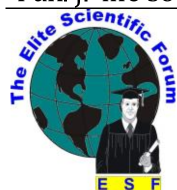
Pak. j. life soc. Sci. (2017), 15(2): 107-113 E-ISSN: 2221-7630;P-ISSN: 1727-4915



Pakistan Journal of Life and Social Sciences

www.pjlss.edu.pk

RESEARCH ARTICLE

Effect of Plant Growth Regulators on Shoot Organogenesis and Somatic Embryogenesis of *Echinacea purpurea* L.

Khalid Masood Ahmad[1*], Zafarullah Zafar[2] and Javed Iqbal Mirza[2]

[1]Department of Horticulture, FAS & T, Bahauddin Zakariya University, Multan, Pakistan

[2]Department of Botany, Bahauddin Zakariya University, Multan, Pakistan.

[3]Department of Biotechnology, Bahauddin Zakariya University Lahore Campus, Pakistan

ARTICLE INFO

Received: May 04, 2016

Accepted: Jul 31, 2017

Keywords

Echinacea

In vitro propagation

Plant regeneration

Somatic embryogenesis

Tissue culture

Medicinal Plants

***Corresponding Author:**

khalidmasood@bzu.edu.pk

ABSTRACT

Echinacea is an indigenous herbal plant genus of North America and occupies an important place among medicinal plants due to its immunostimulatory properties against respiratory ailments. The increasing demand for high-quality plant material has necessitated its true to type, disease-free propagation through tissue culture. *In vitro* regeneration potential of different plant growth substances for *Echinacea purpurea* L. was investigated in this study. The *E. purpurea* was evaluated for its morphogenic potential in different concentrations and combinations of plant growth regulators. Seeds of *E. purpurea* were grown in magenta boxes containing Murashige and Skoog (MS) basal medium under controlled conditions and leaf discs (1.5×2.0 mm) were used as explants in these experiments. Explants were excised and cultured under aseptic conditions on nutritional medium containing MS salts and B_5 vitamins mixed with combinations of 1.0-5.0μM BAP, 1.0-5.0μM IBA, and 0.1-1.0μM TDZ. The cultures were kept in a growth cabinet with cool white light (40-60 mol.m^{-2}.s^{-1}) under 16-hr photoperiod. Regeneration was quantified at 28 days based on the degree of shoot organogenesis and somatic embryogenesis. Different concentrations and combinations of different types of plant growth regulators showed the different response for *in vitro* regeneration of *E. purpurea.* These investigations would aid in the development of a model system for clonal mass propagation and *in vitro* regeneration of *Echinacea spp.*

INTRODUCTION

The genus *Echinacea* is one of the prominent genera of the plants that are utilized in medicinal preparations and drugs. In the year 2000 *Echinacea* products became the top-selling herbal product in North America, accounting for nearly 10% of total sales (Bergeron et al., 2002). However, its increasing demand and popularity as an herbal supplement, *Echinacea* has included among the 19 endangered medicinal plants species considered at risk. Traditionally this genus was used by the First Nations of American Great Plains and Canadian prairies for the treatment of several ailments including respiratory problems, sores, toothaches, tonsillitis, bowel pain, rabies, and several other septic conditions (Mechanda and Baum, 2003). Currently, *Echinacea* products are used mostly for the treatment of infectious disorders, most notably colds and respiratory infections (Mechanda et al., 2004). Although plant-based medicines show much promise and are used extensively, there remain many problems in the industry. One of the biggest problems with plant-based medicines is the variability in chemical concentration as well as their chemical profiles (Murch et al., 2006). Another problem associated with these products is contamination with fungi, bacteria, heavy metals, and other pollutants.

Echinacea is a hardy herbaceous perennial plant that belongs to Composite family, also known as Asteraceae and Daisy family. Most commonly, the members of genus *Echinacea* referred as coneflower, Purple Cone-flower, Sampson Root, Black Sampson, Rudbeckia, Daisy, and Missouri Snakeroot. There are nine species in this genus primarily indigenous to North America (McGregor, 1968). The word *Echinacea* was derived from the Greek word *echinos* meaning hedgehog. *Echinacea* plants have erect stems, stout, hairy and glabrous branches with 60–180 cm height. *Echinacea*

flowers are used as cut flower for display in flowering beds of landscapes and gardens. Fresh or dried aerial parts and roots are the plant parts of interest for the preparations of medicines and drugs for human use.

In vitro propagation offers a solution to the majority of the problems faced by this industry (Koroch et al., 2002a, Lucchesini et al., 2009). The use of plant tissue culture is capable to produce high numbers of genetically similar, disease or contaminant free plant material (Murch et al., 2000). Although tissue culture can provide solutions to these problems but it is crucial that there is a highly efficient method to ensure that it is an economically viable option. Somatic embryogenesis is a phenomenon in which a cell or a group of cells develop into an embryo without going through meiosis and recombination. These results in a somatic embryo, similar in appearance to a zygotic embryo, that is genetically almost identical to the parent plant. These embryos are then germinated and develop into regular plants (Koroch et al., 2002b Lucchesini et al., 2009). A common alternative method of micropropagation is to develop shoots that subsequently develop roots and develop into a plant (Lucchesini et al., 2009). Although both methods result in plants regeneration but somatic embryogenesis is more advantageous (Koroch et al., 2003). Three species of *Echinacea* are commercially and medicinally useful i.e. *Echinacea purpurea*, *Echinacea angustifolia* and *Echinacea pallida*. (Mechanda et al., 2004). *Echinacea purpurea* is commonly known as American coneflower or purple coneflower. *Echinacea purpurea* has long been a popular garden ornamental and included amongst the top selling perennial. A number of chemical compounds have been identified such as caffeic acid derivatives, alkamides, polyalkenes, polyalkynes and polysaccharides. (Federica et al., 2003, Capek et al., 2015, Sutovska et al., 2015, Tacchini et al., 2017). *Echinacea angustifolia* is commonly known as narrow-leaved purple coneflower and black Sampson. *Echinacea pallida* is a hardy, herbaceous perennial with simple or branched stems. *E. pallida* was cultivated mistakenly *E. angustifolia* in Europe for many years. A number of biologically active chemical objects have been identified in *Echinacea pallida* such as caffeic acid derivatives, alkamides, volatile oil, polyalkenes, polyalkynes, and polysaccharides (Federica et al., 2003, Capek et al., 2015, Sutovska et al., 2015, Tacchini et al., 2017).

Somatic embryogenesis is the process in which a bi-polar, embryo-like structure develops from a somatic cell, or group of somatic cells. Most often somatic embryos are formed from pre-embryogenic masses; however, they can be formed directly from competent cells (von Arnold et al., 2002). During somatic embryogenesis, embryo is formed lacking an endosperm without fertilization. The suspensor is likewise not present or severely retarded during somatic embryo development. Other than these differences, the developmental pathway of a somatic embryo parallels that of the zygotic embryo to a large. The regeneration response of different plant species is different in the different set and doses of plant growth regulators in different species. It was hypothesis that different concentrations and combinations of plant growth regulators deliver varying results for the somatic embryogenesis and shoot organogenesis particularly in *E. purpurea.* The present study was conducted to determine the influence of various growth regulators on the somatic embryogenesis and shoot induction of *E. purpurea* from leaf explants.

MATERIALS AND METHODS

Starting plant material

Seeds of *E. purpurea L.* were obtained from a commercial source Sunny View Seed Store and Nurseries, Old Club Road, Lahore, Pakistan and prepared to sow for the provision of plant material for this research project.

Culture medium and chemical compounds

Medium consisted of MS basal salt mixture (Murashige and Skoog, 1962) and B_5 (Gamborg et al. 1968) vitamin solutions were used as a basal medium. MSB (MS Salts + Vitamin B5) was combined with different concentrations and combinations of BAP (6-benzyle amino purine), IBA (Indole butyric acid) and TDZ (Thidiazuron) as per following treatments.

T0 MSB (MS Salts + B5 Vitamins)
T1 MSB + 1µM BAP
T2 MSB + 5 µM BAP
T3 MSB + 10 µM BAP
T4 MSB + 5 µM BAP + 2.5 µM IBA
T5 MSB + 10 µM BAP + 2.5 µM IBA
T6 MSB + 0.01 µM TDZ
T7 MSB + 0.025 µM TDZ
T8 MSB + 0.05 µM TDZ
T9 MSB + 0.1 µM TDZ
T10 MSB + 0.2 µM TDZ

The salts, solutions, sucrose, and agar were purchased from PhytoTechnology Laboratories®, USA. Macro and micro-nutrients were obtained from Sigma Chemical Company. Growth regulators were also purchased from Sigma Chemical Company, Australia.

Petri dishes and Magenta boxes (250 ml) were used for explant culture, shoot organogenesis and somatic embryogenesis. Culture vessels and glassware were mainly purchased from Pyrex®, USA. Pipettes and syringes were purchased from Sigma Chemical Company, Australia.

Preparation of culture medium

MS basal salt mixture (containing macro and micro-nutrients) and B_5 vitamin solution were used as

commercially available powder mix and solution, respectively. For growth regulators, 100X stock solutions were prepared in distilled water. Culture medium was prepared using standard procedures.

Sterilization of culture media and distilled water

Culture media and distilled water were sterilized in conical flasks capped with aluminum foil or in glass bottles with caps using an autoclave at 121 °C temperature at a pressure of 1.06 kg cm-2 (15 psi.). The time adjusted for the sterilization of small (up to 250 ml), medium (250-500 ml) and larger volumes (500-1000 ml) were 20, 25 and 30 minutes, respectively.

Sterilization of instruments

Metallic instruments such as forceps, scalpels, Aluminium foils, cotton wools etc. were sterilized by wet heat using an autoclave at 121°C temperature and 1.06 kg cm^{-2} pressure for 40 minutes. Glassware was sterilized either by wet heat. Before sterilization, all the above dry items were wrapped in two layers of Aluminium foil and put inside sealed glass containers.

Sterility control during manipulation

To avoid contamination, a laminar air-flow cabinet was used for all aseptic operations. The cabinet was allowed to run for at least 30 minutes before starting the work. The work surface and walls of the cabinet were disinfected by spraying with 95% ethanol prior to use. During aseptic operations, forceps and scalpels were kept in 95% ethanol and flamed before each use. The necks of the vessels were also flamed before the explants were transferred.

Germination and establishment of sterile seedlings

The seeds of *Echinacea* obtained from a known commercial sources (Sunny seed Inc., Lahore, Pakistan) to ensure true species and good quality seed. Before sowing, seeds were surface sterilized by washing them in 70% ethanol for 15 seconds followed by a 15-minute soak in 5% hypochlorite containing two drops of tween-20 per liter to reduce the chance of biotic contamination. The sterilized seeds were then rinsed five times with sterilized distilled water to remove any possible residue from the seeds. Many species of *Echinacea* have a high level of seed dormancy resulting in low levels of germination (Macchia et al., 2001). Due to this, they were pre-chilled under continuous light in the presence of Ethephon for one week as described by Macchia et al., 2001. Following this, they were planted on water agar (0.4% w/v). Sterile seeds were germinated in a growth cabinet in 24 hours darkness at 24°C. 100 percent of each seed type was germinated. In order to protect the seedlings from fungal contamination, the germination medium was supplemented with Plant Preservation Medium (PPM-1998) at the concentration of 3 ml per liter of medium. It acted as a biostatic agent for fungal growth.

Plant growth

Once the seeds have germinated, determined by the emergence of the radical, they were transferred into magenta boxes for complete plant growth containing 25 ml of media of MS salts (Murashige and Skoog, 1962), B_5 Vitamins (Gamborg et al., 1968), 3% sucrose w/v, and 0.3% gelrite w/v. 5.7 pH of the media was adjusted by using HCl and NaOH with the help of pH meter. Each box contained four seedlings and maintained at 24°C under a 16-hour photoperiod (30-35 µmol m^{-2} s^{-1}). They were allowed to grow in these conditions for 2 months.

The procedures of plant tissue culture have developed to such a level that any plant species can be regenerated *in vitro* through several methodologies. The rate of plant regeneration in tissue culture varies greatly from one species to another. Various cells, tissues, and organs from numerous plant species can be cultured successfully to regenerate the whole plant. Plant tissue culture relies on the ability of plant cells to differentiate and develops into a new plant. This phenomenon is known as totipotency. Initiating a tissue culture can be a challenge especially if the donor plant comes from an environment that would lead to heavy surface contamination such as field grown plants and if the explant is very small. In theory all living plant cells are capable to differentiate into any type of body cell or whole plant. However, in practice, different cells respond better than others. This variation is seen on many levels, tissue type, genotype, and species. The consequence of this is that a protocol developed to induce somatic embryogenesis may work for a particular tissue and fail to work on another. Similarly, shoot organogesis is a technique that works on one specie may not work on other species, or even another genotype within said species. Zobayed and Saxena (2003) developed a protocol for somatic embryogenesis using *Echinacea purpurea*. This protocol produced a high number of embryos from a relatively small amount of starting tissue. In this experiment, it was determined to verify the versatility of this procedure by evaluating its efficacy for different concentration and combinations of plant growth regulators. It was hypothesized that this protocol enables the shoot induction and proliferation and development of somatic embryos from leaf explant and PGRs to be tested. However, the efficacy of the protocol was found highly variable and can be commercially applicable to specific species, explant type, concentrations and combinations of plant growth regulators.

Plant material used

Leaf explants obtained from six lines of *E. purpurea* developed were used in this study.

Preparation and autoclaving of growth medium

Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962), salts and vitamin B_5 incorporated with 30 gm sucrose and 5 gm gelrite for solidification were used. All the cultures were maintained at 25±2ºC under 16 hours photoperiod with 40 µmol.$m^{-2}s^{-1}$

irradiance provided with cool white fluorescent tubes. The medium was autoclaved at 121ºC temperature for sterilization for 40 minutes at 15 psi pressure.

MS basal medium salts were used in powdered form. Due to extremely hygroscopic nature of the powder media, it was protected from atmospheric moisture by placing in a dry protected chamber at an appropriate temperature. We tried to use all of the package contents immediately after receiving and opening shipment.

For the preparation of 1.00 liter of media, approximately 900 ml distilled water was measured and put in a media flask with the capacity of 2 liters. The powdered medium was added to the flask and stirred until completely dissolved by using stir heaters. 30 grams sucrose, 5 grams gelling agent gelrite, B_5 (Gamborg vitamins) different concentrations and combinations of auxins and cytokinins prepared according to the treatments were dissolved in the medium. Additional distilled water was added to the container to make the desired final volume of media. While stirring on an electric heater pH of 5.8 was adjusted by the use of NaOH, HCl or KOH. After the adjustment of pH, a gelling agent gelrite was added to solidify the media and heated up till the solution became clear and placed in an autoclave for sterilization at 1 kg/cm^2 (15 psi), 121 °C for 40 minutes. The culture medium was cooled prior to use. Then, 15 ml media was dispensed into the Petri dishes used as culture vessels according to different treatments.

Ex-Plant preparation and culture

Plants are generally very responsive to tissue culture manipulation. The basic steps in the initiating tissue culture or *in vitro* culture include separation of explant from rest of the plant, sterilization of the explants obtained from the field grown plants but not in the case of *in vitro* grown plants. And finally the maintenance of explants on a defined medium in controlled conditions.

After two months when the plants were germinated completely and occupied all space of magenta boxes, explants were selected, excised and cultured on growth medium. The leaf discs were excised from the sterile seedling plants of *E. purpurea* and cultured onto the same medium supplemented with various combinations and concentrations of plant growth regulators (PGRs).

Ten treatments (T1 to T10) and one control (T0) were used in the experiment and there were 4 Petri dishes for one treatment and 4 explants of *Echinacea* per petri dish. Treatments were incubated in a growth cabinet maintained at 25±2ºC with 16 hours photoperiod under cool white light (40-60) µmol.m^{-2} s$^{-1.}$ Regeneration was quantified after 14, 21, 28, and 35 days of culture of all explants. After 35 days the regenrants were excised from the explants and sub-cultured onto Murashige and Skoog (MS) basal medium along with B_5 vitamins in Magenta boxes for the development and maturation of regenrants.

Somatic embryogenesis

Plant regeneration occurs via organogenesis and somatic embryogenesis. In the later, many of the developmental stages and structures that occur during zygotic embryogenesis are reproduced in culture. The product of somatic embryogenesis is a bipolar structure having root and shoot meristems. Because there is no vascular connection between the somatic embryos and the explanted tissue, so, embryos can easily be detached from the explant and treated like a completely new individual. These embryos are analogous to zygotic embryos and are capable of germination and developing into whole plants. The somatic embryos can be dried and encapsulated in a gel to obtain what is called an artificial seed. Success with the procedure is determined by the genotype and type and physiological condition of the explant, the composition of the media used and the culture conditions. Somatic embryogenesis is often a multistage process that includes explant production via embryo induction on a medium containing plant growth regulators and embryo and plant development on media without PGRS. The concentration and the ratio of the plant growth regulators in the culture medium, especially auxins and cytokinins, control the pattern of differentiation of tissues in culture.

Shoots and Somatic embryos were produced using the protocol previously optimized by Zobayed and Saxena, (2003). Leaf discs (1.0 ×2.0 cm) explants were taken from the 2-month old seedling of different lines of *E. purpurea* and cultured onto Murashige and Skoog medium along with B_5 vitamins. The culture was maintained at 24˚C with an initial dark period of 14 days. After the dark period, cultures were maintained in a 16-hour photoperiod under cool white fluorescent lamps (30 µmol.m^{-2}s^{-1}) at 24˚C. The number of embryos per explant was recorded.

Statistical analysis

Data were analyzed by using one-way ANOVA in Statistix version 8.1. The means were compared at 5% level of significance.

RESULTS

Generation of shoots

Mean number of shoots from leaf explants of *E. purpurea* is shown in Table 1. Maximum and minimum average number was found in case of treatment 5 (9.75±0.46) and control (0.022±0.45), respectively. All other treatments except treatment 9 produced in the range of 0.35 to 2.57.

Average number of shoot produced by different lines of *E. purpurea* showed that line 4 produced the highest average when line 3 produced the lowest average number of shoots from leaf explants (Table 2).

Table 1: Average number of shoots of *E. purpurea* in different treatment from leaf explants

Treatment	N	Mean	SE
T0	96	0.0000	0.4469
T1	88	1.2561	0.4668
T2	96	1.0833	0.4469
T3	96	1.3854	0.4469
T4	96	9.0625	0.4469
T5	92	9.7528	0.4565
T6	96	0.6146	0.4469
T7	96	0.3542	0.4469
T8	96	2.2812	0.4469
T9	96	2.2500	0.4469
T10	96	2.5729	0.4469

Means are not significantly different P>0.05.

Table 2: Average number of shoots of *E. purpurea* in different lines from leaf explants

Line	N	Mean
EPUR1	176	2.7727[b]
EPUR2	176	3.0625[b]
EPUR3	168	0.7249[d]
EPUR4	176	7.1250[a]
EPUR5	176	1.1648[c]
EPUR6	172	1.8481[c]

Means with different letters differ significantly at P<0.05.

Table 3: Comparison of mean number shoots of *E. purpurea* in different treatments from leaf explants

Treatment	Mean	Treatment	Mean
T0	0.0000[e]	T6	0.6146[de]
T1	1.2561[cde]	T7	0.3542[de]
T2	1.0833[cde]	T8	2.2812[bc]
T3	1.3854[bcd]	T9	2.2500[bc]
T4	9.0625[a]	T10	2.5729[b]
T5	9.7528[a]		

Means with different letters differ significantly at P<0.05.

Table 4: Comparison of mean number shoots of *E. purpurea* in different lines from leaf explants

Line	Mean
EPUR1	2.7727[b]
EPUR2	3.0625[b]
EPUR3	0.7249[d]
EPUR4	7.1250[a]
EPUR5	1.1648[cd]
EPUR6	1.8481[c]

Means with different letters differ significantly at P<0.05.

Analysis of variance and mean comparisons

ANOVA showed highly significant differences among treatments ($F_{10,1028}$= 55.99) and lines ($F_{5,1028}$= 48.51). Comparison of means for an average number of shoot produced by leaf explants of *E. purpurea* for different treatments that five homogeneous groups were formed. Most of the treatments differed significantly. The high impact treatments 5 and 4 were significantly different from all other treatments. Similarly, differences with control were also significant in many cases.

Table 5: Average number of somatic embryo of *E. purpurea* in different treatment from leaf explants

Treatment	N	Mean	SE
T0	96	0.0000	0.7314
T1	88	3.6965	0.7639
T2	96	4.3646	0.7314
T3	96	4.6250	0.7314
T4	96	24.031	0.7314
T5	92	22.513	0.7471
T6	96	2.8021	0.7314
T7	96	2.7500	0.7314
T8	96	4.7292	0.7314
T9	96	5.9375	0.7314
T10	96	10.760	0.7314

Means are not significantly different P>0.05.

Table 6: Average number of somatic embryos of *E. purpurea* in different lines from leaf explants

Line	N	Mean	SE
EPUR1	176	13.244	0.5402
EPUR2	176	8.284	0.5402
EPUR3	168	4.499	0.5529
EPUR4	176	0.903	0.5402
EPUR5	176	6.955	0.5402
EPUR6	172	13.138	0.5464

Means are not significantly different P>0.05.

Table 7: Comparison of mean number somatic embryos of *E. purpurea* in different treatments from leaf explants (N=1028)

Treatment	Mean	Treatment	Mean
T4	24.031[a]	T2	4.3646[cd]
T5	22.513[a]	T1	3.6965[d]
T10	10.760[b]	T6	2.8021[d]
T9	5.9375[c]	T7	2.7500[d]
T8	4.7292[cd]	T0	0.0000[e]
T3	4.6250[cd]		

Means with different letters differ significantly at P<0.05.

Table 8: Comparison of mean number somatic embryos of *E. purpurea* in different lines from leaf explants

Line	Mean
EPUR1	13.244[a]
EPUR2	8.284[b]
EPUR3	4.499[c]
EPUR4	0.903[d]
EPUR5	6.955[b]
EPUR6	13.138[a]

Means with different letters differ significantly at P<0.05.

Comparisons for an average number of shoots produced by different lines of *E. purpurea* showed significant differences. The highly productive line, line 4 significantly differed from all other lines.

Generation of somatic embryo from leaf explants of *E. purpurea*

An average number of somatic embryos produced from leaf explants of *E. purpurea* under different treatments are given in Table 5. Minimum and maximum average

number of somatic embryos were produced by control and treatment 4, respectively. Most of the treatment produced in the range of 2.8 to 10.76 somatic embryos.
Mean number of somatic embryos generated from leaf explants of different lines of *E. purpurea* showed that line 1 produced the most when line 4 produced the least. Differences in lines were obvious.

Analysis of variance and mean comparisons

Analysis of variance gave highly significant differences among treatments ($F_{10,1028}$= 119.82) and among different lines ($F_{5,1028}$= 79.73) of *E. purpurea*.
When compared, means for a number of somatic embryos produced from leaf explant of *E. purpurea* under different treatments showed significant separation falling into five homogeneous groups. All treatment means significantly differed from control (Table 5).
In case of means for different lines, four homogeneous groups were formed showing significant differences (Table 6).

DISCUSSION

Shoot regeneration from leaf explants of *E. purpurea* species depended upon the various concentration of BAP and IBA. A gradual increase in the concentration of BAP from 1 to 10 units showed firstly increase then decrease and finally again increase in number of shoots. When these treatments were supplemented with various concentrations of IBA the yield was increased to a greater extent reaching the highest value. A treatment composing of MSB+10BAP+2.5IBA was the most effective one. By altering the treatment composition by the addition of various concentrations of TDZ also affected the average number of shoots grown from leaf explants but not to the extent of earlier treatments. The most effective combination was in treatment 5 (MSB+10BAP+2.5IBA) in gaining maximum benefit from this species. Line 4 followed by line 2 were the most prolific line of *E. purpurea* with regards to generation of shoots from leaf explants. It was obvious that line differences should be considered while selecting for the number of shoots production from leaf explants in *E. purpurea* species.
In case of somatic embryo production from leaf explants, treatment compositions showed marvelous influences. The MSB alone could not generate any somatic embryo from leaf explants in this specie. When BAP was added at various concentrations, a variable number of somatic embryos were produced at each higher concentration of BAP. Supplementation of IBA produced a surprising increase in number of somatic embryos. An addition of 2.5IBA in MSB+5BAP produced the highest average number of embryos. When 2.5IBA was added to MSB+10BAP, the average number of embryos reduced slightly. Other compositions where TDZ at various concentrations was added to MSB, were also effective but did not produce a comparatively large number of the embryo. Among them, only 0.2 TDZ was the most effective yet it produced the half of the average number of the embryos as compared to treatment 4 (MSB+5BAP+2.5IBA). Lines 1 and 6 of *E. purpurea* were the most productive ones for a number of somatic embryos from leaf explants. Among other lines, lines 2 and 5 also produced the considerable number of somatic embryos from leaf explants. Callus tissues were generated by Koroch et al. (2002) in *E. purpurea* from leaf explants. Shoots were also produced and multiplied using MSB. Their studies differed from present study to some extent because of different treatment composition used. Chae (2016). achieved Shoot organogenesis of *Echinacea angustifolia* DC by different media combinations and used polyamines for shoot induction and proliferation.
Mechanda and Baum (2003) succeeded in developing shoots from leaf tissues of *Echinacea purpurea* using different media and finally achieved viable plants. Their study supported present findings of *E. purpurea*. Recent work of Abbasi et al. (2016) by using MS and B_5 medium along with TDZ also showed promising results of somatic embryogenesis for another plant i.e. *Silybum marianum* L. Study of Zobayed and Saxena (2003) also matched with present study who conducted somatic embryogenesis from leaf explants of *E. purpurea* on MSB, BAP, and IBA containing medium. Young leaves yielded viable tissues for micropropagation (Pan et al., 2004). Shoots were regenerated and complete plants were obtained.

Conclusion

This experiment provided information on the versatility of this protocol for different types oflant and different lines of *E. purpurea*. It proved to be an efficient technique for the induction of shoot organogenesis and somatic embryogenesis for a wide range of genotypes and *E. purpurea* can be propagated successfully by this technique.

Authors' contributions

All authors contributed equally in this manuscript.

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