Plant Cell Reports (2001) 20:183–186
DOI 10.1007/s002990000303

CELL BIOLOGY AND MORPHOGENESIS

V. Sarasan · A.V. Roberts · G.R. Rout

Methyl laurate and 6-benzyladenine promote the germination of somatic embryos of a hybrid rose

Received: 12 July 2000 / Revision received: 23 October 2000 / Accepted: 9 November 2000 / Published online: 20 January 2001

Abstract Globular stage somatic embryos were induced in callus cultures of *Rosa* Heritage × Alister Stella Gray on medium containing 13.5 µ*M* 2,4-dichlorophenoxyacetic acid (2,4-D) and developed to the cotyledonary stage on medium containing 9 µ*M* 2,4-D. Cotyledonary-stage embryos were transferred to germination media with or without 1.5 µ*M* 6-benzyladenine (BA) and with or without 44 µ*M* methyl laurate (Mela). BA and Mela both promoted the development of shoots and roots and increased the frequency of bipolar germinations. An average of 56.5% (SE±4.1%) embryos on medium containing both BA and Mela underwent bipolar germinations compared with less than 20% in treatments where either or both were excluded. The effectiveness of BA and Mela was reduced if Mela was included in the development medium or if the concentration of salts and vitamins in the germination media was sub-optimal. There was evidence that growth at one pole of the somatic embryo promoted development at the other.

Keywords 6-Benzyladenine · Germination · Methyl laurate · *Rosa* Heritage × Alister Stella Gray · Somatic embryos

Abbreviations *BA* 6-Benzyladenine · *Mela* Methyl laurate

Introduction

Somatic embryogenesis in roses (*Rosa* spp.) is used to regenerate plants from genetically modified tissues (Firoozabady et al. 1991; Marchant et al. 1998). This objective can be achieved by either bipolar germination or the unipolar production of shoots. Somatic embryogenesis may also be useful as a method of propagation. For this purpose, bipolar germination is preferred because it provides opportunities for the production of artificial seed. Somatic embryos have been induced on a variety of culture media from in vitro explants of many roses (reviewed by Roberts et al. 1995), but germination frequencies are often low.

Several methyl esters of fatty acids, including methyl laurate (Mela) are used for chemical pruning of field-grown crops. They cause necrosis of the terminal bud and thus release axillary buds from apical dominance (Cathey et al. 1966; Sill and Nelson 1970; Vereecke 1975). The possibility of using these 'chemical pinching agents' in micropropagation was investigated by Voyiatzi et al. (1995). They cultured in vitro shoots of *Rosa* cv. Dr Verhage on multiplication medium containing Mela (400 µ*M*) and obtained an increase in the shoot multiplication rate. After basal clumps of tissue were recultured on multiplication medium without Mela, still higher shoot multiplication rates were observed, which were sustained for a further two recultures.

The main objective of the investigation reported here was to determine whether or not Mela might have a beneficial effect on the development and germination of somatic embryos of *Rosa*. As the effect of cytokinins and concentration of salts and vitamins had previously been found to influence germination, the interaction of Mela with BA, and salts and vitamins (full or half strength) was investigated. These factors were studied in *Rosa* Heritage × Alister Stella Gray, a hybrid raised by David Austin Roses.

Materials and methods

Media for in vitro culture contained sucrose (90 m*M*), salts and vitamins of Murashige and Skoog (1962) (MS) with the modifications and additions specified below. They were adjusted to pH 5.8, solidified with phytagel (2.5 g/l) and autoclaved at 121°C for

Communicated by M.R. Davey

V. Sarasan · A.V. Roberts (✉)
Department of Life Sciences, University of East London,
Romford Road, London E15 4LZ, UK
e-mail: a.v.roberts@uel.ac.uk

G.R. Rout
Regional Plant Resource Centre, Bhubaneswar 751015,
Orissa, India

15 min. Media for shoot multiplication and rooting were dispensed in aliquots of 30 ml to 'minijars' (100 ml capacity, Sigma-Aldrich, UK). Media for the induction, development and germination of somatic embryos were dispensed in 25 ml aliquots to petri dishes (90 mm diameter). Cultures were maintained at 23°C under high-pressure metal halide lamps (HQI-T, Osram, Germany; PPFD 60 µmol/m²/s at the plant level).

Shoots of in vitro plants were multiplied on medium that consisted of full-strength MS salts and vitamins supplemented with 1.5 µ*M* BA. Shoot tips (10 mm) were rooted on half-strength MS medium with no growth regulators. Roots excised from in vitro plants were cultured on an embryo induction medium consisting of half-strength MS salts and vitamins supplemented with 13.5 µ*M* 2,4-D, following the method of Matthews et al. (1991). Globular-stage somatic embryos were observed after 8 weeks and were multiplied by reculture on fresh embryo induction medium for periods of 5 weeks. When sufficient embryogenic tissue had been obtained, pieces (30–50 mm²) of tissue were transferred to development media, which contained 9 µ*M* 2,4-D with or without 44 µ*M* Mela (pre-treatments). After 5 weeks on development medium, cotyledonary-stage embryos were transferred to various germination media (treatments). These contained MS salts and vitamins modified by the replacement of FeEDTA by FeEDDHA (Van der Salm et al. 1994) at full or half strength, with or without 1.5 µ*M* BA and with or without 44 µ*M* Mela. Three petri dishes were initiated per treatment. However, some dishes became contaminated and, for a minority of treatments, data were recorded for only two petri dishes. After 5 weeks on germination media, germination percentages were recorded. At least 30 embryos were observed per petri dish. ANOVAs were carried out on arcsine transformed percentages, so that variances would be independent of the means (Sokal and Rolf 1981).

Results and discussion

After transfer from development media to germination media, somatic embryos formed both shoots and roots (bipolar germinations, Fig. 1), or only shoots or only roots. The frequencies of these three types of germination responses were recorded for each combination of pre-treatment and treatment (Fig. 2). Two-way ANOVAs (Table 1) were carried out to test the effects of BA and Mela in the germination medium. For this purpose, germinations were classified as bipolar, root (root only + bipolar germinations) and shoot (shoot only + bipolar germinations). After pre-treatment on development medium without Mela and transfer to germination media with modified full-strength MS salts and vitamins (Fig. 2A), there were significantly more bipolar, shoot and root germinations in response both to BA and Mela (Table 1), and there were significant, positive interactions between BA and Mela.

After pre-treatment on development medium with Mela and subsequent transfer to germination media with modified full-strength MS (Fig. 2B), significantly more embryos developed roots in the presence of Mela (Table 1). After pre-treatment without Mela and subsequent transfer to germination media with modified half-strength MS (Fig. 2C), significantly fewer embryos developed roots in the presence of BA, and there was a significant negative interaction between BA and Mela on root formation. These results indicate that exposure to 44 µ*M* Mela in both the development and germination media is supra-optimal and that modified half-strength

Fig. 1 Four somatic embryos showing bipolar germination (shown in a 35-mm-diameter petri dish)

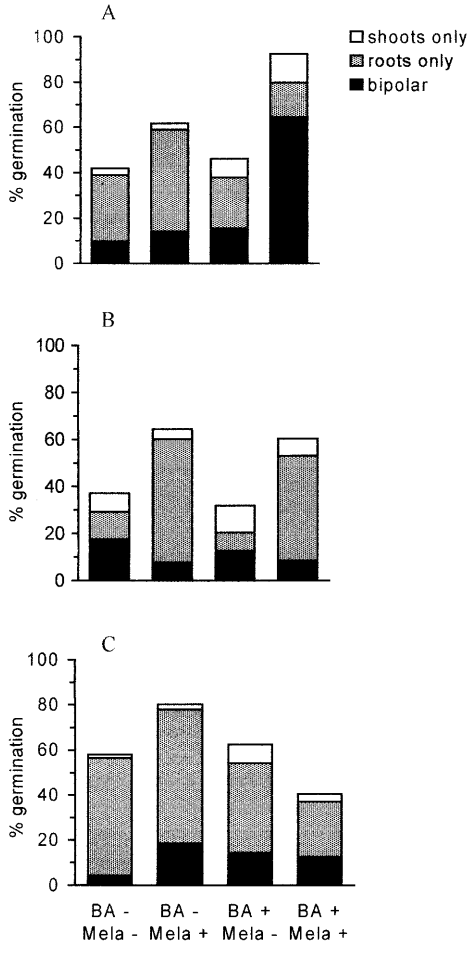

Fig. 2A–C Frequencies of somatic embryos which formed only shoots, only roots or shoots and roots (bipolar germinations) on germination medium with (+) or without (–) BA and Mela. **A** Pre-treatment without Mela and treatment with modified full-strength MS medium, **B** pretreatment with Mela and treatment with modified full-strength MS medium, **C** pretreatment without Mela and treatment with modified half-strength MS medium

Table 1 Summary of two-way ANOVAs testing the effect of Mela (44 μ*M*) and BA (1.5 μ*M*) in the germination media on bipolar, shoot and root germinations

Pre-treatment	Treatment medium	Factors	Significance		
			Bipolar	Root	Shoot
No Mela	Modified full-strength MS	Mela	***	***	***
		BA	***	*	***
		Interaction	**	**	***
Mela (44 μ*M*)	Modified full-strength MS	Mela	n.s.	*	n.s.
		BA	n.s.	n.s.	n.s.
		Interaction	n.s.	n.s.	n.s.
No Mela	Modified full-strength MS	Mela	n.s.	n.s.	n.s.
		BA	n.s.	*↓[a]	n.s.
		Interaction	n.s.	*↓	n.s.

*P<0.05, *P<0.01, ***P<0.001; *n.s.* not significant: P>0.05
[a] ↓ indicates a negative effect on germination

MS is not sufficiently concentrated to elicit the maximum benefit from the presence of BA and Mela.

As the highest frequencies of bipolar germinations occurred after pre-treatment without Mela and subsequent treatment on medium containing modified full-strength MS salts and vitamins, BA and Mela, additional germination tests were carried out on this treatment combination. An average of 56.5% (SE ±4.1%) bipolar germinations was recorded on a total of ten plates, including the three plates represented in Fig. 2A. All other treatment combinations (Fig. 2) gave frequencies of bipolar germination of less than 20%.

The expected frequencies of bipolar germinations were calculated for each replicate of the treatments shown in Fig. 2, and these were plotted in relation to observed frequencies of bipolar germinations (Fig. 3). To minimise the bunching of data points in Fig. 3, we calculated bipolar, shoot and root germinations each as the proportion of total germinations per plate. Expected frequencies were estimated as the product of frequencies of shoot and root germination, on the hypothesis that shoots and roots develop independently. The observed and expected values were closely correlated (r=0.98, df=28, P<0.001), but all the observed values were either equal to or greater than the expected values (y=0.97x+5.89), indicating a positive interaction between root and shoot formation. This interaction may arise from the beneficial influence of hormones produced by shoot or root growth on development at the other pole. This indicates that shoot and bipolar germinations, which can be used for the regeneration of plants from transformed tissues, can both be maximised by the concurrent stimulation of shoots and roots.

Voyiatzi et al. (1995) found that treatments of in vitro roses with 400 μ*M* Mela led to a sustained proliferation of axillary shoots after serial reculture of the basal clumps of tissue on medium without Mela. They attributed the long-term effect of Mela to the initial inactivation of the terminal meristem by Mela and the consequent release of axillary buds from apical dominance. However, they conceded that the after-effects might be due to the retention of Mela in the recultured tissues. Evidence from the present investigation that Mela at much lower concentrations – 44 μ*M* – can directly stimulate meristematic activity indicates that retention of Mela in the tissue clumps may indeed be responsible for the continued production of axillary shoots. As the mechanism of action of Mela that results in the direct stimulation of meristematic activity is obscure, empirical studies are needed to explore potential applications of Mela in plant tissue culture. It is possible that Mela might be used to improve the germination rates of somatic embryos in other species, particularly in woody plants, where low germination rates are a common problem. The promotion of bipolar development of somatic embryos by Mela may be of particular relevance in propagation by artificial seed. The evidence that Mela can stimulate growth in root meristems of somatic embryos suggests that it would also be interesting to investigate the action of Mela on the rooting of micropropagated shoots.

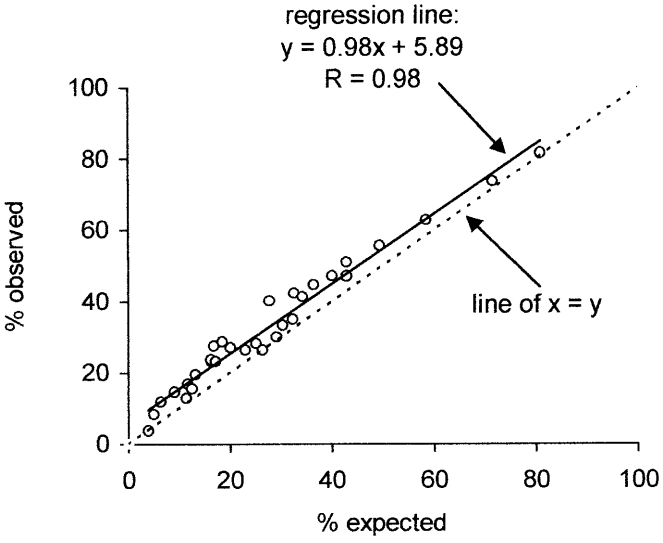

Fig. 3 The observed percentages of germinating embryos that produced both roots and shoots in relation to expected percentages calculated as the product of the frequencies of embryos with shoots (with or without roots) and with roots (with or without shoots)

Acknowledgements The authors thank David Austin Roses for the gift of plant materials, Dr. M Jakobson for helpful discussions and the Ministry of Agriculture, Fisheries and Food for financial support.

References

Cathey HMG, Steffens GL, Stuart NW, Zimmerman RH (1966) Chemical pruning of plants. Science 153:1382–1383

Firoozabady E, Noriega C, Sondahl, MR, Robinson KEP (1991) Genetic transformation of rose (*Rosa hybrida* cv. Royalty) via *Agrobacterium tumefasciens*. In Vitro 27:154A

Marchant R, Davey MR, Lucas JA, Lamb CJ, Dixon RA, Power JB (1998) Expression of a chitinase transgene in rose (*Rosa hybrida* L.) reduces development of blackspot disease (*Diplocarpon rosae* Wolf). Mol Breed 4:187–194

Matthews D, Mottley J, Horan I, Roberts AV (1991) A protoplast to plant system in roses. Plant Cell Tissue Organ Cult 24: 173–180

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco tissue culture. Physiol Plant 15: 473–497

Roberts AV, Yokoya K, Walker S, Mottley J (1995) Somatic embryogenesis in *Rosa* spp. In: Jain S, Gupta P, Newton R (eds) Somatic embryogenesis in woody plants, vol 2. Kluwer, Dordrecht, pp 277–289

Sill LZ, Nelson PV (1970) Relationship between azalea bud morphology and effectiveness of methyl deconate, a chemical pinching agent. J Am Soc Hortic Sci 95:270–273

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, New York

Van der Salm TPM, Van der Toorn CJG, Hanisch ten Cate CH, Dubois LAM, De Vries DP, Dons HJM (1994) Importance of the iron chelate formula for micropropagation of *Rosa hybrida* L. 'Moneyway'. Plant Cell Tissue Organ Cult 37:73–77

Vereecke M (1975) Response of brussels sprouts to chemical pinching with fatty alcohols and methyl esters of fatty acids. HortScience 10:420–421

Voyiatzi C, Voyiatzis DG, Tsiakmaki V (1995). In vitro shoot proliferation rates of the rose cv. (Hybrid Tea) 'Dr Verhage', as affected by apical dominance regulating substances. Sci Hortic 61:241–249