A bioassay screening *Trichoderma* isolates for enhancement of root development in *Impatiens walleriana* cuttings

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Abstract The potential of 62 *Trichoderma* spp. isolates to enhance root development on cuttings was evaluated in a screening bioassay using the ornamental plant impatiens *(Impatiens walleriana)*. Increased root development and consequent growth of cuttings induced by *Trichoderma* treatments were compared to that of a commercial rooting hormone (indole-3 butryic acid) and untreated cuttings. Results recorded after 3 weeks growth indicated sufficient resolution in the bioassay to detect statistical growth differences between treatments. As expected, treatment with IBA (positive control) enhanced root growth in root score, root dry weight, and root to shoot ratio parameters assessed across all three screening experiments. Six individual *Trichoderma* isolates and a commercial mixture of isolates were also identified as significantly improving root growth, with 20-65% increases in the measured growth variables relative to untreated cuttings. The bioassay provided an ideal system for measuring rooting response, and allowed accelerated screening of *Trichoderma* candidates useful for enhancing root development of cuttings.

Keywords *Impatiens walleriana, Trichoderma* spp., cuttings, propagation, bioassay, screening, root development.

INTRODUCTION

The beneficial plant growth promotion effects of *Trichoderma* spp. have the potential to augment plant growth in a commercial nursery setting (Hill et al. 2010). Improved vigour and rooting of economically important plant species has obvious benefits. Vegetatively propagated cuttings that form roots quickly exhibit better growth and more vigorous root systems, than those that are slower to root (Cameron & Thomson 1969). Applied aspects of enhanced rooting and cutting establishment result in maximised stock quality and volume, and decreased hormone, fungicide, and fertiliser inputs (Reglinski & Dick 2005). For horticultural and forestry industries, the ability to quickly produce high quality rooted cuttings whilst decreasing the cost of production can be made possible with *Trichoderma* (Hill et al. 2010).

Some *Trichoderma* isolates are known to enhance growth in a range of plant species. Plant species used to examine *Trichoderma* for increases in root and shoot biomass have included willow (Adams et al. 2007), cucumber (Chaur-Tsuen & Chien-Yih 2002), bean (Hoyos-Carvajal et al. 2009) and lettuce (Ousley et al. 1994). Success using

certain plant species and systems for identification of plant growth promoting *Trichoderma* is often counter-acted by huge resource inputs, spatial limitations, seasonal timing, screening resolution, labour intensity and cost-efficiency. For example, willow cuttings can only be sourced during winter and must be rooted for at least 35 days. Lettuce can only be used from seed or transplant, which prevents source stock from being kept, and new plants then have to be germinated for repeated assays. While these methods may have been successful under particular circumstances, the ability to screen numerous new *Trichoderma* isolates for root growth promotion potential with minimum resource input but high turnover requires refinement of the bioassay constraints.

The ability to effectively screen *Trichoderma* isolates for rooting enhancement properties is the first step in developing an industry product. A fast, effective and simple bioassay with the capacity to screen large numbers of isolates at once is required for this purpose. Easily measurable response variables must also be available to distinguish isolates responsible for improved rooting of cuttings. For the current experiment, the main objective was to develop such an assay using the flowering plant *Impatiens walleriana* Hook (Balsaminaceae), known for its rapid initiation of roots, and ease of culture. A pilot study was completed as a proof-of-concept in October 2009 and positive results led to further development of the bioassay. As a result, it was possible to screen a large number of *Trichoderma* spp. isolates from the Biocontrol Microbial Culture Collection (BMCC, Bio-Protection Research Centre) for their ability to produce increases in plant growth through enhanced root development. This paper presents the results of this bioassay.

MATERIALS AND METHODS

Preparation of *Trichoderma* inoculum

Sixty-two representative isolates of *Trichoderma* species from the BMCC (isolated from soil and roots from localities in New Zealand) were chosen for screening. Cultures were prepared on potato dextrose agar (PDA) (Difco; Sparks, USA) inoculated with a liquid spore suspension, then incubated at 20°C under fluorescent light until sporulation was visible (~7-14 days). The conidial inoculum for each *Trichoderma* isolate was harvested (five plates per isolate) by washing plates with 10 ml sterile distilled water (SDW) and straining the suspension through Mira cloth (22–25 µm) into a 50 ml Falcon tube. The concentration of each suspension was determined via a haemocytometer count and adjusted with SDW to produce the required concentration for inoculation.

Greenhouse assay

Apical cuttings of approximately 5-8 cm in length with between one and four nodes were taken at random from parent plants of *I. walleriana* cv. 'Accent Mystic' (approximately 5 months old), immediately prior to use. Cuttings were set into 550 ml planter bags (PB ¾), one cutting per bag, prepared with a medium of pumice, bulk peat and pine bark (30, 50 and 20%, respectively), and osmocote exact (15-4.0-7.5, 8-9 months).

Indole-3 butyric acid (IBA) (1:10 dilution of LIBA 10,000 at 10 g/litre) and thiram (12 g/litre) treatments were applied immediately prior to setting by way of bulk dip to the basal end of cuttings, for 1 and 4 min, respectively, as per the manufacturer recommendations. AborGuard™, a commercial *Trichoderma* mixture of five isolates in equal proportions, was applied at 1 g/1000 cuttings at 5.5×10^8 CFU/g in a 2 ml aliquot, after the cuttings had been set. *Trichoderma* isolates were applied direct to the potting mix as a spore suspension adjusted to 2.5×10^8 conidia in a 2 ml aliquot per cutting. Cuttings allocated to the untreated control treatment received an equivalent aliquot of tap water.

Following inoculation, the cuttings were maintained for 3 weeks under glasshouse conditions with an 11-12 h photoperiod, temperatures ranging 16-29°C, and twice daily watering. Flower buds were removed from cuttings at time of setting, and again after weeks one and two to encourage leaf and root development.

Each experiment was harvested 21 days after setting. Plant material was uprooted and washed, and the root system of each cutting visually scored for root growth and length. A visual assessment of

the root system enabled a root score to be assigned to each cutting based on a scale of 0-5 (Table 1). Measurement of the longest root was taken. Cuttings were then bagged and dried for 3 days at 65°C, excised at the root/shoot junction and weighed on an analytical scale. The dry weights of shoot and root biomass were recorded.

Experimental design and statistical analysis

Three experiments were conducted on separate occasions, in a randomised block design of ten blocks. Each of the experiments screened 20 different *Trichoderma* isolates (or mixtures of isolates in equal proportions from the BMCC) against controls. The number of replicates in each control treatment (untreated and IBA) was increased to four per block to increase the precision for comparison of each isolate treatment with the controls.

Data were analysed for significance by analysis of variance (ANOVA) and least significant difference (LSD) tests (GenStat, v12). Differences between treatment means and the control treatments with $P<0.05$ were accepted as statistically significant. Variables analysed were root dry weight, shoot dry weight, root to shoot ratio, root score (Table 1) and longest root length.

RESULTS

This bioassay was successfully used to screen large numbers of *Trichoderma* isolates for their ability to enhance root development. The results showed resolution in detecting differences between the negative and positive control treatments (i.e. IBA treated cuttings had significantly better growth than untreated cuttings) and between treatments and controls. Of the 62 *Trichoderma* isolates screened in the three experiments, six isolates significantly improved root or shoot growth when compared to untreated cuttings (Table 2).

As expected, cuttings treated with the commercial rooting hormone IBA exhibited increased root growth by 20-114%. Root dry weights were greatest with the IBA dip, significantly increasing by 92, 33 and 114% in each of the three experiments (Table 2). This resulted in large changes to the root to shoot ratio, while root score was also significantly higher for IBA treated cuttings compared to cuttings rooted without treatment (Table 2). IBA consistently promoted root growth of impatiens cuttings in terms of root score, root dry weight and root to shoot ratio.

Four isolates from the BMCC (IT257, IT160, IT363, IT167), one of which had been previously identified for root growth promotion, and two recently collected isolates (IT216, IT352) significantly promoted root growth relative to the untreated cuttings. IT160 significantly increased the longest root length of cuttings, root dry weight and root to shoot ratio by 20, 42 and 65%, respectively (Table 2). ArborGuard™, a commercial *Trichoderma* formulation utilised in forestry nurseries, gave a 48% increase in root score. Shoot dry weight was influenced by treatment with IBA, although two individual *Trichoderma* isolates from different experiments (IT167 and IT363) significantly enhanced shoot growth by 36 and 37%, respectively. Of all isolates identified as inducing positive root growth, only isolate IT160 demonstrated statistically greater effects than IBA (Table 2).

Table 1 Rooting criteria used for assigning a root growth score to impatiens cuttings.

Rating	Root Growth	
0	No root growth/callus only	no root growth
1	25% of quadrant filled with roots	beginnings of root growth
2	50% of quadrant filled with roots	substantial root growth
3	75% of quadrant filled with roots	significant root growth
4	100% of quadrant filled with roots	very significant root growth

Sample size calculations revealed that for each measured variable, except shoot dry weight, there was 100% power (two-sided test, 0.05 alpha) in detecting an increase between treatments that differed by the mean value, i.e. a 100% increase in growth. The chance of detecting a 50% increase in growth per variable was as follows: root dry weight 65%, shoot dry weight 6%, root to shoot ratio 60%, longest root length 100% and root score 84%.

DISCUSSION

In this impatiens bioassay, all root parameters, at the level of replication used, were effective in detecting large differences between treatments and identifying root growth-promoting isolates.

Table 2 Mean root score, longest root length (cm), root dry weight (g), shoot dry weight (g) and root to shoot ratio of impatiens cuttings at 3 weeks from setting for three separate experiments. Cuttings had been treated with various *Trichoderma* isolates[1] or commercial rooting hormone (IBA). Treatment means that differed significantly from the untreated control are indicated by bolding.

Treatment	Root score	Longest root length	Root dry weight	Shoot dry weight	Root to shoot ratio
Experiment 1					
Untreated	1.55	11.10	0.12	0.11	1.14
IBA	**2.62**	11.21	**0.22**	0.12	**1.94**
Trichoderma IT160	1.70	**13.30**	**0.17**	0.11	**1.89**
Trichoderma IT167	1.80	12.65	0.12	**0.15**	0.85
LSD (P=0.05)					
Isolate vs Untreated/IBA	0.48	1.79	0.05	0.03	0.55
IBA vs Untreated	0.30	1.13	0.03	0.02	0.35
Experiment 2					
Untreated	1.42	10.43	0.09	0.11	0.88
IBA	**2.27**	9.95	**0.12**	0.11	**1.27**
Trichoderma IT216	1.70	9.70	0.10	0.12	**1.30**
Trichoderma IT257	**2.20**	11.90	0.07	0.10	0.77
ArborGuard	**2.10**	9.40	0.10	0.11	1.10
LSD (P=0.05)					
Isolate vs Untreated/IBA	0.58	1.64	0.04	0.03	0.39
IBA vs Untreated	0.36	1.03	0.02	0.02	0.24
Experiment 3					
Untreated	1.27	6.14	0.07	0.13	0.64
IBA	**2.65**	**7.34**	**0.15**	0.14	**1.09**
Trichoderma IT352	**1.94**	6.40	0.07	0.11	0.87
Trichoderma IT363	1.40	6.50	0.09	**0.18**	0.64
LSD (P=0.05)					
Isolate vs Untreated/IBA	0.48	1.24	0.02	0.03	0.27
IBA vs Untreated	0.30	0.78	0.01	0.02	0.17

[1]Only those isolates that showed significant improvements in growth relative to the control are shown in this Table.

In addition, the bioassay provided a relatively quick method of assessment.

The bioassay was found to be successful in identifying differences between isolates and the untreated control, with increases in the measured root growth parameters. Measurement of the longest root length has previously been considered too time consuming for use in a bioassay (Davis & Jacobs 2005), although it should not be dismissed for it has value as a quantitative and morphological indicator of root growth as demonstrated in the present results. Chaur-Tsuen & Chien-Yih (2002) screened more than 2000 *Trichoderma* strains from Taiwan using cucumber, based on measuring the length of the longest root and the number of tap roots formed in 15 days, and were able to identify isolates that successfully promoted root growth. While measuring root and shoot dry weight is a monotonous task, it can be done relatively quickly, and is an efficient predictor of cutting vigour. Root to shoot ratio has given consistent results in predicting root system quality in relation to the plant shoot (Davis & Jacobs 2005). This was confirmed throughout the present bioassay experiments. Shoot dry weight was not a good indicator of root development as the two *Trichoderma* isolates that increased shoot dry weight did not simultaneously increase root growth parameters. Other researchers have also reported problems of accuracy and reliability with the use of the shoot dry weight as a predictor of plant performance (Davis & Jacobs 2005; Aalders et al. 2009).

One possible factor affecting root initiation in cuttings is the number of nodes on a stem inserted below the soil. Cuttings with more stem nodes have higher potential for root initiation compared to smaller cuttings with fewer nodes, as adventitious roots develop from stem primordia. The number of nodes was not taken into account in this study and the differences between cuttings size may have caused variable treatment performance between experiments. For example, a standardised commercial product like IBA would be expected to produce consistent results. However, root growth increases attributed to IBA of 92, 33 and 114% in each of the three experiments suggest a certain level of variation between experiments. If the number of nodes per cutting had been recorded and included as part of the analysis in this study, variation between experiments and within treatments could perhaps have been attributed to the use of smaller cuttings with less nodes, and therefore with less rooting potential.

Ten replicates were used to keep experimental units to a minimum for trial manageability, whilst allowing large differences between treatments to be detected. The effect size likely to be detected is an important consideration for screening bioassays where resources are limited and sample size fixed. As a minimum, isolates should be expected to perform at least 50% better than the mean of untreated cuttings. Defining applicable and accurate ranges of the parameters and effect size of interest, such as a cut-off for growth improvement that is 50 or 100% above that of an untreated cutting, ensures only isolates with significant root promotion potential qualify to proceed to further screening.

The 3-week duration of the experiments allowed for sufficient root system formation on impatiens cuttings, with rooting beginning 8-12 days after setting. This time period was sufficient too, for colonisation and sporulation of most *Trichoderma* isolates. A glasshouse experiment of 21 days duration may not have allowed establishment of slower-growing isolates. Further assays could investigate the effect of increasing trial duration.

Results from the bioassay confirmed that the response of impatiens cuttings to *Trichoderma* treatment was similar to that of IBA treatment, with increases in root growth parameters rather than shoot growth (Table 2). Applied synthetic auxins, such as IBA, are effective at inducing early and vigorous rooting of cuttings (Hussein 2008). A similar mechanism involving production of endogenous auxin, indole-3 acetic acid (IAA), has been suggested to be responsible for root growth stimulated by *Trichoderma* (Contreras-Cornejo et al. 2009). In light of this and other well-documented positive effects on plant health and growth, the benefits of using *Trichoderma*

isolates are of particular interest to the nursery industry. The potential implications of this research could mean that cuttings with poor rooting capability can be propagated much more easily. Rooting response and improvement of quality is of primary importance to commercial propagators of plant material. Time is lost when cuttings root poorly or fail to root at all, and this severely affects profits (Conover & Joiner 1963). The long-term aim of this research is to effectively screen *Trichoderma* isolates for root enhancement of cuttings, and develop potential candidates for plant growth promotion products that hasten the rooting process and improve cutting establishment for nursery grown species. Increased vigour of rooted cuttings results in optimised propagation with reduced fungicide inputs, improved quality and faster stock turnout (Menzies et al. 2001), which are valuable both to forestry and ornamental nurseries.

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