

IN VITRO RESPONSE OF BEAN (PHASEOLUS VULGARIS L.) COTYLEDONARY EXPLANTS TO BENZYLADENINE IN THE MEDIUM

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In vitro production of plantlets derived from excised cotyledons in common bean (Phaseolus vulgaris L.) has not been reported. This study was initiated to investigate the benzyladenine (BA) promotion effect and the potentiality of shoot production from common bean cotyledonary explants from different stages of the seeds. A second objective was to develop a scheme for plantlet production in vitro from different genotypes.

Four dry bean genotypes, 'Harris' Great Northern (GN), 'UI 59', Xan 159 and 'PC 50' were grown in the greenhouse in pots containing 1.8 liter of a 2:2:2:1 soil: sand: sphagnum moss peat: vermiculite medium. Pods were taken after 10-12, 21 and 28 days after pollination. The pods from each stage were surface-disinfested for 15 min. in 0.53% NaOCl before removing the seeds. The basal one-third of the cotyledons, including the proximal notch, was used as the explant. The explants were cultured adaxial side down on the surface of Gamborg's B<sub>5</sub> agar medium containing 0, 5, 10 or 20  $\mu$  M BA. Cultures were incubated at 26°C under continuous light of 26  $\mu$  Mol S<sup>-1</sup> m<sup>-2</sup> from cool-white fluorescent tubes. After 4 weeks of the culture, shoots >1cm were cut and transferred to B<sub>5</sub> medium without BA (OB<sub>5</sub>) for rooting while the multiple buds with shorter shoots were transferred to a second B<sub>5</sub> medium either without BA or with 1  $\mu$  M BA. Data were recorded for percent of explants initiating shoot buds and number of shoots > 1 cm for both the first culture after 4 weeks and the second medium after 3 weeks.

The cotyledonary explants from seeds taken from 10-12 day-old pods developed callus on BA containing medium and no shoots were initiated either on medium with or without BA. The 21-day explants only formed roots on the medium without BA (OB<sub>5</sub>), although 'UI 59' also produced shoots on 30% of the explants. However, all genotypes initiated shoots and roots on OB<sub>5</sub> medium from the 28-day explants (10-50%). On the medium with 5  $\mu$  M BA, 20-50% of the explants initiated shoots in all genotypes from 21-day explants with 'UI 59' exceeding its response on OB<sub>5</sub> medium. 'Harris' exhibited the greatest value (40%) for shoot initiation on medium containing 5  $\mu$  M BA, while 'UI 59' and Xan 159 produced the most shoots on explants (65 and 70%, respectively) on medium containing 20  $\mu$  M BA. 'PC 50' did not show a clear effect of the medium on shoot formation. For the 28-day explants, the maximum shoot formation for 'Harris' and 'UI 59' occurred with only 5  $\mu$  M BA (85 and 100%, respectively). No increase could be detected for Xan 159 even at the BA level of 20  $\mu$  M, while the maximum increase (50%) for 'PC 50' was found on 10  $\mu$  M BA.

This technique provides an efficient, simple and rapid protocol for common bean cloning in vitro. Shoots were initiated only after 7 days and highly significant positive correlation coefficients were found between percent of explants initiating shoot buds and the number of shoots/explant both for 21 and 28-day old explants. This indicates that in a few days it could be possible to decide the medium for best shoot initiation, multiplication and elongation from cotyledonary explants of a given genotype. In this study shoots were proliferated and roots initiated with little difficulty for the dry bean genotypes investigated.