Establishing somatic embryogenesis and genetic transformation systems of *Eustoma grandiflorum*

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Background

Eustoma grandiflorum, also known as prairie gentian and lingering, has a chic flower shape with crinkled petals. Due to its rose-like flower shape and rich variety of colours, it is also called 'thornless rose'. Eustoma grandiflorum is one of the top ten cut flowers in the world now. As an emerging flower in the Yunnan flower industry, it has ranked fourth in terms of production value.

Significance

Eustoma grandiflorum varieties cultivated in China are all F1 hybrids imported from abroad while no cultivar self-bred is released, making the breeding of Eustoma grandiflorum an urgent problem particularly prominent.

The embryonic calli and somatic embryos have been successfully applied in the conservation of rare plant germplasm and cell suspension nursery, as they can be preserved for a long time and multiplied in large numbers.somatic embryos are developed from single cell that are highly efficient in transformation and less prone to chimera formation, making them ideal materials for efficient genetic improvement.

This will shorten the breeding years and realise molecular design breeding for ornamental traits such as flower colour, flower type and flower fragrance, thus accelerating the development of novel varieties of ornamental plants.

process

In this study, cotyledons and hypocotyls of seedlings of different species were cut as explants and 2,4-D at 0.5-3 mg/L was added to induce embryonic cells. After 30-40-day-culture in the dark, loose, yellow and creamy embryonic calli were induced, with up to 100% induction ratio; the embryonic calli were then cultured on medium supplemented with 6-BA, and a large number of somatic embryos were observed after about 30 days. Cultured 20-30 days in the rooting medium, the isolated somatic embryos were rooted and formed into plantlets similar in the morphology of the seedlings germinated from seeds.

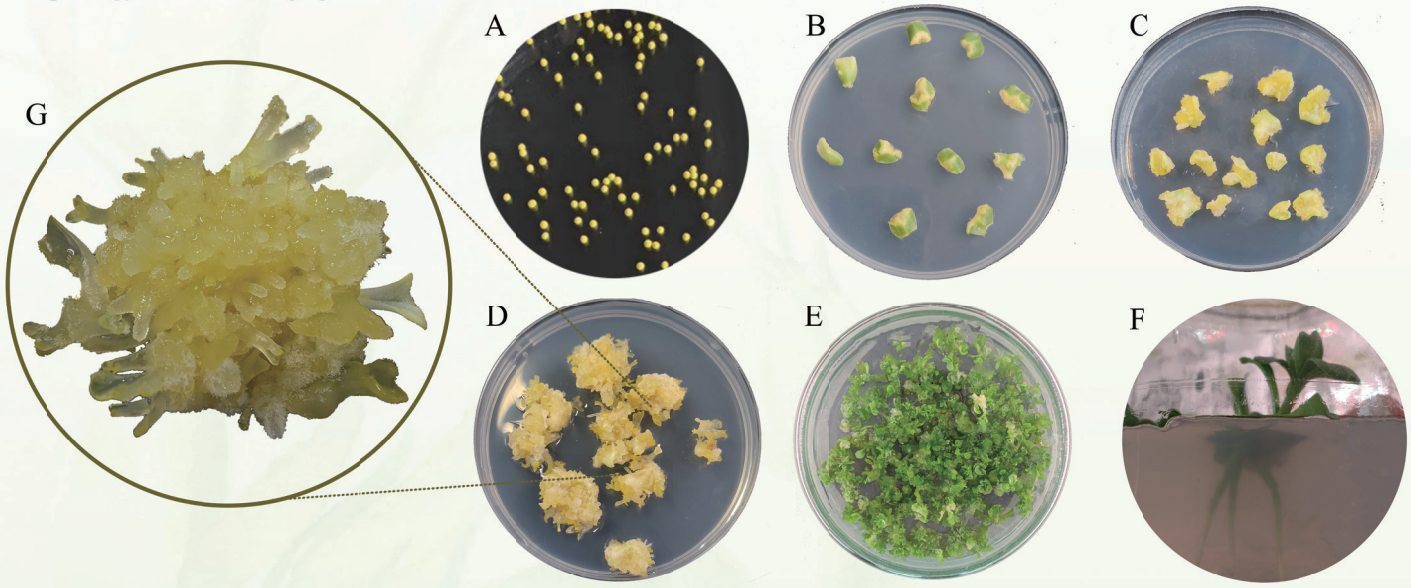

Figure 1. Flow chart of somatic cell embryo induction in Eustoma caryophyllata. A. *Eustoma caryophyllata* seeds green 601 B. Explants after 7 days into the induction medium C. Embryonic guilt tissue after 30 days of induction G. Shoot formation in differentiation culture D. Embryonic healing into differentiation medium to differentiate into germ buds E. Germ proliferation in light culture F. Buds inserted into MS medium to root into seedlings.

result

all varieties respond to induction medium and form somatic embryos after induction. However, the sensitivity to 2, 4-D and 6-BA varied between varieties and between explants of the same variety, and the somatic embryo induction coefficients also varied considerably. The induction efficiency of somatic embryos was the highest in the green variety when using cotyledons as explants and the pink variety when using hypocotyls, with induction coefficients of 15.85 and 6.6, respectively, after 30 days of differentiation. In addition, we found that embryonic calli had the ability to induce somatic embryos for a longer period of time, and after 30 days, a large number of somatic embryos still developed and formed when the culture was maintained.

Figure 2 Development of somatic embryos into seedlings. A. somatic embryo developed from radicle; B. mature and naturally shed germ; C. seedling developed from germ; D. seedling transplanted to cavity tray for seedling training; E. green 601 flowers of Erythrina species.