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Effects of explants and hormone concentrations on regeneration of Myrtle (*Myrtus communis* L.)

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ABSTRACT

Medicinal herb (*Myrtus communis* L.) is a perennial and aromatic herb with different medicinal properties. It grows mostly in the tropical and subtropical regions of the world. This study investigated the effects of important factors including plant explants and hormones concentrations on regeneration of Myrtle. Firstly seeds of Myrtle were sterilized and germinated in the Murashic-Scug medium to use for preparation of aseptic leaf, stem and root explants. So four concentrations (0-1-2-3) mg / L of regeneration hormones including kinetin, BAP, TDZ and BAP + TDZ were used invitro. Leaf explants and concentration of 3 mg / L BAP + TDZ had the highest regeneration. Because of the low germination rate of Myrtle seeds and low repropagation of vegetative protocols in this medicinal plant, our results can be useful for its mass propagation.

Keywords: regeneration, explants, tissue culture, medicinal plant

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Introduction

The Myrtle (*Myrtus communis* L.) belongs to the genus (Myrtus) and belongs to the family (Myrtaceae). The plant grows mostly in the tropical and subtropical regions of South America and Australia. The plant is a small shrub that, in normal conditions, reaches 2–3 m high but in favorable weather reaches more than 2 meter high. Leaves dark green and white. The flowers are large and white and the fruit is two-sided in color. The first state is blackish blue and the second is white until the fruit arrives (Mitrushi, 1955). The Myrtle is one of the important medicinal plants used in traditional medicine in many parts of the world. Its leaf and fruit are widely used as a traditional folk medicine to treat many disorders and diseases. Myrtle oil can be extracted from its leaves. This oil has medicinal properties and is liquid at room temperature. The plant can increase or decrease thyroid activity depending on individual conditions (Stewart, 2005). The fruits and leaves of the mentioned plant have antimutagenic and anti-inflammatory properties and are disinfectant and used for the treatment of internal and local infections (Bonjar,2004;Hayder et al.,2004). The essential oil of the plant is used for the production of natural remedies and its leaves for tea use (Flamini et al., 2004;OgurT,1994). For decades, hornbeam essential oils have been widely used in food, vegetable and fragrance applications (Taylor, 1996). This plant is generally propagated by seed. Some reports also suggest that it can be reproduced with cuttings. In both methods mentioned, reproduction of the Myrtle plant is associated with major difficulties. Long seed dormancy and the production of small and weak seedlings are the disadvantages of seed propagation. Percentage of rooting of cuttings is one of the major limitations of cuttings (Canhoto,1999). Molecular agriculture is one of the new and applied topics in the field of agriculture. Plant cultivation for the production of recombinant proteins, enzymes or secondary metabolites with industrial and therapeutic applications through genetic engineering is called molecular farming (Haque and Ghosh, 2013). Calliogenesis is one of the important processes of molecular agriculture. The first report on tissue culture in the medicinal plant dates back to 1994 by Nobre (Nobre J,1994). In this study, the effects of three explants, concentrations and hormones on regeneration of medicinal plant were investigated

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Materials and methods

The seeds of the medicinal plant were obtained from the live collection of medicinal plants of Zanjan University of Agricultural Research in the fall. Seeds were sterilized in sodium hypochlorite 2%(w / v) for 20 min, in oxygenated water 11% (v / v) for 5 min, and in ethanol 70% (v / v) for 2 minutes. Seeds were rinsed with sterile distilled water 2 times after each step. Gibberellic acid, chilling, scraping and placing in boiling water were used to break the seeds. Then sterilized seeds were cultured in MS medium containing NAA in petri dishes. They were stored on a shelf under a moderate white fluorescent light at a temperature of 25 ° C and a light / dark period of 16/8 hours. After 40 days and after germination, root, shoot and shoot explants were prepared and placed in MS medium containing 2,4-D hormone. Leaf, stem and root microleakage specimens that were callus callus in MS medium containing callus formation hormonewere grown in Murashig-Skog medium containing transgenic hormones Kinetin, BAP, TDZ, and TDZ + BAP were transduced with four levels (0-1-2-3 mg / L). After preparing the Murashig-Skog medium, its pH is adjusted to about 7.5 and the agar is added at (10 gr) to close the medium. To dissolve agar in the Murashig-Skog medium as well as sterilize all the compounds in that medium in an autoclave for 120 minutes at 121 ° C. After placing the explants in the Murashik-Skog environment under controlled light and temperature conditions (average temperature ۲۵ degree and light intensity ۳۰۰۰ luxury) in the growth chamber (one of the most important devices needed in tissue culture laboratories is the Gross Chamber). This machine, with the ability to control humidity and temperature, plays the role of a small cultivation chamber and plays a favorable environment for optimal growth and specimens. All procedures were performed in the culture chamber. This chamber is equipped with a Laminar Airflow hood and a sterilizer. The research data were analyzed by factorial experiment based on completely randomized design with four replicators in three levels.

Results and discussion

In this experiment, three leaf explants, root and shoot explants, which had the highest regeneration rate with 0.5% of the above explants, and the lowest regeneration percentage in the same environmental conditions were used for root explants. The next factor tested was the hormonal levels under investigation. Concentration used at four levels (0-1-2-3) mg / L had the highest regeneration percentage at 3 mg / L and lowest for control (0). The next factor examined in this study was regeneration hormone, which consisted of four hormones: Kinetin, BAP, TDZ and the combination of two hormones BAP + TDZ. Leaf, shoot, and root microenvironment samples were selected in MS medium containing 2,4-D hormone and were subjected to callogenesis and were placed in basal MS medium containing contaminant

hormone. After three weeks, there was no significant change in calluses in this environment. And after four weeks, most of the calluses disappeared. The regeneration rate was very low, so that the leaf specimen was regenerated in a medium containing 3 mg of contaminant only 10%. The highest percentage of regeneration was for BAP + TDZ.

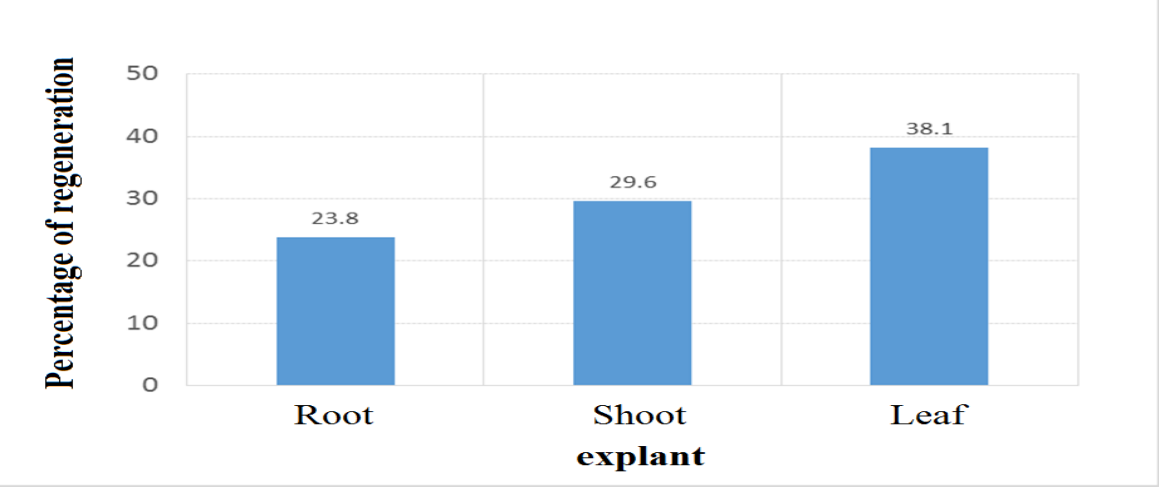

Figure1 - Effect of different explants on regeneration of the case medicinal plant(As can be seen, leaf explant regeneration percentage of 1.38% had the highest regeneration rate compared to other explants).

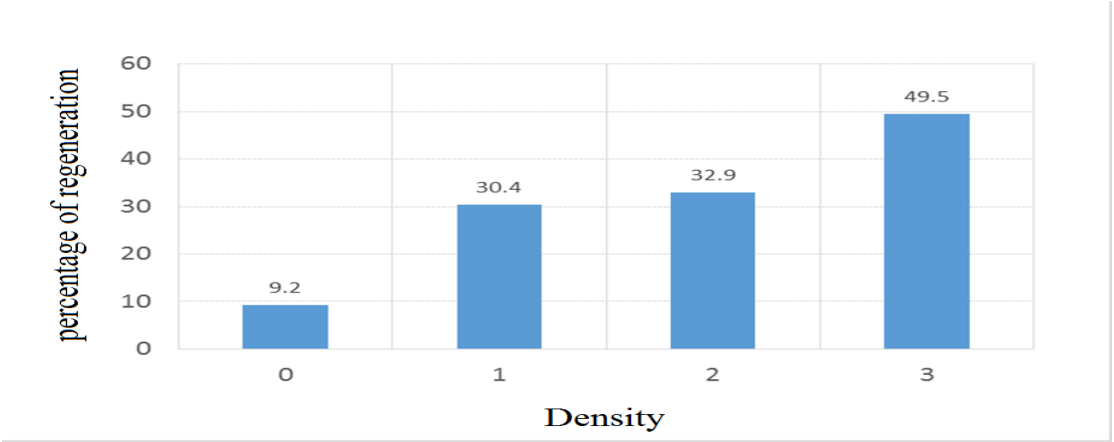

Figure 2 - The effect of different concentrations on regeneration In this experiment, four hormonal levels (types of hormones used in regeneration) were evaluated. Hormonal levels of 3 mg / L had a higher percentage of regeneration than the other levels studied. Hormonal levels of 1 and 2 mg / L did not differ significantly in the rate of regeneration of medicinal plant explants)

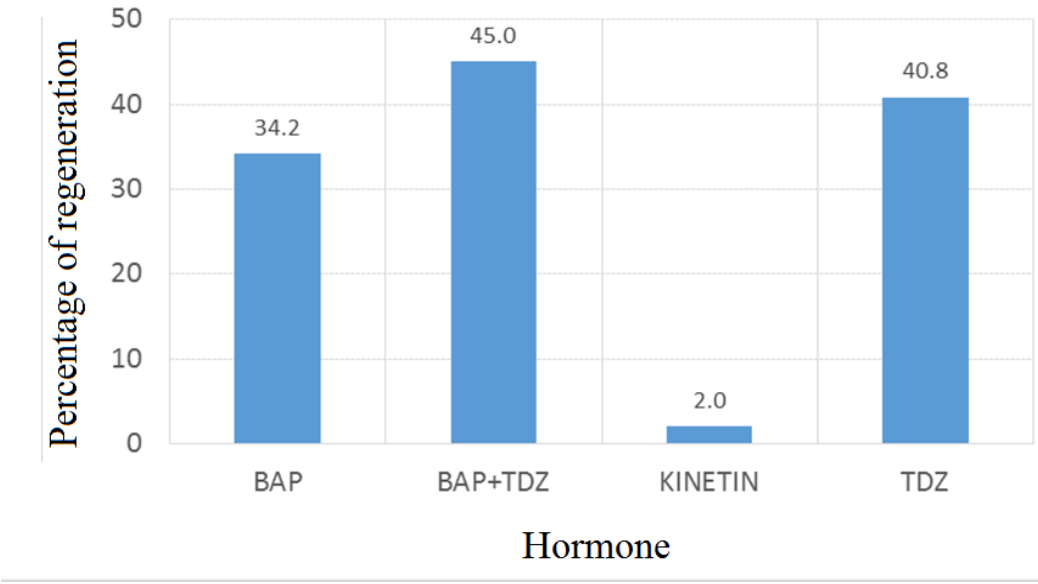

Figure3 - Effect of different hormones on regeneration (BAP + TDZ combined with 45% had the highest regeneration, while Quintine showed the lowest regeneration with 2%)

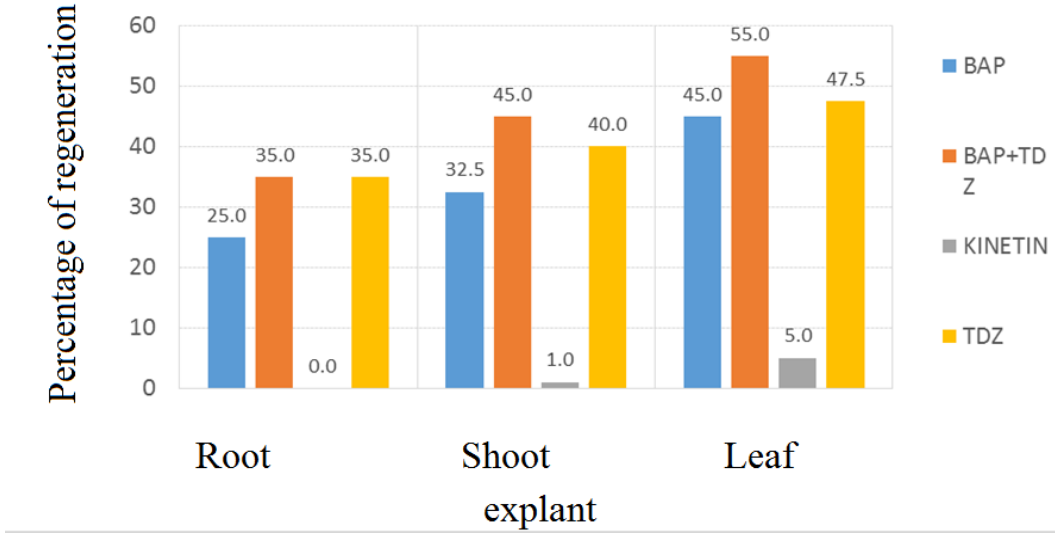

Figure4- Interaction between explants and different hormones used (As shown in Fig. Constant had the lowest percentage of regeneration and was the most successful for leaf explants among the tested explants).

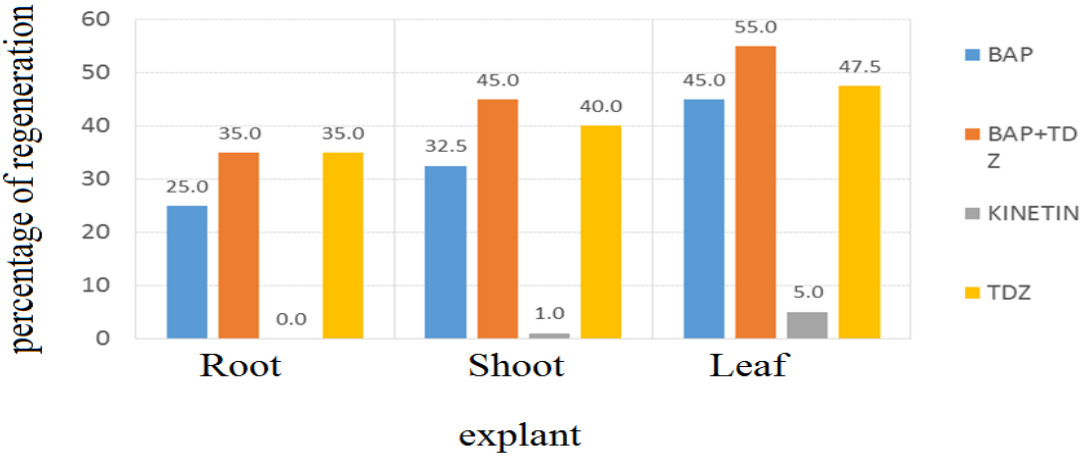

Figure5 - Interactions between the hormones used and the different levels of the hormone (the highest percentage of 3 mg/L concentration is BAP + TDZ)

Conclusion

The main mechanism of the regeneration process is very complex and the percentage of regeneration varies in different plants and hormones used as well as in different media. However, the regeneration potential of the plant is hampered by the difficulty of the plant. The plant growth regulator has had different effects on the regeneration of different plant explants. As the amount of hormone used increases, it has a direct effect on the regeneration of the plant. According to the results of this study, the hormone contaminant was the least effective hormone for regeneration of this plant and TDZ had more effect than both hormone kinetin and BAP. As previously mentioned, the combination of the two hormones 1.5 mg TDZ+1.5mg BAP (3 mg / L BAP + TDZ) had the highest regeneration rate. On the other hand, leaf explants had the highest regeneration success compared to other explants.

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