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A NEW METHOD FOR QUANTIFYING THE EFFECT OF *AZOSPIRILLUM* AND OTHER PGPR STRAINS ON MAIZE AND SUNFLOWER SEEDLINGS

J. FAGES, I. PENOT and J. F. ARSAC

Pioneer France-Mas, Chemin de l'enseigne, F-31840 Aussonne, France.

ABSTRACT

A new germination test on filter paper is proposed to screen for plant-beneficial bacterial isolates on the basis of their effect on plant growth. When used to compare strains inoculated on maize and sunflower, this test allowed the measurement of significant differences between strain effects on growth.

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) have been studied extensively over the past fifteen years. Among them *Azospirillum* (5), and antagonists of plant pathogens (4) appear to have agronomic and economic potential. The purpose of our study was to develop a method of screening rhizobacteria directly for their effect on plant growth.

MATERIALS AND METHODS

1. Cultivars

Sunflower (*Helianthus annuus* L.): Hybrid variety EMIL (Pioneer Hi-Bred Int.). Maize (*Zea mays* L.): Hybrid variety DEA (Pioneer Hi-Bred Int.).

2. Bacterial strains

Forty-five gram-negative bacterial strains isolated by our laboratory from the rhizosphere of sunflower were used for sunflower inoculation (2). Nineteen *Azospirillum* strains isolated from the rhizosphere of maize were used for maize inoculation.

Azospirillum lipoferum CRT 1 isolated from maize roots (3) was used on both plant species.

3. Germination tests

A sheet of filter paper was dipped in a 10^7 CFU/ml bacterial suspension or in a 21.25 mg/L KH_2PO_4 solution (Butterfield's solution) for the control. Three

rows of 10 surface-disinfected seeds were then arranged on this sheet. The seeds were covered with a similarly treated piece of paper. The sheets were then rolled and placed vertically in beakers to avoid cross-contamination. The beakers were then put into buckets. Each bucket contained one control roll and six (or less) inoculated rolls. The buckets were placed in the dark in a germination chamber at 18 °C. Two days after inoculation each roll was reinoculated with 10 ml of the 10^7 CFU/ml bacterial suspension. Control rolls received 10 ml of Butterfield's solution. After another 5 days (for maize) or 6 days (for sunflower) the rolls were opened, five seedlings (dead or abnormal) were eliminated, and growth measured. In the case of sunflower the growth parameter measured was the fresh weight of the 25 remaining seedlings. In the case of corn the primary root length of 15 randomly chosen seedlings was measured. For statistical analysis variance analysis was used followed by Newman and Keuls test when Fisher test probability was less than 5%.

RESULTS AND DISCUSSION

1. Sunflower

In a first series of experiments we compared the strains isolated from the rhizosphere of sunflower (Table 1). According to their effects, the strains could be classified into three groups: (i) strains such as TSL 51 (trial No. 2) and TSL 35 (trial No. 3), exerting a deleterious effect on the growth of sunflower seedlings; (ii) strains whose effects were not significantly different from controls. A majority of isolates belong to this group. Among them, some strains gave a rela-

Table 1. Effect of bacterial inoculation on fresh weight of sunflower seedlings.

Trial No. 1		Trial No. 2		Trial No. 3	
Bacterial strains	weight (g) ¹	Bacterial strains	weight (g)	Bacterial strains	weight (g)
TML 21	10.99 a	CRT 1	12.37 a	TSL 32	11.97 a
TMS 11	10.71 ab	TSL 54	12.09 ab	TSL 34	11.64 ab
TSL 41	10.60 b	TSL 42	11.92 ab	TSL 15	11.61 ab
TSL 42	10.56 b	Control	11.88 ab	Control	11.33 b
TSL 53	10.47 b	TSL 55	11.79 ab	TSL 45	10.86 c
TSL 31	10.44 b	TSL 43	11.59 b	TSL 14	10.76 c
Control	10.31 b	TSL 51	9.30 c	TSL 35	9.53 d

¹Fresh weight of 25 seedlings. Mean of 8 replicates with one bucket per replicate (see Materials and Methods). Results followed by common letter within a trial are not significantly different at $P = 0.05$. Weight variations between trials are due to test duration which may vary by a few hours after 6 days.

tively high increase of fresh weight (over +3.0 %) although the statistical threshold ($P = 0.05$) was not reached, e.g. CRT 1 which produced an increase of 4.1% (trial No. 2); (iii) strains causing a significant increase in the fresh weight of sunflower seedlings, e.g. TML 21 (trial No. 1) and TSL 32 (trial No. 3). Seedling fresh weight is a very simple and useful parameter. Since no photosynthesis occurs during the test, it provides a direct measure of water absorption by the seedlings and is, therefore, directly correlated with the root surface. Seedling dry weight - which is only related to seed weight - and elongation measurements were more time-consuming and less precise (data not shown).

2. Maize

In a second series of experiments we used this test on maize (Table 2). With maize seedlings, fresh weight was a less discriminatory parameter, and we, therefore, decided to measure the primary root length of the seedlings. This parameter, already used in other germination tests (1), confirmed its ability to show differences in the plant response after different microbial treatments. As with sunflower the test differentiated between three groups of bacteria according to the plant response. It can be noted that the same strain, CRT 1, gave a positive effect on plant growth in both plant species.

Despite the weak relative increases measured (due to very early measurements) the accuracy of the test allowed us to differentiate between strain treat-

Table 2. Effect of inoculation with *Azospirillum* strains on the mean of primary root length of maize seedlings.

Trial No. 1		Primary root length (mm)		Trial No. 3	
Bacterial strains	root length (mm) ¹	Bacterial strains	root length (mm)	Bacterial strains	root length (mm)
SAB 2	94.21 a	CLE 2	79.91 a	PAL	82.19 a
CRT 1	84.85 b	CSC 2	79.65 a	CRT 1	81.72 a
CSC 1	83.89 b	CRT 1	78.79 a	SAB 2	81.23 a
SAB 1	80.17 b	EVA 1	78.61 a	DVU 1	80.91 a
Control	73.81 c	DEA	77.69 a	Control	78.41 ab
		Control	75.75 a	DVU 2	75.69 ab
		CLE 1	62.63 b	CSC 1	73.09 b

¹Total primary root length of 15 seedlings randomly chosen. Mean of 5 replicates with one bucket per replicate (see Materials and Methods). Results followed by common letter within a trial are not significantly different at $P = 0.05$. Weight variations between trials are due to test duration which may vary by a few hours after 5 days.

ments. Thus, filter paper germination tests can be used for the screening of PGPR strains with agronomic potential on criteria directly linked with plant growth. Application of this technique is an improvement over classical screening methods which utilize indirect criteria such as biological nitrogen fixation (6). The use of complementary tests in pots and later on in the field are necessary to confirm the beneficial effects observed.

Part of this work was presented at the 5th International Symposium on N₂-Fixation with Non-Legumes.

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