Sarhad J. Agric., Vol. 15 (5), 1999

POTENTIAL OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR ENHANCING THE GROWTH AND YIELD OF POTATO (*SOLANUM TUBEROSUM* L.)

Muhammad Javed* and Muhammad Arshad**

ABSTRACT

Thirty eight rhizobacteria were isolated from the rhizosphere soil, purified and were selected on the basis of their ability to produce auxins (IAA-equivalents) in vitro. Eleven selected isolates were tested for yield promotion of potato (Solarium tuberosum) *in a pot and field trial. In both the trials tuber yield, number of tubers and shoot + root weight were significantly promoted in response to inoculation. Tuber yield was increased upto 47.5% and 25.8% in pot and field trial, respectively in response to PGPR inoculation. Similarly number of tubers and shoot + root weight were enhanced upto 56.2% and 27.6% in pot trial and upto 27.1% and 23.1% in field trial, respectively. The isolates J2, J7 and J24 showed consistent superiority over the other isolates and these could be used as plant growth-promoting rhizobacteria.*

INTRODUCTION

To meet the challenge of ever increasing population of the world, increased agriculture production is an immense need of time. One of the best effort in this regard is the introduction of soil microbes in agriculture for seed and soil inoculation. The successful use of *Azotobacter* and *Rhizobium* for improving agricultural production provides the excited example of this approach. Scientists are agreed on the view that bacterial fertilizers would not replace mineral fertilizers, however, a good combination of bacterial, mineral and organic fertilizers would enhance plant growth more promisingly than their application alone.

The term 'rhizobacteria' was coined for the subset of total rhizosphere bacteria which actively colonize roots, following inoculation. Effects of rhizobacteria on the inoculated host may be neutral, deleterious or beneficial, and the beneficial rhizobacteria are called 'plant growth—promoting rhizobacteria (PGPR)' (Kloepper and Schroth, 1978). However, Frankenberger and Arshad (1995) are of the view that all those rhizobacteria which promote plant growth upon inoculation through any mechanism of action, could be grouped under PGPR.

The role of PGPR for biological disease control has also been well documented. However, the application of PGPR for improving crop production over the optimum fertilizers has not been thoroughly investigated. In China, these beneficial rhizobacteria are termed as yield increasing bacteria (YIB) and are used to inoculate crops for improving yields. After reviewing the work done in China on YIB, Chen *et al.* (1994) concluded that growth promotion was most consistent when the YIB were used in the same geographical area from which they were originally isolated.

The use of PGPR to promote plant growth has increased dramatically over the last few years and a significant yield increases in response to PGPR inoculation have been reported (Chen *et al.*, 1994; Mei, 1989; Xia et al, 1990; Howie and Echandi, 1983; deFreitas and Germida, 1992), but no such work had ever been done in Pakistan. However, the potential of *Rhizobium, Azotobacter* and *Azospirillum* have been investigated by many researchers (Hussain *et al.*, 1983; Hussain *et al.*, 1987; Hussain *et al.*, 1993; Mlaik and Zafar, 1985). Considering the important role of PGPR in growth promotion, a pot and field trial was conducted to investigate the growth enhancing ability of PGPR under soil conditions of Faisalabad, Pakistan.

MATERIALS AND METHODS

The research work reported was conducted at Department of Soil Science, University of Agriculture, Faisalabad, Pakistan during the years 1993-94. Out of 38 rhizobacterial isolates, 11 were selected on the basis of auxin (IAA— equivalents) production in vitro (Table I). Auxin production was measured by using the method described by Sarwar *et al.* (1992). These isolates were tentatively identified as pseudomonad on the basis of physiological and morphological characteristics studied (Table II). In both the pot and field trials eleven selected rhizobacterial isolates were employed for tuber inoculation, in addition to uninoculated control (JO). Potato tubers were inoculated by peat based preparation. Broth culture of each isolate grown for seven days and containing 10^7 to 10^8 cfu mL^{-1} was transferred to sterilized plastic tubs containing autoclaved peat and slurry was prepared with 10% sugar solution. Potato tubers were inoculated with this slurry. Controls were treated with similar slurry without rhizobacteria.

* Directorate of Land Reclamation Canal Bank, Moghalpura, Lahore — Pakistan.
** Department of Soil Science, University of Agriculture, Faisalabad — Pakistan.

TABLE I. AUXIN (MG IAA-EQUIVALENTS L^{-1}) PRODUCTION BY DIFFERENT ISOLATES OF RHIZOBACTERIA

Isolates	IAA without L-TRP	IAA with L-TRP	Isolates	IAA without L-TRP	IAA with L-TRP
JO	0.0	0.0	J1*	9.0	20.0
J2*	21.0	30.5	J3*	12.0	17.5
J4	1.0	4.0	J5	4.0	7.5
J6	2.5	14.0	J7*	17.0	28.5
J8	3.0	16.0	J9	3.1	12.5
J10	5.5	7.5	J11*	25.0	31.5
J12*	21.0	23.0	J13	2.0	2.0
J14	4.0	14.5	J15	7.0	8.5
J16	4.1	13.0	J17*	12.5	44.0
J18	1.5	9.0	J19	3.0	3.5
J20	3.5	16.5	J21	7.0	9.5
J22*	8.0	15.0	J23	6.0	7.5
J24*	22.0	26.0	J25	5.5	6.0
J26*	15.0	20.1	J27	5.5	6.5
J28	8.5	10.5	J29	1.0	1.5
J30*	14.0	19.5	J31	5.0	7.5
J32	6.0	8.0	J33	0.8	3.5
J34	3.0	7.5	J35	7.3	10.5
J36	2.0	7.5	J37	1.0	3.0
J38	6.0	9.8			

* = selected isolates

TABLE II. SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF DIFFERENT RHIZOBACTERIAL ISOLATES

Isolates	Gram staining	Shape of bacteria	Fluorescence	Litmus test	Cytochrome oxidase
J1	-ve	rod	-ve	unchanged	+ve
J2	"	"	"	"	"
J3	"	"	"	"	"
J7	"	"	"	"	"
J11	"	"	"	"	"
J12	"	"	Y. green	"	"
J17	"	"	-ve	"	"
J22	"	"	-ve	"	"
J24	"	"	Y. green	"	"
J26	"	"	-ve	"	"
J30	"	"	-ve	"	"

—ve = Negative
+ve = Positive
Y.green = Yellow green

a) Pot trial

Pot experiment was conducted in the wire—house, Department of Soil Science. Glazed pots were filled with 12 kg of air—dried soil previously passed through 2 mm sieve. A basal dose of NPK @ 125—60—60 mg kg^{-1} soil as urea, single superphosphate and potassium sulphate, respectively was mixed with soil before filling the pots. The treatments were replicated five times using completely randomized design of layout. The potato cv. Cordinal was grown as test crop. Plants were irrigated regularly to avoid any water stress. Plants were also sprayed twice with 0.2% solution of Dithane to protect against early blight. At maturity (90 days old) plants were uprooted and data regarding tuber yield, number of tubers per plant and shoot + root dry weight were recorded.

b) Field trial

Field experiment was conducted in the research area, Department of Soil Science. The treatments were replicated five times using randomized complete block design with plot size of 2.44 x 1.50 m^2. The potato cv. Cordinal was grown as test crop. Fertilizers NPK @ 200—100—100 kg ha^{-1} were applied as urea, single superphosphate and potassium sulphate, respectively. Inoculated and uninoculated potato tubers (as previously described) were sown on ridges keeping row and plant distance of 75 and 30 cm, respectively. Sowing was performed manually and irrigation was applied after sowing. At maturity (102 days) plants were uprooted and data regarding tuber yield, number of tubers and shoot+root dry weight were recorded. The data so collected were statistically analysed (Steel and Torrie, 1980).

RESULTS

Inoculation of potato tubers with rhizobacterial isolates significantly affected the tuber yield in pot trial (Fig. 1). Enhancement in tuber yield was significant in response to inoculation with isolates J2, J7, J11, J12, J17, J24 and J30. Maximum tuber yield (47.5% higher than control) was recorded with isolate J7 and it was followed by isolates J2, J11, J24 and J30, which significantly increased the tuber yields by 28.7, 28.0, 28.6 and 27.7%, respectively over control. Number of tubers $plant^{-1}$ were significantly promoted with isolates J11 and J24 (Fig. 2). Maximum increase in the number of tubers was observed with isolate J24 (56.2% higher than control) and next to it was isolates J11, which increased the number of tubers by 50.0% over control. Inoculation with different isolates significantly affected the shoot + root dry weight of potato plants (Fig. 3). Inoculation with isolates J2, J7, J11, J12, J17 and J24 significantly promoted the shoot+root dry weight over control. Maximum shoot + root dry weight was with isolate J7 (27.6% higher than control), followed by isolates J2 (22.2%), J11 (18.6% and J24 (17.8%) in descending order.

In the field trial all the rhizobacterial isolates except isolate J22 significantly promoted tuber yield compared to control (Fig. 4). Tuber yield was maximum with isolate J7 (25.8% higher than control). Next to it were isolates J1, J2, J3, J11, J12, J17, J24, J26 and J30 which increased the yields by 16.8, 24.8, 22.1, 25.6, 9.4, 21.4, 22.3, 14.1 and 22.7%, respectively over control. Five of the tested isolates significantly increased the number of tubers compared to control (Fig. 5). Maximum number of tubers were recorded with isolate J11 (27.1% higher than control), followed by isolates J17 (20.4%), J3 (14.4%), J26 (13.8%) and J24 (11.0%) in descending order. Shoot+root dry weights of potato plants were significantly increased over control in response to inoculation with all rhizobacterial isolates except J22 (Fig. 6). Maximum shoot+root dry weight was recorded with isolate J7 (23.1% higher than control). Next to it were isolates J2, J11, J24 and J30, which increased the shoot + root weights by 20.6, 18.2, 15.6 and 17.3%, respectively over control.

DISCUSSION

Selected isolates of rhizobacteria significantly promoted yield of potato in pot and field trials. The isolates J2, J7 and J24 showed consistent superiority over the other isolates and these could be used as PG PR. In pot and field trials tuber yield was increased upto 47.5 and 25.8%, respectively with rhizobacterial isolate J7. These increases in yields in response to bacterization correspond to the data of Burr- *et al.* (1978) who found mean tuber yield increases of 14 to 33% due to inoculation with *P.fluorescence* and *P. putida* in five of nine field experiments. Similarly, Kloepper *et al.* (1980) reported mean yield increase of 17% in four of the five field experiments and Vrany *et al.* (1989) reported 23% increase in comparative field experiments in response to bacterial (pseudomonad) treatments. Tuber inoculation with *Pseudomonas* species resulted in significant increases in yields of potato ranging from 30 to 70% (Vrany and Fiker, 1984; Geels and Schippers, 1983). Similar results were also observed by Howie and Echandi (1983).

Significant increases in shoot + root weight and number of tubers were observed in response to inoculation with rhizobacteria in both pot and field trials. Similarly, many workers reported that inoculation of potato tubers with PGPR significantly increased the shoot and root weight (Howie and Echandi, 1983; Burr *et al.*, 1978; Frommel *et al.* 1991) and number of tubers (Geels and Schippers, 1983) compared to uninoculated controls.

The ability of the *Pseudomonas* spp. to rapidly colonize the rhizosphere of most plants makes them an attractive taxonomic group of soil bacteria for bacterization studies. The precise mode of action for commercially used PGPR inoculants are yet not known for all strains (Kloepper, 1994). In general the production of siderophores, antibiotics, several extracellular metabolites and induced systemic resistance are the mechanisms of indirect growth promotion, in addition to direct growth promotion as evidenced by increased root hair development. The most important mechanism of direct growth promotion may be the production of plant growth regulators (Arshad and Frankeflberger, 1991; Brown, 1974). As we have found significant correlations (data not shown) between crop yields and auxin producing ability of isolates, so we can say with some confidence that plant growth regulators are the mechanism of action by PGPR. Since several studies including this have demonstrated the ability of various PGPR to produce plant growth regulators (PGRs) in vitro. However, no direct evidence for in vivo production of PGRs by plant growth-promoting rhizobacteria have been reported.

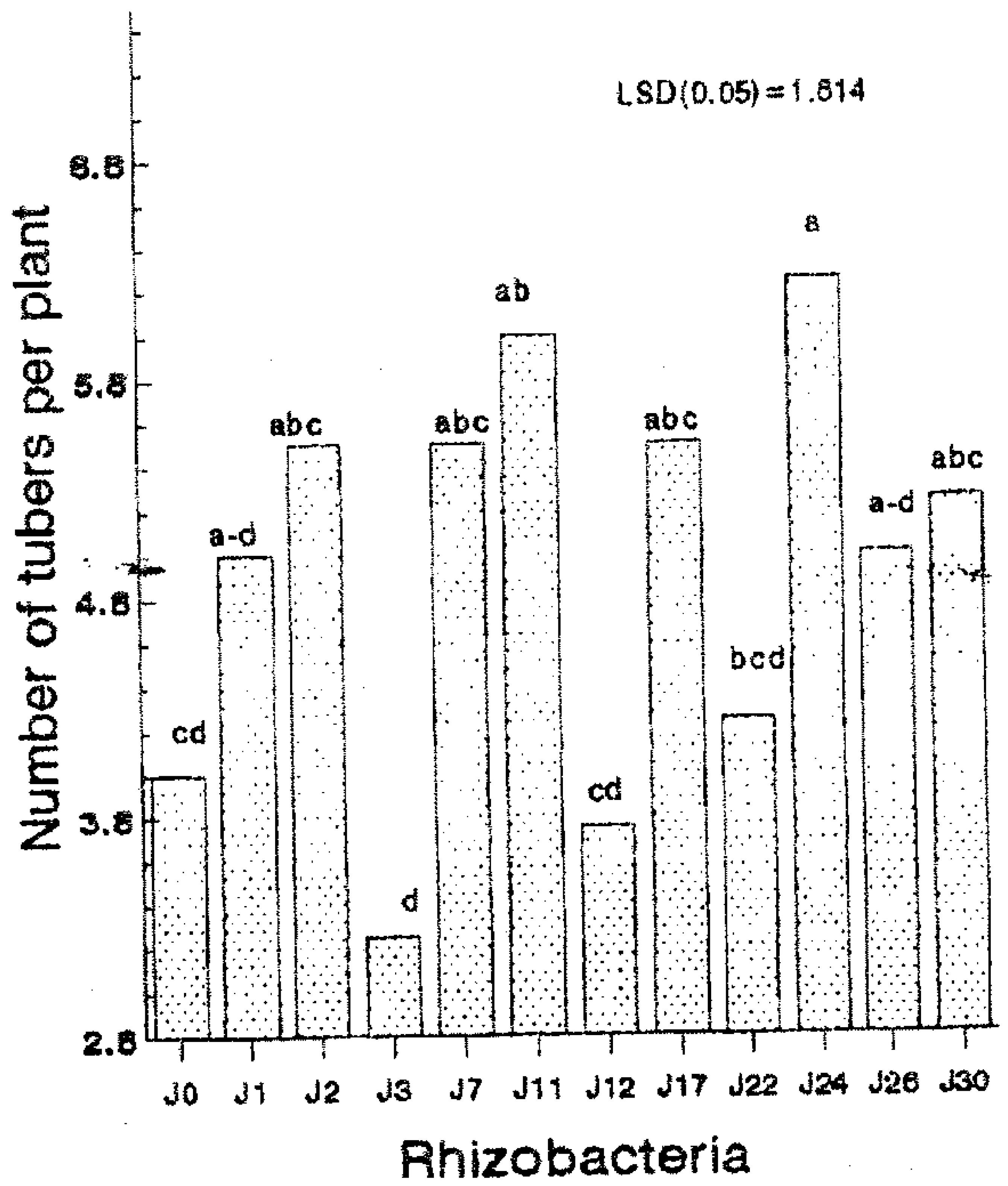

Fig.2 Effect of inoculation with rhizobacteria on number of tubers (pot trial)

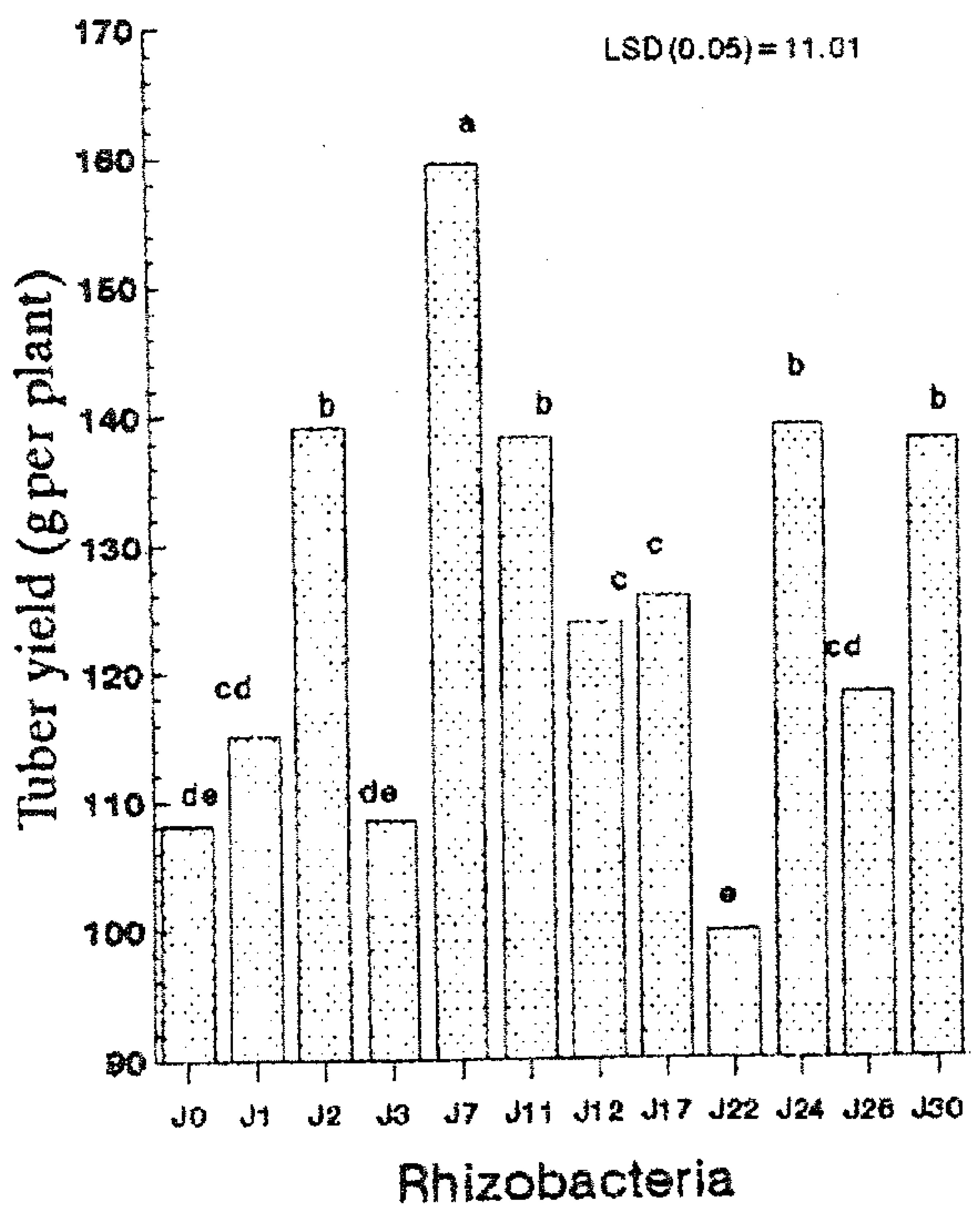

Fig.1 Effect of inoculation with rhizobacteria on tuber yield of potato (pot trial)

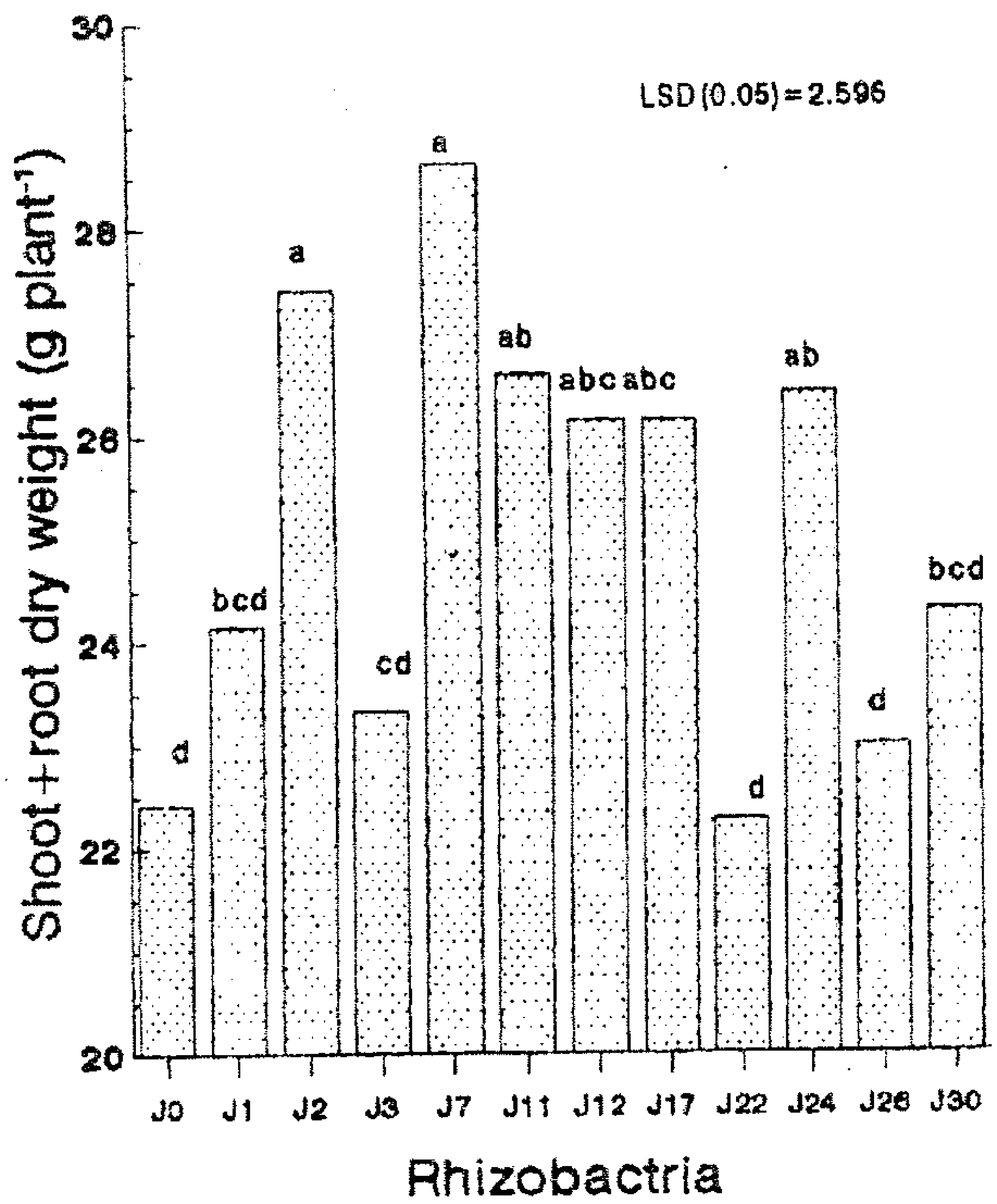

Fig.3. Effect of inoculation with rhizobacteria on shoot + root dry weight of potato (pot trial).

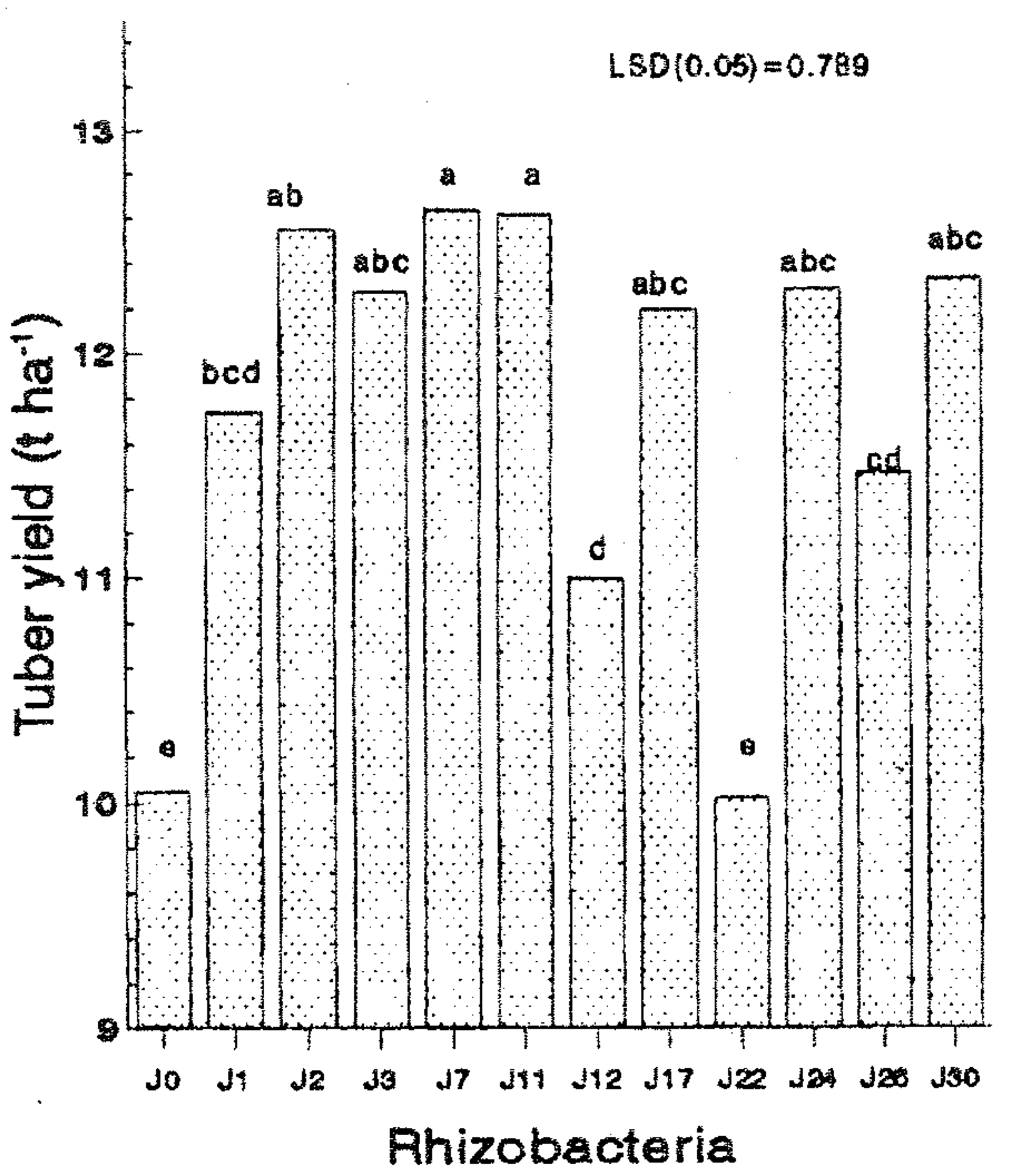

Fig.4 Effect of inoculation with rhizobacteria on tubers yield of potato (field trial)

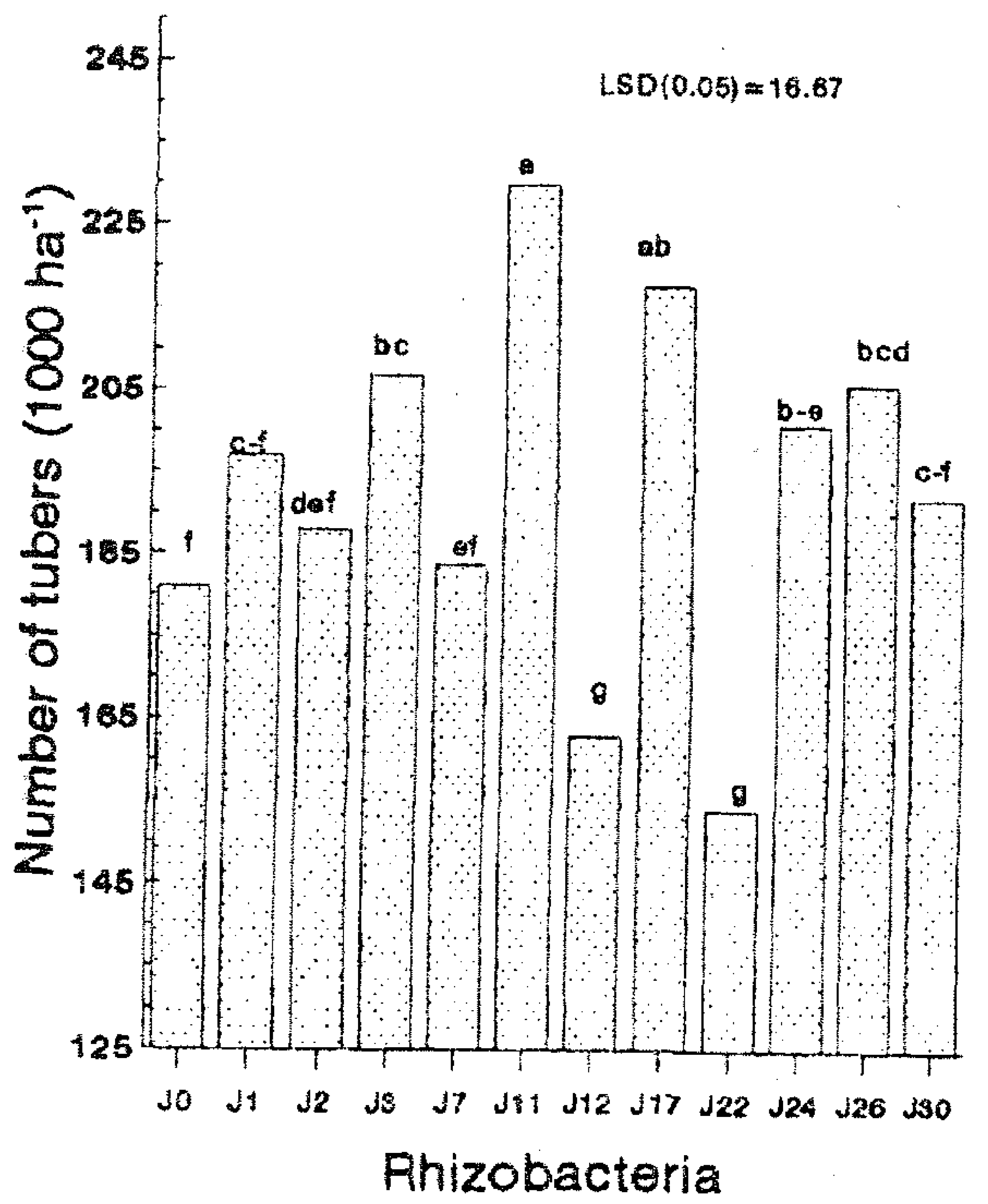

Fig.5. Effect of inoculation with rhizobacteria on number of tubers (field trial)

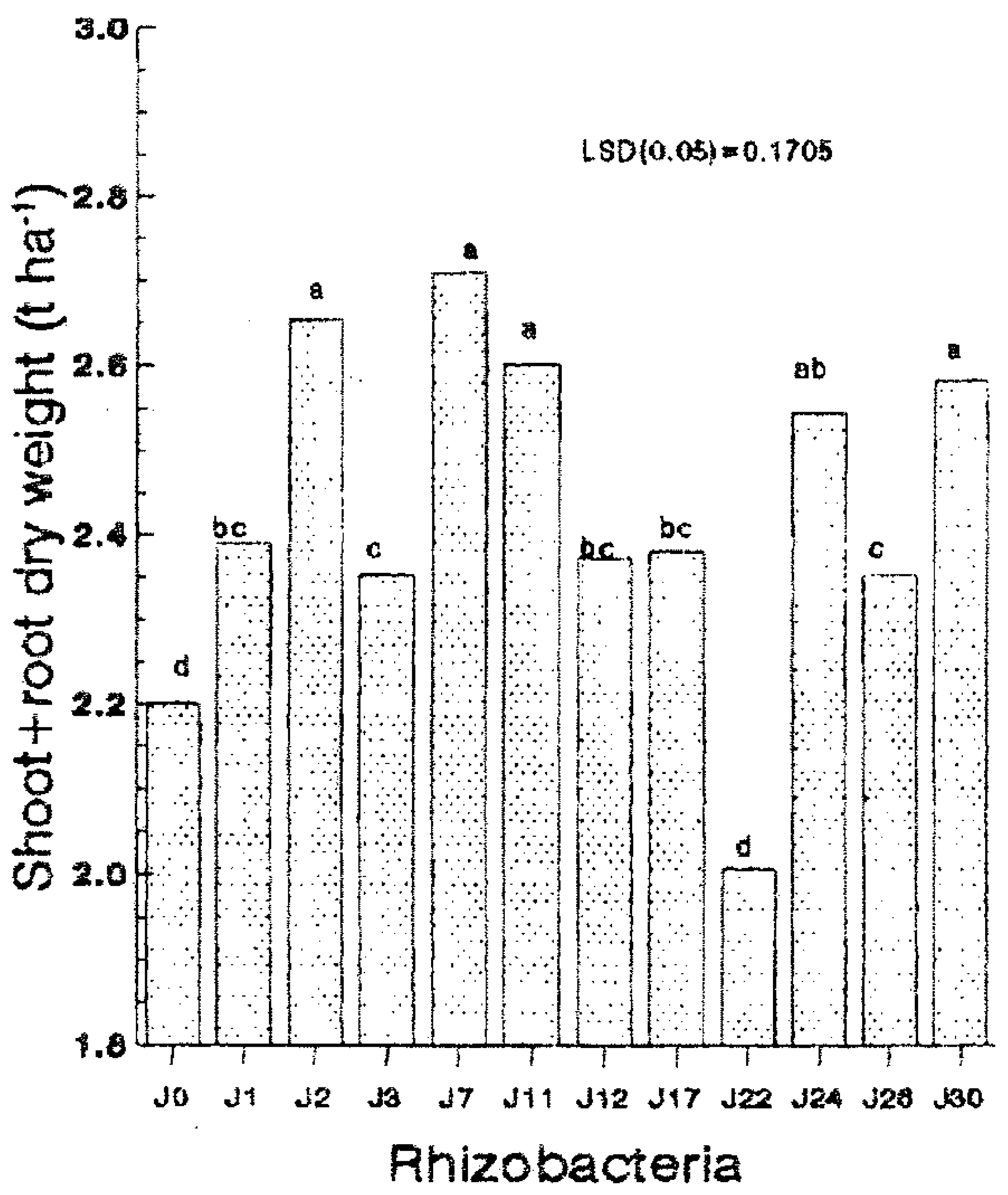

Fig.6 Effect of Inoculation with rhizobacteria on dry shoot + root weight of potato (field trial)

References Cited

Arshad, M. and W. T. Frankenberger Jr. 1991. Microbial production of plant hormones: 327-334. *In* D.L. Keister and P.B. Cregan (eds.) The Rhizosphere and Plant Growth. Kluwer, Dordrecht.

Brown, M. E. 1974. Seed and root bacterization. Ann. Rev. Phytopathol. 12:181-197.

Burr, T. J., M. N. Schroth and T. Suslow. 1978. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescence* and *P.putida*. Phytopathol. 68:1377—1383.

Chen, Y, R. Mei, S. Lu, L. Liu and J. W. Kloepper. 1994. The use of yield increasing bacteria as plant growth-promoting rhizobacteria in Chinese agriculture. *In* V.K.Gupta and R.Utkhede (eds.) Management of Soil-Borne Diseases. M/S Narosa Publishing House, New Delhi, India.

DeFreitas, J. R. and J. J. Germida. 1992. Growth promotion of winter wheat by fluorescent pseudomonad under growth chamber conditions. Soil Biol. Biochem. 24:1127—1135.

Frankenberger, W. T. Jr. and M. Arshad (eds.). 1995. Phytohormones in Soils Microbial Production and Functions. Marcel Dekker Inc., New York.

Frommel, M.L, J.Nowak and G. Lazarovits. 1991. Growth enhancement and developmental modifications of *in vitro* grown potato *(Solarium tuberosum* L.) as affected by a nonfluorescent *Pseudomonas* sp. Plant Physiol. 96:928—936.

Geels, F.P. and B.Schippers. 1983. Reduction of yield depressions in high frequency potato cropping soil after seed tuber treatments with antagonistic fluorescent *Pseudomonas* spp. Phytopath. Z. 108:207—214.

Howie, W. J. and E. Echandi. 1983. Rhizobacteria—influence of cultivar and soil type on plant growth and yield of potato. Soil Biol. Biochem. 15:127-132.

Hussain, A, M.Arshad, A.Hussain and F.Hussain. 1987. Response of maize *(Zea mays* L.) to *Azotobacter* inoculation under fertilized and unfertilized conditions. Biol. Fert. Soils 4:73—77.

Hussain, A, M.Arshad, S.Ali and S. Ahmad. 1983. Prospectives of soybean, wingedbean, mungbean and gram at Faisalabad. Final report of project No. PK-ARS-32. Isolation and Identification of Effective Root Nodule Bacteria for Important Grain Legumes of Pakistan. 73p.

Hussain, A., M.Sarfraz, M.Arshad and M.Javed. 1993. Potential of *Azotobacter* for promoting potato growth and yield under optimum fertilizer application. Pakistan J. Agri. Sci. 30:217—220.

Kloepper, J. W. 1994. Plant growth-promoting rhizobacteria (other systems). p.135-166. *In* Y.Okon (ed.) Azospirillum/Plant Associations. Boca Raton Ann Arbor London, Tokyo.

Kloepper, J. W. and M. N. Schroth. 1978. Plant growth promoting rhizobacteria on radishes. p.879—882. *In* Proc. 4th mt. Conf. Plant Pathogenic Bacteria, Vol. 2, Station tie Pathologie, INRA. Angers (ed.). Gibert-Clary, Tours, France.

Kloepper, J. W., M. N. Schroth and T. D. Miller. 1980. Effect of rbizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. Phytopathol. 70:1078—1082.

Malik, K.A. and Y.Zafar. 1985. Quantification of root associated nitrogen fixation in kallar grass as estimated by N^{15} isotope dilution. p.161-171. *In* K.A.Malik, S.H.M.Naqvi and M.I.H.Aleem (eds.) Nitrogen and the Environment. Nuclear Inst. Agri. Biol., Faisalabad, Pakistan.

Mei, R. (ed.). 1989. Yield increasing bacteria. Agricultural Press, Beijing, 128 P.

Sarwar, M., M. Arshad, D. A. Martens and W. T. Frankenberger Jr. 1992. Tryptophan—dependent biosynthesis of auxins in soil. Plant Soil 147:207—215.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co. Inc., New York, USA.

Vrany, J. and A.Fiker. 1984. Growth and yield of potato plants inoculated with rhizosphere bacteria. Folia Microbiol. 29:248—253.

Vrany, J., K.Dobias and A.Fiker. 1989. Yields of potatoes and their contamination by fusaria after inoculation with bacteria and fungi in field experiments. p.469-473. *In* V.Vancura and F.Kunc (eds.) Interrelationships Between Microorganisms and Plants in Soil (Developments in Soil Sci. 18). Elsevier Amsterdam, Netherlands.

Xia, L., X. Ding, S. Xiao, S. Han and R. Mei. 1990. Mechanism of PGPR. III. Influence of PGPR on physiology, resistance, quality and yield of wheat. Agricultural Sci. in Hunan. 108:26—29.