Temperature and humidity are also important ecological factors affecting the growth and development of *T. hemsleyanum*. This species is suitable for growth in warm and humid environments, with an optimal growth temperature of approximately 25°C. When winter temperature drops to about 10°C, plant growth tends to stagnate, indicating that although it is adapted to warm-temperate evergreen forest environments, it remains sensitive to low temperatures (Guo et al., 2019; Zhu et al., 2020). Higher air humidity helps maintain leaf physiological activity and vine growth, while moderate soil moisture facilitates root absorption of water and nutrients. However, *T. hemsleyanum* prefers humid conditions but does not tolerate long-term waterlogging. If drainage is poor under forest canopies and the soil remains waterlogged for a long period, root hypoxia may occur, increasing the risk of soil-borne diseases such as root rot. Therefore, understory ecological cultivation should make full use of the forest canopy to buffer high temperature, strong light, and water evaporation, while maintaining a rhizosphere environment that is "moist but not waterlogged" through drainage ditches, slope planting, and organic mulching.

2.3 Characteristics of tuberous root formation and medicinal part accumulation in *Tetrastigma hemsleyanum*

The tuberous root of *T. hemsleyanum* is its main medicinal organ and the most economically valuable part in artificial cultivation. Tuberous root formation is not completed at once, but undergoes a continuous developmental process from fibrous roots to bar-shaped roots and then to calabash-shaped tuberous roots (Guo et al., 2019; Ji et al., 2020). Anatomical studies have shown that fibrous roots retain relatively intact vascular bundle structures and contain fewer intracellular inclusions. In the bar-shaped root stage, partial vascular bundle degeneration occurs, accompanied by the initial accumulation of inclusions. Mature calabash-shaped tuberous roots retain only traces of vascular bundles but contain abundant intracellular inclusions, which are considered important storage forms of medicinal substances (Xiang et al., 2021). Therefore, the enlargement of tuberous roots in *T. hemsleyanum* is essentially the result of root morphogenesis, vascular tissue changes, and storage substance accumulation.

Tuberous root formation is closely related to vegetative growth, photosynthate transport, and underground nutrient accumulation. In the early growth stage, *T. hemsleyanum* mainly enhances photosynthesis through the expansion of aboveground stems and leaves, providing a material basis for subsequent underground growth. As the plant enters a relatively stable growth stage, part of the nutrients is gradually transported to the underground organs, promoting tuberous root enlargement and dry matter accumulation. Transcriptome studies have found that tuberous root enlargement in *T. hemsleyanum* is associated with changes in the expression of genes related to cell wall modification, hormone signaling, and membrane components, indicating that root thickening and storage substance accumulation are regulated by complex regulatory networks (Xiang et al., 2021). Because *T. hemsleyanum* grows slowly and requires multiple years to form fully developed tuberous roots, its cultivation cycle is relatively long. Premature harvesting should therefore be avoided in production, as it may affect medicinal material yield and quality (Zhu et al., 2020; Hu et al., 2021).

In terms of medicinal part accumulation, active constituents in *T. hemsleyanum* show obvious tissue specificity. Studies have shown that the tuberous roots are rich in flavonoids, phenolic acids, terpenoids, polysaccharides, and other metabolites. Key indicator compounds such as polydatin, piceatannol, resveratrol, and kaempferol can be stably detected and are often used for medicinal material quality evaluation (Hu et al., 2021; Hu et al., 2023). Integrated metabolomic and transcriptomic studies further show that flavonoids, glycosides, alkaloids, and terpenoids are highly accumulated in tuberous roots, while leaves and fibrous roots are rich in certain flavonols and organic acids, indicating functional differentiation among different tissues in medicinal compound accumulation (Luo et al., 2022; Peng et al., 2025). Spatial metabolomics has also confirmed that some flavonoids, such as vitexin-2″-O-glucoside, are concentrated in the epidermis and xylem of tuberous roots, further supporting the status of tuberous roots as the core medicinal part (Fu et al., 2019; Chen et al., 2024). Therefore, understory ecological cultivation of *T. hemsleyanum* should establish a balance among light regulation, soil improvement, water and fertilizer management, and harvesting age, so as to promote stable tuberous root formation and active compound accumulation.