Selection of Superior Second-Generation Families of Castanopsis hystrix Based on Physiological and Biochemical Indicators (Post-Print)

Authors: Tan Changqiang, Shen Wenhui, Liu Xiu, Cao Yanyun, Huang Zhiling, Hao Haikun

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Abstract

By measuring key substances in the photosynthetic carbon and nitrogen assimilation process (chlorophyll, nitrate reductase activity, glutamine synthetase activity, total ATPase content, PEP carboxylase content, and RUBP carboxylase content) in seedlings from 29 second-generation families of superior Castanopsis hystrix trees, superior families were screened and evaluated using principal component analysis and membership function method. The results showed that significant differences existed among the 29 families for all nine physiological indicators. Chlorophyll a content ranged from 0.13~0.72 mg·g^{-1}Fw, chlorophyll b content from 0.01~0.27 mg·g^{-1}Fw, chlorophyll a+b content from 0.18~0.98 mg·g^{-1}Fw, carotenoid content from 0.03~0.32 mg·g^{-1}Fw, nitrate reductase activity from 1.16~10.26 μg··g^{-1}··h^{-1}, glutamine synthetase activity from 0.30~1.24 A··mg^{-1}protein·h^{-1}, total ATPase content from 0.37~3.55 U·mg^{-1} prot, PEP carboxylase content from 8.42~21.24 IU·L^{-1}, and RUBP carboxylase content from 2.09~9.12 ng·mL^{-1}. Among these, total ATPase content showed the greatest variation, followed by nitrate reductase activity; RUBP carboxylase content showed the least variation. Using principal component analysis and membership function method as comprehensive multi-indicator evaluation methods, 10 superior second-generation families of Castanopsis hystrix were selected, with a repeatability rate of 90%, specifically families B2, B5, P5, A6, P3, P6, R3, D2, and R4. This indicates that both principal component analysis and membership function method can be used to evaluate superior second-generation families of Castanopsis hystrix. Among them, families B2 and B5 showed the best performance.

Full Text

Preamble

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Seedling Selection of Superior Second-Generation Families of *Castanopsis hystrix* Based on Physiological and Biochemical Indices

Tan Zhangqiang, Shen Wenhui*, Liu Xiu, Cao Yanyun, Huang Zhiling, Hao Haikun
Guangxi Academy of Forestry, Nanning 530002, China

Abstract

This study measured nine key physiological indicators related to photosynthetic carbon and nitrogen assimilation in seedlings from 29 superior second-generation families of *Castanopsis hystrix*, including chlorophyll content, nitrate reductase activity, glutamine synthetase activity, total ATPase content, PEP carboxylase content, and RuBP carboxylase content. Principal component analysis and membership function methods were employed to screen and evaluate superior families. The results revealed significant differences among the 29 families for all nine physiological indicators. Chlorophyll a content ranged from 0.13 to 0.72 $\mathrm{mg \cdot g^{-1}}$ FW, chlorophyll b from 0.01 to 0.27 $\mathrm{mg \cdot g^{-1}}$ FW, total chlorophyll (a+b) from 0.18 to 0.98 $\mathrm{mg \cdot g^{-1}}$ FW, and carotenoids from 0.03 to 0.32 $\mathrm{mg \cdot g^{-1}}$ FW. Nitrate reductase activity varied from 1.16 to 10.26 $\mathrm{\mu g \cdot g^{-1} \cdot h^{-1}}$, glutamine synthetase activity from 0.30 to 1.24 $\mathrm{A \cdot mg^{-1}}$ protein $\cdot \mathrm{h^{-1}}$, total ATPase from 0.37 to 3.55 $\mathrm{U \cdot mg^{-1}}$ protein, PEP carboxylase from 8.42 to 21.24 $\mathrm{IU \cdot L^{-1}}$, and RuBP carboxylase from 2.09 to 9.12 $\mathrm{ng \cdot mL^{-1}}$. Total ATPase content showed the greatest variation, followed by nitrate reductase activity, while RuBP carboxylase content exhibited the least variation. Using principal component analysis and membership function methods for multi-index comprehensive evaluation, ten superior second-generation families were selected with a 90% repeatability rate: B2, B5, P5, A6, P3, P6, R3, D2, and R4. Both methods proved effective for evaluating superior second-generation families of *C. hystrix*, with families B2 and B5 demonstrating the best overall performance.

Keywords: *Castanopsis hystrix*, second-generation families, principal component analysis, membership function method, seedling selection, physiological indices

Introduction

Early selection refers to the selection of forest trees before they reach economic maturity. Previous studies have demonstrated that selecting superior individuals and families during the seedling stage not only shortens the breeding cycle but also reduces the scale of test plantations, thereby improving genetic gains and lowering experimental costs. Chlorophyll plays a crucial role in absorbing and transferring light energy, providing the primary energy source for photosynthesis, and its content can reflect plant productivity to a certain extent. Enzymes such as nitrate reductase, glutamine synthetase, total ATPase, PEP carboxylase, and RuBP carboxylase serve as key indicators of plant assimilation capacity, energy transformation efficiency, and cellular metabolic activity. Therefore, evaluating these physiological and enzymatic markers during the seedling stage offers a practical approach for screening superior families of *Castanopsis hystrix*.

Castanopsis hystrix, a member of the Fagaceae family, is a valuable evergreen timber species with strong adaptability and rapid growth. In Guangxi alone, the planted area of *C. hystrix* has exceeded 15,000 hectares. With the implementation of national timber reserve programs for native precious species, increasing market demand for high-grade timber, and the ban on natural forest harvesting, the area of *C. hystrix* plantations is expected to expand further. However, genetic improvement research on this species lags behind production needs. Current studies have focused primarily on growth traits and photosynthetic capacity of first-generation superior families, while early selection of second-generation families based on key substances in the photosynthetic carbon and nitrogen assimilation process remains unreported. This study provides a scientific basis for further breeding of *C. hystrix*.

Materials and Methods

1.1 Experimental Site

The experiment was conducted at the precious species nursery of Guangxi Academy of Forestry in Nanning (108°20 E, 22°55 N). The site features a humid subtropical monsoon climate at an elevation of approximately 80 m. Mean temperatures are 28.2°C in July-August and 12.8°C in January, with extreme highs of 39.4°C and lows of -1.5°C. The annual average temperature is 21.7°C, with over 1,550 sunshine hours annually and accumulated temperature \$ \$10°C of 7,200°C. Frost is virtually absent year-round. Annual precipitation averages 1,300 mm, concentrated mainly from April to September. The soil is lateritic red earth with a pH of 5.6 and moderate fertility.

1.2 Experimental Materials

Seeds were collected from November to December 2016 from a superior tree progeny stand of *C. hystrix* in Pingxiang, Guangxi, representing 29 families. After soaking and sun-drying to remove shells, healthy and plump seeds were selected and sown in sand beds for germination. When 60-70% of seeds had emerged, seedlings with developed plumules or leaves were transplanted into 18 cm × 18 cm nursery bags filled with yellow subsoil. Uniform water, fertilizer, and pest management practices were applied to all seedlings.

1.3 Physiological Measurements

In January 2018, five plants from each family with height and ground diameter near the family mean were selected for physiological measurements. Three healthy, mature but not senescent leaves per plant were sampled, with three replicates per indicator. Measurement methods were as follows: chlorophyll a, chlorophyll b, and carotenoid contents were determined using acetone-ethanol extraction; nitrate reductase activity was measured by the in vivo method; glutamine synthetase activity followed Cai et al. (2010); total ATPase was assayed using a micro-ATPase test kit from Nanjing Jiancheng Bioengineering Institute; and PEP carboxylase and RuBP carboxylase were measured using ELISA kits.

1.4 Data Analysis

Data were processed and analyzed using WPS, DPS 7.05, and SPSS 19.0. The membership function method was used for comprehensive evaluation of different families, calculated as:

(1) u_{ij} = x_{ij} / max(x_j)

where u_{ij} is the standardized value of indicator j for family i, x_{ij} is the original value, and max(x_j) is the maximum value of indicator j across all families. Indicator weights were determined using the standard deviation coefficient method. The standard deviation coefficient v_j was calculated, followed by weight w_j for each indicator. Finally, comprehensive evaluation values were obtained to assess the quality of second-generation seedlings from superior *C. hystrix* trees.

Results

2.1 Physiological Indices of 29 Second-Generation Families

As shown in , significant differences ($P < 0.05$) were observed among the 29 families for all nine physiological indicators: chlorophyll a, chlorophyll b, total chlorophyll (a+b), carotenoids, nitrate reductase, glutamine synthetase, total ATPase, PEP carboxylase, and RuBP carboxylase. This rich variation confirms the feasibility of selecting superior second-generation families at the seedling stage. Specifically, chlorophyll a content ranged from 0.13 to 0.72 $mg \cdot g^{-1}$ FW (mean 0.38), with family P5 showing the highest value (155.8% above the mean). Chlorophyll b ranged from 0.01 to 0.27 $mg \cdot g^{-1}$ FW (mean 0.12), again highest in P5 (209.3% above mean). Total chlorophyll varied from 0.18 to 0.98 $mg \cdot g^{-1}$ FW (mean 0.50), with P5 exceeding the mean by 160.2%. Carotenoid content spanned 0.03–0.32 $mg \cdot g^{-1}$ FW (mean 0.13), peaking in family R2 (222.1% above mean). Nitrate reductase activity ranged from 1.16 to 10.26 $\mu g \cdot g^{-1} \cdot h^{-1}$ (mean 5.00), highest in B5 (182.1% above mean). Glutamine synthetase activity varied from 0.30 to 1.24 $A \cdot mg^{-1}$ protein $\cdot h^{-1}$ (mean 0.54), with P4 showing the maximum (172.8% above mean). Total ATPase content ranged from 0.37 to 3.55 $U \cdot mg^{-1}$ protein (mean 1.36), peaking in A9 (233.8% above mean).

PEP carboxylase varied from 8.42 to 21.24 $\mathrm{IU \cdot L^{-1}}$ (mean 13.57), highest in B5 (94.5% above mean). RuBP carboxylase ranged from 2.09 to 9.12 $\mathrm{ng \cdot mL^{-1}}$ (mean 6.92), with B2 showing the highest value (101.5% above mean).

2.2 Selection Potential of Single Indices

Single-index selection () reveals the maximum genetic improvement potential for each trait. The top 10 families for each indicator overlapped partially but were not identical. Total ATPase content showed the greatest improvement when selecting the top 10 families, followed by nitrate reductase activity, while RuBP carboxylase showed the smallest improvement.

2.3 Comprehensive Evaluation

2.3.1 Principal Component Analysis Based on the criterion of cumulative contribution rate >80%, the first four principal components were selected, accounting for 80.6% of total variance (). These components adequately represent all indicators for evaluating drought resistance among families. The first component (eigenvalue 3.56, contribution rate 39.6%) primarily comprised chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. The second component (eigenvalue 1.47, 16.3%) loaded heavily on nitrate reductase, total ATPase, and PEP carboxylase. The third component (eigenvalue 1.20, 13.3%) included glutamine synthetase and PEP carboxylase. The fourth component (eigenvalue 1.03, 11.4%) encompassed glutamine synthetase, total ATPase, and RuBP carboxylase.

Principal component analysis ranked the 29 families, with the top 10 being: B2 > B5 > P5 > A6 > P3 > P6 > R3 > D2 > P7 > R4 ().

2.3.2 Membership Function Method Using the membership function method, the top 10 families were: B2 > B5 > A6 > D2 > A9 > P3 > P5 > R4 > P6 > R3 ().

2.3.3 Comprehensive Selection of Superior Families Although the two methods yielded slightly different rankings, the overall trends were consistent (and). Families common to both methods were selected as superior: B2, B5, P5, A6, P3, P6, R3, D2, and R4.

Discussion and Conclusion

Plant physiological metabolism results from the combined effects of environmental and genetic regulation. Under controlled conditions, genotypic differences in specific traits manifest as variations in corresponding physiological markers. The significant differences observed among the 29 second-generation families for all nine indicators reflect rich genetic diversity, providing a material basis for seedling selection. Similar findings by Zhao et al. (2013) on physiological characteristics of superior *Quercus variabilis* families support the use of these indicators for early selection of rapid-growth traits.

Single-index selection revealed both overlaps and differences among families across indicators, suggesting correlations among photosynthetic carbon-nitrogen assimilation traits and information redundancy. However, it also indicates that individual indicators play different roles in growth processes, and relying on single traits may introduce selection bias. Therefore, multi-index evaluation methods such as membership function, principal component analysis, and cluster analysis are necessary for more accurate selection. Jiang et al. (2016) applied the membership function method to select superior *Phoebe bournei* families, while Gao et al. (2016) used both membership function and principal component analysis on *Reaumuria soongorica* families, achieving 80% overlap between methods. Our study achieved 90% overlap in the top 10 families selected by both methods (B2, B5, P5, A6, P3, P6, R3, D2, R4), demonstrating that both approaches are effective for evaluating second-generation *C. hystrix* families.

Comparing these results with our previous growth studies, the top 10 families for height (B2, A7, B5, A8, P3, B4, A6, R4, D2, R3) and ground diameter (B2, A7, B5, P3, P2, A3, R4, D2, A6, A2) showed over 60% overlap with those selected by physiological indicators. This confirms that analyzing key substances in photosynthetic carbon-nitrogen assimilation is a viable approach for early selection of superior *C. hystrix* families.

This study utilized nine key physiological and biochemical indicators for seedling selection of second-generation superior families. However, the correlation between these results and long-term family performance requires further validation. Future research should continue monitoring additional growth and physiological indicators (e.g., tree height, DBH, volume, photosynthetic rate) to optimize the evaluation system and enhance the reliability of early selection in *C. hystrix*.

References

CAI HM, XIAO JH, ZHANG QF, et al., 2010. Co-suppressed glutamine synthetase2 gene modifies nitrogen metabolism and plant growth in rice[J]. *Chin Sci Bull*, 55(10): 871-882.

CAI RG, ZHANG M, YIN YP, et al., 2008. Photosynthetic characteristics and antioxidative metabolism of flag leaves in responses to nitrogen application in wheat during grain filling[J]. *Scientia Agricultura Sinica*, 41(1): 53-62.

CHEN LS, 2009. The researches on seed quality and seedling growth characteristics of *Pistacia chinensis* bunge from different provenances[D]. Nanjing Forestry University.

CHEN Y, LIU SL, YANG RJ, et al., 2015. Effects of cadmium on growth, plasma membrane ATPase activity, and absorption of N, P and K in *Solanum nigrum* L. seedlings[J]. *Chin J Appl Environ Biol*, 21(01): 121-128.

DU KB, XU L, SHEN BX, et al., 2009. Genetic analysis and seedling selection of the Poplar progenies of aigeiros section[J]. *J Huazhong Agric Univ*, 28(05): 624-630.

GAO HX, SU SP, LI Y, et al., 2016. Early selection of drought-resistant superior families of *Reaumuria soongorica* based on analysis of osmoregulation substances and chlorophyll[J]. *Chin J Appl Ecology*, 27(01): 40-48.

HAO NB, DU WG, 1991. A comparative study of PEPC activity of green organs in C3 plant[J]. *Acta Bot Sin*, (9): 692-697.

JIANG Y, LI ZH, ZHU JY, et al., 2013. Diurnal variations of photosynthetic characteristics of different provenances *Castanopsis hystrix* A. DC. young plantations[J]. *J Centr S Univ For & Technol*, 33(06): 43-47.

JIANG L, YANG XY, 2005. Applications of physiological and biochemical indexes in tree genetics and breeding[J]. *Hebei J For Orch Res*, (01): 76-79.

JIANG BS, ZHANG Y, WEI XL, et al., 2016. Growth and photosynthetic physiology for two groups of *Phoebe bournei* plus tree seedlings[J]. *J Zhejiang A & F Univ*, 33(01): 51-59.

LIU DY, LI GQ, LI LF, et al., 2009. Seedling selection of superior families and excellent-individuals selection of *Pinus yunnanensis*[J]. *J NW For Univ*, 24(04): 67-72.

LIU SL, YANG RJ, MA MD, et al., 2015. Effects of exogenous NO on the growth, mineral nutrient content, antioxidant system, and ATPase activities of *Trifolium repens* L. plants under cadmium stress[J]. *Acta Physiol Plant*, 37(1): e1721.

PAN RZ, 2010. *Plant Physiology*[M]. Beijing: Higher Education Press.

WANG JH, GU WC, LI B, et al., 2000. Study on selection of *Alnus cremastogyne* provenance/family—analysis of growth adaptation and genetic stability[J]. *Sci Silv Sin*, (03): 59-66.

WU ZF, SUN XW, WANG CB, et al., 2014. Effects of low light stress on rubisco activity and the ultrastructure of chloroplast in functional leaves of peanut[J]. *Chin J Plant Ecol*, 38(07): 740-748.

YANG L, ZHAO HW, LIU JH, 2007. Effects of different nitrogen application on GS activity and yield in different quality spring maize varieties[J]. *J NE Agric Univ*, 38(3): 320-324.

YANG S, LIU ZX, ZHANG HX, et al., 2013. Comprehensive evaluation of salt tolerance and screening identification indexes for three tree species[J]. *Sci Silv Sin*, 49(01): 91-98.

YANG XY, JI KZ, 2004. Early selection in forest tree improvement[J]. *World For Res*, (02): 6-8.

ZHAO R, ZHANG CX, ZHANG WH, 2013. Physiological and biochemical characteristics of *Quercus variabilis* in plus tree progeny[J]. *J NW For Univ*, 28(01): 86-89.

ZHU JY, SHEN WH, JIANG Y, et al., 2014. Genetic variation and superior family selection of *Castanopsis hystrix* families[J]. *J Trop Subtr Bot*, 22(03): 270-280.

ZOU Q, 2000. *Plant Physiology Experiment Instruction*[M]. Beijing: China Agriculture Press: 163-165.

ZHOU ZQ, LI JW, DENG XY, et al., 2009. The ATPase activity in phloem cells and its relation to the accumulation of photo-assimilates in developing caryopsis during wheat grain filling[J]. *Sci Agric Sin*, (42): 2314-2325.

Note: Figure translations are in progress. See original paper for figures.