

Micropropagation of strawberry cultivar Sweet Charlie through axillary shoot proliferation

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Abstract

A protocol for micropropagation of strawberry cv. Sweet Charlie was standardized through axillary shoot proliferation from runner tips. Medium supplemented with TDZ (1 mg/L) alone was favourable for the induction of multiple shoots and daughter runners from runner tips. Such shoots were successfully multiplied for four times on MS incorporated with 0.5mg/L each of BAP, IBA and 1.0 mg/L of GA₃. Rooting of subcultured shoots was achieved on MS medium containing 0.5 mg/L of kinetin alone and along with 0.5 mg/L of IBA. Ex agar plants were harvested regularly after three weeks of growth period for their acclimatization in both cocopeat and soil. The survival rate of tissue cultured plants was 85%.

Key words: Axillary shoot proliferation, strawberry, runner tips, TDZ, daughter runners

Introduction

The cultivated strawberry (*Fragaria x ananassa* Duch.) is an octaploid species ($2n=8x=56$) belonging to genus *Fragaria* of family *Rosaceae* with at least 15 species (Hancock, 1990). Their fruits are rich in bioactive phytochemicals, especially phenolic compounds with high antioxidant capacity, and are known to be beneficial to human health when they are consumed as part of daily diet (Hannum, 2004). These nutritional qualities have ensured economic importance of this crop throughout the world and currently this crop is of primary interest for both research and fruit production. The cv. Sweet Charlie is a hybrid between FL80-456 and Pajaro and tested as FL 85-4925 and is popular for its delicious, sweet and nutritious fruits.

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 a minimum time. Moreover, the ability to regenerate plants is crucial to the successful application of *in vitro* methods (Taji *et al.*, 2002). Strawberries are vegetatively propagated by runners arising from axillary buds on the plant crown in a limited number. The tissue culture plants seemed to produce more runners per mother plant in a short time (Mohan *et al.*, 2005). Plants produced by axillary branching normally retain the genetic composition of the mother plant and this method has proven to be the most applied and reliable method for the production of true-to-types. Successful plant regeneration has been obtained in strawberry from single meristems (Boxus, 1999), node culture (Bhatt and Dhar, 2000), runner tips (Litwinczuk, 2004) and shoot tips (Ko Chien Ying *et al.*, 2009). Thidiazuron (TDZ), a substituted phenylurea (N phenyl-N'-1,2,3-thidiazol-5-yl urea) with its cytokinin- and auxin-like effects, is now among the most active cytokinin like substances (Huetteman and Preece, 1993) used to induce axillary shoot proliferation, shoot organogenesis either alone or in combination with auxins (Passey *et al.*, 2003) in strawberries. An efficient method of micropropagation technique however deemed

essential for supply of reliable plant material of this cultivar to meet the demands of strawberry growers from Mahabaleshwar hill region in Maharashtra. Though, Sweet Charlie is largely cultivated and well adapted variety in India, it is less studied variety. The objective of this study was to investigate the feasible protocol for micropropagation of strawberry cv. Sweet Charlie.

Material and methods

Strawberry plants were collected from "Strawberry Growers Association", Mahabaleshwar and maintained in institutes shade net for further experimentation. Runner tips from the mother plants of strawberry cv. Sweet Charlie were collected from plants maintained in shadenet house of the institute and were treated with Bavistin (2%) for 2 min. followed by surface sterilization with 0.1% mercuric chloride for 1 min. After repeated wash in sterile distilled water and 1 min treatment with 70% absolute alcohol, explants were reared on Murashige and Skoog's medium (Murashige and Skoog, 1962, MS) with sucrose (3%) and agar (0.8%) alone and along with TDZ 0.5, 1.0 and 2.0 mg/L individually and in a different combinations with each of 0.2 and 0.5 mg/L BAP and KN, 0.5 mg/L of IBA, 1.0 mg/L of GA₃. The pH of the medium was adjusted to 6.0. All the cultures were maintained at 26 °C (under 1000 lux) RH 50-60% at 12 hours photoperiod. For each treatment, 12 replicates were used in triplicate. The cultures were maintained for a period of three weeks and at the end of each experiment, cultures/calli were subcultured on a fresh medium for their growth and development. The shoots, roots and somatic embryos were observed using a stereoscopic microscope. The cultures were photographed wherever necessary. The regenerated plantlets via axillary shoot proliferation were successfully shifted to primary hardening in portrays filled with sterilized cocopeat after rinsing in 0.2% Bavistin. The portrays were covered with perforated polythene bags to maintain humidity for two weeks. During third week, the plants were shifted to polybags filled with soil:sand:cocopeat (1:1:1) and were gradually exposed to shadenet condition.

Results

The runner tips were inoculated on MS medium containing two concentration of TDZ (0.5 and 1.0 mg L⁻¹). The induction of multiple shoots occurred within 6 weeks from the runner tips along with the formation of daughter runners (Fig 1a) and the responses are shown in Table 1. The maximum number of stunted shoots, per explants were observed on MS medium supplemented with 1 mg L⁻¹ TDZ.

The separated multiple shoots from induction medium were subcultured on different media containing lower concentration of BAP and KN individually each at 0.5 mg/L and in combination with GA₃ (1 mg/L) and/ or IBA (0.5 mg/L) and the responses are shown in Table 2. The proliferation of multiple shoots was noticed on the medium supplemented with BAP alone and along with GA₃ and /or IBA or KN (Fig. 1b). Whereas, addition of BAP or KN individually and along with GA₃ supported elongation of shoots. On the contrary, incorporation of IBA (0.5 mg/L) supported the growth of both multiple shoots and their elongation (Fig. 1c). Ex agar plants (Fig. 1d) were successfully hardened by gradually lowering the humidity over 3 weeks in cocopeat as protrait plants (Fig. 1e) and in soil as secondary hardened plants (Fig. 1f) after regular three weeks interval period.

Table 1. Response of runner-tip cultures on MS supplemented with TDZ in strawberry cv. Sweet Charlie

TDZ mg L ⁻¹	Cultures responded (%)	Number of shoots/ explant
0.0	50	1.0
0.5	50	11.0
1.0	95	20.2

Discussion

In India, strawberry are cultivated largely in many places. Though cv. Sweet Charlie is cultivated but literature related to this

Table 2. Response of subcultured shoots for plant regeneration or multiple shoots on MS medium supplemented with different types of PGR

BAP	PGR level (mg/L)			Shoots/ culture	Response type	Cultures with multiple shoots (%)
	KN	GA ₃	IBA			
0.5	-	-	-	1.0	Root induction and regeneration of plantlets	11.3
-	0.5	-	-	1.0	Root induction and regeneration of plantlets	16.6
0.5	-	1.0	-	1.0	Elongation of shoots	14.3
-	0.5	1.0	-	1.0	Elongation of shoots	19.7
0.2	0.2	1.0	-	2.0	Shoot multiplication and their elongation	55.3
0.5	-	1.0	0.5	4.0	Shoot multiplication and their elongation	90.7

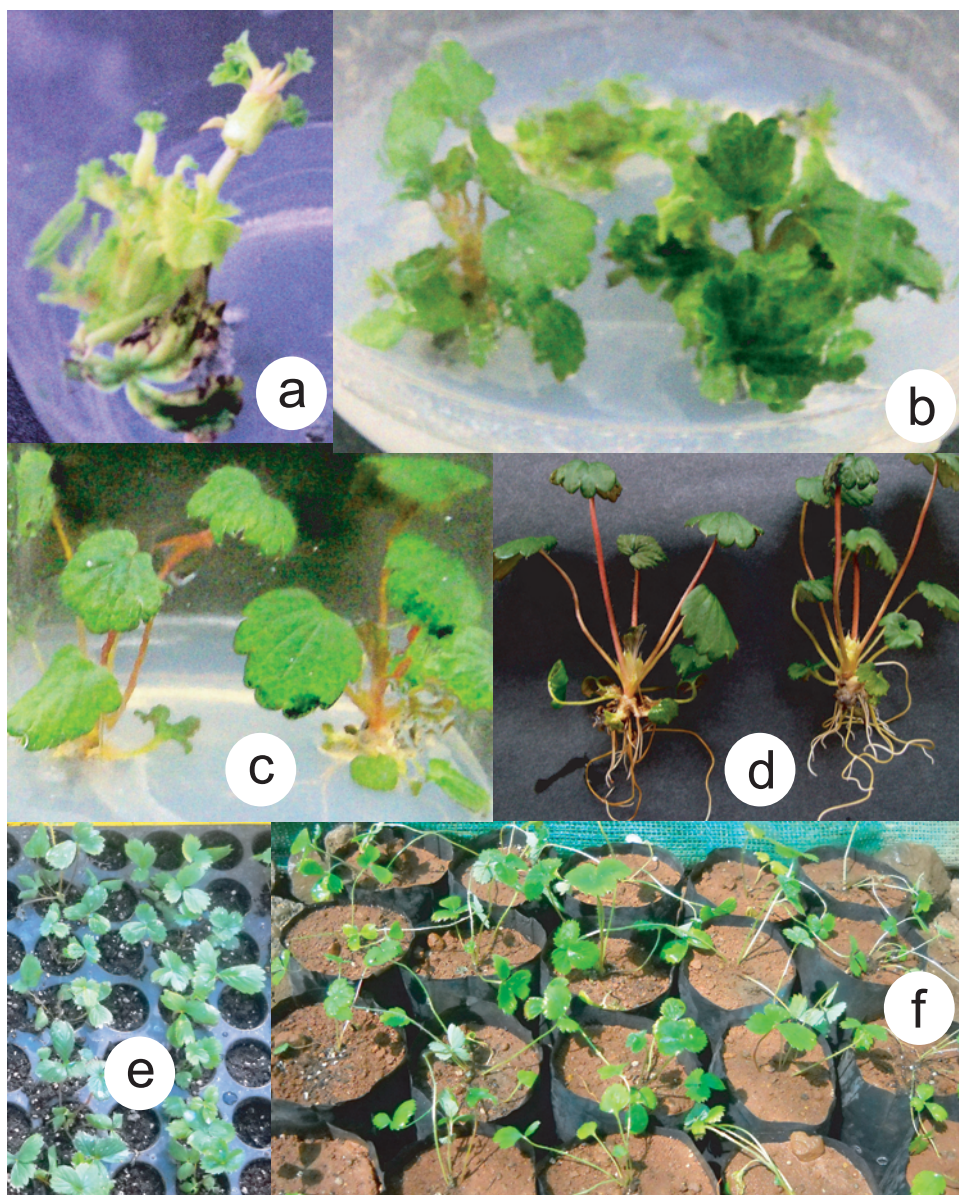


Fig. 1. Micropropagation of strawberry cv. Sweet Charlie from runner tips. a) Runner tip with multiple shoots from clump and *in vitro* runner. b) Separated shoots on multiplication medium. c) Regenerated shoots on rooting medium ready for harvesting

variety is not available. We used TDZ for the first time to get plants from axillary shoots. Explants taken from field-grown plants are difficult to sterilize to establish *in vitro* cultures due to high degree of contamination. It is usually recommended to take explants from plants grown under controlled conditions such as growth room or greenhouse to obtain better results. In the present study, individual TDZ effect was responsible for multiple shoot induction along with daughter runners from runner tips collected from green house maintained mother plants. *In vitro* runner formation was earlier reported in cvs. Gorella and Hakras Romata on Boxus medium with or without GA₃ (Zatykó *et al.*, 1989). The present study also demonstrates formation of daughter runners on MS medium with TDZ. In cv. Camarosa, report on significant effect of TDZ alone and in combination with IAA

for shoot proliferation, as well as at the rooting stage, addition of 1.0 mg/L of TDZ along with charcoal and IAA has been reported (Adak *et al.*, 2009). On the contrary to earlier reports on three strawberry clones other than Sweet Charlie, the highest response of shoot multiplication from nodal segments at the rate of three was obtained in MS containing IBA and KN and the maximum frequency of rooting was produced on medium containing 1.0 mg /L IBA (Sakila *et al.*, 2007).

The present study revealed the potential of successful year round multiplication of strawberry cv. Sweet Charlie from nodal segments to meet the demand for quality planting material in strawberry growing areas.

Acknowledgements

Authors thank Former Principal, Late Dr. R.M. Kothari for his encouragement, Dr. E.A. Singh and Dr. Bipinraj, for assistance in collection of plant material and Professors G.D. Sharma and P.K. Ranjekar for critically going through the MS. The financial support from Bharati Vidyapeeth University, Pune, Maharashtra, India is gratefully acknowledged.

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Received: October, 2010; Revised: November, 2011; Accepted: December, 2011