Efficient Extraction of RNA and Analysis of Gene Expression in a Long-Term *Taxus* Cell Culture Using Real-Time RT-PCR

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A simple, quick and efficient method for isolating total RNA from heavy browning cells was developed by adding polyvinylpyrrolidone, mercaptoethanol and 3 M NaAc during the process of the Trizol (a kind of a widely used RNA extraction buffer) method. High-quality total RNA was isolated and synthesized to cDNA. Transcript levels of four paclitaxel biosynthetic pathway genes: *dxr, hmgr, ggpps* and *dbat* were assayed by real-time RT-PCR. The results demonstrated that the transcript levels of these genes experienced a coincident descent in the past three years as well as a decreasing paclitaxel productivity. According to these results, the possible reason for the descending paclitaxel productivity during long-term *Taxus media* cv. *Hicksii* cell culture maybe due to a decreasing transcripts level of mass genes in close with a gross secondary metabolite level. Gene manipulation emphasized only on key enzyme genes in the paclitaxel biosynthesis pathway may not hamper the somaclonal variation trend of *Taxus media* cv. *Hicksii* cell culture.

Key words: RNA Extraction, *Taxus*, Real-Time RT-PCR