Research Article

www.ekinjournal.com

Ekin
Journal of Crop Breeding and Genetics

(2015) 1-2:65-69

Screening of new varieties of sainfoin with a high potential nitrogen fixation

Galina N. Churkina[1*] **Evgeniya P. Salachenok**[1] **Galiya K. Akhmetova**[1]

[1] A. I. Barayev, Scientific Production Centre for Grain Farming, Shortandy, Kazakhstan
[*] Corresponding author e-mail: galina_churkina@mail.ru

Citation:
Churkina GN, Salachenok EP, Akhmetova GK 2015. Screening of new varieties of sainfoin with a high potential nitrogen fixation. Ekin J Crop Breed and Gen 1-2:65-69

Received: 21.08.2014 ***Accepted: 11.01.2014*** ***Published Online: 30.07.2015*** ***Printed: 31.07.2015***

ABSTRACT

The most common legume in Northern Kazakhstan is sainfoin. Sainfoin has the unique ability to enter into symbiosis with rhizobia, forming nitrogen-fixing nodules which have the ability to absorb 125-480 kg/ha of nitrogen from the air annually. These high yields of cheap vegetable protein are achieved without the use of mineral fertilizers. However, in the arid steppes of northern Kazakhstan root system nodules are not formed due to the moisture deficit in the soil. The objective of the current research is to identify new and promising varieties and sainfoin lines with high nitrogen-fixing ability. To increase the nitrogen-fixing capacity of promising new sainfoin lines, plant seeds were inoculated before sowing with an experimental biopreparation based on nodule bacteria (nitragin) obtained in microbiology laboratories from the local strains of rhizobia to Sainfoin *Rhizobium simplex* (Rs.–5 is for the sainfoin). These studies showed that new lines seeds inoculated with rhizobia promotes more active nitrogen fixation compared to the plants not treated with nitragin. Using the chromatographic method of determination of the nitrogen-fixing ability in new Sainfoin lines K-185 and K-209 it was found that during the growing season fixed nitrogen balance in the atmosphere was respectively 491 and 458.7 mg/ha of the total nitrogen balance of 84 %. This shows that new Sainfoin lines have a more active ability to fix atmospheric nitrogen after inoculation rhizobia than the standard variety of sainfoin. Sainfoin lines K-209 and K-185 proved to be the best forms of sainfoin for nitrogen-fixing conditions in Northern Kazakhstan.

Keywords: sainfoin, *rhizobium simplex*, nitrogen fixation, nitrogen, nitragin

Introduction

The problem of nitrogen deficiency will continue to be one of the main problems of agriculture. Based on a thorough analysis of the history of agricultural development by Pryanishnikov, it was established that the nitrogen level of plants was the main condition determining the average height of the legumes. In the last 2-3 decades, interest in biological nitrogen fixation significantly increased. This is due not only to the determining role of this process in the nitrogen balance of the biosphere, but also the possibility of reductions in the use of mineral nitrogen while reducing energy costs for production, which is very important in the light of current trends in biological farming (Kozhemyakov and Chebotar 2005; Schott 2010; Trepachev 2009). Despite the considerable progress made in the research on this problem, the practical use of techniques enhancing the life of diazotrophes remains scanty, due to the underestimation of the practical significance of the process by production workers, because of insufficient knowledge of many physiological, biochemical and genetic features of the process of nitrogen fixation.

It is especially essential for Kazakhstan conditions since the climate peculiarities, a short vegetative period and short active growth cycle, which lead to more deep undesirable changes in soil properties while using resource saving techniques (Vorobev 1999).

Intensification of the process of symbiotic nitrogen fixation is an urgent priority in creating new and promising varieties of legumes. One of the promising ways to achieve this is to increase the portion of nitrogen symbiotrophic agrocenosis by expanding the range and area of cultivation of legumes and create conditions for the establishment and effective functioning of their symbiosis with appropriate species of nodule bacteria. During the growing season and due to symbiotic nitrogen fixation, legumes herbs are able to accumulate up to 125-480 kg/ha of nitrogen from the air and generate high yields of high-quality environmentally safe food and feed protein, without the use of expensive, energy-intensive and environmentally hazardous mineral nitrogen fertilizers (Zavalin 2005). About 50 % of fixed atmospheric nitrogen is left in the soil with stubble root residues of perennial legumes, which significantly increases the yield of subsequent crops (Bazilinskaya 1988). Taking the above mentioned into account, our task was to evaluate the microbiological studies of nitrogen-fixing ability of new and promising varieties and lines of perennial legumes after rhizobia inoculation.

Materials and methods

Research was done on different crop varieties and lines of perennial legumes (clover, sainfoin) inoculated with nitragin. Soil samples and plants were collected in triplicate in stooling, budding and flowering periods. An experimental nitragin obtained in microbiology laboratories from local strains of rhizobia for clover and sainfoin was used in these experiments. Seeds of plants promising new lines were inoculated in order to increase the nitrogen fixing capacity of sainfoin. An experimental biopreparation of nodule bacteria (nitragin) obtained in the Laboratory of Microbiology from the local strains of nodule bacteria, sainfoin *Rhizobium simplex* (Rs.-5 is. for sainfoin) was used. The amount of symbiotically fixed nitrogen was measured by the chromatographic method. The principle of the method is based on the ability of root nodule bacteria to restore not only the molecular nitrogen, but also a number of other compounds, in particular acetylene (C_2H_2) to ethylene (C_2H_4). It was found that the amount of ethylene formed per unit time is in proportion to the amount of fixed nitrogen of approximately 3:1. The amount of ethylene was calculated by micro gas chromatograph Agilent 3000 and, using the specified ratio, the amount of fixed nitrogen was determined. Multiplying this value by the weight of nodules per unit area, we find the absolute value of fixed nitrogen. Since this figure varies according to the phases of the growing season and time of day, the value of nitrogen fixation for the whole period of vegetation was carried out by multiple measurements in relation to each class. The nitrogen content in the plant mass was determined by the Kjeldahl method (GOST 13496.4-93) (using the unit UDK-142). Field studies were carried out in competitive strain testing. The objects of research are the well-known varieties of sainfoin, "Sandy improved" and promising new lines of sainfoin, K-185, K-209, which were inoculated with nitragin.

Results and discussion

Research done by A. I. Barayev SPCGF breeders of different sainfoin varieties and lines has shown that inoculating new lines seeds with rhizobia promote more active nitrogen fixation than non-treated plants. In order to study the influence of root nodule bacteria biomass on sainfoin nitrogen-fixing ability, the number of nodules formed on the roots of plants was counted. The maximum number of nodules occurred in the stooling phase, in the flowering stage lysis of nodules began due to lack of moisture in the soil. During the sainfoin growing season the maximum number of nodules on the roots of plants was observed on the K-185 line which was treated with nitragin and was up to 31 pcs. per plant (Table 1). The K-185 line uptake of atmospheric nitrogen was 192.8 mg or 83.4 % of total amount of nitrogen. Sandy Improved and K-209 sainfoin both formed more nodules after being inoculated with rhizobia compared to untreated plants. Using the method of determining the balance of nitrogen-fixing ability in Sainfoin new lines K-185 and K-209 fixed 491 and 458.7 mg/ha atmospheric nitrogen, respectively, which was 84 % of the total nitrogen balance, indicating the new lines have a more active ability to fix atmospheric nitrogen than the standard variety of sainfoin. In lines K-209 and K-185 nitragin significantly increased the nitrogen content in the plant mass, which also stimulated the fixation of atmospheric nitrogen.

Observations of the legume-*rhizobium* complex formation in different clover lines showed intense nodulation on the roots of plants during germination and branching. On average during the growing season their number varied from 1.8 to 15.8 nodules per plant. Maximum numbers of nodules were 12.4 and 15.8 pcs per plant from lines D-10 and D-12, respectively. Legumes which grow in a particular area which lacks the specific bacteria needed by the host plant, fail to be a nitrogen accumulator from the air and start to feed on nitrogen from the soil and fertilizers (Kozhemyakov 1988). In addition, nodule bacteria that remains in the soil without the host plant for a prolonged period, as well as in adverse environmental conditions like high soil acidity, drought or flooding, lack of mineral nutrients, energy sources, material, etc. show a reduction in their nitrogen-fixing activity (Buyankin 2005; Gamzikov 2006).

Using nitragin along with the active *Rhizobium* strains stimulates inactive and less active nodule bacteria to provide biological nitrogen to legumes. Less active and inactive strains of nodule bacteria constitute one third or more of nodule bacteria. In areas where this is a problem, the use of nitragin containing high tiers of active breeding strains of nodule bacteria is one of the main methods of increasing not only the yield of legumes, but also the level of accumulation of general and biologically fixed nitrogen in plants and soil.

The highest protein content and dry weight was observed in all lines of clover inoculated with rhizobia and the value ranged from 3.63 to 3.74 % protein and 3.9 to 5.2 % by dry weight of plants. Biological nitrogen fixation from the atmosphere can be the main instrument to solve the problem of producing sufficient vegetable protein. Use of additional atmospheric nitrogen in the biological cycle will produce additional protein. The protein production capacity of a crop capable of symbiotic nitrogen fixation under favourable conditions of symbiosis exceeds many folds the protein productivity of crop plants that don't have this property. Intensive nodulation on the clover roots contributed to the active fixation of atmospheric nitrogen, a high percentage of fixed nitrogen was observed in lines D-2 and D-12 at a rate of 80.2 % and 82 %, respectively.

The amount of fixed nitrogen in the air at the symbiosis period on all variants of the experiment demonstrates the great possibilities of symbiotic systems of studied cultures to provide plants with nitrogen without the use of mineral nitrogen fertilizers. Due to symbiotic air nitrogen fixation energy costs per unit of output is reduced, for example, 1 kg vegetable protein of smooth brome obtained by the use of nitrogen fertilizers is 65 mJ whereas 1 kg of sainfoin protein obtained with biological nitrogen - 21, yellow sweet clover is 14 mJ. Air nitrogen fixation by using nitrogen fertilizers is a very energy-intensive process. Technically, one ton of nitrogen fixation into mineral nitrogen fertilizers requires about 80 gJ of energy. Application of nitragin based on local strains of nodule bacteria provides an increase in fixed nitrogen up to 82% of atmospheric nitrogen in new varieties of sweet clover and sainfoin. Fixed nitrogen concentration in the soil contributes to the accumulation of nitrogen in plant mass. In promising Sainfoin lines K-209 and K-185, nodule bacteria significantly stimulate the processes of nitrogen fixation. Inoculation with nitragin on clover seeds helped increase the fixation of atmospheric nitrogen, which amounted to 174.7 mg (82%).

Studies suggest that in places of systematic cultivation of perennial legumes (host plants), the introduction of appropriate rhizobia populations in the soil will contribute to further fixation of atmospheric nitrogen. Biological nitrogen fixation of air can be the main instrument to solve the problem of vegetable protein production (Mishustin et al. 1980; Kozhemyakov et al. 1989).

Production of additional protein can be obtained by including atmospheric nitrogen into the biological cycle through symbiotic nitrogen fixation under favourable symbiosis conditions. Best of all, production of protein through appropriate bacteria, many times exceeds the protein crop produced using nitrogen fertilizers.

Table1. Effect of rhizobia inoculation of different sainfoin lines on the atmospheric nitrogen fixation, the average value for the vegetation period of 2009 - 2011.

Treatment	The amount of nodules	Dry weight, g	Content of N in seed oil of mass, %	The nitrogen was assimilated, mg		
				The total	Atmospheric	%
Sandy improved control	7.8	5.3	3.82	202.5	164.0	81.0
Sandy improved nitragin	10.6	5.5	3.85	211.7	173.2	81.8
К-185 control	3.4	5.1	3.85	196.3	157.8	80.4
К-185 nitragin	31.0	5.9	4.92	231.3	192.8	83.4
К-209 control	7.0	5.1	3.71	189.2	150.7	79.6
К-209 nitragin	10.2	5.9	5.81	224.8	186.3	82.9

Table 2. The atmospheric nitrogen fixation during clover stooling phase by sowing clover different lines of the second year of life, 2010.

Treatment	The amount of nodules	Dry weight, g	Content of N in seed oil of mass, %	The nitrogen was assimilated, mg		
				The total	Atmospheric	%
Д.1 control	1.8	3.1	3.61	111.9	73.4	65.6
Д.1 nitragin	4.8	4.1	3.75	153.7	115.2	74.9
Д.2 control	1.8	3.0	3.55	106.5	68.0	63.8
Д.2 nitragin	4.0	5.2	3.75	195.0	156.5	80.2
Д.5 control	2.2	2.4	3.64	87.4	48.9	55.9
Д.5 nitragin	3.6	3.9	3.68	143.5	105.0	73.2
Д.10 control	2.6	2.6	3.50	91.0	52.5	57.7
Д.10 nitragin	12.4	4.2	3.63	152.5	114.0	74.7
Д.11 control	0.4	2.4	3.44	82.6	44.1	53.4
Д.11 nitragin	8.4	4.1	3.72	152.5	114.0	74.7
Д.12 control	0.4	3.2	3.67	117.4	78.9	67.2
Д.12 nitragin	15.8	5.7	3.74	213.2	174.7	82

References

Bazilinskaya MV (1988). Associative nitrogen fixation of cereal crops. Russian ed. “Kolos”.

Buyankin NI. (2005). A biological farming and crop production of a promising direction. Mocow, Russia 3rd Ed.

Gamzikov GP. (2006). Nitrogen in the agriculture of Western Siberia. Russia.

Kozhemyakov AP. (1988). Sources of lupine nitrogen nutrition with regard to the dose and timing of nitrogen fertilizer application. Mocow, Russia. 2nd Ed.

Kozhemyakov AP and Chebotar VK (2005). Biological science for agriculture. In the book: Biology in agriculture (Methodology and practice of microorganisms in crop and forage production), Russia, pp: 18 -54.

Kozhemyakov AP, Dorosinsky, LM and Berestetskyi OA. (1989). The efficiency of biopreparation of fixing microorganisms in agriculture. Publishing house “Kolos”.

Mishustin EN, Vostrov IS and Petrov AN (1980). Methods of soil microbiology and biochemistry. Russian publishing house “Mir”.

Schott PR. (2010). Opportunities and prospects of energy and resource optimization with nitrogen and nutrition of crops. Sat energy and resources in agriculture in arid areas, Moscow, Russia.

Trepachev EP. (2009). Agrochemical aspects of biological nitrogen in modern agriculture, Russia.

Vorobev VA (1999). Symbiotic nitrogen fixation and temperature. 2nd Ed.

Zavalin AA (2005). Biologicals, fertilizers and cropping. Publishing house, Russia.