高氮条件下香榧假种皮开裂的生理机制初探

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摘 要:【目的】香榧是我国南方特有的珍稀坚果，具有较高的营养价值和经济效益。香榧假种皮开裂是种子成熟采摘的重要标志，与种子品质密切相关。生产上，常因氮肥施用过多，导致香榧假种皮开裂率降低，严重影响坚果品质。然而，高氮条件下香榧假种皮开裂的生理机制尚不清楚。【方法】通过测定种实发育时期内各处理香榧假种皮开裂率、硬度、解剖结构等生理指标的变化，利用转录组和 RT-qPCR 验证筛选出高氮下参与假种皮开裂过程的关键结构基因和转录因子，并通过双荧光素酶和酵母单杂试验初步明确它们之间的调控效应。【结果】1）与对照处理相比，高氮处理下假种皮开裂率显著降低了 8.3-24.2%，其腐胺显著增多，乙烯合成则明显受阻，且腐胺与乙烯释放量与其开裂率分别呈显著负相关和显著正相关；高氮处理下假种皮的中间薄壁细胞层厚度显著减小了 6.7-22.8%，其水溶性果胶显著降低了 4.9-17.8%，而半纤维素含量显著增加了 6.6-14.2%，且水溶性果胶和半纤维素含量与其开裂率分别呈显著正相关和显著负相关。2）通过转录组分析和 RT-qPCR 结果，发现细胞壁修饰相关基因（*TgEXP*、*Tgβ-Glu*、*TgPME* 和 *Tgβ-Gal*）与其开裂率均呈显著正相关，且 *TgbHLH1* 与 *TgSAMS*、*TgACO*、*TgEXP*、*TgPME* 和 *TgPL* 均呈显著正相性关。3）双荧光素酶结果显示，*TgbHLH1* 能激活 *TgPME*、*TgEXP* 和 *Tgβ-Glu* 的启动子；酵母单杂结果显示，*TgbHLH1* 能与 *Tgβ-Glu* 直接结合，但与 *TgPME*、*TgEXP* 不直接结合。【结论】高氮条件下 TgbHLH1 可能还需要通过结合其他转录因子协同调控香榧假种皮的开裂。

关键词: 香榧；开裂率；细胞壁组分；细胞壁修饰基因

Preliminary study on cracking mechanism of *Torreya grandis* aril under high nitrogen condition

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Abstract:【Objective】 *Torreya grandis* (*T. grandis*) is a rare nut peculiar to southern China, with high nutritional value and economic benefits. Aril cracking of *T. grandis* is an important sign of seed ripening and picking, and is closely related to seed quality. In practice, the excessive application of nitrogen fertilizers often leads to a decrease in the cracking rate of arils, which seriously affects the quality of nuts. However, the physiological mechanisms of seed abscission in *T. grandis* under high nitrogen condition remain poorly understood. 【Method】 The physiological mechanism of aril cracking of *T. grandis* under high nitrogen condition was revealed by measuring the changes of physiological indexes such as the cracking rate, hardness and anatomical structure of *T.*

grandis aril under control (N0) and high nitrogen condition (N30) during seed development. In addition, transcriptome analysis and RT-qPCR were used to verify and screen out the key structural genes and transcription factors accounted for aril cracking under nitrogen deposition, and the regulatory effects between them were preliminarily clarified by double luciferase assay and yeast one-hybrid assay. 【Result】 1) Compared with the control, the cracking rate of aril significantly decreased by 8.3-24.2% under nitrogen deposition treatment, a significant reduction in the putrescine content and ethylene production were also observed, and accompanied by the putrescine content and the release of ethylene negatively correlated and positively correlated with the cracking rate, respectively. The thickness of parenchymatous cell layers of aril significantly decreased by 6.7-22.8% under nitrogen deposition treatment than that of control, and its water-soluble pectin dramatically decreased by 4.9-17.8%, whereas its hemicellulose content significantly increased 6.6-14.2%. Moreover, the water-soluble pectin and hemicellulose content were positively correlated and negatively correlated with its cracking rate, respectively. 2) Transcriptome analysis and RT-qPCR results showed that cell wall modification related genes (*TgEXP*, *Tgβ-Glu*, *TgPME* and *Tgβ-Gal*) were positively correlated with cracking rate, and *TgbHLH1* was positively correlated with *TgSAMS*, *TgACO*, *TgEXP*, *TgPME* and *TgPL*. 3) The result of the double luciferase assay showed that TgbHLH1 could activate the promoters of *TgPME*, *TgEXP* and *Tgβ-Glu* . Furthermore, the results of yeast one hybrid showed that TgbHLH1 positively regulate the *Tgβ-Glu* expression via directly binding the *Tgβ-Glu* promoter, whereas it did not dircetly binding the *TgEXP*, *TgPME*. 【Conclusion】 TgbHLH1 may also be required to synergistically regulate the arils cracking of *T. grandis* by binding other transcription factors under high nitrogen conditions.

Keywords: *Torreya grandis*; Cracking rate; Cell wall component; Cell wall modifcation genes

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