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Plant regeneration from leaves of *Cydonia oblonga* cultivars

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Abstract

Regeneration experiments *in vitro* were started with the aim to create a transformation system for *Cydonia oblonga* Mill. The optimal conditions for microplant regeneration of cvs. K.11, K.16, K.19 of *Cydonia oblonga* were determined. Leaves were isolated from microshoots from *in vitro* culture and grown on Murashige and Skoog nutrient medium supplemented with inosite, vitamins, naphtilacetic acid, sucrose and various amounts of thidiazuron. It was determined that by modifying the thidiazuron concentration in nutrient medium was possible to induce morphogenesis in a sufficient frequency for transformation. The frequency of morphogenesis depended on the plant genotype. The highest output of regenerants was obtained by using *Cydonia oblonga* cv. K.11. The various plant genotypes required different thidiazuron concentrations in nutrient medium. A higher thidiazuron concentration was necessary (32 μM) to produce a given number of regenerants by leaves of cv. K.19 in comparison with cv. K.16. In contrast with plum trees, the morphogenesis of *Cydonia oblonga in vitro* was associated with callus induction.

Key words: *Cydonia oblonga*, plant regeneration, tissue culture.

Introduction

Genetic engineering is a modern tool for plant breeding, especially for vegetatively propagated fruit plants. Successful genetic transformation has been reported for apple (Zhu et al. 2000) and pear (Mourgues et al. 1996). A prerequisite for the transformation is adventitious shoot regeneration in tissue culture from plant organs. The *in vitro* regeneration of shoots from leaf discs of *Cydonia oblonga* Mill. (quince), genotype 'Quince A', was investigated by Dolcet-Sanjuan and co-authors (1991), but the dependence of the morfogenetic reaction of leaf discs on the quince genotype under *in vitro* conditions has not been accessed. The aim of the present work was to investigate the morphogenetic characteristics of *Cydonia oblonga* Lithuanian cultivars in leaf culture and to develop a protocol of shoots regeneration *in vitro* for further genetic transformation.

Materials and methods

The quince (*Cydonia oblonga*) cultivars of Lithuanian origin K.11, K.16, K.19 were used for the investigations. The cultivars were selected from open pollinated seedlings in the middle of 20th century. In total 96 explants were isolated from microshoots developed in *in vitro* culture for each variant of the investigation. The experiment was repeated

three times. The explants were grown on Murashige and Skoog (1962) nutrient medium, supplemented with 100 mg l^{-1} inosite; 0.5 mg l^{-1} thiamine; 0.5 mg l^{-1} pyridoxine; 0.5 mg l^{-1} nicotinic acid; 1 mg l^{-1} ascorbic acid; 0.056 mg l^{-1} naphthylacetic acid and different concentrations (0.2; 2.2; 7.05; 14.1 mg l^{-1}) of thidiazuron (TDZ). The explants were grown in a growth chamber at a temperature 21 to 25 °C and 50 mmol m^{-2} s^{-1} photon flux density illumination for 16 h with luminescent lamps. Morphogenesis was evaluated after 60 days. Significant differences of treatment means were determined by the Duncan's multiple range test.

Results

The investigations showed that by modifying TDZ concentration in the nutrient medium it was possible to induce morphogenesis in quince leaves at the frequency sufficient for transformation experiments. The regeneration frequency depended on the plant genotype and TDZ concentration in the nutrient medium (Table 1). Various plant genotypes required different optimum TDZ concentrations for morphogenesis. The highest number of shoots per explant were regenerated on nutrient medium with 2.2 to 7.05 mg l^{-1} of TDZ. Callus formation occurred at the wound surfaces of leaf explants (Table 1). The frequency of callusogenesis depended on the TDZ concentration. Depending on the genotype of quince, 33.3 to 43.7 % of explants regenerated shoots on the optimal nutrient medium (Table 1).

Discussion

TDZ was the limiting factor for *Cydonia oblonga* shoot regeneration from leaves *in vitro*. The efficiency of TDZ in regeneration of quince may be due to the particularly high cytokinin requirement of this species. The optimal concentration was 2.2 mg l^{-1} TDZ for two *C. oblonga* cultivars of Lithuanian origin used in our investigations, compared to a three times higher (7.05 mg l^{-1}) optimal TDZ concentration for 'Quince A' (Dolcet-

Table 1. Effect of thidiazuron (TDZ) on regeneration of shoots from leaves of *Cydonia oblonga* cultivars *in vitro* (96 explants from four weeks old microshoots were used in three replications). Means followed by the same letter are not significantly different (p=0.05). +, weak callusogenesis; ++, medium callusogenesis; +++, intense callusogenesis

Medium	*Cydonia oblonga* cultivars					
	K.11		K.16		K.19	
	Callus formation	Shoot regeneration (%)	Callus formation	Shoot regeneration (%)	Callus formation	Shoot regeneration (%)
MS 0 mg l^{-1} TDZ	–	0^{c}	–	0^{c}	–	0^{c}
MS 0.2 mg l^{-1} TDZ	+	2.9^{c}	+	12.5^{bc}	+	4.2^{c}
MS 2.2 mg l^{-1} TDZ	+++	43.8^{a}	+++	35.0^{a}	+++	20.8^{b}
MS 7.05 mg l^{-1} TDZ	+++	34.4^{b}	+++	27.8^{a}	+++	33.3^{a}
MS 14.1 mg l^{-1} TDZ	++	29.2^{b}	++	21.9^{ab}	++	4.2^{c}

Sanjuan et al. 1991). The optimal TDZ concentration for K.19 and 'Quince A' resulted in a decreased output of regenerants when used for the cvs. K.11 and K.16. Genotypic differences should be taken into account when developing protocols for shoot regeneration from leaves *in vitro*. A 14.1 mg l^{-1} TDZ concentration decreased the output of regenerants in all the investigated genotypes. In contrast to plum trees (Bassi, Cossio 1994), the regeneration of quince micro shoots from leaves was associated with callus induction.

References

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