Effect of interaction between different plant growth regulators on in vitro shoot multiplication of *Citrus latifolia* Tan. (persian lime)

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Abstract— *In this paper a shoot multiplication is described for Citrus latifolia Tan. (persian lime) using nodal segment explants of young one – old – year trees by two different pathways contain with and without callusing phase. The best result for multiple shoot formation and regenerated shoot formation was 3.2 and 2.6 shoots per explants with 4.44 µM BA plus 0.053 µM NAA and 4.44 µM BA plus 0.049 µM IBA respectively. Alike shoot regeneration, shoot elongation was occurred in medium with 4.44 µM BA and 0.049 µM IBA. Micropropagated and regenerated plants are under other experiments.*

Abbreviation: *BA – 6 benzylaminopurine; IBA – Indole acetic acid; NAA – Naphtalene acetic acid; PGRs – Plant Growth Regulators.*

Key Words: Persian lime; plant growth regulators; shoot multiplication.

I. INTRODUCTION

Accordant worldwide *Citrus* species are the most widely grown fruit crops in Iran. They contain vitamin C that is very useful for human nutrition. Also their fruits are important source of volatile oils, limonene, α-terpinene, β-terpinene, citral cumarins, bioflavonoids, vitamins, and mucilage (Rathore *et al*., 2006). Beside apples and bananas, *Citrus* fruits are the most important fruit crops (FAO 2001). Also it is clear that the sustainable development of the *Citrus* industry is mainly dependent on a continuous supply of new and improved cultivars (Perez – Tornero *et al*., 2010). For the *Citrus* industry to improve fruit quality and reduce biotic and abiotic stresses are major breeding objectives at any time (Wenwu *et al*., 2007). *Citrus* varieties are propagated by both sexual and asexual methods. Generally, rootstocks are propagated sexually through seeds, while most of the commercial varieties are propagated by various asexual methods (Chaudhary 1994). These conventional techniques are also not free from risk of perpetuating in-born pathogens. However in vitro micropropagation technology can overcome some constraints to *Citrus* improvement and cultivation, and can increase fruit quality and resistance to diseases and environmental stresses (Gresser 1994). Also micropropagation systems with high multiplication rates are not only an important asexual method that can be used for the production of clonal plants, but also form the basis for the introduction of genetic variation by genetic transformation or mutagenesis. The genetic transformation of *Citrus* has been widely studied as a tool to generate transgenic plants with enhanced tolerance of biotic (Cardoso *et al*., 2010; He *et al*., 2011 and Ali *et al*., 2012) and/or abiotic stresses (Bunnag and Tangpong 2012). In both cases, is necessary to be able to regenerate viable shoots, which can be propagated, by either organogenesis or somatic embryogenesis (Perez – Tornero *et al*., 2010).

In this paper a protocol for cloning of *Citrus latifolia* Tan. (persian lime) using nodal explant was described. It should be noted that according to Iranian researchers that used graft inoculation and PCR methods, persian lime was tolerant to Candidatus *Phytoplasma aurantifolia*. Candidatus *Phytoplasma aurantifolia* is a serious threat to lime and other susceptible *Citrus* trees in southern Iran (Salehi *et al*., 2005).

II. MATERIAL AND METHODS

This study was conducted in Iranian Research Institute of Plant Protection Department of Plant Diseases, Tehran – Iran

Young trees of *Citrus latifolia* Tan. (persian lime) were collected from Jahrom *Citrus* nursery, Jahrom – Fars – Iran

2.1 Surface sterilization and plant material preparation

One – old – year young tree persian lime (*Citrus latifolia* Tan.) were used as the source of explants from their nodal segments. 30 – 35 – old – day new shoots measuring 12 – 15 cm in length with 4 – 6 nods were cut and collected in plastic bag and transferred to the laboratory from greenhouse for experiments.

2.2 Preparation and sterilization of explants

After defoliation of shoots, and cutting 0.5 – 0.7 cm in length nodal segments (explants) surface sterilized under laminar air flow with dipping in 25% hypochlorite sodium for 5 min. Then were rinsed in distilled water 4 – 5 times.

2.3 Culture media

Culture media consisted of MS (Murashig and Skoog 1962) salts and vitamins plus 3% sucrose that were solidified with 0.75 % agar agar. Also the media were supplemented with BA (0 – 8.88 µM), 0.053 µM NAA and 0.049 µM IBA. The pH of media was adjusted to 5.8 before gelling with 1N NaOH or HCl and after gelling autoclaved for 20 min at 121ºC. Then the media dispensed into 9 – cm diameter petri dishes. The culture was incubated at 25 ± 2ºC and under 16 – h photoperiod.

2.4 Experimental design and data analysis

Experiments were conducted in a completely randomized design with 4 replicates and 5 explants per replicate. The mean number of multiplicated shoots per explants and the mean length of multiplicated shoots assay carried out on MS media with 12 combinations of cytokinin (BA) and auxins (NAA and IBA). Data were analysised by Dunkan Multiple Range Test.

III. RESULTS AND DISCUSSION

Shoot multiplication in the presence of media with different concentrations of BA and 0.053 µM NAA and or 0.049 µM IBA compared after nearly 4 weeks of culture. It can be seen that the highest number of multiplicated shoots (3.2) per explants was observed in the media containing 4.44 µM BA and 0.053 µM NAA (Fig. 1and Fig. 5). Also the highest length of shoot (10 cm) was obtained on medium with 4.44 µM BA and 0.049 µM IBA (Fig. 3 and Fig. 6). It is interesting that in these study observations such as shoot multiplication and growth of shoot (longitudinal growth and foliation) were affected by interaction between different plant growth regulators more rather than plant growth regulators solely. There are reports about positive effect of increasing concentration of BA on shoot multiplication. For example Mehdi Farshad and coworkers in 2014 resulted that BAP alone (from 8.8 µM to 26.6 µM) was significantly effective on shoot multiplication in *Chlorophytum borivilianum*. However there are reports that show shoot proliferation decreased with increasing concentration of BA alone (Komal *et al*., 2013). This experience is in accordance with ours that frequency of response from nodal explants decreased with a progressive increase in the level of BA [8.88 and 4.44 µM BA, 4.75 – 8.20 (cm for length of shoots)] respectively.

As was mentioned earlier, the interaction between plant growths regulators have very important effects on induction of shoots also number of shoot multiplicated. So as was shown in Fig. 2 the mean number of multiplicated shoots on explants increased when BA was used solely (1.2 – 2.66 for 0.044 – 8.88 µM BA). The positive effect of BA on the induction of regenerated shoot is clear. For example the positive effect of BA on shooting in *Cucurbita maxima* Duch (Lee *et al*., 2003), *Ruta graveolunse* L. (Ahmad *et al*., 2010), tomato (*Lycopersicon esculentum* L.) (Rai *et al*., 2012) and Japanese pear (Kadota *et al*. 2001). These results were somewhat similar to our results about the mean number of multiplicated shoots. However it seams 4.44 µM BA either in terms of nature or in terms of level is suitable for shoot multiplication in *Citrus* genus. So that Al-Khayri and Al-Bahrany in 2001 expressed that Best results for multiple shoot formation, 8 shoots per node, were obtained with 4.44 µM BA and 2.32 µM kinetin.

Another interesting result of this study is the circumstance of shoot multiplication. As is clear in figures multiplication shoot in the presence of BA and NAA was direct while multiplication shoot in the presence of BA and IBA was indirect and accompanied by callusing phase (Fig.7 and Fig. 8). So the role of interaction between plant growth regulators in in vitro culture of plants is proved again.

Very different and important roles are demonstrated for plant cell, tissue and organ culture. Briefly shoot multiplication and shoot regeneration are two different kind of organogenesis with two different utilizations. This result implies that rapid plant regeneration system which could be used for the somaclonal variation induction are possible for persian lime, on the other hand in vitro propagation through lateral buds proliferation that is an efficient method for large scale production of true – to – type planting material of important plant (Doo and Iyyakkannu 2016) is practicable for this valuable plant. It is worth noting that for assessment the level of background genetic changes resulting from the tissue culture processes (Munthali *et al*. 1996) and to verify the "true-to-type" genotype of micropropagated plants with shoot multiplication and or shoot regeneration DNA-based marker techniques such as RFLPs (Nelke *et al*., 1993) and or RAPD (Munthali *et al*., 1996) are necessary (Komal *et al*., 2013)

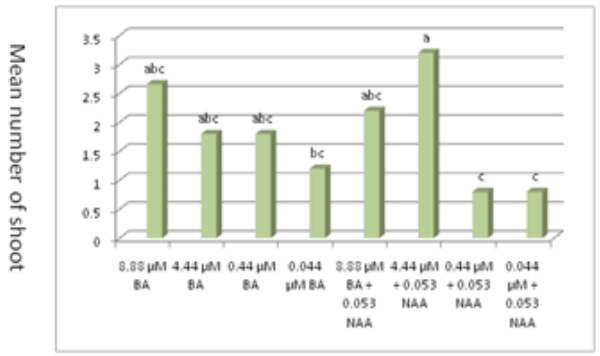

FIG 1. The mean number of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 8.88-0.044 µM BA plus 0.053 µMNAA

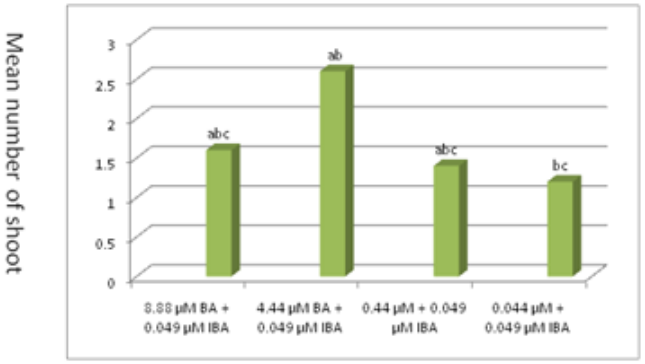

FIG 2. The mean number of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 0.049 µM IBA

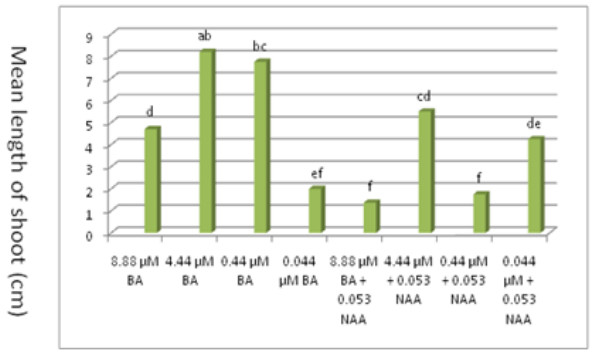

FIG 3. The mean length of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 8.88 – 0.044 µM BA and 0.049 µM NAA

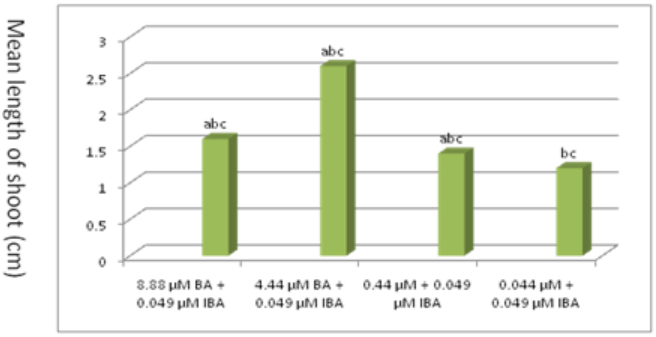

FIG 4. The mean length of multiplicated shoot on medium with 8.88 – 0.044 µM BA and 0.049 µM IBA

Mean followed by same letter(s) are not significantly different

FIGURES 5 – 8. 5 – Multiplicated shoot on medium with 4.44 µM BA and 0.053 µM NAA. 6 – Elongation of multiplicated shoot on medium with 4.44 µM BA and 0.049 µM IBA. 7 and 8 - Multiplicated shoot on medium with 0.44 µM BA and 0.049 µM IBA

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