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Research Article

Effects of Thidiazuran and Zeatin on Plant Regeneration in *Helichrysum pallasii*

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Abstract. The genus of *Helichrysum* comprises many species which have therapeutical effects and used in folk medicine. *H. pallasii* is one of these species, used in the region for traditional medicine and ornamental purposes. Therefore, optimizing alternative micropropagation protocols of tissue culture conditions and secondary metabolite production for these species needs attention. The effect of Thidiazuran (TDZ) combined with Zeatin (ZEA) on shoot regeneration in *H. pallasi* was investigated during this study. The leaf, stem and root parts taken from the seedling germinated under *in vitro* conditions were used as explants. As a result, the root explants were more efficient compared to other explants in inducing plant regeneration using 1 mg L^{-1} Thidiazuran (TDZ) + 0.1 mg L^{-1} Zeatin (ZEA) (88.9%) and 1 mg L^{-1} TDZ treatment (85.7%). The lowest plant regeneration percentage (16.7%) was found in control medium using leaf explants.

Thiadiazuron ve Zeatin'in *Helichrysum pallasii*'de Bitki Rejenerasyonuna Etkisi

Anahtar kelimeler:
Ölmez çiçek, mikro çoğaltım, doku kültürü, thidiazuran, zeatin

Özet. Helichrysum cinsi, tedavi edici etkileri olan ve halk hekimliğinde kullanılan birçok türü içermektedir. *H. pallasii* yörede geleneksel tıp ve süs amaçlı kullanılan bu türlerden bir tanesidir. Bu nedenle, bu türler için doku kültüründe alternatif mikroçoğaltım protokollerinin ve sekonder metabolit üretiminin optimize edilmesi gerektirmektedir. Bu çalışmada Zeatin (ZEA) ile kombine edilmiş Thidiazuran (TDZ)' nın *H. pallasi*'de sürgün rejenerasyonu üzerine etkileri incelenmiştir. *İn vitro* koşullarda çimlendirilen bitkiciklerden alınan yaprak, gövde ve kök kısımları eksplant olarak kullanılmıştır. Sonuç olarak, en yüksek bitki rejenerasyonu kök eksplantlarına uygulanan 1 mg L^{-1} Thidiazuran (TDZ) + 0.1 mg L^{-1} Zeatin (ZEA) (%88.9) ve 1 mg L^{-1} TDZ (%85.7) uygulamalarından elde edilmiştir. En düşük bitki rejenerasyon yüzdesi (%16.7) ise yaprak eksplantları kullanılan kontrol uygulamasından elde edilmiştir.

INTRODUCTION

Plants have been used in the treatment in folk medicines since the earliest times of human history (Cragg *et al*., 1993; Farnsworth, 1993; Eröztürk, 2000). Chemical composition of plants, antimicrobial and other medicinal properties of plants are being investigated in laboratories throughout the world (Nigg and Segler, 1992; Baytop, 1986, Dordevic *et al*., 2013; Ghasemi Pirbalouti *et al*., 2013, Rossi *et al*., 2013; Kalogeropoulos *et al*., 2014). The extracts and essential oils derived from medicinal plants (Li *et al*., 2013; Machado *et al*., 2013; Quassinti *et al*., 2013) are known to display antibacterial and antifungal effects (Soković et al., 2002) and antimicrobial activities (Olmedo *et al*., 2014) which are the basis for many applications such as nutrient preservation, pharmacy, alternative medicine and natural therapy. Immortal flower, which is called "*Helichrysum pallasii*" is a plant of European origin and is widely used for herbal treatment in Turkey (Davis, 1975; Davis, 1988; Lawrence, 1998; Guner *et al*., 2000; Sumbul *et al*., 2003; Angioni *et al*., 2003; Appendino *et al*., 2007). It is necessary to investigate the methods of rapid plant reproduction that can contribute to both the economy of the country and region. Quality products and drugs are one of the aims in breeding and breeding studies and especially in pharmaceutical and spice plants. Important support should be provided for the collection, characterization and registration of genetic material in the area of field crops in Turkey. Therefore, this study aimed to develop a regeneration protocol for the immortal flower using different explants under tissue culture conditions.

MATERIAL AND METHOD

Plant Material

The characterization of collected plant samples was done by Prof. Dr. Serdar Makbul, Faculty of Science, Recep Tayyip Erdogan University in Rize. The seeds of *H. pallasii* were collected from its natural habitat in the Armutlu district of Bayburt province (Turkey) (Figure 1).

Figure 1. General view of immortal plant (*H. pallasii*) during the seed filling stage.
Şekil 1. Ölmez çiçek (H. pallassii)' nin tohum doldurma döneminde görünümü.

Sterilization and Germination

Mature seeds of *H pallasii* were exposed to 70% ethanol for 5 minutes for pre-sterilization. Thereafter, the seeds were treated with Tween-40 for 10 minutes. Sterilization was carried out with 20% commercial bleach (Domestos) for 10 minutes. Then, seeds were rinsed in sterile distilled water for 3×5 min. In order to ensure a high percentage of germination, the seeds were planted on MS medium (Murashige and Skoog, 1962), fortified with 30 g L^{-1} sucrose and 7.5 g L^{-1} agar.

Plant Growth Regulators and Incubation Conditions

MS medium was used as control treatment. (S1), 1 mg L^{-1} Thidiazuran (TDZ) (S2) and 1 mg L^{-1} TDZ + 0.1 Zeatin (S3) were used for regeneration purpose. Regenerated shoots were placed into rooting media supplemented with 1 mg L^{-1} IAA. The explants were cultured under two different incubation conditions of 16 hours light and 8 hours dark conditions at 26 °C, 300 lux light source in growth chamber and dark condition at 26 °C.

Data Analysis

In order to reduce the dimensional variation problems in multivariate analysis is the first step is the transformation of obtained variables into a number of new and uncorrelated variables, which are called principal component (Mebatsion *et al*., 2012). Calculated each component corresponds to a percentage of the total variance in the present data set an allows to visualize the characteristic supporting to the differentiation of investigated material. PCA analysis was performed to clarify the relationship between investigated data and principal component analysis (PCA) to elucidate their relationships using the statistical software package XLSTAT2020.

RESULTS AND DISCUSSION

As explained in Material Methods, seeds of *H. pallasii* were surface sterilized and cultured on MS medium (Figure 2). The surface sterilization and regeneration on MS medium containing different concentrations of plant growth regulators on roots, stem and leaf explants was successful (Figure 2).

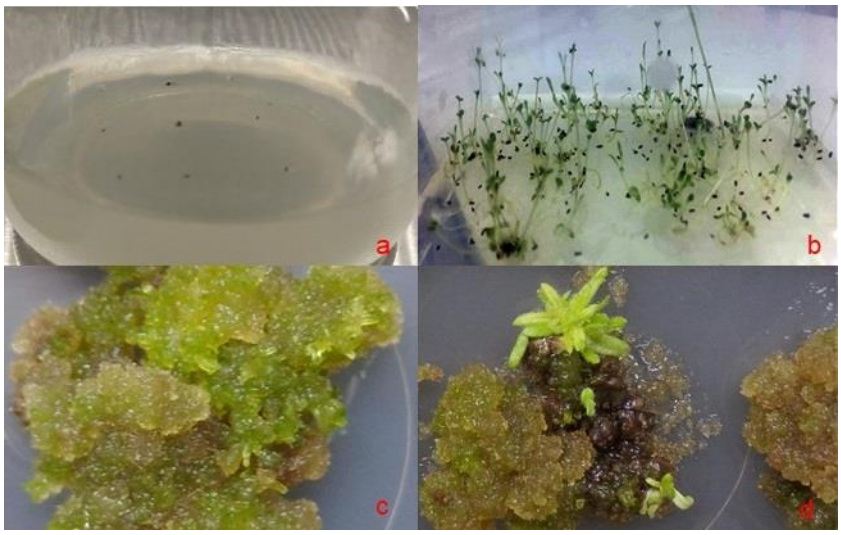

Figure 2. Regeneration of *H. pallasii* under tissue culture conditions; (a) culture of seeds to obtain seedlings, (b) seedlings, (c) callus regeneration and (d) shoot regeneration.

Şekil 2. H. pallasii' nin doku kültürü rejenerasyonu; (a) bitkicik eldesi için tohum kültürü (b) bitkicikler, (c) kallus rejenerasyonu, (d) sürgün rejenerasyonu.

The explants showed variable induction of callus and shoot regeneration (Figure 2a-d)

Callus Regeneration

The highest callus induction percentage (87.5%) was noted on the root explants cultured on S3 medium; while the lowest Callus induction (40%) was determined on S1 medium from the same explant. The highest callus regeneration percentage of 81.3% on stem explant was noted on S2 medium while the lowest percentage of callusing on the explant was noted on S1 medium (35.7%). The highest (51.7%) and the lowest (24%) callus regeneration percentage on leaf explants was noted on S3 medium and S1 medium in the same order (Figure 3, Table 1).

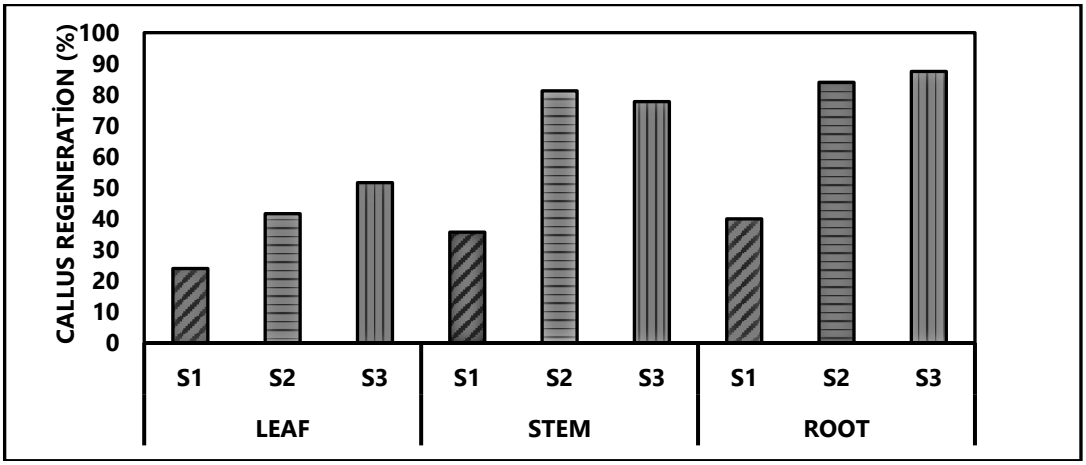

Figure 3. Percentage of regenerated callus obtained from different explant parts using TDZ, TDZ and Zeatin.
Şekil 3. TDZ, TDZ and Zeatin kullanılarak farklı eksplant kaynaklarından elde edilen % kallus rejenerasyonu.

Table 1. Effects of different PGRs treatments on leaf, stem and root explants of *H. pallasii.*
Çizelge 1. Farklı bitki büyüme düzenleyicilerinin H. pallasii de yaprak, gövde ve kök eksplantlarına etkisi.

Explant part	Medium	Number of explants	Number of callus	Number of regenera-ted plants	Callus regenera-tion (%)	Plant regenera-tion (%)
Leaf	S1	50	12	2	24.0	16.7
	S2	60	25	15	41.7	60.0
	S3	60	31	18	51.7	58.1
Stem	S1	70	25	11	35.7	44.0
	S2	80	65	53	81.3	81.5
	S3	90	70	61	77.8	87.1
Root	S1	40	16	8	40.0	50.0
	S2	50	42	36	84.0	85.7
	S3	40	45	40	87.5	88.9

(S1 (Control), S2 (1 mg L^{-1} Thidiazuran) and S3 (1 mg L^{-1} Thidiazuran + 0.1 mg L^{-1} Zeatin).

These results are in agreement with Perrini *et al*. (2009), reporting that TDZ concentrations (0.1 Mg L^{-1} to 2 Mg L^{-1}) were effective in callus regeneration in *H italicum*.

Plant Regeneration

Plant regeneration percentages were highest in root explants. In root explants the highest plant regeneration percentage was determined as 88.9 % in S3 medium (Figure 4, Table 1).

The highest percentage of plant regeneration was found on S3 medium as 87.1%, when using stem explants. The lowest plant regeneration (16.7%) was observed on S1 medium using leaf explants. The highest rate of plant regeneration (60.0%) using leaf explants was observed on S2 medium (Figure 4, Table 1). All regenerated plants were obtained from callus.

Plant regeneration was encouraged by using 0.1 mg L^{-1} zeatin combined with 1 Mg L^{-1} TDZ (S3) Giovannini *et al*. (2003) reported that using 0.91 and 4.56 µM zeatin singly was not effective to stimulate organogenesis. They reported that a combination of zeatin with IAA (1 and 0.5 µM) encouraged shoot proliferation in *H. italicum*.

There are no studies regarding the germination and regeneration of plants under *in vitro* conditions from *H. pallasii*. It is well established that the contamination risk among explants obtained from field conditions is very high compared to the explants taken from plantlets grown under greenhouse or *in vitro* conditions. The results of the study show that the seeds of *H. pallasii* are not difficult to germinate. A large number of seeds could be multiplied in short time.

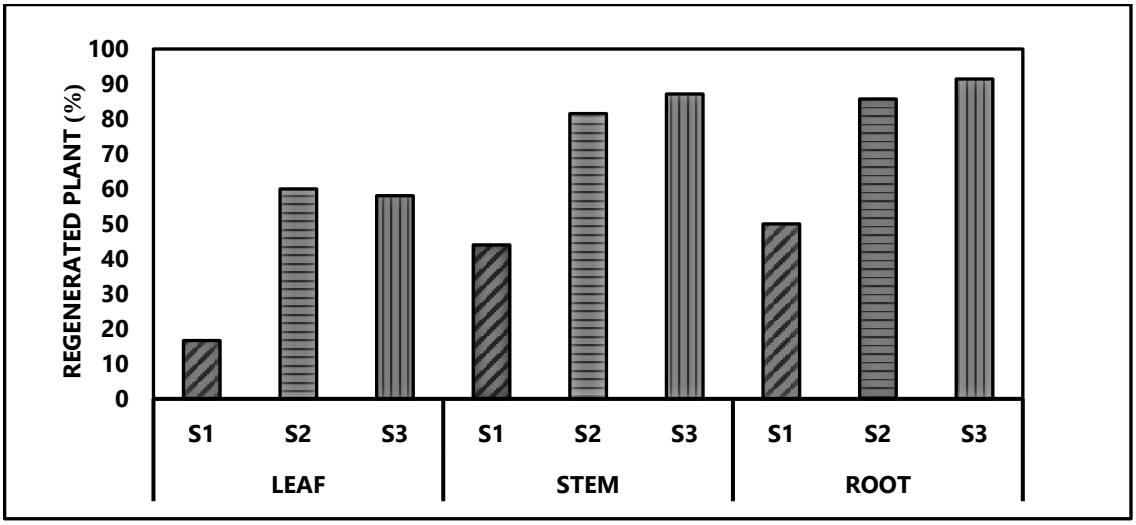

Figure 4. Percentage of regenerated plants taken from different explant parts with TDZ, TDZ and Zeatin.
Şekil 4. TDZ ve TDZ + Zeatin uygulanan farklı eksplantlardan elde edilen % bitki rejenarasyonu.

As can be also seen in Figure 5, PCA of obtained results from the present study revealed that the root explants cultured on S2 and S3 medium were more effective regarding callus regeneration and plant regeneration and stem explants cultured on S2 and S3 medium were effective in obtaining number of regenerated plants and calli.

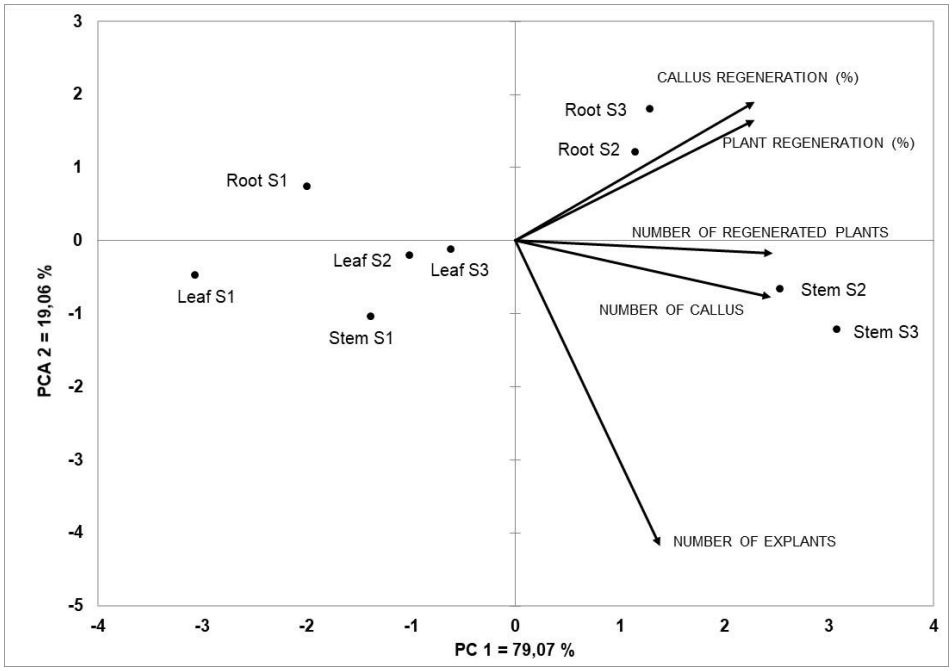

Figure 5. PCA of callus and shoot regeneration of *H. pallasii* tissue culture media containing TDZ, TDZ and Zeatin.
Şekil 5. TDZ ve TDZ + Zeatin içeren besi ortamında yetiştirilen H. pallasii bitkisinde kallus ve sürgün rejenarasyonunun Temel Bileşen Analizi.

CONCLUSION

Based on obtained results, it can be said that the highest percentage of regenerated plants was obtained using stem and root explants on S3 medium and using leaf explants on S2 medium. This study, tested the regeneration ability of immortal flower using three different explants successfully that will contribute to the breeding and secondary metabolites studies in future and the current study will be beneficial in terms of reducing the duration of breeding time.

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