ORGANOGENIC PLANT REGENERATION SYSTEM FOR THE COMMON BEAN (*PHASEOLUS VULGARIS L*).

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INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) cultivars are planned to have good agronomic characteristics (high nutritional quality, resistance to pests and diseases and climatic conditions, among others). However, when genes that provide these characteristics are not available or modification of gene expression is needed, gene insertion represents a suitable approach. For this, it is important to comply with basic requirements such as a consistent in vitro regeneration system which, up to now, has been difficult to establish due to the recalcitrant characteristic of this crop. Here we present a regeneration protocol established in two commercial common bean cultivars.

MATERIALS AND METHODS

Plant Material. Embryonic axis isolated from matured seeds of Flor de Junio Marcela (1) and Flor de Mayo Anita (2) were used as initial explants. Seeds were surface sterilized with the vapours produced by the combination of commercial chlorine (Cloralex®) and HCl 12 N (v/v 5: 0.16) in a vacuum chamber during 17 hrs. Seeds were then soaked in double distilled sterile water for 24 hours for embryo extraction.

Culture media. Basic medium consisted on MS medium (3), amended with myo-inositol 100 mg/l, thiamine 1 mg/l, sucrose 3 %, agar (SIGMA®) 6.8 g/l and pH 5.8 Regeneration induction media consisted of six treatments combining adenine (A) (0, 20, 40 mg/l) and benzyl-amino-purine (BAP) (5 and 10 mg/l).

Elongation medium consisted of the basal MS medium with no hormones and Rooting medium was 50% MS medium. Ten embryonic axis of each cultivar were cultured in three replicates for each of the 6 treatments.

Growing conditions. Explants were incubated in a growth chamber under 16 hrs light with a light intensity of 45 $mmol/m^2$ /seg. Plantlets obtained were transferred to soil and hardened for further development.

RESULTS

The regeneration response in both cultivars consisted of a bud cluster formation. FJM formed this organogenic structures 18 days after initial culture mainly at the internodal and apical areas, whereas FMA formed organogenic clusters 13 days after initial culture only at the apical area of the embryo. Explants were transferred to fresh medium every two weeks for further bud differentiation. Clusters were excised from the embryo after 3 transfers and subdivided in 5 mm segments. Shoot development was obtained after 60 days of initial culture.

FMA formed one bud cluster form every 10 embryonic axes, whereas FJM regenerated 6.3 to 9 bud clusters from the same number of embryonic axes (Table 1). No difference was

observed in FJM shoot formation when adenine was included either with 5 mg/l BAP (P=1.0) or 10 mg/l BAP (P= 0.385). However, presence of adenine was required for FMA shoot formation combined with 10 mg/l BAP (Table 1).

Bud clusters were sub-cultured on elongation medium; these clusters developed at least 1-2 plantlets showing leaves and stems well differentiated. Organogenic shoot and callus formation in hypocotyls and embryonic axes have been reported in *P. acutifolius* and *P. vulgaris* (4, 5, 6, 7, and 8). In this study, bud clusters corresponded to deep green, compact and well developed structures such as leaves and stems.

Individual plantlets were excised from the cluster after 40 days in elongation medium. Finally, they were transferred into rooting medium and incubated for 25 days. Whole plant formation efficiency was 83% for FJM and 50% for FMA (Table 1).

Table 1. Regeneration efficiency of common bean cv. Flor de Junio Marcela (FJM) and Flor de Mayo Anita (FMA).

BAP mg/l	**A mg/l**	**Bud cluster induction**[1]		**Number of shoots**[2]		**No. Of whole plants (Efficiency %)**[3]	
		FJM	**FMA**	**FJM**	**FMA**	**FJM**	**FMA**
5	0	6.3	0.5	13	6	8 (61.5	0
5	20	6.6	1.5	15	10	9 (60.0)	0
5	40	6.6	1.8	12	11	8 (66.6)	3 (28)
10	0	8.6	0.3	14	5	10 (71.4)	0
10	20	8.3	1.2	15	10	9 (60.0)	5 (50.0)
10	40	9	1.3	12	8	10 (83.3)	2 (25.0)

[1] Values represent an average of three Petri dishes with 10 embryos each and three replicates.
[2] Values represent average of shoot formation in 20 bud clusters.
[3] Number of regenerated whole plants / Number of induced shoots x 100.

CONCLUSIONS

- A consistent organogenic regeneration protocol is reported for *Phaseolus vulgaris* from embryonic axes.
- High BAP concentration was determinant for the novo shoot regeneration.
- Shoot formation and whole plant development was adenine dependant for FMA, whereas this cytokinine was not required form FJM shoot regeneration.
- This protocol will be used in transformation experiments due to its convenient regeneration system.

LITERATURE CITED

1. Castellanos-Ramos J.Z. et al. 2003. Crop Sci. 43: 1121-1122.
2. Castellanos-Ramos J.Z. et al. 2003. Rev. Fitotec. Mex. 26: 209-211.
3. Murashige T. and Skoog F. 1962. Physiol. Plant. 15: 473-497.
4. Dillen W. et al. 2000. Acta Hort.521.
5. Franklin C.I. et al. 1991. 24:199-206.
6. Zambre M.A. et al. 1998 17:626-630.
7. Santalla M. et al. 1998. 102:195-202.
8. Mohamed M.F. 1991. Ann. Rep. Bean Improv. Coop. 34:150-151.