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Evaluation of genetic variations in thirteen Iranian, German false chamomile populations using peroxidase isozyme bands pattern

Fatemeh Noori [1], Parvin Salehi Shanjani [*2] , Amir Hossein Bandar Abadi [1], Mohamad Ali Alizadeh [2], Reza Tabaei Aghdaei [2] and Rahim Hadadi [1]

[1]Department of Plant Breeding, Imam Khomeini International University, Ghazvin, Iran

[2] Research Institutes of Forests and Rangelands, P.O.BOX 13185-116, Tehran, Iran

ABSTRACT

Chamomile serves as one of the most important medicinal plants. The most known secondary metabolites in chamomile species are volatile oils of triponoides, poly stilens, flavonoids and phenolic cafeic acid (Krori). These metabolites have wide variety of applications thanks to having medicinal properties of anti-inflammation, antispasmodic and bactericide activity. Also it is used to cure liver disorders and icterus (Zargari). Isozyme has been used successfully as biochemical markers n specific genetic and plant breeding areas. Isozymes serve as different molecular form of enzyme with protein nature, accelerating the same reactions. These molecules appear on electrophorese through pigmented reaction associated into enzyme function. They are products of different alleles located at locus or loci. The present research was conducted to evaluate genetic variation of thirteen chamomile populations and peroxidase enzyme quality. Peroxidase was extracted from fresh leaves and young seedlings. The PAGE approach was used to evaluations. There were three action sites on polyacrylamide gels called PX-A, PX-B and PX-C. based on results of electrophoreses on peroxidase enzyme, the most and least genetic distances were observed between populations Ghazvain-Ts2, Naghadeh-AT1 and Ghazvin-TS2,Ardabil2 respectively.

Key words: Genetic variations, Iranian, German false chamomile populations, Isozyme bands pattern.

INTRODUCTION

Chamomile is one of the important medicinal plants commonly used in Iranian traditional peahen as painkiller and fever treatment (Zargari). Nowadays there is less introduction of synthetic antioxidant mostly due to their toxicity and medicine communities tend to exploits natural antioxidants (Krouri). Indeed, active medicines and drugs may have various quantity and quality

Corresponding Author: Parvin Salehi Shanjani, E-mail: psalehi@rifr-ac.ir

dependent on species, habitat and harvesting period. Peroxidase comprises extended groups of Fe contained enzymes. Peroxidase enzymes such as catalase, breaks oxygen water molecules into oxygen and water, removing its toxic effects. Isozymes are successfully used as biochemical markers in genetic and plant breeding fields. Biochemical markers are proteins resulted from gene expression. Since thirty years ago, researchers found that evaluation protein polymorphism have great deal of importance in genetic, plant breeding, biochemistry and evolution sciences in particular for plant breeding. Similarly, isozyme polymorphism has become a promising new research method. Isozymes are different forms of enzyme appeared in electrophorese and have been used to study breeding materials and natural populations because of unique characteristics (Abdemishani, 1998).

MATERIALS AND METHODS

Seeds were sown in pots having been developed desirably. Out of 130 genotypes belonged to thirteen populations (10 genotypes for each population); about 1 g leave specimens were separated from every genotype. Samples were homogenized within cold mortar in 1:1.5 rations from extraction solution.at the same time; samples got centrifuged in 3000 cycles for 15 minutes. A part of extracts deposited on dross were sampled by sampler, stored within refrigerator. Inspecting polyacrylamide gel showed that peroxidase enzyme is controlled by three loci PX-A, PX-B and PX-C. Data matrices were formed by counting bands created in each locus. Mean bands number in locus, number of bands frequency equals or up to 25% and 50% rare bands(with frequency less than 0.5) and loci heterogeneity and polymorphism by software Gene Alex (Peakel&Smouse, 2006) were calculated. Inter-and intra-specific genetic variation contributions were determined by molecular variance analysis (AMOVA) (Excoffer, 1992) and ARELEQUITIN101 (Scheneider, 1997). Permutation Excoffier 1992 test was used to evaluate importance of each variance components. Genetic distance was estimated for fourteen chamomile population as per equation NEi (1978). Neighbor- joining test by software MEGA and principle component analysis were used to interpret genetic distance matrices.

RESULT AND DISCUSSION

Results obtained by electrophoresis on peroxidase enzyme isozymes for thirteen chamomile false populations as per PAGE method showed that peroxidase enzyme is encoded by three genes locus so that three action areas are appeared on gel. They evaluated as PX-A, PX-B and PX-C in which two alleles in PX-A, two alleles in PX-B and three alleles in PX-C were observed. Kolagari 2004 studied different community's variations by evaluating peroxidase isozymes on polyacrylamides gel. Results of qualitative action of peroxidase enzyme on eleven trees from eleven habitat and bands emergences, showed different iso-enzyme patterns. Cluster analysis was applied to tree grouping in studied habitats in terms of each band frequencies. Intercommunity genetically distance was calculated to estimate differentiation pattern in chamomile populations. Total mean genetic distance estimated in thirteen populations was 0.392.the highest similarity and least genetic distance was related to populations Gazvain2-T and Ardabil-T with genetic distance 0.010.the least similarity and highest genetic distance between populations Ghazvib2-T and Naghadeh1-At with genetic distance 1.449 respectively. Genetic distance among populations

was used to principle component and cluster analysis as per UPGMA approach among others. Given that in principle component analysis nearly three first components account for 85.85% of variations, so these components are considered as the main ones (figure1). The first, second and third components accounted for 64.22, 11.90 and 9.73% of variations respectively. Ephtekhari 2011 reported that there was high polymorphism among chamomile samples. Principle component analysis based on molecular data showed that three components explained up to 48% of total variances. As it can be seen, populations of *T.seveanense* species and those of *A.tinctoria* were located together individually. Each species dedicated one end of dendrogram. Species *M.recutita* was located between both clusters.

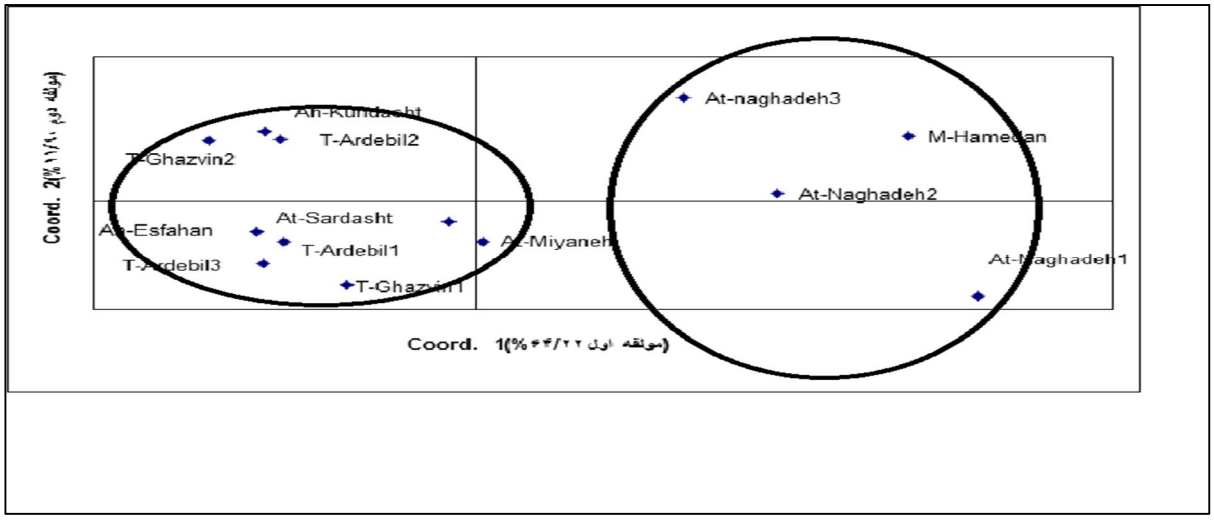

Figure1: ordination graph (PCA) in thirteen chamomile population based on genetic distance resulted from enzyme data

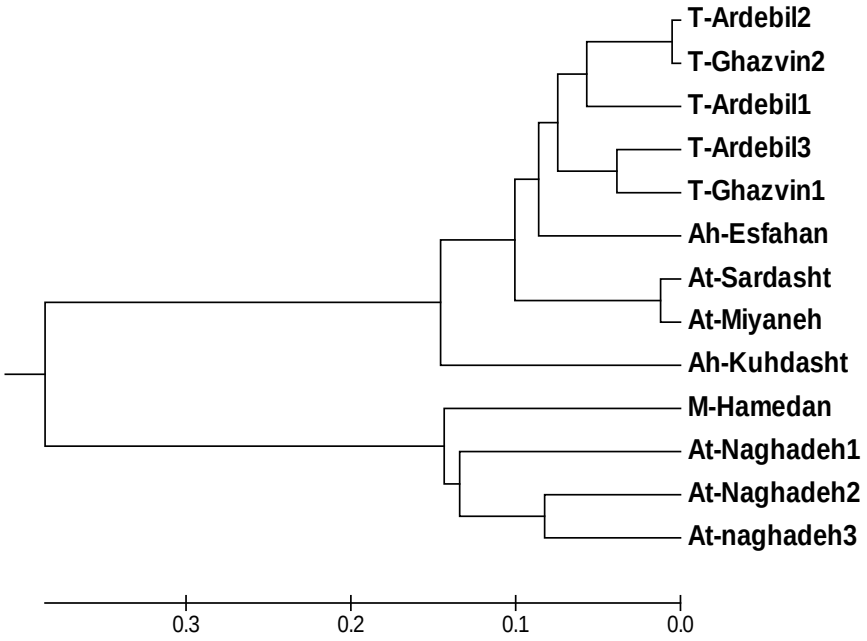

Figure2: dendrogram obtained from cluster analysis as per UPGMA on thirteen chamomile population based on peroxidase enzyme

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