

Genetic and biochemical diversity assessment of promised population thyme collected from Iran

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Abstract

Thymus is the eighth important genera of *Lamiaceae* family with regard to the number of containing species. It's been used as a medicinal, aromatic and spicy plant. Demand for thyme products is growing and is not likely to be supported by collecting from natural populations because of insufficient/irregular rainfall in traditional source areas as well as destruction of its natural habitat. In this investigation nine populations (four population of *Thymus kotchyanous* from different part of Iran, one population of *Thymus daenensis* collected from Zanjan and four cultivars of *Thymus vulgaris*) planted in RCBD design with 3 replicates. Morphologically important traits were recorded to assess the genetic diversity within the *Thymus* genus. Considering dry matter yield, *T. danensis* with 4.1 tons/ha and 60% thymol component in essential oil has been selected as the most recommended population for cultivation in the cold region conditions. In the second rank, *T. kochyanous* code 54 had 3.5 tons/ha dry matter yield and 60% carvacrol content in essential oil samples. In conclusion, *T. daenensis* and *T. kotchyanous_70* could selected for next phase of seed production.

Key words: Thyme, Variation, *Thymol*, Carvacrol, Iran

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Introduction

Thyme is a medicinal herb of the genus *Thymus* L. and family of *Lamiaceae* (Morales, 2002). *Thymus* species, belonging to the family *Lamiaceae*, includes more than 215 species in the world, in which 14 species are distributed in Iran including *T. daenensis*, *T. carmanicus*, *T. fallax*, *T. persicus*, *T. trautvetteri*, *T. migricus*, *T. kotschyanus*, *T. pubesens*, *T. nummularius*, *T. transcaspicus*, *T. eriocalyx*, *T. caucasicus*, *T. transcaucasicus*, and *T. fedtschenkoi* (Jamzad, 2010). That is one of the oldest medicinal plants and possesses various applications in the food, pharmaceutical and cosmetic industries (OmidBeigy, 2007). At present, the demand of essential oils for this herb is raised for perfumery, cosmetic and medicinal use deprived of any breeding programs to select proper cultivars. In traditional herbal medicine, *Thymus* species are greatly used as tonic, antiseptic, antitussive and carminative (Amin, 2005; Ghasemi Pirbalouti et al., 2011). There are studies about thyme, such as genetic variation (Alamdary et al., 2011; Trindade et al., 2009; Khoshokhan et al., 2014), karyotypic (Daftari and Safarnejad, 2011) and in vitro selection (Zia et al., 2011). Regarding the application of thyme in different industries,

the breeding and creating thyme genotypes with high qualitative and quantitative yield is imperative. The main breeding objective of *Thymus* species is to produce plants with higher yield of dry matter, ratio of leaf/shoot, essential oil, *thymol* content, crop uniformity, freezing tolerance in winter, upright form of growth, and finally possibility of mechanizing harvesting (Carlen et al., 2010). In this regard, the simplest way for thyme breeding is evaluation of their populations and then selecting the elite genotypes based on their and chemotype traits (Heydari et al., 2019). Approximately, 60 to 75% of current medicinal crops have been produced through selecting elite genotypes among wild or landraces populations (Bernat, 2000; Nemet, 2000). In terms of dry matter yield, essential oil content, and chemical composition, thyme with higher qualitative traits and uniformity, are highly respected. Progress in plant breeding requires an extended genetic pool (Rahimmalek et al., 2009). Awareness of genetic diversity in crops and their wild relatives is an underlying requirement to improve crop yield (Govindara et al., 2015).

Materials and Method

Plant material

Nine populations and cultivars of *Thymus* sp. has been collected from the previous experiments were conducted by authors. The

species details and their original location characteristics were indicated in the Table 1.

Table 1. Thyme populations and cultivars investigated

Populations	Province	Altitude
<i>T. kotchyanus_5</i>	Ghazvin	2000
<i>T. kotchyanus_22</i>	Ghazvin	1500
<i>T. kotchyanus_54</i>	W.Azerbaijan	1389
<i>T. migricus_59</i>	W.Azerbaijan	1920
<i>T. kotchyanus_70</i>	W.Azerbaijan	1487
<i>T. daenensis_c4</i>	Zanjan	2000
Commercial cultivar	<i>T. vulgaris</i> Wagner <i>T. vulgaris</i> Varico2 <i>T. vulgaris</i> Spain	

Experimental design

To evaluate the genetic and biochemical variation, thyme populations were planted in a RCBD design with ten treatments (thyme populations) and three replications. Each population of thyme comprised 4 rows planted in 50 x 50 cm spaces. Planting and maintenance of plants were conducted as routine cultivation and grow practices. Regards with the traits in the second and third year after planting the seedlings, shoot yield in 50% flowering time, essential oil yield and essential oil composition were

recorded and data analyzed by RStudio Version 1.1.447 – © 2009-2018 RStudio, Inc. The leaf essential oil samples were analyzed by GC-MS. We used an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass selective detector. The GC column was an HP-5 MS fused silica capillary column a film thickness of 0.25 µm, a length of 60 m, and an internal diameter of 0.25 mm.

Results and Discussion

Dry matter Yield

Comparison of different cultivars and populations of thyme in field conditions showed that there is considerable variation in terms of shoot yield and essential oil yield. Among the nine studied populations, in terms of both yield and essential oil criteria, the population of thyme with the code C4 (*T. daenensis*-C4) with 4.1 ton / ha of airway yield and essential oil yield of 1.5 gr / plant in the first place and population Thyme code 54 (*T. kotschyanus*-54) with 3.5

ton / ha yield of aerial part and 1.6 gr / plant essential oil yield in the second place and the population of thyme code 70 (*T. kotschyanus*-70) with 3.3 ton / ha yield Aerial part and 1.4 gr / plant essential oil yield were in the third place (Fig. 1). It seems *T. daenensis* as a landrace could exhibit its potential yield compare to other populations collected from other regions.

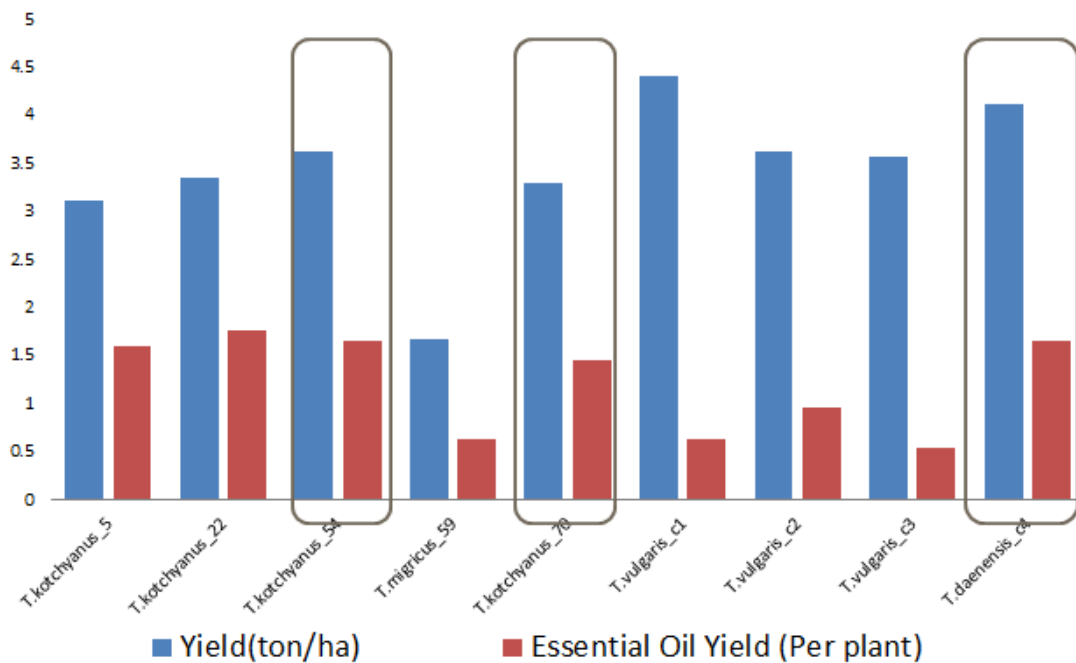


Fig. 1. Comparison of nine thyme promised populations and cultivars regards with the dry matter yield.

Essential oil composition

As shown in Fig. 2, different populations were compared in terms of essential oil composition. At this stage of selection, the

best population of thyme, the content of two isomer compounds of essential oil of thyme plants thymol and carvacrol were compared. For more accurate selection, the sum of the percentages of these two compounds was

used. Accordingly, the population of thyme (*T. kotschyanus*-70) with 75% of the total thymol and carvacrol placed in the first rank and the population *T. daenensis*-C4 with

66% of the content of carvacrol and thymol. In the second place and the population *T. kotschyanus*-54 with essential oil were located in the third place.

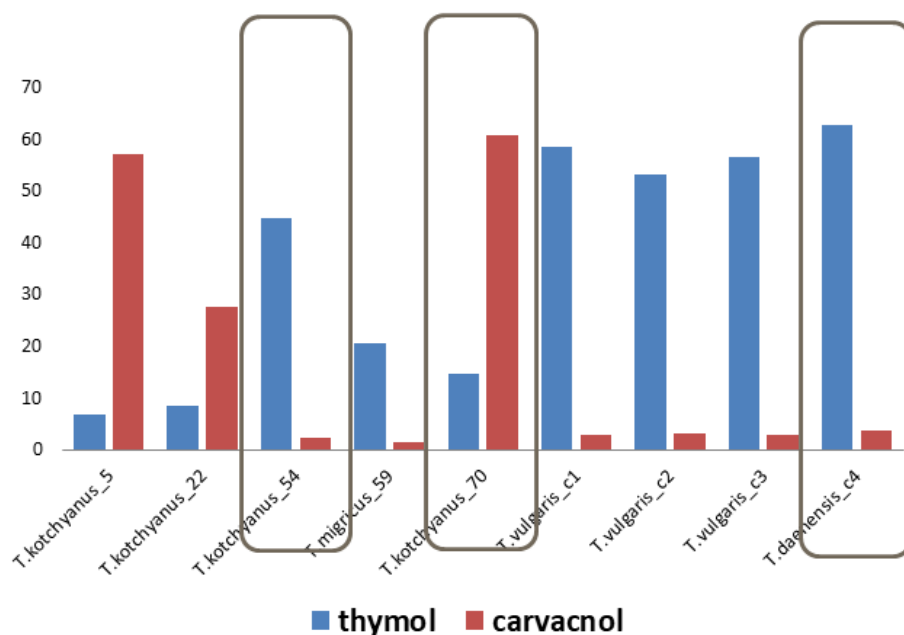


Fig. 2. Comparison of nine thyme promised populations and cultivars regards with Thymol and Carvacrol percentage in shoot essential oil content.

Conclusion

Within the nine-population investigated in the same environmental conditions, three genotypes could be selected as promised lines to propagate, since they have the highest dry matter yield and highest essential oil yield. These lines are including *T. kotschyanous*_54, *T. kotschyanous*_70 and *T. danensis*_C4. Next phase of this

investigation would be the large-scale seed production to release as new cultivars following required standard field trials.

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References

- Alamdary S.B.L, Safarnejad A, Rezaee M, (2011) "Evaluation of genetic variation between *Thymus* accessions using molecular markers" *Journal of Basic and Applied Scientific Research*, 1:2552-2556.
- Amin G, (2005) "Popular medicinal plants of Iran" Tehran University of Medical Sciences Press. Tehran, (In Persian).
- Bernath J, (2002) "Strategies and recent achievements in selection of medicinal and aromatic plants" *Acta Horticulturae*, 10.17660/ActaHortic.2002.576.19
- Carlen C, Schaller M, Carron C.A, Vouillamoz J.f, Baroffio C.A, (2010) "The new *Thymus vulgaris* L. hybrid cultivar (Varico 3) compared to five established cultivars from Germany, France and Switzerland " *Acta Horticulturae*, 860, 161-166.
- Daftari Z, Safarnejad A. Karyotypic, (2012) "Study of four *Thymus* species (*T. pubescens*, *T. fedtschenkoi*, *T. daenensis*, *T. lancifolius*)" *Iranian Journal Rangelands Forests Plant Breeding Genetic Research*, 19:241-250.
- Ghasemi Pirbalouti A, Karimi A, Yousefi M, Enteshari S, Golparvar AR, (2011) "Diversity of *Thymus daenensis* Celak article as a key speaker in the congress. in central and west of Iran" *Journal of Medicinal Plants*, 5:319-323.
- Govindara J.M, Vetriventhan M, SrinivaSan, M, (2015) "Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives" *Genetics Research International*, 2015:431487.
- Heydari A, Hadian J, Esmaeili H, Kanani M.R, Mirjalili M.H, Sarkhosh A, (2019) "Introduction of *Thymus daenensis* into cultivation: Analysis of agromorphological, phytochemical and genetic diversity of cultivated clones" *Industrial Crops and Products*, 131:14-24.
- Jamzad Z, (2010) "*Thymus* and *Satureja* species". Institute Research Forests and Rangeland, 171 pages. (In Persian).
- Khoshsokhan F, Babalar M, Fatahi M, Poormeidani A, (2014) "Assessment of genetic diversity of some wild populations of *Thymus Kotschyanus* using RAPD molecular markers" *Cercetari Agronomice in Moldova*, 3:71-81.
- Morales R, (2002) "The history, botany and taxonomy of genus *Thymus* Elisabeth Stahl- Biskup and Saez, F. (eds). The genus *Thymus*", 1-124.

- Németh E, Szekely G, (2000) "Floral biology of medicinal plants II. Lamiaceae species" International Journal of Horticultural Science, 6:137-140.
- OmidBeigy R, (2007) "Production and processing of medicinal plants" Second Volume. Astan Qods Razavi publications. 280 pages. (In Persian).
- Rahimmalek M, Bahreininejad B, Khorrami M, Tabatabaei S.B.E, (2009) "Genetic variability and geographic differentiation in *Thymus daenensis* subsp. *daenensis*, an endangered medicinal plant, as revealed by inter-simple sequence repeat (ISSR) markers" Biochemical Genetics, 47:831-842.
- Stahl- Biskup E, Saez F, (2002) *Thyme*. 1st ed. Taylor and Francis. England, 346 pages.
- Trindade H, Costa M, Lima AS, Pedro LG, Ana C, Figueiredo J, Barroso G, (2009) "A combined approach using RAPD, ISSR and volatile analysis for the characterization of *Thymus caespitius* from Flores, Corvo and Graciosa islands (Azores, Portugal). Biochemical Systematics and Ecology, 37:670-677.
- Zia A, Rezanejad F, Safarnejad A, (2011) "In vitro selection for NaCl tolerance in *Thymus vulgari* L." Journal of Cell and Molecular Research, 2:86-92.