

accumulation of these bioactive compounds is regulated not only by genetic background but also by cultivation environment and substrate conditions. By altering rhizosphere moisture, oxygen availability, nutrient supply, and the microecological environment, different substrates influence the allocation of resources between primary and secondary metabolism, thereby regulating the biosynthesis and accumulation of dendrobine, flavonoids, and related compounds.

Widely targeted metabolomic analyses comparing *D. officinale* stems cultivated in pine bark, coconut coir, and a 1:1 mixture of the two substrates revealed that although the overall metabolite categories remained similar among treatments, significant differences occurred in metabolite abundance. Among the 529 detected metabolites, flavonoids exhibited the most pronounced variation, with most flavonoid compounds preferentially accumulating in plants grown on pure pine bark. KEGG enrichment analysis further demonstrated that flavonoid biosynthesis was the only pathway significantly affected by substrate-induced metabolic changes, indicating a direct relationship between substrate selection and flavonoid metabolic regulation (Figure 3) (Zuo et al., 2020). These findings suggest that bark-based substrates not only facilitate root attachment and vegetative growth but may also promote the accumulation of specific bioactive compounds through alterations in metabolic pathway activity.

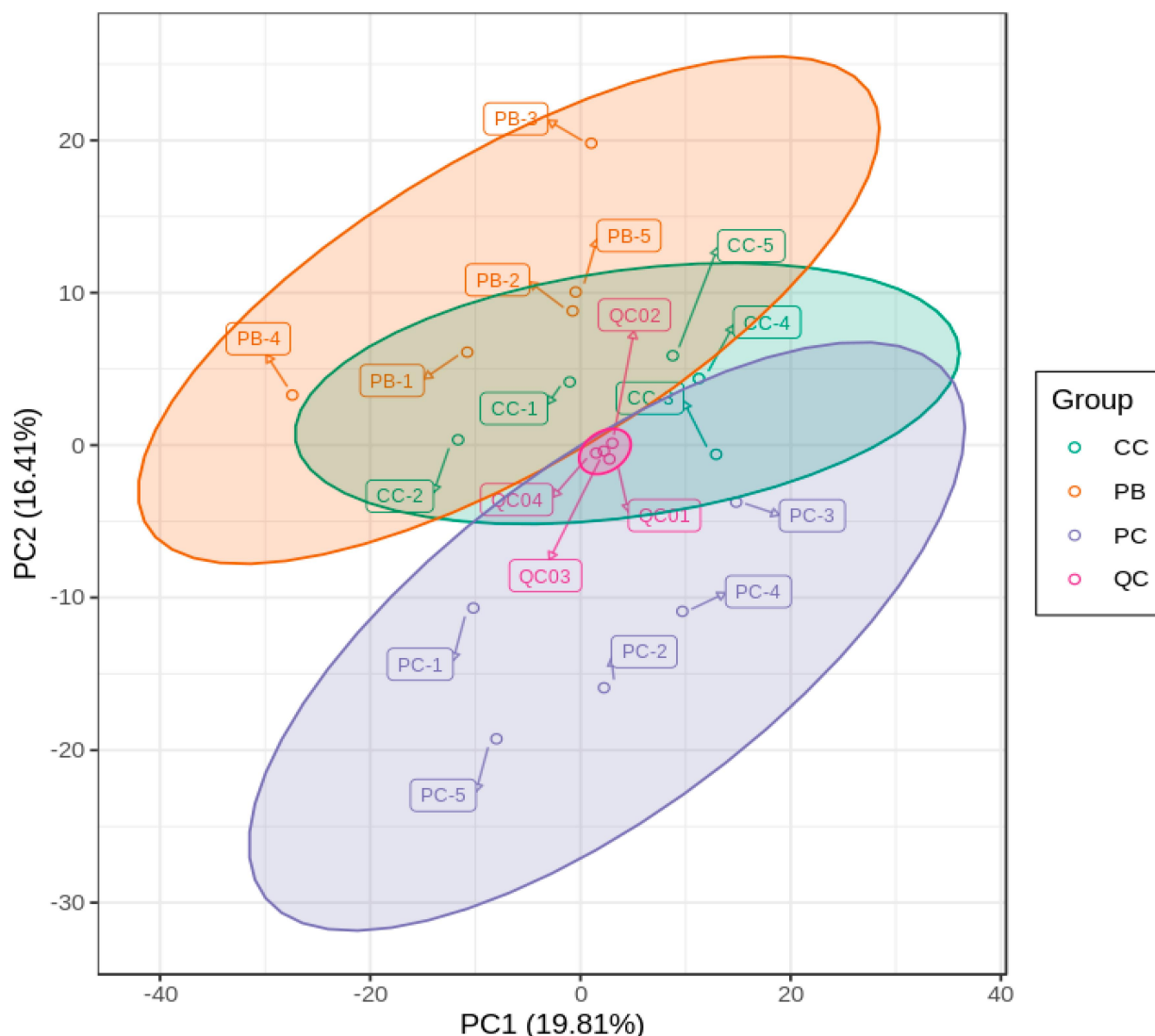


Figure 3 Score plot of principal component analysis (PCA) for *D. officinale* samples (Adopted from Zuo et al., 2020)
Image caption: Planted in pine bark (PB), coconut coir (CC), pine bark: coconut coir 1:1 mix (PC), and quality control (QC) samples; PC1 represents the first principal component and PC2 represents the second principal component (Adopted from Zuo et al., 2020)