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Narrow-leaved forage lupine (*Lupinus angustifolius* L.) breeding aspects

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A lupine breeding program is being performed at Vokė Branch of Lithuanian Institute of Agriculture. During 1995–2005, we selected four narrow-leaved lupine lines using the individual selection method and three low-alkaloid lines using the hybrid cross method. Hybrid lines N1675, N1700, N1702, N1703 have low alkaloid content in the seeds (0.039–0.064%) and green mass (0.030–0.042%). All these lines show a high resistance to fungal diseases, good growth at all plant development phases, a high seed yield (2.3–2.9 t ha^{-1}) and a short vegetation period (88–92 days). These lines are of great value as genetic, breeding and farming material. It will be used in futher lupine breeding programs and new varieties seed production.

Key words: narrow-leaved lupine, lupine breeding, low-alkaloid varieties, resistance to fungal diseases

INTRODUCTION

Various lupine species have been used long since in agriculture as a green manure plant. Scientists Bauer and Prianishnikov first found lupine species with a low alkaloid content in 1926. First alkaloid determination tests were performed by Sengbush in 1928. There were found three yellow lupine and two narrow-leaved mutants without alkaloids [5, 8]. Absence of alkaloids was an inherited feature in breeding. Yield characters of selected non-alkaloid hybrids and lupine plants that had alkaloids showed no significant difference. Selected individuals were used in the breeding, and the first world-known German sweet lupine varieties were selected. Low alkaloid content in lupines is a result of biochemical mutation. First lupine varieties for animal feeding purposes were selected by the individual sampling method from alkaloid lupine populations [14]. Alkaloid content is a dominant feature whose domination is defined by four genes in yellow lupines, five genes in narrow-leaved and eight genes in white lupines [18]. In the breeding work, crossing lupine varieties with a high and low alkaloid levels, F_1 hybrids in the progeny have always a high alkaloid level. Hybrid splitting in the progeny into low- and high-alkaloid in proportion 3 : 1 begins in F_2 generation.

Alkaloids are organic substances, colourless, crystal or amorphous, sometimes liquid, which have toxic and pharmacological features [9]. Low alkaloid levels have no negative influence on the cattle and human health, but higher amounts can cause significant injuries and even animal death. Some authors present information that alkaloids destroy toxic fungi in animal feed and have a positive influence on feed digestibility [4]. Other scientists believe that lupine underground part which accummlates alkaloids takes part in the metabolism, stimulates root growth, makes a natural barrier for microorganisms [11]. There are known a few thousand alkaloids which are classified into protoalkoids, pseudo-alkaloids and natural alkaloids [2, 3]. The main alkaloids found in lupines are lupinine, lupanine, sparteine, lupinidine, hydroxylupanine, anagirine, monolupine, termophsine, puziline, angustifoline and others [2]. The toxicity of alkaloids is different. The most toxic is lupanine, less toxic are sparteine and lupinine. Alkaloid content found in yellow lupine is 0.005–1.7%, in narrow-leaved 0.1–2.8%, in white 0.001–3.5% [8]. According to some researchers, alkaloids operate insecticides and fungicides [1, 5].

In Lithuania, as arable crops two lupines species, yellow feed (*Lupinus luteus* L.) and narrow-leaved (*Lupinus angustifolius* L.), are grown. White lupines (*Lupinus albus* L.) are not grown in Lithuania because they are warm climate plants and in this country do not ripen. Yellow lupines were bred in Lithuania until 1995. The narrow-leaved lupine breeding program was started in 1995. The breeding work is done in three directions: first, low-alkaloid narrow-leaved lupine varieties bred for food industry, second – low-alkaloid narrow-leaved lupines bred for animal feeding, and third – narrow lupines bred for green manure.

The main objective of the present work was to explore new lupine lines in the process of selection of high yielding, early maturity lines resistant to fungal diseases, with a low alcaloids level and suitable for forage and food industry.

METHODS AND CONDITIONS

Breeding trials of narrow-leaved lupines were carried out in a six-field crop rotation, in 1.6 m^2 trial plots. Soil for the trials was haplic luvisoil (IDp), moderately acid, low in humus (2.0–2.1%), with nitrogen reaching 0.096–0.117%, phosphorus 113.2–147.3 mg kg^{-1}, potassium 126/4–153.3 mg kg^{-1}. Soil preparation: deep autumn plough, two spring cultivations. Fungicides: herbicide Gezagard (2–2.5 kg/ha) was applied for weed control, and Kemikar-T (2 l/t seed) was used for seed treatment.

For the primary material breeding of narrow-leaved lupines we used the methods of intervariety straight and reversible crossing and individual selection. Backross was used to intensify one hybrid feature. First generation hybrids were crossed with one of the parents components. Individual plant selection was used for estimation of the selected material.

During vegetation, lupine resistance to fungal diseases was determined at different plant growing phases: sprouting, flowering, shiny pods produce: 1 – very low resistance (affected plants over 50%), 3 – low resistance (affected plants over 26–50%), 5 – medium (affected plants over 11–25%), 7 – high resistance (affected plants over 2.5–10%), 9 – very high resistance (affected plants less than 2.5%). At the full plant germination we counted plants in A and C replications and int the budding–flowering period plants with anthracnosis and fusarium infection. At the full ripeness we counted healthy plants and their yield. At the complete stage, healthy plants were counted and their productivity was estimated. Fungal disease-affected plant percentage was determined according to the formula:

$$P = (n / N) \times 100,$$

where n is the number of affected plants and N is the number of assessed plants [13].

Alkaloid content in field conditions was determined using the colour reaction method [8]. Juice from lupine stems and leave in contact with Dragendorf solution changes colour to brown. Lupine plants that had alkaloids were removed from the trial field.

Alkaloid content in lupine plants was tested by gravimetric methods LST 1560 at the Agrochemical Research Centre. Alkaloid content was calculated as a percentage from dry matter content.

Data on green material and seed yield were processed by statistical methods using the 'Anova' computer program [12].

Hybrid line N1675 was a bred-crossing of a low alkaloid collection sample, N3179, with the alkaloid collection sample N3328, using F_2 individual progeny selection.

Hybrid line N1740 was bred using the backcross method. Variety 'Brianskii-266' which has low alkaloids was crossed with high-yielding, resistant to fungal diseases alkaloid variety 'Timiriazevskii-2', using an individual progeny selection.

Hybrid line N1743 was bred using intervariety crossing between the alkaloid variety 'Brianskii-74' and the low-alkaloid variety 'Brianskii-266'. The selected hybrid in F_2 generation had a low alkaloid content and special phenotypic features – a high content of anthocyans in leaves and an intensive colour of flower.

Low alkaloid content in lupine plants is a result of biochemical mutation. In a few cases, in alkaloid lupine populations can be found non-alkaloids plants. Selection lines N1700, N1684, N1702 and N1703 were selected using individual selection methods from alkaloid lupine varieties.

Selection line N1684 was selected by the individual selection method from variety 'Emir'. The selected hybrid genotype differed from the parent variety in a lower alkaloid content, seed colour and an intensive pink colour of flowers.

Selection line N1700 was selected by individual selection from the alcoloid variety 'Brianskii-74'. The selected genotype had a low alkaloid content, light pink flower colour and light seed colour.

Selection line N1702 was selected by individual selection from the collection sample N3186. The selected genotype had a low alkaloid content and an intensive pink flower colour.

Selection line N1703 was selected from the alkaloid variety 'Timiriazevskii-2'. The hybrid had different flower and seed colour and a low alkaloid content.

RESULTS AND DISCUSSION

In lupine seed production, alkaloidity increases with every generation. It can be determined as alkaloid lupine impurity, physiologic and genetic factors. In crosses with lupine varieties having high and low alkaloid contents, in F_1 lupine breeding generation alkaloid hybrids are received. Alkaloidity in lupine is a dominating feature. In F_2 generation, lupine hybrids split into alkaloid and non-alkaloid in the ratio of 3 : 1 [19].

Lupine breeding results depend to the amount of primary material and its value. A narrow-leaved feeding lupine and narrow-leaved green manure lupine gene bank was collected from Russian N. I. Vavilov Research Institute of Plant Industry. Resistance to fungal diseases, low alkaloid content in lupine plants are recessive features. To sustain these features in the progeny, intervariety backcross and hybrid individual selection were used. As a result of these crossings we obtained three homozygotic lupine hybrid lines: N1675, N1740 and N1743, respectively 0.039–0.055%, 0.035–0.089% (Fig. 1). In plants whose phenotype has a recessive feature, the genotype is homozygotic. In the heterozygotic state, the recesive gene cannot display in the phenotype. In these hybrid lupine lines, plants with high alkaloid levels can be found only after cross-pollination with other plants.

All selected hybrids were tested and described using special lupine descriptors during the 2003–2005 growth period. The control variety was 'Trakiai'. All selected hybrids were tested, their main quality features were determined: resistance to fungal diseases, morphologic features, maturity, yielding and other farming advantages.

In narrow-leafed lupine the flower colour scale is wider in comparison with yellow lupines. An intensive pink flower colour (O_3) have hybrids N1684, N1740, N1743, light pink (O_2) – N1700, N1703, white (O_1) – N1675, N1702, control – an intensive yellow color (O_{12}). From the tested hybrid lines, two (N1740 and N1743) were different from all others: they had dark violet flowers and high levels of antocians in stems and leafs.

In all lupine species, seed yield is an unstable feature. It depends on the species, genetic features, ecological and climatic conditions [10]. Yellow lupine plants produce more green mass than narrow-leaved lupine (Fig. 2). It depends on the lupine spe-

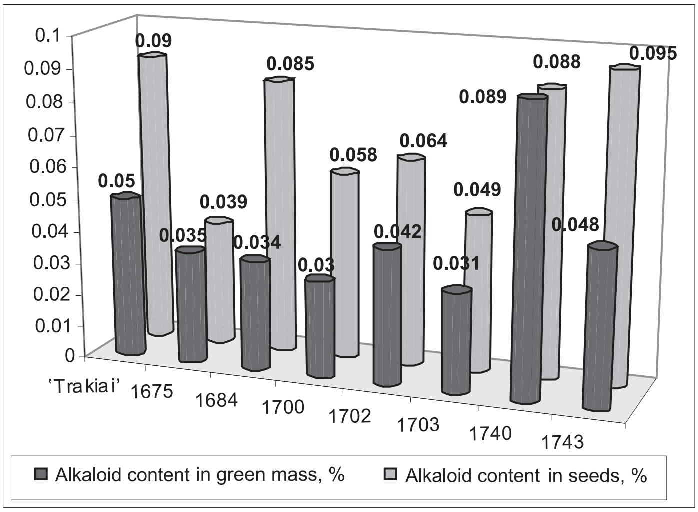

Fig. 1. Narrow-leaved forage lupine alkaloid content in green mass and seeds, %

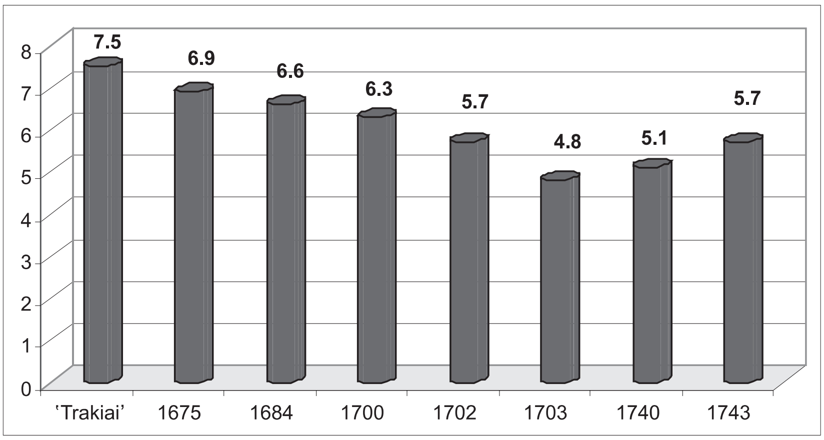

Fig. 2. Narrow-leaved forage lupine green mass yield kg/m², 2003–2005 mean data. R_{05} 0.67

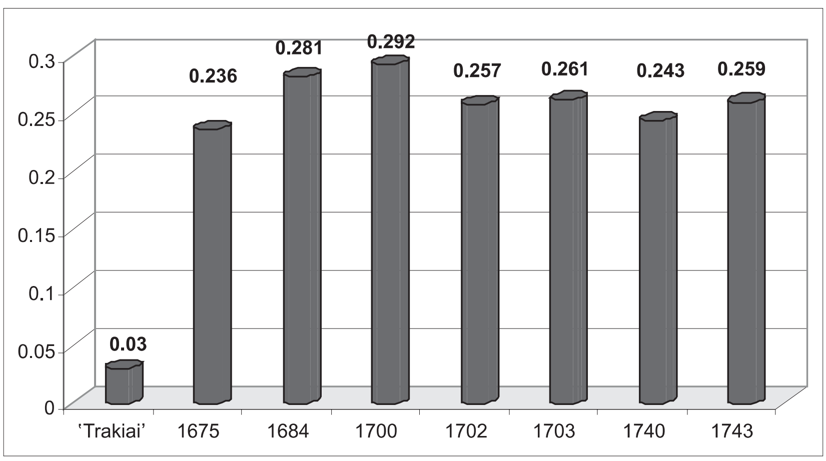

Fig. 3. Narrow-leaved forage lupine seed yield kg/m², 2003–2005 mean data. R_{05} 0.129

cies biological features. Narrow-leaved lupine plant height, depending on agro-meteorological conditions, varies from 48 to 70 cm, while yellow lupine plants reach 90 cm (Table). In narrow-leaved lupines, stem diameter is 5 to 7 mm, while in yellow lupines 10–13 mm. Fungal diseases spread on lupine plants every year. Disease spread depends on variety genetics, meteorological conditions, previous crops, weediness of the field. Lupine anthracnose (*Colletotrichum gloeosporioides* (Penz.) is one of the most harmful diseases which affects all lupine species at any plant growing stage. Yellow lupine species have no resistance to this disease, and farmers had to substitute yellow lupine varieties by narrow-leaved varieties [15, 16]. Lupine grain yield depends on the plant growth stage at which anthracnose affects the plants. The selected narrow-leaved lupine lines show a high resistance to this fungal disease. Conditionally resistant to anthracnosis are hybrid lines in which affected plants were less than 2.5% (Table). In the world lupine gene bank, there are no varieties fully resistant to fungal and viral diseases. But lupine varieties that are partially resistant at a low disease epitophe undergo less infection in the vegetation period. The tested selected lines produced a high seed yield (N1700 2.92 t/ha and N1684 2.81 t/ha) (Fig. 3). The lowest resistance was shown by the standard variety in which infected plants reached 45% and the average seed yield was only 0.3 t/ha.

Table. **Narrow-leaved low alkaloid lupine morphologic features and biologic indeles, 2003–2005 average data**

Hybrid No.	Flower colorva colour	Stem diameter, mm	Plant height, cm	Resistance to *Fusarium*, points	Resistance to anthracnosis, points	Vegetation period, days
'Trakiai'	O_{12}	13.0	90.0	9	3	108
1675	O_1	6.0	57.0	7	9	90
1684	O_3	6.0	63.0	7	9	88
1700	O_2	7.0	61.0	7	9	90
1702	O_1	7.0	70.0	7	9	89
1703	O_2	5.0	48.0	7	9	90
1740	O_3	7.0	63.0	7	7	92
1743	O_3	5.0	62.0	7	7	89

In low-alkaloid lupine varieties, one of the main quality factors is seed quality. Lupines according to their biologic and nutritive value belong to high protein plants. Imported soy beans can be substituted by lupines without decreasing animal growth and meat quality. The main factor is the required amount of amino acids and proteins [6, 7]. In narrow-leaved lupines, protein content is lower than in yellow lupines. In grain dry mass it varies from 28.6 to 35.5% and in the green mass from 17.63 to 23.0% [20]. In all lupine species, amino acids quality and quantity are the same. In proteins are found 18 irreplaceable amino acids, among them 1.45% of lizine and 0.74% of metionine. Lupine grains contain 4.6% to 6% of fat, also vitamins, carotene and mineral nutrients [17].

Lupine seeds, which have a low alkaloid content (0.025–0.099%), can be used for forage and in food industry. Lupine plants accumulate alkaloids in seeds and roots. The alkaloid level is highest in the flowering period [5]. Alkaloid content in lupine green mass depends on the green mass harvesting time. In lupine hybrids N1675, N1700, N1702, N1703 alkaloid content in the green mass was 0.035–0.042%, in hybrids N1743 and N1740 reaching 0.048–0.089% (Fig. 1). Alkaloid content in all narrow-leaved hybrid lines tested was low. The lowest alkaloid content was found in hybrid lines N1675, N1700, N1702 and N1703 – 0.039 to 0.064% in the grain (Fig. 1). Grain taste was similar to that of beans. In the hybrid lines N1740, N1743, N1684 grain alkaloid content was higher (0.085–0.095%), but still they had a good taste.

The selected and tested narrow-leaved lupine hybrid lines showed a fast growth rate in all stages and thus a rather high resistance to anthracnose and high seed yield. The vegetation period of narrow-leaved hybrids is short (88–92 days) (Table) and they can be grown naturally in the whole country without applying special agroengineering measures. Narrow-leaved hybrid lines have a high value in genetic, breeding and farming aspects.

CONCLUSIONS

1. Low-alkaloid narrow-leaved lupine homozygotic lines N1675, N1740 and N1743 were bred using the intervariety backcross-breeding method and individual selection of their progeny. Lupine lines N1700, N1702, N1684, N1703 were bred using the individual selection method from alkaloid varieties.

2. Lupine lines N1675, N1700, N1702 and N1703 had a very low alkaloid content in the grain (0.039–0.064%). Lines N1740, N1743, N1684 had a higher alkaloid content (0.085–0.095%). In all lines, grain taste quality was good.

3. Backcross breeding and individual selection methods were both effective in breeding the new narrow-leaved lupine lines. Lupine breeding methods had no direct influence on progeny quality. Valuable features such as low alkaloid level, high resistance to diseases, yield good and others depended on lupine pedigree genesis.

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SIAURALAPIŲ PAŠARINIŲ LUBINŲ (*LUPINUS ANGUSTIFOLIUS* L.) SELEKCIJOS ASPEKTAI

Santrauka

Lubinų selekcija vykdoma Lietuvos žemdirbystės instituto Vokės filiale. 1995–2005 m. sukurtos 4 individinės atrankos metodu ir 3 tarpveislinio kryžminimo metodu siauralapių mažo alkaloidingumo lubinų selekcinės linijos. Trejų metų tyrimų duomenimis, mažu alkaloidų kiekiu sėklose (0,039–0,064%) ir žaliojoje masėje (0,030–0,042%) išsiskyrė šios selekcinės linijos: N1675, N1700, N1702, N1703. Visos lubinų selekcinės linijos pasižymi dideliu atsparumu grybinėms ligoms, sparčiu augimo tempu visuose augalų vystymosi tarpsniuose, gausiu sėklų derliumi (2,3–2,9 t ha^{-1}), trumpu vegetacijos periodu (88–92 dienos). Tai vertinga genetiniu, selekciniu, ūkiniu požiūriu medžiaga, kuri bus naudojama tolesniame selekciniame darbe, o vertingiausios linijos bus perduotos valstybiniams tyrimams.

Raktažodžiai: siauralapiai lubinai, lubinų selekcija, mažo alkaloidingumo veislės, atsparumas grybinėms ligoms

Зита Макницкене, Алмантас Ражукас

АСПЕКТЫ СЕЛЕКЦИИ УЗКОЛИСТНОГО КОРМОВОГО ЛЮПИНА (*LUPINUS ANGUSTIFOLIUS* L.)

Резюме

Селекция люпина проводится в Вокеском филиале Литовского института земледелия. В течение 1995–2005 гг. созданы 4 методом индивидуального отбора и 3 методом гибридизации малоалкалоидные селекционные линии узколистного люпина. По данным трех лет, низкое содержание алкалойдов в семенах (0,039–0,064%) и в зеленой массе (0,030–0,042%) проявили следующие селекционные линии: 1675, 1700, 1702, 1703. Все созданные селекционные линии прочвили высокую устойчивость к антракнозу, отличились быстрым ростом во всех фазах онтогенеза, высокой урожайностью семян (2,3–2,9 т/га) и коротким вегетационным периодом (88–92 дня). Все селекционные линии являются ценным селекционным материалом, который будет включен в дальнейший селекционный процесс. Перспективные селекционные линии будут переданы в государственные испытания.

Ключевые слова: узколистный люпин, селекция люпина, сорта с низкой алкалоидностью, устойчивость к грибным болезням