

The role of external growth regulators in Optimization of callus production in Red Rubin Basil (*Ocimum basilicum*)

*Mehdi Kakaei¹, Fatemeh Hajmoradi², Mohamad Ali Ebrahimi³ and Mohsen Mansori⁴

1 & 3- Associate Professor and Professor Respectively, Department of Agriculture, Payame-Noor University, Tehran- Iran.

2- Department of Biology, Payame-Noor University, Tehran, Iran

4- Department of Persian Medicine, AJA University of Medical Sciences, Tehran, Iran.

* E-mail (corresponding author): M.kakaei@pnu.ac.ir

Accepted: 2023.05.15

Received: 2022.09.04

Introduction:

Basil (*Ocimum basilicum* L.) is an annual aromatic plant with high medicinal value and belongs to the mint family (*Lamiaceae*). Plant production through tissue culture is very important from the point of view of genetic engineering, therefore, in this research, optimization of tissue culture conditions of the purple basil plant (*Ocimum basilicum*) to produce callus, identification of callus induction percentage, growth rate, growth speed and relative water content of callus through Tissue culture and determining the appropriate hormonal composition for callus production were designed and implemented. Plant cell and tissue culture is a valuable and useful tool in applied and basic studies. (Razavi et al. 2016). Many researchers have used plant explants for seed production in culture (Abbasi et al. 2016 & Abdirad et al. 2011 & Baravardi et al. 2015 & Ghotbzadeh Kermani et al. 2015 & Goravanchi et al. 2012 & Sadatnoori et al. 2012 & Zarei et al. 2020 & Zebarjadi et al. 2014). Phytohormones auxin and cytokinin play a special role in controlling various vital processes including plant growth, development and regulation of response to environmental stimuli. The future study is designed to obtain the technical knowledge of reproduction and optimization of the micropropagation conditions of the purple basil plant from the leaf and stem explants of this plant, as well as choosing the best combination of external growth regulators and choosing the appropriate explant for future studies such as gene transfer to this valuable plant and medicinal species and implemented.

Materials and methods:

factorial optimization of callus formation was carried out in the form of a completely randomized design with four factors, micro samples and levels of benzyl adenine, indole acetic acid and indole butyric acid hormones. First, two stem and leaf explants were used to investigate hormonal treatments for callus formation. Explants on MS medium with hormone treatments, cytokinin source including benzyl adenine (BA), with two levels (1 and 2 mg/L) and auxin sources, indole acetic acid (IAA), with two levels (1 and 2 mg/L) and indole butyric acid (IBA), with two levels (1 and 2 mg/L). This research was conducted in the form of a factorial experiment based on a completely randomized design. Data analysis was done using SAS software, and before analyzing variance, the normality of the data was evaluated. To draw a graph with Excel software, and to perform a comparison of averages, Duncan's multiple range test was used.

Results & discussion:

The results of this research showed that the highest percentage of callus formation was related to the culture medium containing 2 mg/L IBA and 2 mg/L BA. The effect of hormonal compounds on the four investigated traits, percentage of germination, growth rate, relative water content and growth rate (dry weight) had a significant effect at the statistical level ($P \leq 1\%$), which indicates the significant effect of the type of combination of growth regulators that can be used in order to choose the best combination in crop production and optimization of tissue culture and finally breeding purposes, it was used in connection with the mentioned traits. Also, the interaction effect of two factors of explant and hormonal compounds only had a strong significant effect ($P \leq 1\%$) on the growth rate index, which also shows the significant interaction effect of the two factors expressed in the growth rate. The purple basil medicinal plant contains essential oils and basil essence and is used to cure various diseases. Tissue culture micropropagation has made it possible to quickly produce uniform plants for many plants. The effect of cytokinins in tissue culture is very significant and is usually used together with auxin to improve cell division and control organogenesis (Del-Poza et al. 2005). It has been proven that growth regulators interact with each other and can enhance each other's effects. Generally, shoot formation, either directly from explant tissue or indirectly from callus, is regulated by the balance of growth regulators between auxin and cytokinin (Ghasemi et al. 2017). In similar research, an attempt has been made to find the best combination with the most optimal concentration of hormones, Seydi et al. (Seydi et al. 2016). Growth regulators have a

great effect on the main processes of the cell, including the initiation of cell division and cell expansion, acidification of the cell wall, and cell organization in order to create callous tissue or differentiated tissue such as (stem and root) (Gaspr et al. 1996). In general, the results of this research showed that the highest percentage of callus formation was related to the culture medium containing 2 mg/L IBA and 2 mg/L BA, and in general, the averages of stem explants were higher than other explants in most of the hormonal compounds. In fact, this basic research paved the way for the next step in the breeding activities of purple basil researchers, and in other words, the results of examining the response of this plant to calluses will be useful in choosing the levels of hormonal compounds and the type of explant and can promise the possibility of other activities such as regeneration, reproduction, somatic embryogenesis, artificial seed production and targeted gene transfer to this plant are valuable.

Conclusion:

The quality of calluses shows the correct selection of explants and suitable hormonal compounds. Therefore, the amount of callus production is dependent on the number of external growth regulators, and it also seems that the amount of external growth regulators is also related to the type of plant (even the genotype of the plant) and the number of internal plant hormones.

Keywords: *Callus formation, Phytohormones, Micro sample, Tissue culture.*

Conflict of interest

No conflicts of interest are declared by the authors.

Acknowledgement

We are grateful to all the dear ones who cooperated in all stages of this research work.

Statement on ethics:

Statement on ethics We certify that the submission is original work and is not under review at any other publication