovcharov, 1976; Jones and Stoddart, 1982; Nikolaeva, 1982).

In recent years the method of pre-sowing treatment of seeds with gibberellic acid has been applied in industrial cultivation of banewort in Russia. This method replaces the longer process of stratification and increases crop capacity of row material by 10–20% (Geyer,1987; Shain, 1987).

The aim of the present study was to trace the seed laboratory germination under the influence of scarification, variable temperature and gibberellic acid.

Materials and Methods

Seeds collected from two populations of *Atropa bella-donna* – near the Monastery "St. Spas" (Lyulin mountain) in 1994 and 1995 and near the village of Dragomirovo (Konyavska mountain) in 1995 were studied. Seed germination of cultivated plants originating from the Lyulin mountain (1995) and produced by root cuttings was also investigated.

The biological characteristics of the seeds were studied according to the methodological directions of Firsova (1955), Crocker and Barton (1955), Ovcharov (1976) and Nikolaeva (1982).

Seeds were placed into Petri dishes with filter paper moisted with distilled water. The experiments were directed in fourfold replications with 100 viable seeds. A preliminary study was conducted in 1994 to establish the period of seed storage. In 1995 seed germination was studied in the following variants: 1. Scarification with preparative needle; 2. Variable temperature treatment (6 h at 30 °C and 18 h at 15 °C); 3. Treatment with gibberellic acid (concentrations 0.35, 0.50, 1.00, and 1.50 mg/l H_2O at exposition 24 h).

Each variant had its control. All seeds were previously treated with Vitavax (2 g/100 g seeds) for desinfection. The energy of germination was accounted on the 9th day and the germination – on the 50th day. Results obtained were subjected to ANOVA analysis.

Results and Discussion

Atropa bella-dona seeds are small and the 1000 seed weight of different specimens varies from 1.07 to 1.16 g.

Data in Table 1 show that the scarificated seeds start to germinate at the 9th day and reach their maximum at the 50th day – 26%. Non-treated seeds from the control did not germinate in general. This fact can be explained with the hard and cutinizated testa (Dubinskaya, 1949; Jankulov, 1961). In the previous experiments we used sandpaper which harmed significantly the seeds and they got mouldy. Scarification with needle proved more suitable and as a result of this treatment infiltration of water and air was facilitated and they stimulated the biochemical process in the seeds. The method was not very effective because of the small size of seeds and can not be recommended for practical use.

Under the influence of variable temperature both the testa was affected and the ferment action was activized (Ovcharov,1976; Nikolaeva, 1982). As a result of the temperature regime applied (6 h at 30 °C and 18 h at 15 °C) seeds started to germinate rapidly and on the 9th day the energy of germination was 12%. On the 21th day germination reached 60%. The germination process was significantly shortened and maximum number of germinated seeds was counted in the period of 36–39 day (82.5%). There were no germinating seeds in the control. Our data support the available information about the favourable influence of variable temperature (35–15°C) (Dubinskaja, 1949).

Atropa bella-donna seeds are influenced in a different degree by various concentrations of gibberellic acid. Poorest germination was recorded in the treatment with concentration 0.50 mg/l – 49%. The concentrations of 0.35 mg/l and 1.50 mg/l significantly stimulated seed germination – 66% and 67.5%, respectively.

The concentration of 1.00 mg/l at an exposition of 24 h affected the germinative process and produced optimal effect. The maximum germination recorded was 89.5%.

The established concentration of gibberellic acid is significantly lower compared to the reference data 0.7 g/l (Geyer, 1987) and 0.3–2.5 g/l (Shain, 1987).

Banewort seeds are affected variously by the optimal concentration 1.00 mg/l. Seeds originating from Lyulin Mt. with one year of storage have higher germination – 98%, compared to fresh collected seeds – 89.5%. This fact could be explained with the presence of a non-profound physiological rest established for the seeds of this species.

The seeds collected from Konyavska Mt. in 1995 have significantly lower germination (69.5%) in comparison to those from Lyulin Mt. collected at the same year – 89.5%. This is due to the effect of different ecological conditions on the development of mother plants. In the region of Lyulin Mt. because of higher soil and air humidity were developed powerful individuals with abundant fruiting and high seed vitality.

The cultivated plants reproduced by root cuttings produce viable seeds with high germination – 97%.

The results presented in Table 1 show that all variants were statistically significant. The variants with variable temperature and optimal concentration of gibberellic acid (1.00 mg/l) had the highest values of germination. The variants with 0.35 mg/l

Table 1. The influence of different factors on the germination of *Atropa bella-donna* seeds

Variant/Origin \ Day	Germination (%)																
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	50
Scarification																	
1. Lyulin Mt. (1995)	–	–	2.0	2.5	4.5	6.0	7.5	9.5	10.0	11.5	13.0	16.0	19.5	21.0	23.0	25.5	26.0
Variable temperature (6 h 30°C – 18 h 15°C)																	
2. Lyulin Mt. (1995)	–	7.0	12.0	17.0	28.5	56.5	60.0	65.0	69.5	74.0	77.0	82.5	82.5	–	–	–	–
Gibberellic acid (Lyulin Mt., 1995)																	
3. 0.35 mg/l	–	–	–	4.0	11.0	14.5	19.5	22.0	27.0	31.0	36.5	41.5	47.0	54.0	57.0	61.5	66.0
4. 0.50 mg/l	–	–	–	2.5	3.5	5.5	8.0	9.5	13.0	17.5	20.0	24.5	30.5	34.0	38.0	43.0	49.0
5. 1.00 mg/l	–	5.0	9.5	16.0	26.0	29.0	35.0	38.0	43.5	49.0	54.0	62.0	68.0	74.5	79.0	84.0	89.5
6. 1.50 mg/l	–	–	2.0	8.0	13.0	17.0	19.0	22.0	26.5	32.0	37.0	44.5	51.0	55.0	58.5	63.0	67.5
Optimal concentration of gibberellic acid 1.00 mg/l																	
7. Lyulin Mt. (1994)	6.0	12.5	21.5	38.0	49.0	53.0	61.0	65.5	72.0	78.5	82.0	87.0	88.5	92.0	98.0	98.0	98.0
8. Konyavska Mt. (1995)	–	–	1.0	5.0	16.5	18.0	23.5	27.0	34.0	39.0	42.5	48.0	51.5	55.5	60.0	63.0	69.5
9. Cultiv. pl. (Lyulin 95)	–	2.5	7.0	19.5	42.5	50.5	59.0	65.0	70.0	79.0	83.5	89.0	94.5	97.0	97.0	97.0	97.0

$p<0.001$ ***
$LSD_{5\%}$ 1.2
$LSD_{1\%}$ 1.6
$LSD_{0.1\%}$ 2.1

and 1.50 mg/l GA_3 had average values. Lowest germination was recorded in case of scarification and treatment with GA_3 0.50 mg/l.

Conclussions

1. The effect of scarification on seed germination is insignificant – 26% and this method could not be recommended for practical use.

2. Variable temperature (6 h at 30°C and 18 h at 15°C) stimulated significantly seed germination (82.5%) and the germination period was shortened with 10 days.

3. Gibberellic acid had the most favourable influence on germination at concentration 1.00 mg/l and at expossition 24 h (89.5%).

4. The seeds with one year storage, treated with optimal concentration of gibberellic acid (1.00 mg/l) had higher germination compared to fresh and treated seeds.

5. Use of seeds treated with variable temperature or gibberellic acid (1.00 mg/l) can be recommended for seedling production in case of formation of plantations.

Acknowledgements: The study was supported by Grant B-436/1994 from the National Science Fund, Bulgaria. The authors are also grateful to Dr. Iliana Damyanova for the competent advice concerning statistical analysis.

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