Influence of the cold pretreatment and the carbon source on callus induction from anthers in *Phaseolus*

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Information available on the factors that affect the response of anther culture in grain legumes is very scarce, especially for *Phaseolus* species. On the basis of our first results (Muñoz and Baudoin, 1992; Muñoz et al, 1993), we carried out new experiments in order to study the influence of the carbon source (sucrose and maltose) and the cold pretreatment (applied either on buds or on anthers) on callus induction in two *Phaseolus* species.

Phaseolus materials involved two wild forms of *P. vulgaris* originated from Colombia (X1862 and NI922) and one cultivated form of *P. coccineus* from Rwanda (NI15). They were cultivated in a growth chamber with the following climatic parameters: 11h30 day length, 513 µEinstein/m².sec light intensity, 60-70 % relative humidity and 20/24°C night/day temperatures. The flowers buds were collected when the microspores were at the uninucleate stage. The buds were sterilized with alcohol (70% + 0,125% Ca hypochlorite) for 4 minutes then rinsed several times with sterile distilled water. The anthers were then extracted and 40 anthers were cultivated in 55 mm diameter Petri dishes containing 10 ml of medium. The cultures were maintained at 26°C in the dark until callus induction. The medium of Murashige and Skoog (1962) was supplemented with thiamin-HCl (1 mg/l), nicotinic acid (0.5 mg/l), piridoxin (0.5 mg/l), myoinositol (100 mg/l), 2 4,D (2 mg/l), kinetin (2 mg/l) and solidified with agarose (5 g/l). The same composition was utilized for all the experiments. A first experiment was deviced to study the influence of increasing concentrations of maltose and sucrose from 2,5 to 15 % using two genotypes: X1862 (*P. vulgaris*) and NI15 (*P. coccineus*). In a second one, a cold pretreatment was applied for 2 or 4 days, either directly to the flower buds or to the anthers placed in the culture medium. Two wild genotypes of *P. vulgaris* (X1862 and NI922) were tested and only the best concentrations in sucrose or maltose were used. In a third experiment, we combined the best parameters obtained for the media, genotypes and cold pretreatment.

Results In the first experiment the callus induction of the wild *P. vulgaris* genotype was inhibited at the highest levels of sugar concentration, i.e. 10 and 15 % for sucrose or maltose (Table 1). At the 5 % concentration, the percentage of callus induction reached 8.3 % with sucrose and 23.1 % with maltose. The best response was obtained when using the lowest concentration (2.5 %): 34 % for sucrose and 30.3 % for maltose. For NI15, we observed only the callus induction on the medium containing 5 % of maltose. Figure 1 shows the influence of the cold pretreatment on callus induction using the two wild genotypes of *P. vulgaris* (X1862 and NI922), which already showed a good *in vitro* response. A cold pretreatment directly applied to the anthers had a negative effect on callus induction, whatever the duration of treatment (2 or 4 days) and the medium (Fig.1B,1D). On the other hand, when the flowers buds were cold pretreated for 2 days and when the anthers were cultivated in the media containing 2,5 % and 5 % sucrose, an increase in callus induction was observed. But when the cold pretreatment exceeded 2 days or when the media contained maltose instead of sucrose, the percentage of callus induction decreased (Fig.1A,1B). Figure 1E illustrates the results observed in the third experiment. They confirm that better callus induction is obtained with a short cold pretreatment (one day in this case) and with a low sugar concentration.

As a conclusion, in our experimental conditions and for the genotypes evaluated, a 2,5 % concentration of either sucrose or maltose and 1 or 2 days of cold pretreatment directly applied to buds favoured the frequency of callus induction from anthers. Attempts are being made to induce the regeneration of plants from these calli.

References Muñoz & Baudoin (1992) *BIC* **35**, 217-218; Muñoz et al (1993) *BIC* **36**, 18-19
Murashige & Skoog (1962) *Physiol. Plant* **15**, 473-495

Table 1. Influence of different concentrations of sucrose or maltose on callus induction in *P. vulgaris* and *P. coccineus*

Concentration of Sugar %	2.5		5.0		10		15	
	Sucrose	Maltose	Sucrose	Maltose	Sucrose	Maltose	Sucrose	Maltose
Genotype X1862								
Number of anthers	360	360	1440	1440	1360	1400	1320	1320
Callus induction (%)	34.4	30.3	8.3	23.1	2.0	0.0	0.0	0.0
Genotype NI15								
Number of anthers			160	160	160	160	160	160
Callus induction (%)			0.0	4.4	0.0	0.0	0.0	0.0

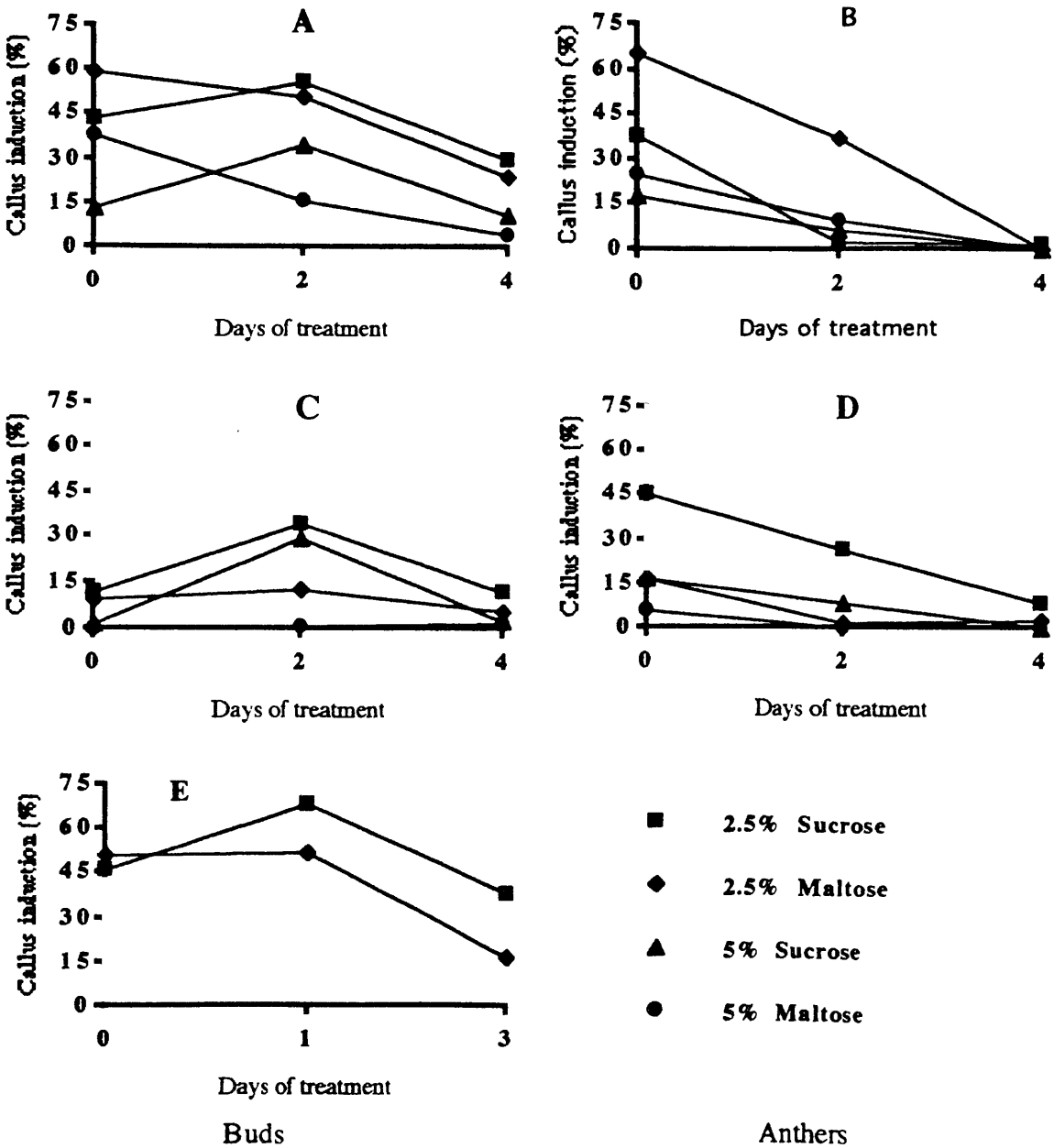

Figure 1. Results in callus induction after a cold pretreatment applied to the buds or to the anthers (A, B, E: wild *P. vulgaris* X1862; C, D: wild *P. vulgaris* NI922).