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Lupinus luteus pdf

Lupinus luteus. Lupinus luteus seeds.

NITROGEN FIXATION B.S. Kurlovich, I. A. Tikhonovich, L.T. Kartuzova, J. Heinänen, A.P. Kozhemykov, S.A. Tchetkova, B.M. Cheremisov and T.A Emeljanenko Biological nitrogen fixation is the problem of the highest priority in biological and agricultural sciences, and in attempts to develop sustainable agricultural production. Development of this trend is a response to ecological and energy problems which agriculture has recently encountered. Legumes in symbiosis with rhizobia are the best known nitrogen fixing system (Hardarson, 1993), and lupin is the best host plant in this system. The genus Lupinus L. totals some hundreds species (Cowling 1984; Gladstones 1974, 1988). By their origin they are divided into two clearly expressed groups (Kurlovich, 1988; Kurlovich et al., 1995): Mediterranean (Subgen. Lupinus), and American (Subgen.

Platycarpos (Wats). Kurl.). All these species are quite variable in most of their characters, including nitrogen fixing intensity. Besides, the intensity of biological nitrogen fixation is considerably predetermined by the efficiency of interaction between lupin plants and different nodule bacteria strains. The genus Lupinus is nodulated by the soil microorganism Bradyrhizobium sp. (Lupinus). Bradyrhizobia are encountered as microsymbionts in other leguminous crops (Argyrolobium, Lotus, Ornithopus, Acacia, Lupinus) of Mediterranean origin (Allen and Allen, 1981; Jordan, 1982; Legocki et al.,1997; Howieson et al. ,1988). Among the genus Bradyrhizobium, the lupin-nodulating form appears to be a taxonomic outline on the basis of fatty acid composition (Graham et al., 1995). Many aspects of the Lupinus-Bradyrhizobium sp. (Lupinus) symbiosis remain till now poorly investigated. In this connection, the purpose of our work was to study biological nitrogen-fixing ability of lupin genetic diversity, to identify the accessions of different lupin species with high nitrogen-fixing ability, effective strains of nodule bacteria and complementary symbioses (plants and nodule bacteria strains) able to effectively accumulate nitrogen from atmosphere, and on this basis to specify definite tasks and programs of lupin breeding for high intensity of symbiotic nitrogen fixation. The 1998-1998 experiments on biological nitrogen-fixing ability involved 1050 accessions belonging to four species of lupin from the Mediterranean area and 16 species from America. The lists of the main accessions and species under investigation are presented in the attached tables together with the results of the experiments. Accessions of diverse eco-geographic origin were assessed against three backgrounds: (1) without inoculating seed with Bradyrhizobium sp. (Lupinus) and mineral nitrogen application (control); (2) under seed inoculation with Bradyrhizobium lupini (biol. nitrogen); and (3) with mineral nitrogen application (mineral nitrogen). Seed were inoculated with commercial strains 363A, 367 and 375A of Bradyrhizobium sp. (Lupinus) produced in the All-Russian Research Institute of Agricultural Microbiology, also known as Rhizotorfin, which was widely used in Russia.

Against the mineral nitrogen background, the ammoniac saltpeter was applied in the amount of 60 kegs of the active substance per 1 hectare. The research was conducted in the fields of Pavlovsk Experiment Station of the N.I. Vavilov Institute of Plant Industry (VIR), 20 km away from St. Petersburg. The soil of the experimental plot was derno-podzolic, light loamy, cultivated, light acid (pH = 6.2 - 6.8). For this experiment such plots were selected where mineral fertilizers had not been applied for several years. Accessions were tested on all backgrounds with uniform density of plants in the field (30 plants per 1 m2). All observations were made according to the unified strategy developed by us and applicable to lupin (Kurlovich et al., 1990, 1995, 2000a; Курлович et al., 1997). The activity of nitrogenase was determined by the acetylene method in compliance with methodical recommendations of the Institute of Agricultural Microbiology (Алисова and Чундерова, 1982). Besides, 7 new strains of nodule bacteria developed in the All-Russian Research Institute for Agricultural Microbiology were tested in greenhouse experiments on two varieties (Nemchinovsky 846 from Russia, and Yandee from Australia) of narrow-leafed lupin (L. angustifolius L.). The strains of Bradyrhizobium sp. (Lupinus) were 1604, 1613 and 1607 selected from root nodules of L. angustifolius L., 1610 from L. luteus L., 1630 and 1635 from L. albus L., and 1625 from L.

polyphyllus Lindl. (Kozhemyakov et al., 1995). When studying nitrogen-fixing ability of Lupinus polyphyllus Lindl. in field experiments (1990-1995), new nodule bacteria strains 1610, 1614, 1625 and 1647 were tested in pure state and in combination with the lentechnin (LT) biostimulant and root diazotrops from the genus Arthrobacter known under the commercial name of Mizorin (MIZ). Results of plant growth and development observations. Our observations have witnessed that seed inoculation with Bradyrhizobium sp. (Lupinus) did not result in any increase in the number of germinated plants or a change in the duration of the vegetation period. However, discrepancies between reference plants (without Bradyrhizobium lupini inoculation or mineral nitrogen application) and inoculated plants were detected early. In the first case, the coloring of lower leaves was yellow, and that of upper ones was yellow-green. At the same time, with application of biological and mineral nitrogen the color of all leaves was bright-green. Such difference in coloring was preserved until the flowering phase. Afterwards, the reference acquired greener color, but its intensity was much lower than in the plants grown against the background inoculated with Bradyrhizobium lupini. The tables with the data and figures on this section are here. White lupin (Lupinus albus L.) In the reference plantings (without inoculation with Bradyrhizobium sp. (Lupinus) all examined accessions had plants without nodules. More frequently these plants were weaker and stunted. However, a majority of plants had from one up to seven large (with maximum diameter up to 20 mm), dense and uneven nodules, located single, on the side roots. Some samples were exceptions. On one of them (k-507 from Egypt), nodules, 23-28 mm in diameter, were observed on the main root and also on side roots. Large number of nodules (up to 45) were found on the plants of three samples (k-1930 from Sudan, and k-2589 and 2617 belonging to the West-European agrogeotype), but their size did not exceed 8 mm. The plantings which had experienced inoculation with Bradyrhizobium sp. (Lupinus) virtually differed from the reference in all morphological parameters.

The plants here exposed a greater number of smooth nodules sized from 2 to 7 mm and placed evenly over the whole root system. On different plants and samples there were different numbers of nodules, but in all cases they were quite numerous. Narrow-leafed lupin (L. angustifolius L.). Accessions of this species predominantly formed nodules also when inoculated with Bradyrhizobium sp. (Lupinus) .

Besides, they were larger, placed as couplings, and half-couplings . Yellow lupin (L. luteus L.). On the background inoculated with Bradyrhizobium sp. (Lupinus), accessions k-2290 and k-2292 from Portugal were noticeable for a large number of nodules. Figure 37 shows the root systems with nodules of k-2292, a wild form of yellow lupin from Portugal (to the right) inoculated with Bradyrhizobium sp. (Lupinus), compared with the roots of commercial cv. Akademichesky 1 (to the left) which was used in our experiment with yellow lupin as control. In the plantings without seed inoculation with Bradyrhizobium sp. (Lupinus) but with application of mineral nitrogen, samples k-2290 and k-2292 were the ones most severely depressed, and nodules did not appear at all. In cv. Augy from Lithuania, nodules were densely located on all main radical root. Fig. 37. Root systems with nodules of the wild form of yellow lupin k-2292 from Portugal (right), compared with the roots of commercial variety Akademichesky 1 (left) under inoculation with Bradyrhizobium sp. (Lupinus). Lupinus pilosus Murr. In the control the uniform distribution on the root system of separate nodules of very large size (up to 15 mm) was characteristic. In the experimental version with inoculation, nodules were pinker and more frequently developed on the main root. Lupin species from America. In the majority of the investigated American species of lupin grown under different nitrogen-supply conditions and on the reference plot, nodules were formed on root systems. There were three exceptions (spp. L. affinis Agardh, L.barkeri Lindl., and L. succulentus Dougl.) whose root systems on the reference plot (without inoculation and mineral nitrogen) were poorly branched and without nodules. Depending on a lupin species and source of nitrogen, nodules were variably sized, shaped and colored. For example, on the plants of L. paniculatus Desr.

the half-couplings and couplings reached 20 mm. More often they presented themselves as elastic, almost smooth thickenings, sometimes raised. Plants of L. douglassii Agardh had marked comblike couplings.

The color of nodules was changeable depending on the species of lupin and version of experiment. Nodules were spread over all root system (L. mutabilis Sweet.), or mostly concentrated on the main root (L. pubescens Benth), or only on the side roots (all species on the sites where mineral nitrogen was applied). It was noticed that on the mineral nitrogen background the root systems were stronger developed. However, application of ammoniac saltpeter slowed down the development of nodules. They were less numerous and occurred chiefly on the side roots, looking more often as beads. One of the important parameters describing the process of biological nitrogen fixation is the activity of nitrogenase (Алисова and Чундерова, 1982; Kurlovich et al.1995; Van Kamen, 1995). The research has shown that this parameter varies in the process of growth and development of plants. In healthy plants the activity of nitrogenase can be rather high up to the phase of seed's green maturity (Tab. 24). However, in the plants affected by Fusarium wilt (L. angustifolius L.), the activity was abruptly reduced before this phase approached. Comparative analysis of this parameter in different experiment variants showed its dependence on a number of factors: versions of experiments, specific and varietal features, belonging to this or that geotype, ecotype or variety type, presence or absence of infection by spontaneous populations of nodule bacteria or commercial strains of Rhizotorfin. Coefficient of variation of the nitrogenase activity level (CV, %) changed depending on a species from 15.1 up to 255.8 %. (Kurlovich et al., 2000a). The highest nitrogenase activity was observed in L.luteus L. and L. mutabilis Sweet., and the lowest in L. succulentus Dougl. Practically all species of lupin manifested great variability of this parameter. For example, in the yellow lupin cv. Augy (k-2956) nitrogenase activity reached 142.70 Mkmol C2 H4h-1 plant-1, while in another cv. Cyt (k-2398) belonging to the same species it varied within the limits of 0.24-16.82 Mkmol C2 H4h-1 plant-1. High nitrogenase activity of cv. Augy enhanced the accumulation of dry matter in plants. Positive correlation between nitrogenase activity and dry substance accumulation, or sometimes also of protein, was observed in many lupin species.

Accumulation of green and dry matter and nitrogen in plants. The efficiency of symbiosis is most completely evaluated on the accumulation of matter and nitrogen in plants. The research has shown that applying preparations of nodule bacteria Bradyrhizobium sp. (Lupinus) in most cases leads to an increase of plant efficiency. For some samples, the increase of dry substance store when inoculated with Bradyrhizobium sp. (Lupinus) bacteria was even greater than when mineral nitrogen was applied. White lupin (L. albus L.). The accessions studied showed variable response to inoculation with Bradyrhizobium lupini. The maximum increase of green and dry matter (6.18 times) was registered in the sample from Greece (k-2864), and the minimum (1.17-1.21 times) in cv. Tambovsky 86 from Russia and sample k-682 from Yugoslavia. In cv. Druzhba from the Ukraine, on the contrary, the content of dry weight under application of Bradyrhizobium sp.

(Lupinus) bacteria was decreased.

Accessions ensuring a considerable yield increase (as compared with the reference) under inoculation of bacteria also included: cvs. Start (k-2644) from Russia, Olezka (k-2980) from the Ukraine, accessions k-2989 and k-3250 from Portugal, and El Harrach-1 (k-3110) from Algeria. Among the investigated samples, the greatest content of dry substance against all backgrounds was observed in k-507 from Egypt, k-1930 from Sudan and k-2986 from Portugal. Narrow-leafed lupin (L.angustifolius L.). Most of the accessions demonstrated increased plant productivity after application of Bradyrhizobium lupini. The best efficiency was observed in cvs.

Ladny and Nemchinovsky 846, Determinant-2 (k-3365) and Determinant-3 (k-3366) from Russia, k-3065 from Australia, Apva (k-2950), Vika 65 (k-2954), DG-94 (k-3351) and DG-95 (k-3352) from Belarus, wild forms k- 3076, k-3079 from Spain, k-3083 from Portugal and k-3093 from Morocco. For example, application of Bradyrhizobium lupini with the commercial Russian cultivar Nemchinovsky 846 increased the dry matter content from 17.8 to 20.5 g. Besides, this cultivar also showed an increase of nitrogen accumulation. In many cases the increase of plant productivity after inoculation of Bradyrhizobium lupini was more significant than after application of mineral nitrogen. However, inoculation failed to increase the productivity of samples and varieties from Palestine (k-288), Greece (k-2866), and some accessions from Australia (k-2632, k-3062). With test purposes, seven new strains of nodule bacteria developed in the Institute of Agricultural Microbiology were engaged in an additional laboratory experiment in vegetative vessels. The new strains were tested on two varieties.

As a result, the highest efficiency of symbiotic nitrogen fixation for the regionally adapted Russian cv. Nemchinovsky 846 was shown by new 1604, 1630 and 1607 strains of Bradyrhizobium lupini. With cv. Yandee from Australia, the highest efficiency was demonstrated by 1604 and 1630 strains. Their application ensured a 1.5-2.0-time increase in accumulation of dry matter in plants. Yellow lupin (L.

luteus L.). The greatest increase of dry substance on the background of nodule bacteria application (99%) was shown by wild forms k-2290 and k-2292 from Portugal, and cv. Cyt (K-2398) from Poland. Cv. Cyt also revealed an increase in nitrogen accumulation (Tab. 26). Figure 37 reproduce the size Root systems with nodules of the wild form of yellow lupin k-2292 from Portugal (right), compared with the roots of commercial variety Akademichesky 1 (left) under inoculation with Bradyrhizobium sp. (Lupinus). However, for cv. Augy from Lithuania and also for samples k-2289 and k-2291 from Portugal, application of 375A strain of bacteria did not lead to an increase in either dry matter or nitrogen. Nevertheless, in spite of it, cv. Augy ensured the greatest content of dry substance among all investigated samples not only with inoculation of Bradyrhizobium lupini but also and especially without it. Accession k-304 of Lupinus pilosus Murr. also showed no increase of green and dry matter against the background infested with the above-mentioned preparation. However, an essential increase in the content and accumulation of nitrogen was observed (Kurlovich et al. 2001a). Species of lupin from America. These species appeared insensitive to application of 367A and 375A strains of nodule bacteria. Maximum accumulation of dry substance and nitrogen was observed almost in all American species when mineral nitrogen had been applied. The exceptions were L. micranthus Dougl.

and L. hartwegii Lindl. which did not respond to inoculation of a commercial bacterial strain and application of mineral nitrogen. The ability to store the greatest amount of nitrogen on all three backgrounds was demonstrated by L. mutabilis Sweet. L. aridus Dougl., L. micranthus Dougl., L. ornatus Dougl. and L. albococcineus Hort. were also of interest as sources for breeding lines with increased nitrogen fixation ability. There were also experiments to study responses of fodder low-alkaloid multifoliate Washington lupin (L. polyphyllus Lindl.) to inoculation with strains of nodule bacteria in pure state and in combinations with lentechnin biostimulator and root diazotrops of the genus Arthrobacter. Best results were obtained after inoculating the said Washington lupin (cv. Truvor) with nodule bacteria strain 1625. Combining inoculation with seed treatment with lentechnin biostimulator was also found to be efficient. The root diazotrops from the genus Arthrobacter did not show great efficiency in this specific combination.

Discussion Our research has shown that evaluation of lupin accessions of diverse origin on different backgrounds with biological and mineral nitrogen was rather effective. As a result of such experiments, a regularity in variation of symbiotic nitrogen fixation parameters was found, the same being dependant on the origin of lupin accessions and methods of their development. In the process of this research, significant differentiation in the response of various lupin species and accessions to inoculation of Bradyrhizobium sp. (Lupinus) was revealed. A majority of the studied accessions showed rather high responsiveness to inoculation of commercial strains of Bradyrhizobium sp. (Lupinus) and ensured an increase of plant yield where these strains had been applied. Low or negative effect of Bradyrhizobium sp. (Lupinus) inoculation on some accessions (especially those from America) can be explained by the mismatch of the used bacterial strains with plant genotypes. Thus, it seems promising to create highly complementary symbiotic pairs (of a plant and microorganism) with the purpose of increasing responsiveness of plants to inoculation. This may be achieved by searching for new advantageous strains of nodule bacteria matchable with the given variety, and by identifying plant genotypes responsive to inoculation of Bradyrhizobium lupini. The necessity to implement such efforts is expressly backed up by the factual results of our investigations which registers a decrease of plant yield after inoculation by same inappropriate strain of Bradyrhizobium sp. (Lupinus). For example, after treatment of the seed of yellow lupin cv. Augy from Lithuania with 376A strain, the efficiency of plants was reduced by 13%. Sample of narrow-leafed lupin k-3062 from Australia, as well as the species from America also showed a reduced efficiency after treatment with 363A and 375A strains. Thus, it is necessary to take into account that without preliminary tests the use of bacterial preparations not only may be ineffective, but can also bring about negative results.

On the other hand, skilled breeding of lupin cultivars and identification of complementary microsymbionts may essentially increase plant yield parameters and soil fertility, and also save expensive mineral fertilizers. The main condition of successful lupin breeding for high nitrogen-fixing ability is the availability of genetically well-defined and diverse materials of both the plant and microorganism. By now, more attention has been paid by the researchers to the second component of symbiosis – nodule bacteria. However, further manipulations with microorganisms will become more and more difficult. In view of this, the research on increasing the efficiency of nitrogen fixation should be put forward on the foreground at the expense of breeding leguminous plants (Четкова and Тихонович, 1986; Kurlovich et al, 1997). Furthermore, these components of symbiosis are not coequal, because leguminous plants can exist and give yield without microorganisms, for example with the help of mineral fertilizers. However, there is no need to target genetic and breeding researches in both directions. The identified promising plant materials should undergo trials against the backgrounds inoculated with different strains of nodule bacteria, and their new strains need to be tested on a rather large set of lupin accessions having different eco-geographic origin. The final outcome of these efforts should be the development of effective complementary symbioses between a plant variety and bacterial strain, which would jointly ensure high symbiotic nitrogen-fixing ability. It is worth mentioning that lupin breeding had started much earlier than the phenomenon of symbiotic nitrogen fixation was discovered.

Moreover, in the regions where lupin had never occurred as a wild plant and was introduced from the outside, there were no lupin-related microsymbionts. This circumstance resulted in spontaneous selection of genotypes with low nitrogen-fixing ability. Thus, we presently possess lupin varieties with rather poor reaction to bacterial inoculation, but at the same time capable to ensure certain yield efficiency without inoculation.

With this in view, one of the most important problems now is to make effective selection of bacterial strains compatible with the existing commercial cultivars of lupin, and consequently to launch further breeding programs toward higher intensity of symbiotic nitrogen fixation. In the existing situation, natural soil populations of microorganisms very frequently do not contain highly active (and even specific) nodule bacteria in their structure. Such situation is most typical when introducing new lupin species into a new geographic region. Utilization of already available commercial strains of bacteria, and also identification and selection of new, more efficient ones, will provide for increasing the efficiency of the culture and for reinforcing the significance of Bradyrhizobium sp. (Lupinus) bacteria as biological nitrogen accumulators. As an example of successful solution of this problem, we have selected an effective strain of bacteria (1625) for the existing fodder cultivar of perennial multifoliate Washington lupin cv. Трувор. This cultivar belongs to the American species Lupinus polyphyllus Lindl. On the basis of the above-mentioned, we are able to make the conclusion that efficient cultivation of Washington lupin is possible only with a combined application of highly efficient preparations of nodule bacteria strains, especially in the regions where lupin has not been cultivated before.

For each new cultivar of lupin it is necessary to select efficient strains of nodule bacteria corresponding to the plant genotype concerned. For promotion of valuable genotypes of lupin, it is also necessary to take into account the activity of the nitrogenase complex which varies in different lupin forms from 0 up to 100 Mmol C2 H4 h-1 plant-1 and more. Even among the plants of one lupin sample there is variability in the given parameter (the coefficient of variation changes from 39.0 up to 94.17 %). It proves that each sample represents a complex population consisting of plants with different activity of nitrogen fixation. In this connection, it seems beneficial to select plants with high parameters of nitrogenase activity and use them directly in the breeding process. Such prospects are justified by the availability of the methods of nitrogenase activity definition without causing any harm to the growing plants (Четкова and Тихонович, 1986). Matching the data on nitrogenase complex activity with the rates of dry matter and protein accumulation in lupin has shown that the correlation between these parameters is traced frequently enough, though not always. For example, the yellow lupin cultivar Augy from Lithuania demonstrated very high activity of nitrogenase (93,10 Mkmol C2 H4h-1 plant-1), and cv. Cyt from Poland had the lowest activity (5,52 Mkmol C2 H4h-1 plant-1).

As a result, the yield of dry matter in cv. Augy on the background without nitrogen and without preparations of nodule bacteria amounted to 24.2 g/plant, while in cv. Cyt it was only 6 g/plant. When a preparation of nodule bacteria (strain 376A) had been applied, the figures were 20.7 and 10.2 g/plant respectively. This example not only confirms a positive correlation between the above-mentioned two parameters, but also serves as a rather clear illustration of the mismatch between the 367A strain of nodule bacteria and the yellow lupin cultivar Augy. Thus, only complex registration of all parameters describing the intensity of biological nitrogen-fixing ability of lupin makes it possible to evaluate the biodiversity of species, identify effective materials for future breeding, and also work out many theoretical assertions useful for introduction and classification of breeding sources. In our research we were guided by Vavilov´s differential systematic geographic method of crop studies based on his law of homologous series in hereditary variation and on the theory of the centers of origin (domestication) of cultivated plants (Vavilov 1920, 1935, 1987). This method provided a possibility to perform targeted search for problems of evolution. This enabled us not only to disclose the diversity of forms, but also reveal a series of regularities in their variation depending on the degree of cultivation, geographic environments and soil conditions. So, our research helped to establish that the samples with high nitrogen-fixing ability more frequently occurred among the materials from primary centers of formation and origin of this or that species of lupin (Kurlovich, 1988). With yellow lupin, rather valuable in this respect was accession k-2292 from Portugal. The territory of this country belongs to the revised center of formation of yellow lupin (Vavilov 1935, 1987; Kurlovich et al, 1995). High productivity of this accession after inoculating it with Bradyrhizobium sp. (Lupinus) is confirmed not only by our data but also by the evidence of other researchers (Черемисов, 1991). Among the accessions of white lupin from Greece (also the center of origin and formation of this species), worth mentioning is the sample k-2864, also with high nitrogen-fixing ability.

We explain this phenomenon, in particular, by admitting that plants and accompanying nodule bacteria in the process of their coevolution in the centers of formation and domestication became more and more adapted to each other, so their separate existence in new places of cultivation experiences difficulties. That is why inoculation with Bradyrhizobium sp. (Lupinus) bacteria in new places of cultivation gives such positive results. The above-indicated forms deserve special attention as the objects of genetic research, as the genes controlling nitrogen-fixing ability have jointly evolved with micro- and macrosymbionts. (Симаров and Тихонович, 1985). Along with introduction and genetic study of the materials available in nature, valuable forms may be obtained with the help of mutagenesis. The chains and methods of radioactive and chemical mutagenesis are widely known and were sufficiently described (Sidorova et al. 1995). The practice however shows that the best results can be achieved by optimum combination of mutagenesis and hybridization. For these purposes, hybridization process must involve mutants, wild forms and, to this or that degree, domesticated forms of lupin of various geographic origins. posted by BK @ 6:10 PM 1 Comments: Anonymous said... Hi! You have executed huge volume of researches! Their results allow to conduct lupin breeding on increase of a biological nitrogen fixation. It will promote development of ecological agriculture!Your colleague from Poland 29 June, 2006 Adhikari KN, Edwards OR, Wang S, Ridsdill-Smith TJ, Buirchell B (2012) The role of alkaloids in conferring aphid resistance in yellow lupin (Lupinus luteus L.). Crop Pasture Sci 63:444–451CrossRef CAS Google Scholar Baird N, Etter P, Atwood T, Currey M, Shiver A, Lewis Z, Selker E, Cresko W, Johnson E (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3:e3376CrossRef PubMed PubMed Central CAS Google Scholar Berger JD, Adhikari K, Wilkinson D, Buirchel B, Sweetingham M (2008) Ecogeography of the Old World lupins.

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