Biotechnology Reports 6 (2015) 79–84

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

# Highly efficient *in vitro* regeneration, establishment of callus and cell suspension cultures and RAPD analysis of regenerants of *Swertia lawii* Burkill

Parthraj R. Kshirsagar [a,*], Jaykumar J. Chavan [b,c], Suraj D. Umdale [a], Mansingraj S. Nimbalkar [a], Ghansham B. Dixit [a], Nikhil B. Gaikwad [a]

[a] Department of Botany, Shivaji University, Kolhapur 416 004, India
[b] Department of Botany, Yashavantrao Chavan Institute of Science, Satara 415 001, India
[c] Department of Biotechnology, Yashavantrao Chavan Institute of Science, Satara 415 001, India

ARTICLE INFO

*Article history:*
Received 29 December 2014
Received in revised form 28 February 2015
Accepted 8 March 2015
Available online 11 March 2015

*Keywords:*
Callus
Cell suspension
Micropropagation
Medicinal herb
RAPD
*Swertia lawii*

ABSTRACT

Highly efficient *in vitro* regeneration system has been developed for *Swertia lawii* Burkill, an important herb used as substitute for *Swertia chirayita*. Shoot tips explants were cultured on MS medium with various phytohormones for multiple shoot production. The best shoot production frequency (100%) and maximum shoots ($10.4 \pm 0.8$) were obtained on MS media containing TDZ ($3.0\ mg\ l^{-1}$) in combination with IBA ($0.3\ mg\ l^{-1}$). Maximum callus induction ($95 \pm 4.8\%$) and callus growth ($1.7 \pm 0.4$ gm) was achieved on MS medium with 2, 4-D ($3.0\ mg\ l^{-1}$). Cell suspension cultures were established and studied for their growth kinetics. Shoots were rooted best ($22.1 \pm 2.5$) in 1/2 MS medium with IAA ($3.0\ mg\ l^{-1}$). The genetic uniformity of the micropropagated clones was assessed using RAPD markers. Out of 405 bands, 400 (98.76%) were monomorphic and rest 5 (1.24%) were polymorphic. High multiplication frequency and low risk of genetic instability ensures the efficacy of this protocol.


## 1. Introduction

*Swertia* L., belongs to the family Gentinaceae, is a group of multipurpose medicinal herbs used in the Indian and Chinese traditional system of medicine since prehistoric time. About 40 species are reported to be available in the eastern and western parts of Indian Himalayas at high altitudes, while Western Ghat harbors about more than eight *Swertia* species [1]. The genus is a rich source of xanthonoids, flavonoids, iridoids and terpenoids [1]. The extracts of a number of *Swertia* species have been used in folk medicine for the treatment of hepatitis, cholecystitis, pneumonia, dysentery, scabies, spasm, pain and neurasthenia [2]. The herbs are extensively used as bitter tonic and febrifuges in ayurvedic system of medicine. Isolated bioactive compounds and various extracts of *Swertia* species possess several pharmacological properties [2–4]. Due to the high medicinal implications of this genus, many species has an established domestic and international market which is increasing at a rate of 10% annually [1,5]. Among all the species, *Swertia chirayita* was the most investigated species in terms of phytochemical analysis and pharmacognosy [4,6]. Almost *Swertia* species (including *Swertia lawii*) are rich source of oleanolic acid and ursolic acid, two pharmacologically important compounds [7–9]. *S. lawii* is an important medicinal herb which is used as adulterant and also as substitute to *S. chirayita* [10–12]. Several xanthone compounds were isolated from various organs of *S. lawii* and it was found that it is rich source of erythrocentaurin, an important bioactive compound [10,13]. Apart from its medical implications, the species has ornamental flowers (Fig. 1a).

Problem associated with *in vitro* plant regeneration is occurrence of somaclonal variations among the sub-clones of parental line, arising as a direct outcome of *in vitro* culture of plant cells, tissue and organs [14,15]. These genetic defects in the regenerants limit the utility of plant tissue culture techniques for large-scale multiplication. However, in recent year's molecular marker techniques such as RAPD and ISSR plays a significant role for detecting the genetic variation in the regenerants.

The *in vitro* propagation studies in *Swertia* species are limited with *S. chirayita* and few other species [16–19]. The establishment of tissue culture protocol will be an important action for multiplication, germplasm conservation and secondary metabolite

* Corresponding author at: Laboratory of Cytogenetics and Plant Breeding, Department of Botany, Shivaji University, Kolhapur 416 004, Maharashtra, India. Tel.: +91 231 2609157; fax: +91 231 2692333.
*E-mail address:* nbgaikwadsuk@gmail.com (P.R. Kshirsagar).

http://dx.doi.org/10.1016/j.btre.2015.03.003

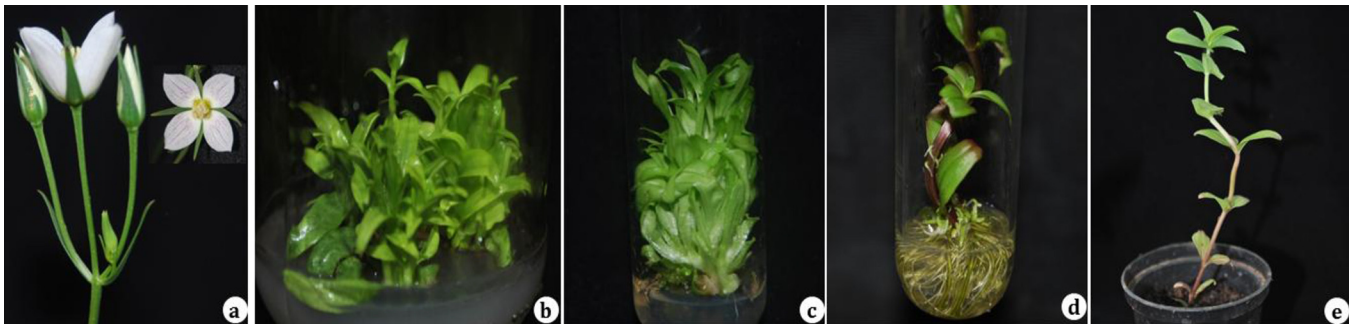


**Fig. 1.** Micropropagation of *Swertia lawii*. a: Flowers, b and c: shoot multiplication (MS + 3.0 mg $l^{-1}$ TDZ + 0.3 mg $l^{-1}$ IBA), d: *in vitro* rooting (2.0 mg $l^{-1}$ IBA), e: hardened plant.

production in *S. lawii*. Thus, the objective of this work was to systematically study new strategies for *in vitro* culture of *S. lawii* aiming at developing efficient plant propagation protocol as well as establishing callus and cell suspension cultures. Moreover, the genetic stability among *in vitro* raised clones was assessed by RAPD markers.

## 2. Experimental

### 2.1. Plant material and culture conditions

Mature fruits of *S. lawii* were collected from Panhala locality of Western Ghats. Seeds were separated and washed with sterile distilled water in vials for 2–3 times. Then the seeds were surface disinfected with aqueous solution of 0.1% $HgCl_2$ for 2 min and finally washed with sterile distilled water for 2–3 times. For *in vitro* germination, seeds were inoculated on the Murashige and Skoog (MS) medium with vitamins, sucrose (3%, w/v) and solidified with 0.2% clarigel (Himedia, India). Before autoclaving at 121 °C for 15 min, the pH of the medium was adjusted to 5.8. All the cultures were maintained at 25 ± 1 °C with photoperiod of 16-h using a photosynthetic photon flux density (PPFD) of 40 μmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent lamps (Philips, India) for 30 days.

### 2.2. Seed germination, primary cultures and shoot multiplication

Surface sterilized seeds were cultured on MS basal medium for *in vitro* germination. In order to obtain cultures, shoot apices were excised from 30 day old seedlings were inoculated on MS medium supplemented with 0.5 mg $l^{-1}$ BAP and incubated under a 16-h photoperiod. In order to optimize shoot multiplication, shoot tips were excised from primary cultures were cultured on MS medium supplemented with different concentrations and combinations of

**Table 1**
Effect of different plant growth regulators on shoot regeneration from shoot tip explants of *Swertia lawii*.

| PGRs | Concentration (mg $l^{-1}$) | Regeneration frequency (%) | Number of shoots/explant (mean ± SE) | Length of shoots (cm) mean ± SE |
|---|---|---|---|---|
| PGR free | 00 | 00 | 0.0 | 0.0 |
| BAP | 1.0 | 100 | 6.3 ± 0.3** | 3.3 ± 0.3** |
| | 2.0 | 100 | 4.8 ± 0.4** | 2.4 ± 0.1** |
| | 3.0 | 100 | 6.8 ± 0.4** | 3.6 ± 0.2** |
| | 4.0 | 100 | 6.1 ± 0.4** | 4.7 ± 0.5** |
| | 5.0 | 100 | 6.2 ± 0.4** | 2.7 ± 0.1** |
| KN | 1.0 | 100 | 4.5 ± 0.2** | 2.9 ± 0.1** |
| | 2.0 | 100 | 4.5 ± 0.3** | 2.8 ± 0.2** |
| | 3.0 | 95 | 3.0 ± 0.2** | 2.3 ± 0.2* |
| | 4.0 | 90 | 3.2 ± 0.3** | 2.8 ± 0.4** |
| | 5.0 | 90 | 2.9 ± 0.2** | 2.2 ± 0.2* |
| TDZ | 1.0 | 60 | 1.7 ± 0.3* | 0.7 ± 0.2$^{ns}$ |
| | 2.0 | 85 | 3.1 ± 0.4** | 2.7 ± 0.5** |
| | 3.0 | 100 | 5.8 ± 0.5** | 3.0 ± 0.2** |
| | 4.0 | 95 | 5.7 ± 0.8** | 1.8 ± 0.1* |
| | 5.0 | 95 | 5.4 ± 0.6** | 2.1 ± 0.2* |
| BAP + IBA | 3.0 + 0.1 | 100 | 6.8 ± 0.4** | 3.5 ± 0.1** |
| | 3.0 + 0.3 | 100 | 7.1 ± 0.3** | 5.9 ± 0.3** |
| | 3.0 + 0.5 | 100 | 6.9 ± 0.4** | 3.4 ± 0.2** |
| | 3.0 + 0.7 | 100 | 6.4 ± 0.4** | 2.9 ± 0.1** |
| | 3.0 + 1.0 | 100 | 6.7 ± 0.6** | 2.3 ± 0.2* |
| TDZ + IBA | 3.0 + 0.1 | 100 | 5.9 ± 0.7** | 2.3 ± 0.2* |
| | 3.0 + 0.3 | 100 | 10.4 ± 0.8** | 3.7 ± 0.4** |
| | 3.0 + 0.5 | 100 | 8.1 ± 0.8** | 3.9 ± 0.3** |
| | 3.0 + 0.7 | 100 | 5.9 ± 0.4** | 3.2 ± 0.2** |
| | 3.0 + 1.0 | 100 | 5.7 ± 0.3** | 2.7 ± 0.1** |

Mean ± S.E. of 30 replicates per treatment and experiments were repeated thrice. The values are significantly different at ns – non significant, *$P < 0.05$ and **$P < 0.01$ level when compared by Dunnett multiple comparisons test using one way ANOVA.