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EFFICIENCY OF STRAIN COMBINATIONS OF DIFFERENT GENERA OF NITROGEN-FIXING BACTERIA ON SUNFLOWER GENOTYPES

Sarić M., Sarić Zora, Govedarica M.,
Faculty of Agriculture, Institute of Biology, 21000 Novi Sad, Yugoslavia.

INTRODUCTION

Although certain microorganisms arecapable of fixing free nitrogen nonsymbiotically, their use in crop production has been much limited.
Investigations aimed at establishing new species of active nitrogen-fixing bacteria and improving the activity of known microorganisms and their use in growing of certain plant species, provide the possibility for a more intensive utilization of atmospheric nitrogen.
In past ten years, attention has been devoted also to a nonsymbiotic nitrogen fixation (Neyra and dobereiner, 1977; Bouton et al., 1979; Van Berkum and Bohlool, 1980; Alberth et al., 1981). The results obtained concerning the future of overcoming the problem of a greater involvement of molecular nitrogen in plant nutrition are promising.
In order to continue the investigations into the problem of genetic aspects of mineral nutrition (Sarić, 1981; Sarić et al., 1987; 1987a) at the level of interaction between plant genotypes and microorganism strains, we included sunflower as an object into our broaden investigations.
The interaction between plant genotypes and microorganism strains is an interesting problem from both theoretical and practical aspects. Therefore, the emphasis of this study is upon the interaction between certain lines and hybrids of sunflower and individual strains of nitrogen-fixing bacteria and their combinations.

MATERIAL AND METHODS

Seed of five inbreds and two hybrids of sunflower (NS-H-26-RM, NS-H-43, RH-26, R-SNRF, L-22, and L-1) was sown in sterilized sand and inoculated with strains of nitrogen-fixing bacteria belonging to six different genera, as well as their combinations:

1. Azotobacter strain 20
2. Escherichia strain 32
3. Klebsiella strain 39
4. Derxia strain 4
5. Beijerinckia strain 4
6. Azospirillum strain 2
7. Combination of eminent Azotobacter strains (20,10,16,8,3,2,5)
8. Combination of eminent Escherichia strains (32, 7)
9. Combination of eminent Klebsiella strains (39, 54)
10. Combination of eminent Derxia strains (4, 8)
11. Combination of eminent Beijerinckia strains (4, 2)
12. Combination of eminent Azospirillum strains (4, 2)
13. Azotobacter strain 2
14. Azotobacter strain 5
15. Azotobacter strain 10
16. Azotobacter strain 16
17. Azotobacter strain 8
18. Combination 1-6
19. Combination 7-12
20. Control

The plants were grown in a greenhouse in sand cultures for 30 days. Noninoculated plants served as the control. Dry mass and concentration and content of nitrogen in shoot and root were determined. Plants were irrigated only with distilled water.

RESULTS AND DISCUSSION

P l a n t m o r p h o l o g y (Figs 1 and 2). Interaction between individual strains and their combinations and sunflower genotypes resulted in an increased growth of all variants of inoculation. A number of examples should be mentioned. The Klebsiella strain 39 affected a remarkable increase in growth of all inbreds and their hybrids. Posibly, this strain is capable of synthesizing certain phytohormones stimulating growth of the plant species. The remaining strains of nitrogen-fixating bacteria showed a more specific influence depending upon plant genotypes. For example, the Azospirillum strain 2 stimualted more strongly the growith of NS-H-26-RM, whereas only a negligible effect was observed in L-22. Similar result was obtained with the Beijerinckia strain 4. The poorest effect upon growth of all sunflower forms under consideration was obtained when the Derxia strain 4 was applied. The strain combinations stimulated to various degree the growth of sunflower plants.

D r y m a s s (Tab. 1). Evident was a positive effect of nitrogen-fixing bacteria upon dry mass of plants. On the average, ten variants of strains of nitrogen-fixing bacteria produced a highly significant positive effect (individual Azotobacter strains 2, 10, 16, and 20, the Escherichia strain 32, Klebsiella strain 39, combination of the eminent Klebsiella strains, Derxia and Beijerinckia strains, as well as a combination of eminent individual strains of all genera). A significant effect was obtained with the combination of the most distinguished strains of the Azotobacter and Azospirillum genera.

Sunflower forms differently responded to nitrogen-fixing bacteria. For example, the greatest number of positive responses was observed in L-22 (seven), then in NS-H-26-RM (six), NS-H-43 /five), RH-26 and L-1 /four), and finally in R-SNRF (only one). No negative effect was recorded in RH-26 and R-SNRF while in other forms only with the combination of the Escherichia strains. These results apparently show the genetic specificity of inbreds and hybrids of sunflower related to certain nitrogen-fixing bacteria. Such a specificity is well documented by the following examples: the Klebsiella strain 39 affected a highly significant increase in L-22 and NS-H-43, a significant in L-1 and NS-H-26-RM, whereas no positive effect in RH-26 and R-SNRF was recorded. Similarly, the Azotobacter strain 20 affected a significant increase in RH-26 and R-SNRF, a highly significant in NS-H-26-RM, whereas no positive effect was recorded in other sunflower forms.

N i t r o g e n c o n t e n t (Tab. 2). On the average, a highly significant positive effect was obtained also with ten variants of inoculation (individual Azotobacter strains 20 and 16, Escherichia strain 32, Klebsiella strain 39, as well as a combination of the strains of Escherichia, Klebsiella, Derxia, and Beijerinckia, and a combination of all individual strains of the genera studied). A significant effect was observed only with the Beijerinckia strain 4.

The greatest number of positive cases was recorded in the NS-H-26-RM form (eight), then in L-22 /seven), NS-H-43 (five), and in L-1 and R-SNRF (two each). In the form RH-26 no significantly positive or negative response was obtained. Only in the form NS-H-26-RM a negative effect was recorded.

Genetic specificity of the sunflower forms relative to the strains of nitrogen-fixing bacteria used was evident not only with individual strains but, also, with the combinations applied. For example, the Azotobacter strain 20 affected positively the form NS-H-26-RM and R-SNRF, strain 2 NS-H-26-RM while strain 16 the form L-1 and L-22. Combinations of Escherichia and Klebsiella strains affected an increase in nitrogen content in most sunflower forms when compared with the individual strains of nitrogen-fixing bacteria. The Escherichia strain 32 affected in increase in NS-H-26-RM and NS-H-43, Klebsiella strain 39 in NS-H-26-RM and NS-H-43 while the Beijerinckia strain 4 only in NS-H-26-RM.

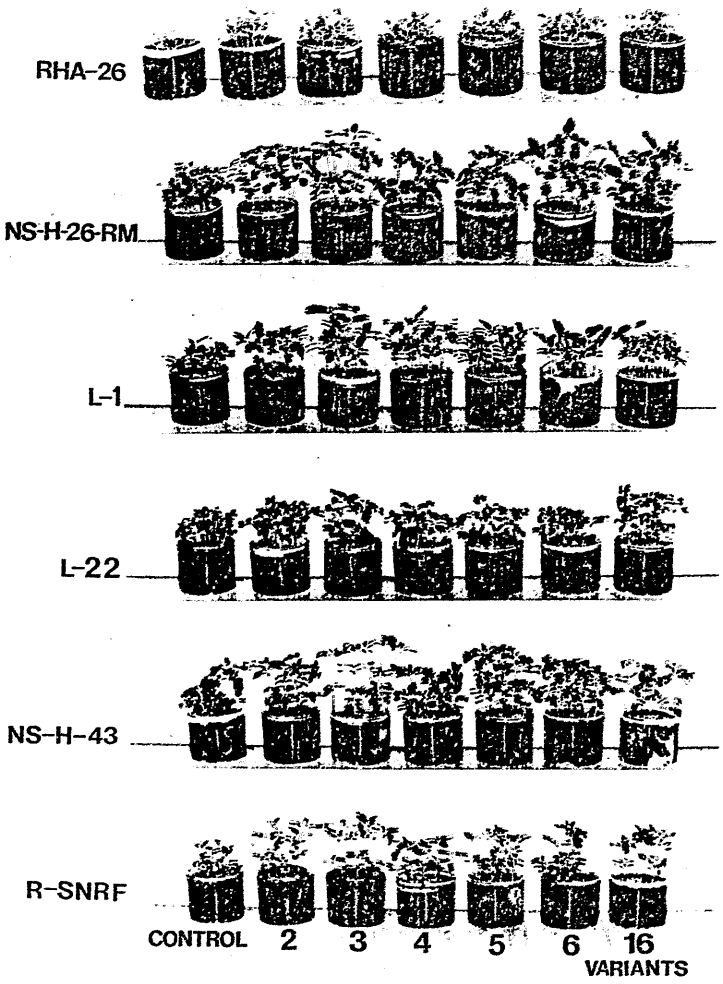
The results obtained show that also in sunflower, the nitrogen-fixing bacteria may cause an increase in both nitrogen content and dry mass. Such an increase

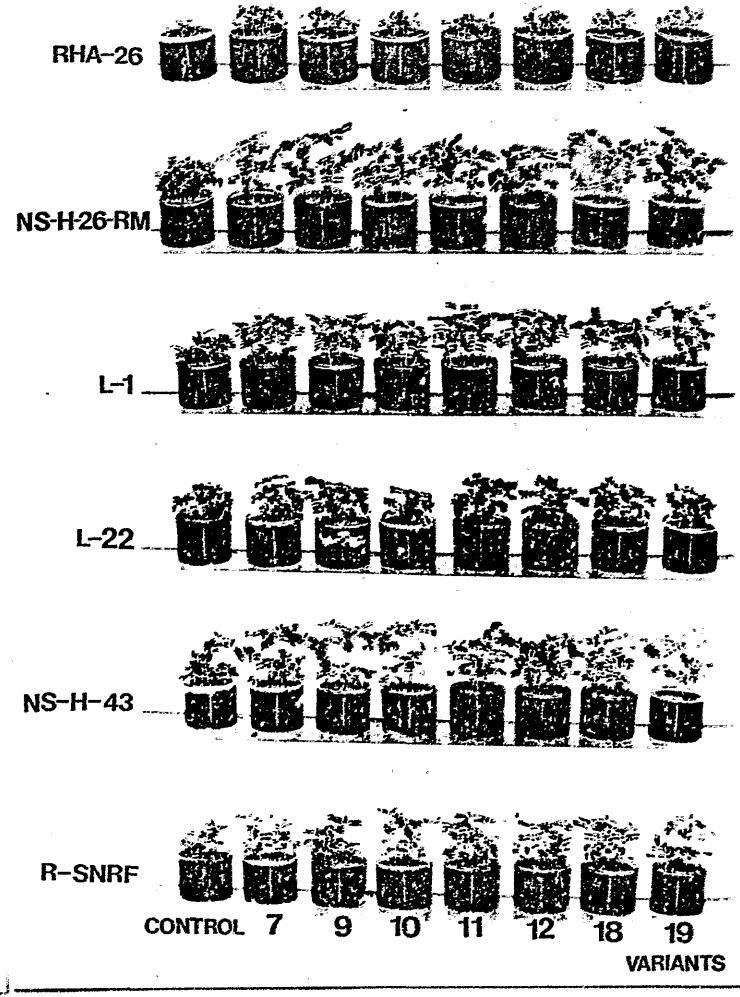
TABLE 1. DRY MASS OF INBREDS AND HYBRIDS OF SUNFLOWER (mg/plant)

COMBINATIONS \ INBREDS	RHA-26	NS-H-26-RM	L-1	L-22	NS-H-43	R-SNRF	AVERAGE	LSD 5%	LSD 10%
CONTROL	66	101	93	61	98	25	74		
1	62	121	102	67	109	43	87		
2	82	116	98	78	110	30	86		
3	76	120	111	84	121	32	91		
4	72	95	89	74	99	27	76		
5	69	108	101	69	99	24	78		
6	68	101	90	72	110	31	78		
7	67	110	98	72	113	32	82		
8	66	70	58	42	71	21	51		
9	72	120	96	78	119	32	86		
10	82	135	95	75	102	27	86		
11	85	113	105	80	108	31	87		
12	74	107	110	77	39	29	82		
13	80	125	94	70	105	32	84		
14	69	104	103	63	97	29	77		
15	76	102	109	64	121	37	84		
16	75	106	121	74	116	34	88		
17	77	103	96	66	84	30	76		
18	74	114	100	78	106	40	85		
19	76	110	95	77	92	34	81		
x	74	104	98	71	104	31	81	3	4
LSD 5%				14			5		
LSD 1%				19			7		

TABLE 2. CONTENT OF NITROGEN IN INBREDS AND HYBRIDS OF SUNFLOWER (mg N/plant)

CONTROL	1.172	1.766	1.723	1.156	1.848	0.412	1.346		
1	1.302	1.999	1.686	1.199	2.037	0.602	1.471		
2	1.276	1.969	1.628	1.278	2.256	0.431	1.473		
3	1.274	2.103	1.881	1.413	1.912	0.477	1.512		
4	1.218	1.801	1.679	1.280	1.748	0.393	1.353		
5	1.277	1.973	1.904	1.304	1.757	0.351	1.428		
6	1.232	1.859	1.547	1.195	1.947	0.408	1.365		
7	1.129	1.759	1.612	1.131	2.165	0.410	1.368		
8	1.307	2.049	1.698	1.437	2.295	0.419	1.534		
9	1.247	2.164	1.601	1.478	2.241	0.482	1.535		
10	1.208	2.139	1.774	1.261	1.935	0.396	1.454		
11	1.329	1.882	1.787	1.404	1.976	0.446	1.470		
12	1.187	1.892	1.870	1.253	1.814	0.397	1.402		
13	1.206	1.965	1.599	1.229	1.871	0.441	1.385		
14	1.264	1.564	1.954	1.176	1.891	0.435	1.381		
15	1.193	1.569	1.821	1.144	2.036	0.444	1.368		
16	1.270	1.746	2.047	1.519	1.731	0.462	1.462		
17	1.250	1.654	1.599	1.384	1.703	0.462	1.342		
18	1.264	1.888	1.888	1.321	2.102	0.620	1.514		
19	1.201	1.925	1.824	1.508	1.984	0.486	1.488		
x	1.240	1.884	1.756	1.304	1.963	0.449	1.432	0.045	0.059
LSD 5%				0.201			0.082		
LSD 1%				0.265			0.108		





Figs 1 and 2. Morphological view of inbreds and hybrids of sunflower plants as related to strain combination of free nitrogen-fixing bacteria.

in both nitrogen content and dry mass. Such an increase in nitrogen content is the result of atmospheric nitrogen fixation since mineral nitrogen was withheld from the nutrien solution. Variability of atmospheric nitrogen fixation as dependent upon plant genotypes and strains of nitrogen-fixating bacteria was found in maize (Sarić et al., 1982: 1987) and in wheat and sugar beet (Sarić et al., 1988). The obtained effect however, considerably depended upon relationships between a certain genotype and bacteria strain.

It is known that in the symbiotic nitrogen fixation the relationship between host plant and Rhizobium ssp. depends upon occurrence of a certain gene. The question to be answered is what is the cause of various efficiency of plant genotypes and microorganism strains when free nitrogen-fixing bacteria are applied? It may be assumed that the relationship between a plant genotype and a strain of microorganism depends upon the following characteristics of partners in the system:

- Quantitative and qualitative composition of root exudates of a certain genotype (amount of exuded organic carbon - sugars, organic acids, amino acids, etc.).
- Specificity of metabolism of a microorganism strain as dependent on the synthesis of certain compounds /acids - lactic, propionic, butyric, pyruvic, succinic, and malic; alcohols - ethanol, methanol, butanol, propanol, and isopropanol; compounds of indole and phenol types).
- Ability of a microorganism strain and a plant genotype to synthesize phytohormones /gibberellin, auxin, cytokinine, abscisic acid, etc.).
- Ability of a microorganism strain to synthesize inhibitors (antibiotics, phenols, various toxins, etc.).
- Influence of the system (plant genotype-microorganism strain) upon an increased strain competitive ability relative to other microorganisms from the rhizosphere and epiphytic seed microflora.
- Ability of a microorganism strain from the system to colonize root surface and penetrate into plant tissue.
- Influence of the system /genotype-strain) upon changes of the ecological factors of rhizosphere /pH, rH_2, pO_2, CO_2, etc.).
- Specificity of a plant genotype related to nitrogen uptake and transport, i.e. different concentration and content of nitrogen in root and shoot tissue.

REFERENCES

Albrecht S.L., Okon J., Lonnquest J., Burris R.H., 1981. Nitrogen fixation by Corn-Azospirillum assotions in a temperate climate. Crop Sci. 21: 301-306.

Bouton J.H., Smith H.L., Schrank S.C., Burton G.W., Tyler M.E., Littele R.C., Gallaher R.N., and Quesenbery K.H., 1979. Response of pearl millet inbreds and hybrids to inoculation with Azospirillum brasilense. Crop Sci. 19: 12-16.

Klucas R.V. and Pefersen W., 1980. Nitrogen fixation associated with roots of sorghum and wheat. Nitrogen fixatin. University Park Press, Baltimore.V.II:243-255.

Neyra C.A. and Dobreiner J., 1978. Nitrogen fixation in grasses. Advances in Agronomy, 29:1-38.

Reynders L. and Vlassak K., 1982. Use of Azospirillum brasiliense of biofertilizer in intensive wheat cropping. Plant and Soil. 66: 217-273.

Sarić M.R., 1981. Genetic specificity in relation to plant mineral nutrition. Jour.Plant Nutrition. 3: 743-766.

Sarić M., Sarić Z., Milić V., Krstić B., Cantar M., 1982. Effect of nitrogen fixation depending on the strain of Azotobacter, genetic character of corn, and nitrogen content in nutritive solution. II Intern.Symp. on N-fixation with non Legumes, 5-10.IX.Banft.Canada.

Sarić M.R., Sarić Z., and Govedarica M., 1987. Specific relations between some strains of diazotrophs and corn hybrids. Plant and Soil. 99: 147-162.

Sarić Z., Sarić M., Govedarica M., and Stanković Ž., 1987a. The effect of maize genotype and nitrogen level on the efficiency of different Azotobacter strains. J.of Plant Nutrition, 10 (9-16), 1779-1786.

Sarić M.R., Sarić Z., and Govedarica M., 1988. Variability of fixation of molecular nitrogen as dependent on plant genotype and diazotrophs strains. III Inter. Symp. on genetic aspects of plant mineral nutrition. Braunschweig, 19-22.VI, 1988.

Van Berkum and Bohlool B.B., 1980. Evoluation of nitrogen fixation by bacteria in association with roots of tropical grasses. Microbiol.Rev. 44: 491-517.