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Multiple Sequence Alignment of Zipper Protein and Defective in Meristem Protein in Four *Prunus* Species

AmirHosein Nazari
9th Grade Student, Toheed Board of Trustees High School, Razi Research Center, District 2, Shiraz, Iran (amirhossein.nazarii2021@gmail.com)*
Sasan Mohsenzadeh
Professor, Department of Biology, Shiraz University of Medical Sciences, Shiraz, Iran
Sara Bostanian
M.Sc. in Developmental–Molecular Biology, Dr. Hesabi Research Center, District 4, Shiraz, Iran
Atefeh Sharafi
Research Supervisor, Dr. Hesabi Research Center, District 4, Shiraz, Iran

Abstract

Prunus is a genus of trees and shrubs in the family *Rosaceae* that naturally occurs in Western Asia and includes plum, cherry, peach, apricot, and almond trees, which contribute to regional climate regulation and play a significant role in biodiversity. To elucidate key characteristics of this genus, the present study focused on two structural proteins of central importance. Zipper proteins, despite their simple architecture, perform essential functions in regulating protein and gene activity. In *Prunus* species, these proteins are critically involved in responses to environmental stress and in the regulation of growth. Their characterization and application in genetic improvement can enhance stress tolerance in plants and lead to the development of more resilient and high-yield cultivars. Defective proteins play a pivotal role in the normal function of the plant meristem, as the meristem comprises proliferating stem cells responsible for plant elongation and radial growth. In this study, the effects of zipper and defective proteins on the resistance of the *Prunus* genus to climatic changes and the extent of their point mutations under different conditions were examined. Sequences were retrieved from the National Center for Biotechnology Information (NCBI) and aligned using the Multalin program. The results indicated the presence of genetic polymorphism attributable to point mutations in the zipper protein sequences of *Prunus dulcis* and *Prunus persica*, and in the defective protein sequences of *Prunus persica*, *Prunus mume*, *Prunus avium,* and *Prunus dulcis*. The findings confirmed that these mutations are beneficial to the plant, increasing resistance to climatic stress, diseases, and pests, and preventing extinction in the face of environmental threats.

Keywords: Prunus, Polymorphism, Genetic, Point Mutation, Leucine Zippers, Meristem

Introduction

Prunus is a genus of trees and shrubs in the family *Rosaceae*, naturally occurring in Western Asia, and includes plum, cherry, peach, apricot, and almond trees. Approximately 200 Prunus species exist, many of which are either deciduous or evergreen. One notable species, wild almond *(Prunus scoparia)*, grows in Iran and exhibits considerable diversity, with 21 wild species identified to date. Notably, the raw fruits of this almond are bitter. Traditional medicine texts refer to this fruit by various names, including *Badamak, Biyu, Arjan, Badamche, Bokhorak, Arjank, Bif, Aqchali,* and *Ghara Chali,* describing its temperament as warm and dry. This species is distributed across Tajikistan, Turkey, Syria, Iran, and Afghanistan.[1]

Due to its hard, woody shell, this plant demonstrates resistance to insects and fungi. Moreover, it contributes to soil erosion control and forest restoration in arid regions, thereby influencing local climate and vegetation.[2]

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Studies have shown that the genetic diversity within and between almond populations is driven by adaptation to their geographic regions.[3]

Proteins perform essential and diverse roles in plants. They act as key cellular components and are involved in various biochemical processes, including metabolism and nutrient transport. Plant proteins generally contain essential amino acids, though their composition may require supplementation from multiple sources to meet plant needs.[4]

Defective proteins play critical roles in plant meristems, which are embryonic tissues responsible for longitudinal growth and formation of new tissues. Key roles of defective proteins in meristems include.[5]

1-Regulation of growth and cell differentiation: Defective proteins modulate the activity of genes involved in growth and cell differentiation, acting as regulatory factors within signaling pathways that enhance or suppress metabolic activity.

2-Cell protection: They may protect meristem cells against environmental stresses such as drought or cold, functioning as antioxidants to prevent oxidative damage.

3-Promotion of cell division: Certain defective proteins stimulate cell division, increasing the number of new cells in meristems, thereby supporting plant growth and development.

4-Hormonal regulation: Defective proteins influence the balance and levels of plant hormones such as auxins and cytokinins, which are critical for growth and development.

Thus, defective proteins serve as key components in plant growth and development, significantly impacting the formation of new structures in meristems.

Zipper proteins constitute a large family of transcription factors in plants, playing a critical role in regulating gene expression in response to various stimuli. These proteins function analogously to the teeth of a clothing zipper, facilitating the association of two proteins to act in a coordinated manner. They possess a specific structural motif within their amino acid sequence that promotes protein dimerization. In this motif, the leucine residue recurs every seventh position, causing the leucines to align on one side of the protein's α-helix. These leucines interact with a corresponding sequence in another protein, forming a structure reminiscent of a zipper. One can conceptualize the leucines as the teeth of a zipper positioned at defined intervals along the helix. When these teeth interlock, the two helices pair, resulting in the formation of an active protein dimer. The subsequent sections discuss the functional roles of zipper proteins.[2,6,7,8]

Zipper proteins are involved in:

1-Response to environmental stress: They regulate expression of genes associated with tolerance to drought, salinity, temperature extremes, and pathogens.

2-Metabolic regulation: They modulate metabolic pathways, including sugar, lipid, and nitrogen metabolism.

3-Plant development: They influence root growth, flowering, and fruit maturation.

4-Hormonal signaling: Certain zipper proteins participate in plant hormone signaling pathways, including *abscisic acid* and ethylene.

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These proteins are crucial for the survival and environmental adaptation of Prunus species, and understanding their functions can inform breeding of stress-tolerant, high-performance plants. Notably, amino acid differences exist among some zipper and defective proteins, corresponding to point mutations. A point mutation is a genetic alteration in which a single nucleotide is substituted, inserted, or deleted in a DNA or RNA sequence. These mutations can variably impact the resulting protein product, with effects generally predictable based on mutation characteristics. Research has shown that in Prunus, a group of bZIP genes becomes active under drought and cold conditions. Genomic studies in almonds have identified over 70 bZIP-related genes, enabling the plant to survive harsh conditions and maintain productivity.[9,10] These bZIP genes can be utilized in almond breeding programs to enhance environmental stress resistance.

To analyze these sequences and genetic mutations, bioinformatics an interdisciplinary field combining computer science, statistics, and biology is employed. Bioinformatics enables identification of patterns and relationships in biological data, including DNA, RNA, and protein sequences, and plays a pivotal role in genetic research, drug development, disease discovery, and understanding biological processes.[6,11]

Analyzing amino acids and proteins requires amino acid alignment, which examines the specific order of amino acids in protein structures. Amino acids, connected via peptide bonds, determine the three-dimensional structure and function of proteins. Changes in their sequence or quantity can significantly affect protein properties and function, potentially leading to disease or genetic disorders. Therefore, amino acid alignment is essential for understanding cellular biochemistry and biology.[12]

Variations observed in amino acid alignments are termed genetic polymorphisms, often resulting from point mutations. Genetic polymorphism refers to diversity in DNA sequences among individuals or populations, manifesting as single nucleotide variations, copy number variations, or larger structural rearrangements. These polymorphisms are key determinants of individual differences, disease susceptibility, and responses to treatments.[13]

Materials and Methods

Initially, six genetic sequences from four Prunus species, each with a unique accession number, were retrieved from the National Center for Biotechnology Information (NCBI) after careful review. The sequences included:

1-Prunus dulcis (XP_034212851)

2-Prunus persica (XP_020417883)

3-Prunus dulcis (XP_034204540)

4-Prunus avium (XP_021814973)

5-Prunus persica (XP_020413631)

6-Prunus mume (XP_008233132)

Among these, sequences 1 and 2 correspond to zipper proteins, whereas sequences 3, 4, 5, and 6 correspond to defective proteins.[14]

After sequence editing to prepare for alignment, multiple sequence alignment was performed using the Multalin program, as illustrated in Figures 1 and 2.[12]

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Results

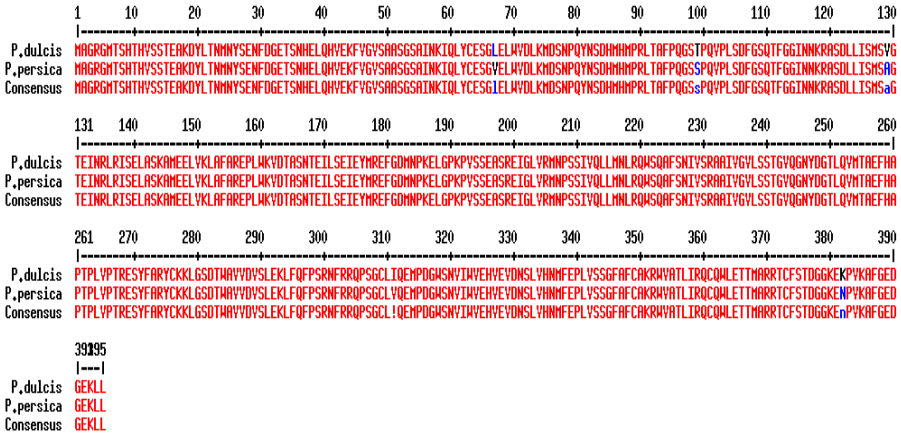

Figure 1. Multiple sequence alignment of zipper proteins in Prunus dulcis and Prunus persica species.

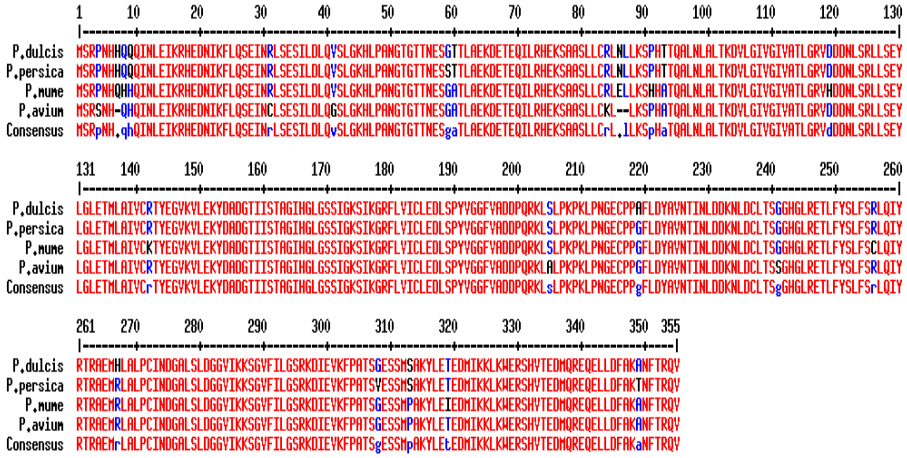

Figure 2. Multiple sequence alignment of defective proteins in the meristems of Prunus dulcis, Prunus persica, Prunus mume, and Prunus avium species.

Table 1. Summary of amino acid sequence alignment results for defective proteins in four Prunus species.

Total Amino Acids	Conserved Amino Acids	Variable Amino Acids	Percent Similarity(%)	Percent Variation(%)	Database	Analysis Tool

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1417	1321	96	93.23	6.77	NCBI	Multialin

Table 2. Summary of amino acid sequence alignment results for zipper proteins in four Prunus species

Total Amino Acids	Conserved Amino Acids	Variable Amino Acids	Percent Similarity(%)	Percent Variation(%)	Database	Analysis Tool
790	782	8	98.988	1.012	NCBI	Multialin

Discussion and Conclusion

Analysis of the zipper protein sequence indicates that, among 790 amino acids, eight exhibit differences (i.e., the sequence contains eight point mutations), corresponding to a 1.012% variation. Zhang et al. investigated and comparatively analyzed the gene family and gene expression under freezing stress during dormancy. Their study demonstrated that environmental factors influence genetic variation, and such alterations enhance adaptation and confer cold resistance.[8] The variation in zipper proteins arises because they respond to environmental stresses; therefore, it is natural that environmental changes induce modifications in zipper proteins. This phenomenon, known as point mutation, contributes to genetic polymorphism.

Considering that plants approximately possess 10,000 genes, the observed 1.012% variation likely reflects alterations in over 100 genes. Notably, these variations, arising from genetic diversity, enhance the plant's adaptability to its environment, which is advantageous because it increases resistance to pests or diseases and prevents extinction.[15,16] Similarly, analysis of the defective protein sequence shows that, among 1,417 amino acids, 96 differ (i.e., the sequence contains 96 point mutations), corresponding to a 6.77% variation. Research indicates that these modifications also enhance environmental adaptation, confer resistance to cold and adverse conditions, and maintain proper growth under varying circumstances. The 6.77% variation likely represents changes in over 670 genes.

The purpose of point mutations in defective proteins, as in zipper proteins, is to promote adaptation through genetic diversity, which is beneficial because it increases resistance to pests or diseases, prevents extinction, and supports continued proper growth.[4]

For future studies, it is recommended to conduct research on other Prunus genes, perform additional molecular investigations on Prunus, and study defective and zipper proteins in other plant species.

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