

A proposal for avoiding fresh-weight measurements when reporting the effect of plant growth-promoting (rhizo)bacteria on growth promotion of plants

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In the recent years, inoculation of plants with plant growth-promoting (rhizo)bacteria (PGPR/PGPB) has been increasingly reported in the scientific literature each year (Bashan et al. 2014; Calvo et al. 2014; Lugtenberg and Kamilova 2009). With this trend, a need arises to compare effects reported by different research groups on how these microorganisms affect plants. To streamline reporting procedures and to increase accuracy, *Biology and Fertility of Soils* initiated a series of policy “letters to the editor” aimed at guiding authors in the reporting of their results and also at avoiding potential methodological mistakes, including mistakes in methods that have already been published. For example, in 2013, a proposal for isolating and testing phosphate-solubilizing bacteria was presented (Bashan et al. 2013), and in 2016, the need for disclosure of the identity of microorganisms, constituents, and application methods when reporting tests with microbe-based or pesticide-based products was presented (Bashan et al. 2016). Following in this tradition, the current letter addresses the suitability of reporting fresh weigh measurements of shoots and roots as a means of assessing plant growth promotion capacity by plant growth-promoting (rhizo)bacteria.

While 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 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). The 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 assessing plant growth induced by PGPR/PGPB strains. In contrast, fresh weight is less precise because it is affected by numerous environmental and technical parameters, including relative humidity, laboratory temperature, air currents during exposure of excised plant parts prior to weighing each sample, the technique used to blot excess moisture from the washed roots and shoots, the total time from collecting and washing samples until weighing, the size of the experiment, and size of the pot (Bashan and de-Bashan 2005; Huang et al. 2016).

These two independent studies were specifically designed to compare fresh and dry weight determinations of the same treatment over variety of crops, bacterial strains, and environmental conditions. Our results of these studies show that fresh-weight determination is inherently faulty while dry weight is, by definition, accurate. This conclusion is based upon the finding that growth promotion effects by PGPR/PGPB are not statistically consistent between fresh and dry weight and that fresh weight lacks the same precision as dry weight.

In summary, we propose that in new manuscripts, only dry weight determination of shoot and roots should be used for plant growth promotion tests and that any reference to plant fresh weights should be deleted.

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