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METHOD ARTICLE

A methodological framework for the standardised evaluation of olive genetic resources: GEN4OLIVE harmonized protocols

[version 1; peer review: 1 not approved]

Hristofor Miho[1], Diego Barranco Navero[1], Isabel Trujillo Navas[1], Pablo Morello Parra[1], Georgios Koubouris[2], Carlos Trapero Ramirez[1], Álvaro López-Bernal[1], Amira Oueslati[3], David Flores Mesas[1], M. Teresa García López[1], Pedro Valverde Caballero[1], R. Efe Deger[1], Esteban Meca[1], Enzo Perri[4], Elena Santilli[4], Juan Moral Moral[1], Cristina Estudillo Cazorla[1], Diego Cabello Pozo[1], Meelad Yousef Yousef[1], Songul Acar[5], Melek Gurbuz-Veral[5], Feliciano Priego Capote[1], Asma Zahiri[6], Sara Oulbi[6], Maria Isabel Márquez Pérez[1], Concepción M. Díez[1]

[1]Universidad de Cordoba, Córdoba, 14071, Spain
[2]ELGO-DIMITRA, Hellenic Agricultural Organization. Institute of Olive tree, Subtropical crops and Viticulture, Chania, Crete, Greece, 73134, Greece
[3]Universidad de Monastir, Monastir, Tunisia, 565000, Tunisia
[4]CREA-OFA, Council for Agricultural Research and Economics, Rende, Italy, 87036, Italy
[5]Olive Research Institute (ORI), Üniversite Caddesi, Izmir, 4335100, Turkey
[6]Institut National de la Recherche Agronomique (INRA), Regional Center of Agricultural Research of Marrakech, Rabat, 415, Morocco

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Abstract

Background

Olive (*Olea europaea* L.) breeding initiatives rely heavily on the extensive and correct characterisation of genetic resources to successfully achieve their goals, such as addressing climate change and emerging diseases challenges. However, the historical lack of standardised phenotyping protocols across multi-environment trials has severely hindered data interoperability and large-scale comparative analyses.

Methods

Within the Horizon 2020 GEN4OLIVE project, five international olive germplasm banks established a consensus-based methodological

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1. **Nunzio D'Agostino**, University of Naples Federico II, Naples, Italy

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framework to systematically evaluate over 500 olive cultivars. We harmonised 14 evaluation protocols covering six fundamental dimensions: phenological and agronomic traits, abiotic stress resilience, biotic stress resilience, olive oil yield and chemical quality, table olive quality assessment, and morphological characterisation and photography. While most protocols were adapted from previously published literature to ensure ease of implementation across different facilities, novel methodologies for frost tolerance and standardised photography were developed *de novo.*

Results

The implementation of these consensus methods across five countries proved highly successful. This methodological framework enabled the generation of the largest harmonised, publicly available dataset on olive genetic resources to date, effectively making possible the correct comparation and ranking of the olive cultivars based on their specific characteristics.

Conclusions

This compendium of methods provides a robust, highly replicable reference point for the standardisation of olive germplasm characterisation and use of shared benchmark cultivars as an effective way for data normalization and comparation. It facilitates future global pre-breeding efforts, ensures international data interoperability, and supports the discovery of resilient cultivars to secure the future of the olive sector.

Plain Language summary

Olive trees face increasing threats from climate change and emerging diseases, making the discovery of resilient olive varieties crucial for the future of agriculture. To find these resilient varieties, scientists conduct “pre-breeding” evaluations. However, researchers in different countries traditionally use different methods to measure tree traits, making it impossible to accurately compare their data. To solve this, the European project GEN4OLIVE brought together five major olive germplasm banks from Spain, Greece, Italy, Turkiye, and Morocco to agree on a single, unified rulebook. We developed and adapted 14 highly standardised protocols to evaluate six key areas: tree growth (phenology and agronomy), drought and frost tolerance, disease and pest resistance, olive oil quality, table olive quality, and the olive cultivars photographing and cataloguing. By applying these easy-to-replicate methods, the international team successfully evaluated over 500 olive varieties, creating one of the largest comparable and public dataset in olive history. This unified protocol framework now serves as a universal standard, allowing researchers worldwide to evaluate olive trees using the exact same criteria.

Keywords
Olea europaea, germplasm evaluation, plant phenotyping, standardisation, benchmark cultivars, multi-environment trials, pre-breeding, plant genetic resources

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Corresponding author: Concepción M. Díez (cmdiez@uco.es)

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1. Introduction

The olive tree (*Olea europaea* L.) is the most important woody fruit crop in the Mediterranean Basin, covering more than 10 million hectares. Olive farming supports thousands of small farms, protects UNESCO-recognized landscapes, and encompasses over 2,000 traditional varieties developed over thousands of years.

Most olive orchards are still cultivated under rainfed conditions, making them entirely dependent on increasingly unpredictable precipitation patterns and other abiotic and biotic stresses. This lack of varietal innovation makes the olive sector vulnerable to environmental pressure, especially since the Mediterranean is a major climate change hotspot. Nevertheless, the olive tree is an excellent candidate for accelerated breeding due to its economic value, wide distribution, and large genetic pool.

The mobilisation of olive (*Olea europaea* L.) genetic resources through pre-breeding activities constitutes a critical strategy to address the emerging agronomic challenges posed by climate change and severe biotic threats. While international germplasm banks safeguard a massive genetic diversity, translating this potential into active breeding pipelines requires the comprehensive and systematic phenotypic characterisation of hundreds of accessions across multiple environments (Rallo, Barranco, et al., 2018a; Rallo, Díez, et al., 2018b).

While numerous scientific methodologies exist to evaluate specific physiological traits, their practical implementation across diverse institutions is frequently hindered by technical complexity and equipment availability. For instance, abiotic stress evaluations, such as frost or drought tolerance, can be quantified through advanced, resource-intensive laboratory techniques (e.g., thermal differential analysis, continuous gas exchange monitoring, or hormonal expression) or through empirical field observations. Consequently, research facilities often adopt different methods based on their specific technical and financial capabilities. This methodological divergence not only restricts replicability but also generates inherently incompatible datasets, as phenotypic results derived from fundamentally different analytical approaches cannot be directly compared or integrated into a unified database (Ćwiek-Kupczyńska et al., 2016; Krajewski et al., 2015; Papoutsoglou et al., 2020). Moreover, the significant genotype-by-environment (GxE) interactions observed in olive phenology, morphology, and stress resilience require uniform evaluation protocols. Without such standardization, it is impossible to compare results across different environments or quantify the true impact of these interactions (Lamoumni et al., 2025; Miho et al., 2021; Yılmaz-Düzyaman et al., 2025).

To evaluate GxE interactions and ensure robust data inter-comparability, multi-environment phenotyping inherently relies on the application of standard protocols along with the systematic use of shared benchmark reference cultivars. These reference genotypes function as universal biological standards that allow researchers to calibrate evaluations, detect seasonal bias, and normalise raw trait values relative to the benchmark performance at each specific site (Li et al., 2025; Mousavi et al., 2019).

To overcome the historical lack of use of standard methods in olive research, the European Horizon 2020 GEN4OLIVE consortium initiated a large-scale international pre-breeding evaluation encompassing over 500 olive varieties. This massive phenotypical characterisation required the establishment of a robust, highly replicable, and unified evaluation framework. Consequently, five participating germplasm banks—the University of Cordoba (UCO, Spain), the Institute of Olive Tree and Subtropical Plants (ELGO DIMITRA, Greece), the Council for Agricultural Research and Economics (CREA, Italy), the Olive Research Institute (ORI, Turkiye), and the Institut National de la Recherche Agronomique (INRA, Morocco)— collaborated to establish a consensus methodology for the accurate evaluation of key agronomic traits in olive germplasm (Web: *GEN4OLIVE Project - H2020*, 2024).

This Methods Article describes the GEN4OLIVE compendium, which integrates 14 protocols, including 12 adapted and two *de novo* designs, for fast and accurate phenotyping. By utilizing shared benchmark cultivars, this standardized methodology generated the largest public olive dataset to date and establishes a new global standard for the scientific community.

2. Methods: Framework harmonisation and setup

2.1 The consensus process and protocol selection

The GEN4OLIVE consortium implemented a rigorous consensus process to standardize the evaluation of over 500 olive accessions, ensuring that all resulting data are fully comparable. Led by the University of Cordoba (UCO), the consortium organised specific working groups comprising the most reputable international experts in each corresponding research area.

During this process, the experts evaluated multiple pre-existing scientific methodologies to quantify each target trait. To definitively select and adapt the final consensus methods and protocols, the consortium strictly applied three primary criteria:

- Large-scale feasibility: The methodologies had to be robust enough to support high-throughput evaluations of massive germplasm collections (>500 varieties), effectively accommodating traits that required continuous monitoring over three consecutive years, such as reproductive phenology.

- Ease of implementation: The experimental procedures needed to be sufficiently straightforward to ensure accurate and reproducible execution by field and laboratory personnel, even those without prior specialised experience in that specific topic. To this end, the protocols were backed up by strict templates and pre-defined spreadsheets to prevent errors in data collection and processing (information available in the Extended Data section of this paper).

- Equipment accessibility: To ensure universal applicability, the protocols strictly avoided the necessity for highly sophisticated, complex, or expensive equipments, acknowledging that not all participating research centres possessed equivalent technical infrastructure or financial resources.

Applying these strict criteria, the designated lead research group for each specific topic prepared an initial methodological draft. Subsequently, all involved consortium members meticulously reviewed, commented on, and refined these initial drafts. This iterative internal review process continued until all partners reached a unanimous agreement, resulting in the establishment of 14 homogeneous and fully harmonised evaluation protocols.

These 14 consensus protocols were strategically grouped into six fundamental research dimensions: I) Phenological and Agronomic Evaluation; II) Abiotic Stress Tolerance Evaluation; III) Biotic Stress Tolerance Evaluation; IV) Determination of Oil Yield and Quality; V) Table Olive Quality Assessment; and VI) Standardised Photographing and Morphological Characterisation.

A comprehensive methodological handbook containing the detailed step-by-step procedures, visual assessment scales, experimental designs, and practical implementation guides for all 14 protocols is provided as Extended Data (Miho et al., 2026).

2.2 Methods origin and adaptation for effective phenotyping

As highlighted previously, physiological and chemical evaluations frequently rely on technically complex or highly resource-intensive methodologies. For instance, frost tolerance is traditionally quantified through advanced laboratory techniques such as Differential Thermal Analysis (DTA) or Electrolyte Leakage (EL) tests (Malyshev et al., 2020; Thalhammer et al., 2020), while robust drought tolerance assessment often demands water potential measurements via Scholander pressure chambers (Baquedano & Castillo, 2007; Scholander et al., 1965). To overcome these operational barriers and facilitate simultaneous multi-site evaluations across five countries, we explicitly prioritised the selection of methods based on their ease of implementation, cost-effectiveness, and strict independence from highly sophisticated or expensive equipments.

Most of the protocols included in this framework were carefully adapted and optimized from previously published scientific literature to meet the specific requirements of effective basic phenotyping in large scale implementation. For example, in the case of drought tolerance evaluation, rather than utilising complex and time demanding equipment, we optimized the minimum leaf conductance protocol to evaluate using a simple precision balance and a digital scanner (Moriana et al., 2002). Similarly, we standardised visual rating scales (0 to 10) for olive aerial diseases to highly accelerate field evaluations without requiring advanced and costly molecular quantification (Estudillo et al., 2025; Moral et al., 2017).

Conversely, specific methodologies lacking scalable precedents in the literature were developed *de novo.* These novel methodologies include the standardised *ex situ* frost tolerance assessment utilising detached leaves exposed to strictly controlled freezing—which successfully bypasses the need for complex EL or DTA equipment (Valverde et al., 2024)—and the specific defined artistic and technical photography protocol designed to generate homogenous databases for GenBank's management. A comprehensive summary of the 14 harmonised protocols, their base references, and specific adaptations is presented in Table 1.

2.3. The crucial role of benchmark cultivars and data standardisation

Given the inherently broad geographic distribution of the GEN4OLIVE trails, environmental heterogeneity posed a major challenge for data integration. Olive traits exhibit significant genotype-by-environment (GxE) interaction,

Table 1. Summary of the 14 harmonised evaluation protocols on olive large scale phenotyping, and base reference methodologies.

Evaluation dimension	Specific protocol	Target trait/ methodology	Adaptation for multi-environment trials	Base reference(s)
I. Phenology & Agronomy	1. Reproductive phenology	Budburst, flowering, and ripening tracking (BBCH & Ferreira scales).	Implementation of a simplified phenological triangle diagram (Fleckinger) to standardise observation dates.	(Fleckinger, 1954; Navero et al., 2017; Sanz-Cortés et al., 2002)
	2. Chilling & thermal units	Mathematical computation of dormancy release and thermal time.	Standardised daily weather data inputs mathematically mapped to unified baseline constants for olive trees.	(De Melo-Abreu et al., 2004)
	3. Agronomic traits	Tree vigour, growth habit, canopy density, flowering/fruiting intensity, and fruit detachment force.	Adoption of 0–5 visual scales for rapid canopy assessment and harmonised hand-held dynamometer application for detachment force evaluation.	(Castillo Ruiz et al., 2018; Ramírez-Santa Pau et al., 2002; UPOV, 2011).
II. Abiotic Stress Tolerance	4. Drought tolerance	Calculation of minimum stomatal leaf conductance.	Replaced complex methodologies for evaluation with a scalable mass-loss technique using detached leaves.	(Moriana et al., 2002)
	5. Frost tolerance	Susceptibility to freezing temperatures (−10 ° C).	*De novo* protocol subjecting detached leaves to controlled freezing and visually evaluating physiological damage.	De novo within GEN4OLIVE project (Valverde et al., 2024)
III. Biotic Stress Tolerance	6. Olive Scab	*Venturia oleaginea* susceptibility.	Standardised 0–10 visual field incidence scale, complemented by a rapid 5% NaOH test to reveal latent infections.	(Moral et al., 2017, p. 20; Viruega et al., 2011)
	7. Cercospora leaf spot	*Pseudocercospora cladosporioides* susceptibility.	Standardised 0–10 visual field incidence scale, complemented by humid chamber incubation to visually differentiate natural leaf senescence from fungal conidia presence.	(Moral et al., 2015)
	8. Anthracnose	*Colletotrichum* spp. fruit rot and branch dieback.	Standardised 0–10 visual field incidence scale and synchronisation of evaluation with the fruit maturity index (MI > 3) to avoid phenological bias.	(Moral et al., 2009, 2017)
	9. Verticillium wilt	*Verticillium dahliae* resistance using a 0–16 rating scale.	Standardised root-dipping procedure (30 min at conidia/mL) applied strictly to acclimatised rooted cuttings.	(López-Escudero et al., 2004)
	10. *Xylella fastidiosa*	Bacterial resistance/ susceptibility and molecular diagnosis.	Dual evaluation combining natural open-field exposure with strictly controlled	('EPPO', 2019; Saponari et al., 2017)

Table 1. *Continued*

Evaluation dimension	Specific protocol	Target trait/ methodology	Adaptation for multi-environment trials	Base reference(s)
			needle-puncture inoculations.	
	11. Olive fruit fly	*Bactrocera oleae* susceptibility.	Harmonised spatial trapping network, visual superficial fruit in field evaluation linked to an in lab destructive interior evaluation.	(Petacchi et al., 2003; Quesada-Moraga et al., 2018)
IV. Oil Yield & Quality	12. Oil extraction and chemical quality	Total oil content, phenolic profile, and fatty acids.	Synchronised harvesting at MI = 2 and highly controlled Abencor extractions (30 min at 28 °C) to preserve phenolic integrity. Implementation of NMR, HPLC and GC instruments for oil content and chemical analysis.	(Castillo-Luna & Priego-Capote, 2024; Miho et al., 2021; Peres et al., 2014)
V. Table Olive Quality	13. Physical traits and bruising	Morphological characteristics, firmness, and olive bruising index (OBI).	Standardised easy to implement protocols for each evaluation: Visual scale indexes and implementation of small instruments for evaluations.	(Jiménez et al., 2016; Morales-Sillero et al., 2014)
VI. Photographing and Morphological Characterisation	14. Artistic and technical photography	Artistic documentation of fruiting shoots and technical composition of leaves, fruits, and endocarps.	*De novo* methodology standardising DSLR camera setups to build multi-site image databases through a dual approach: artistic photography of fruiting shoots (in-field and laboratory), and technical photography utilising a rigid millimetre-marked template to create a highly standardised composition of leaves, fruits, and endocarps.	Developed *de novo* within GEN4OLIVE project by University of Córdoba.

particularly concerning phenology, fruit development, oil quality, and phenotypic stress responses (Lamoumni et al., 2025; Yılmaz-Düzyaman et al., 2023). Consequently, comparing raw trait values across different geographical trials introduces a high risk of misinterpretation (Miho et al., 2021; Mousavi et al., 2019).

To effectively avoid these GxE errors in the cultivar ranking and classifications, we integrated a specific panel of shared benchmark (reference) cultivars into every protocol. These genotypes (e.g., 'Arbequina', 'Frantoio', and 'Picual') acted as universal biological standards across all five germplasm banks that participated in the GEN4OLIVE project. By systematically evaluating these shared references alongside the local accessions, evaluators can detect site or seasonal bias, calibrate sensors in high-throughput phenotyping, and establish a reliable baseline for comparison (Li et al., 2025; Mousavi et al., 2019).

To fulfil with our targeted objective, we conducted data standardization to ensure dataset interoperability. The data standardization is a method of normalizing data when comparisons or classifications are required among cultivars evaluated in different environments or growing seasons. In those cases, researchers may mathematically normalise the raw trait values relative to the benchmark cultivar performance at each specific location. Evaluators should calculate the relative standardised data applying the following mathematical formula:

$$\text{Standardised Trait Value} = \frac{\text{Raw Value of Target Cultivar}_{GB1}}{\text{Raw Value of Reference Cultivar}_{GB1}}$$

(Where GB1 represents the specific Germplasm Bank or local environment where the trial is conducted, and reference cultivar represent the shared cultivars among all the Germplasm Banks or other Experimental Tails).

This relative standardisation process allows researchers to express cultivar performance as a trait ratio, effectively mitigating environmental differential performance across disparate geographic locations. Consequently, the resulting multi-environment datasets become robust, fully interoperable, and highly suitable for advanced genetic association mapping and breeding selection (Bakkali et al., 2013; Wu et al., 2024).

3. Results: Implementation and use case

To validate the efficiency and reproducibility of this 14-protocol methodological framework, the compendium was deployed in a massive multi-environment pre-breeding trial. Between 2021 and 2024, the five participating germplasm banks (UCO, ELGO DIMITRA, CREA, ORI, and INRA) systematically applied these harmonised protocols to comprehensively evaluate over 500 olive accessions across Spain, Greece, Italy, Turkiye, and Morocco.

By rigorously adhering to the defined methodologies—including the *de novo* photographic cataloguing and frost tolerance assessments, and all the methods adaptations for the rest of the traits—evaluators successfully generated robust, inter-comparable phenotypic data. Crucially, the systematic integration of shared benchmark cultivars (e.g., 'Arbequina', 'Frantoio', 'Picual') allowed for the mathematical normalisation of trait values, effectively reducing the environmental variance (GxE interactions) inherent to such disparate geographical locations.

Consequently, this coordinated effort yielded the largest standardised, publicly available dataset on olive genetic resources to date (*GEN4OLIVE Database*, 2025). The successful execution of this international use case demonstrates that the framework overcomes the traditional barriers of technical complexity and data fragmentation, enabling highly reliable, large-scale phenotypic comparisons.

4. Conclusions

The historical lack of standardised phenotyping protocols has long hindered the interoperability of multi-site olive evaluations. By establishing a consensus-based framework of 14 highly replicable protocols, covering six fundamental agronomic and quality dimensions, we provide a robust solution to this critical bottleneck.

This methodological compendium deliberately prioritises cost-effective, high-throughput techniques over highly complex laboratory methods, thereby democratising the evaluation process for diverse research facilities worldwide. Furthermore, the incorporation of benchmark reference cultivars guarantees the successful mitigation of environmental differences and seasonal bias.

Ultimately, this harmonised framework constitutes a new universal standard for olive germplasm banks and researchers. It successfully overcomes data fragmentation, facilitates global data integration, and accelerates the future international pre-breeding initiatives necessary to discover new elite and resilient cultivars.

Ethics and consent

Ethical approval and consent were not required for this study.

Data and software availability

The phenotypic dataset generated from the evaluation of over 500 olive varieties across the five Mediterranean germplasm banks using this compendium of protocols and methodologies, is publicly available via the GEN4OLIVE project repository.

- Repository: GEN4OLIVE Olive Varieties Database.
- Link: https://www.uco.es/ucolivo/gen4olive/olivevarieties (*GEN4OLIVE Database*, 2025).

Extended Data

A comprehensive methodological handbook containing the detailed step-by-step procedures, visual assessment scales, experimental designs, specific templates and practical implementation guides for all 14 protocols is provided as Extended Data.

- Repository: Zenodo
- DOI: https://doi.org/10.5281/zenodo.21379710 (Miho et al., 2026)
- Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Nunzio D'Agostino
University of Naples Federico II, Naples, Italy

This method article addresses an important and timely problem: the lack of harmonised procedures for evaluating olive genetic resources across institutions and environments. The coordination of five germplasm banks and the development or adaptation of 14 protocols have the potential to provide a valuable resource for the olive research and pre-breeding communities. The emphasis on scalability, accessible methods, shared reference cultivars, and open data is particularly relevant.
In its present form, however, the manuscript makes strong claims concerning validation, reproducibility, interoperability, cross-site comparability, and the mitigation of genotype-by-environment interactions without providing sufficient quantitative evidence. Consequently, the paper reads partly as a description of the GEN4OLIVE project rather than as a rigorous methodological study demonstrating the performance of the proposed framework.
The authors should either provide evidence supporting the principal claims or reposition the manuscript more explicitly as a consensus-based protocol and methodological framework.

Major comments
The Results section states that implementation was "highly successful," generated "robust, inter-comparable" data, and reduced environmental variance. These statements are not supported by numerical results, statistical analyses, or quality-control indicators.
To substantiate these claims, the manuscript should report the number of accessions evaluated at each germplasm bank under each protocol, together with the relevant years, seasons, environments, sample sizes, biological replicates, and missing-data rates. It should also identify which cultivars and protocols were common to all five institutions and provide the corresponding protocol-compliance and quality-control results. Evidence of inter-observer and inter-laboratory repeatability is particularly important, as are estimates of measurement error, coefficients of variation, repeatability, or other suitable reliability statistics.
The authors should also demonstrate that standardization improved cross-site comparability. This would ideally involve reporting variance components or applying another suitable analysis capable of separating genotype, environment, and genotype-by-environment effects.
As stated before, if these analyses were not conducted, the manuscript should be reframed as a

consensus protocol or framework paper. In that case, claims of demonstrated validation, guaranteed comparability, interoperability, and reduced genotype-by-environment variance should be removed or substantially moderated.

The consensus process is described as rigorous, iterative, and unanimous, but the information provided is insufficient for readers to assess or reproduce it. The manuscript should explain how the experts and working-group members were selected and report the number and disciplinary composition of the participants in each group. It should also describe the candidate methods considered for each trait, the criteria used to compare them, whether those criteria were weighted, the number and format of the consensus rounds, and the procedure used to resolve disagreements.
The authors should define what constituted consensus or unanimity and clarify whether the protocols were piloted before their final adoption. If pilot testing was undertaken, the manuscript should explain how the resulting feedback affected the final protocols. A flow diagram or supplementary decision table tracing the development of each protocol (from the candidate methods initially considered to the version ultimately adopted) would considerably improve transparency. Subjective expressions such as “the most reputable international experts” should be replaced with objective descriptions of expertise, selection criteria, and representation.

The proposed standardisation formula appears to be based on a simple ratio between the observation and a reference value. Such a ratio may provide a useful form of scaling, but it cannot by itself guarantee data interoperability or remove genotype-by-environment interactions. Its effect is primarily to express measurements relative to a selected reference.
For multi-environment data, mixed-effects models, spatial models, adjusted means, or factor-analytic approaches may be more appropriate, depending on the experimental design and the intended use of the data. At a minimum, the ratio should be presented as a proposed scaling procedure rather than as evidence that environmental effects have been mitigated.
The manuscript also refers to several benchmark cultivars without clearly explaining how they enter the calculation. The authors should specify whether the denominator is based on a single cultivar, the mean of several benchmark cultivars, or a trait-specific reference. They should justify this choice and explain how the procedure handles unstable benchmark performance, missing observations, and values close to zero.

The manuscript does not establish whether the benchmark cultivars were planted or evaluated according to comparable designs at all participating institutions. This information is essential for assessing the proposed calibration system. The authors should provide the complete benchmark panel and explain the criteria used to select its members. They should clarify whether every reference cultivar was present at every site and report the number, age, propagation history, and health status of the trees, together with their experimental arrangement. Information on replication, randomisation, orchard management, and relevant environmental covariates is also required. In addition, the manuscript should explain what procedure was followed when a benchmark cultivar was absent, damaged, diseased, or did not produce fruit, and whether benchmark and target accessions were always evaluated concurrently. Without these details, the validity and practical applicability of the proposed calibration system cannot be properly assessed.

The Results section largely repeats statements already made in the ABSTRACT and INTRODUCTION and does not provide a substantive demonstration of the framework. A convincing use case should show how at least one protocol performed across participating

institutions. For example, the authors could present data distributions before and after standardisation, inter-site correlations for shared cultivars, estimates of repeatability, variance-component analyses, or rates of protocol compliance and missing data. Even a focused demonstration based on a subset of traits would be more informative than broad qualitative statements.

The authors should also explain how the proposed system aligns operationally with MIAPPE and established plant-phenotyping ontologies. These resources are cited as part of the motivation for the study, but their practical incorporation into variable definitions, metadata, identifiers, and data exchange is not sufficiently described.

The criterion of independence from sophisticated or expensive equipment is applied inconsistently. Protocol 12 requires NMR, HPLC, and GC instrumentation, while controlled freezing and molecular diagnosis also depend on specialist facilities that may not be widely accessible. The framework would be clearer and more useful if the protocols were classified according to their infrastructural requirements. The authors could distinguish universally implementable core protocols from methods requiring specialist facilities and from optional advanced analytical modules. A summary table should report, for each protocol, the minimum equipment, required expertise, sample requirements, approximate throughput, and a broad cost category. This would allow prospective users to assess feasibility and would prevent the accessibility claim from being interpreted too broadly.

The manuscript states that 12 protocols were adapted and two were developed de novo. However, the frost method is also attributed to Valverde et al. (2024), which suggests that it may already have been published. The relationship between the present protocol and that earlier publication should therefore be clarified. The photography protocol appears operationally novel, but its scientific validation and intended quantitative applications remain uncertain. The authors should specify whether it is intended primarily for documentation, visual assessment, image-based phenotyping, or a combination of these purposes.
More generally, the novelty of the manuscript should be delineated carefully. The contribution may lie in the coordinated selection, adaptation, harmonisation, and multi-institutional deployment of existing methods rather than in the creation of entirely new protocols. This would still represent a useful contribution, but it should be described accurately. The expression “new universal standard” is premature unless the framework has undergone external validation and achieved broader adoption beyond the GEN4OLIVE consortium.

The phenotype database is referenced through the project website, but the data-access arrangements require further explanation. The manuscript should identify the licence governing the phenotype data, describe the available download formats, and clarify whether bulk machine-readable access is provided. It should also explain the accession-identification system, metadata schema, variable definitions, and versioning procedures, as well as whether both raw and standardised measurements are available. Reliance on a project website alone may not ensure long-term accessibility and reproducibility. The authors should consider depositing the data, protocols, metadata dictionaries, and relevant analysis code in a stable repository with persistent identifiers and explicit versioning.

Minor comments
The manuscript requires comprehensive linguistic and editorial revision. Several specific

corrections should be made. “GEN4OLIVE trails” should read “GEN4OLIVE trials,” and “Experimental Tails” should be corrected to “experimental trials.” The word “comparation” should be replaced with “comparison,” while “equipments” should generally be changed to the uncountable noun “equipment.”

The expression “to fulfil with our targeted objective” also requires rewriting, and “large scale implementation” should be hyphenated as “large-scale implementation.”

Statements employing terms such as “guarantees,” “fully comparable,” “fully interoperable,” and “universal standard” should be avoided unless they are directly supported by evidence. T

Table 1 contains a corrupted symbol for the frost-treatment temperature, which appears to be intended as “−10 °C.” The Verticillium protocol includes the wording “30 min at conidia/mL” but omits the actual inoculum concentration. This omission must be corrected.

For each disease assessment, the authors should clarify whether the reported 0–10 visual scale represents incidence, severity, or a composite index.

The photography protocol needs more detailed technical specifications, including lighting conditions, colour calibration, scale references, file format, image resolution, white balance, naming conventions, and required metadata.

The expression “artistic photography” may be unsuitable in the context of a scientific standard; “documentary photography” or “contextual photography” would be clearer unless an explicitly artistic purpose is intended.

The authors should correct typographical and bibliographic errors, including “Jimémez,” “PROTOCOL,” duplicated words in reference titles, inconsistent punctuation, and the unusual quotation formatting applied to EPPO.

The Introduction contains substantial repetition concerning standardisation, interoperability, and genotype-by-environment effects and could be shortened. Similarly, the
Results portion of the Abstract should either report numerical findings or state more cautiously that the framework was deployed, rather than validated.
Finally, comprehensive English-language editing is required. Numerous grammatical errors, typographical mistakes, and awkward constructions remain despite the declared use of a language-editing tool.

Is the rationale for developing the new method (or application) clearly explained?

Partly

Is the description of the method technically sound?

Partly

Are sufficient details provided to allow replication of the method development and its use by others?

Partly

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Population Genomics, Bioinformatics, Omics sciences

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.