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RESEARCH ARTICLE

BIOLISTIC TRANSFORMATION OF ROSE

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Abstract

A reproducible method has been developed in Biolistic transformation and regeneration of transgenic plant from embryonic callus of rose. DNA delivery was optimized by β glucuronidase (Gus gene).The target explants and supplementation of pre and post bombardment of culture media with 0.25M myo-inositol influenced transformation efficacy.

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INTRODUCTION

Rose is one of the most important commercial flowers worldwide. In China 4.1 billion stems were sold in 2010, accounting for 38% of total sales of cut flowers (data from the Ministry of Agriculture of China).Now a days India rose selling increased roses are usually harvested at the bud stage, which is helpful in reducing physical damage to the petals in post-harvest handling, such as packing and transportation. It is reported that post-harvest loss of cut roses is mainly caused by ethylene, which can result in abnormal flower opening (Ma *et al.*, 2006). Therefore, investigation of the underlying mechanism of how ethylene regulates rose flower opening has been an important issue in post-harvest biology of ornamental plants worldwide. In regulation of plant organ growth, ethylene can function in opposite ways (Pierik *et al.*, 2006). . Senescence which is the main factor affecting on flower quality can be induced by several pre and post harvest factors eg water stress (Sankat and Mujaffar, 1994), amount of carbohydrates (Coorts, 1973; Ketsa, 1989), microorganisms (Van Doorn and Witte, 1991), ethylene effects .The senescence of cut flowers is closely related to the considerable reduction of the energy needed for synthesis reactions (Figueroa et al., 2005).A range of transegene is mostly useful in rose include that of pests, disease resistant , flower colour, morphology etc. Therefore, investigation of the underlying mechanism of how ethylene regulates rose flower opening has been an important issue in post-harvest biology of ornamental plants worldwide.

MATERIAL AND METHODS

Plant material and tissue culture

Somatic embryogenic callus of Rosa hybrida L. cv. Glad Tidings (Tantide, Tantau, Germany) was initiated following an established procedure (Marchant et al., 1996). Briefly, petioles from in Šitro-grown shoots were maintained in the dark at 28³2 °C on preculture (P) medium consisting of Schenk and Hildebrandt's (1972) basal salts and vitamins with 5±0 mg l–" 2,4-D(2,4-dichlorophenoxyacetic acid), 30±0gl–" sucrose, 300±0 mg l–" -proline and 4±0gl–" agarose (pH 5±8). After 14 d culture, explants were transferred to embryo proliferation (EP) medium, of the same composition as P medium, but containing 3±0 mg l–" 2,4-D, and were

maintained under identical conditions. Proliferating calli were subcultured by division every 28 d onto EP medium. Somatic embryogenesis occurred after 42–56 d repeated subculture.

Preparation of tissues for Biolistic gene delivery

Embryogenic calli with globular, white pre-embryos, were subjected to Biolistic gene delivery 14 d after subculture. Pieces of embryogenic callus (approx. 5 mm diameter) were placed in the central 3 cm radius of a 9 cm Petri dish (30 explants per dish), the latter containing 20 ml of EP medium (Marchant et al., 1996). In order to determine the effect of osmotica on the efficiency of gene transfer, the medium was also supplemented, where appropriate, with 0±25 □ myoinositol, 0±125 □ sorbitol and 0±125 □ mannitol, or 0±25 □ sucrose.

Plasmid DNA

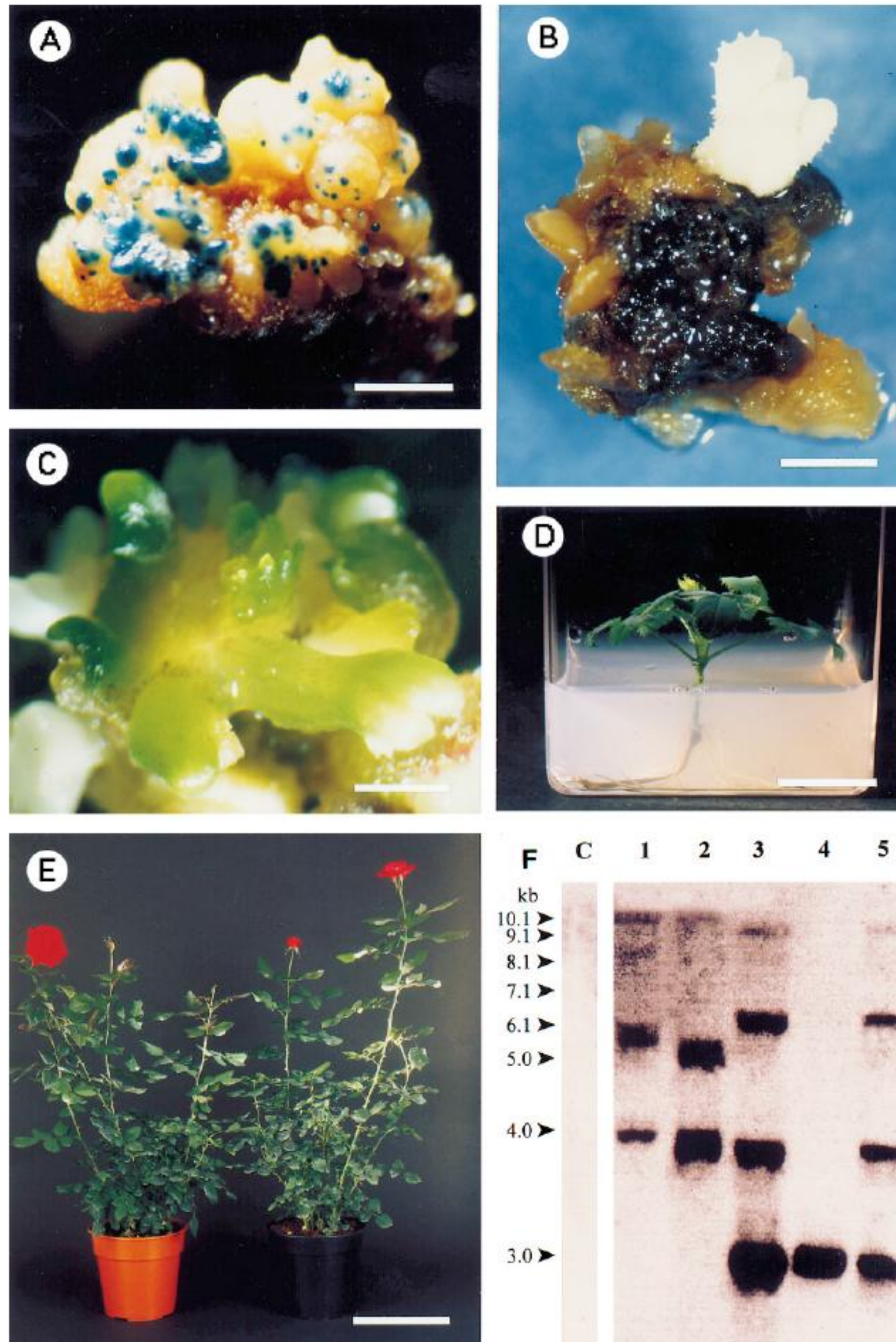
Plasmid pMJD67 was used to optimize the bombardment conditions. This plasmid, described by Rech, Vainstein and Davey (1991), is based on the vector pTZ18 with the CaMV35S promoter upstream of the gus (uid A) coding region. The DNA used for stable transformation was pBl101 (Jefferson, Kavanagh and Bevan, 1987; Clonetech Laboratories Inc., Palo Alto, USA) from which the gus gene and its associated terminator sequence had been deleted, leaving the plasmid containing only the nos promoter upstream of the npt II (neomycin phosphotransferase II) gene and nos terminator. Both plasmids were maintained and amplified in Escherichia coli strain HB101 and isolated and purified using the Wizard Megaprep Plasmid Isolation Kit (Promega, Southampton, UK). Purified plasmid was re suspended in TE buffer [1 m□ Tris HCl (pH 7±8), 0±1 m□ Na# EDTA] and was stored at ®20 °C until required.

Results and Discussion

Optimization of bombardment parameters

Transient gus gene expression 48 h after bombardment was used as an initial indicator of the efficiency of gene transfer (Fig. 1A). These data were exploited to optimize the parameters for gene delivery which were subsequently employed to generate stably transformed rose plants. The effect of firing distance (distance between the stopping screen and explant) and rupture disc pressure are shown in Fig. 2. Irrespective of the rupture disc pressure employed, the level of GUS expression was consistently greater when a firing distance of 70 mm was used compared to a distance of 100 mm. GUS expression was not detected when target material was bombarded at a distance greater than 100 mm. Maximal GUS expression was observed with a rupture disc pressure of 9000 kPa. Effective penetration of the target plant tissue by the micro projectiles carrying the DNA is essential for successful gene delivery (Southgate et al., 1995) and the effects observed in relation to firing distance and rupture disc pressure are likely to reflect the relative velocity of the micro projectiles on reaching the surface of the explants. In some plant transformation systems, successful micro projectile-mediated gene delivery has also been attributed to the osmoticum of the culture medium on which the explants are bombarded or maintained post-bombardment (Vain, McMullen and Finer, 1993; Ye et al., 1994; Southgate et al.,

1995). It has been suggested that the osmoticum induces cell plasmolysis and that the reduced turgor of plasmolysed cells prevents leakage of their protoplasm when cell walls are perforated by the micro projectiles. A comparison was made of the effect of the three osmotica, myo-inositol, sorbitol with mannitol, and sucrose, in the EP medium on which explants were maintained for either 24 h post-bombardment, or for 4 h prior to bombardment and for 24 h post bombardment. Supplementing EP medium by the addition of 0±25 □ myo-inositol more than doubled GUS expression 48 h post-bombardment (Fig. 3). Preconditioning target explants on medium containing myo-inositol for 4 h before bombardment and for 24 h post-bombardment produced a greater increase in gene expression than when the osmoticum was applied for only 24 h post-bombardment (Fig. 3). In order to maximize transformation, all subsequent bombardments were undertaken using 9000 kPa rupture discs with a firing distance of 70 mm; explants were maintained on EP medium supplemented with 0±25 □ myoinositol for 4 h prior to bombardment and for 24 h post bombardment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References

- BZvi MM, Shklarman E, Masci T, Kalev H, Debener T, Shafir S, Ovadis M,Vainstein A. 2012. *PAP1* trans cription factor enhances production of phenylpropanoid and terpenoid scent compounds in rose flowers. New Phytologist 195, 335–345.

- Gudin, S. 1998. Rose : genetics and breeding. Plant Breed. Rev. In press.
- Pertwee, J. 1995. Production and marketing of roses. Second Edition. Pathfast Publishing. Frinton on Sea. U.K. 127p.
- Roberts, A.V. and R. Lewis. 1996. Rose phenotypes , questions of diversity and development. Acta Hort. 424: 313-319.
- Borch, K., M.H. Williams and L. Hoyer. 1996. Influence of simulated transport on postharvest longevity of three cultivars of miniature potted rose. Acta Hort. 424 - 175-180.
- Shepherd, R.E. 1954. History of the rose. Macmillan Publishing Co., Inc. New York. 264 p.
- Zieslin, N. 1996. Influence of climatic and socio-economical factors on mode of cultivation and research of rose plants. Acta Hort. 424 - 21-22.
- Kool, M.T.N. 1996. System development of glasshouse roses. PhD Thesis. University of Wageningen. The Netherlands. 143p.

- *Rh-PIP21*, a Rose Aquaporin Gene, Is Involved in Ethylene-Regulated Petal Expansion.

- Vain P, McMullen MD, Finer JJ. 1993. Osmotic treatment enhances particle Bombardment-mediated transient and stable transformation of maize. *Plant Cell Reports* **12**: 84±88.

- Van der Tern J.G Bouwer $ nish ten Cate CH, Don HJM. 1997**.** Production of *ROL* gene transformed plants of *Rosa hybrida* L. and characterization of their rooting ability. *Molecular Breeding* **3**: 39±47.

- Ye X, Brown SK, Scorza R, Cordts J, Sanford JC. 1994**.** Genetic transformation of peach tissues by particle bombardment. *Journal f the American Society of Horticultural Science* **119**: 367±373.

- Yepes LM, Mittak V, Pang S-Z, Gonsalves C, Slightom JL, Gonsalves. 1995**.** Biolistic transformation of chrysanthemum with the nucleocapsid gene of Tomato Spotted Wilt Virus. *Plant Cell experts 14 694*=98.