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Effects of Nodal Position and Growth Regulators on *In Vitro* Growth of Dog Rose (*Rosa canina*)

M. Shirdel, A. Motallebi-Azar*, M. Matloobi, and F. Zaare-Nahandi
Department of Horticultural Sciences, Faculty of Agriculture, Tabriz University, Tabriz, Iran.

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*Corresponding author's email: motallebiazar@gmail.com

Abstract

Dog rose (*Rosa canina*) has been using as a rootstock for ornamental roses also it is one of the medicinal plants. It can be propagated under in vitro conditions. After removing chilling requirement of buds, axillary wood buds of dog rose were cut in three nodal positions (lower, middle and terminal) on the stem and then explants after decontamination, cultured on Murashige and Skoog medium (MS medium) supplemented with 1 mg l^{-1} GA_3 and 1 mg l^{-1} BAP. For shoot proliferation, MS medium supplemented with 1 mg l^{-1} GA_3 and different concentration of PGRs. The results showed that maximum bud break percentage and highest shoot length were observed in lower nodal position. Minimum bud break percentage and shoot length were observed in middle nodal position. In the media with different concentrations of PGRs the highest shoot length was observed in combinations of BAP 1 mg l^{-1} and Ads 2 mg l^{-1}. The large number of node and maximum axillary shoot percentage were observed in BAP 6 mg $^{-1}$. The growth of dog rose was affected by different explants nodal position and growth regulators on *in vitro*. Assessment of BAP and Ads on axillary shoot percentage and node number was more effective than kinetin + TDZ and BAP + TDZ combination. In media with low concentration of BAP, TDZ and NAA and so kinetin, TDZ and NAA did not produce any axillary shoots or elongated shoots.

Keywords: Dog rose, Growth regulators, *In vitro*, Nodal position, Proliferation.

INTRODUCTION

Dog rose (*Rosa canina*) has been used as a rootstock for ornamental roses (Khosh-Khui and Sink, 1982) while considered as a medicinal plant as well. *Rosa canina* L. (dog rose, rose hip, briar rose) is one of wild roses appreciated for their vitamin c enriched fruits being beneficial to human health (Kazaz, 2009). It can be propagated under *in vitro* conditions. In recent years, several reports have been published on *in vitro* establishment and proliferation of roses, and have shown that tissue culture provides an alternative method for rapid multiplication (Horn *et al*., 1998; Skirvin *et al*., 1990). Micropropagation is a technique used for producing plantlets. It is the foundation of many biotechnological studies, because in many plant biotechnological researches, there is a need for successful establishment of the plants.

Single node culture is an *in vitro* technique which can be used for propagating of some species from axillary buds (Shirdel *et al*., 2011). Apical and axillary buds are used for proliferation of different roses. Effect of plastochrones (explants position along the stem of the mother plant) on optimization of growth on various rose cultivars has been conflicting (Hasegawa, 1979; Bressan *et al*., 1982; Khosh-Khui and Sink, 1982). The role of plant growth regulators (PGRs) is going to be highly important in rose plants propagation and also in controlling of physiological processes (Georg, 1993). Many researchers have reported shoot proliferation of rose on MS medium with varying concentrations of PGRs such as BAP and NAA. Rout *et al*. (1990) indicated that presence of cytokinin in culture medium helps proliferation of shoots in hybrid roses.

The growth and proliferation of axillary shoots in culture medium is usually promoted by incorporating growth regulators in to the growth medium (George, 2007). One of the PGRs that play a major role in growth is Thidiazeron (TDZ), which has been used successfully *in vitro* to induce axillary shoot proliferation (Chin-Yi, 1993). Adenine sulphate (Ads) has been widely used in tissue culture media, but because it mainly gives rise to effects which are similar to those produced by cytokinins (Elliott, 1970). This work focused on the effects of explants nodal position and PGRs on *in vitro* culture of dog rose.

MATERIALS AND METHODS

The experiment was done at the agricultural research station of Tabriz University located at the north western of Iran. After removing chilling requirement of buds in February 2010, axillary wood buds of dog rose (grown at the botanical garden of Tabriz University) were cut at three nodal positions (lower, middle and terminal) on the stem and then were placed under running tap water (for 1 h) and washed with Tween 80 (0.1%) (15 min), and then were decontaminated with 70% ethanol (for 5 min), 0.1% (w/v) solution of mercury (II) chloride (for 2 min) and sodium hypochlorite (for 20 min). Then all explants were washed three times with sterile distilled water. Cefotaxime 250 mg l^{-1} and tetracycline100 mg l^{-1} were used through direct addition on establishment medium for bacterial decontaminations. Explants transferred to establishment medium after being sterilized. MS (Murashige and Skoog's, 1962) basal medium was used for explants establishment and proliferation. Establishment medium was contained 30 g l^{-1} sucrose, 0.8% Agar, 1 mg l^{-1} GA_3 and 1mg l^{-1} BAP and proliferation medium was contained 30 g l^{-1} sucrose, 0.8% Agar, 1 mg l^{-1} GA_3 and different PGRs according to Table 1. The pH of media was adjusted to 5.8. The prepared culture media then was autoclaved at 121°C under 105 kPa pressure for 20 min. Explants were incubated in the culture room at temperature of 25 ±2 °C with 16 h light and 8 h dark cycle. After four weeks, shoot length, node number, axillary shoot percentage, chlorosis leaf number, necrotic leaf number and bottom callus induction percentage were recorded.

All experiments were conducted as a completely randomized design with 8 replications. Data were analyzed with SPSS software Ver. 16 and mean comparison was performed using Duncan's New Multiple Range Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

The effect of nodal position

Analysis of variance showed that the percentage of fungi contamination among the three nodal positions (lower, middle and terminal) was not significant ($p \leq 0.05$) (Table 2). Bacterial contamination was not observed in three nodal positions because of application of two anti-bacterials (cefotaxime and tetracycline) for surface sterilization. In Rosa damascena when cefteriaxon and ofloxacin were used, bacterial contamination was controlled (Nikbakht *et al*., 2005). Bud break percentage was significantly different among three nodal positions ($p \leq 0.05$) (Table 2). Variation of bud break percentage was observed from 17.39 to 30 (%). Maximum bud break percentage in lower nodal position and minimum bud break percentage in middle nodal position were observed.

Three nodal positions significantly influenced shoot length ($p \leq 0.05$) (Table 2). The shoot length varied from 5.75 to 3.37 cm. The longest and shortest shoots were produced by the lower and middle nodal positions, respectively. Growth rate of wood buds after removal of chilling requirement was influenced by the carbohydrate reserves. It was reported that carbohydrates play an important role in providing normal growth and development of shoots emerging from lower nodes and after receiving chilling treatment , it might be the gibberellic acid that exerts the growth initiation on the shoots growing from terminal nodes (Taiz & Zeiger, 2002). However, there are many studies that report the middle part of the stem develops highly proliferating shoots (Bressan *et al*., 1982; Horn *et al*., 1988). Hasegawa (1979) observed no shoot establishment differences between terminal and middle buds. Khosh-Khui and Sink (1982) have reported a higher shoot proliferation of explants taken from shoot-tips than those received from lateral buds.

The effects of growth regulators

Shoot length was significantly influenced by the different treatments ($p \leq 0.01$) (Table 3). The highest shoot length was produced by T10 (Fig. 1a), while the lowest one was occurred in T3 and T4 (Fig. 2). Treatments containing BAP and Ads showed the longer shoots when compared with the other treatments. Additionally, it was indicated that shoot length has been significantly influenced by BAP and Ads concentrations. BAP was usually extremely effective in removing the apical dominance of shoots (Kumar *et al*., 2001). In other treatments there was a negative relationship between BAP concentration and shoot length . Media treated with kinetin, TDZ and higher concentrations of BAP, produced small shoots typically failed to elongate. Treatments influenced significantly influenced node number ($p \leq 0.01$) (Table 3). The number of nodes appeared in T6 was more than that produced in T1 (Fig. 3). No significant difference was observed in node numbers of T1, T2, T3 and T4. These treatments, different concentrations of cytokinins prevented the formation of nodes and removed the apical dominance of shoots influenced. Node numbers in T10 were more than T9 because Ads in high concentration and BAP in low concentration were used in T10 compared to T9. Axillary shoot percentage was significantly influenced by the different treatments ($p \leq 0.05$) (Table3). Maximum axillary shoot percentage was observed in T6.However in T1 and T2 no axillary shoot appeared (Fig. 1b, 4). Increasing the BAP concentration elevated axillary shoot percentage, because BAP enhanced ethylene evolution in culture medium, therefore axillary shoot percentage was increased but in T7 the addition of high concentration of BAP caused axillary shoot formation to be inconsistent. In T1 and T2 due to low concentration of BAP and kinetin the formation of axillary shoot not occurred. In T8, which BAP and Ads concentrations were high, axillary shoot percentage was more than T9, T10 and T11. Assessment of BAP and Ads on axillary shoot percentage was more effective than Kinetin + TDZ and BAP + TDZ combination.

A mixture of more than one cytokinin has also been found to give more effective shoot multiplication in some other species (e.g. *Corylus avellana*, Anderson, 1984; *Cucumis melo*, Kathal *et al*., 1988). The number of leaves with chlorosis was influenced significantly by the different treatments ($p \leq 0.01$) (Table 3). Chlorosis leaf number ranged from 0.15 to 2.5. The highest number

of leaves with chlorosis symptoms was observed in T7 containing higher rates of BAP (Fig. 1c, 5) and similarly in all treatments with BAP and AdS. Low concentrations of BAP and Ads indicated lower rate of leaf chlorosis. BAP enhanced ethylene evolution in culture medium, therefore high BAP concentration was one of the factors that is the reason for ethylene evolution and severe leaf chlorosis (George, 2007). Thomas and Katterman (1986) suggested that TDZ encourage the synthesis of endogenous purine cytokinins or inhibits their degradation and also optimum concentration of TDZ is proper to reduction of leaf chlorosis. Chlorosis leaf number in T1, T2, T3, T4, T5, T8, T9, T10 and T11 was the same but in other treatments significant difference was observed. Necrotic leaf number was influenced significantly by the different treatments ($p \leq 0.01$) (Table 3). Nnecrotic leaf number ranged from 0 to 1.5. The most necrotic leaf number was observed in T7 with high concentration of BAP (Fig. 1d, 6). High concentration of BAP in the culture medium could lead to increasing of ethylene and senescence of textures and causing leaf necrotic (George, 2007). Results showed that in T2, T4, T10 and T11, with low concentration of BAP, necrotic leaf number was not observed. Different treatments significantly affeected feeble callusing percentage at the cut ends of explants ($p \leq 0.01$) (Table 3). Feeble callusing at the cut ends of explants which greatly affects the young seedling's absorption of water and nutrition, and thus inhibit its growth, was not observed in T1 (without BAP) and it was higher in T7 (with high concentration of BAP). In treatments with combination of BAP and Ads no significant difference was observed in feeble callusing percentage at the cut ends of explants (Fig. 1e, 7).

Normally a kind of cytokinin (usually BAP) is generally required for the formation of callus from explants. Addition of BAP (2.0–3.0 mg l^{-1}) as the only growth regulator in the culture medium resulted in feeble callusing at the cut ends of the explants and the shoot elongation was considerably decreased (Rout *et al*., 1999). In this study, effects of PGRs in feeble callusing percentage at the cut ends of explants indicated that callus formation increased by high concentrations of BAP.

CONCLUSION

Based on the results found in the present study, the growth of dog rose was affected by different explants nodal position and growth regulators on *in vitro*. High concentration of BAP in medium had a stranger influence on axillary shoot percentage rather than other growth regulators. In media with low concentration of BAP, TDZ and NAA and so Kinetin, TDZ and NAA did not produce any axillary shoots or elongated shoots.

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Tables

Table 1. Types of plant growth regulators in different treatments.

Treatment	Plant growth regulators
T1	Kinetin 0.5 mg l^{-1} + TDZ 0.1 mg l^{-1} + NAA 0.1 mg l^{-1}
T2	BAP 0.5 mg l^{-1} + TDZ 0.1 mg l^{-1} + NAA 0.1 mg l^{-1}
T3	Kinetin 1 mg l^{-1}+ TDZ 0.1 mg l^{-1}+ NAA 0.1 mg l^{-1}
T4	BAP 1 mg l^{-1}+ TDZ 0.1 mg l^{-1}+ NAA 0.1 mg l^{-1}
T5	BAP 4 mg l^{-1}
T6	BAP 6 mg l^{-1}
T7	BAP 8 mg l^{-1}
T8	BAP 2 mg l^{-1}+ Ads 2 mg l^{-1}
T9	BAP 2 mg l^{-1}+ Ads 1 mg l^{-1}
T10	BAP 1 mg l^{-1}+ Ads 2 mg l^{-1}
T11	BAP 1 mg l^{-1}+ Ads 1 mg l^{-1}

Table 2. Effect of nodal position on *in vitro* growth of dog rose.

	Fungi contamination (%)	Bud break (%)	Shoot length (cm)
Lower nodal position	7.50 a	30 a	5.75 a
Middle nodal position	8.7 a	17.39 b	2.82 b
Terminal nodal position	2.09 a	23.26 ab	3.37 b

Number within columns followed by different letters are significantly different at p<0.05 % level of probably.

Table 3. Analisis of variance for recorded parameters in effect of different growth regulators.

	Shoot length	Node number	Axillary shoot	Chlorosis leaf number	Necrotic leaf number	Feeble callusing
Treatments	42.31**	129.09**	43**	4.03**	1.48**	55**
Erorr	6.2	1.83	0.64	0.14	0.10	9.2

** significant at p<0.01 % level by ANOVA.

Figures

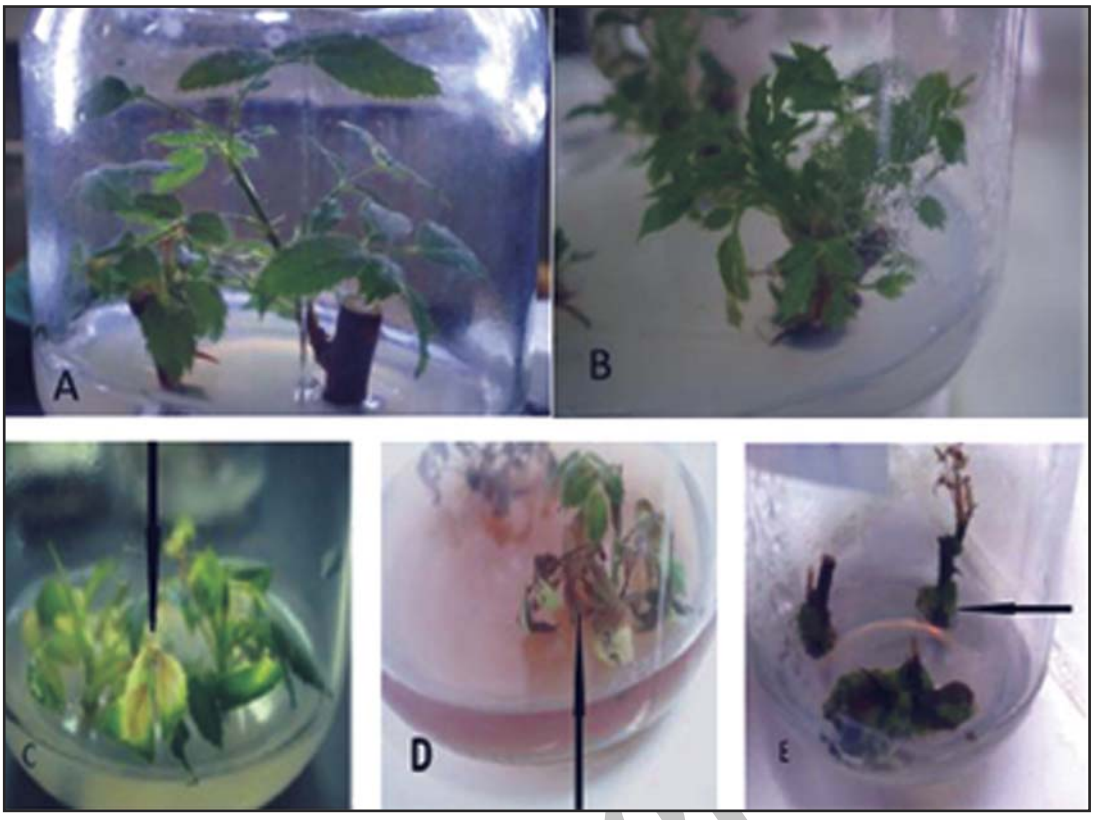

Fig.1. *In vitro* growth of *Rosa canina* on MS medium with different growth regulators. (a) Shoot induction. (b) axillary shoot production. (c) leaf chlorosis. (d) leaf necrotic. (e) feeble callusing.

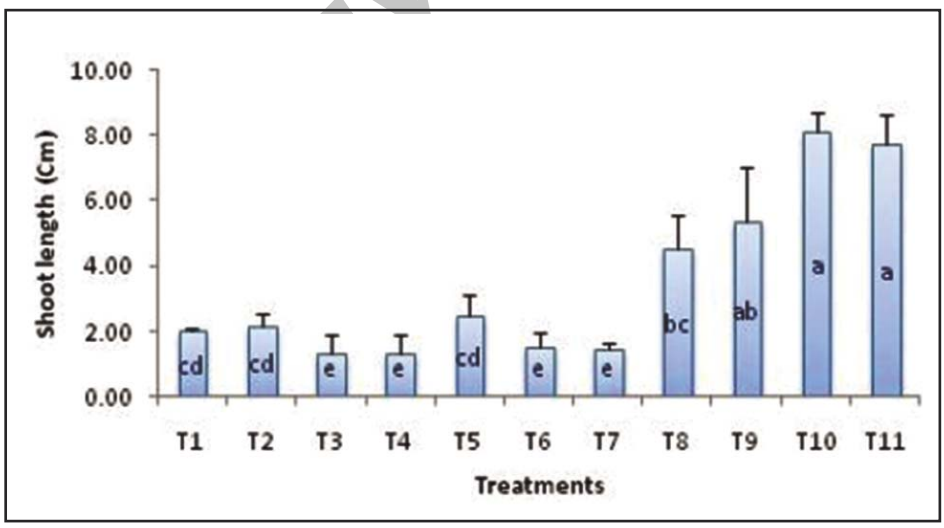

Fig. 2. Effect of different growth regulators on shoot length.

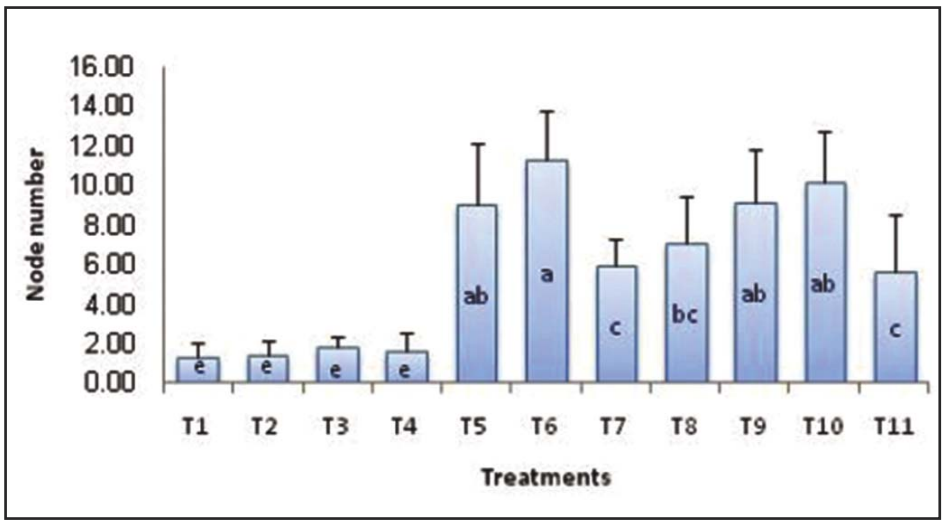

Fig. 3. Effect of different growth regulators on node number.

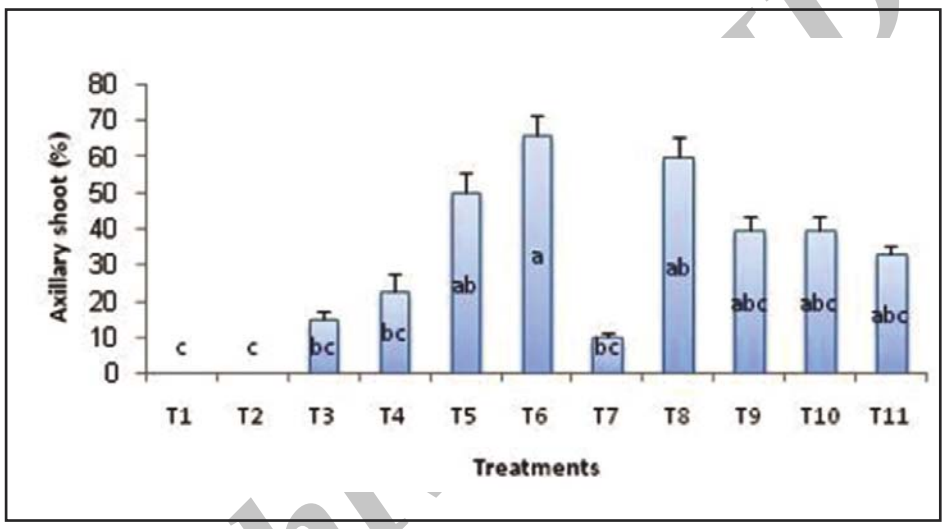

Fig. 4. Effect of different growth regulators on axillary shoot percentage.

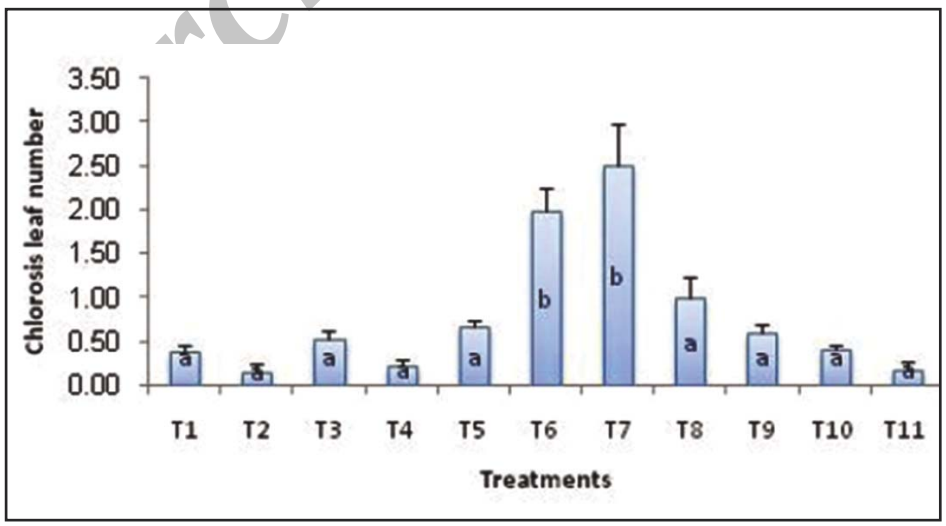

Fig. 5. Effect of different growth regulators on chlorosis leaf number.

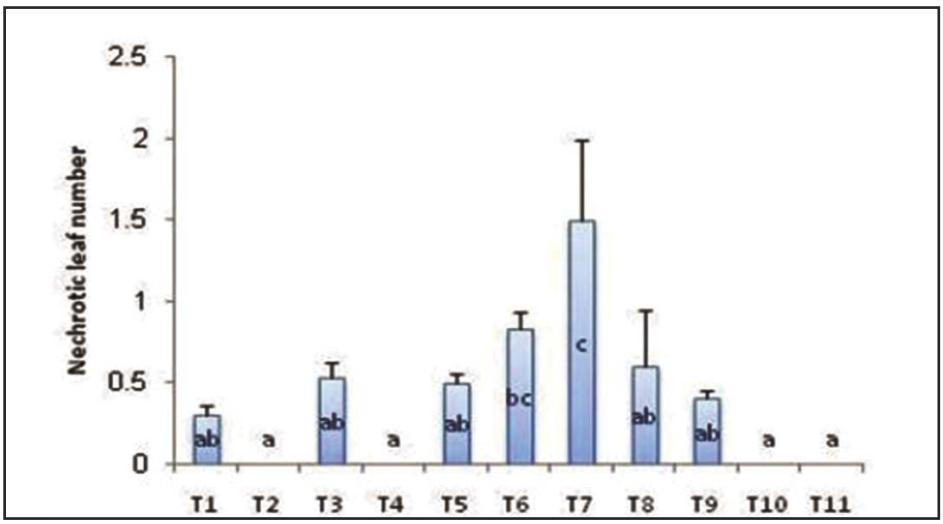

Fig. 6. Effect of different growth regulators on necrotic leaf number.

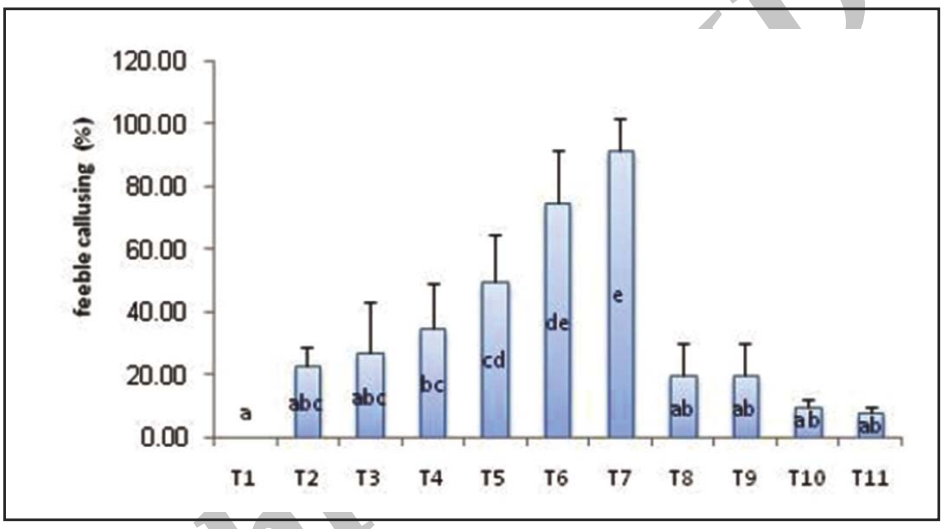

Fig. 7. Effect of different growth regulators on feeble callusing percentage at the end cuts of explant.

T1: Kinetin 0.5 mg l^{-1} + TDZ 0.1 mg l^{-1} + NAA 0.1 mg l^{-1}, T2: BAP 0.5 mg l^{-1} + TDZ 0.1 mg l^{-1} + NAA 0.1 mg l^{-1}, T3: Kinetin 1 mg l^{-1} + TDZ 0.1 mg l^{-1} + NAA 0.1 mg l^{-1}, T4: BAP 1 mg l^{-1} + TDZ 0.1 mg l^{-1} + NAA 0.1 mg l^{-1}, T5: BAP 4 mg l-1,T6: BAP 6 mg l-1, T7: BAP 8 mg l^{-1}, T8: BAP 2 mg l^{-1} + Ads 2 mg l^{-1}, T9: BAP 2 mg l^{-1} + Ads 1 mg l^{-1}, T10: BAP 1 mg l^{-1} + Ads 2 mg l^{-1}, T11: BAP 1 mg. l^{-1} + Ads 2 mg l^{-1}.