INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–1–66–69
http://www.fspublishers.org

Induction of *Ex Vitro* Adventitious Roots on Soft Wood Cuttings of *Centaurea tchihatcheffii tchihatcheffii* Fisch et. Mey using Indole 3-Butyric Acid and α-Naphthalene Acetic Acid

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ABSTRACT

Centaurea tchihatcheffii, with attractive pink red flowers is critically endangered plant found on 15 - 20 km^2 area of Gölbaşi district near Mogan Lake, Ankara, Turkey. Trials were conducted to investigate vegetative propagation of juvenile and mature softwood stem cuttings removed from mother plants. The cuttings were treated with 0, 500, 1000 ppm IBA and NAA for 5, 10 and 15 min and then planted in sand, which served as rooting medium. No rooting was observed on NAA treated cuttings. Rooting was observed on all IBA treatments on juvenile and 500 ppm IBA treated for 5, 10 and 15 min mature cuttings. The highest frequency of rooting, mean number of roots per plant, and root length along with normal flowering were determined from juvenile cuttings treated with 500 ppm IBA for 10 min. When compared with juvenile cuttings; a considerable reduction in mean number of roots per plant and root length with no flowering on mature cuttings was very evident.

Key Words: *Centaurea tchihatcheffii;* Vegetative cuttings; Rooting; *ex vitro;* IBA; NAA

INTRODUCTION

Besides use of conventional propagation methods, endemic and threatened plants can efficiently be conserved with various *ex vitro* strategies (Fay, 1992), which have low impact on wild populations with minimum of plant materials (Krogstrup & Norgaard, 1992; Fay, 1994; Cuenca *et al.,* 1998).

More than 700 species of *Centaurea* flora are found in Mediterranean region, of which more than 178 are found as land race in Turkey. Twelve species of this flora are known to be rare, threatened or endangered and require appropriate germplasm conservation (Ekim *et al.,* 2000 & Ozel, 2002). *Centaurea tchihatcheffii,* is critically endangered (CR) annual endemic species growing wildly on limited area of 15 - 20 km^2 in sandy soils of adjoining Hacilar village and Süleyman Demirel Forest in the Golbasi district of Ankara under strong anthropogenic pressure (Ekim *et al.,* 2000). During vegetative period, the plant is branched from base and grows to height of 30 - 40 cm and bears pinkish red flowers. Involcure is broadly campanulate. During flowering season several inflorescence stems develop from the main stem. Its fertile seeds need long vernalization treatment to break dormancy (Ozel, 2002). They disperse soon after ripening and are difficult to collect under natural conditions. Most of these, though fertile do not germinate due to high dormancy. In order to meet challenge of conserving the plant material of this rapidly consumed and critically endangered (CR) plant, it is important to develop suitable methods for its conservation, improvement and propagation. One possible solution to the problem could be the rooting of softwood cuttings and forcing the plants to bloom during flowering season outside its natural habitat; under green-house conditions to avoid loss of seeds due to dispersal and collect maximum number of seeds, which could be better stored and studied leading to the discovery of factors responsible for rapid extinction of *C. tchihatcheffii*. Although, the procedure is tedious, it would help in conservation of this critically endangered (CR) species. IBA and NAA are known to effect and stimulate rooting more than IAA since 1935 (Arteca, 1996). Since this time there have been numerous reports indicating that IBA and NAA are involved in the initiation of adventitious roots and that division of root initials is dependent either upon the exogenous or endogenous auxin (James & Thurbon, 1979; James, 1983; Blazich, 1988; Iankova *et al.*, 2001; Ercisli *et al.*, 2002; Haynes & Samagula, 2003) The ability to successfully root this plant may lead to the development of new plant products and markets. No information is available about *ex vitro* regeneration and rooting of the plant. Therefore, a study was designed to test the effects of indole 3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) on rooting and flowering of softwood cuttings of *C. tchihatcheffii*.

MATERIALS AND METHODS

Softwood cuttings at juvenile and mature stage were obtained from carefully selected pest and disease free plants during first and last week of April. Each cutting was 9 - 10 cm long with 5 - 6 nodes.

All except the apex leaves were removed to allow easy and uniform treatment with 500 and 1000 ppm (parts per million) each for 5, 10 and 15 min of IBA and NAA contained in 500 mL glass beakers. Thereafter, they were rooted in moist fine river sand contained in metallic boxes (20 x 40 cm) in non-misting shaded green-house, without providing any bottom heat. A control was also planted in each case.

First irrigation was flooding and thereafter, the cuttings were watered with fine jet sprayer once every day, until completion of the experiment. A cutting was considered rooted when it had one or more roots exceeding 0.3 cm. Cuttings were defined as "dead" when severely rotted accompanied by discoloring, drooping or bleaching. The propagation period for the experiment was 15 days. Each treatment was replicated 4 times and contained 7 cuttings in both control and rooting experiments and was repeated twice.

Statistical analysis. Root formation data was lumped for one way ANOVA (analysis of variance) using SPSS for Windows (v. 9. SPSS Inc USA) based on randomized complete block design and the differences between the means were compared by LSD test. Data given in percentages were subjected to arcsine $\sqrt{X}$) transformation (Snedecor & Cochran, 1967) before statistical analysis.

RESULTS AND DISCUSSION

All cuttings were checked periodically for emergence of roots or root primordia. The rooting was observed in two distinct stages (1) formation of root primordia, occuring inside the cuttings and (2) growth occuring outside the cuttings (data not given). Similar observations were recorded by White and Lovel (1984) in rooting of cuttings from *Griselinea litoralis* and *G. lucida*. No visible changes were detected in all treatments after first three days. When checked after 6 days, again most of the cuttings were without roots; however some of juvenile cuttings treated with 500 ppm IBA for 10 min and 1000 ppm IBA for 5 min had visible root primordial out-growths. No rooting was observed from non-treated shoots (control) in each case. After 9 days, variable number of roots were recorded on most of the juvenile cuttings; which developed to required level after 15 days of culture. Miniature roots were observed on mature cuttings treated with 500 ppm IBA for 5, 10 and 15 min; however, root development remained inhibited throughout All mature cuttings treated with 1000 ppm IBA died down (Table I). The highest frequency of 71.43 and 47.57% rooting with maximum of 36.42 and 6.67 roots per cutting were obtained with 500 ppm treatment of IBA for 10 min on both juvenile and mature cuttings, respectively. Root length in each case was also superior over other treatments. Juvenile cuttings took 5.33 days to flower with pink red normal petalled flowers and abundant pollen. No flowering was recorded on mature cuttings. Although, 500 ppm of IBA treatment for 15 min to juvenile and mature cuttings was statistically similar for the frequency of rooting, yet more number of mature cuttings were accompanied by bleached or discolored leaves and rottening on mature cuttings. All treatments of NAA had negative impact on growth and development resulting in rottening of cuttings. This supports the early and late root formation studies in epicotyl cuttings of *Pinus sylvestris* by of Flygh *et al.* (1993), who found that auxins may have either no effect on root formation or they may be inhibitory at higher concentrations. Non-treated shoot cuttings (control) died down with gradual senescence. Rooting potential of *C. tchihatcheffii,* was quite high from juvenile cuttings over mature cuttings in general. It has widely been documented that auxins promote adventitious root development of stem cuttings through their ability to promote the initiation of lateral root primordia (Leakey *et al.,* 1982). We in line with

Table I. Rooting of *C. tchihatcheffii* cuttings treated with IBA at pre and post floral budding stage under greenhouse conditions after two weeks

Concentration (ppm)	Treatment time (min)	Frequency of rooting (%)		Mean number of roots per cutting		Mean root length (mm)		Days to flowering	
		Juvenile cuttings	Mature cuttings	Juvenile cuttings	Mature cuttings	Juvenile cuttings	Mature cuttings	Juvenile cuttings	Mature cuttings
500	5	47.57[1] b[2]	47.57 a	3.68 c	2.33 b	5,94 b	0.98 b	3.33 b	0.00
500	10	71.43 a	47.57 a	36,42 a	6.67 a	12,98 a	1.99 a	5.33 a	0.00
500	15	47.57 b	47.57 a	32.53 a	2.33 b	6,83 b	1.56 a	3.00 b	0.00
1000	5	66.71 ab	0.00 b[3]	7.88 bc	0.00 c	8,73 b	0.00 c	0.00 c	0.00
1000	10	42.86 b	0.00 b	2.11 c	0.00 c	5,97 b	0.00 c	2.00 b	0.00
1000	15	57.14 ab	0.00 b	3.62 c	0.00 c	7,14 b	0.00 c	2.00 b	0.00
Control		0,00 c	0.00 b	0,00 d	0.00 c	0.00 c	0.00 c	0.00 c	0.00
LSD at 0.05		23.86	20.64	3.58	2.29	4.06	0.81	1.55	-

[1] Each value is the mean of 4 replicates with 7 cuttings

[2]Values with in a column followed by different letters are significantly different at 0.01 level of significance using Duncan's Multiple Range Test.

[3] No rooting on non treated control and mature cuttings with 1000 ppm treatment for 5, 10 and 15 min was due to the death of softwood cuttings.

Hartmann *et al.* (1990) and Howard (1994), assume that 1000 ppm of IBA for any duration of time and 500 ppm IBA treatment for 15 min resulted in production of so called "formative effects" typical growth regulator responses, which are not effective in promoting root formation. Associated cutting mortality with increase in IBA concentration on mature cuttings may be attributed to depletion in foliar nutrient contents and the consequent onset of leaf senescence.

The results (Table I) shows that delayed blooming of 5.33 days in juvenile cuttings had positive effect on flowering. The flowers were normal and had abundant pollen with increased seed set. Other concentrations and treatment times were not so helpful and had either reduced number of flowers or aborted pollen with no or reduced seed set.

1000 ppm concentrations of IBA for any duration of time resulted in inhibition of flowering and partially support results of Mesen (1993) who observed inhibition in shooting with increased concentration of IBA in other species. It was observed that reduction in time of flowering not only affected seed yield but also the color of flowers. It is assumed that 1000 ppm concentrations of IBA for any duration of time and 500 ppm IBA treatment for 15 min were partially responsible for change in petal colors, blooming time and pollen availability. Increased number of roots is important for the plants to increase their ability to exploit soil water and nutrients, which in turn increases their overall growth. Roots produced following treatments with IBA were similar in origin to those produced naturally. However, by varying the concentrations and growth regulators, there was variability in the number and characters of roots considerably. Excessive concentrations were either partially or completely fatal and lead the destruction or death of tissues. Enhancing rate of adventitious roots development with auxin application has been found to increase the number of roots initiated per rooted cutting in number of species (Aminah *et al.,* 1995). The water status (Howard, 1986) and age of cuttings (Poupard *et al.,* 1994) effects rooting ability of plants. Our results are in agreement with Felker and Clarke (1981), Klass *et al.* (1987), Ofori *et al.* (1996), Tchoundjeu and Leaky (1996), Mesen *et al.* (1997) and Berhe and Negash (1998) who report differences in rooting frequency depending on the the exogenous auxin or combination of auxins used, with IBA often giving the best results. The loss or reduction in competence to form adventitious roots on mature cuttings as compared to juvenile cuttings is typical phase dependent difference in competence for root initiation in many species. There is no definitive explanation as to why IBA is usually so effectively promotes rooting in many plant species compared to IAA and other auxins (Haissig & Davis, 1994). It was observed that NAA was inhibitory and IBA did not inhibit growth of adventitious roots on softwood cuttings in juvenile phase. Poor or no rooting in mature cuttings indicate that both phases (juvenile & mature) subjected to similar levels of auxins do not undergo similar changes leading to formation of adventitious roots. As the survival and rooting ability of a cutting depend on the positive carbon balance, which in turn is a function of leaf area and leaf photosynthetic vigor. Higher concentrations of IBA resulted in inhibition of shoot production and rottening on mature cuttings and support the findings of Mesen (1993), who observed similar changes in his experiments on Central American hardwoods.

Juvenile and mature phase cuttings did not have the same ability to initiate roots. Juvenile and mature cuttings do not have the same endogenous inductive stimulus to initiate roots. There is difference in endogenous transmissible substances in juvenile and mature cuttings that influence competence to respond to an inductive signal for rooting. Additional research is needed on the rooting mechanism and to know the functions of adventitious roots in the functional biology of the plant. Knowledge of the behavior and relative contribution of these to the total performance of the plant would be beneficial.

In conclusion, it could be said that present work opens a new window of opportunity to rooting *C. tchihatcheffii,* using softwood vegetative cuttings. However, the concentration of IBA must be carefully chosen in order to avoid IBA induced after effects. This study is a step forward towards the regeneration, sustainable utilization and conservation of this critically endangered (CR) indigenous species.

Acknowledgement. The study was funded by University of Ankara and State Planning Organization (DPT) (Project No. 98 K 120640 & 2001 K 120240). The support from the Department of Biological Education, Gazi University, Ankara is also acknowledged.

REFERENCES

Aminah, H., J.M.C.P. Dick, R.R.B. Leakey, J. Grace and R.I. Smith, 1995. Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula. For. Ecol. Manage.,* 72: 199–206

Arteca, R.N., 1996. Rooting. *In*: Arteca, R.N., (ed.) *Plant Growth Substances. Principles and Applications*, pp. 127–45. Chapman and Hall, New York

Berhe, D. and L. Negash, 1998. Asexual propagation of *Juniperus procera* from Ethiopia: a contribution to the conservation of African pencil Cedar. *For. Ecol. Manage.,* 4454: 179–90

Blazich, F.A., 1988. Chemicals and formulations used to promote adventitious rooting, in: "Adventitious root formation by cuttings" *In*: Davis, T.D., B.E. Haissig, N. Sakhla, (eds.) *Advances in Plant Science Sereis Vol 2*, p. 312. Dioscorides Press, Portland

Cuenca, S., J.B. Amo-Marco and R. Parra, 1998. Micropropagation from inflorescence stems of the Spanish endemic plant *Centaurea paui* Loscos ex. Willk. (*Compositae*). *Pl. Cell Rep.,* 18: 674–9

Ekim, T., M. Koyuncu, M. Vural, H. Duman, Z. Aytac and N. Adıguzel, 2000. *Red data book of Turkish plants* (*Pteridophta & Spermatophta*), pp. 1–50. Ankara - Turkey, (In Turkish)

Ercisli, S., O. Anapali, A. Esitken and U. Sahin, 2002. The effects of IBA, rooting media and cutting collection time on rooting of kiwifruit. *Gartenbauwissenschaft,* 67: 34–8

Fay, M.F., 1992. Conservation of rare and endangered plants using *in vitro* methods. *In vitro Cell Dev. Biol. Pl.,* 28: 1–4

Fay, M.F., 1994. In what situation is *in vitro* culture is appropriate to plant conservation? *Biodivers Conserv.,* 3: 176–83

Felker, P. and P.R. Clarke, 1981. Rooting of mesquite (*Prosopis*) cuttings. *J. Range Manage.,* 34: 466–8

Flygh, G., R. Gronroos, L. Gulin and S. von Arnold, 1993. Early and late root formation in epicotyl cuttings of *Pinus sylvestrisıafter* auxin treatment*. Tree Physiol.,* 12: 81–92

Haissig, B.E. and T.D.Davis, 1994. A historical evaluation of adventitious rooting research to 1993. *In:* Davis, T.D. and B.E. Haissig, (eds.) *Biology of Adventitious Root Formation*. pp. 275–331. Plenum Press, N.Y

Hartmann H.T., D.E. Kester and F.T. Davies, 1990. *Plant Propagation-Principles and Practices,* pp. 1–150. Pentice Hall, Englewood, Cliffs, NJ, USA

Haynes, J.G. and J.M. Smagula, 2003. Rooting and establishment of bunchberry stem cuttings as affected by propagation date, K-IBA, rooting duration, and by division or cottage. *Hort Sci.*, 38: 92–6

Howard, B.H., 1994. Manipulating rooting potentials in stock plants before collecting cuttings. *In*: Davis, D. and B.L. Haissig (eds.), *Biology of Adventitious Root Formation. Basic Life Sciences.* Vol 62 pp. 123–42

Howard, B.H., 1986. Factors affecting the rooting response of fruit tree cuttings to IBA treatment. *Acta Hortic.,* 179: 829–40

Iankova, E., M. Cantos, J. Linan, P. Robeva and A. Troncoso, 2001. *In vitro* propagation of Angelica pancicii Vauds, an endangered plant species in Bulgaria. *Seed Sci. Technol*., 29: 477–82

James, D.J. and I.J. Thurbon, 1979. Rapid *in vitro* rooting of the apple root stock M9. *J. Hort. Sci.,* 54: 309

James, D.J., 1983. Adventitious root formation in apple root stocks (*Malus pumila*), I. Factors affecting the length of the auxin sensitive phase in M.). *Physiol. Pl.,* 57: 149

Klass, S., J. Wright and P. Felker, 1987. Influence of auxins, thiamine and fungal drenches on the rooting of *Prosopis alba* clone B_2 V_{50} cuttings. *J. Hort. Sci.,* 62: 97–100

Krogstrup, P. and J.V. Norgaard, 1992. Micropropagation of *Psiada coronopus* (Lam) Benth, a threatened endemic species from the island of Rodrigues*. Pl. Cell Tiss. Org.,* 7: 227–30

Leakey, R.R.B., V.R. Chapman and K.A. Longman, 1982. Physiological studies for tropical tree improvement and conservation. Factors affecting root inititiation in cuttings of *Triplochiton scleroxylon. K. Schum. For. Ecol. Manage.,* 4: 53–66

Mesen, J.F., 1993. Vegetative propagation of Central American hardwoods. *Ph.D Thesis,* University of Edinburgh, Scotland

Mesen, J.F., A.C. Newton and R.R.B. Leakey, 1997. Vegetative propagation of *Cordia alliodora* (Ruiz & Pavon) Oken: The effect of IBA concentration, propagation medium and cutting origin. *For. Ecol. Manage.,* 92: 45–54

Ofori, D.A., A.C. Newton, R.R.B. Leakey and J. Grace, 1996 Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: Effects of auxin concentration, leaf area and rooting medium. *For. Ecol. Manage.,* 84: 39–48

Ozel, C.A., 2002. *In vitro* propagation of Centaurea *tchihatcheffii. M.Sc. Thesis*, Department of Biology, Graduate School of Natural and Applied Sciences, Gazi University Ankara, Turkey. [In Turkish]

Poupard, C., M. Chauvıere and O. Monteuuıs, 1994. Rooting of *Acacia mangium* cuttings: Effect of age with in shoot position and auxin treatment. *Silvae Genet.,* 43: 226–31

Snedecor, G.W. and W.G. Cochran, 1967. *Statistical Methods,* pp. 327–9. The Iowa State University Press, Iowa USA

Tchoundjeu, Z. and R.R.B. Leakey, 1996. Vegetative propagation of African mahogany: Effect of auxin, node position, leaf area and cutting length. *New For.,* 11: 125–36

White, J. and P.H. Lovel, 1984. The anatomy of root initiation in cuttings of *Griselinea litoralis* and *Griselinea lucida. Ann. Bot.,* 54: 7–20

(Received 20 June 2005; Accepted 20 November 2005)