P-64 (206)

CLONING AND IN SILICO CHARACTERIZATION OF GAI GENE AND ITS PROMOTER REGION FROM DWARF/PRECOCIOUS AND VIGOROUS/NON-PRECOCIOUS PERSIAN WALNUT GENOTYPES

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Precocious and low vigor walnut genotypes are valuable materials for studying mechanisms underlying precocity and vigor in Persian walnut (Juglans regia). GAI gene is one of the candidate genes that involved in dwarfism and flowering pathways in plants. Mutation in DELLA domain of this gene affects GA pathway and growth of plants. For detection of any SNPs in this gene, we cloned GAI gene, by gateway cloning method and sequenced it from two precocious/low vigor and non-precocious/vigorous genotypes. No significant mutation was detected in this gene but there were some SNPs in its DNA sequence. Although in silico analysis revealed some differences between two genotypes but these mutations have no significant effect on protein conformation and domains such as DELLA and GRAS domains. Since GAI protein suppressed all of the genes that expressed in response to GA, its promoter region was cloned and sequenced in both precocious and non-precocious genotypes in order to investigate its role in the dwarfism. Expression of GAI gene stimulated by a number of factors and activated by the motifs in its promoter region. Promoter sequence analysis by PLANTCARE software, showed presence of different regulatory elements in this region. We detected some cis-acting elements such as ARE (cis-acting regulatory element essential for the anaerobic), Box-W1 (fungal elicitor responsive element), LTR (cis-acting element involved in low-temperature responsiveness), P-Box (gibberellin-responsive element), TC-reach repeat (cis-acting element involved in defense and stress responsiveness), CGTCA (Cis-acting regulatory element involved in the Methyl Jasmonate) in this region. In conclusion, there is not significant difference between GAI protein function and its promoter region sequence in two type of genotypes. Expression of GAI gene in walnut modified by hormonal signals (Methyl Jasmonate and Gibberellin) and biotic and abiotic stresses (fungi, anaerobic situation and low temperature).

Keywords: GAI gene, Precocity, Dwarf, Promoter analysis, Gene Cloning