新疆野苹果种子 *MsPYR1-like* 基因克隆、表达与亚细胞定位分析

房震[1]，李静[1]，叶春秀[1*]

（1. 新疆农业大学林学与风景园林学院，新疆，乌鲁木齐，830000）

摘 要:PYR/PYL/RCAR 基因家族作为脱落酸（ABA）受体，是参与早期 ABA 的感知和信号传递的三个主要成分之一，种子休眠的调控主要是通过调节赤霉素（GA）和脱落酸（ABA）的代谢和信号传导途径实现的。本研究对新疆野苹果种子 *MsPYR1-like* 基因进行克隆、亚细胞定位与表达分析，为了揭示其参与新疆野苹果种子解除休眠作用机制提供理论依据。基于前期新疆野苹果种子转录组数据库，分析结果获得新疆野苹果种子 PYR1-like 家族基因序列，克隆得到 PYR1-like 基因（暂且命名为 *MsPYR1-like*），并对其进行生物信息学分析，编码蛋白进行序列特征及进化树，构建载体进行亚细胞定位，实时荧光定量（qRT-PCR）分析 *MsPYR1-like* 在新疆野苹果种子不同层积时期中的表达模式。显示获得 *MsPYR1-like* 基因开放阅读框全长为 621 bp，编码 206 个氨基酸，编码的蛋白序列具有 PYR/PYL/RACR 蛋白家族的结构域，*MsPYR1-like* 与欧洲野苹果同源蛋白具有较近的亲缘关系，同源率是 98 %。为不稳定的、不含跨膜结构的亲水性蛋白。亚细胞定位结果显示该蛋白定位于细胞核和细胞质中。qRT-PCR 结果显示，*MsPYR1-like* 基因在层积过程中表达量呈下调表达。*MsPYR1-like* 对新疆野苹果种子休眠解除具有重要的调控作用，为深入了解新疆野苹果种子后续功能分析及研究提供理论基础。

Cloning, expression and subcellular localization analysis of *MsPYR1-like* Genes in *Malus sieversii (Ledeb.) M. Roem.* seeds

FANG Zhen[1], LI Jing[1], YE Chunxiu[1*]

（1. School of Forestry and Landscape Architecture, Xinjiang Agricultural University, Urumqi, Xinjiang, 830000)

Abstract: The PYR/PYL/RCAR gene family, as abscisic acid (ABA) receptors, is one of the three major components involved in early ABA sensing and signaling, and the regulation of seed dormancy is mainly achieved by regulating the metabolic and signaling pathways of gibberellin (GA) and abscisic acid (ABA). In this study, the *MsPYR1-like* gene of *Malus sieversii* seeds was cloned, subcellularly localized and expressed to provide a theoretical basis for its involvement in the mechanism of seed dormancy release in *Malus sieversii* seeds.Based on the transcriptome database of *Malus sieversii* seeds, the PYR1-like family gene sequences were obtained, the PYR1-like gene (tentatively named *MsPYR1-like*) was cloned and bioinformatically analyzed, and the encoded protein was subjected to sequence characterization and evolutionary tree, vector construction for subcellular localization. Using the low temperature lamination method, seeds were evenly mixed with wet sandy soil at a volume of 1:3 and placed in plastic boxes, stored in a refrigerator at 4°C, and seed humidity was maintained. Seeds were selected for 0, 30, 60, 90, and 120 d. The gene expression pattern of *MsPYR1-like* in *Malus sieversii* seeds at different stratification periods by quantitative real-time fluorescence (qRT-PCR) analysis.Showed that the full length of the open reading frame of the obtained *MsPYR1-like* gene was 621bp, encoding 206 amino acids, and the encoded protein sequence had a structural domain of the PYR/PYL/RACR protein family with a hydrophobic ligand-binding deep pocket-like structural domain that specifically binds ABA and mediates its signaling. The physicochemical analysis of the

encoded protein showed that the molecular weight of the encoded protein was 23.14 kDa; the predicted isoelectric point pI was 5.37; the instability coefficient was 41.16, which was classified as an unstable protein; the aliphatic amino acid index was 74.17, which was thermally stable; and the grand average hydrophobicity index was -0.557, which was a hydrophilic protein. With 33 negatively charged residues aspartic acid and glutamic acid (Asp + Glu) and 24 positively charged residues arginine and lysine (Arg + Lys); in the composition of amino acid residues, valine (Val) was high, up to 9.7 %, while hydrophilic amino acids such as glutamic acid (Glu), leucine (Leu), aspartic acid (Asp) and serine (Ser) The content of hydrophilic amino acids such as Glu, Leu, Asp and Ser was also relatively high. The transmembrane structure prediction analysis did not classify it as a transmembrane protein. It was hypothesized that *MsPYR1-like* protein may be localized in the cytoplasm and is a receptor for the cytoplasm by the online prediction software Plant-mPLoc. The subcellular localization results showed that the protein was localized in the nucleus and cytoplasm of the cell. Secondary structure prediction of the *MsPYR1-like* protein showed that it consists of 35.44% α-helix, 3.88% β-turn, 15.05% extended chain and 45.63% irregular coiling. The primary structure of *MsPYR1-like* protein was derived as α-helix and irregular coiling, and the overall secondary structure of the protein consisted of a structure favoring protein stability. The tertiary structure predicted that the *MsPYR1-like* protein had a high similarity to the template at amino acids 13-204 with 76.04% similarity. Evolutionary analysis showed that the obtained *MsPYR1-like* is closely related to the European wild apple homologue, with a homology rate of 98%. The *MsPYR1-like* protein is identical in length to the European wild apple protein, encoding 206 amino acids, but differs in three amino acids, with a high sequence similarity of 98% and the next highest similarity with lentil (94%). The *MsPYR1-like* protein has high sequence identity with different plant PYR1 proteins, indicating that the gene is more conserved in the evolutionary process. qRT-PCR results showed that the expression of the *MsPYR1-like* gene was significantly down-regulated at 30d of low temperature lamination, consistently decreased at 30-90d of lamination, reached a minimum at 90d of lamination and The expression of *MsPYR1-like* gene was significantly down-regulated at 30d of low-temperature lamination, decreased from 30 to 90d of lamination, reached a minimum at 90d of lamination, and was up-regulated from 90 to 120d of lamination. The transcriptome data showed that the expression of *MsPYR1-like* gene was highest at 30 d of lamination, and the expression showed down-regulated expression during the lamination process. The expression of transcriptome and fluorescence quantification both showed down-regulated expression with increasing lamellar time, which showed the same trend of change.*MsPYR1-like* in *Malus sieversii* seeds has an important regulatory role in regulating the dormancy release of seeds. The above study illustrates the dormancy mechanism of *Malus sieversii* seeds, and provides a theoretical basis for understanding the subsequent functional analysis studies in *Malus sieversii* seeds.