



## Phylogenetic relationships among the Iranian *Triticum* diploid gene pool as inferred from the loci *Acc1* and *Pgk1*

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### Abstract

In this study, 129 individuals belonging to the Iranian wild diploid *Triticum* gene pool were screened using PCR-SSCP based on *Acc1* and *Pgk1* loci. Sequencing of the representatives of different SSCP patterns allowed for detection of three haplotypes for each of the loci. The phylogenetic relationships among the Iranian wild diploid *Triticum* haplotypes and related genera were analyzed using DNA sequences from these two single copy loci. Despite previous reports that recommended the usefulness of nuclear single copy genes for phylogenetic inference, the Bayesian and parsimony analyses carried out in this study showed that these two loci were not applicable tools in the phylogeny reconstruction of diploid *Triticum* gene pool. Thus, our findings confirm that phylogenetic relationships among recently diverged species are often difficult to resolve due to insufficient phylogenetic signal in available markers and/or conflict among gene trees.

**Key words:** conflict, phylogeny, *Triticum monococcum* subsp. *aegilopoides*, *T. urartu*

### Introduction

Among the other genera belonging to the Triticeae (Poaceae), *Triticum* Linnaeus (1753: 85) gene pool with three ploidy levels is of vital importance for wheat cultivation. Repetitive hybridizations at different ploidy levels and introgression mean this genus can be considered a polyploid pillar complex (Zohary 1997, Vakhitov *et al.* 2003). Moreover, the multiple polyploidization events between *Triticum* and the other related taxa such as *Aegilops* Linnaeus (1753: 1050) were an important force driving diversification during the evolution of *Triticum* (Tsunewaki 2009, Matsuoka 2011). Diploid *Triticum* einkorn wheat with the genome AA occurs throughout the Fertile Crescent and Iran is of great importance in the origin of cultivated wheat (Vavilov 1992). The A genome of *T. urartu* Thumanjan ex Gandilyan (1972: 176) is particularly significant due to its role in creating *T. turgidum* Linnaeus (1753: 86) (genomes AABB) and *T. aestivum* Linnaeus (1753: 85) (genomes AABBDD) (Dvorak *et al.* 1993).

The availability of large amounts of new DNA sequences has recently led to a significant increase in molecular phylogenetic studies (Soltis and Soltis 1998). The nuclear genes *Acc1* and *Pgk1* are predominantly single copy in grasses and encode plastid acetyl-CoA carboxylase and plastid phosphoglycerate kinase, respectively. These two genes, together or separately, have been frequently used for phylogenetic analyses in wheat and other Poaceae (Huang *et al.* 2002a, b; Kilian *et al.* 2007a; Chalupska *et al.* 2008; Golovnina *et al.* 2007, 2009; Fan *et al.* 2007, 2009, 2012; Chen *et al.* 2013; Adderley and Sun 2014).

The diploid *Triticum* gene pool in Iran comprises *Triticum monococcum* Linnaeus (1753: 86) subsp. *aegilopoides* (Link) Thellung (1918: 470) and *Triticum urartu* (Nasernakhaei *et al.* 2013). They are characterized by two diagnostic characters: anther length and leaf indumentum (Nasernakhaei *et al.* 2013); these two characters are also useful at the generic level. In order to provide an overall summary of the diploid *Triticum* taxa a brief taxonomic key (van Slageren 1994) is reproduced below:

- |    |   |   |
|----|---|---|
| 1- | Wild species, white trichomes present at the base of the spikelets, leaf blade surface with trichomes .....         | 2   |
| 1- | Cultivated species, white trichomes absent at the base of the spikelets, leaf blade surface without trichomes ..... |   |
|    | ..... <i>T. monococcum</i> subsp. <i>monococcum</i>   |   |
| 2- | Leaf blade surface loosely covered with long whitish trichomes, anthers 5–6 mm long.....                            | <i>T. monococcum</i> subsp. <i>aegilopoides</i> |
| 2- | Leaf blade surface densely covered with very short whitish trichomes, anthers 2–3 mm long .....                     | <i>T. urartu</i>                                |

Acc1 results indicated a sister relationship between *Ae. tauschii* and *Triticum* species (Fig. 4) as in Kellogg and Apples (1995) and Mason-Gamer and Kellogg (1996) while the unresolved position in *Ae. tauschii* and *Triticum* species in P<sub>gk1</sub> tree is congruent with the previous molecular analysis, e.g. nrDNA ITS (Hsiao *et al.* 1995) and chloroplast DNA (Yamane and Kawahara 2005).

Our analyses (Fig. 4 and Fig. 5) placed *S. cereale* and *H. vulgare* in an unresolved sister position to the *Triticum-Aegilops* clade. This result is in agreement with Kellogg and Apples (1995) which used 5S RNA genes for investigating phylogenetic relationship among Triticeae.

This study confirms the common difficulty in resolving relationships among closely related, recently diverged lineages (Maddison and Knowles 2006). The problem can partly be explained by the fact that DNA fragments commonly chosen for sequence analysis lack sufficient phylogenetic resolution for the lowest taxonomic levels. Such limited amounts of data result in phylogenetic inaccuracy that can be explained by systematic errors or inability to distinguish between gene histories and species histories, as discussed by Page and Charleston (1997).

Additionally, major evolutionary mechanisms which include incomplete lineage sorting and horizontal gene transfer complicate the task of interpreting phylogeny as a bifurcating history (Maddison 1997). From this point of view, incomplete lineage sorting can be estimated based on a statistical noise, arising from stochastic coalescence that can mask the distinguishing styles of true phylogenetic divergence events. On the other hand, horizontal gene transfer both in the form of introgressive gene flow and hybrid speciation can result in a reticulate evolution (Eaton and Ree 2013). Above processes might have had a higher prevalence between closely related species and populations characterized by incomplete reproductive isolation in diploid *Triticum* gene pool.

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