BIOGLOBIA Vol. 7 (2): 92 –99, 2020 **ISSN: 2349-5626**

Colchiploids of *Asparagus racemosus* Willd. (Liliaceae)

PALLAWI KUMARI

Department of botany, Patna Science College , Patna University, Patna- 800005, Bihar, India
Author's e-mail: palak251079@gmail.com
Received: 10.08.2020 **Revised: 30.08.2020** **Accepted: 25.09.2020**

ABSTRACT

Asparagus racemosus is an endangered medicinal plant with vital chemical constituents like saponins, asparagenine, isoflavones, imparts medicinal properties. Colchicine is a known polyploidizing agent used to alter morphology of the plant for better agronomic purposes. Colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants were done by Manzoor *et al*.,2019. In this study the application of colchicine was done at multiplication stage with concentration of (150-300mg/l). 25 anthers/ medium were inoculated to initiate cultures in four different compositions of N_6 media i.e. (CPM_1-CPM_4) with BA, IAA, 2.4-D, NAA and Kin (0.25-2.0) mg/l) concentrations to initiate the cultures. 80% cultures formed calli in CPM_4 after 45 days of inoculation. The embroygenic calli were then transferred to six different MS media (EMM_1EMM_6) with IAA, BA, GA_3, Ancymidol, NAA and Kin at conc. (0.1-1.5 mg/l) for the maturation of embryos. 75% embryos matured in EMM_6 after 30 days of transfer. Embryos were germinated in EGM_1-EGM_7 MS media with combinations of BA, IAA, 2,4-D and GA_3 at the concentrations of (1.0-2.0 mg/l). Colchicine was added in EGM at six different dose levels, i.e. (100, 150, 200, 250, 300, 350 mg/l) for 18h and 36h. Media without colchicine was treated as control. Dose levels of (100-250) mg/l gave positive results and the higher doses drastically reduced the doubbling efficiency of the regenerates. 75.9% shoots formed roots in RA_4 media. 73.5% and 56.6% plants were acclimatized and successfully established in the field. The variation due to application of colchicine is estimated in one month old plant. 250 mg/l. 60.01% and 56.02% of regenerated plants were obtained when colchicine (250mg/l) treatment was given for 18 h and 36h resp in EGM_5 but 18h treatment was more effective. Highest number of DHs i.e. 45% was obtained with 250 mg/l colchicine. Highest frequency of DHS (45%) was obtained with 250mg/l colchicine. Lower percentage also induced polyploidization but at lower frequency whereas higher concentration >250mg/l appeared to be harmful for the investigating herb. Rooting was done in ½ MS media. The present observations are valuable in terms of agronomical traits.

Keywords: *Agronomic traits, GR, Colchiploids, Asparagus racemosus, better genotype,Dose level.*

INTRODUCTION

Roots were found to possess gastrointestinal effect (Karr *et al*., 1991), anti-tumor and anticancer activities (Shao *et al.*,1996 and Diwanay *et al*., 2004), anti-ulcerogenic and anti-secretory activities (Data *et al.,* 2002). Galactogogues effect (Gautama *et al*., 2004), anti-stress, anti-diarrhoeal, anti-dyspepsia, adaptogenic action, cardio protection. Ethnopharmacology, Phytochemistry and Bioactivity of the herb were undertaken by Nagar *et al*., (2011). The investigated herb had many medicinal properties but it is in endangered condition due to overexploitation of the herb. Although micropropagation has been done by several workers, but anther and pollen cultures have not been undertaken so far in the selected plant species. Polyploidy has the importance in Horticulture for the development of specific ornamental varieties with desirable morphological changes of utility *i.e.*intense color of leaves, flower, leaf thickness, compactness and restored fertility (Manjoor *et al.,* 2019).

Colchicine is used widely as mitotic inhibitor for the induction of polyploidy in plants. Polyploids can be induced by doubling the no of chromosome in somatic cell. By observing morphological and physiological traits the ploidy level could be determined in plants. External use of colchicine is not found to be fruitful as it yield large no of cytochimera. In the present study colchicine was added at multiplication level. Different plant organs like seeds, apical meristems, flower buds, and roots can be used to induce polyploidy through many application methods such as dipping/soaking, dropping or cotton wool. In this study it was done at multiplication stage. Anther culture provides an attractive biotechnological tool to produce Hs, DHs, Polyploids, which has significant impact on agricultural system. Due to the immense importance of polyploidy, it has been artificially induced in many economically important crops, however the majority of success has been reported in ornamental industry. Chromosome doubling through colchicine by using different application methods has been obtained in many ornamental crops such as lily, salvia, phlox, gladiolus, petunia and marigold etc.

MATERIALS AND METHODS

Germplasms (seeds) were obtained from CIMAP, Lucknow, Uttar Pradesh and were multiplied in the Polyhouse, Department of Botany, Patna University, Patna. The germplasm were multiplied in the polyhouse thus, the environmental factors bringing about variations have either been eliminated or minimised and the screened genotypes expressed their true genetic constitution. N_6 media was used for culture initiation and establishment of calli. MS media was used for maturation and germination of embryos, multiplication and rooting of shoots.

Preparation of Explant:

Buds were surface sterilised with (70%) alcohol (3min), then washed with ddw. Excess water was blotted with sterile blotting paper, wrapped in aluminium foil, kept in plastic bag, and given chilling treatment in Refrigeration unit for 24h at (57°C) temp. After pre-culture treatment, all the experimental works were done under aseptic conditions in the inoculation chamber under laminar air flow unit. The floral bud were surface sterilized with (70%) ethanol (3 min) followed by (2.5%) sodium hypochlorite (20 min), rinsed thrice with ddw and placed in sterilized petridish. The pedicels of buds were cut by pair of sterilized scissors (Figure 1).

Culture Initiation:

Anthers (6/bud) were gently extracted out from each bud by sterilised forcep and filaments were aseptically removed with sterilized blade. . Anthers at uni-to bi-nucleate stages were inoculated. Petridishes were sealed with three layers of parafilm (to prevent desiccation of medium), kept in dark at (32 ± 2^0 C) for 3-4 weeks, then shifted to (25 ± 2^0 C) temp and photoperiodic regime of (16h light and 8h dark) for 3-4 weeks and were regularly observed. All the experiments were done in triplicate and total of 90 anthers were inoculated. When the calli attained (2-3 mm) diameter, were transferred in fresh CPM_4 medium. After 6-8 weeks, the calli containing ELS were transferred, kept under florescent light (approx. 3000 lux), (25 ± 2^0C), photoperiod (16 hrs light and 8 hrs dark) for 4 weeks, which developed into putative embryos.Anthers with microspores at the late uni nucleate stage has the highest frequency of total and embryogenic calli formation. 25 anthers/ medium were inoculated to initiate cultures in four different compositions of N_6 media i.e. (CPM_1-CPM_4) with BA, IAA,

2.4-D, NAA and Kin (0.25-2.0) mg/l) concentrations to initiate the cultures. 80% cultures formed calli in CPM_4 after 45 days of inoculation. The embroygenic calli were then transferred to six different MS media (EMM_1-EMM_6) with IAA, BA, GA_3, Ancymidol, NAA and Kin at conc. (0.1-1.5 mg/l) for the maturation of embryos. 75% embryos matured in EMM_6 after 30 days of transfer. Embryos were germinated in EGM_1-EGM_7 MS media with combinations of BA, IAA, 2,4-D and GA_3 at the concentrations of (1.0-2.0 mg/l).

Colchicine Administration:

The different concentrations of colchicine (100-350 mg/l) and duration 18 and 36 h were used in EGM (Figure 3) embroyos (0.20.3 cm) long, were separated from callus, incubated for a period of 18h and 36h in EGM (with and without colchicine), then transferred to fresh EGM media without colchicine (Figure 2). One set (without colchicine) was treated as control *i.e*. EGM_1. Colchicine was added in EGM at six different dose levels, (100, 150, 200, 250, 300, 350 mg/l) for 18h and 36h. Media without colchicine was treated as control. Treated embryos were transferred in fresh EGM.Germinated embryos were then transferred to liquid rooting media (RA_1- RA_4) with various combinations of IBA, BAP, NAA, BA and BAP at the concentration of (0.3-2.0 mg/l). The variation were recorded on the morphological ,anatomical and cytological basis.

RESULTS AND DISCUSSION

Mature embryos were transferred to embryo germination media (EGM_1 - EGM_7). 60.01% and 56.02% of regenerated plants were obtained when colchicine (250mg/l) treatment was given for 18 h and 36h resp. in EGM_5 but 18h treatment was more effective. Dose levels of (100-250) mg/l gave positive results and the higher doses drastically reduced the doubling efficiency of the regenerates. Highest number of DHs (45%) was obtained with 250 mg/l colchicine. In control plantlets 20% HS and 7% DHS appeared. Highest frequency of DHs (45%) was obtained with 250mg/l colchicine (Table 1). Lower concentration also induced polyploidization but at lower frequency whereas colchicine concentration more than >250 mg/l exhibited deleterious effect. 3.0% Hs, 6.0% mixoploids and 1.0% albino plantlets were spotted. However, there are still several factors that hinder the application of anther and microspore culture. For this purpose, variations with better gene pool, heterotic vigour, better adaptability and performance needs to be developed by various cost effective techniques. Microspore embryogenesis has greater potential for DH production, due to the abundance of microspores per flower compared to the single ovule per floret. However, there are still several factors that hinder the application of anther and microspore culture at a commercial level specially the low rates of embryogenesis and regeneration, and the high frequency of albinism among regenerants and low frequency of chromosome doubling in some cultivars. One month old plants were screened for gametoclonal variation. Plants were categorized on the basis of phenotypic appearance like size of internode, cladode, color of cladode, tuber color, number and size of tubers, confirmed by anatomical, epidermal, cytological studies. Length of cladode recorded was 3.5, 7.5 and 11.2 mm in scarce, normal and vigorous plants (Table 2, Figure 4). Internode length (0.89, 1.5 and 1.61cm) observed in scarce, normal and vigorous plants. Number of tubers/crown (2.8, 8.2 and 14.9) were found whereas tuber size varied from (1.97 x 0.75cm), (3.8 x 0.8cm) and (4.25×1.5cm) in scarcey, normal and vigorous regenerates. Anatomical studies revealed that amount of vascular tissues were more

in vigorous plant than in other counterparts. Frequency of stomata recorded were 19.23, 18.2, 14.4 in 40x magnification and whereas their size recorded variation (9.1×2.5µm), (7.8×1.7µm) and (4.0×1.1µm) in all the three types of plants. Initial screening for variations was done on the basis of general appearance of the plant followed by morphological traits and confirmed by anatomical, epidermal and cytological studies. Internode 0.75 ± 0.01 cm in vigorous normal and scarce plant. In control plantlets 20% Hs and 7% DHs appeared .The results were discussed in the light of media used, combination and concentrations of the GRs, culture conditions, mode of regeneration, genomic stability. The variations observed in the regenerated plants are presented in Table 2. Regenerates were analyzed for their ploidy status. Plants were categorized on the basis of phenotypic appearance-(i) Scarce; thin and light green (ii) Normal, healthy and dark green (iii) Vigorous. From the observations, it is evident that in vigorous plants the internode size number and tuber size is more than normal and scarce plants. Anatomical studies revealed that amount of vascular tissues was more in vigorous plants than the other counterparts. Frequency and stomata size also recorded variation. It varied from (14.4± 0.02), (18.2±0.01), and less than (9.23±0.02) in vigorous, normal and scarce plants. Colchicine concentrations (100mg/l for 32&48 h) and (100mg/l for 48h) has been used for ploidy induction in *Asparagus Lettuce* by Wu and Zhang, 2012. Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants . Manzoor *et al*.,2018 and Mohammadi *et al*.,2011 used 100, 200, 250, 350 and 400mg/l colchicine in the pre-treatment media for 3, 6 and 9 days duration in *Zea mays* by treating Hs with colchicine. Dunwell *et al.* (2010) obtained DH and mixoploids for commercial scale cultivation in oil palm. Some workers while working with microspore culture of *Brassica napus* var. *napus* remarked that in general colchicine treatment induces diplods, polyploids and mixoploids, so some modifications of culture protocol should be done to obtain fully fertile DH plants. Soriano *et al*., (2013) investigated the effect of colchicine on microspore culture of *Triticum aestivum*. They applied colchicine during mannitol stress pretreatment or during the first 48h of culture at concentrations 150mg/l and 300mg/l. When colchicine was applied during stress pre -treatment, the percentage of doubling depended on genotype and concentration. The duration of colchicine exposure had a considerable effect on chromosome doubling, induction and the survival rate. The present experiment showed that a treatment duration of 18h at the 250mg/l colchicine conc has greater positive effect on the doubling efficiency whereas lower doses were not very effective. Our findings are in accordance with the previous reports which indicate that the best embryo induction can be achieved by the combination of genotype, concentration of colchicine in the medium, duration of treatment, time of incorporation and composition of CPM. The concentration and duration of colchicine treatment used in this study can be effectively applied for *in vitro* chromosome doubling. Polyploidization can be effectively and conveniently applied for conservation of the endangered species along with productivity of the valuable tubers. Anatomical studies revealed that amount of vascular tissues were more in vigorous plant than in other counterparts. Frequency of stomata recorded were 19.23, 18.2, 14.4 in 40x magnification and whereas their size recorded variation (9.1×2.5µm), (7.8×1.7µm) and (4.0×1.1µm) in all the three types of plants. In control plantlets 20% Hs and 7% DHs appeared .The results were discussed in the light of media used, combination and concentrations of the GRs, culture conditions, mode of regeneration, genomic stability.

Table 1
Colchicine Response in the Culture

Culture response	Growth regulators (GRs)	Media	Culture response	Colchicine added (250mg/)	Hs (%)	DH (%)	Polyploids (%)
Callus formation	B.A(1.0) + N.A.A(20 + Kin(0.1)	N6	80%	_			
Embryo-maturation	N.A.A(0.1) + KIN(0.1) + Ancymidol(0.65)	MS	75%	_			
Embryo germination-in control	I.A.A(1.0) + GA3(1.0)+ B.A(1.0)+ 2,4D(2.0)	MS	31.8%	--	20.0 %	7.0%	--
Embryo-germination	I.A.A(1.0) + GA3(1.0) + B.A(1.0) + 2,4D(2.0)	MS	60.01%	18hr	3%	45%	6%
			56.02%	36hr	5%	42%	5%
Rooting	IBA(2.0) + I.A.A(2.0)	1/2MS	75.9%				

73.5% plants are acclimatised and 56.6%were successfully established in the field. One month old plant was analysed on morphological, anatomical and cytological basis. Some plants are mixoploids.

Table 2
Variation in One Month Old Plant

Parameters	HS(scarce)	DH(Normal)	Polyploids (vigorous)
Cladode length(cm)	3.5±0.01	7.5±0.04	11.2±0.03
Internode length(cm)	0.89±0.03	1.5±0.1	1.6±0.2
Number of tubers / crown	2.8±0.02	8.2±0.04	14.9±0.01
Tuber length(cm)	1.97±0.23	3.8±0.02	4.25±0.75
Tuber width(cm)	0.75±0.01	0.8±0.02	1.5±0.01
Stsomata frequency (40x)	19.23±0.02	18.2±0.01	14.4±0.02
Size of Stomata (µm) (LxW)100x	9.1 x 2.5	7.8 x 1.7	4.0 x 1.1

Figures 1-4 representing Flower, Anthers, Embryo Germination, Colchicine Treatment and Variations.

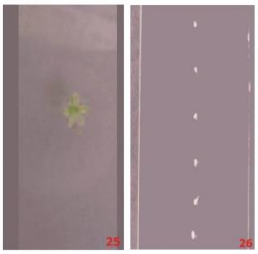

Figure 1. Flower & Anther of *Aspapragus racemosus*

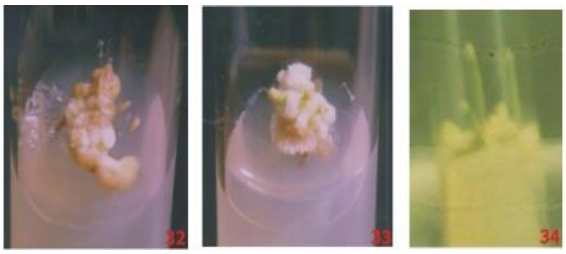

Figure 2. Germinating embryos in EGM media

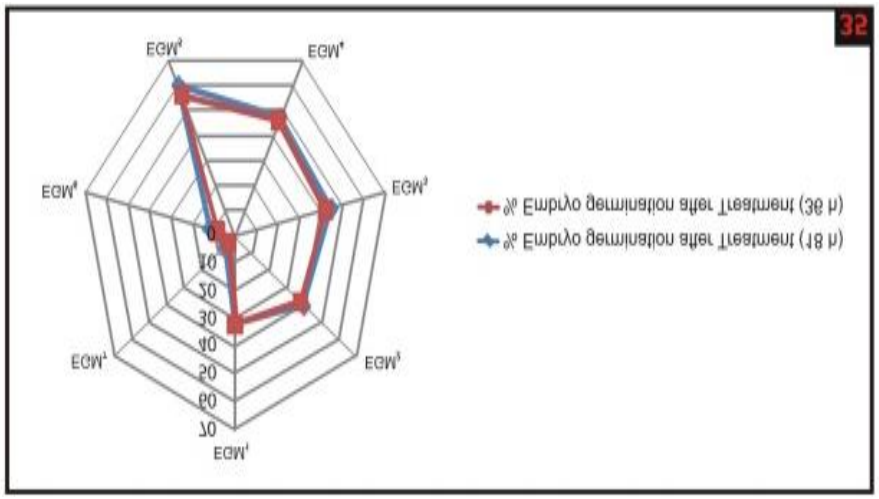

Figure 3. Embryo germination after colchicine treatment

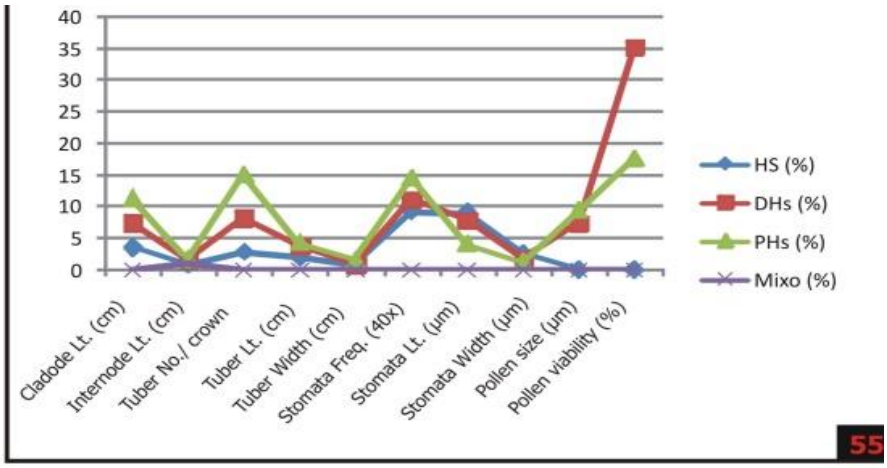

Figure 4. Effect of colchicine treatment on ploidy status of the regenerate.

Morphological alteration stomatal frequency, vascular bundles amount were parameters for ascertaining polyploidization in colchicine treated population. Beck *et al*., (2003) and Cohen and Yao (1996) have observed and reported that size and stomata may change significantly due to chromosome doubling compared to the diploids of vine zantedeschia cultivars and *Acacia mearnsii* respectively. Chemically induced polyploidy is

an important tool for improving plant physiology and production, therefore, induced polyploidy should be produced to overcome the problem of low yield and limited rainfall in tea growing areas. Induced polyploidy as a tool for increasing Tea *Camellia sinensis L.* production. (Hasnain *et al.*, 2015). In Mulberry plants colchicine concentration at 0.4% recovered dwarf, stout, thick, greenish leaves, leaf yield increased to 10.11% and number of chloroplast ranged from 23-27 compared to control (44.00) in C1 generation (Ramesh and Yogananda , 2014).For creating new brown leaved tetraploid cultivar by treating seedlings with treatment with solution of colchicine in range from 0.1 to 2.5%. He found some morphological changes like leaf coloration, flower shapes in colchiploids differed between the genotypes. Some mixoploids have loss of coloration , failure of blooming (Jadrna *et al*.,2010). The present observations are valuable in terms of agronomical traits. Anther culture of the species has been done for the first time and encouraging results are scored. The technique needs to be further modified by more intense and in depth studies for utilization and conservation of the precious medicinal plant .In control plantlets 20% HS and 7%DHS appeared. Highest frequency of DHS (45%) was obtained with 250mg/l colchicine. Lower concentration also induced polyploidization but at lower frequency whereas higher concentration>250mg/l appeared to be harmful for the investigating herb. The present observation are valuable in terms of agronomical traits. The results were encouraging .The technique need to be further modified by more intense and in depth studies for utilization and conservation of the precious medicinal herb.

CONCLUSION

Efforts had been made to make colchiploids of *Asparagus racemosus* and to evaluate better agronomic traits. The altered genotype could be established as a cultivar in subsequent breeding programms. The study would be beneficial for systematic yield trails which would culminate into to a better commercial genotype.

ACKNOWLEDGEMENTS

I am thankful to Head of the Botany Department for providing 'Tissue Culture lab facilities'. I am greatly obliged to my mentor Dr. Chander Prabha, Associate Professor, Botany, Patna Science College, Patna University, Patna for her incessant presence and guide.

REFERENCE

1. Diwanny, S., Chitre, D. and Partwardhan, B. 2004: Immunoprotection by botanical drug in cancer chemotherapy. *S. Ethenopharmacol*.90:1:49-55.
2. Dutta, T. K., Sahoo, P. K., Nawab Sing., Rao, S. B and Chaudhary U. B.2002.Partial replacement of concentrate mixture with *Leucanea leucocephala* leaves in potted feed of goates.*India J.Sci*.,729:820-822.
3. Hasnain, A., Muhammad, R. and Salahuddin. 2015. Induced Polyploidy as a Tool for Increasing Tea (Camellia sinensis L.) Production. *J Northeast Agricultural University*, 22(3): 43-47.
4. Jadrna, P., Plavocova, O. and kobza, F. 2010: Morphological changes in colchicine treated *pelargonium x hortorum L.H*. Bailey greenhouse plant. Horticultural Sci, 37(1): 27–33.
5. Manzoor, A., Ahmad, T., Bashir, A. M., Hafiz, A. I. and Sylvestri, C. 2019: Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. *Plants (Basel)*, 8(7): 194.
6. Manzoor, A., Ahmad, T., Bashir, M. A. 2018: Induction and identification of colchicine induced polyploidy in *Gladiolus grandiflorus* 'white prosperity. *Folia Horticulturae*, 30(2):307-319.

7. Mohammadi, P. P. and Mooieni, A. 2011. Doubled haploid plant following colchicine treatment of microspore derived embryos of oil reed rape *Brassica napusL. Plant cell Tiss organ cult*: DOI 10/S.36-40

8. Mohammadi, P. P., Mooieni A. J. J. M., 2007: colchicine induced embryogenesis and doubled haploid production in maize (*Zea mays L.*) anther culture: *Iranian J. Biotechnology*,5 :3.

9. Ramesh, H. L. and Murthy, V. N. Y. 2014. Induction of colchiploids in mulberry(*Morus)* variety kajali in C1 generation. *Ind. J. Adv. Res*, 2(4): 468-473.

10. Nagar, B. P., Dutt, G. V. and Dhiman, A. 2011. Ethanopharmacologty phytochemistry and bioactivity of *Asparagus recemosus. Pharmacology line*, 2: 979-994.

11. Negi, S. J. 2010.Chemical constituent of Asparagus Northeast: Agric. Univ. 22: 43–47.

12. Prabha, C., Sing, A. and Rolly. 2012. Induction of haploids and doubled haploid in *Chlorophytum Borivilianum Santa pau & Farnandes*: An endangered medicinal crop: ICBLS.

13. Shalaby, T. A., Haneklaus, S. and Schung. 2003.Influence of growth regulators and cultivar on callus and embryo induction in anther culture of *Asparagus, Asparagus officinalis Landbauforsch voelkenrode* 53: 217-221.

14. Sharma, K. and Bhatnagar, M. 2011. *Asparagus recemosus* (Satavari): A versatile female tonic. *Int. J. Pharma Bio. Arch*, 2(3):855-863.

15. Wolyn, D. J. and Teng, X. R. 1991. High frequency production of haploid embryos in *Asperagus* anther culture*: Plant cell Reports*,11:574-578.

16. Zhao, F. C., Nilenthi, D., Yang, Y. S. and Wu, H.2006.Anther culture and haploid plant regeneration in purple coneflower (*Echinacea purpuria* L.). *Plant cell Tissue organ Cult*,85:55-62.