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Variability of the Traits of Cones and Seeds in Different Larch Clones II. The Energy and Capacity of Germination of Seeds

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Abstract. Germination rates of seeds belonging to seven clones of larch with different provenance from Romania were investigated by using different chemical treatments. The highest germination rate was obtained for the seeds immersed in an aqueous solution with 9% naphthaleneacetic acid (NAA) for eight hours; they were followed by the seeds immersed in a aqueous solution that contains copper sulphate (1g/0.5 l water) for 24 hours and by the seeds immersed in a aqueous solution with potassium permanganate (1.25g/0.5 l water) for 24 h. Low germination rates were obtained for the seeds immersed in an aqueous solution with sodium nitrophenol (1.8%) for eight hours, the seeds immersed in an aqueous solution of 20 g of calcium hydroxide in 0.5 l of water for 18 h and for those immersed in tap water. The Sinaia provenance showed the lowest percentage of germination energy and germination capacity for all the applied treatments, while the Bv. Valea Cetăţii, Bv. Valea Popii and Latoriţa showed the highest percentage of germination energy and germination capacity. As the process of obtaining new plants of larch is very difficult, a database designed to improve larch seed germination using different chemical treatments can be extremely useful for seed production industry.

Keywords: auxin, copper sulphate, *Larix*, germination capacity, germination energy

INTRODUCTION

Nevertheless, from among silvicultural methods that can significantly affect forest yield and profitability, seed transfer trials to identify better-adapted and faster growing alternatives to local provenances remain one of the most important (Nilsson, 2011).

The life cycle of any plant is divided into different phases among which seed germination is the basic stage in the growth of plants. Plants propagation through seeds has an important role in maintaining population genetic diversity. Recent studies regarding variability, seeds life cycles, and applications of new techniques for improving seed germination have been carried out (Sorg, 2010).

It is well known that individual species of *Larix* Mill. are characterized by the extremely high percentage of non-vital, empty, seeds and therefore (despite the appreciable importance of this genus in forestry and landscape ecology) their breeding, seed management and artificial regeneration are complicated. Concurrently, *Larix* species are characterized by large, heavy and wingless (so-called non-saccate) pollen grains, whose mobility is considered relatively low in most cases (Chalupa, 1985; Spus *et al.*, 1991). Seeds of larch species, with the exception of *Larix lyallii* Part., have mild dormancy, which can vary within a species. In genetic tests, prompt and complete emergence is desirable, to reduce subsequent variability in plant size and to minimize selective effects in the nursery (Campbell and Sorensen 1984).

However, according to the studied literature data, the chronically high percentage of empty seeds produced by *Larix* is not caused by a single factor, but it is to be considered as

the result of numerous accidents and irregularities in various processes of the reproductive cycle (Kosiński, 1989; Shin and Karnosky, 1995).

Different treatments for larch seed germination were reported by Carlson (1993), who obtained a high germination rate while stratifying the seeds for 30 days in a slightly acidic medium. Shearer *et al.* (1996) improved the germination by immersing the seeds in 1% hydrogen peroxide medium for 6 to 24 h. Moreover, treating the seeds with giberelinic acid increases the germination percentage for *Pinus sylvestris* and *Larix decidua*.

Gorian and Pasquini (2007) pointed the importance of light upon the percentage of seeds germination. Li *et al.* (1994) showed that light may reduce germination for stratified seeds of larch and had no effect on unstratified seeds. Sorensen (1990) recommended short stratification periods for germination in a warm greenhouse specifying that longer periods will improve uniformity of emergence.

Based on the well-known fact that larch seeds have a very low germination percentage, of only 30-50% (David, 2002), the goal of this study was to create a database with effective treatments, which can help to improve seeds germination. The results of this approach can be extremely useful in seed production, to improve germination and development of the seedlings, which is a problematic issue regarding larch species.

MATERIALS AND METHODS

Plant material and experimental conditions

The plant material consisted of larch clones, obtained through grafting, from plus trees, were selected from different natural or artificial populations from Romania. The provenances of the study were: Gura Humorului, Valea Cetatii, Sacele, Valea Popii, Sinaia, Anina and Latorita.

Larch cones were collected in November 2010 and were stored indoors for drying, at temperatures of 20-25ºC. Seeds were manually extracted from the dried cones. All germination experiments were performed in laboratory, at temperatures of 16-25ºC and natural daylight conditions.

Six treatments were conducted to study germination energy and germination capacity: V1-seeds were sown in Linhardt dishes and soaked in tap water; V2-seeds were immersed in aqueous solution that contained sodium nitrofenolate (1.8%), for eight hours; V3-seeds were immersed in aqueous solution that contained 9% naphthaleneacetic acid (NAA), for eight hours; V4-seeds were immersed in an aqueous solution of copper sulphate 1g/0.5 l water, for 24 h; V5-seeds were immersed in an aqueous solution with potassium permanganate 1.25 g/0.5 l water, for 24 h; V6- seeds were immersed in an aqueous solution of calcium hydroxide with a final concentration of 20 g in 0.5 l water, for 18 h.

One hundred seeds per replicate and dish, at a distance of 0.5-1.5 cm between them, in four replicates, were established for each variant.

Investigated parameters

Two parameters were considered for this study: germination energy and germination capacity. The germination energy represents the percent of seeds that germinate within a given period (defined as the energy period) under optimum or stated conditions, also called the minimum time required for the seeds to start germinating. The germination energy period for larch is defined as being 7-10 days (David, 2002). The germination capacity refers to the percentage of seeds capable to germinate in 17-20 days. The criterion used to consider a seed as germinated was radicle emergence from the testa.

Statistical analyses

In order to compare the effect of interaction treatment x genotype, the influence of the genotype and the effect of the treatment on germination energy and capacity, ANOVA test has been used, considering the mean of the experiment as control.

RESULTS AND DISCUSSION

Germination energy

Taking into account the interaction provenience x treatment (Tab. 1), the following values of germination energy were recorded (given from the highest value to the lowest one): Bv. Valea Cetăţii (15.5%), Bv. Valea Popii (13.8%) when treated with tap water (V1), Bv. Valea Cetăţii (18.5%), Bv. Valea Popii (23.8%) and Anina (14.8%) treated with ANA (V3), Bv. Valea Cetăţii (34.0%), Bv. Valea Popii (34.8%) and Latoriţa (29.8%) treated with copper sulphate (V4), Bv. Valea Cetăţii (18.3%), Bv. Valea Popii (31.8%) and Latoriţa (27.5%) treated with potassium permanganate (V5) and the provenances Bv. Valea Cetăţii (5.5%) and Latoriţa (27.5%) treated with calcium hidroxide.

When the influence of the genotype on the germination energy of seeds was analyzed (Tab. 2), the lowest percentage of germination energy was recorded in Sinaia provenance (0.7%) and the highest percentage in Bv. Valea Popii (20.4%). All treated variants showed statistically differences while comparing with the mean of experience (Tab. 3). When treating the seed with auxinic-ANA (V3), copper sulphate (V4), as well as the treatment with potassium permanganate (V5), germination energy values were very significant. In treatments with tap water (V1), aqueous solutions of sodium (V2) and calcium hydroxide (V6), the germination energy percentage was negative, with a value of 6.3% lower than the mean of experience.

Germination capacity

The interaction genotype x treatment (Tab. 1) regarding to the germination capacity has the amplitude of variation between 0.0% (Sinaia, treated with water) and 47.8% (Bv. Valea Cetăţii treated with NAA), compared with the mean of experience (17.2%). In the first five treatments, the provenances Bv. Valea Cetăţii and Bv. Valea Popii showed the highest values for the germination capacity.

Analyzing the influence of provenance on seed germination capacity (Tab. 2), data shows that the lowest percentage of germination capacity was recorded in provenance Sinaia (1.0%) and the highest in provenance Bv. Valea Popii (33.0%).

When treatments considered for this study were compared (Tab. 3) statistically values were obtained in the experimental V1, V3, V4, V5 and V6 variants. Significant values for the germination capacity were obtained while treating the seeds with NAA, copper sulphate and potassium permanganate.

Tab. 1

The influence of treatment x provenance interaction on germination energy and germination capacity of larch species

Crt. No.	Provenience and treatment	Germination energy (%)		Germination capacity (%)	
		Average	Signif. difference	Average	Signif. Difference
1.	O.S. Gura Humorului V1	10.8	-	12.5	-
2.	O.S. Gura Humorului V2	4.5	ooo	5.5	Ooo
3.	O.S. Gura Humorului V3	11.8	-	16.0	-
4.	O.S. Gura Humorului V4	11.3	-	14.5	-
5.	O.S. Gura Humorului V5	9.0	-	12.8	O
6.	O.S. Gura Humorului V6	1.5	ooo	2.8	Ooo
7.	O. S. BV. Valea Cetăţii V1	15.5	**	39.8	***
8.	O. S. BV. Valea Cetăţii V2	3.8	ooo	35.5	***
9.	O. S. BV. Valea Cetăţii V3	18.5	***	47.8	***
10.	O. S. BV. Valea Cetăţii V4	34.0	***	37.3	***
11.	O. S. BV. Valea Cetăţii V5	18.3	***	23.3	***
12.	O. S. BV. Valea Cetăţii V6	5.5	oo	9.8	Ooo
13.	O. S. Săcele V1	1.5	ooo	5.3	Ooo
14.	O. S. Săcele V2	9.5	-	17.5	-
15.	O. S. Săcele V3	10.3	-	21.0	*
16.	O. S. Săcele V4	11.5	-	14.0	-
17.	O. S. Săcele V5	11.0	-	15.8	-
18.	O. S. Săcele V6	2.3	ooo	4.8	Ooo
19.	O. S. BV. Valea Popii V1	13.8	(*)	42.3	***
20.	O. S. BV. Valea Popii V2	6.8	o	26.5	***
21.	O. S. BV. Valea Popii V3	23.8	***	35.5	***
22.	O. S. BV. Valea Popii V4	34.8	***	39.5	***
23.	O. S. BV. Valea Popii V5	31.8	***	39.3	***
24.	O. S. BV. Valea Popii V6	11.5	-	15.0	-
25.	O. S. Sinaia V1	0.0	ooo	0.0	Ooo
26.	O. S. Sinaia V2	0.8	ooo	1.0	Ooo
27.	O. S. Sinaia V3	0.3	ooo	0.8	Ooo
28.	O. S. Sinaia V4	0.8	ooo	1.0	Ooo
29.	O. S. Sinaia V5	1.8	ooo	2.3	Ooo
30.	O. S. Sinaia V6	0.8	ooo	1.0	Ooo
31.	O. S. Anina V1	1.3	ooo	1.3	Ooo
32.	O. S. Anina V2	8.5	-	20.5	-
33.	O. S. Anina V3	14.8	*(*)	32.0	***
34.	O. S. Anina V4	2.0	ooo	3.3	Ooo
35.	O. S. Anina V5	3.0	ooo	5.0	Ooo
36.	O. S. Anina V6	2.5	ooo	3.8	Ooo
37.	O. S. Latoriţa V1	2.0	ooo	2.5	Ooo
38.	O. S. Latoriţa V2	8.0	-	10.5	Ooo
39.	O. S. Latoriţa V3	11.0	-	16.8	-
40.	O. S. Latoriţa V4	29.8	***	31.8	***
41.	O. S. Latoriţa V5	27.5	***	31.5	***
42.	O. S. Latoriţa V6	19.8	***	24.5	***
Mean of experiment (Control)		10.6		17.2	
	LSD 5% =	3.1		3.7	
	LSD 1% =	4.2		4.9	
	LSD 0.1% =	5.3		6.3	

Note: V_1-seeds were sown in Linhardt dishes and soaked in tap water; V_2-seeds were immersed for eight hours in aqueous solution that contained sodium nitrofenolate (1.8%); V_3-seeds were immersed for eight hours in aqueous solution that contained 9%. naphthaleneacetic acid (NAA); V_4- seeds were immersed in an aqueous solution of copper sulphate 1g/0.5 l water for 24 h; V_5-seeds were immersed in an aqueous solution with potassium permanganate 1.25g/0.5 l water for 24 h; V_6- seeds were immersed in an aqueous solution of calcium hidroxide with a final concentration of 20 g in 0.5 l water for 18 h.

Tab. 2

The provenances influence on germination energy and germination capacity of larch species

Crt. No.	The provenances	Germination energy (%)		Germination capacity (%)	
		Average	Signif. difference	Average	Signif. Difference
1.	O.S.Gura Humorului	8.1	ooo	10.7	Ooo
2.	O. S. BV. Valea Cetăţii	15.9	***	32.2	***
3.	O. S. Săcele	7.7	ooo	13.0	ooo
4.	O. S. BV. Valea Popii	20.4	***	33.0	***
5.	O. S. Sinaia	0.7	ooo	1.0	ooo
6.	O. S. Anina	5.3	ooo	11.0	ooo
7.	O. S. Latoriţa	16.3	***	19.6	**
Mean of experiment (Control)		10.6	-	17.2	-
LSD 5% =		1.3		1.5	
LSD 1% =		1.7		2.0	
LSD 0.1% =		2.2		2.6	

Tab. 3

The influence of treatments on germination energy and germination capacity of larch specie

Crt. No.	Treatment	Energy germination (%)		Capacity germination (%)	
		Average	Signif. difference	Average	Signif. Difference
1.	V1	6.4	ooo	14.8	oo(o)
2.	V2	6.0	ooo	16.7	-
3.	V3	12.9	***	24.3	***
4.	V4	17.7	***	20.2	***
5.	V5	14.6	***	18.5	*
6.	V6	6.3	ooo	8.8	ooo
Mean of experiment (Control)		10.6	-	17.2	-
LSD 5% =		1.2		1.4	
LSD 1% =		1.6		1,9	
LSD 0.1% =		2.0		2.4	

Note: V_1-seeds were sown in Linhardt dishes and soaked in tap water; V_2-seeds were immersed for eight hours in aqueous solution that contained sodium nitrofenolate (1.8%); V_3-seeds were immersed for eight hours in aqueous solution that contained 9% naphthaleneacetic acid (NAA); V_4- seeds were immersed in an aqueous solution of copper sulphate 1g/0.5 l water for 24 h; V_5-seeds were immersed in an aqueous solution with potassium permanganate 1.25g/0.5 l water for 24 h; V_6- seeds were immersed in an aqueous solution of calcium hidroxide with a final concentration of 20 g in 0.5 l water for 18 h.

A positive result was obtained after treating the seeds with auxins and microelements, as expected, knowing all the their positive effects on a number of plant activities, including: development of the embryo, leaf formation, apical dominance, fruit development, root initiation and development (Ni *et al.*, 2001; Grodnitskaya, 2008; Thakur, 2008).

It can be concluded that treating the seeds of *Larix decidua* specie with auxins, copper sulphate and potassium permanganate, significantly improves the germination energy and germination capacity.

In all the treatment variants, provenance Sinaia recorded the lowest percentage of germination energy and germination capacity. The provenances Bv. Valea Cetăţii, Bv. Valea Popii and Latoriţa showed higher germination percentage for both germination energy and germination capacity.

The low rate of *Larix decidua* seed germination can be explained by the high percentage of empty seeds. The time of pollination is critical for the development of viable and high-quality western larch seeds (Owens *et al.,* 1994). Pâques (2000), Owens (1995), Kosiński (1989, 2003) had observed that normally, more than 70% of the seeds are empty; of these, 17% are due to the lack of pollination, 9% are due to disturbances in megasporogenesis, 17% are due to failure of pollen, and 30% are due to embryo degeneration is much increased by selfing. Larch flushes early, which makes it liable to frost damage and makes the trees vulnerable to diseases (Taylor, 1964, cited by Larsson-Stern, 2003). In areas with frequent spring frosts, larch seeds can be seriously affected. Submerging the seeds in cold water for 15-20 minutes improves the germination.

Even more, an excellent option to improve seed germination of larch is to use growth regulators such as naphthaleneacetic acid (NAA), copper sulphate and potassium permanganate.

CONCLUSIONS

Out of the six different treatments used in this study for improving the germination capacity of larch seeds, only three showed positive results: water treatment, sodium nitrophenol and calcium hydroxide variants. The germination energy and germination capacity of larch seeds were significantly improved when using the treatment with a minimum concentration (9%) of NAA, with aqueous solution containing copper sulphate (1g/0.5 l water) and with aqueous solution containing potassium permanganate (1.25/0.5 l water). Creating a database, in which to reckon the new techniques, in order to improve larch seed germination, can be a useful tool for studying seed production and seed propagation.

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