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Clonal variation of the basic resistance factors (POD, SOD, CAT, MDA, PRO) in *Michelia chapensis*

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Abstract

Background: *Michelia chapensis* Dandy is a rare and endangered evergreen woody species endemic to China, with high ecological, horticultural, and medicinal values. However, it is threatened by climate change, especially temperature fluctuations, and human activities. Enhancing its stress resistance is crucial for conservation and breeding, yet intraspecific clonal variation in basic resistance factors, peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and proline (PRO), under natural conditions remains unclear.
Aims: This study aimed to quantify variation in five resistance factors among *M. chapensis* clones and identify elite clones for stress-resistance-oriented breeding.
Methods: Leaves of 109 clones from five provinces grown in a common garden were analyzed for POD, SOD, CAT, MDA, and PRO. Variation among clones and provinces was assessed using *ANOVA*, principal component analysis (PCA), and stress-resistance ranking.
Results: *ANOVA* showed highly significant differences ($P < 0.001$) in all factors among clones; POD had the highest variability ($CV = 61.71\%$), and CAT the strongest clonal differentiation ($F = 160.29$). Only SOD and PRO differed significantly among provincial origins ($P < 0.05$), with Guizhou clones having the highest mean SOD (767.06 U g^{-1} FW and PRO (159.23 μg g^{-1} FW). PCA revealed PC1 (27.05%) and PC2 (21.25%) explained 48.30% of total variance, reflecting trade-offs between POD/MDA and SOD/CAT. Thirty-six high-resistance clones were identified, with five top clones (*e.g.*, GDSX03, GZLP01, GDLC16, GXRS02, and GXYF04) showing high antioxidant enzyme activities and low MDA.
Conclusions: Pronounced clonal variation in basic resistance factors underpins resistance-oriented breeding and guides selection of resilient *M. chapensis*.

Keywords: antioxidant enzymes, breeding, clonal variation, *Michelia chapensis*, non-enzymatic components.

Introduction

Michelia chapensis Dandy, a rare and endangered evergreen woody species of the genus *Michelia* (Magnoliaceae), is endemic to China, with only scattered natural communities (Zhou et al., 2023). First reported from Lechang City, Guangdong Province in 1929 (Dandy, 1929), it is mainly distributed across southern China, with smaller populations in Vietnam (Sima et al., 2020). Valued for its fragrant flowers, medicinal properties, timber, and strong adaptability to diverse soil and climatic conditions (Ao, 1986; Chen et al., 2005; Wang et al., 2009; Liu et al., 2018; Zhou et al., 2023), *M. chapensis* also plays an important role in providing ecosystem services in both natural and urban environments (Cao et al., 2011; Chen, 2020).

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Abbreviations: CAT - catalase; MDA - malondialdehyde; PCA - principal component analysis; POD - peroxidase; PRO - proline; ROS - reactive oxygen species; SBRF - score of basic resistance factors; SOD - superoxide dismutase.
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However, the species now faces mounting pressures from environmental change and anthropogenic activities. Increasing abiotic stresses, including drought, heat waves, cold spells, air pollution, and soil degradation, combined with the excessive harvesting of wild individuals for ornamental and timber purposes, have contributed to pronounced population declines (Zhou et al., 2023). Climate change is predicted to exacerbate these challenges by altering precipitation patterns and increasing the frequency of extreme weather events, thereby amplifying physiological stress on existing *M. chapensis* populations (Jiang, 2006; Shen et al., 2025). This change has further reduced the availability of suitable growth sites, threatening not only the genetic diversity and long-term viability of wild populations but also their ecological functions and landscape value.

Given these increasing threats, enhancing the stress resistance of *M. chapensis* has become a critical conservation and breeding priority. Insights can be drawn from the broader Magnoliaceae family, to which the species belongs. The Magnoliaceae family, which includes numerous genera such as *Magnolia* (Huyen et al., 2025), *Michelia* (Liao et al., 2022), and *Schisandra* (Chen et al., 2024), harbors a wide diversity of species renowned for their ornamental, ecological, and medicinal significance. One of the common characteristics is that many produce abundant bioactive secondary metabolites, such as flavonoids, phenolic acids, lignans, and essential oils, which exhibit strong antioxidant activities (Liao et al., 2022). These pharmacologically relevant compounds may also contribute to an inherently robust *in vivo* antioxidant system, enabling plants to counteract reactive oxygen species (ROS) generated under environmental stress (Llauradó Maury et al., 2020). In plant physiology, such a coordinated antioxidant defense network is recognized as a central determinant of stress tolerance (Hasanuzzaman et al., 2011), offering a promising physiological basis for improving the adaptive capacity of *M. chapensis*.

The antioxidant defense system in plants is complex and multifaceted, comprising both enzymatic and non-enzymatic components (Irato and Santovito, 2021). Among these, enzymatic antioxidants play crucial roles in mitigating oxidative damage caused by ROS (Rajput et al., 2021). Enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) form the primary defense network against ROS (Huchzermeyer et al., 2022). SOD serves as the first line of defense by catalyzing the dismutation of superoxide radicals into hydrogen peroxide (McCord and Fridovich, 1969), which is subsequently neutralized by CAT (Aebi, 1974), preventing oxidative injury to proteins, lipids, and nucleic acids. POD primarily catalyzes H_2O_2 reduction *via* electrons from various donors in their regular cycle (Cosio and Dunand, 2009). In non-enzymatic components, osmolytes such as proline (PRO) play multifaceted roles in osmotic adjustment, stabilization of cellular structures, and scavenging of hydroxyl radicals, especially under drought, salinity, and temperature extremes (Szabados and Savouré, 2010). Malondialdehyde (MDA), on the other hand, is not a protective molecule but an oxidative stress marker; it is a by-product of polyunsaturated fatty acid peroxidation and is widely used to assess the degree of membrane lipid damage (Morales and Munné-Bosch, 2019). Together, these physiological indices, including POD, SOD, CAT, PRO, and MDA, are often referred to as "basic resistance factors" and have been broadly applied as reliable markers for evaluating plant stress tolerance (Fujita and Hasanuzzaman, 2022).

In plants, genetic variation in these basic resistance factors provides the physiological foundation for selecting superior genotypes with enhanced adaptability (Saed-Moucheshi et al., 2021). Considerable clonal variation in antioxidant-related has been documented in various types of plants, including perennial herb *Iris pumila* (Vuleta et al., 2016), perennial vine *Actinidia arguta* (Latocha et al., 2013), bush *Punica granatum* (Melgarejo-Sánchez et al., 2015), broad-leaved tree *Populus alba* (Vuksanović et al., 2023), and conifer *Pinus halepensis* (Djerrad et al., 2015). Such variation is often structured, with certain genotypes consistently exhibiting higher enzyme activities and lower oxidative damage, thereby conferring superior performance under stress conditions. These physiological differences have been successfully applied in plant breeding programs to improve tolerance. To date, research on the antioxidant capacity of *M. chapensis* has primarily focused on interspecific comparisons within the Magnoliaceae family (Pan et al., 2020; Shen, 2020; Shen et al., 2020; Liao et al., 2022). In contrast, the extent and pattern of intraspecific clonal variation in key resistance factors of *M. chapensis*, particularly under natural growth conditions, remain largely unexplored. One of the most important explanations for this knowledge gap is the lack of sufficiently large germplasms of the species.

Therefore, this study aimed to comprehensively assess the physiological variation among germplasms of *M. chapensis* using materials recently collected from 109 germplasms (He, 2025; He et al., 2025). We evaluated 109 clones under natural growth conditions, quantifying five basic resistance factors (POD, SOD, CAT, PRO, MDA). Specifically, our objectives were to (1) assess the extent of germplasm variation in these resistance factors, (2) explore the multivariate structure of resistance factors, and (3) identify clones with superior overall resistance profiles to inform selection strategies. By elucidating the physiological variation among *M. chapensis* germplasms, this work aims to establish a scientific basis for resistance-oriented breeding, guide the selection of planting materials for large-scale cultivation, and ultimately enhance the resilience and sustainable utilization of this ecologically and horticulturally important species in the face of ongoing environmental change.

Materials and methods

Plant material and growth conditions: This study was based on a basic breeding population of the *M. chapensis* Dandy breeding program of Guangdong, China. The whole population comprised 109 elite

genotypes. These germplasms had divergent geographical origins covering the main breeding regions of China, including Guangdong (n = 60; GD), Guangxi (n = 13; GX), Hunan (n = 18; HN), Jiangxi (n = 12; JX), and Guizhou (n = 6; GZ). They were grafted for the breeding program with 3 repeats (3 ramets per genotype) since 2022 in Longshan State Forest Farm (Guangdong, China, 25°11'N, 113°27'E, 288 - 322 m above sea level). The trees were maintained using standard commercial practices. The area has a south-facing slope with a gradient of 12 - 15°, red soils, a mean annual temperature of 25.6°C, average annual precipitation of 1 150 mm, and a mean relative humidity of 55%. Leaf samples were collected from 2.5-year-old *M. chapensis* clonal lines in August 2024. In Guangdong, August generally corresponds to the peak of summer, characterized by intense solar radiation and vigorous plant growth. This timing minimizes confounding effects of leaf developmental stage or senescence, thereby providing a robust basis for assessing the inherent differences in antioxidant defense among *M. chapensis* genotypes. For each genotype, three grafted ramets were used, and five representative mature leaves were sampled from the middle-upper canopy of each ramet. To ensure consistency across samples, fully expanded, non-senescent leaves from the 2^{n}d to 4th node of the current-year shoots were collected. Leaves collected from the three ramets were pooled together, from which three independent subsamples were randomly taken for subsequent analyses. All samples were frozen rapidly with liquid nitrogen and then transported to the laboratory of the Guangdong Academy of Forestry, and immediately stored at –80°C until further analysis.

Enzyme activity and content detection: The fresh leaf tissues (0.1 g per replicate) were homogenized on ice in the extraction buffer provided with the corresponding assay kit, and the homogenates were centrifuged at 8 000 *g* for 10 min at 4°C to obtain the supernatant for subsequent enzyme activity determination. The activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as the contents of malondialdehyde (MDA) and proline (PRO), were determined using specific assay kits supplied by *Suzhou Comin Biotechnology Co., Ltd.* (Suzhou, China). All assays were conducted in accordance with the manufacturer's instructions. Briefly, SOD activity was determined based on the inhibition of nitroblue tetrazolium (NBT) reduction, measured at 560 nm (Giannopolitis and Ries, 1977). POD activity was assessed by monitoring the increase in absorbance at 470 nm, which corresponds to the oxidation of a specific substrate (guaiacol) in the presence of H_2O_2 (Chance and Maehly, 1955). CAT activity was determined using a molybdate colorimetric method (Aebi, 1984). In this assay, residual H_2O_2 reacts with ammonium molybdate to form a yellow stable complex, $(H_2MoO_4 \cdot xH_2O)_n$, which exhibits a strong absorbance peak at 405 nm. The decrease in absorbance at 405 nm reflects the consumption of H_2O_2 by CAT and is linearly related to the enzyme's catalytic activity. MDA content was estimated using the thiobarbituric acid (TBA) method (Heath and Packer, 1968). MDA reacts with TBA to form a red adduct with a maximal absorbance at 532 nm. Non-specific turbidity was corrected by subtracting the absorbance at 600 nm, and the final MDA content was calculated based on the difference ($A_{532} - A_{600}$). Proline content was determined using the sulfosalicylic acid (SA) extraction method (Bates et al., 1973). Following heating, proline reacts with acidic ninhydrin to form a red chromophore. After extraction with toluene, absorbance was measured at 520 nm. All measurements were performed using a multifunctional microplate reader, *SpectraMax M2* (*Molecular Devices*, San Jose, USA). Three biological replicates were analyzed for each treatment. Each biological replicate was further analyzed with three technical replicates to ensure the accuracy and reproducibility of the results.

Comprehensive evaluation method: The comprehensive score value of 109 germplasms of *Michelia chapensis* for resistance factors (POD, SOD, CAT, MDA, PRO) was calculated based on principal component analysis (PCA) combined with subordinate function approach (Wang et al., 2022). The standardized data for the resistance factors were analyzed using *SPSS version 26.0* (*IBM Corp.*, Armonk, NY, USA) and processed with the subordinative function (SF) to evaluate the basic resistance level of the 109 germplasms examined here. The evaluation of basic resistance is based on the various SF indices and weighted value from principal component factor.

(1) SF value

For the positive correlation, including POD, SOD, CAT, and PRO, the form as follow:

$$u(x_{ij}) = \frac{x_{ij} - x_{j\,\min}}{x_{j\,\max} - x_{j\,\min}}$$

For the negative correlation, MDA, the form as follow:

$$u(x_{ij}) = 1 - \frac{x_{ij} - x_{j\,\min}}{x_{j\,\max} - x_{j\,\min}} (j = 1,, 2, 3, ..., n)$$

Here, i is a particular accession, j is a particular index, x_{ij} is the testing value of the index j of accession i, $x_{j\,min}$ is the minimum value of index j for all accessions, $x_{j\,max}$ is the maximum value of index j of all accessions, $u(x_{ij})$ is the SF value of accession i, and index j that relates to basic resistance.

(2) Weighted value

$$w_j = \frac{p_j}{\sum p_j} (j = 1,, 2, 3, ..., n)$$

p_j indicates the contribution rate of the j-th principal component factor for the dataset. w_j represents the importance (or weight) of the j-th principal component factor in all the comprehensive indicators.

(3) Score of basic resistance factors (SBRF)

$$SBRF_i = \sum \left[u\left(x_{ij}\right) \times w_j \right]$$

$SBRF_i$ indicates the SBRF value of the i-th *Michelia chapensis* clone.

Statistical analyses: Analysis of variance (*ANOVA*) was performed using *SPSS version 26.0* (*IBM Corp.*, Armonk, NY, USA).

Results

Significant clonal variation in basic resistance factors: *ANOVA* revealed highly significant differences ($P < 0.001$) among the 109 *M. chapensis* clones for all five basic resistance factors (Table 1). POD activity exhibited the broadest variation, ranging from 9 733.33 to 117 333.33 U g^{-1} FW, accompanied by the largest coefficient of variation (*CV* = 61.71%), indicating pronounced divergence among clones. SOD and CAT also showed wide ranges (120.96 ~ 2 005.33 U g^{-1} FW and 61.13 ~ 481.36 U g^{-1} FW, respectively), with relatively high *CV*s (50.42% and 48.21%). In contrast, PRO (85.54 ~ 263.72 µg g^{-1} FW, *CV* = 26.69%) and MDA (13.59 ~ 77.06 nmol g^{-1} FW, *CV* = 35.24%) exhibited narrower ranges and lower variability. Among these traits, CAT yielded the highest *F* value (160.29), reflecting the strongest clonal differentiation, whereas PRO had the lowest *F* value (10.20), suggesting moderate but still significant variation.

At the geographical-origin level, however, a different pattern emerged (Table 2). Only SOD activity and PRO content differed significantly among geographical origins, whereas POD, CAT, and MDA showed no significant differences. Notably, the GZ clones exhibited the highest mean SOD activity (767.06 U g^{-1} FW) and PRO content (159.23 µg g^{-1} FW), along with relatively high variation (*CV*s of 52.95% and 38.91%, respectively). These results suggest that POD and CAT variation is primarily distributed among individuals at the whole-population level, whereas SOD and PRO exhibit more structured differences at the geographical-origin level.

Multivariate analysis reveals divergence in basic resistance factors among clones: Scores plot based on principal component analysis (PCA) distinguished clear multivariate divergence in basic resistance factors among the 109 clones (Fig. 1*A*). PC1 (27.05%) and PC2 (21.25%) together explained 48.30% of the total variance. Clones from GZ (black dots) clustered tightly, indicating highly consistent resistance profiles, whereas GX and JX clones were more dispersed, suggesting greater within-group variation. Hierarchical clustering of z-score-normalized data (Fig. 1*B*) revealed distinct resistance expression patterns. Several GD clones (GDZC04, GDRH05, and GDSX01, *etc.*) exhibited consistently high SOD and CAT levels, potentially conferring superior stress tolerance. Column clustering indicated coordinated variation among PRO, POD, and MDA. Collectively, these multivariate analyses corroborate the *ANOVA* results, indicating that clonal differences reflect structured divergence in resistance profiles and providing a basis for identifying high-performing clones (*e.g.*, high SOD + CAT, low MDA) for targeted breeding.

Principal component and correlation analyses reveal patterns of variation among resistance factors: The PCA loadings (Table 3) and loading plot (Fig. 2*A*) indicate that POD (0.505) and MDA (0.487) contributed most strongly to PC1, with positive loadings, whereas CAT (–0.300) and SOD (–0.213) loaded negatively. PRO showed a moderate positive contribution (0.334) to PC1. PC2 was primarily influenced by SOD (0.623) and CAT (0.608), both with strong positive loadings, while POD, PRO, and MDA had smaller positive effects. These results suggest that PC1 mainly reflects variation between oxidative stress-related damage (MDA) and defense enzyme activity (POD), whereas PC2 captures variation driven by enzymatic antioxidant capacity (SOD and CAT).

The correlation matrix (Fig. 2*B*) revealed generally weak pairwise correlations among the five basic resistance factors, with the highest being a modest positive correlation between POD and MDA ($r = 0.21$, $P < 0.05$). CAT and SOD showed negligible associations with other factors, except for a weak positive relationship ($r = 0.11$). The absence of strong correlations suggests that these resistance factors vary largely independently across clones, reinforcing the rationale for a multivariate approach to assess overall resistance profiles.

Comprehensive evaluation and resistance grouping of clones: Based on PCA combined with the subordinate function approach, the comprehensive resistance score (SBRF) of 109 clones ranged from 0.185 to 0.738,

Table 1. Analysis of basic resistance factors in 109 clones of *Michelia chapensis*. ** indicates significance levels at $P < 0.01$. Values are the means ± standard deviation (mean ± SD) ($n = 3$), and *CV* represents the coefficient of variation. POD, SOD, CAT, PRO, and MDA refer to peroxidase, superoxide dismutase, catalase, proline, and malondialdehyde, respectively. FW - fresh weight. *F*-value: a ratio of between-group variance to within-group variance; larger values suggest greater differences between group means. *P*-value: the probability of observing the calculated *F*-value (or larger) if all group means are equal; $P \leq 0.05$ typically indicates significant group differences.

Basic resistance factors	Mean ± SD	Amplitude of variation	*CV* (%)	*F*	*P*
POD (U g^{-1} FW)	26 993.27 ± 16 657.76	9 733.33 ~ 117 333.33	61.71	50.64**	0.000
SOD (U g^{-1} FW)	634.79 ± 320.07	120.96 ~ 2 005.33	50.42	25.97**	0.000
CAT (U g^{-1} FW)	193.43 ± 93.26	61.13 ~ 481.36	48.21	160.29**	0.000
PRO (µg g^{-1} FW)	132.34 ± 35.32	85.54 ~ 263.72	26.69	10.20**	0.000
MDA (nmol g^{-1} FW)	36.07 ± 12.71	13.59 ~ 77.06	35.24	60.00**	0.000

Table 2. Analysis of basic resistance factors of *Michelia chapensis* with different geographical origins. * indicates significance level at $P < 0.05$. Values are the means ± standard deviation (mean ± SD) ($n = 3$), and *CV* represents the coefficient of variation. POD, SOD, CAT, PRO, and MDA refer to peroxidase, superoxide dismutase, catalase, proline, and malondialdehyde, respectively. FW - fresh weight. *F*-value: a ratio of between-group variance to within-group variance; larger values suggest greater differences between group means. *P*-value: the probability of observing the calculated *F*-value (or larger) if all group means are equal; $P \leq 0.05$ typically indicates significant group differences.

Basic resistance factors	Geographical origins	Mean ± SD	Amplitude of variation	*CV* (%)	*F*	*P*
POD (U g^{-1} FW)	GD	27 680.01 ± 15 303.86	9 733.33 ~ 93 333.33	55.29	1.179	0.324
	GX	21 911.11 ± 8 708.68	10 266.67 ~ 34 133.33	39.75		
	GZ	24 457.14 ± 10 419.60	14 266.67 ~ 39 333.33	42.60		
	HN	23 955.56 ± 11 702.99	9 866.67 ~ 45 333.33	48.85		
	JX	34 677.78 ± 30 311.96	14 400.00 ~ 117 333.33	87.41		
SOD (U g^{-1} FW)	GD	691.56 ± 323.01	120.96 ~ 2 005.33	46.71	2.816*	0.029
	GX	415.11 ± 131.43	204.01 ~ 612.39	31.66		
	GZ	767.06 ± 406.13	386.88 ~ 1 589.69	52.95		
	HN	598.51 ± 238.00	312.38 ~ 1 119.84	39.77		
	JX	547.90 ± 304.73	262.32 ~ 1 182.87	55.62		
CAT (U g^{-1} FW)	GD	196.95 ± 95.95	61.13 ~ 443.09	48.72	0.309	0.871
	GX	192.32 ± 137.11	79.67 ~ 481.36	71.29		
	GZ	200.05 ± 68.42	98.66 ~ 316.85	34.20		
	HN	198.53 ± 82.04	75.37 ~ 346.82	41.32		
	JX	165.39 ± 52.13	86.94 ~ 271.61	31.52		
PRO (μg g^{-1} FW)	GD	127.84 ± 26.56	85.54 ~ 213.80	20.78	3.044*	0.020
	GX	151.99 ± 43.91	92.07 ~ 263.72	28.89		
	GZ	159.23 ± 61.95	96.55 ~ 258.60	38.91		
	HN	129.08 ± 20.22	90.66 ~ 172.20	15.66		
	JX	124.37 ± 27.79	92.45 ~ 189.22	22.34		
MDA (nmol g^{-1} FW)	GD	35.45 ± 11.74	17.89 ~ 73.44	33.12	1.351	0.256
	GX	40.06 ± 11.60	25.11 ~ 59.17	28.96		
	GZ	30.12 ± 9.61	15.31 ~ 341.45	31.91		
	HN	34.32 ± 12.00	13.59 ~ 51.94	34.97		
	JX	42.25 ± 17.93	18.23 ~ 77.06	42.44		

indicating marked variability in resistance potential (Fig. 3*A*). Using the 33.33% and 66.67% percentiles as thresholds, 36 clones were classified as high-resistance (SBRF > 0.422), 37 as moderate-resistance (0.332 - 0.422), and 36 as low-resistance (SBRF ≤ 0.332). High-resistance clones, such as GDSX03, GZLP01, GDLC16, GXRS02, and GXYF04, demonstrated superior performance across multiple resistance factors. The high-, moderate-, and low-resistance groups each comprise clones from five different geographical-origin provinces, indicating significant variation in basic resistance both among and within provenances. Notably, heatmap analysis showed that the top five clones exhibited generally higher antioxidant enzyme activities and PRO levels, along with lower MDA contents, indicating that these five clones are elite basic resistant clones (Fig. 3*B*).

Discussion

As an endangered species (*M. chapensis*) facing environmental degradation and habitat loss (Zhou et al., 2023), identifying trait variation at the clonal level is critical for enhancing its resilience. To our knowledge, this study represents the first large-scale, systematic assessment of clonal variation in physiological resistance factors of *M. chapensis* under natural conditions. The pronounced clonal variation in the five resistance factors (POD, SOD, CAT, MDA, PRO) among the 109 *M. chapensis* clones is of paramount importance for the species' conservation and breeding strategies. The high coefficient of variation in POD (61.71%) and significant *F* values across all factors ($P < 0.001$) suggest that there is substantial genetic variation available for selection in breeding programs (Yoshida et al., 2003). This is in line with the general understanding that intraspecific variation is crucial for a species' ability to adapt to changing environments (O'Dell and Rajakaruna, 2011).

Antioxidant defense efficiency differs among plant species and genotypes (Hasanuzzaman et al., 2020). Our findings support this view by revealing highly significant differences among the 109 evaluated clones in individual basic resistance factors, including POD, SOD, CAT, PRO, and MDA. At the clonal level, SOD and CAT activities

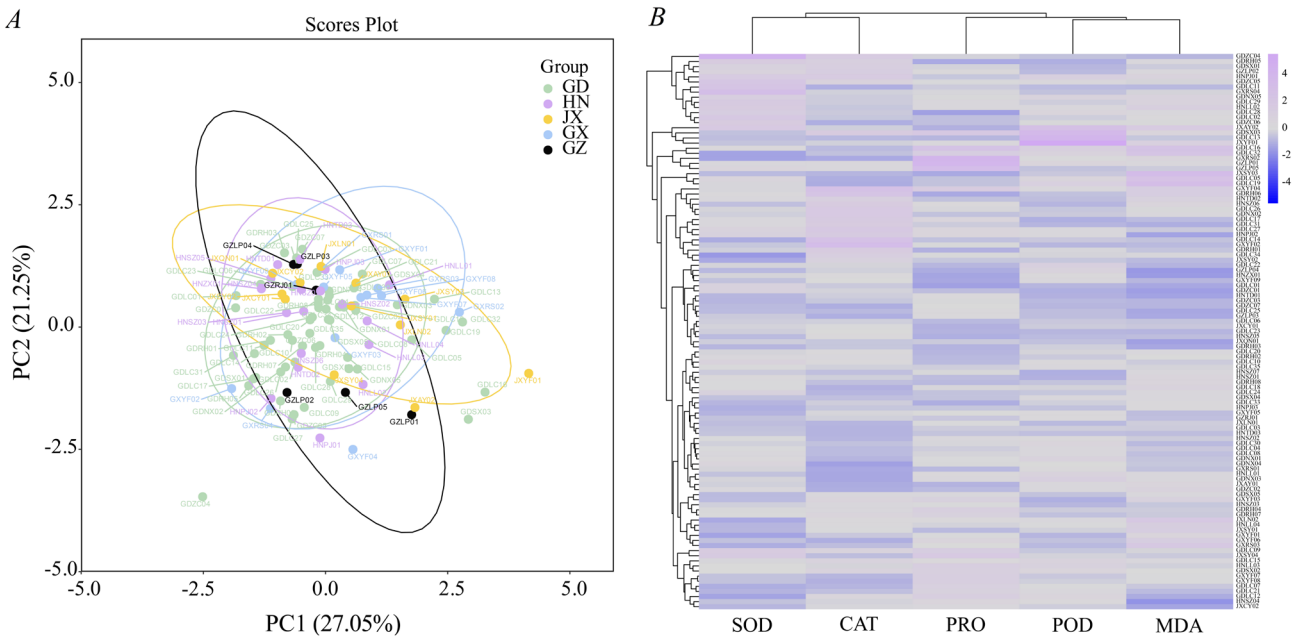

Fig. 1. Principal component analysis (PCA) scores plot (*A*) of five basic resistance factors (POD, SOD, CAT, MDA, PRO) based on samples from different geographical populations (GD, HN, JX, GX, GZ). Scatter points with different colors represent samples of corresponding populations, and ellipses delineate the distribution range of samples within each population at a 95% confidence level (0.95). Cluster heatmap (*B*) of five basic resistance factors in different samples. The darker blue (near 0.00) indicates a lower value of the index; the lighter purple (near 1.00) indicates a higher value of the index.

Table 3. Principal component analysis of basic resistance factors in *Michelia chapensis*. POD, SOD, CAT, PRO, and MDA refer to peroxidase, superoxide dismutase, catalase, proline, and malondialdehyde, respectively. FW - fresh weight.

Basic resistance factors	Principal component loadings		
	PCA1	PCA2	PCA3
POD (U g^{-1} FW)	0.505	0.181	–0.237
SOD (U g^{-1} FW)	–0.213	0.623	–0.530
CAT (U g^{-1} FW)	–0.300	0.608	0.392
PRO (μg g^{-1} FW)	0.334	0.281	0.676
MDA (nmol g^{-1} FW)	0.487	0.267	–0.207
Eigenvalue	1.352	1.063	1.010
Contribution rate (%)	27.048	21.252	20.203
Accumulating contribution rate (%)	27.048	48.300	68.503

in *Iris pumila* reached 50 000 - 280 000 and 50 000 - 400 000 U g^{-1} FW, respectively, with MDA contents of 2 - 10 nmol g^{-1} FW (Vuleta et al., 2016). In white poplar, PRO ranged from 207 to 460 μg g^{-1} FW (Vuksanović et al., 2023). By contrast, *M. chapensis* showed much lower SOD (120.96 - 2 005.33 U g^{-1} FW) and CAT (61.13 - 481.36 U g^{-1} FW) activities but higher MDA contents (13.59 - 77.06 nmol g^{-1} FW). Its PRO levels (85.54 - 263.72 μg g^{-1} FW) partially overlapped with poplar but were generally lower. These patterns suggest that *M. chapensis* maintains a distinct antioxidant and osmotic adjustment profile, which likely reflects adaptive responses to its ecological habitats (Wang et al., 2009). Within *M. chapensis*, our large-scale (109 clones) survey fills gaps in prior small-scale studies: 61 clones exceeded 550 U g^{-1} FW in SOD (*vs.* <550 U g^{-1} FW in Huang et al., 2016) and 99 exceeded 300 U g^{-1} FW (*vs.* <300 U g^{-1} FW in Pan et al., 2020; 2021); POD's lowest value (9 733.33 U g^{-1} FW) was far higher than Shen et al. (2020)'s 799.20 U g^{-1} FW, while PRO (85.54 ~ 263.72 μg g^{-1} FW) was higher than their 38.32 μg g^{-1} FW. This extensive variation highlights the value of our germplasm panel for capturing intraspecific diversity, which is key to targeted breeding.

Variation in antioxidant factors among the *M. chapensis* clones was evaluated under uniform cultivation conditions, minimizing environmental influences and indicating that the observed differences primarily reflect genetic control (Song et al., 2014). CAT activity exhibited marked differentiation (F = 160.29), consistent with inherent genetic capacity for H_2O_2 decomposition and oxidative stress mitigation (Smirnoff and Arnaud, 2019). The wide variation in CAT activity among clones may therefore reflect differences in their genetic ability to cope with oxidative stress, which could be exploited in breeding for stress-tolerant varieties (Song et al., 2014). Significant variation was also observed in SOD activity and PRO content among provinces of origin, with Guizhou (GZ) clones displaying the highest mean values (SOD: 767.06 U g^{-1} FW; PRO: 159.23 μg g^{-1} FW). Given the uniform growth environment, these differences are likely attributable to intrinsic genotypic variation, potentially shaped by historical selection in their native habitats rather than by current environmental conditions. For example, Guizhou's higher elevation, compared

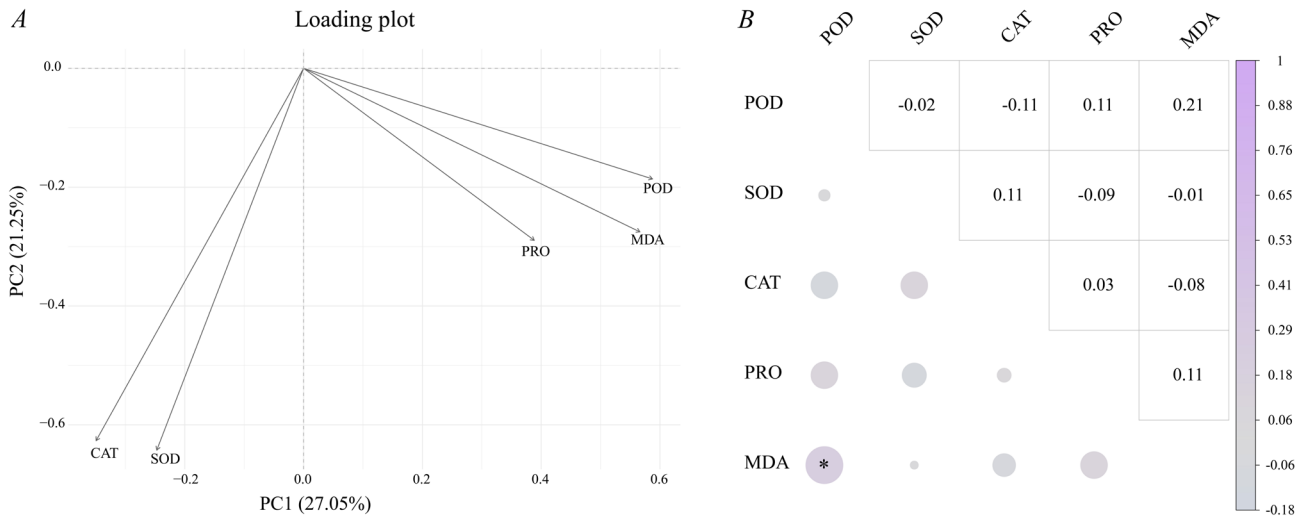

Fig. 2. Principal component analysis (PCA) loading plot (*A*) of five basic resistance factors (POD, SOD, CAT, MDA, PRO) based on samples from different geographical populations (GD, HN, JX, GX, GZ). Correlation (*B*) among five basic resistance factors based on *Pearson* coefficient.

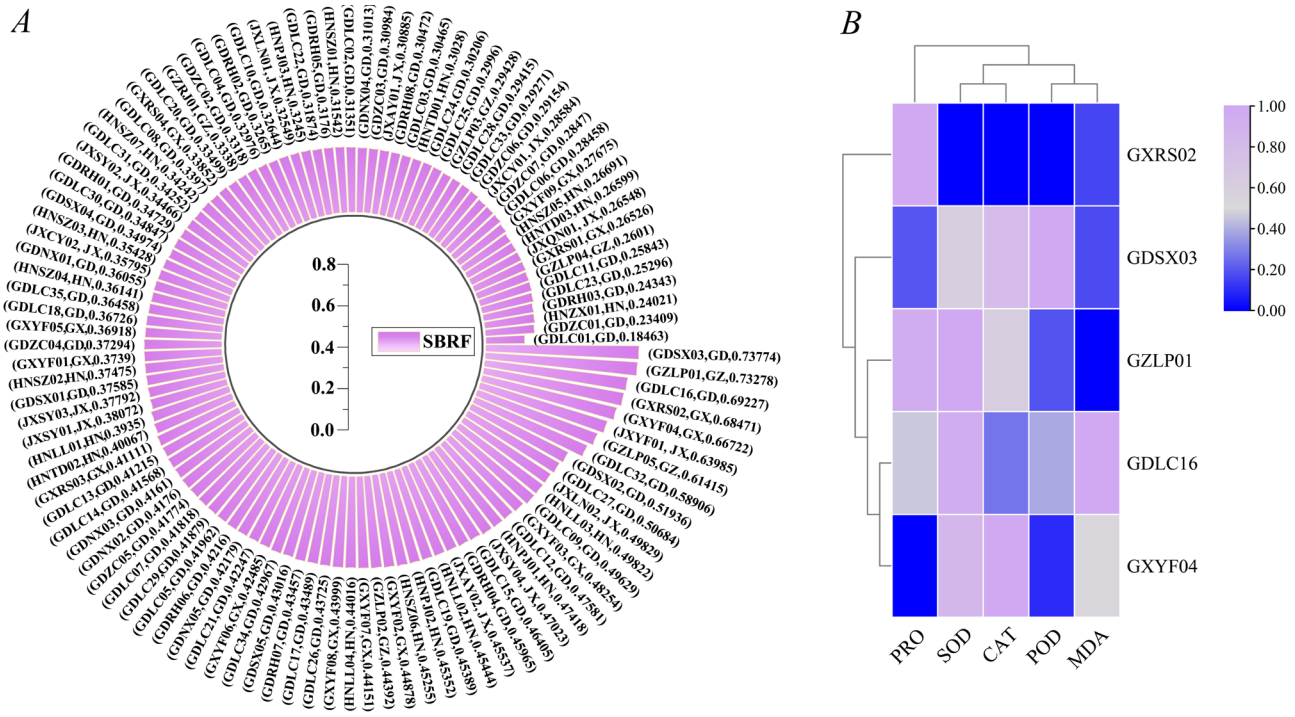

Fig. 3. Comprehensive score value of 109 clones in *Michelia chapensis*. (*A*) Radial bar plot of SBRF for 109 *Michelia chapensis* clones, derived from five basic resistance factors (POD, SOD, CAT, MDA, and PRO). (*B*) Heatmap of top five clones based on SBRF. SBRF - score of basic resistance factors. The darker blue (near 0.00) indicates a lower value of the index; the lighter purple (near 1.00) indicates a higher value of the index.

with provinces such as Guangdong, creates unique environmental selection pressures, including greater exposure to ultraviolet-B radiation (UV-B) (Jiao et al., 2022). SOD, as the first line of defense against ROS, is crucial for scavenging superoxide radicals ($O_2^{\cdot-}$) generated under stress conditions such as UV-B exposure (Fu and Shen, 2017). The higher SOD activity in GZ-origin clones may therefore represent a genotypic legacy of adaptation to UV-B stress. Similarly, PRO, which functions as an osmolyte, contributes to maintaining cellular osmotic balance under stress (Hayat et al., 2012). Recently, Wei et al. (2025) predicted that the distribution of this species was mainly constrained by water and heat, both of which are tightly linked to elevation. The elevated PRO levels observed in GZ-origin clones may thus be associated with enhanced stress tolerance, as PRO concentrations are generally higher in stress-tolerant than in stress-sensitive plants (Ashraf and Foolad, 2007). Collectively, POD

and CAT variation appears to reflect fine-scale genetic differentiation among individuals within geographical-origin provinces, whereas SOD and PRO may retain genotypic signatures shaped by ancestral adaptation among geographical-origin provinces.

PCA revealed two distinct antioxidant strategies in *M. chapensis* clones. PC1, which explained 27.05% of the total variance and was characterized by positive loadings of POD and MDA and negative loadings of CAT and SOD, represents a strategy where clones prioritize POD-mediated damage mitigation. POD is involved in the polymerization of phenolics and lignin synthesis, which can help repair cell wall damage caused by oxidative stress (Mnich et al., 2020). Higher MDA levels in these clones may indicate more significant oxidative damage, but the elevated POD activity could be a compensatory mechanism (Li et al., 2013). In contrast, PC2, explaining 21.25% of the variance with strong positive loadings of SOD and CAT, reflects a strategy focused on ROS prevention. Clones with high scores on PC2 are likely better equipped to prevent the accumulation of ROS in the first place, thereby reducing the potential for oxidative damage. The weak pairwise correlations among the five resistance factors (Fig. 3*A*) justify the use of a multivariate approach for comprehensive resistance assessment. Importantly, these two antioxidant strategies can inform breeding recommendations tailored to different stress environments. Clones with strong PC2 performance (high SOD/CAT activity) are likely more suitable for regions prone to acute oxidative bursts, such as areas with frequent heat waves, or high-intensity UV radiation, where rapid ROS scavenging capacity is critical. In contrast, clones exhibiting a PC1-type response (high POD and lower MDA) may offer advantages in environments where oxidative stress is more chronic or sustained, such as sites experiencing long-term moderate drought, as their enhanced cell wall repair and phenolic-based defense mechanisms support long-term damage tolerance. Thus, integrating both antioxidant strategies into breeding programs will facilitate the selection of genotypes with context-appropriate stress resistance.

The top five elite clones (GDSX03, GZLP01, GDLC16, GXRS02, and GXYF04) identified in this study exhibited a combination of high SOD/CAT/POD activities and low MDA content (Fig. 3*B*). Notably, these elite clones originate from multiple provinces (*e.g.*, GDSX03 from Guangdong, GZLP01 from Guizhou; GXYF04 from Guangxi). This implies that high resistance is not limited to a single provenance. In breeding programs, mixing clones from different provenances can maintain genetic diversity while improving population-level stress resilience (Engelhardt et al., 2014). By incorporating clones with different genetic backgrounds and antioxidant strategies, the resulting population will be more adaptable to a wider range of environmental stressors.

This study has several limitations that warrant consideration. First, all sampling was conducted at a single site and within one season, which constrains the ability to evaluate clonal variation under diverse environmental and seasonal conditions. Second, the absence of molecular marker or epigenetic analyses precludes distinguishing heritable genetic differences from environmentally induced or epigenetic effects. Third, the assessment was limited to five antioxidant-related resistance factors, without integrating other relevant physiological or metabolic traits that may contribute to stress tolerance. Future work should therefore expand sampling across sites and seasons, incorporate genomic and epigenomic approaches, and adopt a broader set of traits to provide a more comprehensive understanding of clonal variation and its breeding potential in *M. chapensis*.

Conclusion

This study demonstrates pronounced clonal variation in antioxidant resistance factors of *M. chapensis*, with CAT and POD showing strong genetic differentiation and SOD and PRO reflecting regional adaptations. PCA revealed two complementary antioxidant strategies: POD-mediated damage mitigation and SOD/CAT-driven ROS prevention. Several elite clones (GDSX03, GZLP01, GDLC16, GXRS02, and GXYF04) combined high SOD, CAT, and POD activities with low MDA, indicating robust antioxidant defense and minimal cellular damage. Their distribution across multiple provinces suggests that stress-tolerant genotypes are not restricted to a single provenance. However, the unbalanced sample sizes across provinces, particularly the small number of clones from GZ, may introduce bias in estimating mean SOD and PRO levels, and the observed geographical structuring should therefore be interpreted with caution until validated with more balanced sampling. Collectively, these findings provide a genetic and physiological basis for selecting elite clones and integrating complementary antioxidant strategies to enhance the conservation and breeding of this endangered species. Future work should aim to integrate molecular-level data, such as transcriptomic and genomic analyses, and explore additional physiological traits to deepen our understanding of the regulatory mechanisms underlying these antioxidant strategies and further guide breeding programs.

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