IN VITRO STUDIES ON CALLUS INDUCTION IN BOTH VEGETATIVE AND GENERATIVE PARTS IN *ALSTROEMERIA* FOR FURTHER APPLICATION TO TRANSFORMATION

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Abstract

We compared both vegetative and generative parts of *Alstroemeria* in friable embryogenic callus induction, and subsequent production of somatic embryos and regeneration of plants because there exist only a few protocols with low efficiency for application to transformation. Nodal parts of the plant showed the best performance in callus induction, production of somatic embryos and regeneration as compared to internodes and all seven generative parts tested. Thus, it can be concluded that the nodal explants can be used as a source for transformation in combination with the MS medium containing 1 mg/l 2,4-D, 0.25 mg/l BAP, 3% sucrose (w/v) and 0.75% (w/v) microagar for the production of a high level of compact callus and somatic embryos. In the future, more research will be needed on the acceleration of the plant production process from somatic embryos, to use this system in an optimised way for transformation experiments.

1.Introduction

In *Alstroemeria*, only a few reports on callus induction and regeneration have been published, whereas the knowledge in these fields is considerably important for further application of *Alstroemeria* transformation (Ziv *et al*., 1973; Gonzalez-Benito and Alderson, 1992; Hutchinson *et al*., 1994; Van Shaik *et al*., 1996; Lin *et al*., 2000a). Most of these reports did not show the ideal results for the next step to genetic modification. Furthermore, establishment of an efficient regeneration system from induced friable embryogenic callus is required to successfully transfer foreign gene(s) into the plant genome (Taylor *et al*., 1996; Raemakers *et al*., 1997; Lin *et al*., 2000a). Therefore, we focused on comparison of both vegetative and generative parts of the plant for the induction of friable embryogenic callus (FEC) and somatic embryos (SE) in order to establish a regeneration and transformation system in *Alstroemeria*.

2. Materials and methods

Seven different generative tissues (flower stalk, anther, filament, ovary, petal, ovule, stigma) were taken from field grown VV024 plants, whereas vegetative tissues (node, internode) were taken from *in vitro* grown VV024 plants of *Alstroemeria*. Surface sterilization was carried out according to Lin *et al*. (1997). The tissues were cultured on Murashige and Skoog (1962) medium containing 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 mg/l benzylaminopurine (BAP), 3% (w/v) sucrose and 0.75% (w/v) microagar (MS30) for callus induction. The medium was adjust to pH 6.0 before autoclaving at 121^{o}C for 20 min. After 4 weeks of culture, induced calli were transferred to the second induction medium: MS30 supplemented with for 1 mg/l 2,4-D and 0.25mg/l BAP for growth of callus and further development into somatic embryos. Culture condition for callus induction was at 18 ^{o}C in the dark. Proliferating calli were removed

from the explants and cultured in Petri dishes containing 25 ml of PCA medium (Sofiari *et al*., 1998). For regeneration, somatic embryos were transferred to regeneration medium. That is composed of MS medium supplemented with 0.5 m/l BAP, 2.5 g/l Gelrite, 4 % (w/v) sucrose. The culture room was maintained at 18 ^{o}C with a 16-h photoperiod of white fluorescent lamps.

3.Results

The initiation of callus formation was observed about 1 week after inoculation for anther tissue, 3 weeks for ovary tissue and 2 weeks for node and internode tissue. Other tissues showed no callus formation in the beginning of culture. A small amount of white friable calli were formed in the generative explants (Figure 1C), whereas white and yellow callus appeared in node tissues (Figure 1A). Later on, callus color in generative parts changed into a more yellowish color. Compact and friable calli were observed in these experiments (Figure 1A, B). The compact type of calli were produced in large quantities in all explants cultured (result not shown) and shown to posses a high regeneration capacity compared to friable calli (data not shown) . Regarding to callus induction, among the generative explants, the ovary tissue showed the best result with 25%, whereas node tissue presented more promising results with 45% (Table 1). Furthermore, the formation of somatic embryos occurred only in anther, ovule and ovary of the generative parts and in all vegetative tissues after 8 weeks of culture on callus induction medium, although callus induction was obtained in all explants types tested here (Table 1). For generative parts, anther tissue showed the best result with 12.4% in somatic embryo formation (Figure 1D). Nodal tissue showed the best result with 22.5% of somatic embryo production (Figure 1E). Compact calli produced new compact calli and globular embryos on PCA medium (see materials and methods) after 8 weeks of culture. After 12 weks of culture, well-developed somatic embryos isolated from compact calli were transferred to MS medium with BAP at 0.5 mg/l for another 8 weeks in order to germinate. As a result of this, 36.5% of the somatic embryos from node-derived callus produced multiple shoots (Table 1). Some parts of embryos developed into a cotyledon (Figure 1 E).After another 4 or 6 weeks, somatic embryos generated small plantlets (Figure 1F).

4.Discussion

For improving a crop by using biotechnological methods, tissues with high efficiency in proliferation and regeneration must be easily obtained (Taylor *et al*., 1996; Raemakers *et al*., 1997; Lin *et al*., 2000a). To do this, first of all, selection of suitable explants should be conducted in terms of callus induction, proliferation and plant regeneration. Then, more detailed experiments can be tested. During our experiments, compact and friable calli were produced in all generative and vegetative parts cultured. Compact callus formed new compact callus during culture on callus induction medium. Moreover, friable callus can be obtained by subculturing compact calli on PCA medium. Furthermore, friable callus cultured on PCA medium developed via compact callus into plants. However, friable callus showed less regenerability than that of compact callus, although friable callus has a possibility to be used in transfromation experiments due to its superior proliferation rate (Van Schaik, 1998; Lin *et al*., 2000b). So far, both compact and friable calli have maintained for more than 1 year on BA-containing medium without losing their capacities to regenerate plants. In summary, nodal segments have proven to be a considerable source for further application to transformation, due to their easy accessibility, high regeneration and callus induction capacity in these experiments. Therefore, vegetative tissues such as nodes can be used in a transformation system as starting material. To achieve this, more research on several factors in the establishment of high efficiency regeneration protocols of FEC and compact callus will be performed in the future.

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Tables

Table 1. Comparison of the *in vitro* response between generative and vegetative parts in VV024 of *Alstroemeria.*

Explant Type	No. of Explant	Percentage of Explants with callus*	Percentage of Explants with somatic Embryos*	Percentage of somatic Embryos with shoots**	Level of Browning***	Color of Formed Callus*
Node	120	45.0	22.5	36.5	++	Yellow
Internode	120	30.0	8.3	15.0	+++	White
Anther	210	21.9	12.4	0.0	++	Yellow
Ovule	100	16.0	3.0	0.0	+++	Yellow
Petal	125	2.4	0.0	0.0	+++	White
Ovary	200	25.0	4.5	0.5	++	White
Flower stalk	100	3.0	0.0	0.0	+++	White
Stigma	60	5.0	0.0	0.0	++	White
Filament	250	0.8	0.0	0.0	+++	White

*: These data were collected after 8 weeks of culture on callus induction medium
**: These data were collected after 12 weeks of culture on regeneration medium
***: +++ Severe browning ++ normal browning + weak browning

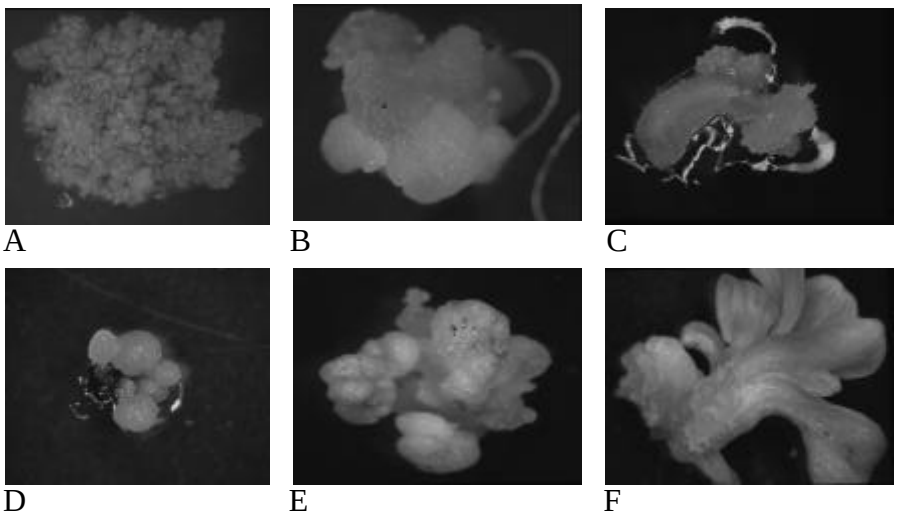

Fig.1. Different stages of regeneration process of calli from all vegetative and generative in VV024 of *Alstroemeria*
A: Friable embryogenic clump from node B: Compact callus from node
C: Friable calli on ovule D: Embryo structures from anther tissue
E: Germination of somatic embryos from node F: Plant regeneration