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Review Article

THYMUS PERSICUS (RONIGER EX REACH. F.) JALAS: A MINI-REVIEW

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ABSTRACT
Thymus persicus (Ronniger ex Rech.f.) Jalas (Lamiaceae) is a *Thymus* species endemic to Iran. The essential oil derived from the vegetative and reproductive aerial parts, which tend to be rich in carvacrol and thymol, have antimicrobial and anti-insecticidal activity. The *in vitro* propagation of *T. persicus* has been achieved through shoot tip culture or shoot regeneration from leaves. Polyploid induction is possible through the use of colchicine. RAPD analysis has been used to verify the clonal stability of *in vitro* regenerants. Betulinic, oleanolic and ursolic acids have been found to exist in *in vitro* plants at levels equivalent to plants growing in the wild. The only molecular work conducted on *T. persicus* has been the use of cluster analysis based on internal transcribed spacers of nuclear ribosomal DNA. Given the important chemical constituents of the essential oil of this plant, and its threatened status in Iran, biotechnology would be a suitable way to mass propagate this aromatic and medicinal plant.

Keywords: *Biotechnology, Essential Oil, Microtuber, Plant Growth Regulator, Tissue Culture*

INTRODUCTION
Biological Properties of Essential Oil
Thymus persicus (Ronniger ex Rech.f.) Jalas (Lamiaceae; The Plant List 2016) is a *Thymus* species endemic to parts of the northwest of Iran (Rechinger, 1982).
Thymus species are appreciated for their essential oils (EOs) that are used in the spice, perfumery and cosmetic industries. Sefidkon *et al*. (2002) found that the main constituents in the EO of aerial parts of *T. persicus* collected from Mahneshan (Zanjan province) in the vegetative and flowering stages following hydrodistillation were: carvacrol (39.0%, 27.1%), geraniol (15.7%, 9.4%), *p*-cymene (7.5%, 10.2%), thymol (6.5%, 11.9%), γ-terpinene (6.1%, 6.5%) and geranyl acetate (5.3%, 5.3%). Rasooli and Mirmostafa (2003) noted that the main constituents of the EO of *T. persicus* leaves collected from Damavand (Mazandaran province) and hydrodistilled were (vegetative stage; flowering stage): carvacrol (38.96%, 27.07%), thymol (6.48%, 11.86%), *p*-cymene (7.51%, 10.16%), γ-terpineol (0%, 9.51%), nerol (15.66%, 9.41%), γ-terpinene (6.11%, 6.51%), and thymol acetate (5.29%, 5.30%). The values of the EO constituents reported by Rasooli and Mirmostafa (2003) were strangely near-identical to the values reported by Sefidkon *et al.* (2002) while Rasooli (2005) represents a duplication of the Rasooli and Mirmostafa (2003) data. Talei and Meshkatalsadat (2007) found that the main constituents in the EO from the hydrodistilled leaves of plants (developmental stage not indicated) collected from Zagross Mountain (Lorestan province) were: thymol (10.71%), carvacrol (25.71%), γ-terpinene (5.63%), α-pinene (1.14%), β-pinene (1.02%), limonene (11.65%) *trans*-sabinene hydrate (7.78%) and 1-borneol (4.07%). The same data had already been reported by Meshkatalsadat *et al.* (2006), and also by Sfaei-Ghomi *et al.* (2009), although the relative composition of several compounds were oddly different, while others were identical. Sajjadi (2002) found that the hydrodistilled aerial parts during the flowering phase of *T. persicus* collected near Hammadan and Zanjan (north of Iran) and Isfahan (center of Iran), showed the following constituents in the EO: thymol (42.3%), *p*-cymene (23.9%), γ-terpinene (9.8%), carvacrol (4.7%), linalool (2.3%) and borneol (1.9%). However, no other detailed results were presented, nor were differences between EO constituents among the three geographic locations indicated. All these studies used GC-MS to assess the volatile constituents. EOs accumulate in the glandular capitate trichomes (Tavan *et al*., 2015). *T. persicus* EO showed an antibacterial effect against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* when diluted four-fold (vegetative), against *Klebsiella pneumonia* when diluted two-fold,

E. coli, *S. aureus*, and *K. pneumonia* when diluted four-fold (flowering), and *B. subtilis* when diluted 16-fold (flowering) (Rasooli and Mirmostafa, 2003). The *T. persicus* EO isolated by Talei and Meshkatalsadat (2007) showed strong antibacterial activity against *Pseudomonas aeruginosa*, even at a 1280-fold dilution.

Mirjalili *et al.* (2016) found 856.9, 480.6 and 941.7 mg/100 g dry weight of plant of betulinic acid, oleanolic acid and ursolic acid, three pentacyclic triterpenoids with multiple biological activities, in lyophilized and powdered aerial parts of the flowering stage of *T. persicus* collected from Baderloo (Takab, West Azerbaijan province).

Saroukolai *et al.*, (2010) found that the EO derived from the hydrodistillation of the dry leaves and flowers of *T. persicus* collected from Zagros Mountains in Lorestan province, and rich in carvacrol (44.69%) and thymol (11.05%), could inhibit the adults of two storage insect pests, the red flour beetle, *Tribolium castaneum* (Herbst), and the rice weevil, *Sitophilus oryzae* (L.).

Tissue Culture and in Vitro Micropropagation

Using seeds of *T. persicus* plants collected from Baderloo (Takab, West Azerbaijan province), Bakhtiar *et al.* (2014) first surface disinfected them by soaking in 70% ethanol for 1 min, then in 5% sodium hypochlorite for 8 min, and a wash in three rinses of sterilized distilled water. Using half-strength (Murashige and Skoog, 1962) basal medium with 1% sucrose, seeds were germinated in the dark at 25°C, with a 85% germination success rate. After three days, seedlings were elongated in the light (16-h photoperiod; 40 µmol m^{-2} s^{-1}) on full-strength MS medium. The response of shoot tips derived from *in vitro* seedlings and surface sterilized wild plants was compared. MS supplemented with 3% sucrose and 8.9 µM 6-benzyladenine (BA; see notes in Teixeira da Silva, 2012) resulted in highest shoot multiplication: 83.3% of shoot tips proliferated, forming an average of 4.4 shoots/explant. The use of kinetin and thidiazuron was also successful, but resulted in fewer shoots per explant (maximum of 2.8 and 2.5, respectively). When 8.9 µM BA was combined with an auxin (2.7 µM 1-naphthalene-acetic acid (NAA)), 7.1 shoots could form per explant. When NAA was substituted for other auxins (2,4-D, IAA or indole-3-butyric acid (IBA)), shoot development was at most half the amount when NAA was used, but IBA at 2.5 µM in half-strength MS medium resulted in most root formation from shoots. Rooted plantlets showed 80% survival in a substrate of peat moss and perlite (1:1). *In vitro*-derived plantlets showed little genetic variation when assessed by RAPD analysis, but banding pattern differed from seedling tissue. *In vitro* plantlets were able to produce betulinic, oleanolic and ursolic acids. Thus, this protocol allows for the mass production of *T. persicus*, which is considered to be an endangered plant in Iran (Jalili and Jamzad, 1999).

Using the Bakhtiar *et al.* (2014) protocol as base, Bakhtiar *et al.* (2016) induced callus from 100% of leaves and stems of *in vitro* plantlets in MS medium in the presence of 2.0 mg L^{-1} NAA and 0.5 mg L^{-1} kinetin. 96% of callus could then be induced to form shoots in the presence of 2.0 mg L^{-1} BA and 1.0 mg L^{-1} NAA. The genetic stability of *T. persicus* plantlets derived from this indirect route was not shown, nor was the production of betulinic, oleanolic and ursolic acids proved.

Tavan *et al.* (2015) used colchicine to alter ploidy in *T. persicus* shoot tips derived from *in vitro* plantlets using the Bakhtiar *et al.* (2014) protocol. Whereas the chromosome number of mother plants was $2n = 2x = 28$ (2C DNA = 1.20 pg), colchicine-induced autotetraploids had $2n = 4x = 56$ (2C DNA = 2.39 pg). When 960 shoot tips were exposed to 0.3% colchicine for 12 h, 7.8% were tetraploid while 1.04% were mixoploid. Tetraploids were slightly stunted, and diploid, tetraploid and mixoploid plants had much lower (half to one-sixth) the levels of betulinic, oleanolic and ursolic acids than from plants assessed by Bakhtiar *et al.* (2014) and Mirjalili *et al.* (2016).

Molecular Biology

Abdolahinia *et al.* (2011) compared the sequences of two internal transcribed spacers (ITS), ITS1 and ITS2, of nuclear ribosomal DNA (nrDNA) of four *Thymus* species (*T. daenensis* subsp. *daenensis*, *T. persicus*, *T. pubescens* and *T. trautvetteri*) in a bid to differentiate them from other thyme species using GenBank, following ITS sequence alignment using ClustalX. Curiously, the ITS sequences of these four *Thymus* species collected from Azarbaijan province were identical, with stable and conserved secondary

structures even though morphological parameters showed wide variation. However, two of the four *T. persicus* accessions could be differentiated in a consensus tree after maximum parsimony analysis of ITS sequences.

Sonboli *et al*. (2013) also used nrDNA ITS, ITS4 and ITS5A, to differentiate 25 *Thymus* accessions from other genera. Using agronomic and climatic variables (type of bed rock, pH, and soil structure), Asbaghian *et al.* (2011) differentiated eight *Thymus* species, including *T. persicus*, using UPGMA-based cluster analysis.

CONCLUSION

The biotechnology of *T. persicus* is still very underexplored most likely because of its geographically restricted area, with all research conducted thus far being in Iran. The research of this medicinal plant would benefit from an understanding of *in vitro* flowering (Teixeira da Silva *et al.*, 2014), organogenesis induced by ultrasound or sonication (Teixeira da Silva and Dobránszki, 2014) by magnetic fields (Teixeira da Silva and Dobránszki, 2015), or by TCL technology (Teixeira da Silva and Dobránszki, 2013; Teixeira da Silva *et al.*, 2015).

Conflicts of Interest

The author declares no conflicts of interest.

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