Plant Cell Tiss Organ Cult (2010) 101:371–376
DOI 10.1007/s11240-010-9692-x

RESEARCH NOTE

Shoot regeneration and free-radical scavenging activity in *Silybum marianum* L.

Bilal Haider Abbasi · Mubarak Ali Khan · Tariq Mahmood · Mushtaq Ahmad · Muhammad Fayyaz Chaudhary · Mir Ajab Khan

Received: 18 May 2009 / Accepted: 18 January 2010 / Published online: 2 February 2010

Abstract The morphogenic potential and free-radical scavenging activity of the medicinal plant, *Silybum marianum* L. (milk thistle) were investigated. Callus development and shoot organogenesis were induced from leaf explants of wild-grown plants incubated on media supplemented with different plant growth regulators (PGRs). The highest frequency of callus induction was observed on explants incubated on Murashige and Skoog (MS) medium supplemented with 5.0 mg l^{-1} 6-benzyladenine (BA) after 20 days of culture. Subsequent transfer of callogenic explants onto MS medium supplemented with 2.0 mg l^{-1} gibberellic acid (GA_3) and 1.0 mg l^{-1} α-naphthaleneacetic acid (NAA) resulted in 25.5 ± 2.0 shoots per culture flask after 30 days following culture. Moreover, when shoots were transferred to an elongation medium, the longest shoots were observed on MS medium supplemented with 0.5 mg l^{-1} BA and 1.0 mg l^{-1} NAA, and these shoots were rooted on a PGR-free MS basal medium. Assay of antioxidant activity of in vitro and in vivo grown tissues revealed that significantly higher antioxidant activity was observed in callus than all other regenerated tissues and wild-grown plants.

Keywords *Silybum* · Callus · Regeneration · Antioxidant · DPPH · Gibberellic acid

B. H. Abbasi (✉) · M. A. Khan · M. F. Chaudhary
Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan
e-mail: bhabbasi@qau.edu.pk

T. Mahmood · M. Ahmad · M. A. Khan
Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan

Abbreviations

BA	6-Benzyladenine
DPPH 1	1-Diphenyl-2-picrylhydrazyl
FRSA	Free-radical scavenging activity
GA_3	Gibberellic acid
Kn	Kinetin
MS0	MS medium without plant growth regulators
½ MS	Half-strength macro-nutrients of MS medium
NAA	α-Naphthaleneacetic acid
PGRs	Plant growth regulators
Zn	Zeatin

Fruits of *Silybum marianum* (L.) Gaertn (milk thistle, Asteraceae), contain isomeric mixtures of flavonolignans, including silychristin, silydianin, silybin, and isosilybin, collectively known as silymarin (Kurkin et al. 2001; Morazzoni and Bombardelli 1995). Silymarin is used as a hepatoprotector for oral treatment of toxic liver damage and of chronic inflammatory liver diseases and liver cirrhosis (Valenzuela et al. 1986; Flora et al. 1998). Aside from its antioxidant properties and its role in stimulating protein synthesis and cell regeneration (Tawaha et al. 2007), silymarin may also reduce incidence of certain forms of cancer (Katiyar et al. 1997), and has been among the most investigated plant extracts with known mechanisms of action (Becker and Schrall 1977; Cimino et al. 2006; Sanchez-Sampedro et al. 2007). Although cell cultures of *S. marianum* are capable of producing silymarin, amounts produced are lower than those produced in fruits (Ferreiro et al. 1991; Cacho et al. 1999; Alikardis et al. 2000; Sanchez-Sampedro et al. 2005).

Currently, all commercially available silymarin is obtained from intact fruits of wild plants. The increasing

worldwide demand for silymarin is endangering the sparse populations of *S. marianum* in the Mediterranean region (Ahmad et al. 2008). Moreover, knowledge of yield and chemical composition is limited (Khan et al. 2009). The efficiency of seed germination and seedling growth in some species of Asteraceae is low, inconsistent, and is highly dependent on various biological and environmental factors (Abbasi et al. 2007).

So far, there are no reports on in vitro regeneration of wild-grown plants of *S. marianum*. However, several protocols for regeneration of *S. marianum* from in vitro derived plantlets have been reported (Iqbal and Srivastava 2000; Hetz et al. 1995; Radice and Caso 1997). Recently, biosynthesis of silymarin has been reported in cell suspension cultures of *S. marianum* seedlings (Tumova and Tuma 2009; Hasanloo et al. 2008; Sanchez-Sampedro et al. 2007).

In a recent study, it has been demonstrated that the protective effect of silymarin is most likely due to its free-radical scavenging activity (FRSA) (Wallace et al. 2008). Although there are few reports on FRSA of wild-grown *Silybum* (Wojdyło et al. 2007; Tawaha et al. 2007; Ligeret et al. 2008), there are no reports on the influence of different in vitro culture systems of *S. marianum* on FRSA. In this study, in vitro cultures of *S. marianum* were established from leaf explants of wild-grown plants. Assays were conducted to evaluate FRSA of the main secondary metabolites in different in vitro derived tissues and compared with those of wild-grown plant material.

Approximately, 28 day-old leaves of wild-grown *S. marianum* were collected from plants growing at the Quaid-i-Azam University (Islamabad, Pakistan). Leaves were surface-sterilized by immersion in 70% (v/v) ethanol for 60 s, 0.2% (w/v) mercuric chloride ($HgCl_2$) solution for 2 min, and rinsed three times with sterile distilled water. Leaf explants were incubated on a Murashige and Skoog (MS) (1962) medium containing 3% sucrose, and solidified with 0.8% (w/v) agar (Agar Technical LP0013, Oxoid, Hampshire, England). Different plant growth regulators (PGRs) were added to the medium, and the pH was adjusted to 5.8. All media were autoclaved at 121°C for 20 min. All cultures were maintained in a growth room at 25 ± 1°C under a 16 h photoperiod with a light intensity of ~40 µmol m^{-2} s^{-1} provided by cool-white fluorescent tube lights.

For callus initiation and shoot regeneration, immature (~28 days old) leaves were cut into ~1 cm^2 segments, and placed onto MS medium containing different concentrations (0.25, 0.5, 1.0, 2.0, 5.0, and 10 mg l^{-1}) of either 6-benzyladenine (BA) or gibberellic acid (GA_3) with or without 1.0 mg l^{-1} NAA. A PGR-free medium (MS0) was used as control for callus and shoot regeneration experiments. About six leaf segments were incubated in a single Petri-plate. After 20 days of culture, number of explants developing callus were recorded. Yellowish friable callus was excised, and transferred to MS medium containing similar combinations of PGRs for shoot organogenesis. Data on number of shoots per culture flask and mean shoot length were recorded after 30 days following subculture of callus cultures. Subsequently, elongated shoots were transferred to either MS0 or ½ MS medium without any PGRs, and after 30 days, rooted plantlets were removed, rinsed in distilled water to remove medium, and transferred to potting soil mixture, acclimatized, and grown under greenhouse conditions. Survival rates of transplanted plantlets were determined after 20 days following transfer to in vivo conditions.

Callus, regenerated shoots, seed derived in vitro plantlets, and field-grown wild plants were also used for determining antioxidant activities (FRSA) and assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity as described by Liu et al. (2004). Briefly, 30–40 mg of tissue was weighed, macerated in 0.5 ml 80% ethanol for 5 min, and crude extract was obtained by centrifugation for 3 min at 10,000 rpm. A series of dilute crude extract solutions were prepared prior to initiating the reaction by adding 0.5 ml diluted test sample to 0.5 ml of 2000 µmol l^{-1} of a DPPH solution for free-radical scavenging quantification. The test tube was kept for 20 min at 25°C, and the absorbance of the reaction mixture was measured at 520 nm using UV–Visible spectrophotometer (Agilent 8453, CA USA). The volume of sample extract required to result in a 50% decrease in absorbance relative to the control (100%) was then calculated. When the range of dilution was optimized, the reaction was repeated until the proper value was obtained. The FRSA was expressed as fresh weight of tissues required for 50% reduction in radical production, which was calculated from the added volume of the crude extract solution (Takahata et al. 2001).

Each treatment consisted of 10 culture Petri-plates/culture flasks and all experiments were repeated twice. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used for comparison among treatment means. Petri plates were used for callus induction from leaves and flasks were used for inducing organogenesis, shoot regeneration, and rooting of shoots.

The contamination of *S. marianum* explants was high (~80%) as leaf explants were obtained from in vivo-grown plants. Treatment of leaf explants with 70% ethanol and 0.2% mercuric chloride significantly reduced the level of contamination (≤5%, data not shown). A similar protocol has been reported to surface-sterilize explants of *Plumbago* and *Lysimachia* spp. (Selvakumar et al. 2001; Zheng et al. 2009). Recently, the biocide PPM (plant preservation mixture) has been reported to inhibit microflora of in vitro cultures of *Echinacea* (Lucchesini et al. 2009). George and

Tripepi (2001) reported undesired effect of decontamination protocol on regeneration of birch, rhododendron and chrysanthemum.

The effects of various PGRs such as kinetin (Kn), zeatin (Zn), BA, GA_3, and NAA on explant response in preliminary experiments on callus induction from leaf explants were evaluated (data not shown). However, leaf explants incubated on MS medium supplemented with varying levels of BA/GA_3 with or without 1.0 mg l^{-1} NAA promoted callus induction, shoot organogenesis, and shoot proliferation (Fig. 1). Callus development was observed within 20 days following culture (Figs. 1a, 2). Frequency of callus induction was highest when explants were incubated on medium containing either 5 mg l^{-1} BA (88% of explants) or 2 mg l^{-1} BA with 1.0 mg l^{-1} NAA (84% of explants) (Fig. 2). The callus was initially pale in color and exhibited slow growth, but after 25 days, it became brownish, friable, and grew rapidly (Fig. 1a). Previously, Cimino et al. (2006) induced callus from cotyledons of *S. marianum* incubated on B5 medium (Gamborg et al. 1968) supplemented with 0.05 mg l^{-1} BA and 0.5 mg l^{-1} of 2,4-dicholorophenoxyacetic acid (2,4-D). However, Iqbal and Srivastava (2000) induced callus from leaf, shoot apex and nodal explants of seedlings of *S. marianum* incubated on medium containing NAA, BA, and Zn. In this study, organogenic regions on callus were observed after 15 days following subculture of callus to fresh medium of the same composition of PGRs to induce shoot organogenesis. Previously, it has been reported that successive passages of calli were reported to inhibit shoot organogenesis in *Echinacea* spp. (Lucchesini et al. 2009). A similar response was observed in this study as well when callus cultures

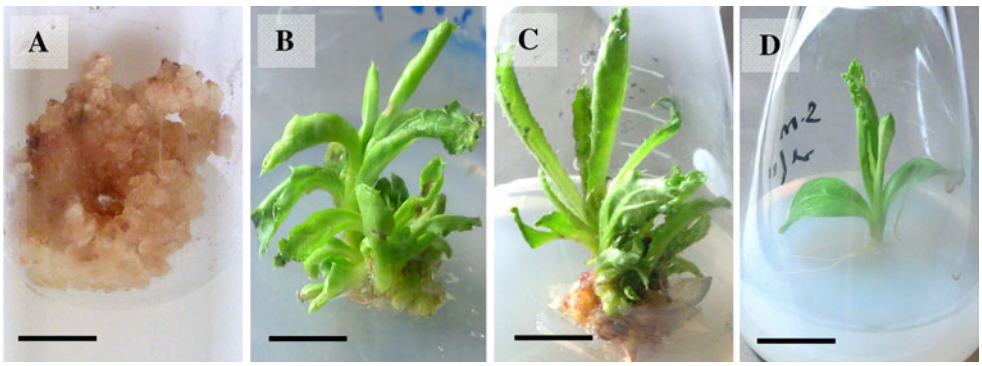
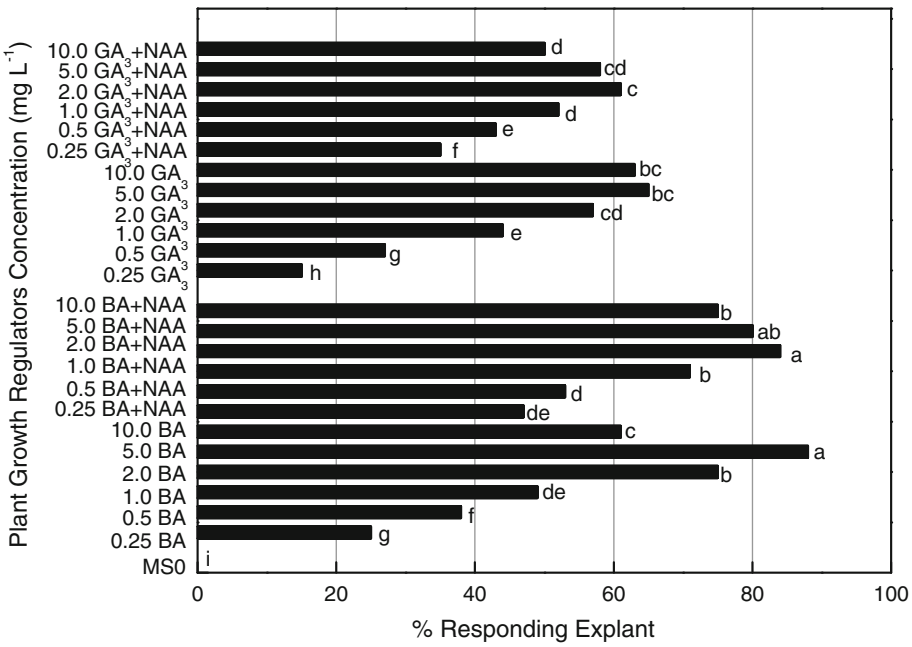


Fig. 1 Plant regeneration from leaf explants of *Silybum marianum*. **a** Callus formation from leaf explant, *Bar* 5 mm. **b** Shoot organogenesis from leaf explants, *Bar* 0.7 cm. **c** Proliferation and elongation of regenerated shoots, *Bar* 1.2 cm. **d** Rooting of regenerated shoots, *Bar* 2.5 cm

Fig. 2 Effects of various concentrations of BA and GA_3 with or without 1 mg l^{-1} NAA on response of *Silybum marianum*. Data were collected after 20 days of culture. Values are means of 10 replicates. Columns with *common letters* are not significantly different at $P < 0.05$



underwent several passages of culture. Moreover, lower frequencies of organogenesis were observed when callus cultures were incubated on medium containing GA_3 rather than BA, and no callus was observed when explants were incubated on MS0 medium. The callus produced was soft, brown, and slow growing, but was organogenic (Fig. 2). Recently, Zheng et al. (2009) reported leaf explants from in vitro-grown plants of *Lysimachia* were more responsive to callogenesis and organogenesis than those obtained from in vivo growth conditions. In this study, leaf explants from wild-grown plants showed high frequency of callogenesis (88%) and organogenesis (76%).

However, callus production may be important for studies of indirect morphogenesis (Radice and Caso 1997) or for studies of production of secondary metabolites in medicinal plants (Meratan et al. 2009; Cimino et al. 2006). Frequency of organogenesis was determined after 30 days of sub-culture and the mean number of shoots per culture flask was significantly higher (25.5 ± 2.0 shoots per flask) when explants were incubated on medium containing 2.0 mg l^{-1} GA_3 and 1.0 mg l^{-1} NAA than all other PGR treatments tested (Figs 1b, 3 and 4). Previously, Iqbal and Srivastava (2000) found best shoot regeneration steps for *S. marianum* on MS medium supplemented with NAA and Zn. However, Radice and Caso (1997) found MS0 medium optimum for shoot regeneration. No shoot regeneration response was observed on explants incubated on MS0 (Figs. 3 and 4).

Comparatively, the presence of NAA along with GA_3 and BA increased the number of shoots per culture flask as compared to BA or GA_3 alone (Fig. 3). Our results support the findings that the synergistic combination of auxin with other PGRs promoted shoot regeneration (Sagare et al. 2000). Moreover, the promoting effect of auxin and cytokinin combinations on organogenic differentiation has been well documented for some members of Asteraceae (Koroch et al. 2002). Regenerated shoots were separated and sub-cultured on fresh MS medium with similar combinations of PGRs for further shoot elongation (Fig. 4). BA is considered as one of the most useful cytokinins for shoot proliferation (Stfaan et al. 1994; Lucchesini et al. 2009). Nonetheless, in this study, 0.5 mg l^{-1} BA with 1.0 mg l^{-1} NAA promoted the longest shoots, 3.5 ± 0.1 cm, after 30 days of sub-culture (Fig. 1c).

Multiple shoots grown on shoot regeneration medium were transferred to MS0 and ½ MS for rooting. On MS0 medium, highest frequency (73.3 ± 5.4%) of rooting was observed with a mean of 4.2 ± 0.34 roots per shoot. On the other hand, shoots incubated on ½MS showed 64.7 ± 7.1% frequency of rooted shoots with a mean of 3.4 ± 0.21 roots per shoot. The emergence of white roots was observed after 15 days of sub-culture (Fig. 1d). These findings are in agreement with those of Hetz et al. (1995). Iqbal and Srivastava (2000) obtained rooting of *S. marianum* regenerated shoots on MS medium supplemented with NAA and Zn. Plantlets with well-developed roots were successfully transferred to soil. Of 30 plantlets transferred to in vivo condition, 22 survived (74%) acclimatization and growth in the greenhouse. In this study, enhanced regeneration was achieved using the same medium for callus and shoot regeneration; while, optimum rooting of shoots was obtained on basal MS medium.

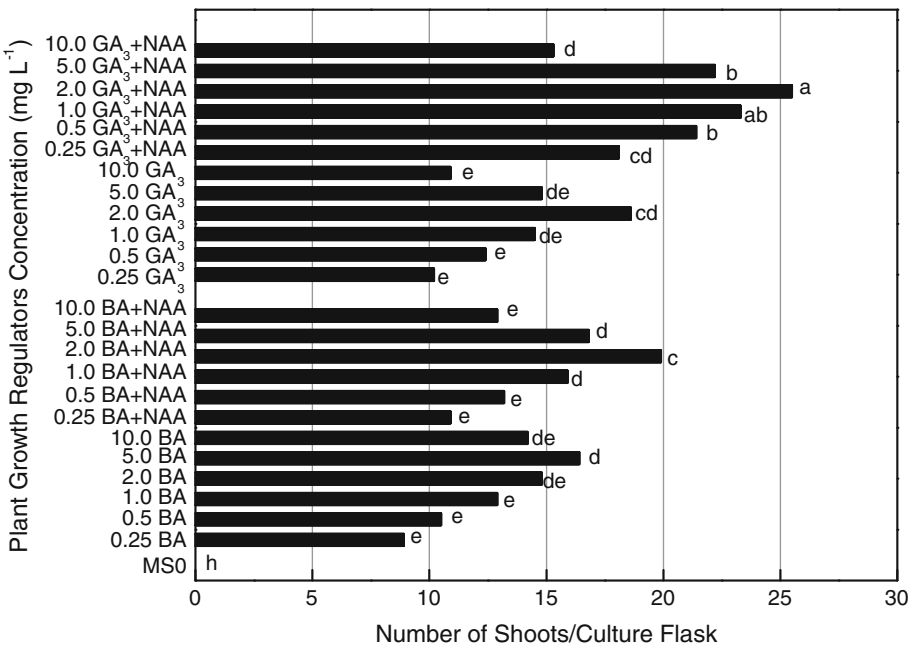


Fig. 3 Effects of various concentrations of BA and GA_3 with or without 1 mg l^{-1} NAA on number of shoots per culture flask of *Silybum marianum*. Data were collected after 30 days of sub-culture to MS media with similar composition of plant growth regulators. Values are means of 10 replicates. Columns with *common letters* are not significantly different at $P < 0.05$

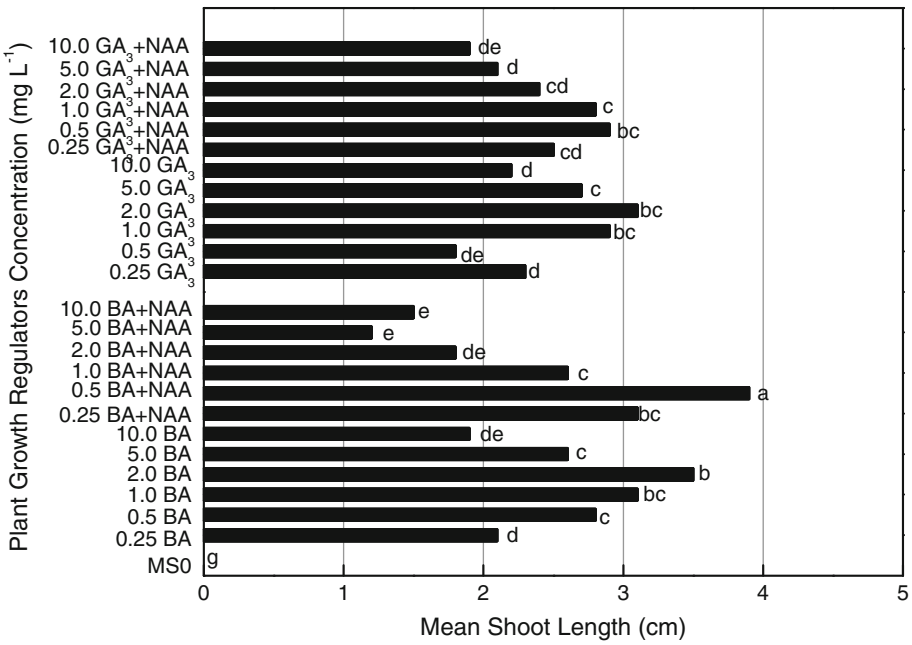


Fig. 4 Effects of various concentrations of BA and GA_3 with or without 1 mg l^{-1} NAA on mean shoot length of *Silybum marianum*. Data were collected after 30 days of sub-culture to MS media with similar composition of plant growth regulators. Values are means of 10 replicates. Columns with *common letters* are not significantly different at $P < 0.05$

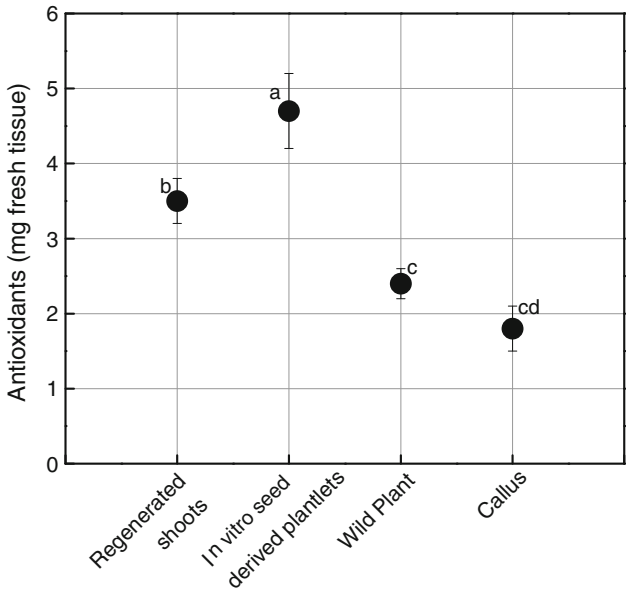


Fig. 5 Antioxidant activity in *Silybum marianum* L. tissues at different development stages (callus, in vitro shoots after 45 days, in vitro plantlets after 45 days, wild grown plants after ~45 days). Note: Antioxidant is expressed as the weight of tissue required for a 50% reduction in free radical generation. Therefore, a lower value indicates a greater antioxidant activity. Values are means of triplicates with standard deviation. Means with *common letters* are not significantly different at $P < 0.05$

Free-radical scavenging activity (FRSA) was determined to evaluate the antioxidant potential of regenerated tissues, and these were compared with levels in wild-grown plants. Callus had significantly higher capacity to detoxify DPPH free radicals than other tissues collected from wild and in vitro conditions (Fig. 5). Callus cultures of *S. marianum* were also reported as potential sources of milk clotting peptidases (Cimino et al. 2006). Positive linear correlation between antioxidant activity and total phenolic content for alcoholic extracts of *S. marianum* was reported by Tawaha et al. (2007). Several studies suggested that the phenolic compounds contributed significantly to the antioxidant capacity of 112 wild grown Chinese herbs (Cai et al. 2004). However, this is first report on the anti-oxidant activity of regenerated tissues of *S. marianum*. Our results are consistent with numerous previous findings in which positive correlation between total phenolic content and antioxidant activity for *Silybum* and other related plant species was reported (Koksal et al. 2009; Vaknin et al. 2008; Wallace et al. 2008; Zheng and Wang 2001). Iqbal and Srivastava (2000) concluded that yield of silybin content in cultured tissues of *S. marianum* varied with age and composition of the medium.

The above results demonstrated that wild-grown leaf explants of *S. marianum* are amenable to in vitro culture. Further, the results highlight the efficiency of BA and GA_3 on wild-grown leaf explants for regeneration for the first time. Plantlets derived from leaf explants can be useful sources of tissues for biochemical characterization of medicinally active constituents and for selection and cloning of superior individual genotypes. As FRSA levels were high in callus culture, this suggested that commercial production of callus-derived cultures of *S. marianum* for biologically active compounds were possible.

Acknowledgments Support of Pakistan Science Foundation (PSF) is highly acknowledged. Authors also appreciated Dr. Saleem A. Bokhari for his critical reading of manuscript.

References

Abbasi BH, Saxena PK, Murch SJ, Liu CZ (2007) *Echinacea* biotechnology: challenges and opportunities. In Vitro Cell Dev Biol-Plant 43:481–492

Ahmad M, Khan MA, Hasan A, Zafar M, Sultana S (2008) Chemotaxonomic standardization of herbal drugs milk thistle and globe thistle. Asian J Chem 20:4443–4459

Alikardis F, Papadakis D, Pantelia K, Kephalas T (2000) Flavonolignan production from *Silybum marianum* transformed and untransformed root cultures. Fitoterapia 71:379–384

Becker H, Schrall R (1977) Callus und suspesionkulturen von *Silybum marianum*. Planta Med 31:185–192

Cacho M, Moran M, Corchete P, Fernandez-Tarrago J (1999) Influence of medium composition on the accumulation of flavonolignans in cultured cells of *Silybum marianum* (L.) Gaertn. Plant Sci 144:63–68

Cai Y, Luo Q, Sun M, Corker H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 74:2157–2184

Cimino C, Cavalli SV, Spina F, Natalucci C, Priolo N (2006) Callus culture for biomass production of milk thistle as a potential source of milk clotting peptidases. Elec J Biotech 9:3

Ferreiro P, Pais MSS, Cabral JMS (1991) Production of silybin-like compounds in cell suspension cultures of *Silybum marianum*. Planta Med 57:2–3

Flora K, Hahn M, Rosen H, Benner K (1998) Milk Thistle (*Silybum marianum*) for therapy of liver disease. Am J Gastroenterol 93:139–143

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension culture of soybean root cells. Exp Cell Res 9:213–218

George MW, Tripepi RR (2001) Plant preservation mixture can affect shoot regeneration from leaf explants of chrysanthemum, European birch, and rhododendron. HortScience 36:639–644

Hasanloo T, Kharai-Nejad RA, Majidi E, Ardakani MRS (2008) Flavonolignan production in cell suspension culture of *Silybum marianum*. Pharma Biol 46:876–882

Hetz E, Huancaruna Perales E, Liersch R, Schieder O (1995) Plant generation from mesophyll and suspension protoplasts of *Silybum marianum*. Planta Med 61:554–557

Iqbal SM, Srivastava PS (2000) In vitro micropropagation of *Silybum marianum* L. from various explants and silybin content. J Plant Biochem Biotech 9:81–87

Katiyar SK, Korman NJ, Mukhtar H, Agrawal R (1997) Protective effects of silymarin against photocarcinogenesis in a mouse skin model. J Natl Cancer Inst 89:556–566

Khan MA, Blackshaw RE, Marwat KB (2009) Biology of milk thistle (*Silybum marianum*) and the management options for growers in north-western Pakistan. Weed Biol Manag 9:99–105

Koksal E, Gulcin I, Beyza S, Sarikaya O, Bursal E (2009) In vitro antioxidant activity of silymarin. J Enz Inhibit Med Chem 24:395–405

Koroch A, Juliani HR, Kapteyn J, Simon JE (2002) In vitro regeneration of *Echinacea purpurea* from leaf explants. Plant Cell Tiss Organ Cult 21:525–530

Kurkin VA, Zapeschnaya GG, Volotsueva AV, Avdeeva EV, Pimenov KS (2001) Flavolignans of *Silybum marianum* fruit. Chem Nat Prod 37:315–317

Ligeret H, Brault A, Vallerand D, Haddad Y, Haddad PS (2008) Antioxidant and mitochondrial protective effects of silibinin in cold preservation-warm reperfusion liver injury. J Enthopharm 115:507–514

Liu CZ, Murch SJ, El-Demerdash M, Saxena PK (2004) *Artemisia judaica* L.: micropropagation and antioxidant activity. J Biotech 110:63–71

Lucchesini M, Bertoli A, Mensuali-Sodi A, Pistelli L (2009) Establishment of in vitro cultures from *Echinacea angustifolia* D.C adult plants for the production of phytochemical compounds. Sci Hort 122:484–490

Meratan AA, Ghaffari SM, Niknam V (2009) In vitro organogenesis and antioxidant enzymes activity in *Acanthophyllum sordidum*. Physiol Plant 53:5–10

Morazzoni P, Bombardelli E (1995) *Silybum marianum* (*Cardus marianum*). Fitoterapia 66:3–42

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Radice S, Caso OH (1997) Somatic embryogenesis and organogenesis in cultured cotyledons of *Silybum marianum* (L) Gaertn. Biocell 21:59–64

Sagare AP, Lee YL, Lin TC, Chen CC, Tsay HS (2000) Cytokinin-induced somatic embryogenesis and plant regeneration in *Corydalis yanhusuo* (Fumariaceae)- a medicinal plant. Plant Sci 160:139–147

Sanchez-Sampedro MA, Fernandez-Tarrago J, Corchete P (2005) Enhanced silymarin accumulation is related to calcium deprivation in cell suspension cultures of *Silybum marianum*. J Plant Physiol 162:1177–1182

Sanchez-Sampedro A, Kim HK, Choi YH, Verpoorte R, Corchete P (2007) Metabolomic alterations in elicitor treated *Silybum marianum* suspension cultures monitored by nuclear magnetic resonance spectroscopy. J Biotech 130:133–142

Selvakumar V, Anbudurai PR, Balakumar T (2001) In vitro propagation of the medicinal plant *Plumbago zeylanica* L. through nodal explants. In Vitro Cell Dev Biol-Plant 37:280–284

Stfaan P, Werbrouck O, Debergh PC (1994) Applied aspects of plant regeneration. In: Dixon RA, Gonzales RA (eds) Plant cell culture: a practical approach. Oxford University Press, Oxford, pp 127–145

Takahata Y, Ohnishi-Kameyama M, Furuta S, Takahashi M, Suda I (2001) Highly polymerized procyanidins in brown soybean seed coat with a high radical-scavenging activity. J Agric Food Chem 49:5843–5847

Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T (2007) Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem 104:1372–1378

Tumova L, Tuma J (2009) Affecting production of secondary metabolites in *Silybum marianum* cell culture by Paraquat elicitor treatment. Chemicke Listy 103:503–510

Vaknin Y, Hadas R, Schafferman D, Murkhovsky L, Bashan N (2008) The potential of milk thistle (*Silybum marianum* L.), an Israeli native, as a source of edible sprouts rich in antioxidants. Intl J Food Sci Nutri 59:339–346

Valenzuela A, Guerra R, Videla LA (1986) Antioxidant properties of flavonoids silybin and silibinin, an active constituent of milk thistle: comparison with silymarin. Cancer Lett 147:77–84

Wallace S, Vaughn K, Stewart BW, Viswanathan T, Clausen E, Nagarajan S, Carrier DJ (2008) Milk thistle extracts inhibit the oxidation of low-density lipoprotein (LDL) and subsequent scavenger receptor-dependent monocyte adhesion. J Agric Food Chem 56:3966–3972

Wojdyło A, Oszmianski J, Czemerys R (2007) Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem 105:940–949

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 49:5165–5170

Zheng W, Xu XD, Dai H, Chen LQ (2009) Direct regeneration of plants derived from in vitro cultured shoot tips and leaves of three *Lysimachia* species. Sci Hort 122:138–141