Non-leguminous Plant Genotypes and Associative N_2 Fixation

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The N_2-fixation associations between bacteria and non-legumes have been studied mainly at the microbiological level, while the host-plant role in activity determination and control of the association remains essentially unstudied. The pioneer works in this field appeared little more than ten years ago /DÖBEREINER, 1977; FUJII et al., 1979; NEAL and LARSON, 1976/. To date there is little information available on this subject, so the situation is anything but satisfactory. Rhizobium-legume symbiosis, which seems to be the most closely related and well studied association analogue, shows the process of symbiosis formation to be genetically controlled by both partners with the host-plant playing a major role /SIMAROV and TIKHONOVICH, 1985/. In the last few years it has been proved that non-legumes maintain a bacterial population in the rhizosphere and rhizoplane by means of root exudates, but it is not clear whether this process is controlled genetically by the host--plant. A number of works demonstrate the genus, species and cultivar variability of cereals in associative N_2-fixation /RODYNYUK, 1935; HIROTA et al., 1978; LETHBRIDGE et al., 1982; LETHBRIDGE and DAVIDSON, 1983; RENNIE and LARSON, 1979/. Some attempts have been made to select plants for their ability to support associative N_2-fixation /N_2-fixation supportive trait-nis/ and to cross plants with different associative N_2-fixation activities /BOUTON et al., 1985; ELA et al., 1982/. The practical aspect of these works is also of great interest, since plants were shown to satisfy 1/3-2/3 of their N_2 requirements by associative N_2-fixation. All this proves the vital importance of nis-trait research. This paper examines the distribution of nis-ability in species, cultivar groups and lines of wheat /Triticum L./.

Methods

The associative N_2-fixation activity was studied in wheat samples /genus Triticum/ from the World Collection of the N. I. Vavilov All-Union Institute of Plant Industry /Leningrad/. Monosomic wheat lines of the cultivar Saratovskaya 46 were kindly given by Dr. V. A. KRUPNOV /Scientific--Industry Union "Elita Povolzh'ja"/. Seeds were placed on 1.2% agar in Petri dishes for germination. Germinating seeds were transferred to tubes measuring 150 mm, with a diameter of 15 mm, containing 5 ml of agar medium, as recom-

mended by THOMAS-BAUZON et al. /1982/. On the 3-6th day of germination, after the preliminary rejection of bacterially and fungally infected seedlings, the rest of them were inoculated with a soil suspension. Brown soil taken from irrigated fields at the Ershovs Experimental Station /Saratov region/, cultivated permanently for three years with wheat, was used for seedling inoculation. The inoculation dose was 0.5 ml of soil-water suspension /soil : water = 1 : 2/ per plant. Before inoculation the soil-water suspension was treated at 5000 rpm for 5 minutes with an MPW-302 homogenizer /Poland/ to ensure the desorption of microorganisms from the surface of the soil particles. Six to twenty-six plants were studied in each experiment. The cultivar Saratovskaya 29 was used as a standard. The plants were grown in a VKSh-I climate box /USSR/ with a daylength of 16 hours, a day temperature of +25-30 °C and a night temperature of +15-20 °C. The acetylene reduction method was applied to determine the N_2-fixation activity in two-week-old seedlings using an LHM-8MD gas chromatograph /USSR/. Acetylene was added at a rate of 5-10% from the gaseous phase for 24-72 hours. Statistical data processing was performed using an "Electronica D3-28" minicomputer /USSR/.

Results and discussion

The process of nitrogen absorption consists of two components: the passive and active absorption of mineral nitrogen from the soil and the absorption of biologically fixed nitrogen. The latter is induced by plants due to the lack of mineral nitrogen in the soil. It would seem that changes occurring in the course of wheat evolution necessitated the development of a trait whereby the plant could be supplied with biological nitrogen. A number of wheat cultivars with different ploidy levels: diploid /2n = 14/, tetraploid /2n = 28/, hexaploid /2n = 42/ and octoploid /2n = 56/, were investigated for the nis trait, since polyploidy appears to be one of the evolutionary trends. The experimental data are shown in Table 1. If the nis-ability of the diploid /the most ancient/ level of Triticum organization is taken as the basic unit, transfer to the tetraploid level causes an average increase in nis-ability of 37% /averaged over two groups of tetraploids with AB and AG genomes/. The associative N_2-fixation in cultivars with the hexaploid level of genome organization showed a 59% higher level of nis-ability than in cultivars with the diploid chromosome number. In both cases the difference was significant /$P < 0.01$/. The acetylene reduction activity in octoploid T. timonovum was 38% higher than in the control /with a significance of 99.88%/. Thus the data from Table 1 show an increase in nis-ability as the multiplicity of the chromosome content increases.

The nis-ability distribution in wheats with various ploidy levels is close to the normal distribution with the exception of the octaploid level, where the nis-ability distribution is close to the Maxwell distribution. A normal distribution of nis-ability is found in four of nine species /T. monococcum, T. dicoccum, T. dicoccoides, T. timopheevii/. The nis-trait distribution in T. militinae is similar to the Maxwell distribution and the nis-ability distribution in the other four species is close to the theoretical Parretto distribution /Table 2/. The distribution characteristic of the majority of the cultivars tested indicates the heterogeneity of the wheat population for the associative N_2-fixation trait, allowing selection for this trait.

It should be noted that the data in the table are given on a logarithmic scale, so the differences between cultivar groups with various ploidy levels will be more significant on a linear scale. The tendency for nis-ability to increase in the wheat polyploid series can be traced quite clearly from the

Table 1

Associative N_2-fixation in wheats with different ploidy levels /lg ng C_2H_4/g roots/24 hours/ taking the diploids as the control

Group of species	Number of cultivars /n_1/	Number or samples /n_2/	Genomes	Ploidy /n=7/	x^a	s^b	w^c	p^d	$(Q_F)^e$ /%/	$(P_{t_d})^f$ /%/
Diploids	6	90	A	2n	1.74	0.91	52.1	5.29		
Tetraploids	9	223	AB	4n	2.25	0.65	29.0	2.62	99.4	99.9
Tetraploids	2	22	AG	4n	2.54	0.67	26.4	5.63	94.2	99.9
Hexaploids	8	66	ABD	6n	2.77	0.78	28.3	3.49	89.7	100.0
Octoploids	1	8	AAGG	8n	2.41	0.49	20.6	7.30	95.2	99.8

[a]Arithmetic average; [b]Average quadratic deviation; [c]Variation coefficient; [d]Index of experimental accuracy; [e]Significance of differences between dispersions by Fisher's test; [f]Significance of differences between arithmetic averages by Student's test

Table 2

Associative N_2-fixation activity in different wheat species /lg ng C_2H_4/g roots/24 hours/ taking Triticum monococcum as the control

Genomes	Ploidy /n=7/	Wheat species	Number of samples /n_1/	Number of cultivars /n_2/	x^a	s^b	w^c	p^d	$(Q_F)^e$ /%/	$(P_{t_d})^f$ /%/
A	2n	T.monococcum	93	6	1.73	0.89	53.1	51.2		
AB	4n	T.durum	6	1	2.47	0.17	7.0	2.8	99.9	100.0
AB	4n	T.dicoccum	51	5	2.28	0.60	26.3	3.6	99.8	99.9
AB	4n	T.dicoccoides	64	4	2.12	0.62	29.3	3.6	99.8	99.7
AG	4n	T.militinae	7	1	2.78	0.52	18.7	7.0	ND	ND
AG	4n	T.timopheevii	15	2	2.43	0.72	29.7	7.6	80.6	99.6
ABD	6n	T.vavilovii	23	5	2.55	0.49	19.5	4.0	99.8	ND
ABD	6n	T.aestivum	31	5	3.09	0.91	29.5	5.3	59.5	100.0
AAGG	8n	T.timonovum	8	1	2.29	0.25	10.9	3.3	99.9	99.0

[a-f] See Table 1; ND = non-determined

data in the table /correlation coefficient of genome ploidy N_2-fixation is 0.9724/, but the question of which genome is responsible for this increase remains unclear.

To answer this question, wheat species with A, B, D and G genomes in different combinations were studied for associative N_2-fixation activity. The nis-ability of the wheat species T. monococcum, possessing seven chromosome pairs related to genome A, was taken as 100%. The appearance in the tetraploid wheats T. dicoccoides, T. timopheevii and T. dicoccum of chromosomes from genomes B and G resulted in a significant increase /29 and 46%, respectively/ in the ability of wheat to support associative N_2-fixation in soil bacteria /Table 2/. In wheat, the transfer to the hexaploid level of genome organization, with the introduction of chromosomes originating from Ac. tauschii Coss /genome D/, was accompanied by an increase in associative N_2-fixation /on 58%/. The duplication of genomes AG in octoploid T. timonovum did not result in an increase in N_2-fixation activity compared with T. timopheevii /genomes AG/. The data obtained suggested that the hexaploid level of wheat genome organization was optimum for the associative N_2-fixation trait.

Up to the present wheat has not been selected for increased biological N_2 utilization ability. Therefore, screening for associative N_2-fixation ability and the consolidation of this trait could perhaps be linked with that of other agronomically important traits, such as seed quality and productivity. Previous authors /BERLYAND-KOZHEVNIKOV and DOROFEEV, 1977/ proved that wild and cultivated wheats had almost equal seed productivity on rich agrophones, irrespective of the ploidy level. Various wheat species do not differ greatly as regards nis-ability /Table 2/. The lowest associative N_2-fixation activity was registered in T. monococcum, while the maximum activity was found in T. aestivum. The latter is widely cultivated suggesting that cultivars of this species should be sought which combine nis-ability with a number of other important traits. It should be noted that Triticum aestivum was represented partly by cultivars selected long ago /Rusak, Poltavka/ which possess the highest associative N_2-fixation activity. It may be that only these old cultivars are able to serve as donors of high associative N_2-fixation activity. At the same time, cultivars selected more recently may show a significant reduction in or even a complete loss of nis-ability against the background of nitrogen fertilization /RENNIE, 1981/. This means it will be necessary to study cultivars selected at various times in order to characterize nis-ability in species used in selection for a long time.

According to the data, wheat species possessing more complex genomes than T. monococcum have significantly higher associative N_2-fixation activity /with the exception of T. dicoccoides and T. timopheevii/. The trait variation coefficient /w/ characterizes the heterogeneity of the population. In the majority of wheat cultivars tested the variation coefficient exceeded 10%, indicating the significant cultivar heterogeneity of the nis-trait. The highest variation coefficients, were observed in cultivars of Triticum monococcum. Great variability in the nis-trait within the same cultivar, in legumes as well as in wheat, has been noted by many investigators /AVIVI and FELDMAN, 1982; RENNIE, 1981; PROVOROV et al., 1987; SADYKOV et al., 1986; SMETANIN et al., 1985, 1987/.

Attention is currently focussed on the existence of a certain number of plants /10 to 90% in different cultivars/ with "zero" nis-ability. This has been noted by other investigators /JAIN and RENNIE, 1986/ but not explained till now. Since the soil bacteria population used for inoculation in the present experiment was a standard one, the existence of "plant-bacteria" associations with "zero" N_2-fixation activity may reasonably be attributed to the host-plant genotype.

The nature of nis-ability distribution in monosomic lines of the wheat cultivar Saratovskaya 46 suggests that this trait may be controlled polygenically. On average, the greater disruptions of associative N_2-fixation were observed in lines 1A-7A /Table 3/. Proceeding from this, a conclusion may be drawn on the major role of genome A in affecting the associative

Table 3

Associative N_2-fixation activity /acetylene reduction - lg ng C_2H_4/g/roots/24 hours/ in monosomic lines of wheat cultivar Saratovskaya 46

Line	N_2-fixation activity	Line	N_2-fixation activity	Line	N_2-fixation activity
1A	2.99 ± 0.95	1B	ND	1D	3.79 ± 0.77
2A	3.08 ± 1.34	2B	3.75 ± 0.72	2D	3.09 ± 1.37
3A	2.71 ± 0.25	3B	3.86 ± 1.31	3D	3.82 ± 0.80
4A	3.26 ± 1.37	4B	3.51 ± 1.31	4D	3.61 ± 0.54
5A	3.83 ± 0.66	5B	4.08 ± 1.07	5D	3.65 ± 0.91
6A	4.09 ± 1.44	6B	3.85 ± 0.83	6D	2.32 ± 0.41
7A	3.61 ± 0.54	7B	3.51 ± 0.53	7D	4.06 ± 0.59

Cultivar Saratovskaya 46 was used as the control /4.47 ± 1.48/
ND = non-determined; significant differences with control /$P < 0.5$/

N_2-fixation activity of hexaploid wheats. On the basis of genome effects on nis-ability, the monosomic lines may be arranged in the following order: A ≥ D > B. The absolute value of nis-ability was the lowest in lines 6D and 1A.

Thus, an increase in wheat ploidy up to the hexaploid level is accompanied by a significant increase in the associative N_2-fixation supportive ability of the soil bacteria. The nis-trait in hexaploid wheats is inherited polygenically. Trait distribution within the same cultivar is close to normal. A considerable heterogeneity is observed in cultivar populations with respect to associative N_2-fixation. Differences in associative N_2-fixation activity between winter and spring cultivars have not been registered. The maximum level of associative N_2-fixation activity in the nine wheat species of the genus Triticum tested was recorded in T. aestivum.

References

AVIVI, Y. and FELDMAN, M., 1982. The response of wheat to bacteria of the genus Azospirillum. Isr. J. Bot. 31. 237-245.

BERLYAND-KOZHEVNIKOV, V. M. and DOROFEEV, V. F., 1977. Some features of cultural wheat species evolution. Agricultural Biology /In Russian/ 12. 860-868.

BOUTON, J. H., ALBRECHT, S. L. and ZUBERER, D., 1985. Screening and selection of pearl millet for root associated bacterial nitrogen fixation. Field Crops Res. 11. 131-140.

DÖBEREINER, J., 1977. Plant genotype effects on nitrogen fixation in grasses. In: Genetic Diversity in Plants. /Eds.: MUHAMED, A., AKSEL, R. and VON BORSTEL, R. C./ 325-334. Plenum, New York.

ELA, W. W., ANDERSON, M. A. and BRILL, W., 1982. Screening and selection of maize to enhance associative bacterial nitrogen fixation. Plant Physiol. 70. 1564-1567.
FUJII, T. et al., 1979. Nitrogen fixation in the rhizosphere of rice. Annu. Rept. Nat. Inst. Jap. 29. 101-103.
HIROTA, Y. et al., 1978. Nitrogen fixation in the rhizosphere of rice. Nature. 276. 416-417.
JAIN, D. K. and RENNIE, R. J., 1986. Use of spermosphere model for the screening of wheat cultivars and N_2-fixing bacteria for N_2-fixation. Can. J. Microbiol. 32. 285-288.
LETHBRIDGE, G. and DAVIDSON, M., 1983. Root-associated nitrogen-fixing bacteria and their role in the nitrogen nutrition of wheat estimated by ^{15}N isotope dilution. Soil Biol. Biochem. 15. 365-374.
LETHBRIDGE, G., DAVIDSON, M. S. and SPARLING, G., 1982. Critical evaluation of the acetylene reduction test for estimating the activity of nitrogen--fixing bacteria associated with the roots of wheat and barley. Soil Biol. Biochem. 14. 27-35.
NEAL, J. L. and LARSON, R. I., 1976. Acetylene reduction by bacteria isolated from the rhizosphere of wheat. Soil Biol. Biochem. 8. 151-155.
PROVOROV, N. A. et al., 1987. Variation of cultivated alfalfa species as regards the ability of symbiotic nitrogen fixation. Agricultural Biology. /In Russian/ 6. 29-32.
RENNIE, R. J., 1981. Potential use of induced mutations to improve symbioses of crop plants with N_2-fixing bacteria. In: Induced Mutations - a Tool in Plant Research. 293-321. International Atomic Energy Agency, Vienna.
RENNIE, R. J. and LARSON, R. I., 1979. Nitrogen fixation with disomic chromosome substitution lines of spring wheat. Can. J. Bot. 57. 2771--2775.
RODYNYUK, I. S., 1985. Wheat genotype effect on establishment of effective associations with N_2-fixing microorganisms. Bulletin of the All-Union Research Institute for Agricultural Microbiology /In Russian/ 42. 54-56.
SADYKOV, B. F. et al., 1986. Associative nitrogen fixing activity in rhizosphere of wheat. Agricultural Biology. /In Russian/ 12. 34-36.
SIMAROV, B. V. and TIKHONOVICH, N. A., 1985. Genetical bases of Rhizobium--legume symbiosis. In: Mineral and Biological Nitrogen in the USSR Agriculture. /Ed.: MISHUSTIN, E. N./ 165-174. Nauka. Moscow.
SMETANIN, N. I., RODYNYUK, I. S. and SHUMNY, V. K., 1985. Polymorphism of populations of legumes according to the level of nitrogen fixation. Proceedings of Siberian Division of the USSR Academy of Sciences Series Biological. /In Russian/ 3. 38-41.
SMETANIN, N. I. et al., 1987. Polymorphism for nitrogen fixing activity in wild pea species. Agricultural Biology. /In Russian/. 9. 40-43.
THOMAS-BAUZON, D. et al., 1982. The spermosphere model. 1. Its use in growing, counting and isolation of N_2-fixing bacteria from the rhizosphere of rice. Can. J. Microbiol. 28. 922-928.