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INTEGRATIVE HIGH-THROUGHPUT PHENOTYPING AND MOLECULAR ANALYSES REVEAL NOVEL INSIGHTS INTO HEMP SEED QUALITY

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Cannabis sativa is a multi-use crop characterized by extensive varietal diversity resulting from a long domestication history and selection for distinct industrial applications. While this heterogeneity constitutes a resource for crop improvement, it also poses a significant challenge for the identification of reliable seed quality markers essential for germplasm characterization and breeding programs. To facilitate the identification of seed quality markers in hemp, the present study adopted an integrative approach that combines high-throughput phenotyping with molecular analyses.

Six hemp seed lots belonging to two commercial varieties and two breeding lines were used in this study. The seed lots were produced in different years and locations, and corresponding climatic data were retrieved from ARPA database. Seed quality was initially assessed through germination and viability assays, revealing marked differences both between varieties and seed lots. Subsequently, multi-level analyses were conducted. These included multispectral imaging (MSI) for high-throughput phenotyping, volatilome profiling, measurement of ROS and DNA

damage, and gene expression profiling for the identification of quality markers. All data were used to compare high- and low-quality lots within each genotype and subsequently evaluated across genotypes to identify conserved markers.

The results of this integrated approach indicated that the environmental conditions experienced by plants during seed development in the field strongly influenced seed quality. Morphometric data showed that high-performing seed lots presented greater seed area, length, and width compared to lower-performing lots. Additionally, reflectance properties at 365 nm and the MSI mean value resulted significantly different within most varieties and seed lots.

PTR-ToF-MS analysis allowed to identify several volatile biochemical markers consistently associated with seed quality, with terpene emission showing the strongest correlations with germination indices. Terpene emission was further investigated through SPME-GC–MS approach, identifying α- and β-pinene as the volatile compounds mostly associated with low seed quality. Gene expression profiling highlighted genes involved in dormancy regulation, cell-cycle control, and ROS homeostasis as potential candidate markers of seed quality.

Overall, the identified markers represent valuable tools for the evaluation of hemp germplasm and may support breeding strategies aimed at improving seed quality and crop establishment.

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