QTL mapping for hull color identified one major QTL through four different phenotyping methods in Foxtail millet[27]

Shaohua Chai[1], Xing Shi[1], Yan Guo[1], Shuqing Guo[1], Hui Song[2], Qinghua Yang [1], Jinfeng Gao[1], Xiaoli Gao[1], Baili Feng [1], and Pu Yang[1,*]

(*[1] College of Agriculture, Northwest A&F University/State Key Laboratory of Crop Stress Biology in Arid Areas, Yangling 712100, China; [2] Institute of Millet Crops, Anyang Academy of Agricultural Sciences, Anyang 455000, China)*

Abstract: 【**Objective**】 Foxtail millet (*Setaria italica* L.) is a highly nutritious crop with significant value, especially in arid and semi-arid regions. Hull color is a crucial characteristic that helps assess the nutritional value and economic potential of foxtail millet. However, since the color is a complex trait, accurately and efficiently identifying hull color is not possible through a single detection method alone. Additionally, the genetic mechanisms governing hull color in foxtail millet are not yet fully comprehended. As such, the objective of the present study is to unravel the genetic mechanism behind hull color in foxtail millet. 【**Method**】 The F_6 recombinant inbred line population (RIL), consisting of 250 families generated from the crossing of Yugu 18 and Hongjiugu, was chosen as the focus of this study. To analyze the quantitative trait locus (QTL) associated with hull color in foxtail millet, four phenotypic detection methods (I: grouping method, II: visual colorimetry method, III: Lab-based method, IV: RGB-based method) were utilized in four different environments. Additionally, we chose a RIL line with a light yellow hull (LY) and another with dark yellow hull (DY) for RNA sequencing analysis at three distinct stages. 【**Result**】 Our analysis using the four phenotypic detection methods identified a total of 36 QTL associated with hull color. Among these QTLs, 13 QTLs were repeatedly mapped in multiple environments, with four major QTL ($R^2 \geq 10\%$) and nine minor QTL explaining 1.09% to 69.63% of the phenotypic variation. By conducting RNA sequencing analysis, we identified 3183 differentially expressed genes (DEGs) related to hull color. Previous studies suggested that it was found that lignin metabolism and flavonoids are important factors that affect hull color. We obtained 43 and 11 DEGs from the phenylpropanoid metabolic pathway and flavonoid biosynthesis pathway pathways, respectively. 【**Conclusion**】 The results of this study lay a foundation for further cloning of hull color genes, and help to elucidate the regulatory mechanism of foxtail millet hull color synthesis.