during short-term trials; however, their pore structure, water-holding capacity, salinity levels, and nutrient-release patterns may change over extended cultivation periods, thereby influencing subsequent plant growth and quality formation. In addition, climatic conditions, cultivation systems, and irrigation and fertilization practices differ among regions, meaning that the same substrate may perform differently under various production environments. Therefore, future research should establish unified physical, chemical, and biological evaluation standards while providing detailed descriptions of irrigation, fertilization, and pest management conditions. Such efforts would facilitate the interpretation of substrate performance under commercial production conditions and provide comparable and reliable scientific evidence for substrate selection and practical application in *D. officinale* cultivation (Barrett et al., 2016).

6.2 Insufficient understanding of quality formation mechanisms

Current studies on the effects of cultivation substrates on the quality of *D. officinale* primarily focus on changes in the contents of bioactive compounds such as polysaccharides, dendrobine, and flavonoids, whereas the underlying regulatory mechanisms remain insufficiently understood. Existing research generally suggests that substrates promote the accumulation of bioactive constituents by improving the rhizosphere environment, regulating water and nutrient availability, and influencing rhizosphere microbial communities. However, systematic evidence is still lacking regarding how substrate-derived signals affect metabolic pathways, enzyme activities, and gene expression associated with quality formation. With advances in molecular biology and omics technologies, the quality formation of *D. officinale* has increasingly been recognized as the result of interactions among genetic background, environmental signals, and metabolic regulation. Nevertheless, the specific roles and regulatory pathways of substrate-related factors remain poorly characterized.

Significant progress has been made in elucidating the molecular mechanisms underlying key quality traits in *D. officinale*. For example, integrated metabolomic and transcriptomic analyses demonstrated that high light intensity induces the expression of *DoHY5*, which subsequently activates genes involved in flavonoid and polysaccharide biosynthesis, resulting in increased accumulation of anthocyanins and mannose-rich polysaccharides. Other studies have clarified the regulatory role of the MYB-bHLH transcriptional complex in anthocyanin biosynthesis and identified candidate genes associated with the organ-specific accumulation of polysaccharides, alkaloids, and flavonoids (Wang et al., 2021; Zhao et al., 2025). Comparative omics analyses between *D. officinale* and *Dendrobium nobile* further revealed that differences in polysaccharide and alkaloid accumulation are closely associated with differential expression of glycosidase-related genes and terpene biosynthesis pathways (Zhang et al., 2024).

Despite these advances, most studies have focused on genetic and signaling regulation under controlled in vitro conditions, light treatments, or elicitor-induced responses, while substrate-driven rhizosphere factors have rarely been integrated into the analysis. Research on soilless cultivation has shown that substrate type, nutrient dynamics, and rhizosphere microbial communities can substantially alter root-zone environments; however, knowledge regarding microbial community structure, functional stability, and their regulatory effects on plant metabolism within organic substrates remains limited (Tuxun et al., 2025). In *D. officinale*, systematic links among substrate physicochemical properties, root-microbe interactions, carbon-nitrogen metabolic balance, and the activation of specific biosynthetic genes or transcription factors have not yet been established. Therefore, future studies should integrate transcriptomic, metabolomic, proteomic, and microbiome analyses to elucidate how different substrates regulate the biosynthesis of polysaccharides, dendrobine, flavonoids, and other bioactive constituents, thereby providing a theoretical basis for the precise improvement of medicinal quality.

6.3 Insufficient integration of substrate formulations with industrial applications

Although numerous novel and composite substrate formulations have been developed in recent years, many remain at the experimental stage and have not been widely adopted in commercial production. On one hand, some substrate formulations are overly complex, relying on unstable raw material sources, high-cost components, or labor-intensive processing methods, making large-scale implementation difficult. On the other hand, many studies