Saudi Journal of Biological Sciences (2016) **23**, 488–494

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Development of an efficient callus proliferation system for *Rheum coreanum* Nakai, a rare medicinal plant growing in Democratic People's Republic of Korea

Song-Chol Mun [a,*], **Gwan-Sim Mun** [b]

[a] *Department of Pharmacy, Pyongyang Medical College, Kim Il Sung University, Pyongyang, Democratic People's Republic of Korea*
[b] *Department of Medical Plant Resources, Institute of Pharmaceutics, Academy of Medicine Sciences, Pyongyang, Democratic People's Republic of Korea*

Received 6 March 2015; revised 25 May 2015; accepted 25 May 2015
Available online 6 June 2015

KEYWORDS

Anthraquinone;
Plant growth regulators;
Rheum coreanum Nakai;
Rhizome;
Rhubarb;
Tissue culture

Abstract A clonal mass propagation to obtain mountainous sources of *Rheum coreanum* Nakai, a rare medicinal plant in Democratic People's Republic of Korea was established by rhizome tissue culture. Whole plants were selected and collected as a vigorous individual free from blights and harmful insects among wild plants of *R. coreanum* grown on the top of Mt. Langrim (1.540 m above the sea) situated at the northern extremity of Democratic People's Republic of Korea. Induction of the callus was determined using four organs separated from the whole plant and different plant growth regulators. The callus was successfully induced from rhizome explant on MS medium containing 2.4-D (0.2–0.3 mg/l). In the MS medium supplemented with a combination of BAP (2 mg/l) and NAA (0.2 mg/l), single NAA (0.5 mg/l), or IBA (0.5 mg/l), a higher number of shoot, root and plantlets was achieved. The survival rate on the mountainous region of the plantlets successfully acclimatized (100%) in greenhouse reached 95%, and yields of crude drug and contents of active principles were higher than those obtained by sexual and vegetative propagation. This first report

Abbreviations: 2,4-D, 2,4-dicholorophenoxy acetic acid; BAP, 6-benzylaminopurine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; KT, kinetin; MS, Murashige and Skoog culture medium; NAA, a-naphthalene acetic acid.

* Corresponding author at: Department of Pharmacy, Pyongyang Medical College, Kim Il Sung University, St. Sungri, Pyongyang, Democratic People's Republic of Korea.
E-mail address: muchmu123@163.com (S.-C. Mun).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.05.017

of *R. coreanum* tissue culture provides an opportunity to control extinction threats and an efficient callus proliferation system for growing resources rapidly on a large scale.

1. Introduction

Rheum coreanum Nakai is a perennial plant indigenous to DPR of Korea (Hoang et al., 1997). The rhizome and root of this plant have been generally used a stomachic, antidote, cathartic and hemostatic in the traditional medicine of DPR of Korea. On the other hand, Rheum species is useful in the treatment of hemorrhoid (Çakılcıoğlu and Türkoğlu, 2007), inflammation (Hu et al., 2014), pass kidney stones (Çakılcıoğ lu and Türkoğlu, 2008), and diabetes disease (Kaval et al., 2014; Raafat et al., 2014). It also shows anti-allergic (Matsuda et al., 2004), antidyslipidemic (Mishra et al., 2013), antifungal (Agarwal et al., 2000), antimicrobial (Kosikowska et al., 2010), antioxidant (Öztürk et al., 2007), anti-platelet (Aburjai, 2000), antiviral (Chang et al., 2014), cytotoxic (Kubo et al., 1992), hepatoprotective (Ibrahim et al., 2008), and hypoglycemic (Naqishbandi et al., 2009) activities, and immuno-enhancing (Kounsar et al., 2011), and renal function improving (Azhar Alama et al., 2005) effects. Its demand has increased in connection with the antivirus and anticancer activities of anthraquinone derivatives, which have recently been known (Li et al., 2009; Martens et al., 2014). The contents of free anthraquinones, total anthraquinones and combined anthraquinones in roots were higher than in other organs, that is, their contents in the underground part were much more than those of the aerial parts. A previous study reported the content of anthraquinone derivatives in the rhubarb processed with *R. coreanum* was higher than the rhubarb processed with other Rheum species such as *Rheum palmatum*, *Rheum officiale* and *Rheum undulatum*. (Ohshima et al., 1988) However, natural resources of *R. coreanum* were exhausted due to the lower propagation rate of this plant distributed at limited areas of high mountain tops (more than 1.500 m above the sea) and over collection for a long time. The plant, a polar plant, is damaged by insects (Kurosaki et al., 1992; Walkey and Matthews, 1979) and changed into metamorphosis when cultivated in lower regions. Rheum species may be propagated by sexual reproduction with seeds that produce hereditary non-uniformity generations since it is the cross-fertilization plant and by vegetative reproduction with single lateral buds that are propagated very slowly (Ohshima et al., 1988; Schulte et al., 1984).

Plant tissue culture techniques offer a powerful tool for germplasm conservation and mass multiplication of many threatened plant species, and also offer an alternative to manufacture economically important secondary metabolites such as flavors and pharmaceuticals within controlled laboratory environments (Dakah et al., 2014; Guo et al., 2007; Palacio et al., 2012; Ozel et al., 2015; Wang et al., 2011). An efficient callus proliferation system for *Rheum franzenbachii* Munt., a rare medicinal plant, has been developed because of its demand (Palacio et al., 2012; Wang et al., 2011). Therefore, we designed that resources of *R. coreanum* would be created at mountainous regions (1.000–1.500 m above the sea, 10–18 °C in summer), which adapt to the growth condition of *R. coreanum*. The aim of this work is to study the possibility of rhizome tissue culture technology for the clonal mass propagation of *R. coreanum* on mountainous regions.

2. Materials and methods

2.1. Materials

Whole plants were selected and collected as vigorous individuals free from blights and harmful insects among wild plants of *R. coreanum* grown on the top of Mt. Langrim (1.540 m above the sea) in the northern extremity of Province of Jagang, DPR of Korea and were used as initial materials in this study.

2.2. Preparation of an explant segment

Stem tip, young leaf, rhizome and root tip were separated from the whole plants. After washing the separated parts of the plant under running tap water, the explants were surface-sterilized by washing with 75% ethanol for 30 s and undiluted sodium hypochlorite with the pH adjusted to 6.8–7.0 using hydrochloric acid (HCl) for approximately for 3 min. Subsequently, the Stem tip, young leaf, rhizome and root tip were washed five times with sterile distilled water and cut it into the size of 10–15 mm^3 in capacity.

2.3. Induction of callus on the explant segments of different organs

Explants were placed to germinate in Petri dishes containing MS medium with half strength of macronutrients, 0.8% agar and 3% sucrose (pH 6). The medium also contained different kinds and concentrations of plant growth regulators. The pH of the media was adjusted to 5.6 prior to autoclaving for 15 min at 120 °C. Subsequently, they were incubated at (25 ± 1) °C and 60–70% relative humidity under a 14 h photoperiod by cool-white fluorescent lights (36.0 μmol m^{-2} s^{-1}) for 25 days.

Table 1 Comparison of inducing rate of the callus on different organs of *Rheum coreanum* (n = 30).

Parts of plant	Inducing rate of callus according to kinds and concentrations of plant growth regulators (%)					
	2.4-D/mg/l		NAA/mg/l		Kinetin/mg/l	
	0.2	0.5	0.2	0.5	0.5	1.0
Stem tip	85	80	58	65	12	7
Leaf	36	25	92	30	5	3
Rhizome	95	82	75	80	15	13
Root top	60	73	42	55	16	10

2.4. Callus multiplication culture of rhizome segments

The rhizome segments were incubated in MS liquid medium containing various kinds and concentrations of plant growth regulators. Cultures were kept in a growth chamber at (25 ± 1) °C and 30 rotations/min under a 14 h photoperiod by cool-white fluorescent lights (36.0 μmol m^{-2} s^{-1}) for 28 days in order to form the callus. For fresh weight determination (FW), twelve callus pieces were harvested from each treatment and weighed, with the dry (DW) being determined after lyophilization. These parameters were recorded after 4 weeks of culture. Then multiplication rate of the callus was measured.

2.5. Differentiation of callus

Callus segments cut into the size of 15–20 mm^3 in capacity were transplanted on MS medium (pH 6) containing 0.8% agar and 3% sucrose, supplemented with the different kinds and concentrations of plant growth regulators 0.2 mg/l and 0.5 mg/l NAA in combination with three concentrations of BAP (1, 2 and 3 mg/l) or each BAP alone (1 and 2 mg/l) and cultivated at (25 ± 1) °C under a 14 h photoperiod by cool-white fluorescent lights (36.0 μmol m^{-2} s^{-1}) for 28 days. Plantlets were transplanted on root different medium and cultivated at the same condition for 30 days, then the number and the length of a root were measured.

2.6. Transplantation of plantlet to soil

The stopper of a cultured bottle was open for 3–4 days, then the plantlets with 3–5 roots (length 2–4 cm) were taken off from the cultured bottle and transplanted on a nursery with sterilized soil, and were adapted to low humidity and sunshine for about 20 days. Adapted plantlets were transplanted at a distance of 1 m between the plants to the mountainous region (more than 1.000 m above the sea).

2.7. The extraction and analysis of anthraquinones

0.3 g of samples was extracted using the mentioned method with a slight modification (Farzami Sepehr and Ghorbanli, 2002). The extract was cooled rapidly and filtered, acidified with concentrated hydrochloric acid and refluxed for 1 h. The solution was cooled and extracted with chloroform. The anthraquinones in the chloroform layer were used for an analysis. Determination of total anthraquinone and sennoside by spectrometry was performed at 500 nm with 1, 8-dihydroxyanthraquinone as standard substance.

2.8. Statistical analysis

All the above experiments were independently repeated three times (each with 30 explants) under the same conditions. Data were evaluated using an analysis of variance from which mean ± standard error values were computed for comparison between treatments. An analysis of variance (ANOVA) followed by Duncan's multiple range test for mean comparison at a 5% probability level was used to test statistical significance.

3. Results and discussion

3.1. Induction of callus from different organs of the plant

Rhizome among various organs of the whole plant is an excellent explant for callus induction. A combination of plant growth regulators had successfully induced the formation of callus culture from many higher plants (Han et al., 2009; Li et al., 1999).

Manipulation by plant growth regulators is essential to optimize callus induction. In this study, the effect of different concentrations of auxins (2.4-D, NAA and IBA) and cytokinin (BAP and kinetin) on callus induction was investigated.

To determine the inducing rates of the callus on different organs of *R. coreanum*, and kinds and the optimal concentrations of plant growth regulators, four organs including stem tip, leaf, rhizome and root top, and three plant growth regulators with different concentrations were used. The concentration of 0.2 mg/l 2.4-D in the medium reached the optimal inducing rate in rhizome but the maximum inducing rate of leaf was obtained in the medium with 0.2 mg/l NAA. The inducing rate of stem tip was more than 80% in the medium with the concentrations of 0.2 mg/l and 0.5 mg/l 2.4-D (Table 1). The use of plant growth regulators is of fundamental importance in directing the organogenic response of any plant tissue or organ under in vitro conditions (Sugiyama and Imamura, 2006).

3.2. Effects of plant growth regulators on callus growth of rhizome segment

Several studies had been reported regarding the effects of plant growth regulators on callus growth of different plant species (Nadia and Li, 2011). When using different concentrations in single auxin treatment, the addition of 2.4-D increased the growth in the rhizome callus of *R. coreanum* with the maximum at 0.2 mg/l of 2.4-D, but increasing the levels of 2.4-D suppressed the callus growth (Table 2).

A combination of cytokinin BAP at 0.2 mg/l with the best auxin treatment (0.2 mg/l 2.4-D), produced profuse amounts of whitish and friable callus (Table 2). When kinetin was used instead, the amount of biomass recorded lowered than that of the best BAP (0.2 mg/l) (Table 2).

Table 2 Effects of different concentrations of 2.4-D and combination with cytokinin on callus growth of rhizome segment (n = 30).

Concentration of plant growth regulators in MS liquid medium/mg/l	Fresh weight of segment*/g, $x \pm s$	Fresh weight of callus/g, $x \pm s$	Multiplication rate/%
–	20 ± 1.6	0.2 ± 0.02	11
2.4-D 0.1	20 ± 1.5	1.5 ± 0.28	75
2.4-D 0.2	20 ± 1.7	3.3 ± 0.45	158
2.4-D 0.3	20 ± 1.2	3.0 ± 0.38	150
2.4-D 0.5	20 ± 1.4	2.3 ± 0.28	109
2.4-D 0.2 + BAP 0.2	20 ± 1.7	2.9 ± 0.26	140
2.4-D 0.2 + kinetin 0.2	20 ± 1.3	2.6 ± 0.50	130
2.4-D 0.2 + kinetin 0.5	19 ± 1.4	2.2 ± 0.36	117

* Segment size is 15–20 mm^3 in capasity.

Table 3 Multiplication rate of the callus of *R. coreanum* on generation times.

Number of generation times	Multiplication rate of callus/%
Primary culture	158
2nd subculture	173
3rd subculture	152
4th subculture	165

The callus multiplication rate of explant segments and callus segments induced from rhizome reached 150 times more than in the fresh weight of the callus, when cultivated in MS liquid medium containing 0.2–0.3 mg/l of 2.4-D at (25 ± 1) °C and 30 rotations/min under light exposure for 28 days. In this study, although the control was cultured in medium without plant growth regulators, after 28 days of culture, multiplication rates were somewhat recognized. A particular interaction was observed between 0.2 mg/l 2.4-D and 0.2 mg/l BAP.

The accumulation of biomass in the cultured callus yielded the highest amount of biomass, 2nd subculture from the primary culture (Table 3). From the start to the end of 2nd subculture, the growth curve revealed that there was no increase in the amount of cells in the culture, indicating that the culture was going through a lag phase. There was a reduction of biomass profile on 3rd subculture.

3.3. Study of callus organogenesis and plant regeneration

After 1 week in the callus induction medium, the explants started to swell. For those growing in the MS basal medium without auxin (control), no little callus was induced even after 30 days, indicating that plant-growth regulators are required for callus induction. Single BAP or the combination of BAP (1, 2 and 3 mg/l) and NAA (0.2 mg/l) in medium promoted the differentiation of shoot from the callus, but increase in the concentration of NAA slightly suppressed the differentiation.

The medium with BAP and NAA produced differentiated shoots from the callus. In the 2 mg/l BAP and 2 mg/l NAA culture medium assayed, differentiated shoots from the callus appeared after 4 weeks of culture, and developed adventitious shoots (Table 4).

Table 4 Effect of BAP and NAA on shoot formation from callus of *R. coreanum*.

Concentration of plant growth regulators/mg/l		Number of callus	Number of callus differentiated shoot	Number of shoot/a callus	Number of leaf/a shoot
BAP	NAA				
–	–	5	–	–	–
1	–	5	5	2–3	1–2
2	–	5	5	2–3	2–3
1	0.2	5	4	2–4	2–3
2	0.2	5	5	4–5	3–4
3	0.2	5	5	4–5	2–3
1	0.5	5	3	3–5	1–2
2	0.5	5	5	3–4	1–2
3	0.5	5	4	3–4	1–2

Table 5 Effect of plant growth regulators on root formation of shoot (plantlet) of *R. coreanum*.

Concentration of plant growth regulators		Inductive rate of root formation/%	Number of root/plantlet	Average length of roots/mm
Kind	mg/l			
2.4-D	0.2	68	1–2	12
	0.5	55	1–3	14
	1.0	47	1–2	11
NAA	0.2	75	2–4	13
	0.5	91	3–5	11
	1.0	50	2–3	15
IBA	0.2	74	2–3	16
	0.5	85	3–3	18
	1.0	62	1–2	18
KT	0.0	35	1–2	7
	0.5	38	1–2	7
	1.0	40	2–3	5
BAP	0.2	30	1–3	5
	0.5	27	1–2	7
	1.0	23	1–2	7

Table 6 Combination effect of NAA and IBA on root formation of shoot (plantlet) of *R. coreanum*.

Concentration of NAA and IBA/mg/l	Inductive rate of root formation/%	Number of root/plantlet	Average length of roots/mm
NAA 0.2 ± IBA 0.2	87	3–4	17
NAA 0.2 ± IBA 0.5	100	3–5	20
NAA 0.2 ± IBA 1.0	94	3–4	18
NAA 0.5 ± IBA 0.2	100	3–5	15
NAA 0.5 ± IBA 0.5	100	3–4	19
NAA 0.5 ± IBA 1.0	78	2–3	17
NAA 1.0 ± IBA 0.2	72	2–4	16
NAA 1.0 ± IBA 0.5	80	3–4	17
NAA 1.0 ± IBA 1.0	67	2–3	15

The optimum condition of shoot formation on rhizome callus segments was to culture on the MS agar medium containing BAP 2 mg/l and NAA 0.2 mg/l. The adventitious shoot development was also observed after 28 days of culture (Table 4). In the present experiment, BAP alone was able to induce adventitious shoot regeneration, whereas a combination of BAP and NAA of a high concentration (0.5 mg/l) appeared to have a suppressive effect on shoot differentiation (Table 4). These results confirm the strong propensity of callus pieces grown on media with a high BAP concentration to develop organized structures. BAP plays a key role in plant regeneration in vitro (Guo et al., 2005; Xu et al., 2008).

Small callus pieces were cultured separately in the MS medium supplemented with various auxin and cytokinin in combination with three concentrations. Auxin exerts more favorable influence than cytokinin on rooting of shoots. NAA and IBA among auxin induce strong rooting of shoots. Different types and concentrations of plant growth regulators

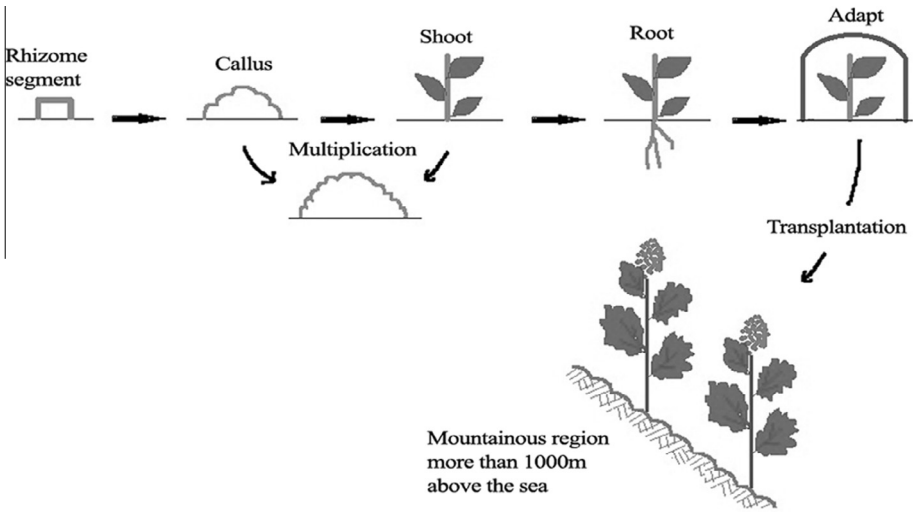

Figure 1 Diagram of tissue culture propagation of *Rheum coreanum*.

are known to have different effects on growth and developmental processes (Ramachandra Rao and Ravishankar, 2002).

Table 5 shows the plant growth regulators have capacity and tendency in the callus growth to develop organogenesis. After 30 days, the callus became whitish-yellow and friable, with this indicating that types and concentrations of the different auxin and cytokinin played important roles in callus induction and proliferation (Table 5).

Finally, for the effect of the NAA and IBA on cell growth and inductive rate, the ratio of NAA to IBA was varied in the basal medium. The results showed that the optimum culture medium of root formation on shoots and plantlets induced from the rhizome callus was the medium containing NAA 0.2–0.5 mg/l and IBA 0.5 mg/l. Further investigation on the ratio of NAA to IBA showed that when IBA was added into the single auxin of different concentrations, inductive rates of root formation were different (Table 6).

We found that MS supplemented with single auxin, 2.4-D at 0.2 mg/l, produced the highest biomass for compact callus while the combination of NAA 0.2–0.5 mg/l and IBA 0.5 mg/l yielded the highest inductive rate of root formation.

3.4. Transplantation of plantlet to soil and anthraquinone compound production

Transplantation of plantlet to soil was observed. Established rate on the mountainous region of plantlets induced from rhizome explant segments has reached 95%. The diagram of tissue culture propagation of *R. coreanum* rhizome performed in this study is shown in Fig. 1. Different carbon sources including sucrose and sugar were best for anthraquinone yields in cell suspension cultures of various plants (Zenk et al., 1975; Suzuki et al., 1984). Sucrose is commonly the most favorable carbon source for cell growth (Farzami Sepehr and Ghorbanli, 2002). In addition, both increasing levels of myoinositol and vitamin free medium suppressed anthraquinone formation, but the addition of increasing concentrations of vitamins promoted callus growth (Farzami Sepehr and Ghorbanli, 2002). A comparison of yield and content of anthraquinone in crude drug processed from *R. coreanum* produced by multiplication methods was observed (Table 7). When rhizome of *R. coreanum* was cultured, the total anthraquinone yield was higher than in other treatments. This observation showed that there was an absolute requirement for plant growth regulators for anthraquinone production. The other plant species, from the tissue culture of *Cassia tora* L. were isolated and identified three anthraquinones and reported that the maximum anthraquinones content in a fresh weight basis was 0.334% which is higher than the content of total anthraquinones in dry seeds (Tabata et al., 1975).

Table 7 Comparison of yields and contents of anthraquinone in crude drug processed from *R. coreanum* produced by different multiplication methods.

Item	Multiplication method		
	Rhizome tissue culture	Seeding	Lateral buds propagation
Average dry[1] weight of crude drug per plant (n = 100)/kg	1.08	0.63	0.85
Content of total anthraquinone (free + glycoside)/%	4.56 (0.42 + 4.14)	3.87 (0.38 + 3.49)	3.19 (0.32 + 3.87)
Content of sennoside/%	0.95	0.84	0.91

Note: [1]Data measured with triennial plant.

4. Conclusion

For a rapid creation of mountainous resources of *R. coreanum*, a rare medicinal plant growing in DPR of Korea, an efficient callus proliferation system was developed by rhizome tissue culture of it.

The results reported here indicated that the clonal mass propagation of *R. coreanum* could be obtained by rhizome tissue culture. MS supplemented with single 2.4-D (0.2 mg/l)

produced the highest biomass for the compact callus. The shoot formation on rhizome callus segments was created by the optimum condition, which was cultured on the MS agar medium containing BAP (2 mg/l) and NAA (0.2 mg/l), while the combinations of NAA (0.2–0.5 mg/l) and IBA (0.5 mg/l) yielded the highest inductive rate of root formation. The plantlets transplanted on mountainous region were successfully acclimatized and contained the high levels of anthraquinones than those obtained by the other propagation methods. Generally it could be concluded that mass-propagated hereditary uniform, virus-free young plants can be obtained by only rhizome tissue culture, and mountainous resources of *R. coreanum* can be made by transplantation of young plants. This study might provide new opportunities for clonal propagation of *R. coreanum* and in vitro-formed rhizomes might be an important source of disease-free planting material.

Acknowledgements

The authors thank the National Regulatory Authority, Democratic People's Republic of Korea for help in determination of anthraquinones.

References

Aburjai, T.A., 2000. Anti-platelet stilbenes from aerial parts of *Rheum palaestinum*. Phytochemistry 55, 407–410.

Agarwal, S.K., Singh, S.S., Verma, S., Kumar, S., 2000. Antifungal activity of anthraquinone derivatives from *Rheum emodi*. J. Ethnopharmacol. 72, 43–46.

Azhar Alama, M.M., Javedb, K., Jafria, M.A., 2005. Effect of *Rheum emodi* (Revand Hindi) on renal functions in rats. J. Ethnopharmacol. 96, 121–125.

Çakılcıoğlu, U., Türkoğlu, I., 2007. Plants used for hemorrhoid treatment in Elazığ central district. Int. Med. Aromat. Plants Conf. Culinary Herbs 826, 89–96.

Çakılcıoğlu, U., Türkoğlu, I., 2008. Plants used for pass kidney stones by the folk in Elazığ. Herb J. Syst. Bot. 14, 133–144.

Chang, S.J., Huang, S.H., Lin, Y.J., Tsou, Y.Y., Lin, C.W., 2014. Antiviral activity of *Rheum palmatum* methanol extract and chrysophanol against Japanese encephalitis virus. Arch. Pharm. Res. http://dx.doi.org/10.1007/s12272-013-0325-x.

Dakah, A., Zaid, S., Suleiman, M., Abbas, S., Wink, M., 2014. In vitro propagation of the medicinal plan *Ziziphora tenuior* L. and evaluation of its antioxidant activity. Saudi J. Biol. Sci. 21 (4), 317–323.

Farzami Sepehr, M., Ghorbanli, M., 2002. Effects of nutritional factors on the formation of anthraquinones in callus cultures of Rheum ribes. Plant Cell Tiss. Org. 68, 171–175.

Guo, D.P., Zhu, Z.J., Hu, X.X., Zheng, S.J., 2005. Effect of cytokinins on shoot regeneration from cotyledon and leaf segment of stem mustard (*Brassica juncea* var. *tsatsai*). Plant Cell Tiss. Org. 83, 123–127.

Guo, B., Gao, M., Liu, C.Z., 2007. In vitro propagation of an endangered medicinal plant *Saussurea involucrate* Kar et Kir. Plant Cell Rep. 26, 261–265.

Han, H., Zhang, S.G., Sun, X.M., 2009. A review on the molecular mechanism of plants rooting modulated by auxin. Afr. J. Biotechnol. 8 (3), 348–353.

Hoang, H.D., knüpffer, H., Hammer, K., 1997. Additional notes to the checklist of Korean cultivated plants (5). Consolidated summary and indexes. Genet. Resour. Crop Evol. 44, 349–391.

Hu, B.Y., Zhang, H., Meng, X.L., Wang, F., Wang, P., 2014. Aloe-emodin from rhubarb (*Rheum rhabarbarum*) inhibits lipopolysaccharide-induced inflammatory responses in RAW264.7 macrophages. J. Ethnopharmacol. http://dx.doi.org/10.1016/j.jep.2014.03.059i.

Ibrahim, M., Khaja, M.N., Aara, A., Khan, A.A., Habeeb, M.A., Devi, Y.P., Narasu, M.L., Habibullah, C.M., 2008. Hepatoprotective activity of *Sapindus mukorossi* and *Rheum emodi* extracts: in vitro and in vivo studies. World J. Gastroenterol. 14 (16), 2566–2571.

Kaval, İ., Behçet, L., Çakılcıoğlu, U., 2014. Ethnobotanical study on medicinal plants in Geçitli and its surrounding (Hakkari-Turkey). J. Ethnopharmacol. 155, 171–184.

Kosikowska, U., Smolarz, H.D., Malm, A., 2010. Antimicrobial activity and total content of polyphenols of *Rheum* L. species growing in Poland. Cent. Eur. J. Biol. 5 (6), 814–820.

Kounsar, F., Rather, M.A., Ganai, B.A., Zargar, M.A., 2011. Immuno-enhancing effects of the herbal extract from Himalayan rhubarb *Rheum emodi* Wall. ex Meissn. Food Chem. 126, 967–971.

Kubo, I., Murai, Y., Soediro, I., Soetarno, S., Sastrodihardjo, S., 1992. Cytotoxic anthraquinones from *Rheum palmatum*. Phytochemistry 31 (3), 1063–1065.

Kurosaki, F., Nagase, H., Nishi, A., 1992. Stimulation of anthraquinone production I in rhubarb tissue culture by an ethylene-generating reagent. Phytochemistry 31 (8), 2735–2738.

Li, J.R., Liu, M.X., Chen, H.R., Wu, Z.B., Wang, J.J., 1999. Callus initiation and subculture of *Taxus chinensis*. J. For. Res. 10 (1), 11–14.

Li, W.Y., Chan, S.W., Guo, D.J., Chung, M.K., Leung, T.Y., Yu, P.H.F., 2009. Water extract of *Rheum officinale* Baill. induces apoptosis in human lung adenocarcinoma A549 and human breast cancer MCF-7 cell lines. J. Ethnopharmacol. 124, 251–256.

Martens, S., Mateus, N., De Freitas, V., 2014. Special issue on anthocyanins. Planta. http://dx.doi.org/10.1007/s00425-014-2185-0.

Matsuda, H., Tewtrakul, S., Morikawaa, T., Yoshikawa, M., 2004. Anti-allergic activity of stilbenes from Korean rhubarb (*Rheum undulatum* L.): structure requirements for inhibition of antigen-induced degranulation and their effects on the release of TNF-a and IL-4 in RBL-2H3 cells. Bioorg. Med. Chem. 12, 4871–4876.

Mishra, S.K., Tiwari, S., Shrivastava, A., Srivastava, S., Boudh, G.K., Chourasia, S.K., Chaturvedi, U., Mir, S.S., Saxena, A.K., Bhatia, G., Lakshmi, V., 2013. Antidyslipidemic effect and antioxidant activity of anthraquinone derivatives from *Rheum emodi* rhizomes in dyslipidemic rats. J. Nat. Med. http://dx.doi.org/10.1007/s11418-013-0810-z.

Naqishbandi, A.M., Josefsen, K., Pedersen, M.E., Jäger, A.K., 2009. Hypoglycemic activity of Iraqi *Rheum ribes* root extract. Pharm. Biol. 47 (5), 380–383.

Ono, Nadia N., Tian, L., 2011. The multiplicity of hairy root cultures: prolific possibilities. Plant Sci. 180, 439–446.

Ohshima, Y., Takahashi, K., Shibata, S., 1988. Tissue culture of rhubarb and isolation of sennosides from the callus. Planta Med. 54 (1), 20–24.

Ozel, C.A., Khawar, K.M., Unal, F., 2015. Factors affecting efficient in vitro micropropagation of *Muscari muscarimi* Medikus using twin bulb scale. Saudi J. Biol. Sci. 22, 132–138.

Öztürk, M., Aydoğmus-Öztürk, F., Duru, M.E., Topçu, G., 2007. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): an edible medicinal plant. Food Chem. 103, 623–630.

Palacio, Lorena, Cantero, Juan J., Cusidó, Rosa M., Goleniowski, Marta E., 2012. Phenolic compound production in relation to differentiation in cell and tissue cultures of *Larrea divaricata* (Cav.). Plant Sci. 193–194, 1–7. http://dx.doi.org/10.1016/j.plantsci.2012.05.007.

Raafat, K., Aboul-Ela, M., El-Lakany, A., 2014. Alloxan-induced diabetic thermal hyperalgesia, prophylaxis and phytotherapeutic effects of *Rheum ribes* L. in mouse model. Arch. Pharm. Res. http://dx.doi.org/10.1007/s12272-014-0372-y.

Ramachandra Rao, S., Ravishankar, G.A., 2002. Plant cell cultures: chemical factories of secondary metabolites. Biotechnol. Adv. 20, 101–153.

Schulte, U., El-Shagi, H., Zenk, M.H., 1984. Optimization of Rubiaceae species in cell culture for the production of anthraquinones. Plant Cell Rep. 3, 51–54.

Sugiyama, M., Imamura, K., 2006. Dose-, time- and tissue-dependent effects of 5-bromo-2′-deoxyuridine on the in-vitro organogenesis of *Arabidopsis thaliana*. Plant Cell Tiss. Org. 87, 17–25.

Suzuki, H., Matsumoto, T., Mikami, Y., 1984. Effects of nutritional factors on the formation of anthraquinones by *Rubia cordifolia* cells in suspension culture. Agric. Biol. Chem. 48 (3), 603–610.

Tabata, M., Hiraoka, N., Ikenoue, M., Sano, Y., Konoshima, M., 1975. The production of anthraquinones in callus cultures of *Cassia tora*. Lloydia 38 (2), 131–134.

Walkey, D.G.A., Matthews, K.A., 1979. Rapid clonal propagation of rhubarb (*Rheum rhaponticum* L.) from meristem-tips in tissue culture. Plant Sci. Lett. 14, 287–290.

Wang, J.L., Lu, Y., Wang, Q., Liu, K., Song, Y.F., Bi, K.L., 2011. An efficient callus proliferation protocol and rhaponticin accumulation of *Rheum franzenbachii* Munt., a medicinal plant. J. Plant Biochem. Biotechnol. 20 (2), 252–257.

Xu, Z., Um, Y.C., Kim, C.H., Lu, G., Guo, D.P., Liu, H.L., Bah, A.A., Mao, A., 2008. Effect of plant growth regulators, temperature and sucrose on shoot proliferation from the stem disc of Chinese jiaotou (*Allium chinense*) and in vitro bulblet formation. Acta Physiol. Plant. 30, 521–528.

Zenk, M.H., El-Shagi, H., Schulte, U., 1975. Anthraquinone production by cell suspension cultures of *Morinda citrifolia*. Planta Med. 28, 79–101.