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ORIGINAL PAPER

Evidence that fresh weight measurement is imprecise for reporting the effect of plant growth-promoting (rhizo)bacteria on growth promotion of crop plants

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Abstract Eight greenhouse experiments were performed to compare the effect of seven plant growth-promoting (rhizo)bacteria (PGPR/PGPB) on fresh and dry weights of four crop plants. This has been done to validate if fresh weight measurements of plant variables can serve as reliable values when reporting the effect of these bacteria on plant growth. These experiments show that the growth promotion effects by the tested PGPR/PGPB, including *Bacillus amyloliquefaciens* GB03, *Bacillus subtilis* IN-937B, *Bacillus altitudinis* INR7, and *Pseudomonas mandelii* 89B-27 in corn and cucumber and *Azospirillum brasilense* Cd, *A. brasilense* Sp 245, and *Azospirillum lipoferum* Br 17 in pepper and tomato, varied significantly between fresh and dry weights of shoot, root, and/or whole plant in the repeated greenhouse experiments. These results support our hypothesis that using fresh weight determination for assessing plant growth promotion by beneficial bacteria is inherently faulty. Therefore, it is recommended that dry weight determination rather than fresh weight determination is used for plant growth promotion tests.

Keywords *Azospirillum* · *Bacillus* · Plant growth promotion · PGPR · PGPB · Fresh weight · Dry weight

Introduction

Growth promotion of crop plants using inoculation with plant growth-promoting (rhizo)bacteria (PGPR/PGPB) is an emerging agricultural and environmental technology with many small- and medium-scale inoculant companies already in the marketplace (Bashan et al. 2014, 2016; Calvo et al. 2014). Although many research approaches target the final yield of the plant, for initial studies revealing the agronomic potential of these PGPR/PGPB strains, short-term in vitro studies and medium-term greenhouse studies are commonplace. Both types of studies typically assess the effects of the bacteria on parameters of plant growth, including most commonly weights of shoots and roots. These weights are often expressed in the scientific literature as fresh weight, dry weight, or both (Bashan and de-Bashan 2005).

Precise determination of weight is fundamental for duplication and repetition of experiments done with the same PGPR/PGPB strain in different laboratories. This is specifically important when the study has been done in a public research facility (university, research institute) and the intended users are commercial companies. Numerous studies have shown that dry weight determination is an accurate measurement for many effects on plant growth induced by PGPR/PGPB strains, while in contrast, fresh weight is less accurate because it is affected by numerous environmental and technical parameters including relative humidity, laboratory temperature, and air currents during exposure of excised plant parts prior to weighing each sample Additional factors affecting fresh weight determination are the technique used to blot excess moisture from the washed roots, the total time from collecting

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and washing samples until weighing, the size of the experiment, and the size of the pot (Bashan and de-Bashan 2005). Most fresh weight determinations when presented in a manuscript during the peer review process are usually eliminated by reviewers and are discouraged by editors. Yet, fresh weight continues to be reported in studies with PGPR/PGPB (Ali et al. 2009; Choi et al. 2008; Compant et al. 2005; Domenech et al. 2006; Feng et al. 2006; Gravel et al. 2007; Gutiérrez-Luna et al. 2010; Jha and Kumar 2009; Jha et al. 2012; Joo et al. 2005; Kang et al. 2007; Khan et al. 2008; Li et al. 2008; Ortíz-Castro et al. 2008; Rajendran et al. 2008; Ribaudo et al. 2006; Ryu et al. 2005; Shaharoona et al. 2006; Shoebitz et al. 2009; Vespermann et al. 2007; Yildirim et al. 2006; Zahir et al. 2008), and sometimes, both fresh weights and dry weights are reported (Abbasi et al. 2011; Arkhipova et al. 2007; Banchio et al. 2008; Çakmakçı et al. 2007; Chen et al. 2010; Gamalero et al. 2008; Ganesan 2008; Kumar et al. 2008; Ma et al. 2009, 2011; Rajkumar and Freitas 2008; Saravanakumar et al. 2011; Saravanakumar and Samiyappan 2007; Tank and Saraf 2010; Zahid et al. 2015).

We hypothesized that fresh weight determination is inherently faulty while dry weight is, by definition, accurate. If fresh weight determination was solid and accurate, then growth promotion effects by PGPR/PGPB would be consistent between fresh and dry weights, and theoretically, water content in plant tissues, correlation coefficients (r), and slopes should be consistent between non-inoculated control and inoculated treatments. To test our hypothesis, we conducted a series of specific experiments on selected PGPR and PGPB strains, which was repeated twice, using common row crop (corn) and vegetables (cucumber, pepper, and tomato). For all experiments, both fresh and dry weights were determined, and water content was calculated for shoot, root, and whole plant. All data were subjected to analysis of variance, together with correlation and regression analyses between fresh weight and dry weight, to show the solidity of each type of determination.

Materials and methods

Organisms, experimental design, and treatments

Four PGPR strains that were selected for growth promotion tests in corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) included *Bacillus amyloliquefaciens* GB03, *Bacillus subtilis* IN-937B, *Bacillus altitudinis* INR7, and *Pseudomonas mandelii* 89B-27. Three PGPB strains that were evaluated in pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicon* L.) included *Azospirillum brasilense* Cd, *A. brasilense* Sp245, and *Azospirillum. lipoferum* Br 17. The four PGPR strains were from the PGPR culture collection in the Department of Entomology and Plant Pathology at Auburn University, and the three PGPB strains were from Bashan's research group, CIBNOR, Mexico. A total of eight pot experiments, which were repeated twice, were conducted in spring 2016 in a greenhouse at the Plant Science Research Center, Auburn University, Auburn, AL. Temperature within the greenhouse was maintained at 27 ± 2 °C with an average relative humidity of 85% throughout the experiments. The experimental design was a randomized complete block design with 9 (corn and pepper) or 10 (cucumber and tomato) replicates for each treatment where a single pot was a replicate. In addition to the selected bacterial strains treatments, one water blank was also included as non-inoculated control in each experiment.

Preparation of transplants

Prior to seeding, Speedling transplant trays (36, 5-cm square cells per tray) were filled with Sunshine Mix #8 (Sun Gro Horticulture, Agawam, MA). Single seeds of cucumber cv. 'Diva' or pepper cv. 'California Wonder' or tomato cv. 'Juliet' (Park Seed, Greenwood, SC) were sewn into each cell at a depth of 1.25 (pepper and tomato) or 2.54 cm (cucumber). After seeding, the trays were watered each morning to provide enough moisture for germination and growth until transplanting. At 2 weeks after seeding cucumber and 3 weeks after seeding pepper and tomato, the seedlings were about 10-cm tall and ready for transplanting to larger pots. Seeds of corn cv. 'Viking 60-01' (Albert Lea Seed, Albert Lea, MN) were directly sewn as described later.

Preparation of bacterial inoculants

All bacterial inoculants and inoculation procedures were prepared following established guidelines (Bashan et al. 2016). Specifically, all selected bacterial strains were transferred from cryovials maintained at −80 °C for long-term storage to plates of tryptic soy agar (TSA; Hardy Diagnostics, Santa Maria, CA) and were incubated at 28 ± 2 °C for 48–72 h. Then a loopful of a single colony of each strain was serially diluted in distilled water. Bacterial suspensions were adjusted to a concentration of 10^6 cfu ml^{-1}. This solution served as the liquid inoculant immediately (Bashan and de-Bashan 2015).

Pots and growth medium

Round black plastic pots, 16.2 cm in diameter and 18.4-cm deep, were filled with 2.3 kg of thoroughly mixed soils (dry weight basis), which were taken from a field at the E.V. Smith Research Center, Field Crop Unit of the Alabama Agricultural Experiment Station near Tallassee, AL. The soil analysis was performed by Waters Agricultural Laboratories (Camilla, GA) using Mehlich-1 extraction method. The soil was a loamy sand, with a texture of 81.2% sand, 2.0% clay, and 16.8% silt, containing 11 mg kg^{-1} NO_3-N, 14 mg kg^{-1} P, 56 mg kg^{-1} K, 203 mg kg^{-1} Ca, 49 mg kg^{-1} Mg, 9 mg kg^{-1} S, 0.1 mg kg^{-1} B, 3.2 mg kg^{-1} Zn, 12 mg kg^{-1} Mn,

Table 1 Fresh and dry weights of shoot, root, and whole plant of corn at 4 weeks after inoculation with one of the four PGPRs

Experiment no.	Bacterial inoculant treatment	Shoot weight (g)		Root weight (g)		Whole plant weight (g)	
		Fresh	Dry	Fresh	Dry	Fresh	Dry
1	Non-inoculated control	16.33 ± 0.59	1.379 ± 0.052	4.41 ± 0.35	0.476 ± 0.048	20.74 ± 0.62	1.854 ± 0.088
	Bacillus amyloliquefaciens GB03	15.14 ± 0.97	1.359 ± 0.088	5.48 ± 0.35*	0.537 ± 0.048	20.62 ± 1.05	1.896 ± 0.088
	Bacillus subtilis IN-937B	15.39 ± 0.59	1.310 ± 0.088	5.65 ± 0.46*	0.572 ± 0.075	21.04 ± 1.05	1.882 ± 0.135
	Bacillus altitudinis INR7	16.14 ± 0.59	1.431 ± 0.052	6.37 ± 0.46***	0.571 ± 0.048	22.51 ± 1.05	2.002 ± 0.088
	Pseudomonas mandelii 89B-27	14.49 ± 0.59	1.358 ± 0.052	5.69 ± 0.35**	0.694 ± 0.075*	20.18 ± 0.62	2.052 ± 0.135
2	Non-inoculated control	19.92 ± 0.91	2.247 ± 0.093	5.48 ± 0.32	0.604 ± 0.033	25.40 ± 1.03	2.851 ± 0.119
	B. amyloliquefaciens GB03	19.61 ± 0.91	2.261 ± 0.093	7.11 ± 0.32**	0.673 ± 0.040	26.72 ± 1.03	2.934 ± 0.119
	B. subtilis IN-937B	16.41 ± 1.27	1.874 ± 0.126	6.03 ± 0.48	0.538 ± 0.040	22.43 ± 1.70	2.412 ± 0.158
	B. altitudinis INR7	18.05 ± 0.91	2.164 ± 0.093	7.66 ± 0.32***	0.686 ± 0.033	25.71 ± 1.03	2.850 ± 0.119
	P. mandelii 89B-27	19.20 ± 0.59	2.278 ± 0.093	8.43 ± 0.48***	0.681 ± 0.033	27.64 ± 1.03	2.959 ± 0.119

*$p < 0.05$, **$p < 0.01$, and *** $p < 0.001$, compared with control of each experiment

± standard error of the mean

17 mg kg^{-1} Fe, 0.5 mg kg^{-1} Cu, 23.5% field capacity (dry basis), and 1.25% organic matter; pH 5.1.

Planting and application of inoculant treatments

Prior to planting, soil water content was adjusted to a 60% field capacity in all pots. At planting, one corn seed was placed into the center of each pot at a depth of 3.8 cm or one transplant of cucumber, pepper, or tomato was transplanted into each pot. Inoculation was by applying 1 ml of the appropriate bacterial inoculant over the top of each corn seed or pouring 25 ml of the appropriate bacterial inoculant as a drench around each transplant, then covering the seed or transplant with soil. After planting, soil water content was adjusted and maintained to an 80% field capacity in all pots. To maintain the soil as close as possible to an 80% field capacity during the 4-week-long experiments, pots were watered each morning. This was done by weighing 10 randomly selected pots from each experiment and adding the used water. To further precise the irrigation regime and to minimize the effects of watering on the growth of plants, every 7 days, all pots were weighed and watered.

Measurements of fresh and dry weights

Four weeks after inoculation, shoots of corn, cucumber, pepper, and tomato were cut at their base, and roots were carefully washed using tap water to reduce root loss. Fresh weights of shoots and roots were measured on a scale (±0.01 g) with an ambient temperature of 21 ± 2 °C. Excess moisture on the roots was blotted with brown paper towels (Boardwalk Paper Supply Co., Myrtle Beach, SC) before measuring root fresh weight. Then, the samples were dried in an oven at 70 °C for 72 h when constant weight was reached. Dried shoots and roots were weighed on a scale (±0.001 g). In addition, water content of shoot, root, and whole plant was calculated.

Statistical analysis

Fresh and dry weight data and water content data were subjected to analysis of variance for each experiment independently using SAS v9.4 PROC GLIMMIX procedure (SAS Institute 2009, Cary, NC). Water content data in percentage were transformed to arcsine before analysis. The critical p value of 0.05 was used as cutoff for testing treatment effect, and determination of differences in least-squares means between non-inoculated control and inoculated treatments was based on adjusted p values obtained by using the option ADJUST=DUNNETT in the LSMEANS statement (SAS Institute 2009). All mean values of fresh and dry weight data in the tables were accompanied by standard errors. Back-transformed 95% confidence intervals were provided for results of water content data that were arcsine transformed before analysis.

In addition, correlation and regression analyses between fresh weight (predictor variable) and dry weight (response variable) of shoot, root, and whole plant were performed for each experiment separately and bacterial inoculant treatments using PROC CORR and PROC REG procedures in SAS, respectively. Then, R. A. Fisher's z test (r-to-z transformation) (Fisher 1921) and t test were used for comparing correlation coefficients (r) and slopes between non-inoculated control and inoculated treatments, respectively.

Results

Fresh and dry weights of four crop plants inoculated with PGPR/PGPB

The growth promotion effects by the tested PGPR/PGPB on shoot, root, and total dry weight of corn, cucumber, pepper, and tomato plants were different from the effects recorded using fresh weight in repeated greenhouse experiments (Tables 1, 2, and 3; Supplementary Table S1). Specifically, inoculation of corn seeds with *B. amyloliquefaciens* GB03, *B. subtilis* IN-937B, *B. altitudinis* INR7, or *P. mandelii* 89B-27 significantly increased root fresh weight of corn at 4 weeks after inoculation when compared to non-inoculated control, but the same effects were not observed on root dry weight of corn (Table 1). One exception was that inoculation of corn seeds with *P. mandelii* 89B-27 significantly enhanced both fresh and dry weights of corn roots in the first experiment (Table 1). Yet, the significance level was higher for the growth promotion effects on root fresh weight of corn ($p < 0.01$) than for the effects on root dry weight of corn ($p < 0.05$).

Similar to corn, inoculation of cucumber transplants with one of the four tests PGPR significantly increased shoot, root, and/or total fresh weight of cucumber 4 weeks after inoculation when compared to the non-inoculated control (Table 2). Yet, the same effects were either not observed on dry weight of cucumber or the significance level of the statistical analysis changed between fresh and dry weights (Table 2). One exception was that the inoculation of cucumber transplants with *B. amyloliquefaciens* GB03 significantly enhanced both fresh and dry weights of cucumber roots with the same significance level of 0.01 in the fourth experiment (Table 2).

The differences in growth promotion effects by the tested PGPB on fresh and dry weights were also recorded on pepper and tomato. Inoculation of pepper transplants with *A. lipoferum* Br 17 increased shoot fresh weight, root dry weight, and total fresh weight of pepper at 4 weeks after inoculation in the fifth experiment but did not increase shoot dry weight, root fresh weight, and total dry weight of pepper in the same experiment (Table 3). Inoculation of tomato transplants with *A. brasilense* Cd, *A. brasilense* Sp 245, or *A. lipoferum* Br 17 increased root dry weight and/or total dry weight of pepper at 4 weeks after inoculation in the eighth experiment but did not increase root fresh weight and/or total fresh weight of pepper in the same experiment (Supplementary Table S1). Moreover, some PGPR treatments that increased both fresh and dry weights of pepper and tomato did not have the same significance level (Table 3; Supplementary Table S1). In summary, the growth promotion effects by tested PGPR/PGPB varied significantly between fresh and dry weights of tested crops in this study.

Table 2 Fresh and dry weights of shoot, root, and whole plant of cucumber at 4 weeks after inoculation with one of the four PGPRs

Experiment no.	Bacterial inoculant treatment	Shoot weight (g)		Root weight (g)		Whole plant weight (g)	
		Fresh	Dry	Fresh	Dry	Fresh	Dry
3	Non-inoculated control	14.62 ± 0.69	1.692 ± 0.095	3.98 ± 0.33	0.240 ± 0.014	18.60 ± 0.92	1.932 ± 0.098
	Bacillus amyloliquefaciens GB03	17.03 ± 0.50*	1.890 ± 0.072	5.97 ± 0.52**	0.281 ± 0.019	23.00 ± 0.92**	2.171 ± 0.083
	Bacillus subtilis IN-937B	16.37 ± 0.69	1.751 ± 0.072	5.64 ± 0.52*	0.255 ± 0.019	22.01 ± 0.92*	2.006 ± 0.083
	Bacillus altitudinis INR7	16.54 ± 0.50	1.829 ± 0.095	5.62 ± 0.33***	0.253 ± 0.014	22.16 ± 0.62**	2.082 ± 0.098
	Pseudomonas mandelii 89B-27	16.85 ± 0.50*	1.949 ± 0.095	5.72 ± 0.33***	0.275 ± 0.014*	22.58 ± 0.62**	2.224 ± 0.098
4	Non-inoculated control	17.97 ± 0.47	1.772 ± 0.060	3.96 ± 0.26	0.282 ± 0.022	21.93 ± 0.55	2.054 ± 0.068
	B. amyloliquefaciens GB03	18.83 ± 0.50	1.806 ± 0.063	6.36 ± 0.55**	0.399 ± 0.023**	25.32 ± 1.16*	2.204 ± 0.072
	B. subtilis IN-937B	20.46 ± 0.99	1.933 ± 0.063	4.50 ± 0.55	0.266 ± 0.023	25.09 ± 1.16	2.199 ± 0.072
	B. altitudinis INR7	19.98 ± 0.47*	1.790 ± 0.033	4.87 ± 0.26*	0.274 ± 0.009	24.85 ± 0.55***	2.064 ± 0.037
	P. mandelii 89B-27	19.67 ± 0.93	1.847 ± 0.060	5.94 ± 0.52**	0.307 ± 0.022	25.61 ± 1.10*	2.153 ± 0.068

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared with control of each experiment

± standard error of the mean

Table 3 Fresh and dry weights of shoot, root, and whole plant of pepper at 4 weeks after inoculation with one of the three PGPBs

Experiment no.	Bacterial inoculant treatment	Shoot weight (g)		Root weight (g)		Whole plant weight (g)	
		Fresh	Dry	Fresh	Dry	Fresh	Dry
5	Non-inoculated control	8.89 ± 0.40	1.161 ± 0.064	3.39 ± 0.23	0.262 ± 0.024	12.24 ± 0.49	1.423 ± 0.034
	Azospirillum brasilense Cd	9.93 ± 0.52	1.224 ± 0.064	3.66 ± 0.34	0.301 ± 0.024	13.58 ± 0.74	1.526 ± 0.098
	A. brasilense Sp 245	9.87 ± 0.52	1.270 ± 0.064	3.92 ± 0.34	0.293 ± 0.024	13.79 ± 0.74	1.563 ± 0.098
	Azospirillum lipoferum Br 17	10.01 ± 0.40*	1.296 ± 0.064	4.34 ± 0.34	0.341 ± 0.019*	14.35 ± 0.49**	1.637 ± 0.034***
6	Non-inoculated control	10.00 ± 0.59	1.290 ± 0.095	3.73 ± 0.43	0.313 ± 0.034	13.73 ± 0.82	1.603 ± 0.109
	A. brasilense Cd	10.84 ± 0.36	1.451 ± 0.051	4.10 ± 0.43	0.337 ± 0.034	14.93 ± 0.57	1.788 ± 0.063
	A. brasilense Sp 245	10.13 ± 0.36	1.336 ± 0.051	4.38 ± 0.51	0.352 ± 0.034	14.52 ± 0.57	1.688 ± 0.063
	A. lipoferum Br 17	11.53 ± 0.59	1.378 ± 0.095	4.77 ± 0.51	0.309 ± 0.039	16.30 ± 0.82*	1.687 ± 0.109

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared with control of each experiment

± standard error of the mean

Table 4 Water content of shoot, root, and whole plant of corn at 4 weeks after inoculation with one of the four PGPRs

Experiment no.	Bacterial inoculant treatment	Water content (%)		
		Shoot	Root	Whole plant
1	Non-inoculated control	(91.12, 91.99)	(87.90, 90.25)	(90.48, 91.60)
	Bacillus amyloliquefaciens GB03	(90.37, 91.49)	(89.41, 91.04)	(90.19, 91.32)
	Bacillus subtilis IN-937B	(91.01, 92.10)	(88.73, 91.53)	(90.60, 91.71)
	Bacillus altitudinis INR7	(90.83, 91.50)	(90.79, 91.82)*	(90.54, 91.66)
	Pseudomonas mandelii 89B-27	(90.30, 90.99)**	(86.49, 89.54)	(89.28, 90.46)*
2	Non-inoculated control	(88.07, 89.32)	(88.40, 89.62)	(88.27, 89.26)
	B. amyloliquefaciens GB03	(87.81, 89.07)	(89.83, 91.21)*	(88.52, 89.50)
	B. subtilis IN-937B	(87.36, 89.37)	(90.37, 91.71)**	(88.40, 89.86)
	B. altitudinis INR7	(87.35, 88.63)	(90.48, 91.60)***	(88.52, 89.26)
	P. mandelii 89B-27	(87.48, 88.75)	(91.57, 92.21)***	(88.78, 89.75)

Values in the parentheses are back-transformed 95% confidence interval

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared with non-inoculated control of each experiment

Table 5 Water content of shoot, root, and whole plant of cucumber at 4 weeks after inoculation with one of the four PGPRs

Experiment no.	Bacterial inoculant treatment	Water content (%)		
		Shoot	Root	Whole plant
3	Non-inoculated control	(88.00, 88.76)	(93.51, 94.26)	(89.21, 89.93)
	Bacillus amyloliquefaciens GB03	(88.27, 89.50)	(94.69, 95.69)***	(90.18, 90.87)**
	Bacillus subtilis IN-937B	(88.65, 89.86)	(95.17, 95.66)***	(90.31, 91.44)**
	Bacillus altitudinis INR7	(88.40, 89.62)	(95.04, 95.86)***	(90.19, 91.10)**
	Pseudomonas mandelii 89B-27	(87.69, 89.19)	(94.86, 95.37)***	(89.59, 90.75)
4	Non-inoculated control	(89.70, 90.54)	(92.59, 93.20)	(90.30, 90.99)
	B. amyloliquefaciens GB03	(90.24, 90.70)	(93.01, 94.16)	(91.06, 91.50)*
	B. subtilis IN-937B	(90.07, 90.98)	(93.51, 94.26)***	(90.72, 91.61)
	B. altitudinis INR7	(90.64, 91.44)*	(94.08, 94.63)***	(91.23, 92.10)**
	P. mandelii 89B-27	(89.83, 91.21)	(94.28, 95.15)***	(90.95, 92.04)

Values in the parentheses are back-transformed 95% confidence interval

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared with non-inoculated control of each experiment

Table 6 Water content of shoot, root, and whole plant of pepper at 4 weeks after inoculation with one of the three PGPBs or not inoculated

Experiment no.	Bacterial inoculant treatment	Water content (%)		
		Shoot	Root	Whole plant
5	Non-inoculated control	(86.12, 87.47)	(91.94, 92.58)	(88.06, 88.70)
	Azospirillum brasilense Cd	(87.41, 87.93)	(91.28, 91.94)	(88.38, 89.14)
	A. brasilense Sp 245	(86.39, 87.74)	(91.61, 93.20)	(88.26, 89.02)
	Azospirillum lipoferum Br 17	(86.73, 87.27)	(91.61, 92.27)	(88.26, 88.89)
6	Non-inoculated control	(86.94, 87.34)	(91.17, 91.72)	(88.19, 88.45)
	A. brasilense Cd	(86.46, 86.87)	(91.45, 92.00)	(87.74, 88.26)
	A. brasilense Sp 245	(86.60, 87.00)	(91.39, 92.37)	(88.06, 88.58)
	A. lipoferum Br 17	(87.93, 88.32)***	(93.20, 93.70)**	(89.45, 89.94)***

Values in the parentheses are back-transformed 95% confidence interval

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared with non-inoculated control within each experiment

Water content in plant tissues of four crop plants inoculated with PGPR/PGPB

The variations in growth promotion effects between fresh and dry weights could be explained by the inconsistent water content in plant tissues between non-inoculated control and inoculated treatments. Our results show that shoot, root, and/or total water content of the four tested crops varied between non-inoculated control and inoculated treatments in repeated experiments. Variation in water content

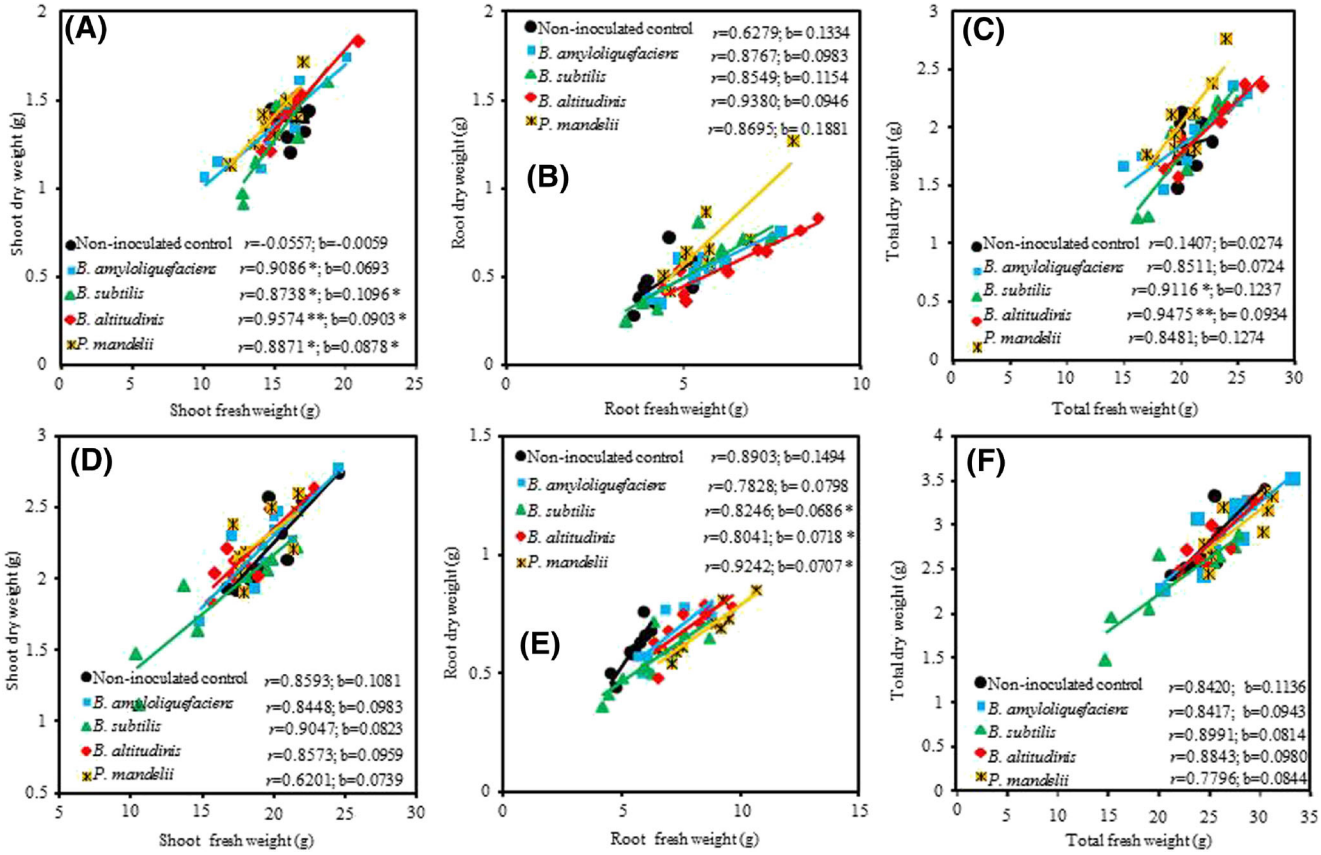

Fig. 1 Relationships between fresh and dry weights of shoot, root, and whole plant of corn at 4 weeks after inoculation with one of the four PGPRs or not inoculated in repeated experiments. **a** Relationship between shoot fresh and dry weights of corn in the first experiment. **b** Relationship between root fresh and dry weights of corn in the first experiment. **c** Relationship between total fresh and dry weights of corn in the first experiment. **d** Relationship between shoot fresh and dry weights of corn in the second experiment. **e** Relationship between root fresh and dry weights of corn in the second experiment. **f** Relationship between total fresh and dry weight of corn in the second experiment. *$p < 0.05$, **$p<0.01$, and ***$p<0.001$, compared correlation coefficients (r) and slopes (b) of the four PGPR treatments with non-inoculated control within each experiment

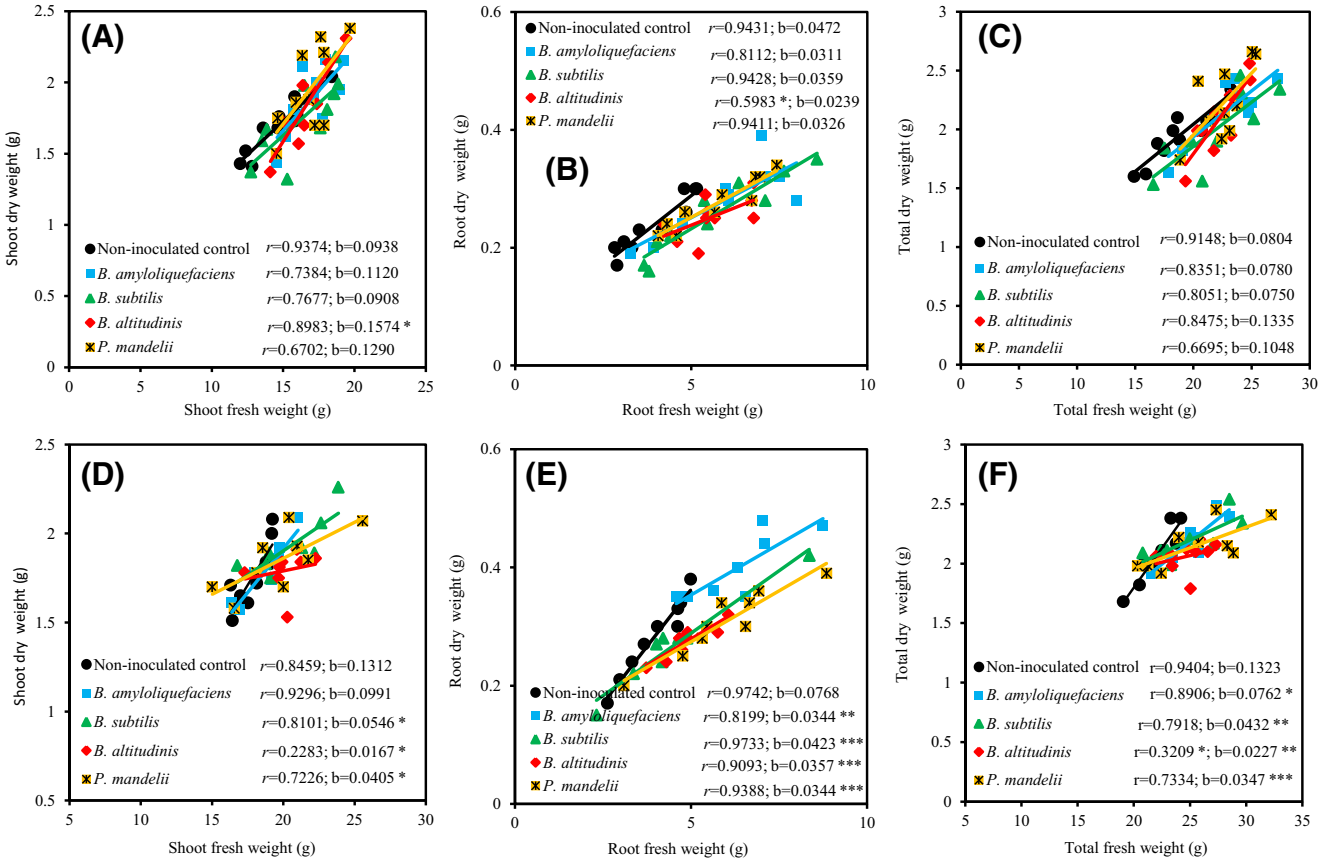

Fig. 2 Relationships between fresh and dry weights of shoot, root, and whole plant of cucumber at 4 weeks after inoculation with one of the four PGPRs or not inoculated in repeated experiments. **a** Relationship between shoot fresh and dry weights of cucumber in the third experiment. **b** Relationship between root fresh and dry weights of cucumber in the third experiment. **c** Relationship between total fresh and dry weights of cucumber in the third experiment. **d** Relationship between shoot fresh and dry weights of cucumber in the fourth experiment. **e** Relationship between root fresh and dry weights of cucumber in the fourth experiment. **f** Relationship between total fresh and dry weights of cucumber in the fourth experiment. $*p < 0.05$, $**p<0.01$, and $***p<0.001$, compared correlation coefficients (r) and slopes of the four PGPR treatments with non-inoculated control within each experiment

was not fully consistent with the variations between the treatments between fresh and dry weights (Tables 4, 5, and 6; Supplementary Table S2).

Correlation and regression analyses between fresh weight and dry weight of shoot, root, and whole plants

To further explain how the growth promotion effects by tested PGPR varied between fresh and dry weights when compared to non-inoculated control, correlation and regression analyses between fresh and dry weights of shoot, root, and whole plant were performed per experiment and per bacterial inoculant treatment, separately. Then, correlation coefficients (r) and slopes of inoculated treatments were compared with that of non-inoculated control to illustrate the relationship difference between fresh and dry weights. Our results show that correlation coefficients and/or slopes for predicting dry weight from wet weight of shoot, root, and/or total of the four tested crops varied between non-inoculated control and inoculated treatments in repeated experiments while not fully consistent with the variations between fresh and dry weights (Figs. 1, 2, and 3; Supplementary Fig. S1).

Discussion

The sole purpose of this study was to design a set of experiments specifically addressing evaluation of fresh weight and dry weight plant data, following inoculation of PGPR/PGPB on plants. A comparison was made over repeated, identically designed, eight greenhouse experiments comparing results of fresh and dry weights in response to inoculation This was done to determine which variables are more reliable and should be reported in future publications on this topic. Our study demonstrated that the growth promotion effects by the four PGPR strains in corn and cucumber and the three PGPB strains in pepper and tomato vary between fresh weight and dry weight of shoot, root, and/or whole plant. These results support other studies, where plant growth promotion effects by most of the tested PGPR or PGPB were also not consistent

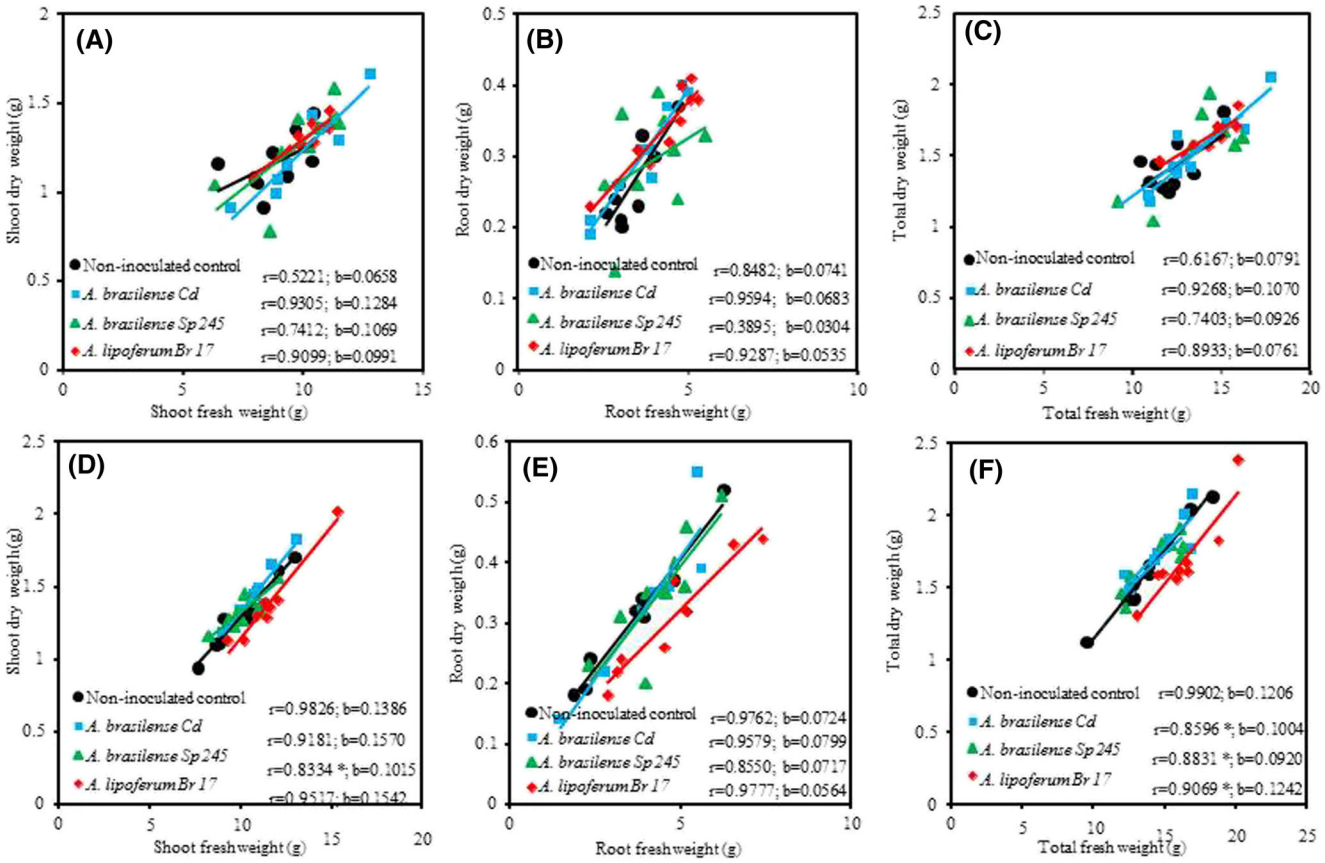

Fig. 3 Relationships between fresh and dry weights of shoot, root, and whole plant of pepper at 4 weeks after inoculation with one of the three PGPBs or not inoculated in repeated experiments. **a** Relationship between shoot fresh and dry weights of pepper in the fifth experiment. **b** Relationship between root fresh and dry weights of pepper in the fifth experiment. **c** Relationship between total fresh and dry weights of pepper in the fifth experiment. **d** Relationship between shoot fresh and dry weights of pepper in the sixth experiment. **e** Relationship between root fresh and dry weights of pepper in the sixth experiment. **f** Relationship between total fresh and dry weights of pepper in the sixth experiment. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, compared correlation coefficients (r) and slopes of the three PGPB treatments with non-inoculated control within each experiment

between fresh and dry weights (Abbasi et al. 2011; Arkhipova et al. 2007; Banchio et al. 2008; Çakmakçı et al. 2007; Chen et al. 2010; Gamalero et al. 2008; Ganesan 2008; Kumar et al. 2008; Ma et al. 2009, 2011; Rajkumar and Freitas 2008; Saravanakumar et al. 2011; Saravanakumar and Samiyappan 2007; Tank and Saraf 2010; Zahid et al. 2015).

This variation in growth promotion effects between fresh and dry weights could be explained by the inconsistent water content in plant tissues between non-inoculated control and inoculated treatments. Although not fully parallel with variations between fresh and dry weights, water content of shoot, root, and/or whole plant of the four tested crops varied between non-inoculated control and inoculated treatments in repeated experiments. This is probably caused by variations in environmental and technical factors, such as temperature, relative humidity, air currents, size of the experiment, and blotting excess moisture from tissues during harvest and/or preparation of tissues for weighting (Bashan and de-Bashan 2005).

The notion that water content is a major parameter in determining effects of PGPR/PGPB on plant growth is supported by this study. Our results show that in few tested PGPR, treatments that did not vary in growth promotion effects between fresh and dry weights had also statistically the same water content, correlation coefficients, and slopes when compared to non-inoculated control. This indicates that fresh weight determination for growth promotion effects by these PGPR/PGPB treatments can sometimes be as accurate as dry weight determination. This suggests that if the environmental and technical factors that affect fresh weight determination were minimum or were well managed, as done in our experiments and collection of data, the accuracy of fresh weight determination for plant growth promotion tests could be improved. With this exception notwithstanding, it is unlikely to eliminate the erroneousness of fresh weight determination for plant growth promotion tests demonstrated for most treatments.

Conclusions

Our study investigated the fundamental question whether or not fresh weight determination for plant growth promotion tests has

the same precision and is solid as dry weight determination. Our results showed that the growth promotion effects by the tested PGPR/PGPB, including *B. amyloliquefaciens* GB03, *B. subtilis* IN-937B, *B. altitudinis* INR7, and *P. mandelii* 89B-27 in corn and cucumber and *A. brasilense* Cd, *A. brasilense* Sp245, and *A. lipoferum* Br 17 in pepper and tomato, varied significantly between fresh and dry weight of shoot, root, and/or whole plant of corn, cucumber, pepper, and tomato in the repeated greenhouse experiments. This is explained by the variation in water content in plant tissues between non-inoculated control and inoculated treatments, which is probably caused by variations in environmental and technical factors that were documented in our previous study. In addition, comparisons of correlation coefficients and slopes reveal that the relationship difference between fresh and dry weights varied between non-inoculated control and inoculated treatments. Collectively, the results support our hypothesis that fresh weight determination for plant growth promotion tests is inherently faulty. Therefore, it is recommended that dry weight determination rather than fresh weight determination is used for plant growth promotion tests.

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