

***IN VITRO* MICROPROPAGATION OF GARDEN THYME (*THYMBRA SPICATA* L. VAR. *SPICATA* L.) COLLECTED FROM SOUTHEASTERN TURKEY USING COTYLEDON NODE**

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ABSTRACT

Thymbra spicata L. from Labiaceae family is perennial plant that prefers dry sunny hillsides and high dry meadows. It bears beautiful purple white flowers and bloom from June to August. The essential oils found in different parts of *Thymbra spicata* make it an important antibacterial and antioxidant natural source. It is used in food industry as spice and for treatment of asthma and bronchitis in the traditional system. Micropropagation can help to produce large number of true to type plants in short period of time. The study reports micropropagation from cotyledon node explants obtained from *in vitro* grown plants. These were cultured on MS medium containing 0.05 mg/l TDZ (Thidiazuron), 0, 0.01, 0.02, 0.04, 0.08 and 0.12 mg/l NAA (α naphthalene acetic acid). Developing shoots were rooted on MS medium supplemented with 0.5 mg/l IBA (Indole-3-butyric acid). All plants were acclimatised in the greenhouse.

Keywords: aromatic plant, *in vitro*, micropropagation, TDZ, NAA

Introduction

The genus *Thymus* has about 400 species, all of which are mainly low spreading evergreen perennial sub shrubs. About 40 species are found around the Mediterranean region and in countries of Western Europe to North Africa and in countries lying eastward to Japan. *Thymbra spicata* L. var. *spicata* L. family Labiaceae is perennial plant that prefers dry sunny hillsides and high dry meadows. The height of plants varies between 15-40 cm and the plants bear beautiful purple white flowers (7).

They have considerable ornamental value and bloom from June to August. They are also popular with herbalists because the oil contains thymol, carvacrol and γ -terpinene, phenols with important range of important biological activities and pharmacological properties. The essential oils found in different parts of *Thymbra spicata* make it an important antibacterial and antioxidant natural source (1). The plant is used in the traditional medicinal system of Turks, Greeks, Egyptians and Romans to treat asthma and bronchitis besides the use in food industry for flavour, aroma and preservation.

In general, collectors uproot thyme in the belief that this prepares the ground for smaller plants to grow. However, indiscriminate uprooting in an area containing thyme species of limited distribution could lead to their extinction. This is believed to be the case with many species of *Thymus*. Therefore, the study reports an efficient and reproducible protocol for rapid and large-scale propagation of *T. spicata* by shoot proliferation from cotyledon node explants.

Materials and Methods

Mature seeds were obtained from Assoc. Prof. Dr. Suleyman Kizil, Department of Field Crops, Dicle University, Diyarbakir, Turkey. The Seeds were treated with 50% commercial bleach for 15 min followed by 3x5 min rinsing with sterile distilled water and germinated on MS medium (4). The seed germination started after 17-20 days of culture. The cotyledon node explants were obtained from 9-10 days old *in vitro* germinated seedlings and cultured on MS (4) medium (control) or MS medium containing 0.05 mg/l TDZ, 0, 0.01, 0.02, 0.04, 0.08 and 0.12 mg/l NAA. Developing shoots were rooted on MS medium supplemented with 0.5 mg/l IBA.

The pH of all media was adjusted to 5.6-5.8 with 1 N KOH or 1 N HCl before autoclaving at 121°C, 118 kPa for 20 min. All cultures were incubated at 24±1°C under cool white fluorescent lights (30 000 lux) with 16 h light photoperiod.

Observations and Statistical analysis

Each treatment had 4 replicates (Petri dishes) containing 5 explants and all experiments were repeated twice (4x5x2=40 explants). Data was analysed with SPSS 16.0 using one way ANOVA and the post hoc tests were performed using Duncan's Multiple Range test. Data given in percentages were subjected to arcsine transformation (6) before statistical analysis.

Results and Discussion

The results showed that thidiazuron acted as an effective regeneration material in the presence or absence of any concentration of NAA (Table 1). Induction of shoot regeneration started after one week of culture with initiation of shoot meristems followed by growth of shoot buds thereafter. Shoot regeneration was recorded on all MS regeneration media containing 0.05 mg/l TDZ with or without NAA.

TABLE 1

Effects of TDZ-NAA on axillary shoot regeneration from cotyledonary node explants of *T. spicata*

Treatments		Frequency of shoot regeneration	Number of shoots per explant	Shoot length (cm)
TDZ (mg/l)	NAA (mg/l)			
MS (control)		0.00	0.00	0.00
0.05	0.00	43.33 b	2.67 d	0.40 c
0.05	0.01	86.67 a	5.00 c	0.44 c
0.05	0.02	86.67 a	7.33 b	0.77 b
0.05	0.04	93.33 a	9.67 a	1.21 a
0.05	0.08	100.00 a	10.33 a	1.36 a
0.05	0.12	100.00 a	7.71 b	0.41 c

Values with in column followed by different small letters are significantly different at the 0.05 level by Duncan's test. Each value is the mean of 3 replications each with 10 plants.

However, very low frequency of shoot regeneration (43.33%) was recorded on MS regeneration medium without NAA. Thereafter, each increase in the concentration of NAA in the regeneration medium resulted in increase in the frequency of shoot regeneration but had statistically similar effect. No regeneration was recorded on MS medium without plant growth regulators (control).

The number of shoots per explants was in the range of 2.67-10.33. The smallest (0.40 cm) and the longest (1.36 cm) shoots were recorded on MS medium containing 0.05 mg/l TDZ and 0.05 mg/l TDZ-0.08 mg/l NAA (Fig. 1a), respectively. Addition of NAA in the regeneration medium had promotory effect and each increase in the concentration of NAA up to 0.08 mg/l resulted in corresponding increase in the number of shoots per explants and shoot length. Thereafter, at 0.05 mg/l TDZ-0.12 mg/l NAA, a sharp decline in the number of shoots per explant and shoot length was very evident.

Rooting

Irrespective of the concentration of TDZ-NAA in shoot regeneration media, regenerated shoots started rooting in the rooting medium containing 0.5 mg/l IBA within 10 days. All shoots developed roots in three weeks time (Fig. 1b). They were transferred to pots successfully and acclimatized in the greenhouse (Fig. 1c).

Efficient micropropagation of *T. spicata* under *in vitro* conditions is an important step toward improvement of this neglected but important medicinal plant. The protocol provides an alternative and rapid mean for improvement and multiplication of *T. spicata* through tissue culture. Cotyledon node culture provides a powerful tool to produce healthy and vigorously growing planting material, which can also help to produce true to type plants genetically similar to their parent plants.

Shoot regeneration behavior of cotyledon node used in the experiment showed variation under the influence of TDZ-NAA concentrations. This is in agreement with Mendes and Romano (3), who found that *in vitro* shoot proliferation of *T. mastichina* was strongly dependent on cytokinin supplied to the medium. However, the results are not in agreement with Saez et al. (5), who found that increase in the concentration of BAP and IAA did not improve the proliferation rate.

Least shoot regeneration and shoot length were recorded on MS medium containing 0.05 mg/l TDZ. That might be due to inhibitory effect of TDZ when used alone, which ultimately resulted in lower shoot regeneration, number of shoots per

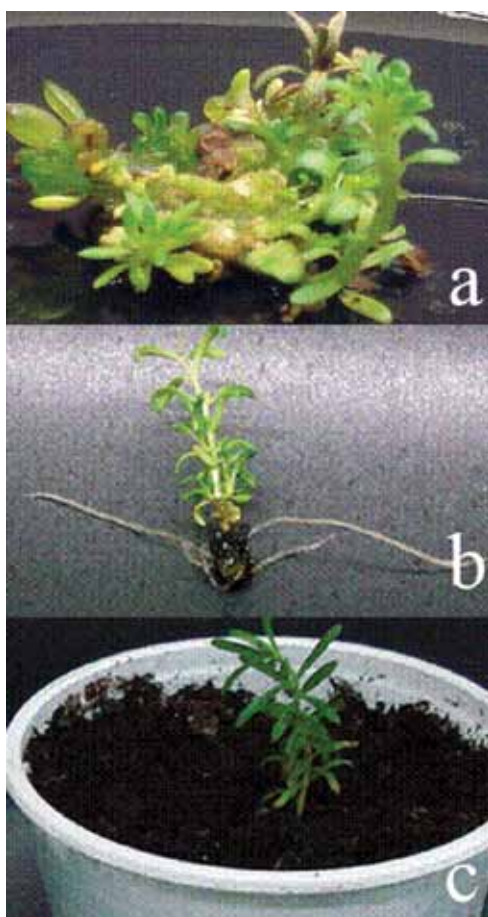


Fig. 1. Micropropagation of *Thymbra spicata* (a) shoot regeneration from cotyledonary node explant on MS medium containing 0.05 mg/l TDZ and 0.08 mg/l NAA (b) rooting on MS medium containing 0.5 mg/l IBA (c) acclimatization in the greenhouse.

explant and shoot length. However, adding any concentration of NAA in the regeneration medium seemed to neutralise negative effects of TDZ. This is in agreement with Le (2) in *T. vulgaris*, Saez et al. (5) in *T. piperella*, Mendes and Romana (3) in *T. mastachina*. All of them observed high percentage of rooting without any difficulty using various auxins.

Conclusions

On the basis of results, it is concluded that this difficult to regenerate plant could be easily regenerated from cotyledonary nodes. This protocol could also be easily utilised for genetic improvement of this important medicinal and ornamental plant for future use in *in vitro* mutation breeding.

REFERENCES

1. **Dorman H.J.D. and Deans S.G.** (2000) J. Appl. Microbiol., **88**, 308-316.
2. **Le C.L.** (1989) Microbouturage Vitic. Arboric., **21**, 355-358.
3. **Mendes M.L., Romano A.** (1999) Acta Hort., **502**, ISHS.
4. **Murashige T., Skoog F.** (1962) Physiol. Plant., **15**, 473-497.
5. **Saez F., Sanchez P., Piqueras A.** (1994) Plant Cell Tiss. Organ Cult., **39**, 269-272.
6. **Snedecor G.W., Cochran W.G.** (1967) The Iowa State University Press, Iowa, USA.
7. **Tubives** (2009) <http://www.tubitak.gov.tr/tubives> dated 4 April 2009.