

Investigations into rhizosphere microflora. VII.

Effect of seed bacterization on root region microflora

V. B. SRIVASTAVA* and R. R. MISHRA

Department of Botany, University of Gorakhpur, India

(Received: February 4, 1973)

Abstract

The effects of bacterization of *Triticum aestivum* L. and *Hordeum vulgare* L. with *Azotobacter chroococcum* was studied.

The changes in the bacterial population during vegetative period are described.

Also the positive effect of bacterization of seeds on growth and health of the plants was observed.

INTRODUCTION

Although it has been shown that root developing from nonsterile seed in sterilized sand or liquid culture develops an abundant rhizosphere population (Rovira and Bowen 1966), it is not certain whether these could develop to any significant level in competition with responsive soil bacteria.

A beneficial effect of bacterization of seeds with *Azotobacter* or *Phosphobacterium* on crop yield has been reported by some workers (Garretson 1948; Sperber 1957; Cooper 1959; Brown, Burlingham and Jackson 1964), but the ability of seed inoculants to move from the germinating seed to the root has been put in doubt. In the present investigation an attempt was made to study the effect of seed bacterization of *Triticum aestivum* L. and *Hordeum vulgare* L., with *Azotobacter chroococcum* on the rhizosphere and rhizoplane microflora.

MATERIALS AND METHODS

Healthy seeds of *T. aestivum* and *H. vulgare* were surface-sterilized by treating them with 0.1% aqueous mercuric chloride solution for 2

* Present address: Pilot Plant Laboratories, IDPL, Virbhadra, Rishikesh, Dehradun, U.P., India.

minutes. These seeds were thoroughly washed with sterilized distilled water and soaked in a bacterial suspension which carried 80 million cells of *Azotobacter chroococcum* per ml. Seeds were treated with the bacterial suspension for 18, 24 and 30 hours and then dried in the shade. The inoculated (bacteriated) seeds were sown in earthen pots containing unsterilized garden soil. Twenty pots, with five plants in each, were used in each combination. Plants developed from untreated sterilized seeds served as control. The rhizosphere soil samples were collected at intervals of 15, 30, 70 and 100 days. For both the plants the rhizosphere and rhizoplane microbial populations were estimated by the methods suggested earlier (Mishra and Srivastava 1969; 1970). Martin's modified, meat-extract agar, and Warcup's (pH 4) methods were used for isolation of rhizospheric fungi and bacteria, and rhizoplane fungi, respectively. The data collected were subjected to statistical analysis of variance.

RESULTS

The findings are compiled in tables 1—2 and figures 1 and 2.

The dominant fungal species in the rhizosphere and rhizoplane regions of the treated and untreated combinations of wheat and barley varied at different sampling periods. In wheat, the dominants in the rhizosphere were in general: *Syncephalastrum racemosum*, *Aspergillus nidulans*, *A. flavus*, *Cladosporium herbarum*, and *Alternaria tenuis* in the 18 hrs. bacteriated combination; *Rhizopus nigricans*, *Aspergillus nidulans*, *A. flavus*, *Cladosporium herbarum* and *Fusarium nivale* in the 24 hrs. treated combination; *Aspergillus flavipes*, *A. flavus*, *Paecilomyces fusisporus* and *Cladosporium herbarum* in the 30 hrs. treated combina-

Table 1

Mycoflora in the rhizosphere and rhizoplane regions at different stages of plants' growth

	Plants age	<i>T. aestivum</i>				<i>H. vulgare</i>			
		Bacterization period in hours							
		18	24	30	Control	18	24	30	Control
Total number of species	15 days	7(6)	8(5)	13(3)	20(7)	19(8)	17(5)	19(5)	16(7)
	30 days	11(5)	9(3)	11(3)	14(5)	18(9)	14(8)	14(9)	21(7)
	70 days	15(4)	16(3)	8(3)	25(7)	19(6)	17(5)	12(4)	26(4)
	100 days	15(5)	12(4)	15(7)	19(5)	15(3)	13(3)	14(5)	18(6)
Mean number of colonies per plate	15 days	(3)	(2)	(2)	(3)	(3)	(3)	(3)	(4)
	30 days	(3)	(3)	(2)	(3)	(4)	(3)	(4)	(4)
	70 days	(4)	(3)	(3)	(4)	(4)	(3)	(3)	(3)
	100 days	(5)	(5)	(4)	(5)	(5)	(4)	(5)	(5)

Digits in parenthesis stand for rhizoplane region.

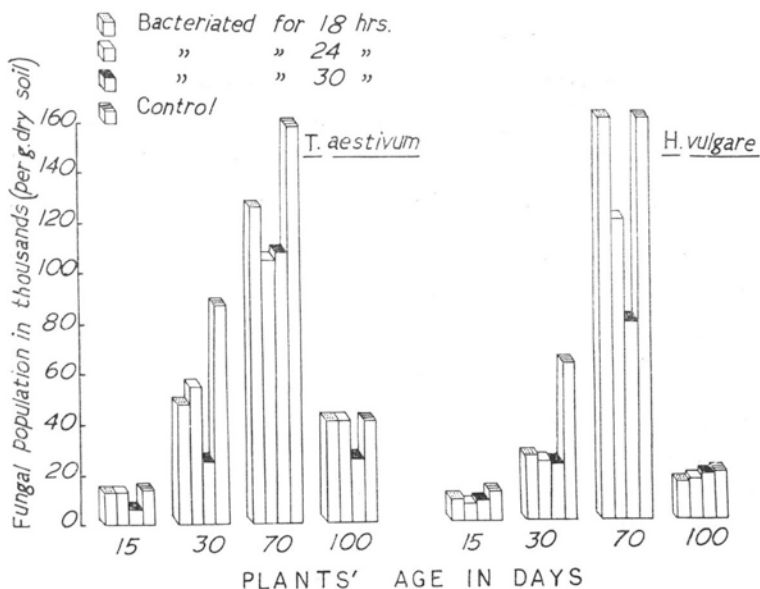


Fig. 1. Distribution of fungi in the rhizospheres of *Triticum aestivum* L. and *Hordeum vulgare* L. at young, flowering, and post-flowering stages

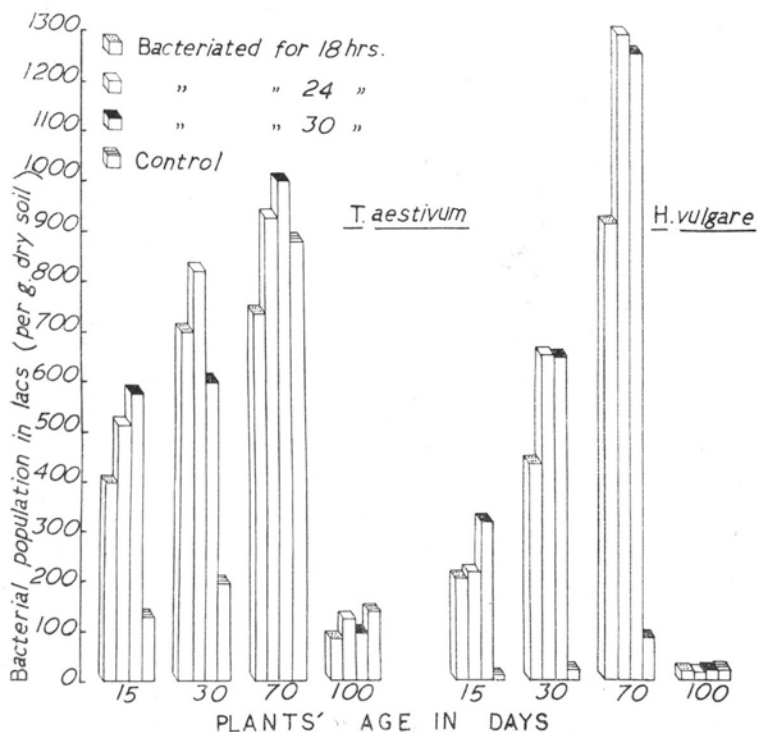


Fig. 2. Distribution of bacterial population in the rhizospheres of *Triticum aestivum* L. and *Hordeum vulgare* L. at young, flowering, and post-flowering stages

Table 2

Statistical analysis of the data

Variation due to:	Tabulated F		Calculated F			
	1%	5%	<i>T. aestivum</i>		<i>H. vulgare</i>	
			Fungi	Bacteria	Fungi	Bacteria
Treatment	6.99	3.86	4.22*	9.05**	0.90+	0.95+
Age	6.99	3.86	51.7**	8.91**	21.59**	4.02*

* Significant value at 5% level.

** Significant value at 1% level.

+ Insignificant value.

tion; and *Aspergillus nidulans*, *A. flavus*, *A. terreus*, *Cladosporium herbarium*, *Fusarium nivale* and white sterile colonies in the control. Certain specific forms occurrence were: *Cunninghamella echinulata*, *Chaetomium indicum*; *Aspergillus niveus*, *A. tamaraii*, *A. terreus* var. *africanus*, *Penicillium digitatum*, *P. spiculisporeum*; *Cladosporium epiphyllum*, *Curvularia pallescens*; and *Cunninghamella bertholletiae*, *Thielavia terricola*, *Aspergillus candidus*, *A. ochraceus*, *Paecilomyces varioti*, *Stachybotrys atra* and Yellow sterile colonies in 18, 24 and 30 hrs. inoculated (bacteriated) and control combinations, respectively. Dominants in the rhizoplane region were *Rhizopus nigricans*, *Aspergillus sydowi*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium oxysporum*; *Mucor racemosus*, *Aspergillus sydowi*, *Fusarium oxysporum*, black sterile colonies; *Rhizopus nigricans*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium nivale*, *F. oxysporum* in 18, 24, 30 hours, bacteriated and control combinations, respectively. Black sterile colonies were restricted in the rhizoplane of the 18 hrs. inoculated (bacteriated) combination, while the remaining three sets did not exhibit any specific fungal species.

As in the case of wheat, a number of fungi were dominant in the root regions of 18 hrs. treated barley. *Aspergillus nidulans*, *A. versicolor*, *Cladosporium herbarum*; these 3 forms together with *Fusarium avenaceum* in 24 hrs.; *Aspergillus nidulans*, *A. versicolor*, *Acremonium vitis*, *Paecilomyces fusisporus* and *Fusarium nivale* after 30 hrs. and *Aspergillus versicolor*, *Cladosporium herbarum*, *Fusarium nivale*, *F. oxysporum* and white sterile colonies were dominant in the control combination. Fungal species restricted only to individual combinations were *Gongronella butleri*, *Scoleocobasidium constrictum*, *Alternaria humicola*, *Torula* sp.; *Cephalosporium curtipes*, *Penicillium luteum*, *P. notatum*, *Pestalotia monorhinca*, *Epicoccum nigrum*, *Rhizoctonia solani* and *Mortierella* sp., *Aspergillus ochraceus*, *Penicillium terrestre* and *Myrothecium roridum* in 18 hrs, 30 hrs. bacteriated and control combinations respectively, while there were no specific species in the 24 hrs. bacter-

iated combination. The dominant species in the rhizoplane region were *Penicillium rugulosum*, *Cladosporium herbarum*, *Fusarium oxysporum* and White sterile colonies in 18 hrs.; *Aspergillus nidulans* and Black sterile colonies in 24 hrs.; *Aspergillus sydowi*, *Fusarium oxysporum* and White sterile colonies in 30 hrs. bacteriated combinations and *Cladosporium herbarum*, *Fusarium nivale*, *F. oxysporum* and White sterile colonies in the control. No species was found to be specific in this region except *Aspergillus flavipes* in the case of the 18 hrs. bacteriated combination.

The rhizosphere of treated plants of the two crops harboured a lesser number of fungal species than the control combination. No such trend was observed in the rhizoplane region where sterile forms together with *Fusaria* were more numerous during the senescent stage.

The fungal population (expressed per 1g dry soil) in the rhizosphere of both the plants was always more numerous on 70th day and the smallest at the beginning. Generally a larger population was recorded from the rhizosphere of 18 hrs. bacteriated plants. The population, however, was more numerous in control combinations than the bacteriated ones.

The size of the bacterial population reached maximum on the 70th day in all the combinations, and minimum on the 100th day, except in the control ones where the minimum was recorded at the beginning. The bacterial population was mostly low in the control combinations.

In the rhizoplane region of the different combinations, the mycoflora (average no. of colonies per plate) was always at minimum in the seedling stage (15th day), it gradually increased and reached maximum during the post-maturation stage.

The plants obtained from bacteriated seeds were healthier and taller than those developed in untreated control combinations.

The variation in the micro-population associated with the different rhizospheres developed by different treatments and age was highly significant except in one case of barley where the microbial variation due to treatment was not significant (Table 2).

DISCUSSION

The maximum population on the 70th day in the rhizosphere of the plants is obviously due to more root leakage in the form of total free amino acids. At the beginning and the end when the plants were in seedling and post-maturation stages the smaller population was due to lesser exudation (Srivastava 1969). The results indicate that the bacteria from the seed coat could establish successfully even during the young stage and multiply abundantly in the presence of a suitable

environment in the rhizosphere region of both the plant species and attained the highest peak on the 70-th day.

A larger fungal population in the control combination indicates a possible depressive effect of the bacterial flora on the fungi in the treated combinations. The effect may be either of antagonistic type through the secretion of metabolites unfavourable for fungal growth or by competition for space and nutrients, in which the bacterial flora prevails over the fungi owing to their rapid rate of multiplication and utilization of a wider range of nutrients. During the flowering stage (70th day), when the maximum root leakage was obtained, the competition for nutrition and also space was least and the microflora (both fungi and bacteria) were at their maximum. However, the nature of microbial interrelationships is more complex and further detailed work, by culture, or other suitable methods, is necessary before a convincing and suitable explanation may be put forth.

The same is true as regards the cause of better growth of treated plants. The microorganisms in the root region are supposed to facilitate the availability of various nutrients in the soil to the plant, unavailable otherwise or available to a lesser degree. It has been suggested by various workers (Okina 1940; Macura 1956; Cooper 1959; Sundra Rao and Sin'ha 1963) that the rhizosphere bacterial flora stimulates plant growth. Besides nutrient supply, other factors responsible for stimulating growth have also been suggested. Secretion of antibiotics, enzymes, hormones and vitamins by rhizosphere microorganisms has been reported by various workers. These substances in turn contribute to a better growth of the plants in various ways.

In earlier stages, when the epidermal cells are undamaged, the rhizoplane mycoflora was small. Later the epidermal cells are damaged and open the way for fungal species to enter and consequently higher numbers of colonies per plate were obtained during the post-maturation stage. These forms in association with other microorganisms play an important role in the decomposition of plant remains in the soil.

SUMMARY

The present paper deals with seed bacterization of *Triticum aestivum* L. and *Hordeum vulgare* L. with *Azotobacter chroococcum* and its effects on rhizosphere and rhizoplane microflora. In the rhizosphere of all the treated combinations the bacterium could settle and multiply successfully up to the flowering stage after which the microflora decreased. The bacterial population was mostly larger in the treated combinations than in the control ones. The mycoflora population in rhizoplane region was the smallest at the beginning and the highest during the post-maturation of the plants. All the plants developed from bacteriated seeds exhibited healthier and better growth than those obtained from untreated seeds. The micro-population was at its peak during maximum vegetative growth.

Acknowledgements

The authors are grateful to Professor K. S. Bhargava, Head of the Department of Botany, University of Gorakhpur, for providing laboratory facilities.

REFERENCES

- Brown M. E., Burlingham S. K. and Jackson R. M., 1924. Studies on *Azotobacter* species in soil. III. Effect of artificial inoculation on crop yields, Plant and Soil 20: 194—214.
- Cooper R., 1959. Soil and Fertilizer, 22:327.
- Garretson F. C., 1948. The influence of microorganisms on the phosphate intake by the plants, Plant and Soil 1: 51.
- Macura J., 1956. Sborn. Csl. Akad. Zemed. 29:957.
- Mishra R. R. and Srivastava V. B. 1969. Rhizosphere fungal flora of certain legumes, Ann. Inst. Pasteur 117: 717—723.
- Mishra R. R. and Srivastava V. B., 1970. Variation in the rhizosphere microflora of certain crops, Proc. Nat. Acad. Sci. India, 40 (8): 195—202.
- Okina E. G., 1940. Compt. Rend. Acad. Sci (U.S.R.R.) 27: 157.
- Rovira A. D. and Bowen G. D., 1966. The effect of microorganisms upon plant growth. II. Detoxication of heat sterilized soil by fungi and bacteria, Plant and Soil 25:129—142.
- Sperber J. I., 1957. Nature 180:994.
- Srivastava V. B. 1969. Investigations into rhizosphere microflora of certain crop plants, Ph. D. thesis, University of Gorakhpur, India, 1—256.
- Sunda Rao W. V. B. and Sinha M. K., 1963. Ind. Jour. Agric. Sci. 33: 272.

Authors' address:

Dr. V. B. Srivastava
Pilot Plant Laboratories, IDPL
Virbhadra
Rishikesh
Dehradun, U.P., India

*Badania nad mikroflorą rhizosfery. VII.**Wpływ traktowania nasion bakteriami na mikroflorę obszaru korzeniowego*

Streszczenie

Badano wpływ zakażenia nasion pszenicy i owsa bakteriami — *Azotobacter chroococcum*.

Opisano zmiany w populacji mikroflory w trakcie rozwoju tych dwóch roślin. Wykazano pozytywny wpływ traktowania nasion bakteriami na wzrost i zdrowotność roślin.