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Indirect somatic embryogenesis from petiole segment in strawberry cv. Sweet Charlie

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ABSTRACT

A study was undertaken on somatic embryogenesis from petiole segments in strawberry (*Fragaria* x *ananasa* Duch.) cv. Sweet Charlie. The simple and feasible protocol for micropropagation *via* somatic embryogenesis from petiole segments from shadenet house maintained mother plants has been standardized. The petiole segments induced nodulated friable embryonic callus on Murashige and Skoog (MS) medium containing 1.0 mg/l each of thidiazuron (TDZ) and 2,4-dichlorophenoxy acetic acid (2,4-D). The development and maturation of somatic embryos was achieved on MS medium supplemented with abscisic acid (0.5 mg/l). Successful conversion of somatic embryos into plantlets was observed on hormone-free half-strength MS medium. The regenerated plantlets were successfully acclimatized in pre-sterilized cocopeat at primary hardening stage. The survival rate of tissue cultured plants was 65.0%. Further trials are on under shadenet and greenhouse conditions to judge their *ex vitro* performance.

Key words: Strawberry, somatic embryogenesis, petiole segment.

INTRODUCTION

Strawberry plant belongs to the Rosaceae family as the third economically important cultivated crop. Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is a hybrid between Scarlet or Virginia strawberry (*F. virginiana* Duch.) and the pistillate South American strawberry *F. chiloensis* (L.) Duch (Debnath *et al*., 2). It is popular for its nutritious fruits and is cultivated in plains as well as in hills up to an elevation of 3,000 m in humid or dry regions. *In vitro* techniques are important tools for modern plant improvement programs to introduce new traits into selected plants, to multiply elite selections and to develop suitable cultivars in the minimum time. The ability to regenerate plants is crucial to the successful application of *in vitro* methods.

Efficient micropropagation system of somatic embryogenesis can be used to genetic transformation of plant cells and to identify and/or induce somaclonal variants, which could accelerate cultivar development programmes. Somatic embryogenesis research with strawberries is however, still in a preliminary stage and some more efforts are required to develop the technology (Graham, 4). Thidiazuron (TDZ), a the substituted phenylurea (N-phenyl-N'-1,2,3-thidiazol-5-y-lurea) with its cytokinin- and auxin-like effects, is now among the most active substances for plant tissue culture (Huetteman and Preece, 6). It has been used to induce shoot organogenesis in *Fragaria* petiole explants of different cultivars (Catherine and Letouze, 1; Folta *et al*., 3; Passey *et al*., 10; Zhao *et al*., 12). The induction and plant regeneration *via* somatic embryogenesis from leaves of cvs. Selva and Comarosa (Kordestani and Karami, 8), cv. Chandler (Husaini and Aquil, 7) have already been reported. The work on cv. Sweet Charlie is restricted to only shoot organogenesis from leaf explants (Singh and Pandey, 11). Hence, the present study was undertaken.

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MATERIALS AND METHODS

Petiole segments from fully grown leaves (1.5-2 mm dia, 1.0-1.2 cm length) of strawberry cv. Sweet Charlie were collected. Exlants collected from shadenet house maintained plants were treated with 2% carbendazim (Bavistin) for 2 min. followed by surface sterilization with 0.1% mercuric chloride for 1 min. followed by final dip in 70% ethanol for 15 sec. After four repeated washings in sterile distilled water the explants were air-dried and cultured MS medium (Murashige and Skoog, 9) with sucrose (3%) and agar (0.8%) along with 0.5, 1.0 and 2.0 mg/l thidiazuron (TDZ), kinetin (KN), 2,4-dichlorophenoxy acetic acid (2,4-D) individually and in different combinations. The medium pH was adjusted to 6.0 before autoclaving. All the cultures were maintained at 26°C under 1,000 lux light intensity and RH 50-60%. For each treatment, 12 replicates were used and the experiment was repeated thrice. The cultures were maintained for a period of three weeks and at the end of each experiment, culture/calli was sub-cultured on to fresh medium for further growth and development.

Globular stage embryos developed into cotyledonary ones when embryogenic callus was sub-cultured onto half-strength MS medium containing abscisic acid (0.5,

1.0 and 2.0 mg/l) and in combination with 5% coconut water (v/v). The organogenic response in terms of shoot, root and somatic embryo formed on calli were observed using inverted stereoscopic microscope. For callus, sub-culture at three week period was sufficient. Squash preparation of calle were made permanent after passing through acetic acid-n-butyl alcohol series and mounting in DPX regularly. The regenerated plantlets after germination of somatic embryos were acclimatized in cocopeat and transferred to soil. The data was analysed following ANOVA.

RESULTS AND DISCUSSION

Petiole explants cultured on MS medium supplemented with TDZ (individually) alongwith other auxins were found to be most responsive. Greenish compact callus induction started at the cut ends within first week which turned into nodulated hard masses with brownish appearance restricting to cut ends. On the contrary, white friable soft callus was induced on media supplemented with combinations of TDZ and 2,4-D. Cytological studies of such calli revealed the presence of oval to round parenchymatous cells with prominent nuclei and pro-embryos during second week of culture. Same study after three weeks revealed the presence of globular late globular somatic embryos and their growth was restricted to only those stages in culture medium. Development of somatic embryos from the globular to cotyledon stage was not observed on the induction media even after three weeks. Prolonging the cultures in the same medium caused them to be converted to non-embryogenic calli. The effect of different concentration of TDZ and 2,4-D on the percentage of callus induction are shown in Table 1. Medium containing 1 mg l^{-1} each of TDZ and 2,4-D gave the maximum response (61%) and the maximum callus induction per explant (96%). Both higher and lower concentrations reduced the percentage of callus induction as well as organogenic response.

The sub-culture of embryogenic callus showed maximum conversion of globular embryos into cotyledonary stage somatic embryos on MS medium supplemented with 0.5 mg/l of abscicic acid alone gave 74.2% success (Fig. 1). Cotyledonary somatic embryos were converted to entire plantlets within two weeks, when the clusters of somatic embryos were transferred onto hormone-free half-strength MS medium. However, the regenerated plantlets showed luxuriant growth when 1.0 mg/l kinetin was incorporated. The regenerated plantlets *via* somatic embryogenesis were successfully shifted to primary hardening in portrays filled with sterilized cocopeat after rinsing in 0.2% carbendazim. The portrays were tightly covered with polythene bags to maintain humidity for two weeks. After third week, the plants were shifted to polybags filled with sterilized soil and were gradually exposed to shadenet condition with 65.0% survival and are under observation for their further growth and development in pots.

The micropropagation using embryogenic cell suspension culture and the bioreactor with a temporary immersion system has immense practical significance. Adventitious shoot regeneration was reported earlier in petiole segments on TDZ and 2,4-D supplemented medium (Passey *et al*., 10) in different strawberry cultivars. The present study revealed the pathway of regeneration as somatic embryogenesis in cv. Sweet Charlie on medium containing 1 mg/l each of TDZ and 2,4-D. Similar result in cv. Chandler was reported with 4 mg/l TDZ from leaf disc explants. Somatic embryo development was restricted at globular stage on induction medium but their germination and conversion into plantlet was observed on half-strength MS medium supplemented with 0.5 mg/l. This result is in concurrence with that on cv. Chandler (Husaini and Aquil, 7). The different stages of indirect regeneration protocol are presented in Fig. 2. The protocol for indirect somatic embryogenesis using petiole segments on half-strength MS medium will be helpful for reducing the cost of production of strawberry plants, which can be adapted for commercial exploitation by using suspension cultures for clonal multiplication by using bioreactors, artificial seed production, crop improvement and germplasm conservation.

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Table 1. Effect of different concentration of TDZ and 2,4-D on callus induction (3 wk).

TDZ (mg/l)	2,4-D (mg/l)	Explant survival (%)	Callusing (%)
0.5	0.5	33.0	60.0
1.0	1.0	61.0	96.0
2.0	2.0	55.0	70.0
$CD_{0.05}$		5.87	4.85

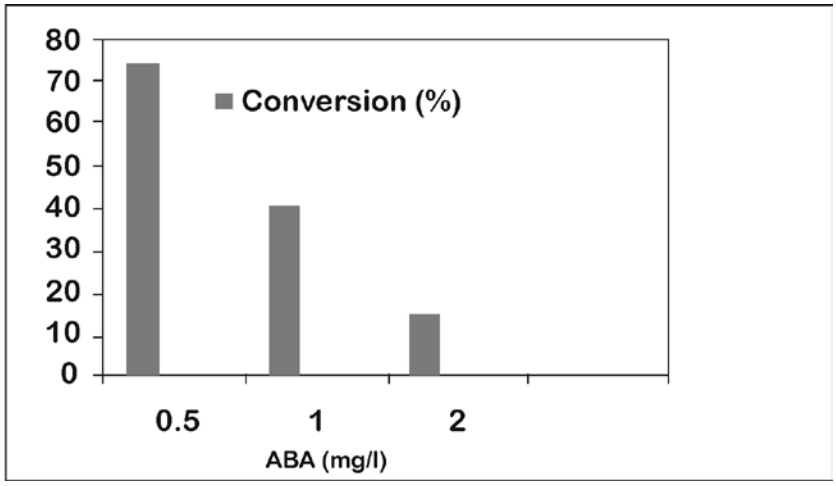

Fig. 1. Conversion of somatic embryos into plantlets on half-strength MS medium supplemented with ABA.

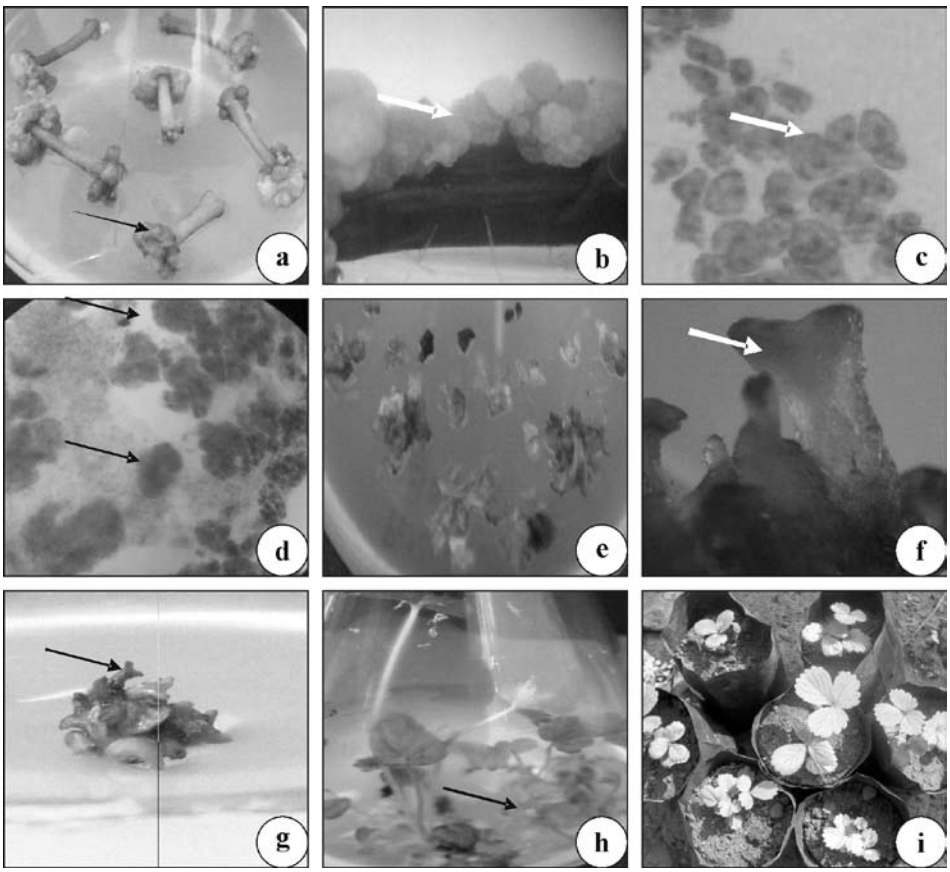

Fig. 2. Micropropagation *via* indirect somatic embryogenesis from petiole segments in strawberry cv. Sweet Charlie. (a) Compact, nodular hard callus at cut edges of explant on TDZ (1.0 mg/l) supplemented medium, (b) Friable whitish callus from the cut end and surface of the explant, (c & d), Cytological observation of callus showing pro-embryos, (e) Callus sub-cultured on embryo development and maturation medium; (f) Stereoscopic observation of growing somatic embryos, (g) Magnified picture of somatic embryos at cotyledonary stage, (h) Conversion of somatic embryos into plantlets, and (i) Acclimatized regenerated plants *via* somatic embryogenesis ready for field transfer.

REFERENCES

1. Catherine, F. and Letouze, R. 1987. *In vitro*: Regeneration of strawberry plants from petiole segments and flower buds. *Biol. Plant.* **29**: 409-14.
2. Debnath, S.C. and Jaime, A. Teixeira da Silva. 2007. Strawberry culture. *In vitro*: Applications in genetic transformation and biotechnology. *Fruit, Veg. Cereal Sci. Biotech.* **1**: 1-12.
3. Folta, K.M., Dhingra, A., Howard, L., Stewart, P.J. and Chandler, C.K. 2006. Characterization of LF9, an octoploid strawberry genotype selected for rapid regeneration and transformation. *Planta,* **224**: 1058-67.
4. Graham, J. 2005 *Fragaria* Strawberry. **In**: Litz, R. (Ed.) *Biotechnology of Fruit and Nut Crops*. Biotechnology in Agriculture Series No. 29, CAB International, Wallingford, UK, pp. 456-74.
5. Hanhoneva, K.J. and Karenlampi, S.O. 2007 Production of strawberries by temporary immersion bioreactor system and verification by TAIL-PCR. *BMC Biotech.* **7**: 11-12.
6. Huetteman, A. and Preece, E.J. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant Cell, Tiss. Org. Cult.* **33**: 105-19.
7. Husaini, A.M. and Aquil, S. 2008. A high-efficiency direct somatic embryogenesis system for strawberry (*Fragaria* x *ananassa* Duch.) cv. Chandler. *J. Crop Sci. Biotech.* **11**: 107-10.
8. Kordestani, G.K. and Omid Karimi. 2008. Picloram-induced somatic embryogenesis of strawberry (*Fragaria ananassa* L.). *Acta Biol. Series Botanica*, **50**: 69-72.
9. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant*. **15**: 473-97.
10. Passey, A.J., Barrett, K.J. and James, D.J. 2003. Adventitious shoot regeneration from seven commercial strawberry cultivars (*Fragaria* × *ananassa* Duch.) using a range of explant types. *Plant Cell Rep.* **21**: 397-401.
11. Singh, A.K. and Pandey, S.N. 2004. Genotypic variation among strawberry cultivar for shoot organogenesis. *Acta Hort*. **662**: 277-80.
12. Zhao, Y., Liu, Q.Z. and Davis, R.E. 2004. Transgene expression in strawberries driven by a heterologous phloem-specific promoter. *Plant Cell Rep.* **23**: 224-30.

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