The Formation of Symbiotic Potential and Yields of Soybean Depending on Elements of Growing Technology

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Abstract: The study of the nitrogen-fixing activity of nodule bacteria of soybean plants and features of the formation of the number and mass of nodules depending on variety, sowing method and seeding rate influenced by water regime of the soil and different lighting intensity.

Methods: Field and laboratory experiments, evaluation and comparison. A series of laboratory experiments in sand culture have explored the influence of water logging and dehydration of the root system and light intensity on soybean nitrogenase activity and the release of CO_2 by nodules of plants. According to the results of field research it was found that the intensity of nodule formation was increased from the phase of the third trifoliate leaf prior to flowering. At a sowing rate of 500 seeds it made from 20,0 PCs. for the variety Ustya to 27,4 in the variety of Romance. The increase in seeding rate contributed to the increase in the number of nodules to 28,1 per piece (Romance), 27,5 pieces (Ustya) and 25,5 units (Vorskla). The mass of active nodules was within 75% of the total number. Seed rate more than method of sowing influenced the amount of soybean yield. Increase the seeding rate to 800 thousand/ha of viable seeds, especially of sowing on later dates, have not contributed to a significant increase in productivity. Therefore it is most expedient to sow soybean normal line (15 cm) or in wide (45 cm) methods with a seeding rate of 700 thousand/ha of viable seeds. In the line way of sowing increasing the seeding rate contributed to the increase in the number of nodules to 28,1 per piece (Romance), 27,5 pieces (Ustya) and 25,5 units (Vorskla). In wide way of sowing these figures were less than 4,5% to 10,6%. The mass of active nodules was within 75% of the total number. It is advisable to sow soybean normal line (15 cm) or in wide (45 cm) methods with a seeding rate of 700 thousand/ha of viable seeds.

Keywords: Soybean, Water deficiency, Light intensity, Nitrogen fixation, Symbiotic potential, Yield.

INTRODUCTION

As leguminous soybeans are capable for symbiosis with the nodule bacteria. Due to this in biological cycle is released a huge amount of atmospheric nitrogen. Biologically bound nitrogen can be up to 60-70 % of total nitrogen yield, in addition, a significant amount remains in the soil, which makes soy a valuable precursor for subsequent crops in the rotation. As a result of symbiosis between bacteria and soy rises not only grain yields, but also improves the quality of harvest – increases the content of protein, vitamins, etc. Among the factors of formation of world food resources biological nitrogen fixation is a global process that ensures the existence of life on Earth. The binding of soybean molecular nitrogen in the air occurs as a result of the symbiosis of plants with a specific group of nodule bacteria *Rhizobium japonicum* [1].

For leguminous crops conditions of soil nutrition of plants with nitrogen are of great importance in the formation of the crop. The need for soybeans in nutrients is determined by its biological characteristics. At the beginning of the growing season it develops very slowly, from seedlings to flowering uses a small amount of nutrients. The greatest need for soybeans in the nutrients is observed during the flowering-pouring of beans, absorbing at this time up to 65-70% of nitrogen, phosphorus and potassium [2].

Bean plants are very negatively perceived as a moisture deficit as excess moisture in the root zone. There are many data on the susceptibility of root nodules in literature, along with the process of symbiotic nitrogen fixation to water deficit [3]. The lack of moisture in the soil can cause both reversible and irreversible damage to the symbiotic nitrogen-fixing system, which leads to damage to the structure of nodules as a result of mechanical effect of desiccation [4]. The drought may stop the flow of the products of photosynthesis to the nodules under the condition of dehydration plants. Progressive consumption of nitrogen fixation activity in soybean nodules for drying the surface associated with a reduction in the rate of diffusion of oxygen into the nodules [5]. In the absence of water, plants that grow on symbiotic nitrogen are killed in the first place, compared with plants that receive mineral nitrogen as fertilizers [6].

Nitrogen-fixing nodules need sufficient oxygen. But in the field the oxygen contents in the rhizosphere can be reduced through absorption by roots, soil aerobic organisms and limited gas exchange [7]. Especially significant decrease of oxygen content in the soil when

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E-ISSN: 2311-858X/22

is waterlogged soil. This leads to a reduction of nitrogen fixation activity of nodules, which depends on many factors. It is – generic, specific and varietal differences of plants, the Rhizobium strain, the phases of plant development, duration of flooding, temperature of the environment [8].

In case of flooding of the root system of soybean the physiological processes in nodules, the activity of photosynthesis in the leaves and movement of assimilates from leaves to roots and nodules are delayed [9]. Anaerobic conditions directly affect the respiratory system of the cells of the nodules, reducing the formation of ATP required for the process of nitrogen fixation. With a significant lack of oxygen in the environment, the cells of the nodules switch to anaerobic metabolism, resulting in increased formation of ethanol and carbon dioxide [10, 11]. New roots are formed on such roots, the mass of which exceeds the mass of soybeans, which have grown under normal moisture [12]. In over wet soil in soybean nodules, the development of superficial placing, tightly folded elongated cells is observed, which does not delay the free movement of gases in the middle of the nodule and in the opposite direction [13].

Factors that increase photosynthesis (light, CO2 concentration, temperature) under conditions of nitrogen 'starvation' did not have a positive effect on the intensity of photosynthesis [14]. However, it has been proven on soybean plants that photosynthesis in conditions of nitrogen deficiency in the soil can be increased by inoculation with the active Rhizobium strain [15]. The positive effect of high-intensity illumination on the growth of nodules is associated not only with the increase in the intensity of photosynthesis, but also with an increase in the outflow of organic matter into the root system and nodules [16]. Only 26% of the carbon absorbed during photosynthesis remains in the aboveground part, 42% is transformed to the roots, and 32% goes directly to the tubers [17].

Analysis of the literature shows that the process of nitrogen fixation provides a sufficient amount of nitrogen for the normal growth and development of plants. Many researchers recommend the introduction of 'starting' doses of mineral nitrogen in soybean crops. The lack of nitrogen is especially evident in soybeans at the beginning of the growing season, as the nodules form very slowly and become active only after flowering plants [18]. During vegetative growth, soybean in the field captures only 10% of nitrogen (from total nitrogen fixation), 90% is absorbed in the reproductive period [19].

The growth and development of this culture can take place without the application of nitrogen fertilizers, as the symbiosis of plants with nitrogen-fixing bacteria ensures their normal nutrition and high yield. If legumes receive nitrogen from mineral fertilizers, they absorb it, like all other types of plants, but at the same time biological nitrogen fixation from the atmosphere is equivalent to reduction, under such a condition nitrogen application is a loss of fertilizers [20].

The aim of our research was to study the effect of soil water regime and illumination of different intensity on the nitrogen-fixing activity of soybean nodules bacteria, as well as peculiarities of formation of number and weight of nodules depending on the variety, sowing method and seeding rate. Laboratory experiments were carried out in the laboratory of the Institute of Crops and Grassland science in the University of Hohenheim (Germany, Stuttgart), thanks to the receiving grant of a DAAD research by the author (Shevnikov M.Ya.) and in the laboratory of the Department of Plant Growing Poltava State Agrarian Academy.

MATERIALS AND METHODS

To identify the reasons of the reduction of nitrogen-fixing activity in soybean nodules under different conditions of water deficit, a series of laboratory experiments in sandy culture in the conditions of water deficiency of 2 and 3 days without watering and subsequent watering has been conducted. Studied the effect of moistening of the root system on nitrogenase activity and allocation of CO_2 in nodules of soybean plants by flooding of plants from 1 to 4 days.

Three varieties of soybeans were studied in the field experiment, they are Vorskla, Romance, Ustya. They were sown in two ways: the usual row with 15 cm rows and wide rows, 45 cm. Soybean sowing rate in the experiments was 500, 600, 700 and 800 thousand similar parts per 1 ha, soybean sowing time was determined by establishing a constant soil temperature at the depth of seed yield within + 12 °C. This temperature regime of the soil was observed in calendar terms from April 26 to May 10 in different years of research. The total area of the plot is 25 m^2, the repetition of the experiment is four times. The effect of these agrotechnical measures on the total number of nodules formed, their activity and weight, as well as on the yield of soybean seeds was studied.

The soil of the experimental site was podzolic medium-textured loam chernozem with humus content of 3.7%, pH (saline) - 5.6. Meteorological conditions during the years of the experiments were varied and fully characterized the climate of the area.

RESULTS AND DISCUSSION

In order to identify the effect of individual environmental factors on soybean yield, it was found that the sum of effective temperatures in the conditions of the left-bank forest-steppe is quite sufficient for the cultivation of early-ripening and middle-ripening soybean varieties. A more significant factor is the unstable and uneven soil moisture during the growing season. Therefore, it is necessary to study the effect of soil water regime, namely excessive moisture and lack of water, on the nitrogen-fixing activity of soybean nodule bacteria.

To clarify the causes of the decrease in the nitrogen-fixing activity of soybean nodules under conditions of water deficiency, a series of laboratory experiments in sand culture were conducted (Table **1**).

It was found that in the first two days after the cessation of irrigation, the saturation of the sandy substrate decreased by 38%, but the water content of the nodules decreased by only 1%. The subsequent decrease in the water content (3 days after the cessation of irrigation) led to a sharp decrease in its content in the nodules. One day after watering was restored, the water saturation deficit of water-potatoes recovered to a control level of only 7.0%.

The effect of the drought is primarily reflected in the nodules rather than the leaves. The water content of the first fully expanded leaf 2 days after the cessation of irrigation was practically the same as on the control. During the first two days, no signs of drying of soybean leaves were observed due to the lack of moisture. On the 3rd day after watering was stopped, the water content of the leaves changed significantly, and the photosynthesis was completely suppressed. The effectiveness of lightening and darkening of different intensities on the nitrogen-fixing activity of soybean nodule bacteria was studied. In studies, the soybeans were grown at an intensity of 60; 120 W/m^2. The effect of light on the accumulation of total biomass, nodule mass and nitrogen fixation are more dependent on the age of the plants (Table **2**).

Table 1: Characteristics of Water Regime of the Soybean Soil in the Conditions of Water Deficit and Subsequent Watering

Variant	The moisture content of the sand, % of total capacity	Water content in nodules, %	The deficit of water saturation of nodules, %	Dry substance, %	
				In nodules	In leaves
Control	56,2	68,9	2,9	25,2	24,7
2 days without watering	12,8	69,9	22,0	30,7	27,8
3 days without watering	7,8	55,9	38,9	39,9	38,7
2 days without watering + 1 day with watering	28,8	68,5	7,0	27,7	26,2

Table 2: The Effect of Light Intensity of Soybean on Fixation of Atmospheric Nitrogen

Phase of plant development	The light intensity in W/m^2	Dry weight, g		The activity of nitrogen fixation 1 plants C_2H_4 nmol/min.
		Plants	Nodules	
Budding	60	1,21	0,02	6,0
	120	1,79	0,04	14,0
Blossoming	60	2,97	0,04	48,9
	120	3,39	0,14	101,0
The green ripening of beans	60	6,22	0,13	47,0
	120	7,63	0,30	46,0

The above data shows that under conditions of increasing light intensity, the growth of nodules are significantly increased with no change in the specific activity of nitrogen fixation, but the total nitrogen fixation on the plant is increased. According to the results of field studies it was established that the dynamics of formation of symbiotic apparatus in soybean varieties had a positive character of development, namely: the number of formation of common and active nodules increased from the phase of the third trigeminal leaf to flowering.

Subsequently, from flowering to seed formation, there was a decrease in the number of nodules. On the average, over the years we have found that the maximum number of nodules was in the flowering period (Table **3**). Analyzing the above indicators, we found that for row sowing the average number of nodules was higher. For the seeding rate of 500 thousand similar seeds, it ranged from 20.0 pcs. in sort Ustya to 27.4 in sort Romance. Increase in seeding rate helped to increase the number of nodules to 28.1 pcs. (Romantika), 27.5 (Ustya) and 25.5 pcs. (Vorskla). For row sowing, these values were less than this value by 4.5–10.6%, indicating better conditions for the microbiological activity of the soybean root system than row sowing.

In addition to counting the number of nodules on the roots of soybean plants, we also determined their weight. Depending on the factors of the experience, the accumulation of the mass of nodules had its own

Table 3: The Number Nodules in Soybean Plants Depending on Variety, Sowing Method and Seeding Rate (Average Over 2013-2015)

Sort	Method of sowing	The norm of seeding, thousand/ha	The total number of nodules	The number of active nodules
Romantika	Row method of sowing, 15 cm	500	27,4	20,0
		600	27,8	20,3
		700	27,8	21,0
		800	28,1	20,6
	Wide-row method of sowing, 45 cm	500	26,3	18,7
		600	26,7	19,6
		700	26,5	18,9
		800	26,8	19,6
Ustya	Row method of sowing, 15 cm	500	26,3	19,5
		600	26,4	20,0
		700	26,8	20,3
		800	27,5	21,2
	Wide-row method of sowing, 45 cm	500	24,4	18,1
		600	25,8	18,4
		700	24,8	18,1
		800	25,1	19,0
Vorskla	Row method of sowing, 15 cm	500	23,7	18,1
		600	25,1	18,5
		700	25,5	18,9
		800	24,5	20,1
	Wide-row method of sowing, 45 cm	500	24,1	17,1
		600	24,4	17,8
		700	25	15,6
		800	24,7	18,1

SSD_{05}, t/ha, for a factor: A − 0,09; B – 0,07; C – 0,11; ABC – 0,07.

characteristics in each of the variants of the experiment (Table **4**). A greater mass of nodules was observed in plants with a row spacing of 15 cm, which is explained by the greater number of them in these areas. According to the seeding rate of 500 thousand / ha, the mass of nodules from 10 plants ranged from 3.91 g (Vorskla) to 4.21 g (Romantika), at the same time in the areas of wide-sowing these indicators were less important - 3.79 and 4,08 g. At an increased seeding rate of up to 600 thousand / ha, the mass of nodules had values from 3.91 g (Vorskla) to 4.28 g (Romance).

Wide-row sowing contributed to a smaller mass of nodules from 10 plants - 3.83 and 4.14 g, respectively. This trend was characteristic of other increased seeding rates. Comparing the varieties according to this indicator, we came to the conclusion that the largest mass of nodules had plants of the variety Romantika, slightly less important were Ustya and Vorskla. The mass of active nodules was within 75% of their total number. The sowing rate influenced to the soybean yield value more than the sowing method (Table **5**).

Increasing the seeding rate to 800 thousand / ha of similar seeds, especially when sowing in late terms, did not contribute to a significant increase in yield. Therefore, it is most expedient to sow soybean in ordinary row (15 cm) or wide row (45 cm) ways with a seeding rate of 700 thousand / ha of similar seeds. Early ripening variety Romantika had the highest seed yield for row sowing with a sowing rate of 800 thousand /

Table 4: The Mass of Nodules of 10 Soybean Plants (g) Depending on Variety, Sowing Method and Seeding Rate (Average over 2013-2015)

Sort	Method of sowing	The norm of seeding, thousand/ha	The total number of nodules	The number of active nodules
Romantika	Row method of sowing, 15 cm	500	4,24	3,24
		600	4,28	3,23
		700	4,20	3,30
		800	4,28	3,29
	Wide-row method of sowing, 45 cm	500	4,08	3,10
		600	4,14	3,09
		700	4,13	3,11
		800	4,14	3,13
Ustya	Row method of sowing, 15 cm	500	4,07	3,20
		600	4,13	3,19
		700	4,14	3,23
		800	4,15	3,22
	Wide-row method of sowing, 45 cm	500	3,85	2,99
		600	3,89	3,04
		700	3,88	2,95
		800	3,91	3,01
Vorskla	Row method of sowing, 15 cm	500	3,91	3,00
		600	3,91	3,24
		700	3,88	3,27
		800	3,93	3,28
	Wide-row method of sowing, 45 cm	500	3,79	2,87
		600	3,83	2,90
		700	3,81	2,85
		800	3,83	2,86

SSD_{05}, t/ha, for a factor: A − 0,08; B – 0,08; C – 0,08; ABC – 0,06.

Table 5: Yield of Soybean Seeds Depending on the Variety, Seeding Rates, Term and Method of Sowing (the Average for 2013-2015 Years)

Sort	Method of sowing	The norm of seeding, thousand/ha	Yield, t / ha		
			First term of sowing	Second term of sowing	Third term of sowing
Romantika	Rowmethod 15 cm	500	1,95	2,46	2,24
		600	2,20	2,88	2,35
		700	2,33	2,91	2,33
		800	2,38	3,07	2,40
	Wide-rowmethod, 45 cm	500	1,68	2,38	2,05
		600	1,94	2,60	2,43
		700	2,48	2,70	2,95
		800	2,40	2,78	2,77
Ustya	Rowmethod, 15 cm	500	1,61	2,17	1,82
		600	2,16	2,29	1,94
		700	2,16	2,50	2,21
		800	2,34	2,72	2,31
	Wide-rowmethod, 45 cm	500	2,04	2,11	2,16
		600	2,30	2,25	2,28
		700	2,45	2,64	2,75
		800	2,53	2,81	2,38
Vorskla	Rowmethod, 15 cm	500	1,73	2,12	1,90
		600	1,81	2,31	2,28
		700	2,25	2,85	2,39
		800	2,16	2,75	2,47
	Wide-rowmethod, 45 cm	500	1,75	2,32	1,89
		600	2,07	2,48	2,21
		700	2,40	2,60	2,65
		800	2,54	2,77	2,45

SSD_{05}, t/ha, for a factor: A − 0,07; B – 0,08; C – 0,08; ABC – 0,05.

ha for the second sowing period - 3.07 t / ha (first sowing period - 2.38 t / ha, third term - 2.40 t / ha).

The highest seed yield in the fast-growing variety Ustya was for row sowing with a seeding rate of 800 thousand / ha for the second sowing period - 2.72 t / ha (the first sowing period - 2.34 t / ha, the third one - 2.31 t / ha). In the case of wide-row sowing (45 cm) and the given rate of sowing, the yield indicators were 2.81 t / ha (2.53 and 2.38 t / ha). The reduction of seed rate for row sowing from 700 to 500 thousand / ha led to a decrease in seed yield from 8.1 to 20.2%, for wide-row sowing - from 6.1 to 25.0%.

The fast-growing variety Vorskla was the most productive for row sowing with a seeding rate of 700 thousand / ha for the second sowing period - 2.85 t / ha (the first sowing period - 2.25 t / ha, the third one - 2.39 t / ha). For wide-row sowing (45 cm) and seeding rate of 800 thousand / ha, the yields were 2.77 t / ha (2.54 and 2.45 t / ha). The reduction of seed rate for row sowing from 700 to 500 thousand / ha led to a decrease in seed yield from 19.0 to 25.7%, for wide-row sowing - from 3.8 to 16.3%.

CONCLUSIONS

In the conditions of high intensity of lighting the growth of nodules are increased without change in the specific activity of nitrogen fixation, but the total nitrogen fixation to the plant increases. The number of formed nodules are increased from the third phase of

the third trifoliate leaf prior to flowering. For row sowing with a seeding rate of 500 thousand seeds, the number of nodules ranged from 20.0 pcs. (Ustya) to 27.4 (Romantika). Increasing in seeding rate helped to increase the number of nodules to 28.1 pcs. (Romantika), 27.5 pcs. (Ustya) and 25.5 pcs. (Vorskla). In the case of wide-row sowing, these figures were less by 4.5–10.6%. The weight of the nodules was also greater than that of ordinary sowing. The largest mass of nodules had plants of the variety Romantika, slightly less important - Ustya and Vorskla. The mass of active nodules was within 75% of their total number.

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Received on 30-08-2022 Accepted on 07-10-2022 Published on 16-11-2022

DOI: https://doi.org/10.12974/2311-858X.2022.10.05