metabolite production. Similarly, diurnal temperature fluctuation treatments increased polysaccharide and total flavonoid contents in protocorm-like bodies and induced numerous differentially expressed genes enriched in secondary metabolite and flavonoid biosynthetic pathways. Stress-related genes, including heat shock proteins (HSPs) and heat shock transcription factors (HSFs), were strongly induced, indicating a close relationship between mild temperature stress and the accumulation of quality-related metabolites (Chen et al., 2024).

Root-zone water status and osmotic conditions, which are strongly influenced by substrate characteristics, also play important roles in regulating secondary metabolism. In vitro studies have shown that osmotic stress simulated by PEG-6000 disrupts water metabolism and antioxidant systems in *D. officinale* seedlings, increases cell membrane permeability, and induces physiological disorders such as hyperhydricity, indicating that unfavorable osmotic conditions can damage tissues and alter metabolic processes (Gao et al., 2020). Reviews of dynamic changes in functional constituents of *D. officinale* have further emphasized that environmental factors such as light, temperature, and water availability significantly affect the biosynthesis and accumulation of polysaccharides, alkaloids, flavonoids, and phenolic compounds, with characteristic accumulation peaks occurring at specific developmental stages (Wang et al., 2025). Moreover, metabolomic comparisons among plants cultivated on different substrates revealed that although the overall metabolite profile remains relatively stable, flavonoids are highly sensitive to substrate conditions and preferentially accumulate under pine bark cultivation, where flavonoid biosynthesis was identified as the only significantly enriched KEGG pathway (Zuo et al., 2020). Collectively, these findings indicate that substrate-induced stresses-including salinity, temperature fluctuations, water imbalance, and nutrient stress-can function as regulatory signals that activate hormonal and stress-response networks, thereby enhancing or redirecting secondary metabolite biosynthesis and ultimately altering the medicinal quality of *D. officinale*.

6 Current Challenges in Research

6.1 Lack of unified standards for substrate evaluation

In recent years, the expansion of large-scale cultivation of *Dendrobium officinale* has led to a substantial increase in studies investigating different cultivation substrates. However, a unified framework for evaluating substrate performance has yet to be established. Considerable variation exists among studies in terms of evaluation criteria, experimental duration, and management practices, making direct comparisons difficult. Some studies primarily focus on growth-related parameters, such as plant height, stem diameter, root development, and biomass accumulation, whereas others emphasize the contents of bioactive compounds, including polysaccharides, alkaloids, and flavonoids. Consequently, a comprehensive evaluation system that simultaneously incorporates physicochemical properties, plant growth performance, quality attributes, economic costs, and ecological benefits has not yet been developed. Research on substrates for orchids and other epiphytic plants has also indicated that growers often formulate substrate mixtures empirically to balance aeration, water retention, pH, and cost. Since no single material can adequately satisfy all these requirements, substantial variation exists in substrate formulations and evaluation methods.

From a broader perspective, the lack of standardized evaluation criteria has become a major obstacle to the application and dissemination of research findings in soilless cultivation systems. Plant performance in soilless horticulture is often assessed under different experimental designs, management regimes, and evaluation parameters, ranging from visual quality ratings to biomass, yield, and product quality. Such inconsistencies make it difficult to compare results across studies and hinder the development of widely applicable cultivation recommendations (Barrett et al., 2016). Moreover, substrate properties are strongly influenced by raw material sources, processing methods, and composting conditions. This is particularly evident for peat substitutes and organic substrates, whose nutrient contents, biological activities, and physicochemical stability may vary considerably, further complicating the establishment of universal fertilization and management protocols (Atzori et al., 2021; Cacini et al., 2021).

Another limitation in current substrate evaluation research for *D. officinale* is the emphasis on short-term performance while neglecting long-term stability. Some substrates exhibit promising growth-promoting effects