Effect of plant growth regulators on axillary shoot proliferation and multiplication of several genotypes from mature Himalayan cedar [*Cedrus deodara* (Roxb.ex Lamb) G. Don.] trees

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**Abstract** This paper describes a protocol for the *in vitro* establishment and shoots proliferation of mature Himalayan cedar [*Cedrus deodara* (Roxb.ex Lamb) G. Don.] trees. Four genotypes of *Cedrus deodara* were cultured in vitro. The effects of different basal media (LP, MS and WPM) were evaluated and shoot length by the utilization of two types of explants (leafy and defoliated shoot-tips). In this study, defoliated explants were cultured on WPM supplemented with cytokinins [benzyladenine (BA) and thidiazuron (TDZ)] alone or in combination with auxin, α naphthaleneacetic acid (NAA). Each genotype reacted differently to applied cytokinins. Of the two cytokinins tested for shoot culture establishment, medium supplemented with 0.8 μM TDZ and 2 μM NAA produced the highest average number of induced axillary shoots per explant (4.31), and the longest axillary shoots were achieved when 0.8 μM TDZ was applied (11.20 mm). There were significant differences between genotypes for shoot proliferation at all TDZ concentrations. Among two cytokinins used for shoot multiplication, TDZ was more effective than 2ip. Elongation of axillary shoots happened after their transfer to growth regulator-free half strength WPM medium containing 15 gl-1 sucrose and 3 gl-1 activated charcoal. Neither the auxin treatments nor other tested methods, including ancillary compounds, were effective in inducing root initiation. This protocol is considered as the first successful report on culture establishment of several genotypes of mature *C. deodara* trees.

*Keywords*: Conifer, Pine, Auxin, Micropropagation, Cytokinin, Culture establishment

**Introduction**

There is a critical need for wide afforestation projects in the world. For this purpose, several ways of regeneration are possible. Among them common regeneration through seeds is quite slow and undependable. The amount of viable seeds achieved from seed orchards is not always consistent, grafting is not economically appropriate on a wide scope and cuttings from mature trees root difficulty (Malabadi 2004). The characteristic of trees such as highly heterozygote, long life cycles and self-incompatible, make commercially the fixation of an allele very hard and requiring a lot of time (Campbell et al. 2003). Maturation of trees is a major limiting factor for the use of micropropagation in afforestation projects. In addition, the financial worth of a tree can only be appraised after it reaches maturity, this causes changes in meristematic behavior and decreasing the propagation potential of the tree (Von Aderkas and Bonga 2000).The risk of mutation during organogenesis is low, because the clones taken from axillary buds sustain genetic stability (Lin et al. 1991). Thus, *in vitro* propagation of preferred mature trees could improve afforestation management and breeding projects. Also, because the genotype plays a significant role in reproduction through *in vitro* protocols (Sul and Korban 1994), its effect must be evaluated. The Himalayan cedar [*Cedrus deodara* (Roxb.ex Lamb) G. Don.], which belongs to the Pinaceae family, is an elegant, evergreen, ornamental tree growing widely on the gradient of the Western Himalayas (Champion et al. 1965). Deodar cedar is commonly propagated by seeds. However, seed production is inconsistent and commercial seed bearing of deodar cedar starts only from 30-45 years of age, and good seed crops taking place every 3 years (Tewari 1994). There are few reports on *in vitro* propagation from mature conifers in the last 20 years (Gupta and Durzan 1987, Dumas and Monteuuis 1995, Parasharami et al. 2003, Andersone and Ievinsh 2005, Malabadi and van Staden 2005, Cortizo et al. 2009, De Diego et al. 2010). A few reports on *in vitro* propagation of cedars (*Cedrus libani* A. Rich. and *C. atlantica* Manetti) are available (Piola and Rohr 1996, Piola et al. 1998, 1999, Renau-Morata et al. 2005). There are only two reports on the *in vitro* culture of deodar cedar with the sole use of seed (Bhatnagar et al. 1983, Tamta and Palni 2004). To our best knowledge, no reports document the *in vitro* vegetative regeneration of this species from mature tissues. Our goal was to build methods for the *in vitro* propagation of *C*. *doedara* from adult trees, and to show the effect of genotype on its micropropagation.

**Materials and methods**

Experiment 1

*Plant material*

Actively growing shoots (4–6 cm long) were collected from mature 10-12 year-old *C. deodara* trees in a seed orchard near the Agricultural College of Shiraz University, Iran. This was done from September 2010 to November 2011. The explants were wrapped in wet paper and then placed in plastic bags and kept at 4◦C until 1 day before use.

*Surface sterilization of explants*

In this experiment, two kinds of explants with the same length were used. The first type of explants retained their needles (leaves) to full length (Fig.1a). In the second type of explants, leaves were trimmed to a quarter of their initial length (Fig. 1a). Both types of explants were soaked in tap-water for 2 h, submerged in an aqueous solution of 2% benomyl for 30 min and then treated with 70% ethanol for 2 minutes followed by 15% Clorox (containing 5.25% sodium hypochlorite) with 0.2% Tween-20 for 15 minutes for surface sterilization and then rinsed three times with sterile distilled water. Both kinds of explants were cut into 1-2 cm pieces under sterile conditions and subsequently cultured on different nutrient media.

*Culture establishment*

The explants were cultured on three different culture media: LP (Quorin and Lepoivre 1977), WPM medium (Lloyd and McCown 1980) and MS (Murashige and Skoog’s medium 1962), namely the induction medium (IM1), was supplemented individually with 0.62 to 5.00 µM benzyladenine (BA) at 5 concentrations. All media were supplemented with 3 gl-1 sucrose and 8 gl-1 agar. The pH of all media was adjusted to 5.8 by 0.1 normal HCl before autoclaving for 15 minutes at 121 °C and 147 kPa pressure. 50 ml of the culture medium was poured into separate glass jars. Cultures were kept for up to 6 weeks inside a growth chamber at 25 ± 2 °C under cool white fluorescent light (30 μm m–2 s–1), for 16 h each day.

*Elongation of induced shoots*

Axillary shoots formed on explants were isolated and transferred to elongation media (EM1). The composition of EM1 was half as strong as that of IM1 but lacked cytokinins. It was supplemented with 2 gl-1 activated charcoal (AC), 45 gl-1 sucrose and 8 gl-1 agar. The control was not supplemented with any activated charcoal. The explants were maintained in the medium culture, and then subcultured into glass jars (150 ml) containing 40 ml of the fresh medium EM1. The act of subculturing into glass jars was repeated every 4 weeks.

*Rooting*

In the first trial of the experiment, elongated microshoots (≥ 2 cm) were transferred to the medium. The composition of the medium was a quarter and a half-strength basal WPM, supplemented with 0, 6 and 12 μM indol-3-butyric acid (IBA) alone or in combination with 0, 5 and 10 μM α-naphthaleneacetic acid (NAA). The microshoots were kept in this medium at 20 °C in the dark for 7 days, before being moved to the light. They were then cultured into media that were free of growth regulators but were supplemented with 15 and 30 gl-1 sucrose, 2 gl-1 activated charcoal (AC) and 8 gl-1 agar. In the second trial, the elongated shoots were dipped in IBA (2.5 or 5 mM) in the presence or absence of 1 mM NAA for 1 s and 5 min, respectively. They were then cultured on media similar to that defined previously.

Experiment 2

*Plant material*

To examine the effect of genotype, 100 to 130 actively growing shoots (4–6 cm long) were collected from four mature 10-12 year-old *C. deodara* trees (genotypes CD1 to CD4) in a seed orchard near the Agricultural College of Shiraz University, Iran and kept in the same conditions as in ‘‘Experiment 1’’. Defoliated shoots (1–2 cm in length) were used as initial explants.

*Aseptic culture, Culture establishment*

Surface sterilization was carried out in the same manner as in experiment 1. The explants were intended for shoot induction and proliferation, cultured on WPM medium supplemented with growth regulators [BA, NAA and thidiazuron (TDZ)]. The medium supplemented with BA (2.5 to 20.0 µM) and TDZ (0.4 to 1.6 µM) alone or in combination with NAA (1 to 3 µM) was called induction medium 2 (IM2). The effect of TDZ and BA on shoot proliferation of four genotypes (CD1 to CD4) was investigated. To study the effect of harvest time on culture establishment, the explants were collected in spring, summer, autumn and winter and cultured on Medium WPM supplemented with BA (2.5 to 20.0 µM) and TDZ (0.4 to 1.6 µM) alone or in combination with NAA (1 to 3 µM). The conditions, under which the cultures were incubated, were similar to those of experiment 1.

*Elongation of* *induced shoots*

Induced shoots were excised and cultured into glass jars (150 ml) containing 40 ml of growth regulator-free medium supplemented with 3 gl-1 activated charcoal and 15 gl-1 sucrose. The control was not supplemented with any activated charcoal. This medium, which was designated as elongation medium 2 (EM2), was solidified with 8 gl-1 agar. Microshoots were transferred once a month to fresh EM2 medium and maintained in the same culture conditions as in the beginning phase.

*Shoot multiplication*

After 1 months, the shoots grown on Medium EM2 were cut to the same length and then transferred to shoot multiplication medium containing TDZ (0.1 to 0.8 µM) and 2iP (0.1 to 0.5 µM) in combination with 2.5 µM BA. Total culture period was 6 weeks. These growth regulators were assessed for effect on multiplication of shoots.

*Rooting*

In the first trial of experiments, the elongated shoots from EM2 that were intended for rooting, cultured in medium containing a half-strength of the initial WPM, 3 gl-1 sucrose and 8 gl-1 agar. They were treated with 0, 0.5, 1, 1.5, 3, 6 and 12 μM IBA and indoleacetic acid (IAA) and also their combination, along with 0, 1, 2, 3, 4 and 5 mg l-1 β-cyclodextrin, with or without 3 gl-1 active charcoal. In the second trial, [putrescine and spermidine were used by 0.1, 0.2, 0.4, 0.8 and 0.16 μM concentrations along with 2.5, 5 and 10](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CDYQFjAB&url=http%3A%2F%2Fwww.merriam-webster.com%2Fdictionary%2Fputrescine&ei=OD3kTvK1IOWjsQKt9JnsBQ&usg=AFQjCNGszOoK3t0SkcWWzeuNQC0t-0DRLg&sig2=LuqtPpWT4N9zcT4Tx5IYtw) μM IBA. The culture medium was autoclaved for 15 minutes at 121°C under 147 kPa pressure. After cooling down to 50 °C, putrescine and spermidine were filtered out and subsequently added to the medium. In the third trial, the explants were cultured on liquid medium containing 2.5, 5 and 10 μM IBA in combination with 0.25 and 0.5 mgl-1 paclobutrazol.

Statistical analysis

All experiments were conducted as factorial using a completely randomized design with four replications for each treatment. Each replication had four explants. All experiments were repeated twice. Shoot proliferation rate, shoot length, and visual quality were recorded at the end of fourth week. SPSS statistical software was used for analyzing data, and Least Significant Differences (LSD) test (*P* ≤ 0.05) was used for comparing means. Three-way ANOVA was applied to examine the effect of genotype and growth regulators.

**Results**

Experiment 1

The effects of different culture media (WPM, MS and LP) on two types of explants were investigated to determine the amounts of proliferation and shoot length. In all three culture media, the explants without leaves (needles) showed the best results regarding shoot proliferation and had a significant difference, when compared to the explants with leaves (almost threefold or greater in most cases). Overall, leafy explants did not show a good response. Leafless explants cultured on media containing 2.5 μM BA had the most success in shoot proliferation and resulted in the longest shoots. The lowest frequency in shoot proliferation belonged to the leafy explants in MS medium (0.06) (Table 1). The comparison of results obtained from the different culture media (WPM, LP and MS) generally revealed that WPM medium delivered the highest frequency of proliferated shoots, and these shoots possessed the longest lengths (Table 1, 2). Axillary shoots of the proliferated material were dissected and cultured in EM1 media. No shoot proliferation was observed in EM1. The same trend was observed on shoot elongation in different mediums, explants and BA concentrations, though there was less different between leafy and leafless explants, and BA had more significant effects at different concentrations (Table 2). After 4 subcultures, suitable microshoots were transferred to rooting media for the commencement of rooting. No adventitious roots were generated in this experiment.

Experiment 2

After the identification of the best medium culture, leafless subcultures were exposed to treatments of different growth regulators. Among the utilized cytokinins, TDZ had the greatest effect on the induction of proliferated shoots both in terms of number and length in comparison to BA. In general, shoots that developed on TDZ-containing media were more vigorous than those developed on media containing different concentrations of BA. The highest mean number of axillary shoots per explant (4.31) was obtained when 0.8 μM TDZ was used in combination with 2 μM NAA and the highest mean length of axillary shoots (11.20 mm) was obtained when 0.8 μM TDZ was applied alone (Tables 3, 4, Fig. 1b). Moreover, most of the explants that were cultured on media supplemented with NAA were more vigorous, when compared to those cultured on media without this supplementation. However, increasing the concentration of NAA to 3 μM, reduced the number and length of proliferated shoots (Tables 3, 4). Furthermore, the combination of NAA with different concentrations of BA proved to be effective for the proliferation phase. When explants transferred to EM2, shoots induced in lower concentrations of BA (2.5 - 5 μM) showed better elongation rates compared to those induced in higher BA concentrations (10 - 20 μM) which were stunted (the result has not been shown). The effect of cytokinins on shoot multiplication was tested by using different concentrations of TDZ and 2ip with 2.5 μM BA in the shoot multiplication medium (Fig. 2). The highest mean number of axillary shoots per explant (4.06) and mean length of axillary shoots (10.36 mm) were obtained when 0.8 μM TDZ was applied (Table 5). No axillary bud proliferation was induced on the microshoots cultured in EM2. It should be noted that the shoots which were transferred to EM2 developed faster than in EM1 (Fig. 1c). After 3 subcultures, they reached a suitable length to be cultured on rooting media. Microshoots had more dense foliage, were longer and more vigorous. Unfortunately, no rooting was observed during this experiment as well.

After studying the results of different cytokinin treatments on proliferation of shoots, it was found that there was the significant differences in the interaction between genotype and TDZ concentrations (p>0.5), whereas the differences in BA × genotype interaction were not significant (Table 6). Also, significant differences on the number of proliferated shoots were detected between TDZ concentrations. Results for shoots proliferation showed significant differences between BA concentrations. The same result was obtained for genotype (Table 6). Among all genotypes, CD1 and CD2 showed best response to growth regulators (Table 7). The best result for shoot proliferation in TDZ obtained for CD1 genotype at concentration of 0.8 μM (Fig. 3). The same result was obtained for CD1 genotype at BA 2.5 μM (Fig. 4). The proliferative capacity of explants of each season was examined. The best and least results for proliferation and length of shoots were obtained in summer and winter, respectively (data not shown).

**Discussion**

Propagation of conifers is hardly accomplished by the utilization of mature explants. In general, most studies on induction of embryogenesis and/or organogenesis in conifers includes culture of seed or zygotic tissues (Tang et al. 2006, Humánez et al. 2011). These origins of explants are very heterozygote, and hence regenerates are possible to present variability. In addition, direct regenerations have attributes such as great stability, simplicity and taking less time to regenerate a wide number of plants with low frequency of somaclonal variation and chromosomal abnormalities (Piola et al. 1999).To our best knowledge, this is the first report of successful induction of axillary shoot formation on explants taken from adult *C. deodara.* There have been few reports on the effect of different kinds of basal media on axiliary bud induction in micropropagation of coniferous mature tissues (Andersone and Ievinsh 2002, De Diego et al. 2010, Renau-Morata et al. 2005). In the present investigation, Shoot proliferation rates were generally greater on basal WPM than on basal LP and MS media. Shoot buds cultured on MS medium presented the lowest organogenic response, and this was perhaps as a result of the comparatively high nitrate concentration as compared to the LP medium or WPM. Tuskan et al. (1990) displayed that the extra nitrate could have a negative effect on the organogenic response during micropropagation. Therefore, low nitrogen content of the medium is a major factor for promoting organogenesis in conifer species (Tang et al. 2001, Schestibratov et al. 2003). Explant type was an important factor affecting axillary bud proliferation in *in vitro* culture. Piola and Rohr (1996) reported that axillary and apical buds of *in-vitro*-propagated cuttings of *C. libani* are unable to burst at 24°C, but this inhibition was overcome at 30°C. Subsequently, Piola et al. (1998) demonstrated that abscisic acid (ABA) accumulation in needles at 24°C is greater than at 30°C. In addition, when needles were removed, bud growth release was achieved at 24°C. The accumulation of ABA in needles seems to be the major cause of bud dormancy in micropropagation of *C. libani* (Piola et al. 1998). Results obtained from our research also show that the dissection of leaves (needles) from explants leads to a tremendous effect by increasing bud proliferation levels in *C. deodara*. Although the cytokinin type and concentration suitable for microprogation of woody plants may depend on plant species, this study showed that adding TDZ at 0.8 µM with 2 µM NAA was the most suitable treatment for shoot proliferation of *C. deodara*.

Genotype is important factor to determine appropriate medium for micropropagation. In *in vitro* bicentennial cedar micropropagation, it was found that the accomplishment of the protocol depends on genotype (Renau-Morata et al*.* 2005). Among two cytokinin treatments examined, TDZ induced the highest number of proliferated shoots for all genotypes. After the effect of three TDZ concentrations on shoot proliferation of four genotypes was investigated, the significant difference for their interaction was found. Genotype CD1 and CD2 showed the best response over all TDZ concentrations tested and it showed genotypic differences for shoot proliferation of *C. deodara*. However, in our investigation, BA showed no genotypic significant differences for shoot proliferation. In general, TDZ increases shoot formation of several woody plant species more efficiently than purine adenine derivatives. It facilitates efficient micropropagation of many recalcitrant woody species. Low concentrations of TDZ (<1µM) can induce great axillary shoot proliferation in many other woody plants. TDZ has an apical dominance release potential that accelerates axillary bud formation on explants (Huetteman and Preece 1993). In addition, it can highly stimulate and balance endogenous auxin/cytokinin levels (Visser et al. 1992). TDZ has gotten more attention in recent years due to its ability to assist *in vitro* regeneration of conifers (Mathur and Nadgauda 1999, Sul and Korban 2004, Renau-Morata et al. 2005, Tang and Newton 2005a, Cortizo et al. 2009, De Diego et al. 2009, Humánez et al. 2011). TDZ can decrease the enzymes activity related to oxidative stress during formation of adventitious shoot (Tang and Newton 2005a). Mathur and Nadgauda (1999) found that a combination of TDZ (0.05 mM) and BA (2.5 mM) yielded good results of adventitious bud induction from mature zygotic embryos of *Pinus wallichiana* A.B. Jacks. It was also reported that TDZ-induced regeneration from mature zygotic embryos in *Pinus strobus* L. via organogenesis is more efficient than BA and 2iP (Tang et al*.* 2006). Recent reports on mature stone pine also displayed the superiority of TDZ over other cytokinins in advancing axillary shoot proliferation (Cortizo et al. 2009). It was showed that high concentrations of cytokinin in the medium, particularly BA led to the low regeneration response, which may be attributed to toxic effects of high concentrations of cytokinins (Sarmast et al. 2012). In this report, the presence of NAA with either TDZ or BA improved the incidence of shoot organogenesis in adult tissues of *C. deodara*. This has been also observed in other conifer species (Sul and Korban 2004, Zhu et al. 2010). This study showed that the incorporation of AC into a culture medium could have been the factor that enhanced shoot elongation of *C. deodara*, as has been reported in other pine species (Sul and Korban 2004, Humánez et al. 2011, Zhu et al. 2010). The AC was advantageous in the micropropagation procedure of mature conifer trees as shown by Abdullah et al. 1987, Dumas and Monteuuis 1995, De Diego et al. 2009 and Parasharami et al. 2003. The exact function of charcoal is unknown, but it has been shown to act by absorbing materials such as phenolic metabolites and residual plant growth regulators (Pan and Van Staden 1998). Successful micropropagation of mature conifers is frequently limited by low rooting percentages (Cortizo et al. 2009, Dumas and Monteuuis 1995, Renau-Morata et al. 2005) and *C*. *deodara* was no exception. It seems that the cause of their reluctance to form adventitious roots is the loss of competence in mature explants, which has been ascribed to progressive specialization of the tissue, which decreases the flexibility and ability of the cells to dedifferentiate (Abdullah et al. 1987). A variety of secondary compounds such as paclobutrazol, β-cyclodextrin and polyamines (Bettaieb et al. 2008, George et al. 2008, Tang and Newton 2005b) can enhance rooting capacity in recalcitrant species. In our studies, all the variables failed to induce rooting in shoots of *C*. *deodara* axillary buds*.* In spite of the fact that our studies were not successful in root induction, further attempts can be made in this field. Using other treatments such as different temperature regime, media or growth regulators, and *Agrobacterium rhizogenes*- mediated transformation can be recommended for further investigations.

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**The aim of study**

Conifer forestry contributes to the economic and social well being of many countries, and the use of plantation forests has a significant positive impact on natural forests and world climate balance. The demand for forest products such as pulp, paper and timber is ever increasing. There is an urgent need for large-scale afforestation programmes. The Himalayan cedar [Cedrus deodara (Roxb.ex Lamb) G. Don.], which belongs to the Pinaceae family, is a graceful evergreen ornamental tree growing extensively on the slopes of the Western Himalayas. Deodar forests are found in the principal valleys of the Himalayas and exist on lands from Afghanistan (Hindu Kush) and Pakistan (Karakoram) to India (Kashmir). C. deodara is the main source of pulp, timber, fuel and other valuable products such as wood oil. So, I tried to micropropagate Cedrus deodara for purpose of plantation forests.

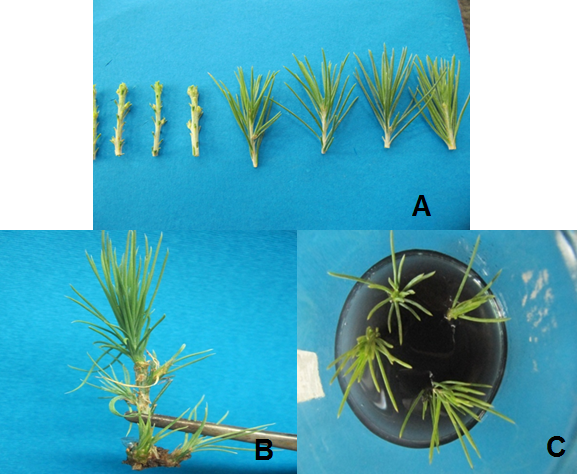


Fig. 1. *In vitro* multiplication of *Cedrus deodara* (Roxb.ex Lamb) G. Don**.** through axillary bud proliferation from shoot apices of mature trees:(A) Different types of explants: explants retained their needles (leaves), defoliated explants; (B) Axillary shoot proliferation from explanted shoots cultured on WPM medium supplemented with 0.8 μM TDZ and 2 μM NAA (after 4 weeks); (C) Elongation of axillary bud on EM2 medium after 50 days.



Fig. 2. Multiple shoot formation on subculture of Cedrus deodara on WPM with 0.8 μM TDZ in combination with 2.5 μM BA.

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Fig. 3. Effect of TDZ concentrations on shoot proliferation of four genotypes of *Cedrus deodara*.

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Fig. 4. Effect of BA concentrations on shoot proliferation of four genotypes of *Cedrus deodara*.

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| Table 1. Comparison of the number of proliferated shoots and interaction of leafy and leafless explants of *Cedrus deodara* in different mediums (LP, MS and WPM) containing different concentrations of BA. | | | | | | | |
| BA concentration (µM) | Medium | | | | | | Main effect of BA on proliferation |
| WPM | | LP | | MS | |
| Leafy | Leafless | Leafy | Leafless | Leafy | Leafless |
| 0.000 | 0.31± 0.06 lm | 2.75± 0.00 bc | 0.19± 0.06 mn | 2.38± 0.07 ef | 0.06± 0.06 n | 1.94± 0.06 j† | 1.27 A |
| 0.625 | 0.44± 0.06 l | 2.87± 0.07 ab | 0.19± 0.06 mn | 2.38± 0.07 ef | 0.06± 0.06 n | 2.12± 0.07 hi | 1.34 A |
| 1.250 | 0.44± 0.06 l | 2.81± 0.06 ab | 0.06± 0.06 n | 2.50± 0.00 de | 0.12± 0.07 mn | 2.19± 0.06 gh | 1.35 A |
| 2.500 | 0.62± 0.07 k | 2.94± 0.06 a | 0.31± 0.06 lm | 2.75± 0.00 bc | 0.19± 0.06 mn | 2.25± 0.00 fg | 1.51 A |
| 5.000 | 0.25± 0.00 mn | 2.62± 0.07 cd | 0.12± 0.07 mn | 2.31± 0.06 fg | 0.06± 0.06 n | 2.00± 0.00 ij | 1.23 A |
| Mean values (effect of medium and Explant type) | 0.41 D | 2.80 A | 0.17 E | 2.46 B | 0.10 E | 2.10 C |  |
| Mean values  (effect of medium) | 1.61 A | | 1.32 AB | | 1.10 B | |  |
| † Means with same letters (small letters for interactions and capital letters for main effects) has not significant difference according to the LSD test at 5%. | | | | | | | |

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| Table 2. Comparison of the length of proliferated shoots (mm) and interaction of leafy and leafless explants of *Cedrus deodara*  in different mediums (LP, MS and WPM) containing different concentrations of BA. | | | | | | | |
| BA concentration (µM) | Medium | | | | | | Main effect of BA on shoot length |
| WPM | | LP | | MS | |
| Leafy | Leafless | Leafy | Leafless | Leafy | Leafless |
| 0.000 | 3.00± 0.00 f-k | 4.50± 0.29 b-i | 1.25± 0.43 k | 5.75± 0.48 a-e | 1.50± 1.5 jk | 6.00± 0.35 a-d† | 3.66 AB |
| 0.625 | 2.25± 0.25 h-k | 5.87± 0.43 a-e | 2.75± 0.92 g-k | 4.25± 0.14 c-i | 1.25± 1.25 k | 4.75± 0.25 a-h | 3.52 B |
| 1.250 | 3.00± 0.35 f-k | 4.75± 0.14 a-h | 2.25± 2.25 h-k | 5.50± 0.2 a-f | 1.37± 0.8 jk | 4.00± 0.2 d-j | 3.47 B |
| 2.500 | 3.75± 0.14 d-k | 7.25± 0.44 a | 3.25± 0.14 e-k | 6.75± 0.25 abc | 1.90± 0.67 ijk | 6.25± 0.14 abc | 4.85 A |
| 5.000 | 3.25± 0.14 e-k | 7.00± 0.41 ab | 3.75± 2.17 d-k | 5.75± 0.25 a-e | 1.25± 1.25 k | 5.15± 0.15 a-g | 4.35 AB |
| Mean values (effect of medium and Explant type) | 3.05 B | 5.87 A | 2.65 B | 5.60 A | 1.45 C | 5.23 A |  |
| Mean values  (effect of medium) | 4.46 A | | 4.12 AB | | 3.34 B | |  |
| † Means with same letters (small letters for interactions and capital letters for main effects) has not significant difference according to the LSD test at 5%. | | | | | | | |

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| Table 3. Effects of the interaction of TDZ, BA and NAA growth regulators on the number of proliferated shoots in *Cedrus deodara*. | | | | | | |
|  |  | NAA | | | |  |
|  | Conc. (µM) | 0 | 1 | 2 | 3 | Mean values |
| Control | 0.0 | 2.41± 0.02 jkl | 2.16± 0.02 n-q | 2.31± 0.05 k-n | 1.97+0.01 q† | 2.21 F |
|  |  |  |  |  |  |  |
| TDZ | 0.4 | 3.75± 0.00 b | 3.31± 0.03 d | 3.44± 0.03 cd | 3.06± 0.03 e | 3.39 B |
| 0.8 | 3.39± 0.03 d | 3.50± 0.00 cd | 4.31± 0.03 a | 3.63± 0.03 bc | 3.70 A |
| 1.6 | 2.25± 0.00 l-o | 2.50± 0.00 h-k | 2.63± 0.03 ghi | 2.00± 0.00 pq | 2.34 EF |
|  |  |  |  |  |  |  |
| BA | 2.5 | 2.69± 0.03 fgh | 3.06± 0.03 e | 3.40± 0.03 d | 2.50± 0.00 h-k | 2.91 C |
| 5 | 2.38± 0.03 j-m | 2.44± 0.03 i-l | 2.44± 0.03 i-l | 2.56± 0.03 hij | 2.45 DE |
| 10 | 2.44± 0.03 i-l | 2.88± 0.03 ef | 2.81± 0.03 fg | 2.31± 0.03 k-n | 2.61 D |
| 20 | 2.25± 0.00 l-o | 2.38± 0.03 j-m | 2.19± 0.03 m-p | 2.06± 0.03 opq | 2.22 F |
|  |  |  |  |  |  |  |
| Mean values |  | 2.66 AB | 2.71 AB | 2.87 A | 2.45 B | 2.67 |
| † Means with same letters (small letters for interactions and capital letters for main effects) has not significant difference according to the LSD test at 5%. | | | | | | |

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| Table 4. Effects of the interaction of TDZ, BA and NAA growth regulators on the length of proliferated shoots (mm) in *Cedrus deodara*. | | | | | | |
|  |  | NAA | | | |  |
|  | Conc. (µM) | 0 | 1 | 2 | 3 | Mean values |
| Control | 0.0 | 5.80± 0.06 gh | 5.55± 0.06 g-j | 4.88± 0.04 jk | 4.50± 0.09 klm† | 5.18 D |
|  |  |  |  |  |  |  |
| TDZ | 0.4 | 8.75± 0.05 c | 6.92± 0.01 f | 8.12± 0.09 cd | 7.12± 0.12 ef | 7.73 B |
| 0.8 | 11.20± 0.14 a | 7.75± 0.07 de | 9.56± 0.05 b | 6.80± 0.22 f | 8.83 A |
| 1.6 | 4.96± 0.07 ijk | 5.24± 0.08 g-k | 5.20± 0.08 g-k | 4.67± 0.02 kl | 5.02 D |
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| BA | 2.5 | 6.82± 0.16 f | 7.32± 0.10 ef | 5.57± 0.10 g-j | 5.08± 0.08 h-k | 6.20 C |
| 5 | 7.15± 0.19 ef | 7.43± 0.10 ef | 5.93± 0.11 g | 5.18± 0.15 g-k | 6.42 C |
| 10 | 5.65± 0.16 ghi | 5.93± 0.08 g | 4.07± 0.09 lmn | 3.12± 0.08 o | 4.69 D |
| 20 | 3.44± 0.12 no | 3.84± 0.17 mn | 3.90± 0.13 mn | 2.16± 0.07 p | 3.34 E |
|  |  |  |  |  |  |  |
| Mean values |  | 6.62 A | 6.19 A | 5.79 A | 4.79 B | 5.84 |
| † Means with same letters (small letters for interactions and capital letters for main effects) has not significant difference according to the LSD test at 5%. | | | | | | |

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| Table 5. The effect of cytokinins on shoot multiplication Comparison of mean proliferation and shoot elongation (mm) of *Cedrus deodara*, under different levels of TDZ and 2ip in combination with 2.5 µM BA. | | | |
| Treatment | Conc. (µM) | Number of proliferated shoots | Length |
| Control | 0.0 | 2.44± 0.07 f | 6.69± 0.12 g† |
|  |  |  |  |
| TDZ | 0.1 | 3.38± 0.16 cd | 7.26± 0.16 f |
| 0.2 | 3.44± 0.21 cd | 7.80± 0.27 de |
| 0.4 | 3.88± 0.16 ab | 9.93± 0.24 a |
| 0.8 | 4.13± 0.13 a | 10.38± 0.11 a |
|  |  |  |  |
| 2ip | 0.1 | 2.50± 0.20 f | 6.58± 0.11 g |
| 0.2 | 2.69± 0.12 ef | 7.38± 0.18 ef |
| 0.3 | 3.00± 0.10 de | 7.93± 0.21 cd |
| 0.4 | 3.25± 0.18 cd | 8.38± 0.15 bc |
| 0.5 | 3.56± 0.12 bc | 8.88± 0.14 b |
| † Means with same letters in each column has not significant difference according to the LSD test at 5% | | | |

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| Table 6. Effects of genotype, TDZ and BA on shoot proliferation in *Cedrus deodara*. | | |
| Source of variation | df | Sig. |
| Genotype | 3 | 0.0000\*\* |
| TDZ | 2 | 0.0000\*\* |
| BA | 3 | 0.0003\*\* |
| TDZ × genotype | 6 | 0.0010\*\* |
| BA × genotype | 9 | 0.7143 |
| \*\* Statistically significant at p = 0.05 | | |

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| Table 7. Effects of genotype on the number of proliferated shoots in *Cedrus deodara*. | | |
| Genotype | TDZ | BA |
| CD1 | 4.35± 0.16 a | 3.40± 0.11 a† |
| CD2 | 4.17± 0.16 a | 3.30± 0.11 a |
| CD3 | 1.92± 0.16 b | 1.73± 0.11 b |
| CD4 | 2.10± 0.16 b | 1.75± 0.11 b |
| † Means with same letters has not significant difference according to the LSD test at 5%. | | |