An advanced genotyping tool to inspect grapevine variability: the Axiom®Vitis22K SNP array

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1) SNP selection strategy

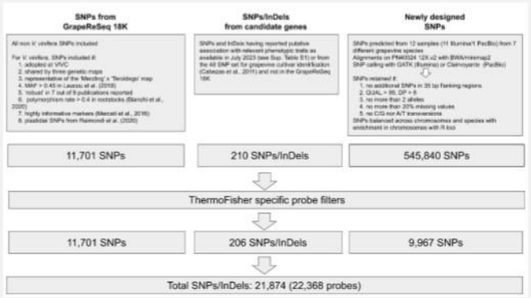
The **Axiom®Vitis22K SNP array** variants (SNPs/InDels) were combined from **three sources**:

I. **GrapeReSeq 18K Vitis chip** (Le Paslier et al., 2013): all non-*vinifera* SNPs (*V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. labrusca*, *V. lincecumii*, and *M. rotundifolia*) were kept unfiltered due to limited data. In contrast, *V. vinifera* SNPs were filtered from 13,560 available markers using partially overlapping criteria to **maximize informativeness** (Costantini-Micheletti et al., 2026).

II. **Trait-associated variants**: **289 SNPs/InDels** selected from literature available up to July 2023 based on phenotypic associations.

III. **Novel variants**: Newly identified SNPs from resequencing 12 samples across seven grapevine species.

2) SNP choice pipeline



Strategy for variant selection and final number of SNPs and InDels tiled in the Axiom®Vitis22K SNParray.
Costantini-Micheletti et al., 2026

3) Pilot SNP array hybridization

The pilot hybridization experiment was carried out on **two ad hoc panels** from the Fondazione Edmund Mach (FEM) germplasm collection (Bettinelli et al., 2024), after Trueness-to-type verification via nine universal SSR markers (Maul et al., 2012).

BPP
Breeding Parental Panel
95 genotypes
(two somatic variants)

FSP
Flower Sex Panel
48 genotypes

Phenotypes for **four target traits** were assigned using visual observations, unpublished data, and literature/VIVC records to enable **SNP validation**.

FLOWER SEX | **SEED DEVELOPMENT** | **BERRY SKIN COLOR** | **BERRY TASTE**

Genotyping was performed at the FEM Sequencing and Genotyping Platform: for the **Axiom®Vitis22K SNP array**, on the GeneTitan™ MC Instrument following the Axiom™ 2.0 Assay 96-Array Format Manual Workflow; for the GrapeReSeq 18K Vitis chip, on the Illumina HiScan Reader. Raw SNP clustering was performed using Axiom Analysis Suite (AxAS, call rate < 0.85) or GenomeStudio v2011.1 (GenTrain threshold < 0.70, call rate < 95), respectively.

4) Technology comparison and PCR validation

To assess **reproducibility** between the GrapeReSeq 18K Vitis chip and the Axiom®Vitis22K SNP array platforms, **27 genotypes** were compared across common SNPs on the concordance between homo and hetero calls. The concordance raised after curating the Axiom dataset via the **AxioSAFE pipeline** (Spina et al., 2026) with default parameters.

To **validate** trait-associated SNPs on the Axiom® Vitis22K array, BPP and FSP panel genotypes were characterized using PCR markers for flower sex (VVIB23) and berry color (VvMybA1) following original protocols (Lijavetzky et al., 2006; Battilana et al., 2013).

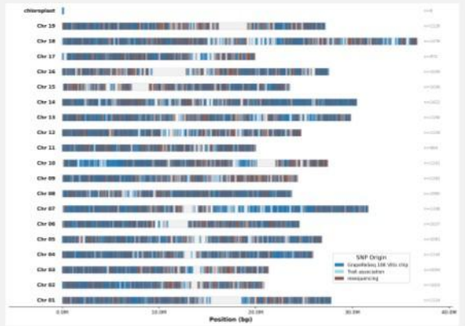
5) Overall SNP statistics

The array includes **22,368 probes** targeting 21,874 variants (excess probes capture dual-side variants, A/T and C/G transversions, or indels).

Variant Breakdown:
- GrapeReSeq 18K chip: 11,701 variants (7,201 *V. vinifera*, 4,509 non-*vinifera*), including 89 trait-associated markers.
- Sequencing data: 9,967 variants from 12 *Vitis* species and clones.
- Literature based: Remaining markers linked to phenotypic traits.

Mapping & Distribution
(upon AxAS v5.4 & AxioSAFE curation):
- 21,610 variants mapped to PN40024.T2T (v5).
- 209 variants mapped to Alternative chromosomes.
- 55 variants unpositioned or in unanchored regions.

Distribution of the variants showed a **uniform coverage** across chromosomes, though centromeric regions are markedly less enriched than adjacent regions.

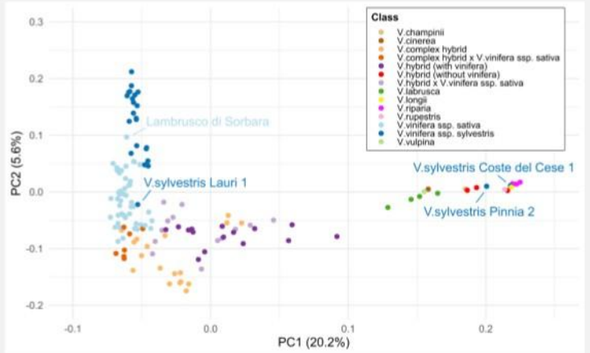


Distribution of the 21,610 variants of the Axiom®Vitis22K SNParray that have a unique position on the PN40024.T2T (v5) genome colored by SNP origin.

Species	No. of Accession	Average ObsHet	Min ObsHet	Max ObsHet	Average Calls
V. champinii	1	0.15	0.15	0.15	10,103
V. cinerea	1	0.08	0.08	0.08	9,903
V. hybrid	56	0.26	0.09	0.33	10,281
V.labrusca	4	0.09	0.06	0.15	10,104
V. longii	1	0.08	0.08	0.08	10,195
V. riparia	4	0.08	0.08	0.08	10,115
V. rupestris	1	0.09	0.09	0.09	10,171
V. vinifera ssp. sylvestris	22	0.2	0.11	0.24	10,267
V. vinifera ssp. sativa	49	0.25	0.2	0.3	10,299
V. vulpina	1	0.1	0.1	0.1	10,083

Observed heterozygosity metrics across *Vitis* species.

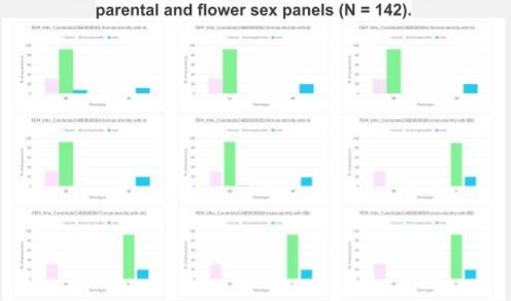
6) Scouting the genetic diversity for breeding purposes



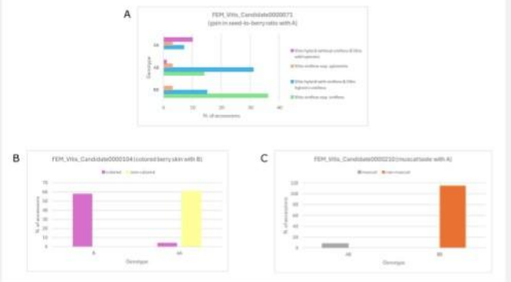
Genetic diversity of the studied panel based on SNP Principal component analysis (PCA). Colors indicate the taxonomic classes defined in this study. PC1 (20.2% variance) separates wild vs. cultivated *Vitis* and their hybrids; PC2 (5.6%) separates *V. vinifera* ssp. *sativa* and *sylvestris*. Labeled genotypes resulted as misclassified base on PCA.

7) Validation of candidate polymorphisms for specific traits

Nine SNPs associated with flower sex in the breeding parental and flower sex panels (N = 142).



Polymorphisms associated with seed-to-berry ratio (A), berry color (B) and muscat taste (C), (N = 123).



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Finanziato dall'Unione europea NextGenerationEU | Ministero dell'Università e della Ricerca | Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA | agritech National Research Center for Technology in Agriculture

Funded by the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022)