

6-Tuliposide B (PosB), a major secondary metabolite that accumulates in tulip (*Tulipa gesneriana*), is converted to the antibacterial lactone, tulipalin B (PaB), by PosB-converting enzyme (TCEB). *TgTCEB1* and *TgTCEB-R*, which encode TCEB, are specifically expressed in tulip pollen and roots, respectively, but are hardly expressed in other tissues (e.g. leaves) despite the presence of substantial PosB-converting activity, suggesting the existence of another TCEB isozyme. Here, we describe the identification of *TgTCEB-L* ("L" for leaf), a paralog of *TgTCEB1* and *TgTCEB-R*, from leaves via native enzyme purification. The enzymatic characters of *TgTCEB-L*, including catalytic activity and subcellular localization, were substantially the same as those of *TgTCEB1* and *TgTCEB-R*. However, *TgTCEB-L* did not exhibit tissue-specific expression. Identification of *TgTCEB-L* explains the PosB-converting activity detected in tissues where *TgTCEB1* and *TgTCEB-R* transcripts could not be detected, indicating that tulip subtilizes the three *TgTCEB* isozymes depending on the tissue.