Efficient plant regeneration of *Viola cornuta* "Lutea Splendens" L. using seedlings explants

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Introduction

Viola cornuta, also known as horned pansy, is a valuable evergreen perennial ornamental plant belonging to the *Violaceae* family. Horned pansy grows naturally in the high Pyrenees in Spain and France. Plants grow up to 30 cm in height, with flowers consisting of four petals pointing upwards and one pointing downwards. There are more than 25 cultivars and hybrids with flower colors ranging from yellow to violet. The present study was undertaken to develop an efficient protocol for *in vitro* plant regeneration for yellow horned pansy, *V. cornuta* L. "Lutea splendens", with the aim of subsequently developing a transformation system for modifying the flower color of this species.

Material and methods

Plant material

Well-developed seedlings (10 cm) were used as starting material for experiments. In the first set of experiments the leaves, petioles and hypocotyls were used as initial explants while in second set of experiments hypocotyl explants are used as initial explants.

Shoot induction

Explants were excised from seedlings, cut into small pieces (5-10 mm long), and cultured four/eight weeks on solid Murashige and Skoog medium with half-strength mineral solution, full strength of vitamins, 2% sucrose, 0.7% agar, inositol 100 mgl^{-1}, tyrosine 100 mgl^{-1}, adenin sulphate 80 mgl^{-1} and plant growth regulators, 2,4-D (0.1 mgl^{-1}) and BA (2 mgl^{-1}). Cultures were grown in the culture room under light conditions (16 h light/8 h dark, photosynthetic flux density (PPFD) within the range of 80-100 $\mu mol\ m^{-2}\ s^{-1}$, 24 ± 2°C) or in darkness. After four/eight weeks the explants were transferred on ½MS media supplemented with NAA (0.2 mgl^{-1}), GA_3 (2.0 mgl^{-1}) and TDZ (1.0 mgl^{-1}) for another four weeks.

Shoot multiplication

Developed shoots were multiplied on ½MS medium supplemented with NAA (0.5 mgl^{-1}) and BA (1.0 mgl^{-1}).

Shoot rooting

For root induction, 2.0 cm long apical part of the shoots grown on media for shoot multiplication were cut and transferred on ½MS medium without plant growth regulators.

Acclimatization

Rooted plants were washed with water to remove adhering agar and planted in a soil mix of peat and perlite (3:1) in plastic pots (30 cm x 20 cm) and grown under glasshouse conditions til flowering.

Conclusions

- We developed efficient plant regeneration protocol, useful for mass propagation by culture *in vitro* and genetic transformation studies for a yellow cultivar of *Viola cornuta* "Lutea Splendens" using seedlings explants.
- The highest frequency (12%) of shoot induction was obtained from petiole seedlings explants while the highest frequency callus formation (97%) was achieved on hypocotyl explants (Fig. 1).
- In culture of hypocotyl explants the highest shoot induction was achieved when the upper part of hypocotyls (53%) was used and explants were cultured at light conditions (30%) first four weeks of culture (Fig. 2, Tab. 1, Tab. 2).
- Regenerated *V. cornuta* shoots were successfully rooted, acclimatized in a greenhouse conditions and flowered in next flowering season (Fig. 4).

Results

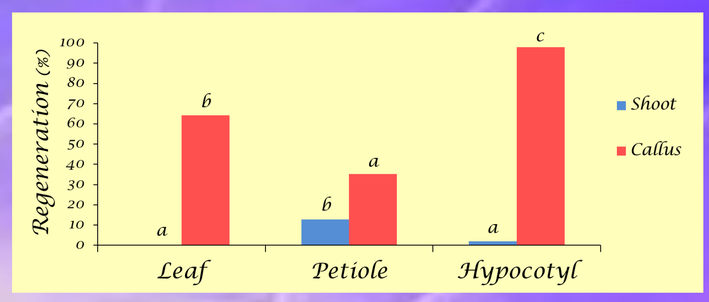


Figure 1. The effect of explants type (leaf segments, petiole, hypocotyl) on frequency of shoot induction (%) and callus formation (%) of *V. cornuta* cv. "Lutea Splendens" in culture of seedlings explants.

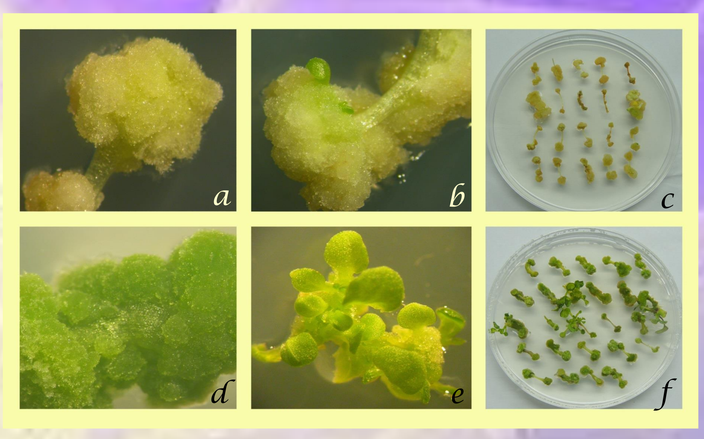
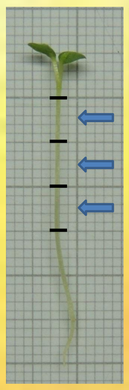


Figure 2. Shoot induction and callus formation in culture of hypocotyl explants of *V. cornuta*. a-c) Callus formation and shoot induction under dark conditions; c-e) Callus formation and shoot induction under light conditions.

Table 1. The effect of culture conditions on frequency of shoot induction (%) and callus formation (%) of *V. cornuta* cv. "Lutea Splendens" in culture of hypocotyl explants.

Culture conditions	Shoots (%)	Callus (%)
Darkness	8.97 ± 3.22 a	100.00 ± 0.00 a
Light	29.63 ± 6.29 b	100.00 ± 0.00 a

Table 2. The effect of hypocotyl segment position on frequency of shoot induction (%) and callus formation (%) of *V. cornuta* cv. "Lutea Splendens".



Segment position	Shoot induction (%) after 4 weeks	Shoot induction (%) after 8 weeks
a	53,12 ± 8,96 b	59,37 ± 8,82 b
b	15,62 ± 6,52 a	37,50 ± 8,69 ab
c	15,62 ± 6,52 a	21,87 ± 7,42 a

Figure 3. *Viola cornuta* "Lutea Splendens" seedling

Figure 4. Multiplication, rooting and acclimatization of regenerated plants. a) Shoot multiplication on ½MS with NAA and BA (0.1, 1.0 mg/l, respectively); b) Shoot regenerated on ½MS without plant growth regulators; (c,d) Flowering of *V. cornuta* "Lutea Splendens" plantlets regenerated by culture of seedlings explants.

Acknowledgement

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