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Factors affecting the use of mung bean (*Vigna radiata* L. Wilczek) cuttings as a bioassay for root-promoting substances

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SUMMARY

Cotyledons were shown to be a major source of inherent root promotion in mung bean cuttings. Cuttings from seeds of different size and seedlings of different ages therefore gave a variable rooting response in bioassays for root-promoting activity. A consistent and sensitive bioassay was achieved by removing the cotyledons from germinating seedlings four days after sowing.

INVESTIGATIONS into the endogenous factors controlling the rooting of cuttings require a sensitive, uniform and quick test to measure root-promoting or inhibiting activity in plant extracts. It is desirable that a rooting bioassay, in which a solution of the extract is applied to cuttings whose resulting root development acts as a measure of extract activity, uses cuttings that are readily available, uniform in growth and easily handled. Especially important is the need for a consistent and sensitive response with treatment effects unobscured by inherently high or variable background rooting in the bioassay.

Following early work by Went (1934) using etiolated pea cuttings and by Hemberg (1951) using *Phaseolus vulgaris*, Hess (1957) developed the forerunner of the bioassay most widely adopted today using cuttings prepared from uniformly germinating and quick-growing mung bean (*Vigna radiata* L. Wilczek) seedlings. His original bioassay used five-day-old etiolated seedlings, the cotyledons and small leaves being removed 24 h before harvest to reduce their content of endogenous rooting factors. Seven-cm lengths of hypocotyl were then harvested and placed in vials containing test solutions for between 24 and 92 h before being moved into distilled water for the remaining 5-d rooting period. Using this test, Hess (1962) recorded between 9 and 18 roots per cutting after treatment in a test solution of 5×10^{-6} M IAA.

In later tests for rooting cofactors of IAA 9- to 10-day-old light-grown seedlings were used. Cuttings made from these consisted of 3 cm of hypocotyl, the epicotyl, the primary leaves and a trifoliate bud; cotyledons were removed if they had not already abscissed. The number of roots produced per cutting in a solution of 5×10^{-6} M IAA varied between 18 and 30 (Hess, 1964a) or 10 and 16 (Hess, 1964b).

In addition to the somewhat variable results obtained by Hess, Munoz and Villalobos (1977) reported a background level of rooting in mung beans ranging from 5 to 22 roots per cutting. Fadl and Hartmann (1967) recorded between 10 and 21 roots for cuttings rooted under similar conditions.

Using *Phaseolus vulgaris* 'Contender' cuttings, Poapst *et al.* (1967) successfully reduced the inherent variability in root numbers by removing epicotyl, leaves and cotyledons, leaving only the hypocotyl. Normal leafy cuttings had an average root number of 24 and a standard deviation of 11, whereas hypocotyl cuttings rootes very uniformly with 5 ± 2 roots per cutting.

There have been few attempts to reduce the variability and inherent rooting level in the mung bean bioassay. Heuser and Hess (1972) decapitated etiolated seedlings 12 to 48 h prior to using them but resulting root numbers were still

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very high in the presence of 10^{-5} M IBA with more than 40 roots per cutting after 24 h prior decapitation.

The following experiments were aimed at identifying and eliminating the sources of natural variability between cuttings, thus increasing both the uniformity and sensitivity of the mung bean bioassay response.

MATERIALS AND METHODS

Mung bean seeds were sown thickly over a 3-cm bed of vermiculite and barely covered to about their own depth. A typical sowing procedure involved 25 ml of dry seeds spread in a seed tray measuring 25 × 20 × 4 cm. Trays were watered to saturation with tap water, covered with a glass sheet which was removed after three days and then placed under warm white fluorescent tubes at 700 lm ft^{-2} in a 16-h photoperiod (21.2 W m^{-2} PAR); ambient air temperature was *c.* 23°C.

After the required number of days from sowing, uniform seedlings were selected from the tray and the hypocotyl cut with a razor blade 4 cm below the cotyledonary node. Four cuttings were placed in a 5-ml vial containing between 4 and 5 ml of the test solution which was replenished with distilled water every three days. All roots and visible root primordia were counted and other effects noted after seven days.

RESULTS

Sources of variability

Seed source

A comparison of seeds from six sources obtained from retail shops and seed houses showed considerable variation in weight (Table I). When cuttings from these seed sources were rooted in the presence of the ammonium salt of IBA at 10^{-5} M (2 ppm) a clear positive linear relationship (regression $y = 1.597 + 0.117x$, $P < 0.001$) between seed weight and $\sqrt{\text{roots}}$ per cutting was obtained (Figure 1).

The largest proportion of the seed mass is cotyledon tissue, being from 81% to 84% of the total dry weight in small and large seed sources

TABLE I
Comparison of mung bean seeds from six sources

Source	Weight of 500 seeds (g)					
	A	B	C	D	E	F
	28.0	21.2	17.1	19.4	26.3	18.0

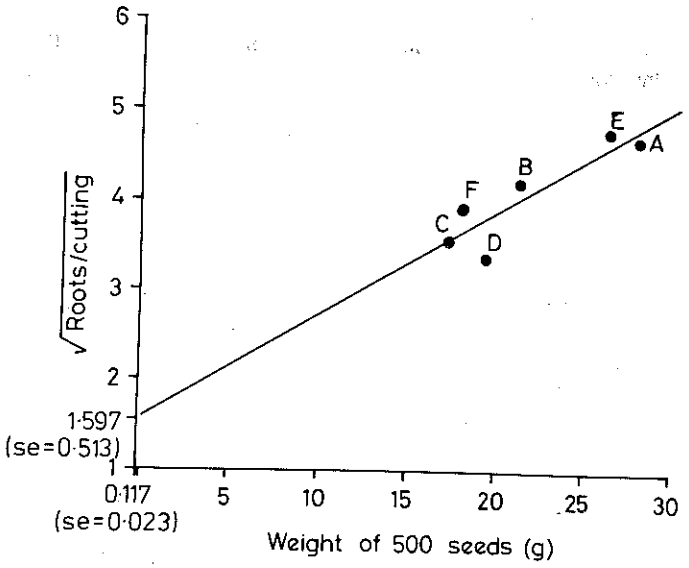

FIG. 1
Relationship between mung bean seed weight and rooting of cuttings

respectively. Therefore, the effect on rooting of removing cotyledons when preparing four-day-old cuttings was tested as a means of reducing the variability found in different seed sources. The removal of cotyledons greatly reduced root numbers and a significant interaction ($P < 0.001$) showed that rooting differences between seed sources in the presence of cotyledons (root number range 11.8 to 24.0) were much greater than those when cotyledons were removed (root number range 4.3 to 6.9) (Figure 2).

To investigate further the relationship between seed size and cotyledon removal the obviously larger and smaller seeds from two distinctly different sources (E, large and C, small) were separated visually from the remainder of the pop-

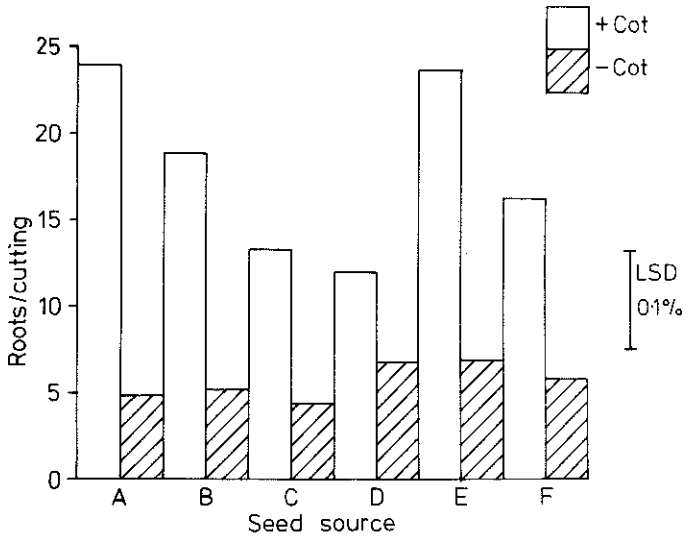

FIG. 2
Rooting of mung bean cuttings with and without cotyledons removed (+ 10^{-5} M IBA).

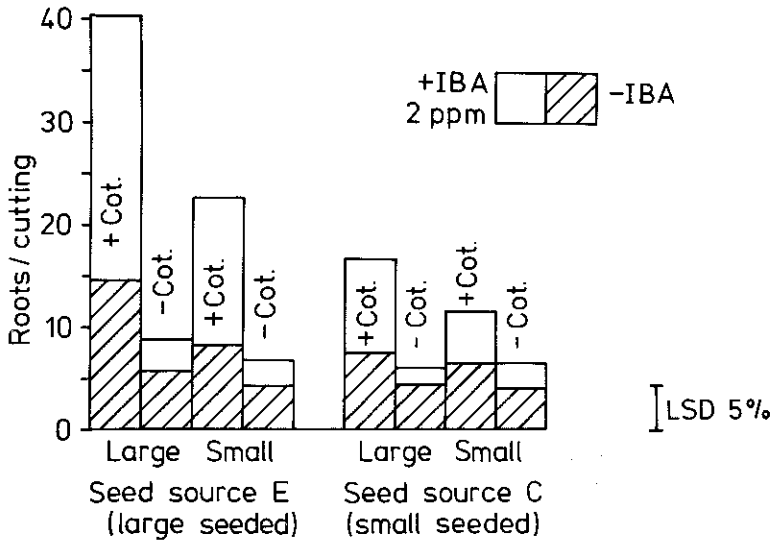

FIG. 3
Effects upon rooting of mung bean cuttings of different seed size within and between sources in the presence or absence of cotyledons and IBA (10^{-5} M).

ulation and the resulting cuttings from larger and smaller seeds within each seed source were rooted with and without cotyledons and IBA. The effect of seed size on rooting was similar both within and between seed sources, but was nearly eliminated when either the cotyledons were removed or IBA omitted (Figure 3). IBA interacted with the cotyledons synergistically to promote rooting.

Age of cutting

The effect of age (in days from sowing) of the seedlings before they were made into cuttings was investigated. Cuttings from seedlings 2, 3, 4, 5 and 7 days from sowing were compared after a seven-day rooting period in the presence and absence of IBA (Figure 4). There was considerable difference in rooting with difference in age especially between three- and four-day cuttings which gave 23 and 36 roots respectively. Days two to four span a period of rapid growth of the seedling from a light green 4-cm 'hook' with embryonic leaves and large cotyledons to a straight 8-cm dark green seedling with expanded primary leaves and smaller slightly shrivelled cotyledons.

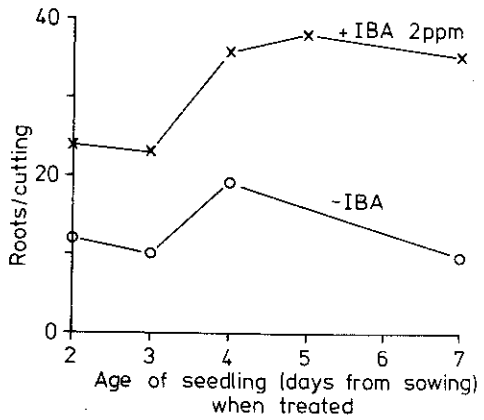

FIG. 4
Effects of mung bean cutting age on rooting in the presence or absence of IBA (10^{-5} M).

The effect on rooting of rapidly changing cotyledon and leaf condition with increasing seedling age was investigated in the presence of 2 ppm IBA. During the seven-day time span of the experiment leaves grew rapidly and their removal from cuttings of increasing age showed the converse effect from that of cotyledon removal. Leaf removal from two-, three- and four-day-old cuttings did not depress rooting, but for five-day and older cuttings their removal depressed rooting significantly as did removal of cotyledons from younger cuttings (Figure 5). It is also apparent that when leaves or cotyledons showed the greatest root promotion, they also increased the standard error and thus the variation within the test.

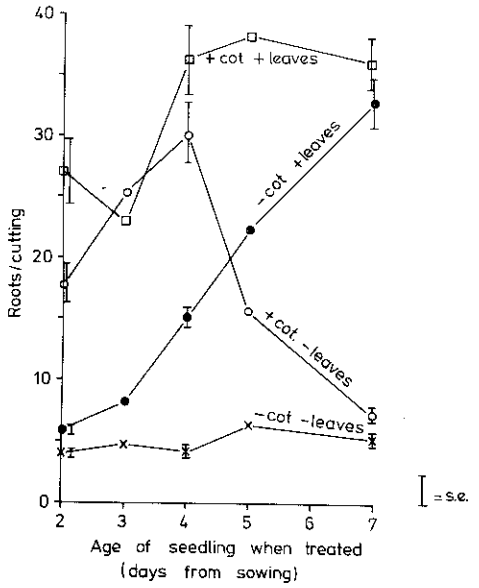

FIG. 5
Effect of cotyledon and leaf removal at different times on rooting of mung bean cuttings (+ 10^{-5} M IBA).

Testing the sensitivity of 4-day-old cotyledonless cuttings

The aqueous residues of methanol extracts from two-day-old mung bean cotyledons were tested on four-day-old mung bean cuttings without their cotyledons as well as other cotyledonless cuttings. It was confirmed that cotyledons contain root-promoting substances and that young cotyledonless cuttings are sensitive to their presence (Table II).

A further indication of the increased sensitivity of four-day-old cotyledonless cuttings was obtained by comparing cuttings with and without cotyledons in the presence of substances shown to be root promoting (Bassuk *et al.*, 1981). IBA (10^{-5} M), phloridzin (2×10^{-3} M) and polyphenol oxidase enzyme (PPO, 400 units) were tested in all combinations on

cuttings with or without cotyledons. In the presence of cotyledons (Figure 6), the addition of PPO (column 14) to IBA and phloridzin (column 12) did not give significantly more roots per cutting. However, in cuttings without cotyledons (columns 11 and 13) the addition of PPO caused a significant difference ($P < 0.001$).

DISCUSSION

Both the level of rooting and the differences between seed sources were greatly reduced after cotyledon removal. This indicates that the cotyledons were a major source of endogenous root promoters and suggests that their varying sizes, and thus by implication their

TABLE II

The effect of mung bean cotyledon extract on rooting in mung bean, French bean and cucumber

	Mean root No per cutting		
	Mung bean	French bean	Cucumber
Control (10^{-5} M*)	7.9	39.7	31.7
12 cotyledon-equivalent extract + IBA 10^{-5} M	24.9	77.3	52.3
LSD ($P < 0.001$)	7.98	38.2	10.3

*2 ppm

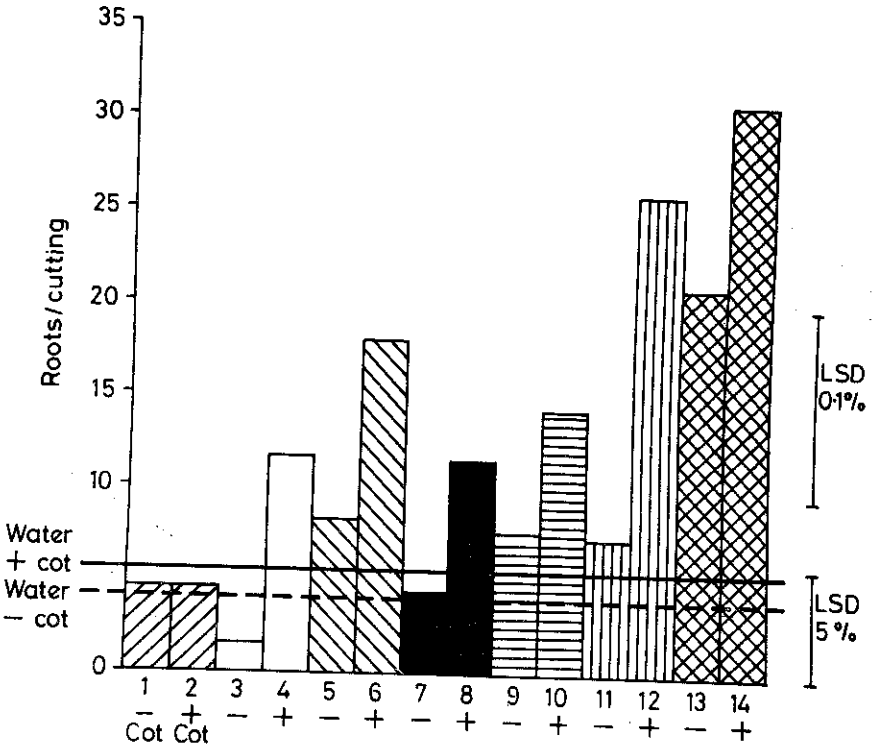

FIG. 6

Rooting response of mung bean cuttings with and without cotyledons to IBA, PPO and phloridzin

1 & 2 PPO 400 units
3 & 4 Phloridzin 2×10^{-3} M
5 & 6 IBA 10^{-5} M
7 & 8 PPO + phloridzin
9 & 10 PPO + IBA
11 & 12 IBA + phloridzin
13 & 14 IBA + PPO + phloridzin

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to rooting factors (cuttings with both cotyledons and leaves removed responded poorly). This was effectively achieved by removing cotyledons from seedlings only four days after sowing and using the cuttings at that stage. At this point the majority of hypocotyls were just straightened out, the entire seedling being about 8 cm tall. Epicotyls should be no longer than 0.5 cm with primary leaves not yet fully expanded and held in a near-vertical poise.

The presence of partly-developed transpiring leaves can be expected to assist in the uptake of the test solution. Such cuttings, when raised from medium-sized seeds, rooted in distilled water consistently producing between four and five roots per cutting while cuttings given IBA at 2 ppm produced between seven and 11 roots per cutting.

These results are taken from a thesis by Nina L. Bassuk, accepted by London University in partial fulfilment of the Ph.D. degree.

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