

## **Article**

## Berry texture QTL and candidate gene analysis in grape (Vi i i ife a L.)

Hong Lin

(PerB). The high-density linkage map was constructed using whole-genome resequencing based on 151 F

1 individuals originating from

intraspecific hybridization between the firm-flesh cultivar 'Red Globe' and soft-flesh cultivar 'Muscat Hamburg'. The total length of the consensus map was 1613.17 cM, with a mean genetic distance between adjacent bin markers of 0.59 cM. Twenty-seven quantitative trait loci (QTLs) for berry MesF, PPH, and PerB were identified in linkage groups (LGs) 1, 3, 4, 6, 8, 9, 10, 11, 14, 16, and 17, including twelve QTLs that were firstly detected in LGs 6, 11, and 14. Fourteen promising candidate genes were identified from the stable QTL regions in LGs 10, 11, 14, and 17. In particular, VvWARK2 and VvWARK8 refer to chromosome 17 and are two promising candidate genes for MesF and PPH, as the VvWARK8 gene may increase pectin residue binding with WARK for high berry firmness maintenance and the allele for VvWARK2 carrying the 'CC' and 'GA' genotypes at Chr17:1836764 and Chr17:1836770 may be associated with non-hard texture grape cultivars. In addition, real-time quantitative polymerase chain reaction (RT-qPCR) verification revealed that the promising candidate transcription factor genes VvMYB4-like, VvERF113, VvWRKY31, VvWRKY1, and VvNAC83 may regulate cell wall metabolism candidate gene expression for grape berry texture changes.

## Introduction

Grape (Vitis vinifera L.), which belongs to family Vitaceae, is an economically important fruit tree cultivated worldwide. Grape berry texture has high agronomic relevance because of its relationships with the quality parameters and marketing requirements of table, raisin, and wine grapes [1], and the flesh firmness and peel

found two stable QTLs associated with berry firmness near SSR



the present study (n=151). The female parent 'Red Globe' has a firm-flesh texture after maturation, and the male parent 'Muscat Hamburg' has a soft-flesh texture after maturation. Intraspecific hybridization was conducted in May 2011, and hybrid seeds were collected in October 2011. The offspring and parents were cultivated at Shenyang Agricultural University with commercial vineyard management and pruning. Genomic DNA (gDNA) was extracted from young leaves of the parents and offspring. Clusters were harvested from each plant at maturity from 2017 to 2019, 30 similarly sized berries were used for texture evaluation immediately, and 10 berries were frozen with liquid nitrogen for the following experiment after sampling.

## Grape berry texture determination

The berry mesocarp (flesh) firmness (MesF), pericarp (peel) puncture hardness (PPH), and pericarp brittleness (PerB) were determined using a texture analyzer (TA. XT Express, Stable Micro System, Godalming, UK) according to a previous method, with some modifications [64]. MesF indicated the average force (g) required to puncture the berry flesh, PPH indicated the force (g) used from the probe touching the peel until the peel is punctured and PerB indicated the displacement distance (mm) from the probe touching the peel to puncture peel. Berry puncture determination

- 12. Grattapaglia D, Sederoff R. Genetic linkage maps of Eucalyptus grandis and Eucalyptus urophylla using a pseudo-testcross: mapping strategy and RAPD markers. Genetics. 1994;137:1121-37
- 13. Weeden NF, Hemmatt M, Lawson DM. et al. Development and application of molecular marker linkage maps in woody fruit crops. Euphytica. 1994;77:71-5
- 14. Carreño I, Cabezas JA, Martínez-Mora C. et al. Quantitative genetic analysis of berry firmness in table grape (Vitis vinifera L.). Tree Genet Genomes. 2015;11:818
- 15. Guo DL, Zhao HL, Li Q. et al. Genome-wide association study of berry-related traits in grape [Vitis vinifera L.] based on genotyping-by-sequencing markers. Hortic Res. 2019;6:11
- 16. Wang H, Yan A, Sun L. et al. Novel stable QTLs identification for berry quality traits based on high-density genetic linkage map construction in table grape. BMC Plant Biol. 2020;20:411
- 17. Herzog K, Schwander F, Kassemeyer HH. et al. Towards sensorbased phenotyping of physical barriers of grapes to improve resilience to botrytis bunch rot. Front Plant Sci. 2022;12:808365
- 18. Park M, Vera D, Kambrianda D. et al. Chromosome-level genome sequence assembly and genome-wide association study of Muscadinia rotundifolia reveal the genetics of 12 berry-related traits. Hortic Res. 2022;9:uhab011
- 19. Brummell DA. Cell wall disassembly in ripening fruit. Funct Plant Biol. 2006;33:103-19
- 20. Posé S, Paniagua C, Matas AJ. et al. A nanostructural view of the cell wall disassembly process during fruit ripening and postharvest storage by atomic force microscopy. Trends Food Sci Tech. 2019;87:47-58
- 21. Nunan KJ, Sims IM, Bacic A. et al. Descriptive profiling of new and commercial British Columbia table grape cultivars. Plant Physiol. 1998;118:783-92
- 22. Brummell DA, Harpster MH, Civello PM. et al. Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell. 1999;11:
- 23. Gwanpua SG, van Buggenhout S, Verlinden BE. et al. Pectin modifications and the role of pectin-degrading enzymes during postharvest softening of Jonagold apples. Food Chem. 2014;158:
- 24. Vicente AR, Saladie M, Rose JK. et al. The linkage between cell wall metabolism and fruit softening: looking to the future. J Sci Food Agric. 2007;87:1435-48
- 25. Atkinson RG, Sutherland PW, Johnston SL. et al. Down-regulation of POLYGALACTURONASE1 alters firmness, tensile strength and water loss in apple (Malus x domestica) fruit. BMC Plant Biol. 2012;12:129
- 26. Nunan KJ, Davies C, Robinson SP. et al. Expression patterns of cell wall-modifying enzymes during grape berry development. Planta. 2001;214:257-64
- 27. Yakushiji H, Sakurai N, Morinaga K. Changes in cell-wall polysaccharides from the mesocarp of grape berries during veraison. Physiol Plant. 2001;**111**:188–95
- 28. Li W, He C, Wei H. et al. VvPL11 is a key member of the pectin lyase gene family involved in grape softening. Horticulturae. 2023;9:182
- 29. Ma Y, Wang C, Gao Z. et al. VvPL15 is the core member of the pectate lyase gene family involved in grape berries ripening and softening. Int J Mol Sci. 2023;24:9318
- 30. Barnavon L, Doco T, Terrier N. et al. Involvement of pectin methyl-esterase during the ripening of grape berries: partial cDNA isolation, transcript expression and changes in the degree of methyl-esterification of cell wall pectins. Phytochemistry. 2001;58:693-701

- 31. Kuhn N, Guan L, Dai ZW. et al. Berry ripening: recently heard through the grapevine. J Exp Bot. 2014;65:4543-59
- 32. Li S, Xu H, Ju Z. et al. The RIN-MC fusion of MADS-box transcription factors has transcriptional activity and modulates expression of many ripening genes. Plant Physiol. 2018;176:891-909
- 33. Waseem M, Li Z. Overexpression of tomato SlbHLH22 transcription factor gene enhances fruit sensitivity to exogenous phytohormones and shortens fruit shelf-life. J Biotechnol. 2019;299:

XTcM[2-9.7Ze310.3stion fact.0002.4(s)3M[(PhABT)M-As)3M[(J)-278iosyn67(sDM-.0erif)s

- 50. Tello J, Roux C, Chouiki H. et al. A novel high-density grapevine (Vitis vinifera L.) integrated linkage map using GBS in a halfdiallel population. Theor Appl Genet. 2019;132:2237-52
- 51. Wang H, Yan A, Wang X. et al. Identification of QTLs and candidate genes controlling berry size in table grape by integrating OTL and transcriptomic analysis. Sci Hortic. 2022;305:111403
- 52. Blois L, de Miguel M, Bert PF. et al. Genetic structure and first genome-wide insights into the adaptation of a wild relative of grapevine, Vitis berlandieri. Evol Appl. 2023;16:1184-200
- 53. Vervalle JA, Costantini L, Lorenzi S. et al. A high-density integrated map for grapevine based on three mapping populations genotyped by the Vitis18K SNP chip. Theor Appl Genet. 2022;135: 4371-90
- 54. Shi X, Cao S, Wang X. et al. The complete reference genome for grapevine (Vitis vinifera L.) genetics and breeding. Horticulture Research. 2023;10:uhad061
- 55. Fu P, Tian Q, Lai G. et al. Cgr1, a ripe rot resistance QTL in Vitis amurensis 'Shuang Hong' grapevine. Hortic Res. 2019;6:67
- 56. Sapkota S, Chen LL, Yang S. et al. Construction of a high-density linkage map and QTL detection of downy mildew resistance in Vitis aestivalis-derived 'Norton'. Theor Appl Genet. 2019;132:
- 57. Wang N, Fang L, Xin H. et al. Construction of a high-density genetic map for grape using next generation restriction-site associated DNA sequencing. BMC Plant Biol. 2012;12:148
- 58. Seifert GJ, Blaukopf C. Irritable walls: the plant extracellular matrix and signaling. Plant Physiol. 2010;153:467-78
- 59. Kohorn BD, Kohorn SL. The cell wall-associated kinases, WAKs, as pectin receptors. Front Plant Sci. 2012;3:88
- 60. Liu F, Dou T, Hu C. et al. WRKY transcription factor MaWRKY49 positively regulates pectate lyase genes during fruit ripening of Musa acuminata. Plant Physiol Biochem. 2023;194:643-50
- 61. Liu GS, Li HL, Grierson D. et al. NAC transcription factor family regulation of fruit ripening and quality: a review. Cell.
- 62. Wu B, Shen F, Wang X. et al. Role of MdERF3 and MdERF118 natural variations in apple flesh firmness/crispness retainability and development of QTL-based genomics-assisted prediction. Plant Biotechnol J. 2021;19:1022-37
- 63. Zhang WW, Zhao SQ, Gu S. et al. FvWRKY48 binds to the pectate