Variation in the rooting of mung bean (Vigna radiata) seedling stem cuttings

P.J. Wilson, H.M. Dicks and J. van Staden

Introduction

Mung bean [Vigna radiata (L.) Wilczek] seedling stem cuttings are easy to culture, uniform in appearance and able to root in aqueous solution, and have been widely used in rooting bioassays (Wilson & Van Staden 1990) and in other studies of adventitious rooting (Jarvis & Ali 1985; Jarvis & Shaheed 1986). While the validity of the traditional bioassay is open to question (Wilson & Van Staden 1990, 1991), more critical rooting studies in mung bean are likely in view of the increasing importance of vegetative propagation.

Sources of variation which have been recognized in mung bean propagation trials, and which are generally kept constant if possible, include the quantity of boron in the test solution (Middleton et al. 1978), the length of the hypocotyl retained on the cutting (Hess 1964), the age of the assay plants (Blazich & Heuser 1979) and the environment in which the assay plants are grown and tested. Nevertheless, rooting varies from cutting to cutting within a trial and the results of repeated trials can differ appreciably (Wilson & Van Staden 1991).

In this paper some further sources of variation in mung bean cuttings are evaluated, and the statistical properties of mung bean root counts are described, in order to assist the design and analysis of future propagation trials.

Materials and Methods

Mung bean seeds were surface-sterilized in 3% hypochlorite solution for 10 – 20 min, then rinsed and soaked in tap water for 4 – 8 h. They were sown in vermiculite at a spacing of 1.8 × 1.6 cm (3 cm² per seed) or at other specified spacings (see below). The seedlings were cultivated in a constant-environment cabinet at 25°C with 20 h of light (250 μmol m⁻² s⁻¹). Bean seedlings were harvested when the cotyledons had begun to abscise and could be dislodged without injury to the stem. (This was done so that the cotyledons would not drop into and contaminate the test solutions.) Unless otherwise stated, cuttings were severed at the root–shoot junction, put in vials (5 × 2 cm i.d.), filled to 4 cm with tap water, and returned to the cabinet under the conditions specified for seedlings. Vials were topped up with de-ionized water every 24 h and roots were counted after 7 days. Vials were placed at 4.4 × 4.4 cm in randomized complete blocks, except in trials 2A and 2B which were simply randomized trials. In trials 1A, 1B, 3A and 5, analyses of variance were nested: treatments, vials within treatments, and cuttings within vials, so that variation due to blocks and vials was confounded. Rooting ability was recorded as roots per cutting. In the standard regime described above, no cuttings failed to root.

Trials 1A and 1B

1A. The proximal ends of freshly harvested cuttings were placed in solutions of catechol in tap water: at 0, 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶ or 10⁻⁷ M and incubated for 24 h. They were then rinsed and placed in de-ionized water. There were 3 vials per treatment (i.e. 3 blocks) and 6 cuttings per vial. 1B: The trial was repeated except that there were 3 vials per treatment and 8 cuttings per vial.

Trials 2A and 2B

2A. The air-dry weights of 48 seeds were recorded before sowing at 2 × 2 cm spacing (within two surround rows). At harvest, fresh weights and hypocotyl lengths of the cuttings were also recorded before they were placed 1 per vial. The three variables, seed weight, fresh weight and hypocotyl length, were related to the untransformed number of roots per cutting in simple linear regressions. Seed weight was also related to cutting fresh weight. 2B: The trial was repeated except that the beans were sown at 1 × 1 cm².

Trials 3A and 3B

3A. The rooting ability of cuttings severed at the root–shoot junction (mean hypocotyl length 6.2 ± 0.1 cm) was compared with that of cuttings severed 3 cm below the insertion of the cotyledons. There were 6 vials per treatment, 8 cuttings per vial. 3B: Seeds were sown broadcast at a high mean density and the basal cut was made either at the root–shoot junction, 2 mm below, or 2, 4, 6 to 16 mm above. There were 10 vials per treatment, 5 cuttings per vial.
Trial 4
Beans were sown at spacings to give areas per bean of 0.125, 0.25, 0.5, 1, 2, 4 and 8 cm² (a logarithmic series). Only non-edge cuttings were harvested. There were 12 vials per spacing, 3 cuttings per vial. Five leaves per treatment (1 leaf per cutting) were harvested from surplus plants to estimate mean leaf area.

Trial 5
After severance, cuttings were allowed to dry out on a laboratory bench for up to 5 h at 29°C before being rehydrated and placed in vials. There were 9 treatments (0, 1, 2, 2.5 to 5 h), 3 vials per treatment and 6 cuttings per vial.

Trial 6
Mung bean cuttings take up water through their bases when immersed. Water level (tap water topped up with tap water) was maintained at the depths 5, 10, 15 to 1 mm by putting only 1 cutting per vial and topping up frequently. There were 16 vials per depth. Leaf area per cutting was measured and roots per cutting counted in 64 cuttings in eight blocks.

All trials
Root counts were log-transformed [if necessary: \( Y = \log (X + 1) \)] before analysis, but untransformed means are given in the text. \( F \) values in the text were significant at the \( P < 0.01 \) and **0.005 levels.

Results
Trial 1
In an analysis of variance of trials 1A and 1B combined, rooting was significantly affected by the concentration of the treatment solution (\( F = 23.6^{**} 6, 24 \) df) and was highest in catechol at \( 10^{-3} \)M in both trials (Table 1). However, cuttings had higher overall rooting ability in trial 1A, and the variation due to occasion (\( F = 84.2^{**} 1, 4 \) df) and the occasion x treatment interaction (\( F = 14.4^{**} 6, 24 \) df), were both highly significant. The log transformation stabilized the variance, as reflected in the SE (means), with the possible exception of the highest concentrations, in which there was a tendency for rooting to be promoted in some cuttings within a treatment but inhibited in others. In trial 1A the range in the number of roots per cutting was 7 – 32 at the weakest concentration of catechol and 1 – 46 roots per cutting at the strongest. In trial 1B the equivalent ranges were 4 – 19 and 2 – 33 roots per cutting. The variation due to vials (confounded with blocks) was not significant in either trial 1A (\( F = 0.3; 14, 105 \) df) or trial 1B (\( F = 1.0; 14, 147 \) df).

Table 1 Untransformed and log-transformed mean numbers of roots per mung bean cutting, and the standard errors of the means based on the log-transformed data

| Trial | 10⁻³ | 10⁻³.25 | 10⁻³.5 | 10⁻⁴ | 10⁻⁴.3 | 10⁻⁵ | C |
|-------|------|--------|-------|------|--------|------|---|
| Untransformed mean number of roots per cutting | | | | | | | |
| 1A | 18.9 | 24.0 | 29.8 | 21.2 | 17.9 | 17.8 | 19.2 |
| 1B | 15.9 | 17.2 | 17.5 | 6.1 | 6.6 | 6.0 | 9.8 |
| Log-transformed mean number of roots per cutting | | | | | | | |
| 1A | 2.42 | 3.03 | 3.32 | 2.99 | 2.78 | 2.79 | 2.86 |
| 1B | 2.62 | 2.77 | 2.72 | 1.73 | 1.84 | 1.72 | 2.25 |
| Standard errors of the mean (transformed data) | | | | | | | |
| 1A | 0.312 | 0.137 | 0.086 | 0.084 | 0.121 | 0.100 | 0.112 |
| 1B | 0.131 | 0.079 | 0.110 | 0.085 | 0.062 | 0.077 | 0.054 |

The cutting bases were immersed for 24 h in aqueous solutions of catechol at the concentrations \( 10^{-3} \) – \( 10^{-5} \)M before being transferred to water. Control (C) cuttings were in water throughout. The trial was conducted on two occasions (trials 1A and 1B) with 18 and 24 cuttings per treatment, respectively.

Trial 2
In trial 2A individual seed weight, cutting fresh weight and hypocotyl length were unrelated to the untransformed numbers of roots per cutting in simple linear regressions (\( r^2 = 0.01, 0.02 \) and 0.00, respectively; 47 df), while seed weight and cutting fresh weight were well related (\( r^2 = 0.79^* 47 \) df). At a higher sowing density (trial 2B) virtually identical results were obtained. In both trials 2A and 2B there were 13.1 roots per cutting, and coefficients of variation (CVs) were 32 and 33% respectively (untransformed data), and 12 and 13% respectively (transformed data).

Trial 3
In trial 3A, reducing the length of the hypocotyl from 6.2 to 3.0 cm increased the mean number of roots per cutting from 14.6 to 17.6 (\( F = 7.1^{**} 1, 80 \) df), while the CVs in the full-length and reduced cuttings were similar, at 14 and 12% respectively (transformed data). The variation due to blocks/vials was not significant (\( F = 0.5; 14, 80 \) df). In trial 3B, in which seedlings were grown at a higher mean density, reducing the hypocotyl length of cuttings by increasing the height of the severance cut (\( H \)) tended to have the opposite effect, reducing the mean number of roots per cutting (\( N = 7.34 - 0.19 H; r^2 = 0.86^* 9 \) df). The predicted number of roots was 7.3 when cuttings were severed at the root–shoot junction and 4.3 when severed 16 mm above. The decrease in rooting ability was partly due to an increase in the percentage of cuttings, from 0% (root–shoot junction) to 20% (height 16 mm), which failed to root, and this also resulted in an increase in the CVs of roots per cutting from 36 to 65% (untransformed data) and from 17 to 60% (transformed data).

Trial 4
From the highest to the lowest sowing density \( (S) \) the mean number of roots per cutting \( (N) \) increased from 5.7 to 7.6 roots per cutting \( (N = 6.40 + 0.94 \log S; r^2 = 0.85^* 5 \) df). The CVs (untransformed data) decreased with decreasing sowing density from 60% \( (0.125 \) cm² per bean) and 64% \( (0.25 \) cm² per bean) to 24% \( (8 \) cm² per bean), and from 30 and 25% to 10% (transformed data). The ranges in the numbers of roots per cutting were 4 – 12 at the spacings giving 4.0 – 8.0 cm² per bean \( (n = 72) \) and 0 – 24 at the spacings giving 0.125 – 0.25 cm² per bean \( (n = 72) \). Two cuttings in the latter group failed to root. Cuttings grown at the highest densities were more spindly than the more open-grown cuttings. Mean leaf area per plant at harvest (two fully developed primary leaves) was 10.6 ± 0.6 cm².

Trial 5
Temporary dehydration of cuttings, for 2 to 5 h after severance, increased the number of roots per cutting from 6.2 roots (control) to 10.2 – 14.1 roots \( (F = 5.5^{**} 8, 135 \) df). There was no trend in time in rooting beyond 2 h. Rooting in cuttings dehydrated for only 1 h \( (6.8 \) roots per cutting) was similar to the control. The variation due to blocks/vials was not significant \( (F = 1.0; 18, 135 \) df).

Trial 6
The depth of immersion of the base of the cutting had no effect on roots per cutting \( (F = 0.8; 7, 105 \) df), the eight depth means ranging from 7.6 to 8.9 roots per cutting. Variation due to blocks was not significant \( (F = 0.9; 15, 105 \) df). There was no linear relationship between leaf area per cutting and the number of roots per cutting \( (r^2 = 0.02; 63 \) df).

All trials
In treatments representing the standard culture regime in all trials (except 3B which was sown broadcast), the mean number and standard deviation of roots per cutting, unpoole
trials, was 11.3 and 3.760 (untransformed data) and 2.3 and 0.327 (transformed data). To detect a true deviation from the mean of 10%, if it existed, would require 88 ($P < 0.05$) or 150 ($P < 0.01$) cuttings per treatment without transformation, or 18 and 31 cuttings, respectively, with transformation (Freese 1967). In these treatments and trials the mean CV was 33% (untransformed data) and 14% (transformed data).

**Discussion**

Poapst et al. (1967) and Bassuk and Howard (1981) reduced variation in root counts of *Phaseolus vulgaris* L. and mung bean by reducing the mean number of roots per cutting. They used hypocotyls instead of leafy cuttings, and removed the cotyledons before the primary leaves had fully expanded, respectively. However, the means and standard deviations of mung bean root counts are more or less linearly related (Wilson & Van Staden 1991), so reducing the mean need not increase the sensitivity of an experiment. Correlation between means and standard deviations of mung bean root counts was overcome by using the log transformation (Jackson & Harney 1970; Wilson & Van Staden 1991) and, in this study, the log transformation at least halved coefficients of variation of roots per cutting. However, variation was still relatively high in presumably injurious treatments (trials 1A, 1B, Table 1), which promoted rooting in some cuttings but was prejudicial in others, and the transformation failed to stabilize the variance when many cuttings failed to root (3B).

Parent plants and cuttings were propagated in an environment cabinet in which there was no significant spatial variation (due to blocks) within a trial, or due to vials when there were 6 – 8 cuttings per vial. However, variation due to occasion was considerable (cf. 1A and 1B), suggesting that replication in time would be advisable, at least when the environment cannot be fully specified (as in the present study). In the traditional practice of testing eluates from a chromatogram in a bioassay the variation between chromatograms ('occasions') was also very large (Walker et al. 1958).

Variation in rooting between cuttings (genotypes) within a trial was high but could not be predicted from individual morphological traits. The number of roots per cutting was unrelated to the weight of the seed the cutting had grown from, cutting fresh weight, hypocotyl length (2A, 2B) or leaf area (6). Seed weight and cutting fresh weight were well related because cuttings were made when the cotyledons had shrivelled completely so that differences in the initial weight of the seeds, mostly cotyledonary tissue, were largely translated into differences in the fresh weight of the cuttings. In contrast, the rooting ability of cuttings with only slightly shrivelled cotyledons increased with seed weight both within and between seed lots (Bassuk & Howard 1981). In these cuttings, those with larger cotyledons might have sustained themselves better, resulting in more rooting, particularly as at that stage the primary leaves were probably not fully developed. Bassuk and Howard (1981) noted that the relationship between seed size and subsequent rooting ability was much weaker if the cotyledons were removed from such cuttings.

Reducing the hypocotyl length of the cutting either had little effect on the coefficient of variation of root counts in robust cuttings (3A), or increased it in spindly cuttings (3B). Cuttings should therefore be severed at the root–shoot junction (the easier practice) rather than at a fixed distance below the insertion of the cotyledons, as was recommended by Hess (1964). Reducing hypocotyl length increased rooting in robust cuttings, possibly because mutual shade decreased (Jarvis & Ali 1985). The shorter cuttings protruded less from their vials and their leaves were therefore more densely clustered. Mutual shade in the intact seedlings, when grown at close spacings, may also have increased rooting in a small proportion of cuttings (4).

Cuttings withstood temporary dehydration after severance (5) and were insensitive to variation in the depth of the liquid in which their bases were immersed (6). In trials/treatments representing the standard culture regime, the mean number of roots per cutting and mean coefficient of variation (transformed data) was 11.2 roots and 13% when there was one cutting per vial (2A, 2B, 6), and 12.5 roots and 15.5%, respectively, when there were 6 – 8 cuttings per vial (1A, 1B, 3A, 5).

Thus, there was a small difference (if real), given that, when there were 6 – 8 cuttings per vial, total leaf area was 3 – 4 times the ground area (leaf area per cutting, 10 cm²; area per vial, 19 cm²). In parent plants even more leaf overlap (at 1 cm² per plant; cf. 2A and 2B) did not result in an increase in the coefficient of variation, but there was a steep increase at spacings closer than this (4).

In designing mung bean propagation trials some latitude in the culture of the parent plants and cuttings is possible, but high sowing densities and broadcast sowing should be avoided. When all cuttings root, 'roots per cutting' is a satisfactory criterion of rooting ability which can be log-transformed to increase the sensitivity of statistical analyses. When many cuttings fail to root, separate analyses of the proportion of cuttings which rooted and the number of roots per rooted cutting should be considered. In the seed source used in the present study there was high individual variation in rooting. Genetic variation could be eliminated by clonal propagation, but other sources of variation would then operate between individual parent plants within the clone, between shoots within plants, etc., whose magnitude is unknown.

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