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Formation of seedlings of white lupin, *Lupinus albus* L. and blue lupin, *Lupinus angustifolius* L. in laboratory and field experiments

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ABSTRACT

Modern varieties of white lupin can be an alternative to soy in overcoming vegetable protein deficiency: to produce or import? Unlike soybeans, lupin seeds do not contain trypsin inhibitors; they don't need heat treatment to be used in feed. Lupin has a high attachment of beans. It is drought-resistant, adapted to environmental conditions with insufficient moisture. The formation of seedlings is a critical period in the ontogeny of lupin, preceding sprouting. At this time, parameters of seedlings such as the speed and vigor, density, distribution uniformity on the soil surface are determined. The objective is to determine the dynamic characteristics of seedlings of white and blue lupin seeds by alterations in the wet and dry weight of the seedling and its individual organs in laboratory and field experiments. In the laboratory experiments, seeds of white and blue lupins were germinated on sifted and calcined sand, moistened to 80% of the total moisture capacity at 20 °C. On the 4th and 7th days, the wet and dry weight per 100 seedlings, morphological alterations in the seedling bodies and their mass were determined, as well as the expenditure of seed reserve nutrients for the formation of seedlings was calculated. In field experiment, the same parameters of seedlings were determined, considering their laboratory germination (according to the number of viable seeds per 1 m row). The formation features of seedlings of white and blue lupins in laboratory and field experiments have been studied. The certain features influencing the parameters of seedlings in field during the critical "sowing-seedlings" period have been determined.

Keywords: White lupin (*Lupinus albus* L.), Blue lupin (*Lupinus angustifolius* L.), Critical sowing-seedlings period, Formation of seedlings.

Article type: Research Article.

INTRODUCTION

There is a global tendency towards an increase in the consumption of meat and other livestock products per capita. This is especially obvious in developing countries, including highly populated China and India. The development of intensive animal husbandry presupposes an increase in the production of high-protein fodder products (Abraham *et al*. 2019; Zotikov 2020). There are many reports about seedling of some plants around the world (Radmard *et al*. 2019, Alexsandrovna Galikhanova & Arnoldovna Timofeeva 2020; Mirsaleh Gilani *et al*. 2020; Salehi *et al*. 2021). Among leguminous crops, the leaders in protein content and its amino acid composition are soybeans and cultivated species of lupin (white, yellow, and blue): they contain twice as much protein in seeds than other leguminous crops (Struti *et al*. 2020). There are two ways to overcome a deficiency of vegetable protein in the country. The deficiency of vegetable protein can be replenished either by domestic production of high-protein crops or by their import (De Visser *et al*. 2014). Among other leguminous crops, soybeans are distinguished by a high protein content in seeds (35-40%), as well as oil (16-18%). The major soybean producers are 3 countries of the American continent. In 2019, Brazil, the United States, and Argentina accounted for 35.6%,

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30.2%, and 17.2% in the total volume (321 million tons) of soybeans produced in the world. The gross harvest of soybeans in these countries is 83% of the global (FAO). Soybean production in these countries has increased dramatically in recent years due to the production and growing of transgenic varieties (GMOs) resistant to continuous action herbicides, glyphosates. The countries of the European Union depend heavily on imports of soybeans from the American countries. Based on the analysis of the problem and the results of the joint EU-supported research project, it has been reported that soybean imports to the EU countries account for 23% of the total global soybean production. As an in-depth study of the problem has shown, European countries, heavily dependent on soy imports, have missed the opportunity to receive income from the development of their production of high-protein crops (De Visser *et al.* 2014; Zander *et al.* 2016).

The EU reports emphasize an integrated positive effect of legumes in crop rotation. They leave a certain amount of nitrogen in the root and crop residues. Cereals that supersede legumes typically have 25% higher yield than other predecessors. They interrupt the cycle of spreading diseases, pests of crops. Australian farmers consider it profitable to grow lupin even with a yield of 120 kg ha^{-1}, as in this case the yield of wheat sown after lupin and the protein content in its grain increase significantly (Gataulina & Belyshkina 2017). Legumes usually have lower yields than cereals. ***Fixation*** needs a lot of energy. The plant spends its assimilates to fix and instead uses nitrogen fixed by bacteria. The fixation of 1 g of nitrogen requires approximately 3 g of carbohydrates. This largely explains why the potential yields of legumes are lower than those of cereals. The soybean yield of 3 ton ha^{-1} is considered to the same extent high as the corn grain yield of 6 ton ha^{-1} (Berger *et al.* 2017).

Lupin alternative to soya

White lupin, *Lupinus albus* L. has the highest production potential, 4-5 ton ha^{-1}, among cultivated forage species of lupin. The seeds contain 35-40% protein and 10-12% fat (Lucas *et al.* 2015; Chiofalo *et al.* 2012). Unlike soybeans, lupin seeds do not contain trypsin inhibitors, thus they don’t need heat treatment to be used in feed (Gataulina & Nikitina 2016). The production of lupin in the Central Black Earth Region of our country requires stable ripening, technological, high-yielding varieties with a high protein content. They also must be disease-resistant and adapted to local conditions. Such varieties were bred at RSAU Moscow Timiryazev Agricultural Academy: Start, Manovitsky, Gamma, Delta, Dega, Deter 1, Timiryazevsky, and Gana (FSBSI 2020). These varieties differ in the duration of the growing season and economically valuable traits. They all mature steadily in the northern part of the Central Black Earth Region. The growing season from germination to full ripening takes 95-120 days, the protein content in the seeds is 35-42%, the first bean attachment height is 42-50 cm, the seed yield is up to 5 ton ha^{-1}, wet weight is up to 65 ton ha^{-1}, depending on varieties and meteorological conditions of the year (Gataulina & Nikitina 2016). Thus, growing leguminous crops is a real way to solve the problem of vegetable protein production. The use of modern varieties of white lupin, adapted to specific conditions, makes it possible to obtain cheap protein due to nitrogen fixation without the need for expensive and energy-intensive nitrogen fertilizers, and to introduce air nitrogen into agricultural production (Baddeley *et al.* 2013). At the same time, the plant is environmentally friendly, does not deplete the soil nitrogen, and increases the yield of aftercrops. Germination is the most important period in the life of plants, when the seed embryo turns into a seedling through a series of successive changes in the presence of moisture and heat. In the field, germination occurs in the soil and continues from sowing of seeds to emergence of seedlings. In annual species of lupin, including white lupin, *Lupinus albus* L. and blue lupin, *Lupinus angustifolius* L., as a result of germination, the hypocotyl carries the cotyledons to the surface. Under light exposure, they turn green and start photosynthesis; this is when the germination phase proceeds. Information about the peculiarities of field germination of varieties of white and blue lupin usually refers to the determination of the field germination of these crops. Successful germination of legumes primarily requires seed swelling. Opinions about the required amount of absorbed moisture for swelling of leguminous seeds are contradictory (from 150 to 80% of the mass of seeds). Thus, the germination rate depends on the temperature regime and moisture content at the seeding depth (Reckling *et al.* 2016; Chen *et al.* 2016; Annicchiarico 2014). The objective is to determine the dynamic characteristics of seedlings of white and blue lupin seeds by changes in wet and dry weight and individual bodies of the seedling in laboratory and field experiments.

MATERIALS AND METHODS

Objects of research are Start, RSAU-MAA bred variety of white lupin with a determinant type of growth, compared with varieties of blue lupin. Ladny variety is a joint breeding variety of the RSAU-Moscow Agricultural

Academy and the All-Russian Research Institute of Agriculture of the Non-Chernozem Zone (Nemchinovka) with determination on the leader (without side shoots) and Nemchinovsky 97 variety with the usual type of branching. Germination was studied in field and laboratory conditions. In the control and seed laboratory, seeds were germinated at 20 ºC in sifted and calcined sand, moistened up to 80% of full moisture capacity. On the 4th and 7th day, the wet and dry weight per 100 seedlings, morphological changes in the seedling bodies and their mass were determined, as well as the expenditure of seed reserve nutrients for the formation of seedlings was calculated.

Similar studies were conducted in the field. The soil of the experimental plot was sod-podzolic, medium loamy. The arable layer contained on average 2.1-2.4% humus, 18.6 mg P_2O_5 and 10.1 mg K_2O per 100 g of soil. Soil acidity was 5.8-5.9. One hundred seeds of each variety were sown, considering their laboratory germination (according to the number of germinating seeds per 1 m row) in quadruplicate to determine biometric changes in the seedling, wet and dry weight and water content of the seedling on the 4th and 7th day. The consumption rate of dry seed mass for seedling formation per 100 seedlings was determined. Data were statistically processed in MS Excel.

RESULTS AND DISCUSSION

Seed germination in laboratory and field conditions

For the laboratory germination of seeds, optimal conditions were created: temperature regime (20 ºC) and the sand moistening up to 80% of the full moisture capacity. After swelling, biochemical and morphophysiological alterations begin in the seeds, and the root of the embryo starts to grow. The mass of seedlings changes (Table 1).

Table 1. Wet and dry matter, g per 100 seedlings.

Variant	Dry weight, g per 100 seeds	Dry seedling weight		Wet seedling weight	
		day 4	day 7	day 4	day 7
Laboratory germination of seeds					
Start	33.2	30.3 ± 1.21	29.8 ± 0.77	79.6 ± 2.07	130.8 ± 5.89
Ladny	12.6	8.1 ± 0.45	8.1 ± 0.30	22.7 ± 0.84	34.3 ± 1.41
N-97	13.1	11.3 ± 0.40	11.5 ± 0.55	32 ± 1.54	44.1 ± 1.54
Field germination of seeds					
Start	33.2	28.5 ± 2.57	27.7 ± 4.16	65 ± 9.10	121.4 ± 14.6
Ladny	12.6	9.6 ± 0.96	9.3 ± 0.74	43.7 ± 6.99	45.7 ± 4.57
N-97	13.1	10 ± 1.20	8.2 ± 0.90	54 ± 4.86	70.5 ± 5.64

Wet weight of white lupin seedlings (Start) on the 4th and 7th day was 240% and 390% of the dry weight of the seeds, respectively. Blue lupin N-97 showed 240% and 350%, respectively, although the absolute moisture absorption of 100 seeds of white and blue lupins was 46 g and 19 g, respectively. In the case of field germination, the dry weight of the seedlings decreased by 8-14% on the 4th day, and did not change significantly on the 7th day. Wet weight of Start seedling on the 4th day 2 times exceeded the weight of dry seeds, while the varieties of blue lupin exceeded it by 3.5 and 4 times, respectively. The seeds of blue lupin had faster water absorption rate, % of the seed weight, than large seeds of white lupin. Khabibov *et al.* (Khabibov *et al.* 2009) studied the increase in wet weight on the 3rd, 6th and 9th day in seedlings of samples with different weights of seeds of 9 species of leguminous crops *in vitro*. The growth rates of raw biomass varied unequally depending on the species, variety and place of its cultivation. The authors suggested a tendency for a higher relative green weight gain in varieties with a lower seed weight. Khabibova *et al.* (Khabibov *et al.* 2009) did not study dry weight of seedlings.

Table 1 shows a decrease in the dry weight of seedlings on the 4th day in comparison with the dry weight of seeds in our study. This is due to the mobilization and changes in the biochemical composition of nutrients concentrated in the cotyledons. These changes are aimed at enhancing respiration and morphophysiological changes in the germinating seed, which determine the growth and development of the seedling (Chiofalo *et al.* 2012). Dry weight of white lupin (Start) seedlings on the 7th day did not change significantly compared to the 4th day, while their wet weight increased twice from the 4th to the 7th day during laboratory germination, while the blue lupin varieties showed an increase by 1.4-1.5 times. Field studies showed a continuing absorption of moisture by Start seedlings on the 7th day; while varieties of blue lupin were already in germination phase at this time. Obviously, the moisture content (water content) is one of the important criteria for seedling development (Table 2). On the 4th day of laboratory germination, when the germinative power of seeds is determined, the moisture content of seedlings of blue and white lupin was within 62 - 65% and did not differ significantly. On the 7th day, the water content of seedlings increased and averaged 76%. Note, however, that the average error in white lupin was greater than in blue lupin varieties. Field germination showed similar results; however, the limits of deviations from the mean were significantly higher compared to laboratory experiments. Temperature conditions and moisture content at the seeding depth and in the root growth zone largely depend on weather conditions during this period. The field "sowing-seedlings" period is critical for leguminous crops carrying cotyledons to the surface (Abraham *et al.* 2019; Struti *et al.* 2020; Chiofalo *et al.* 2012; Gataulina & Nikitina 2016). On the 4th day of field germination, white lupin (Start) lagged behind blue lupin in terms of moisture absorption and seedling development. Thus, dry weight of seedlings in lupin species and varieties day did not change significantly on the 7th in comparison with the 4th day, while wet weight continued to grow intensively. At this time, the seedling bodies developed (Table 3).

Table 2. Changes in moisture content in seedlings of white and blue lupin.

Variant	Moisture content in seedlings (%)		Wet-to-dry weight ratio	
	day 4	day 7	day 4	day 7
Laboratory germination of seeds				
Start	62 ± 2.98	77 ± 4.24	1.6 ± 0.08	3.3 ± 0.09
Ladny	64 ± 1.66	76 ± 1.52	1.8 ± 0.04	3.2 ± 0.15
N-97	65 ± 2.28	74 ± 1.92	1.9 ± 0.10	2.8 ± 0.11
Field germination of seeds				
Start	56 ± 7.84	77 ± 5.78	1.3 ± 0.18	3.3 ± 0.40
Ladny	78 ± 7.80	80 ± 10.4	3.5 ± 0.35	4 ± 0.32
N-97	82 ± 7.38	86 ± 15.5	4.6 ± 0.41	6.1 ± 0.67

Table 3. Dry weight of bodies, % of the whole seedling.

Variant	Dry weight, cotyledons* (%)		Dry weight, roots (%)	
	day 4	day 7	day 4	day 7
Laboratory germination of seeds				
Start	96 ± 4.61	86 ± 3.87	4 ± 0.13	14 ± 0.53
Ladny	90 ± 2.61	83 ± 2.24	10 ± 0.41	17 ± 0.46
N-97	93 ± 3.44	88 ± 2.11	7 ± 0.32	12 ± 0.62
Field germination of seeds				
Start	93 ± 4.65	85 ± 4.25	7 ± 0.35	15 ± 0.75
Ladny	75 ± 3.75	64 ± 3.20	25 ± 1.25	24 ± 1.20
N-97	80 ± 4.00	49 ± 2.45	20 ± 1.00	37 ± 1.85

*Note: the term "cotyledons" in this and further tables includes cotyledons and seed shell.

Dry weight of the cotyledons in the varieties decreased by 5-10%, while the wet weight by 15-25%. Notably, the dry weight of the seedling did not change between the 4th and 7th days. At this time, there was a redistribution of plastic substances contained in the cotyledons between the organs of the developing seedling, and the roots grew intensively. Table 4 shows wet weight of the seedling organs.

Table 4. Wet weight of bodies, rate (%) of the whole seedling.

Variant	Dry weight, cotyledons (%)		Dry weight, roots (%)	
	day 4	day 7	day 4	day 7
Laboratory germination of seeds				
Start	91 ± 2.37	66 ± 1.32	9 ± 0.36	34 ± 0.88
Ladny	81 ± 3.00	66 ± 2.71	19 ± 1.05	34 ± 1.70
N-97	86 ± 4.13	72 ± 2.52	14 ± 0.49	28 ± 0.73
Field germination of seeds				
Start	75 ± 6.98	62 ± 11.1	25 ± 0.84	38 ± 1.80
Ladny	56 ± 7.50	36 ± 4.80	44 ± 2.75	43 ± 2.64
N-97	56 ± 8.00	29 ± 4.41	44 ± 2.40	57 ± 2.78

Varieties of blue lupin had significantly higher wet and dry weight of roots, % of the seedling, on the 4th day of the laboratory experiment (in terms of wet weight - by 1.5-2 times). On the 7th day, the share of roots in the seedling mass increased to 28-34%. During this period, the rate of root growth in white lupin was higher than that of blue lupin varieties. The share of roots on the 4th and 7th days of the field germination was significantly higher than in laboratory conditions. As the field soil moisture content decreases, seedlings enhance their root growth.

CONCLUSION

Seedling formation is largely determined by the genome of the species and variety. A number of authors report this relationship, as well as the environmental influence of the place of formation of leguminous seeds. Unfavorable, stressful environmental factors during the field germination period cause changes in the development of seedlings (Abraham *et al.* 2019; Struti *et al.* 2020; Reckling *et al.* 2016; Chen *et al.* 2016). The sowing-to-germination period is critical in the formation of not only a seedling but also a further yield of seeds, as its final stage determines the vigor and uniformity of germination and the density of seedlings. During this period, the seeds swell and seedlings develop. Seed germination has shown similar changes in the development of the seedling of white and blue lupin varieties (carrying the cotyledons to the surface) in laboratory and field experiments.

Changes in wet and dry weight and moisture content of the seedling are essential parameters of its development. At a seed moisture content of 60-65%, an important stage in the formation of a seedling ends. Up to this moment, the development of seedlings was due to the mobilization of nutrients contained in the cotyledons. The speed of reaching this stage depends on the species, variety and, if in the field, on the temperature and water conditions at the seeding depth and in the root zone. It has been reported that by the 4th day of germination, dry weight of the seedling decreases by 8-14% compared to dry weight of the seed; further, the dry weight of the seedling does not change, and the wet weight continues to grow rapidly. By this time, the seedling reaches a stable dry weight and a new stage begins - the redistribution of nutrients between the seedling organs. At the end of germination, the moisture content of the seedlings is 75-80%. These processes occurred faster in the varieties of blue lupin than in white lupin. Seed germination in field is subject to limiting factors. In our field studies, blue lupin seedlings developed much faster. White lupin lagged behind in the development of seedlings: the noted critical moisture content occurred later than in the varieties of blue lupin. However, on the 7th day of germination, wet weight of the roots of white lupin was greater than that of blue lupin; the seedlings intensified root growth with a decrease in the field soil moisture.

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