Progress in Nutrition 2017; Vol. 19, N. 1: 92-96 - DOI: 10.23751/pn.v19i1.5305

Original Article

Efficient callus formation and these callus antibacterial activities of a valuable medicinal plant *Stachys cretica* L. subsp. *garana* (Boiss) Rech

Fethi Ahmet Ozdemir[1*], *Gulden Kocak*[1], *Murat Kursat*[2]

[1]Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, Bingol, Turkey - E-mail: ozdemirfethiahmet23@yahoo.com; [2] Department of Biology, Faculty of Science and Art, Bitlis Eren University, Bitlis, Turkey

Summary. An effective in vitro callus regeneration was developed for *Stachys cretica* L. subsp. *garana* (Boiss) Rech., a valuable medicinal plant. Callus formation in *in vitro* of *S. cretica* subsp. *garana* was induced on hypocotyl explants cultured on 2,4-D with and without TDZ (9 combinations). The findings of the results showed that callus formation percentage of the treatments ranged from 83.66±0.13 to 46.33±1.23. Maximum callus formation percentage was observed on explants cultured on MS medium having 4 mg/l 2,4-D. Minimum callus formation percentage was seen on MS medium having 2 mg/l 2,4-D + 0.2 mg/l TDZ. Callus weight of the treatments ranged from 352.32±0.63 to 163.12±1.56. Increase concentration of TDZ significantly decrease in callus weight. Antibacterial activity was evaluated inhibition of growth *Staphylococcus aureus* COWAN 1 and *Bacillus subtilis* var. *niger* ATCC 10 in the callus extracts determined by disc diffusion methodology. No antibacterial activities were detected in control group, acetone extracts and hexane extract of callus regenerated on MS medium having 4 mg/l NAA. Antibacterial activity for *Staphylococcus aureus* COWAN 1 and *Bacillus subtilis* var. *niger* ATCC 10 was noted on methanolic callus extracts. Moreover, the present study results showed that the increase of TDZ concentration in the callus regeneration environment resulted in a increase of antibacterial activity. The results of the present finding showed that extracts of calli developed through influenced therapeutic potential by the plant growth regulators treatments. The *in vitro* induced *S. cretica* subsp. *garana* Calli can be used as healing and curing agent in pharmaceuticals.

Key words: *Stachys cretica* subsp. *garana*, callus formation, *Staphylococcus aureus* COWAN 1, *Bacillus subtilis* var. *niger* ATCC 10

Introduction

There are more than 270 species in genus *Stachys* L. (1) and is it is the largest genera among the Labiatae. This genus is generally found in the temperate Irano-Turanian and the Mediterranean regions (2). With 83 recorded species and a level of 48% endemism, Turkey is one of the richest countries in *Stachys* taxa. Many members of this genus find use in traditional medicine of Anatolia. They are used the same purpose as sage to treat skin infections, digestive problems and respiratory disorder (3). Similar folkloric uses of many other species that possess antiphlogistic, cholagogic, sedative which are used for the treatment of coughs, kidney diseases, tumors and throat pains have also appeared in the world literature (4,5). *Stachys* taxa at least nine natural product chemical which including alkaloids (6), iridoids (7), terpenoids (8,9), steroids, flavonoids (10), phenylpropanoid glycosides, as well as carbohydrates, essential oils and lipids (11-13).

Plant micropropagation and tissue culture is most popularly used to conserve rapidly propagate plants under *in vitro* conditions to produce secondary metabolites (14, 15). These cultures are well known in the medicine and pharmacology. These cultures can also help in bioactive extraction of natural products contin-

uously without waiting for a specific season. Therefore, this plant species could be benifically used for extraction of useful medicinally important metabolites.

The objective of present research was to develop a protocol for *in vitro* callus induction using hypocotyls of *S. cretica* subsp. garana and to determine antibacterial activity of *S. cretica* subsp. garana which may have a greater application and potential in treatment of infectious diseases against *Staphylococcus aureus* COWAN 1 and *Bacillus subtilis* var. *niger* ATCC 10.

Materials and Methods

Plant material and callus regeneration

The seeds of *S. cretica* subsp. *garana* were provided by the Department of Biology, Bitlis Eren University, Bitlis, Turkey. Voucher specimens are deposited at the Faculty of Science Herbarium at Firat University, Elazig, Turkey. The seeds were sterilized with 5% NaOCl from Ace® Turkey branded commercial bleach by continuously stirring for 20 min. It was followed by 3 × 3 min. sterilized distilled water rinsing. The sterilized seeds were germinated by culturing on agar solidified MS medium (16) contained in Petri dishes (100 ×10 mm) that was supplemented with 3% sucrose at 4 ± 1°C. Thereafter 7 weeks, the hpocotyl explants were excised from the seedlings and taken to culture on 1, 2, 4 mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid) containing MS medium with and without 0, 0.1, 0.2 mg/l TDZ (Thidiazuron). All cultures contained 3% (w/v) sucrose and 0.65% (w/v) plant agar (Duchefa), them under 16 h light photoperiod (35µmol $m^{-2}s^{-1}$) in Aralab versatile growth chamber at 24 ± 1°C. All media were autoclaved for 20 min. at 121°C and 1.4 kg cm^{-2} pressure. The pH of all media was adjusted to 5.7± 0.1 with 1N NaOH or 1N HCl.

Antibacterial Activity

The calli regenerated after each treatment were lavigated under aseptic conditions. Each of the lavigated calli (5 g) were extracted using 25 ml hot water (control), acetone, hexane and methanol (99%) solvents by using a rotary shaker (100 rpm) for 24 h. The solvents used in the study were removed with the help of a rotary vacuum evaporator at 35°C. Whatman filter paper were used for filtering. Thereafter, 25 µl extracts were syringed onto empty antibiotic paper discs (6 mm) (Bioanalyse). The experimental studies were repeated triplicate. Antibacterial activities for *Staphylococcus aureus* COWAN 1 and *Bacillus subtilis* var. *niger* ATCC 10 strains of bacteria were studied. Therafter, 100 µl bacterial solution (10^6 cells/ml) were inoculated into petri plates containing Mueller Hinton Agar (Difco) using disc diffusion method for antibacterial activity. Six (6) mm diameter discs were used containing to pour 25 µl (500 µg) of the extracts (20 mg/ml) that were placed on the inoculated Mueller Hinton Agar (Difco). The Petri dishes were incubated at 4°C for 2 h. Thereafter, the inoculated plates were incubated at 37 °C, for 24 h (for bacterial strains). Antibacterial activity was evaluated by measuring zone diameters in mm (17).

Statistical analysis

In each treatment 30 explants were used and divided into 3 replications. Experimental values for each parameter were compared using One Way Anova of IBM SPSS 22 for Windows. Standard error was computed using descriptive statistics function. Means were compared using Tukey's b test. All values expressed as 0.00 were not subjected to statistical analysis.

Results

Callus induction on MS medium containing 2,4-D + TDZ

Callus induction was noted for all treatments on hypocotyl explants (Table 1). The findings of the results showed that 100% calli were induced on hypocotyl explants irrespective of 2,4-D concentrations with or without TDZ in the culture medium. The result showed that the plant growth regulators had significantly different effect on callus formation percentage (Table 1). Callus formation percentage of the treatments ranged from 83.66±0.13 to 46.33±1.23. Maximum callus formation percentage was noted on MS medium containing 4 mg/l 2,4-D followed 4 mg/l 2,4-D + 0.1 mg/l TDZ. Minimum callus formation percentage was recorded on MS medium containing 2 mg/l 2,4-D + 0.2 mg/l TDZ (Table 1). The callus weight was also affected by the growth regulators com-

binations. Callus weight of the treatments ranged from 352.32±0.63 to 163.12±1.56. Maximum callus weight was noted using 4 mg/l 2,4-D in MS medium. It was followed weight of 312.08±0.19 and 301.68±0.74 using 2 mg/l 2,4-D, 1 mg/l 2,4-D in MS medium. Each increase in the concentration of 2,4-D (with or without any concentration of TDZ) was accompanied with significant increase in callus weight but increased TDZ concentrations significantly decreased the callus weight (Table 1).

Antibacterial activity

Antibacterial activity was detected in *in vitro* callus of *S. cretica* subsp. garana are given in Table 2. No antibacterial activities was detected using extracts obtained from control group, acetone extract and hexane extract from develope on MS medium containing 4 mg/l NAA. The methanol extracts of *S. cretica* subsp. garana calli exhibited inhibitory action against both bacterial strains variably.

When we compared the antibacterial activity of the methanol extracts of the calli developed on MS medium containing 4 mg/l 2,4-D with those on 2,4-D + 0.10 or 0.20 mg/l TDZ induced callus extracts it was noted that the obtained on MS medium containing 2,4-D had less antibacterial activity. Methanolic extracts of various callus showed bioactivity against *Staphylococcus aureus* COWAN 1 and *Bacillus subtilis* var. *niger* ATCC 10 bacteria. The increase of TDZ concentration in the callus production environment resulted in an increase of antibacterial activity.

Discussion

S. cretica subsp. *garana* herbaceous plant that contain a large number of volatile and aromatic compounds (18). We studied callus induction in hypocotyl explants raised from seeds and tested their crude callus extracts for antibacterial activity.

Synergistic effects in callus formation is dependent on cytokinin-auxin in *in vitro*. Comparative impacts were additionally seen in a few other species (19-21). The outcomes demonstrate that the blend of 2,4-D and TDZ were critical for significantly callus development in *S. cretica* subsp. *garana*. The combined favorable impact of cytokinin-auxin was similar to the response observed in other lant species (19, 22, 24). The present research showed that callus induction in *S. cretica* subsp. garana is reliant on the sort and blend of phytohormones. Comparable results have likewise been reported for other plant species (19, 20). The nu-

Table 1. The effect of 2,4-D and TDZ concentrations on callus formation percentage and callus weight of hypocotyl explants.

Plant growth regulator concentrations and combinations		Callus formation percentage (%)*	Callus weight (mg)*
2,4-D (mg/l)	**TDZ (mg/l)**		
1	0.0	56.37±0.24e	301.68±0.74c
2	0.0	67.87±0.52b	312.08±0.19b
4	0.0	83.66±0.13a	352.32±0.63a
1	0.1	52.33±1.42f	205.61±0.35f
2	0.1	57.87±0.87d	243.21±0.96e
4	0.1	63.33±1.02c	262.17±0.43d
1	0.2	48.67±1.36g	163.12±1.56i
2	0.2	46.33±1.23h	187.63±1.07h
4	0.2	51.66±1.07f	194.79±1.43g
Control (MS medium)		0.00 ± 0.00	0.00 ± 0.00

**All values in a single column showed by different small letters are statistically different at 0.05 level of significance ± = Standard error*

Table 2. Effects of extracts from 2,4-D + TDZ induced calli on antibacterial activity against *S. aureus* and *B. subtilis*

Plant growth regulator combinations	Extract type	Antibacterial activity	
	S. aureus	***B. subtilis***	
4 mg/l 2,4-D	Acetone	Nil	Nil
	Hexane	Nil	Nil
	Methanol	5	8
	Control	Nil	Nil
4 mg/l 2,4-D + 0.10 mg/l TDZ	Acetone	Nil	Nil
	Hexane	Nil	10
	Methanol	5	11
	Control	Nil	Nil
4 mg/l 2,4-D+ 0.20 mg/l TDZ	Acetone	Nil	Nil
	Hexane	9	10
	Methanol	8	12
	Control	Nil	Nil

Inhibition zone diameters were measured in mm.

trition needs for the growth of tissue under *in vitro* conditions can fluctuate among species and genotypes and also with the impact of endogenous phytohormones level in respective plant species.

Investigation of bioactivity of callus and plant tissues is needed to bolster its utilization in customary pharmaceuticals (25). By and large, micropropagated and plants propagated in fields can not be utilized therapeutically potent as naturally grown plants. However, this case is not generally precise (26, 27). In the present research antibacterial bioactivity was noted on *in vitro* induced callus against *S. aureus* and *B. Subtilis*. The outcomes show that bioactivity is subject to treated plant growth regulators and the plant tissue.

This acquisition is likewise effective for *Scutellaria orientalis* subsp bicolor, *Drimia robusta, Catha edulis and Cotyledon orbiculata* (19, 24, 27, 28). Antibacterial properties were influenced by auxiliary compounds (19, 24, 29). The bioactivity in plants may have been affected with secondary compounds which controlled by PGRs amid *in vitro* cultures and acclimatization. Be that as it may, the system of biochemical changes should be researched in this species. In conclusion this is the first report of *in vitro* callus development and antibacterial movement in *S. cretica* subsp. *garana*. This paper demonstrated that *in vitro* callus induction of *S. cretica* subsp. *garana* contains therapeutic ability, which can be utilized as a part of conventional medicines. Additionally, the convention will be useful for callus induction of bioactive compounds for future research.

References

1. Mabberley DJ. The plant book. Cambridge University Press, Cambridge, New York, Melbourne.
2. Bhattacharjee R. Taxonomic Studies in Stachys: II. A New Infrageneric Classification of Stachys L. 1980. Edinburgh: Notes from the Royal Botanical Garden.
3. Yesilada E, Sezik E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey V. Folk medicine in the iner Taurus Mountains. Journal of Ethnopharmacology, 1999; 64: 195-210.
4. Maleki-Dizaji N, Nazemiyeh H, Maddah N. Screening of extracts and fractions from aerial parts of Stachys schtschegleevii Sosn. for anti-inflammatory activities. Pakistan Journal of Pharmaceutical Sciences, 2008; 21: 338-343.
5. Kartsev VG, Stepanichenko NN, Auelbekov SA. Chemical composition and pharmacological properties of the genus Stachys. Chemistry of Natural Compounds, 1994; 30: 645-654.

6. Pulatova TP. Presence of alkaloids in some plants of the family Labiatae. Khimiya Prirodnykh Soedinenii, 1969; 5: 62-63.
7. Toshihiro M, Endo Y, Miyase T, Yoshizaki F. Iridoid glycoside constituents of Stachys lanata. Journal of Natural Products, 2008; 71: 1768-1770.
8. Kotsos MP, Aligiannis N, Mitakou S. A new flavonoid diglycoside and triterpenoids from Stachys spinosa L. (Lamiaceae) Biochemical Systematics and Ecology, 2007; 35: 381-385.
9. Soliman SM, El-Dib R, Shalaby NMM, Duddeck H, Simon A, Toth G. Isolation and structure determination of compounds from Stachys yemenensis Hedge. Natural Product Communications, 2007; 2: 977-980.
10. Ahmad VU, Arshad S, Bader S, Ahmed A, Iqbal S, Khan A, Khan SS, Tareen RB. A new triterpenoidal saponin and a flavone glycoside from Stachys parviflora. Natural Product Communications, 2007; 2: 889-894.
11. Radulović N, Lazarević J, Ristić N, Palić R. Chemotaxonomic significance of the volatiles in the genus Stachys (Lamiaceae): Essential oil composition of four Balkan Stachys species. Biochemical Systematics and Ecology, 2007; 35: 196-208.
12. Radulović N, Lazarević J, Stojanović G, Palić R. Chemotaxonomically significant 2-ethyl substituted fatty acids from Stachys milanii Petrović (Lamiaceae). Biochemical Systematics and Ecology, 2006; 34: 341-344.
13. Giuliani C, Pellegrino RM, Tirillini B, Bini LM. Composition of essential oils from leaves and flowers of Stachys germanica subsp. salviifolia (Ten.) Gams (Labiatae) and related secretory structures. Natural Product Communications, 2009; 4: 831-834.
14. Sajc L, Grubisic D, Vunjak-Novakovic G. Bioreactors for plant engineering: an outlook for further research. Biochemical Engineering Journal, 2000; 4:89–99.
15. Georgiev V, Ivanov I, Berkov S, Pavlov A. Alkaloids biosynthesis by Pancratium maritimum L. shoots in liquid culture. Acta Physiologiae Plantarum, 2011; 33:927–933.
16. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 1962; 15: 473–494.
17. Collins CM, Lyne PM. Microbiological methods butter morths & Co (Publishers) Ltd. London, 1987; 450 pp.
18. Serbetci T, Demirci B,Bozkurt Guzel C, Kultur S, Erguven M, Baser KHC. Essential oil composition, antimicrobial and cytotoxic activities of two endemic Stachys cretica Subspecies (Lamiaceae) from Turkey. Natural Product Communications, 2010; 5(9): 1369-1374.
19. Ozdemir FA, Kilic O, Atalan E. In vitro callus propagation and antibacterial activities of callus an edible endemic and medicinal plant Scutellaria orientalis L. subsp. bicolor. Progress in Nutrition, 2016; 18(1): 81-86.
20. Parmaksiz I, Khawar KM. Plant regeneration by somatic embryogenesis from immature seeds of Sternbergia candida Mathew et T. Baytop, an endangered endemic plant of Turkey. Propagation of Ornamental Plants, 2006; 6 (3): 128-133.
21. Ozel CA, Khawar KM, Karaman S, Ates MA, Arslan O. Efficient in vitro multiplication in Ornithogalum ulophyllum Hand.-Mazz. from twin scale explants. Scientia Horticulturae, 2008; 116(1): 109-112.
22. Distabanjong K, Geneve RL. Multiple shoot formation from cotyledonary node segments of Eastern redbud. Plant Cell Tissue Organ Culture, 1997; 47: 247–254.
23. Daksa J, Abera B, Taddese T. Micropropagation of Phytolacca dodecandra L 'Herit (Endod var. E-44). African Journal of Biotechnology, 2015; 14: 108–118.
24. Kumari A, Baskaran P, Van Staden J. In vitro propagation and antibacterial activity in Cotyledon orbiculata: a valuable medicinal plant. Plant Cell Tissue Organ Culture, 2016; 124: 97–104.
25. Baskaran P, Chukwujekwu JC, Amoo SO, Van Staden J. Anticholinesterase and mutagenic evaluation of in vitro-regenerated Agapanthus praecox grown ex vitro. In Vitro Cellular & Developmental Biology - Plant , 2014; 50: 271–275.
26. Garcı´a-Pe´rez E, Gutie´rrez-Uribe JA, Garcı´a-Lara S. Luteolin content and antioxidant activity in micropropagated plants of Poliomintha glabrescens (Gray). Plant Cell Tissue Organ Culture, 2012; 108:521–527.
27. Baskaran P, Singh S, Van Staden J. In vitro propagation, proscillaridin A production and antibacterial activity in Drimia robusta. Plant Cell Tissue Organ Culture, 2013; 114: 259–267.
28. Kumari A, Baskaran P, Van Staden J. Enhanced HIV-1 reverse transcriptase inhibitory and antibacterial properties in callus of Catha edulis Forsk. Phytotherapy Research, 2015; 29: 840–843.
29. Banasiuk R, Kawiak A, Krolicka A. In vitro cultures of carnivorous plants from the Drosera and Dionaea genus for the production of biologically active secondary metabolites. BioTechnologia, 2012; 93: 87–96.

Correspondence:
Fethi Ahmet Ozdemir
Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, 12000, Bingol, Turkey
Tel No: +90 0 426 216 00 12
Fax No: +90 0 426 216 00 22
E-mail: ozdemirfethiahmet23@yahoo.com