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Protection and valorisation of Apulian Fruit Vegetables

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Tutela e valorizzazione di specie orticole pugliesi da frutto

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Summary

Italy is the European country with the greatest biological richness, hosting over a third and almost half of European fauna and flora, respectively. This biodiversity represents a key factor for sustainable development, both from an environmental and socio-economic perspective. Nevertheless, this wealth is at risk, as numerous breeds and varieties continue to disappear year after year. One of the sectors most affected by the loss of genetic resources is horticulture, where new varieties (so-called "improved varieties" or "hybrids") are introduced to the market annually, replacing those selected over generations by farmers (known as "local varieties" or "landraces").

In Apulia, an Italian region, most varieties used by local farmers derive from improved varieties developed by breeders from other Italian regions or, more frequently, from other European countries. Indeed, the Common Catalogue and the National Register of vegetable species varieties include only 17 vegetable varieties registered by Apulian breeders and seed companies, compared to nearly 2,000 improved varieties registered in Italy and approximately 22,000 across Europe. Therefore, given this scenario, it becomes necessary to research, conserve and protect local vegetable varieties at risk of erosion or extinction. These represent a heritage that must be safeguarded for various reasons, ranging from the possibility of using these resources for genetic improvement programmes, to the possibility of exploiting the unique characteristics of these varieties to challenge current climate change.

This thesis started from this basis to address different strategies aimed at protecting and enhancing the vegetable biodiversity of Apulia. Processes useful for these purposes were identified, starting from the recovery and characterization of local varieties of regional fruit vegetables to the analysis of national and EU regulations aimed at their protection.

The research activities were carried out as part of the "Biodiversity of Apulian Fruit Vegetables" (BiodiverSO Karpos - Regione Puglia, Rural Development Program 2014–2022) at: Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro; Experimental Farm "La Noria" (in the countryside of Mola di Bari, BA); Agricultural Cooperative "BioSolequo" (in the countryside of Ostuni, BR).

With regard to the activities of recovery and characterization of fruit vegetables landraces, the main focus was placed on landraces of immature melons, mainly "Barattiere" and "Carosello" (*Cucumis melo* L.). In this regard, nine local varieties of *C. melo* were characterized by analysing their morphological and productive characteristics according to the latest guidelines in the field. Unique characteristics were identified, useful for the identification and potential commercial exploitation of these landraces.

The analyses allowed the identification of bitter fruit samples in one of the landraces of *C. melo* considered: the 'Carosello Scopatizzo'. In collaboration with the Department

of Chemistry of the University of Bari, an extraction and analysis protocol was developed to detect the presence of cucurbitacin molecules in 'Scopatizzo' fruits, which are generally responsible for bitterness in fruits of plants in the Cucurbitaceae family. The research enabled the identification – for the first time in the literature – of cucurbitacin R, D, B, and 23,24-dihydro-cucurbitacin B in fruits.

From a legal and regulatory perspective, this thesis analysed two useful tools for protecting and enhancing vegetable landraces: the conservation varieties regime (Directive 98/95/EC) and the recognition of Italian Traditional Agri-food Product (TAP) (Legislative Decree 173/1998).

The first policy was introduced in Europe in 1998 with the aim of making the European Common Catalogue of varieties of vegetable species more flexible and reducing the rate of genetic erosion of European landraces. The conservation varieties regime allows those varieties – mainly landraces – that are in danger of genetic erosion and do not meet the Distinctness, Uniformity and Stability (DUS) requirements to be listed in a special section of the Common Catalogue. This thesis analysed the results obtained from this regime, considering the Italian case study as an example to identify its strengths and weaknesses. It shows how the conservation varieties regime has not fully achieved its objectives, suggesting that changes should be made to simplify registration procedures and expand the marketing territory of registered varieties.

Traditional Agri-food Product (TAP) recognition, on the other hand, is an exclusively Italian measure, also introduced in 1998 and aimed at promoting the peninsula's traditional agri-food products. Through consumer surveys and comparisons with European Geographical Indications, strengths, weaknesses, criticalities and opportunities of this recognition applied to traditional Italian horticultural products were evaluated. In agreement with the SWOT analysis, TAP recognition was not found to be particularly commercially effective. Nevertheless, it represents a useful promotional tool for traditional Italian vegetable products, representing a useful first step in the designation of traditional Italian products to the European Geographical Indications regimes (56.94% conversion rate).

The strategies and tools analysed and described in this thesis represent a solid basis for the protection and enhancement of local vegetable varieties. In addition, the study contributed to the development of a new research paradigm. The protection of horticultural agrobiodiversity, both at the Italian and Apulian levels, requires an approach that goes beyond the mere recovery and conservation of genetic resources, favouring instead the active cultivation of landraces. This approach makes it possible to create the conditions for these varieties to readapt and evolve over time. To summarize, it can be said that to protect biodiversity, it is necessary to increase biodiversity.

Riassunto

L'Italia è il Paese europeo con la maggiore ricchezza biologica: ospita oltre un terzo della fauna e circa la metà della flora europee. Tale biodiversità rappresenta uno dei fattori chiave per lo sviluppo sostenibile, sia dal punto di vista ambientale che socioeconomico. Ciononostante, tale ricchezza è a rischio e numerose razze e varietà continuano a scomparire di anno in anno. In questo scenario, uno dei settori in cui si perde il maggior numero di risorse genetiche è l'orticoltura, con varietà sempre nuove (c.d. "varietà migliorate" o "ibridi") che ogni anno sono immesse sul mercato, soppiantando le varietà selezionate negli anni da generazioni di agricoltori (c.d. "varietà locali"). In Puglia la maggior parte delle varietà utilizzate dagli agricoltori derivano da varietà migliorate, selezionate da costitutori di altre regioni italiane o, ancora più spesso, di altri Paesi europei: nel Catalogo Comune e nel Registro Nazionale delle varietà delle specie orticole sono presenti solamente 17 varietà orticole registrate da ditte sementiere pugliesi, a fronte delle quasi 2000 varietà migliorate registrate in Italia e circa 22.000 varietà europee. A fronte di questo scenario, diviene dunque necessario recuperare, caratterizzare e conservare le varietà locali ortive a rischio di estinzione e/o erosione; un patrimonio da salvaguardare per molteplici aspetti, che vanno dalla possibilità di sfruttare tali risorse come fonte di geni da utilizzare per programmi di miglioramento genetico, alla possibilità di sfruttare le caratteristiche uniche di tali varietà per contrastare i cambiamenti climatici in atto.

La presente tesi è partita da queste basi per trattare differenti strategie finalizzate a tutelare e valorizzare la biodiversità orticola della Puglia. Sono stati individuati processi utili a tali fini, partendo dal recupero e caratterizzazione di varietà locali di ortaggi da frutto regionali, fino all'analisi di normative nazionali e comunitarie finalizzate alla tutela delle stesse.

Le attività di ricerca sono state svolte nell'ambito del progetto "Biodiversità delle specie orticole pugliesi da frutto – BiodiverSO Karpos" (Regione Puglia, Programma di Sviluppo Rurale 2014-2020) presso: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti dell'Università degli Studi di Bari Aldo Moro; Azienda sperimentale "La Noria" (in agro di Mola di Bari, BA); Cooperativa Agricola BioSolequo (in agro di Ostuni, BR).

In merito alle attività di recupero e caratterizzazione di varietà locali di ortaggi da frutto, è stata posta l'attenzione principalmente su varietà locali di meloni immaturi, principalmente barattiere e carosello (*Cucumis melo* L.). In tal senso, sono state caratterizzate nove varietà locali di meloni immaturi, analizzandone caratteristiche morfologiche e produttive secondo le più recenti linee guida di settore. Sono stati individuati caratteri unici e peculiarità, utili all'identificazione e alla potenziale valorizzazione commerciale di tali varietà locali.

Le analisi hanno permesso di individuare campioni di frutti amari in una delle varietà locali di *C. melo* considerate, il carosello 'Scopatizzo'. In collaborazione con il Dipartimento di Chimica dell'Università degli Studi di Bari, è stato quindi sviluppato un protocollo di estrazione e analisi in grado di individuare la presenza di molecole di cucurbitacina nei frutti di 'Scopatizzo', generalmente responsabile dell'amaro nei frutti di piante della famiglia delle Cucurbitacee. La ricerca ha permesso di individuare, per la prima volta, molecole di cucurbitacine (cucurbitacina R, D, B e la 23,24-diidro-cucurbitacina B) in frutti di varietà locali di *C. melo*.

Sotto il profilo normativo-giuridico, la presente tesi ha analizzato due strumenti utili per tutelare e valorizzare le varietà locali di ortaggi: il regime delle varietà da conservazione (Direttiva 98/95/CE) e il riconoscimento di Prodotto Agroalimentare Tradizionale (PAT) italiano (D.lgs. 173/1998).

Il primo strumento è stato introdotto in Europa nel 1998 con l'obiettivo di rendere più flessibile il Catalogo Comune europeo delle varietà delle specie di ortaggi e ridurre il tasso di erosione genetica di varietà locali europee. Il regime delle varietà da conservazione consente l'iscrizione in apposita sezione del Catalogo Comune di quelle varietà – principalmente varietà locali – a rischio di erosione che non soddisfano i requisiti di Distinguibilità, Uniformità e Stabilità (DUS). La tesi analizza i risultati ottenuti da tale regime, considerando il caso studio italiano come esemplificativo per individuarne punti di forza e criticità. Si evidenzia come il regime delle varietà da conservazione non abbia pienamente raggiunto gli obiettivi prefissati, suggerendo l'opportunità di apportare modifiche volte a semplificare le procedure di iscrizione e ad ampliare il territorio di commercializzazione delle varietà registrate.

Il riconoscimento di PAT è invece uno strumento esclusivamente italiano, avente come obiettivo la promozione della tradizionalità dei prodotti agroalimentari della penisola. Attraverso indagini tra i consumatori e comparazioni con le Indicazioni Geografiche europee, sono stati valutati punti di forza, debolezza, criticità e opportunità di tale riconoscimento applicato ai prodotti della tradizione orticola italiana. D'accordo con l'analisi SWOT, il riconoscimento PAT non è risultato essere particolarmente efficace sotto il profilo commerciale. Ciononostante, esso rappresenta un'utile leva di promozione per i prodotti orticoli della tradizione italiana, rappresentando un primo passo utile per la designazione dei prodotti tradizionali italiani ai regimi delle Indicazioni Geografiche europee (tasso di conversione del 56,94%).

Le strategie e gli strumenti analizzati e descritti nella tesi rappresentano una solida base per la tutela e la valorizzazione delle varietà locali di ortaggi. Inoltre, lo studio ha contribuito allo sviluppo di un nuovo paradigma di ricerca: la protezione dell'agrobiodiversità orticola richiede un approccio che superi il mero recupero e la conservazione statica delle risorse genetiche, privilegiando invece la coltivazione attiva delle varietà locali. Tale approccio consente di creare le condizioni affinché queste varietà possano

meglio adattarsi ed evolvere nel tempo. In sintesi, è possibile affermare che per proteggere la biodiversità è necessario aumentare la biodiversità.

Introduction

It has been forty years since the work of researchers focused on biodiversity, a relatively young concept introduced by Walter G. Rosen at the 'National Biodiversity Forum', organised from 21 to 24 September 1986 in Washington [1]. What about before this date? The term biodiversity – like the term agrobiodiversity – did not exist. However, in the last hundred years, we have lost about 75% of the genetic diversity of plants used by farmers and 30% of livestock breeds are threatened with extinction [2]. At the same time, it is necessary to consider that the world's food needs are satisfied by only five animal species and twelve plant species, of which three species – rice, maize and wheat – are responsible for providing about 60% of the world's food needs [2].

These numbers have turned the spotlight on the topic of agrobiodiversity: in the following years, both the United Nations – with the Convention on Biological Diversity (1992), the Nagoya Protocol (2010) and the 2030 Agenda for Sustainable Development (2015) – and the European Union – with the Farm to Fork Strategy (2020), the EU Biodiversity Strategy 2030 (2020) and the EU Strategies for Adaptation to Climate Change (2021) – have developed plans and actions to protect and preserve European and global biodiversity. The EU Biodiversity Strategy 2030, in particular, states that «[...] there is a need to reverse the trend of genetic variety erosion, for example, by facilitating the use of traditional crops and breeds: this would also benefit for (human) health.» [3,4].

In terms of biodiversity and, in particular, agrobiodiversity, an important role is played by landraces of vegetables. They are defined as «variable populations, which are well identifiable and usually have a local name, and which have not been subject to an organised programme of genetic improvement.» [5]. Moreover, landraces have a high capacity to adapt to the environmental and growing conditions of specific areas [6,7]. These characters are important in terms of: (i) genetic improvement, as these varieties offer useful genetic bases for the development of new improved varieties [8–10] and (ii) farming under special agro-environmental conditions such as, for example, organic farming regimes, cultivation developed in marginal areas or areas strongly influenced by climate change processes [11–15]. It is therefore necessary to recover landraces – often subject to a high risk of genetic erosion and preserved by elderly farmers in small home gardens [16] – and develop appropriate protection and valorisation processes for them. These processes cannot ignore characterisation activities, which have a twofold objective: on the one hand, it is necessary to study these local varieties from a morphological point of view, because this information is essential to begin the protection procedures identified by Italian national and regional laws (e.g., National Law 1 December 2015, no. 194, and Regional Law of Apulian Region of 8 April 2013, no. 39) and necessary to differentiate landraces unambiguously and avoid, for example, cases of homonymy and synonymy [17–20]; on the other hand, it is necessary to characterise local resources from

an agronomic and nutritional point of view, in order to find useful information to cultivate, valorise and reintroduce these varieties in commerce [21–24].

On this basis, Chapter 1 reports the morphological and agronomic characterisation study of nine local Apulian immature melon varieties (*Cucumis melo* L); Chapter 2, however, reports the discovery of Cucurbitacins in one of the *C. melo* varieties, a result that follows the chemical-nutritional characterisation of this population.

With reference to vegetable landraces, one of the main causes of extinction of plant genetic resources is the progressive abandonment of local varieties in favour of modern varieties (e.g. F1 hybrids) [16,25,26]. This is emphasised by the rules for the marketing and commercial production of seeds of varieties of vegetable species, which state that seeds of a variety may only be marketed if that variety is registered in the Common Catalogue of varieties of agricultural plant and vegetable species [27,28]. In order to be registered in the Common Catalogue, varieties of plant species must meet the requirements of Distinctiveness, Stability, and Uniformity (DUS), characteristics that are not satisfied by landraces [6,29,30]. In response, the European Council introduced two derogation schemes in 1998: conservation varieties and varieties with no intrinsic value, whose aim is to allow the registration in the Common Catalogue of varieties otherwise excluded [12,14,31]. In particular, varieties (i) at risk of genetic erosion, (ii) characterised by a high level of genetic diversity, and (iii) traditionally linked to a specific area of origin may be included in the conservation varieties scheme [27]. The conservation variety regime, therefore, allows the registration of landraces in the Common Catalogue, facilitating the marketing of propagation material and, thus, an increased protection of these varieties [32,33]. Nevertheless, until today, the number of conservation varieties registered in Italy and Europe is extremely limited [12,14,33]. This has two major consequences: on the one hand, a decreasing use of local varieties in the field; on the other hand, a low degree of differentiation of European vegetable crops and, therefore, an increasing erosion of agrobiodiversity [16,25]. Understanding and exploiting the existing derogation regimes - conservation varieties and varieties with no intrinsic value - can, instead, help to halt this erosion and, thus, protect and enhance local vegetable varieties. On this basis, Chapter 3 shows a work dedicated to conservation varieties regime, aimed at analysing the results obtained from this instrument of protection and its possible future developments.

Lastly, an important step in the process of protecting and valorising the resources of agrobiodiversity is the product proposal to the consumer. In this respect, an important lever for valorisation is offered by the European Geographical Indications (GIs): Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), Traditional Speciality Guaranteed (TSG) [34–36]. However, the awarding of such marks often requires long waiting times and the identification of consortia or other collective protection bodies; these requirements, in many cases, do not match the characteristics and

needs of the micro-small farms (often individual farmers) that cultivate landraces [16,37,38]. Although in Italy there is a supplementary recognition that is potentially useful for the promotion of landraces and, more generally, of traditional products; in 1998, in fact, Legislative Decree No 173/1998 introduced into Italian law the recognition of "Traditional Agrifood Product" (TAP), attributed to those products «whose methods of processing, storage and maturing […] are carried out on their territory in a homogeneous manner and according to traditional rules and protracted over time; however, for a period not less than twenty-five years.» [39,40]. TAP recognition - which provides a category dedicated exclusively to plant products in their natural or processed state, including horticultural landraces recognised as traditional - represents therefore a useful tool to disseminate and promote traditional local products [41,42].

Chapter 4 is dedicated to the analysis of the strengths, weaknesses, opportunities and threats (SWOT analysis) of the TAP recognition applied to vegetable landraces. The work was carried out through a special consumer questionnaire and a specific regulatory analysis dedicated to TAP and European GIs.

Finally, in Chapter 5, starting to national constitutional charts and international laws aimed at preserving biodiversity, the role of bioresearch in biodiversity conservation was revisited. The roles of landraces and modern varieties were analysed in light of recent developments in horticulture, and future objectives for the conservation and valorisation of agrobiodiversity were outlined.

Based on these considerations, the PhD thesis aims to present tools and systems for the protection and valorisation of local fruit vegetable varieties, following the ideal path identified by the Italian National Plan on Biodiversity of Agricultural and Food Interest. This process began with the recovery and characterisation of vegetable landraces in the reference area, Apulia, an Italian region rich in agrobiodiversity, as part of the Project "Biodiversity of Apulian Fruit Vegetables" (BiodiverSO Karpos - Regione Puglia, Rural Development Program 2014–2022), on the basis of the previous Project "Biodiversity of Apulian Vegetable Species" (BiodiverSO - Regione Puglia, Rural Development Program 2014–2020) and the work developed in the previous years. For example, the BiodiverSO Project is responsible for the recovery and enhancement of a (now) famous Apulian landraces: 'Carota di Polignano' (*Daucus carota* L.). This landrace, until ten years ago, was at very high risk of extinction; during the Project, this carrot was characterized, cultivated and reintroduced on the local markets, activities that have enabled it to achieve its current reputation [21,43–45]. The same strategy was applied for the work presented in this PhD thesis, with a specific focus on local varieties of immature melons (*C. melo*), with morphological, agronomic and nutritional characterisation activities. In particular, the activities were concerned on a specific *C. melo* landrace, the 'Carosello Scopatizzo', on

the basis of other research which identify this landrace as particularly interesting in terms of production and nutrition profiles [17,23,46].

In addition, new research fields have been explored in the literature, such as, for example, the use of TAP recognition for the valorisation and promotion of vegetable landraces. At the same time, a further protection and valorisation process for Apulian vegetable landraces - the conservation variety regime - has been analysed in order to overcome the problem of supplying propagation material. The work carried out is consequently focused on profound reflections on the role of research regarding the protection and valorisation of vegetable landraces and, more generally, of biodiversity.

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Chapter 1

Yield, Morphological, and Qualitative Profile of Nine Landraces of Unripe Melon from the Puglia Region Grown in Open Field

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Article

Yield, Morphological, and Qualitative Profile of Nine Landraces of Unripe Melon from the Puglia Region Grown in Open Field

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Abstract: In recent years, increasing attention in regional and national markets has been given to the Puglia region's traditional landraces of unripe melon (*Cucumis melo* L.). However, distinguishing these landraces is challenging due to their significant variability. A detailed morphological characterization is crucial to identify the unique features of each variety, while also assessing their productive potential. This study evaluated nine Puglia landraces of *C. melo*: '*Barattiere*', '*Carosello leccese*', '*Carosello scopatizzo*', '*Cucumbr* di Martina Franca', '*Carosello* di Polignano', '*Carosello* striato tondo di Massafra', '*Spuredda bianca*', '*Spuredda nera*', and '*Spuredda fasciata*'. The aims of the work were to identify specific and distinctive characters for these landraces, subdivided into traditional macro-groups ("*Barattiere*", "*Caroselli*", and "*Spuredde*"), and to evaluate productive and quality traits that could be interesting for future commercial promotion. The main findings revealed distinct characteristics among the "*Barattiere*" group and the other two macro-groups across all the parameters considered. The differentiation of the "*Caroselli*" and "*Spuredde*" macro-groups, on the other hand, was more challenging because of similar intragroup characteristics. In particular, a case of synonymy was found between the landraces '*Carosello leccese*' and '*Spuredda bianca*', and a high degree of dissimilarity was identified between '*Carosello* di Polignano' and the other landraces.

Keywords: agrobiodiversity; characterization; *Cucumis melo* L.; *Carosello*; *Barattiere*

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1. Introduction

The Puglia region (Southern Italy) is a treasure trove of horticultural agrobiodiversity [1–3]. Among the most important vegetables grown in this region—such as the tomato (*Solanum lycopersicum* L.), artichoke (*Cynara cardunculus* L.), rapini (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.), broccoli and cauliflower (*Brassica oleracea* L. var *italica* and *Brassica oleracea* L. var. *botrytis*), and fennel (*Foeniculum vulgare* Mill.) [4]—Puglia is particularly renowned for its melon production (*Cucumis melo* L.), especially unripe melons (Figure 1), traditionally consumed as an alternative to cucumber (*Cucumis sativus* L.) [5,6]. Its fruits are harvested when the seeds are only faintly outlined and sold as "whole-edible" fruits so that the exocarp and placental parts, apart from the mesocarp [5,7], can be consumed.

Figure 1. Fruits of some unripe melon (Cucumis melo L.); landraces of the Puglia region (Southern Italy).

From a nutritional point of view, the ability to eat all parts of the fruit is remarkable considering that the placenta is rich in polyphenols and tocopherols, especially α-tocopherols. These compounds provide health benefits, in particular for the cardiovascular system [7–10].

In the Puglia region, hundreds of hectares are committed to the cultivation of landraces producing unripe melons (*Cucumis melo* L.) [11]. These are locally classified into three macro-groups: the "*Caroselli*" and "*Barattiere*" groups, primarily cultivated in the Bari and Taranto areas of Puglia, and the "*Spuredde*" group, characteristic of the Salento zone, corresponding to the Brindisi and Lecce areas in the south of Puglia [5,11,12]. Regarding the first two groups, a study from 2017 [13] showed that "*Barattiere*" and "*Caroselli*" belong to two distinct genetic subpopulations, confuting the assumption that "*Barattiere*" is a variant of "*Caroselli*". In particular, the landraces belonging to the macro-group "*Caroselli*" were classified under the taxonomic group *chate* [13]. However, the taxonomic classification of the macro-groups "*Barattiere*" and "*Spuredde*" remains unclear, as does the distinction between "*Caroselli*" and "*Spuredde*". These unripe melons, as local populations selected annually by small farmers, are identified by dialectal names that often vary from farmer to farmer [12,14]. Consequently, the classification of a landrace into one macro-group or the other depends more on socio-territorial factors than on morphological or productive difference, with frequent occurrences of homonyms and synonyms [6,12,15].

These unripe melon landraces are characterized by a high degree of intraspecific variability [6,13,16,17], typical of *C. melo* landraces [18,19]. For this reason, these landraces do not meet the minimum requirements for inclusion in the Common Catalogue of varieties of vegetable species, which is the catalogue of marketable varieties in Europe [20–23].

These populations can be placed on the market as a "commercial" category, for which registration in the Common Catalogue is not required, and are commercially identified only by the species (the producer is free to specify a variety denomination, if they deem it appropriate). In fact, these landraces are labelled with a specific label by seed companies that report essential data to protect the farmer (including germination and purity of the seed). The large intraspecific variability and high fragmentation of the production areas, in addition to the lack of detailed identification on commercial labels, has led to confusion among producers and consumers [6].

On this basis, it is essential to identify the diverse landraces through morphological and agronomic traits to provide clear and unambiguous information [24–26]. This is in line with the proposals of the main programmes for the protection of agricultural biodiversity such as the Italian National Plan for Biodiversity of Agricultural Interest (PNBA). Among the essential actions for the protection of agrobiodiversity, PNBA reserves a fundamental role for the morphological characterization of landraces according to common principles and techniques [27].

Furthermore, although most unripe melon landraces are cultivated on small plots and by individual farmers, there has been increasing interest in recent years from both productive-commercial and historical-traditional perspectives. Regarding the first aspect, several studies have focused on the yield and quality of *C. melo* landraces [7,28–30]. For instance, Somma et al. [6] and Palmitessa et al. [30] demonstrated that certain unripe melon landraces can achieve production levels comparable to those of commercial cucumber (*C. sativus* L.) varieties. Additionally, some *C. melo* landraces exhibit tolerance to specific pathogens, highlighting their potential use as rootstocks for other susceptible cucurbit crops [31,32]. From a cultural and traditional perspective, it is important to highlight that some unripe melon landraces have recently been recognized as Traditional Agrifood Products (TAP) of the Puglia region . This designation formalizes their intrinsic link to the local population, customs, and traditions of the region where they are cultivated, and represents a valid tool for the promotion of agrifood products [33,34].

On the other hand, some of Puglia's landraces of unripe melon are at a serious risk of genetic erosion or extinction today, as demonstrated by the inclusion of some of them in regional catalogues and national registers aimed at protecting plant biodiversity [35,36]. It is therefore essential to study their characteristics and properties to preserve vegetable biodiversity and the traditions of their areas of origin.

The aims of this research, with reference to nine landraces of the Puglia region's unripe melons, were as follows: (i) to identify the specific and distinctive characters able to univocally distinguish the landraces; (ii) to evaluate productive and quality traits that could be interesting for future commercial promotion; and (iii) to explore possible differences between macro-groups of these unripe melons ("*Barattiere*", "*Caroselli*", and "*Spuredde*").

2. Materials and Methods

2.1. Experimental Location

The experiment was carried out between May and July 2022 (spring–summer) at the experimental farm "La Noria" of the Institute of Sciences of Food Production,

National Research Council, located in Mola di Bari (BA, Puglia region, Southern Italy, 41.062156° N, 17.066914° E) in open-field conditions.

2.2. Plant Material and Growing Conditions

Nine landraces of *C. melo* traditionally cultivated in the Puglia region (Italy) were tested: '*Barattiere*' (BT); '*Carosello leccese*' (CAL); '*Carosello scopatizzo*' ('*Scopatizzo*'—CAS); '*Cucumbr* di Martina Franca' (CUM); '*Carosello* di Polignano' ('*Tomentoso*'—CAP); '*Carosello* striato tondo di Massafra' (CAM); '*Spuredda bianca*' (SB); '*Spuredda nera*' (SN); and '*Spuredda fasciata*' (SF).

Seedlings were provided by a local plant nursery using seeds self-produced by local farmers. Transplant was carried out at the two-true-leaves stage on 9 May 2022 at 100 cm between rows and 40 cm within row (with a final density of 2.5 plants/m^2). The main stem of each plant was vertically trained. Based on the pruning approach, two different growing systems were tested for each landrace: "wild" and "cut". The wild treatment did not involve any topping but rearing of the main stem and free growth of the primary, secondary, and possible tertiary side shoots. The cut treatment involved rearing of the main stem and topping of the side shoots after the second node bearing a female or hermaphrodite flower. For the landrace BT, the treatment was developed by topping the main stem at the second node and growing one of the primary side shoots in vertical as the main stem. This treatment was developed in accordance with local cultivation practices and the sexual expression of BT, in which the main stem does not produce fruiting flowers [5,6,11,37].

Plants were grown according to local practices and irrigated daily with a micro-irrigation system.

Daily temperature and relative humidity values were recorded at 15 min intervals for the entire duration of the experiment. Day/night temperatures were 29 ± 7.0/26 ± 5 °C and day/night relative humidity was 71.0 ± 23/66 ± 22%.

2.3. Experimental Design

The experimental design was a split-plot with three repetitions, considering the growing system as the main plot and the nine varieties as the sub-plot. The elementary unit consisted of five experimental plants.

2.4. Morpho-physiological Descriptors

The landraces of *C. melo* were described according to the descriptor model of the GIBA (Gruppo di Lavoro Nazionale sulla Biodiversità Agraria, i.e., the Italian "National Working Group on Agricultural Biodiversity") for melon (Rif. CPVO TP/104/2) and the guidelines to conduct tests of distinctness, uniformity, and stability of the International Union for the Protection of New Varieties of Plants (UPOV, TG/104/5 Rev. 2) [27,38]. These guidelines were integrated with further morphological descriptors of the International Plant Genetic Resources Institute (IPGRI) and other studies focused on the morphological characterization of *C. melo* varieties [39]. The following plant organs were considered: seeds, true leaves, flowers, young fruits, and harvested fruits.

2.5. Seed Biometrics

For each landrace, biometric descriptors were recorded on an adequate seed sample, namely seed length, seed width, seed thickness, and the ratio between length and width.

2.6. Leaf Biometrics and Chlorophyll Content

Leaves between the fifth and eighth node of the vertically grown stem (the main stem for "*Carosello*" and "*Spuredde*" groups and a primary side shoot for BT) of the plants with at least eleven nodes were considered for analysis and data collection, in accordance with the reference guidelines [27,38,39]. The following descriptors were measured on fully grown leaves at the end of the experimental cycle: leaf area (LA, calculated considering only the leaf blade excluding petiole), leaf petiole length, fresh weight (FW), dry weight (DW), dry matter (DM), and specific leaf area (SLA). The DW was measured after drying fresh samples in a forced draft oven at 65 °C until constant weight was reached. The dry matter content (DM) of leaves was calculated as a percentage of the DW in FW; the SLA was calculated as a ratio of leaf area to leaf DM. Furthermore, the chlorophyll content of 54 plants (six plants for each landrace) was measured using an Apogee chlorophyll metre (MC-100, LI-COR instrument, Ecosearch s.r.l., Montone, Perugia, Italy) on fully expanded leaves.

2.7. Fruit Yield and Biometrics

The growing cycle lasted eleven weeks, from 9 May 2022 (transplantation date) to 25 July 2022; fruits were harvested three times a week, approximately every two days. The unripe fruits of about 200–350 g were treated as the stage of commercial maturity, according to the local practices.

The following yield descriptors were measured: number of fruits harvested per plant (in weight and in number), fruit fresh weight (FW) and dry weight (DW). The DW was measured after drying fresh samples (half of each fruit) in a forced draft oven at 65 °C until constant weight was reached. The dry matter content (DM) of fruit was calculated as a percentage of the DW in FW. Subsequently, the biometric characteristics of the fruits were measured, also considering the morphological descriptors requested by the descriptive guidelines: equatorial diameter (diameter of the maximum cross section of the fruit), polar diameter (diameter of the cross section of the fruit near the peduncular extremity, approximately 1 cm from the peduncular extremity), fruit length (distance between the two poles of the fruit in longitudinal section), endosperm length and width, mesocarp thickness, and peduncle length [6,7]. Moreover, five descriptors characterizing the fruits' exocarp were considered: colour, rind hairiness, grooves, wrinkling, and the presence of patches.

2.8. Fruit Quality and Commercial Parameters

To determine the quality and commercial values of the nine landraces of *C. melo*, the following descriptors were analysed: total soluble solids content, measured using a digital refractometer (DBR 55-0/55 Brix; Giorgio Bormac s.r.l, Napoli Italy) (TSS), fruit firmness (FF), measured using a portable analogic durometer (53207, Turoni, Forlì, Italy), and colour descriptors. The colour analysis was carried out on the exocarp and mesocarp of 12 freshly cut fruits of each landrace using a portable colorimeter (Minolta Chroma Meter CR-400; Minolta

Camera Co. Ltd., Osaka, Japan), expressing the results using the CIELAB colour scale (L, a*, b*). This scale consists of three coordinates: "lightness" (L), whose value varies between 0 (black) and 1 (white); "red/green chromaticity or redness" (a*), where positive values tend to red and negative values tend to green; and "yellow/blue" chromaticity or yellowness" (b*), where positive values tend to yellow and negative values tend to blue. Furthermore, derived colour descriptors were calculated: hue angle ($h° = \tan^{-1} b^*/a^*$), indicating the dominant colour, and colour saturation or chroma ($C = [(a^*)^2 + (b^*)^2]^{½}$. The colorimeter was calibrated with a standard reference having L, a* and b* values of 104.34, 0.07, and 2.39, respectively.

2.9. Statistical Analysis

All data underwent an analysis of variance (ANOVA) using the General Linear Model procedure of SAS software (SAS version 9.1, SAS Institute, Cary, NC, USA). According to the research objectives, the means were compared using orthogonal contrasts with one degree of freedom (eight contrasts): (i) BT vs. others; (ii) ("*Caroselli*": CAL, CAM, CAP, CAS, CUM) vs. ("*Spuredde*": SB, SF, SN); (iii) SB vs. (SF and SN); (iv) SF vs. SN; (v) (CAL and CAP) vs. (CAM, CAS, CUM); (vi) CAL vs. CAP; (vii) CAS vs. (CAM and CUM); and (viii) CAM vs. CUM. Since none of the results were statistically influenced by the pruning system and the interaction between landrace and pruning system, the means of the two growth systems are reported in the tables.

For a visual analysis of the data, a Principal Component Analysis (PCA) (XLStat, version 2023.2.1414, Addinsoft, Paris, France) was performed on the mean centred and standardized (unit variance scaled) data prior to the analysis. The data matrix submitted to the PCA was made up of nine observations (one for each landrace) and 32 variables (fruit yield per plant; number of fruits per plant; fruit fresh weight; fruit dry matter; fruit length; fruit equatorial diameter; fruit polar diameter; endosperm length; endosperm width; mesocarp thickness; fruit peduncle length; mesocarp brightness; mesocarp redness; mesocarp yellowness; mesocarp hue angle; mesocarp saturation; exocarp brightness; exocarp redness; exocarp yellowness; exocarp hue angle; exocarp saturation; leaf area; leaf petiole length; leaf fresh weight; leaf dry matter; specific leaf area; leaf chlorophyll content; seed length; seed width; seed thickness; soluble solids content; epicarp hardness). The results of the PCA are shown as biplots of scores (landraces) and loadings (variables). Finally, the Agglomerative Hierarchical Clustering (AHC) method was used to group the elements according to their similarity, starting from a bottom-up approach. The data were analyzed to quantify the dissimilarity between the observations. Subsequently, clusters were iteratively formed by joining the two most similar groups at each step, using a linkage criterion such as the single, complete, or average linkage method. The result was represented by means of a dendrogram, which illustrates the hierarchical structure of the clusters.

3. Results and Discussion

3.1. Pruning effectiveness

In this research, the pruning practice was tested to fit the plants in a vertical training system at a higher density compared to the common non-trained system in open field. Shortening the lateral shoots at two nodes was a common

practice also observed in previous work [6,28,29] that largely advantaged the cultural practices and the manual harvest compared to non-pruned plants. The results in terms of morphology, production, or fruit quality vary from variety to variety and depending on the cultivation technique [40–42]. In this research, no substantial differences were found between pruned and unpruned plants in any of the descriptors considered. Thus, in light of the benefits in terms of facilitating harvesting, aeration of the foliage, and reduction in shading in the row, the pruning system used made it possible to improve operations without affecting harvesting results.

3.2. Morphological Analysis

The complete morphological characterization of the nine landraces is reported in the Supplementary Materials (Table S1).

The landraces of *C. melo* present a high level of intraspecific distinctness, which determines numerous individual differences at the phenotypic level; nevertheless, some characteristics of the populations may be very similar to each other. In this regard, with the support of the morphological characterization tools, the varieties studied were described and compared with each other in order to identify their distinctive characteristics.

3.2.1. Plant Morphology

Melon plants showed prostrate growth habit. With the exception of BT plants, the *C. melo* landraces showed the formation of shortened internodes and female flowers in the first growth phase, commonly called "basal rosette" (Figure 2).

Figure 2. Example of the "basal rosette" phase in '*Spuredda bianca*' (SB), a Puglia region unripe melon (*Cucumis melo* L.) landrace.

The number of shortened internodes in the basal rosette phase was approximatively up to five nodes for CAM, CAS, CUM, and SF; seven nodes for CAL, SB, and SN; and ten nodes for CAP. The length of the elongated internodes on the vertically trained main stem (primary side shoot for BT) was on average 6.75 cm for CAL, CAM, CAS, CUM, SF, and SB and 9.20 cm for CAP, SN, and BT. Fruits of *C. melo* landraces were generally developed on the shortened internodes in the basal rosette phase, on the main stem, and at the first or second node of side shoots.

3.2.2. Seeds

The seeds generally had a so-called "pine-nut" shape, with the apex tending to be rounded (Figure 3). The only exception were CAS seeds, which had a more pronounced apical protrusion, clearly visible in lateral section (Figure 3). The colour of the seed tegument varies between creamy-white and creamy-yellow; specifically, BT and CAS seeds tend to the latter colour compared to the others (Figure 3).

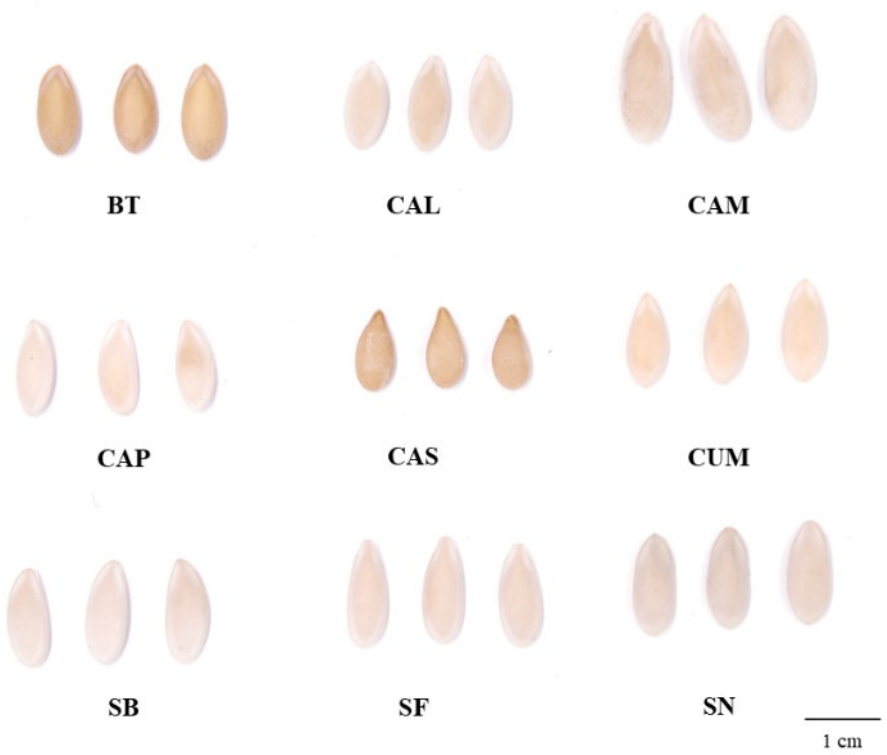

Figure 3. The seeds of nine unripe melon (*Cucumis melo* L.) landraces: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca'; SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; SN, '*Spuredda nera*'.

BT seeds were 10% shorter and 3% wider than "*Caroselli*" and "*Spuredde*" seeds (Table 1); similarly, seeds from the "*Caroselli*" group were found to be 2% wider than seeds from the "*Spuredde*" group (Table 1). Moreover, BT seeds were 7% thinner and had a length/width ratio 12% lower than the other two groups (Table 1). These differences were also found when comparing "*Caroselli*" and "*Spuredde*", with the seeds of the former group being 20%

thicker and having a 2% lower length/width ratio than the seeds of the latter group (Table 1).

Within the "*Spuredde*" group, SB presented a seed length 5% greater than SF and SN while SF, in turn, exhibited the same percentage difference compared to SN (Table 1). In addition, a 20% lower seed thickness was found in SB, compared to SF and SN (Table 1). An 8% variation in thickness was also observed between SF and SN (Table 1). Considering the "*Caroselli*" group, CAP and CAL seeds were less thick than CAM, CAS, and CUM seeds (1.89 and 2.18 mm, respectively). Moreover, CAL seeds were 20% thicker than those of CAP (Table 1). Appreciable differences were found in the comparison of CAS vs. (CAM and CUM), where CAS seeds were 30% shorter and 13% tighter than CAM and CUM seeds and presented a 19% lower length/width ratio (Table 1). CAS seeds were 20% thicker than the other two "*Caroselli*" landrace seeds (Table 1). Finally, the seed sizes of CAM (length, width, thickness, and length/width ratio) were always greater than those of CUM (28%, 14%, 8%, and 12%, respectively (Table 1). The other differences between groups and landraces, all being <10%, were not treated as significant as they could result from different fruit ripening stages or plant stress conditions.

Table 1. Morphological data of seeds of nine unripe melon (*Cucumis melo* L.) landraces: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca' (CUM); SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. The term "others" refers to the group comprising the landraces CAL, CAM, CAP, CAS, CUM, SB, SF, and SN.

	Length	**Width**	**Thickness**	**Length/Width Ratio**
Landraces	**mm**	**mm**	**mm**	
BT	11.16 ± 0.54	5.20 ± 0.24	1.80 ± 0.16	2.15 ± 0.14
CAL	12.34 ± 0.53	4.85 ± 0.11	2.11 ± 0.16	2.55 ± 0.14
CAM	15.56 ± 0.49	5.79 ± 0.24	2.13 ± 0.15	2.69 ± 0.11
CAP	12.07 ± 0.50	4.84 ± 0.20	1.68 ± 0.13	2.50 ± 0.14
CAS	9.71 ± 0.45	4.72 ± 0.15	2.44 ± 0.16	2.06 ± 0.09
CUM	12.13 ± 0.50	5.07 ± 0.18	1.98 ± 0.16	2.39 ± 0.11
SB	12.79 ± 0.39	5.18 ± 0.22	1.47 ± 0.15	2.47 ± 0.10
SF	12.43 ± 0.46	4.94 ± 0.19	1.77 ± 0.16	2.52 ± 0.12
SN	11.87 ± 0.29	4.81 ± 0.19	1.92 ± 0.16	2.47 ± 0.06
Significance				
BT vs. others	***	***	***	***
(CAM, CAP, CAS, CAL, CUM) vs. (SB, SF, SN)	ns	*	***	*
SB vs. (SF and SN)	***	***	***	ns
SF vs. SN	***	ns	*	ns
(CAP and CAL) vs. (CAM, CAS, CUM)	*	***	***	***
CAP vs. CAL	ns	ns	***	ns
CAS vs. (CAM and CUM)	***	***	***	***
CAM vs. CUM	***	***	**	***

Significance of contrasts: ***, ** and *, for p≤0.001, p≤0.01 and p≤0.05, respectively; ns = not significant.

Based on these data, it can be stated that visually distinguishing the seeds of these landraces is challenging; however, some of them exhibit distinctive seed characteristics that facilitate such identification. Specifically, BT and CAS seeds can be distinguished by their characteristic colour and shape; in the case

of CAS, they can be distinguished by their smaller size as well. Furthermore, the data suggest the potential for identifying CAM seeds, which are significantly larger compared to those of the other varieties. Indeed, CAM seeds were longer and wider by at least 22% and 11%, respectively, than those of other varieties.

3.2.3. Leaves

BT leaves showed a chlorophyll content 24% higher than "*Caroselli*" and "*Spuredde*" (Figure 4a), while the leaf area of BT was 13% lower than that of "*Caroselli*" and "*Spuredde*" (Figure 4b). In contrast, the leaf area of SB was 37% higher than SF and SN (Figure 4b). Within the "*Caroselli*" group, CAP and CAL reported a 49% higher leaf area than CAM, CAS, and CUM; CAS presented an 11% lower value than CAM and CUM (Figure 4b). BT also showed a petiole 33% shorter compared to the "*Caroselli*" (19.3 cm, on average) and "*Spuredde*" (17.0 cm, on average) groups (Figure 4c). The petiole was longer in CAP than in CAL by 18%, and both presented an average petiole length 48% longer than the other "*Caroselli*" landraces (CAM, CAS, CUM; Figure 4c). Moreover, the leaf DM of BT was 22% higher than the other two macro-groups and 19% lower in SF than SN (Figure 4d). Finally, leaf FW was 39% higher for CAP and CAL compared to the group formed by CAM, CAS, and CUM (Figure 4d); the complete data are available in Table S2.

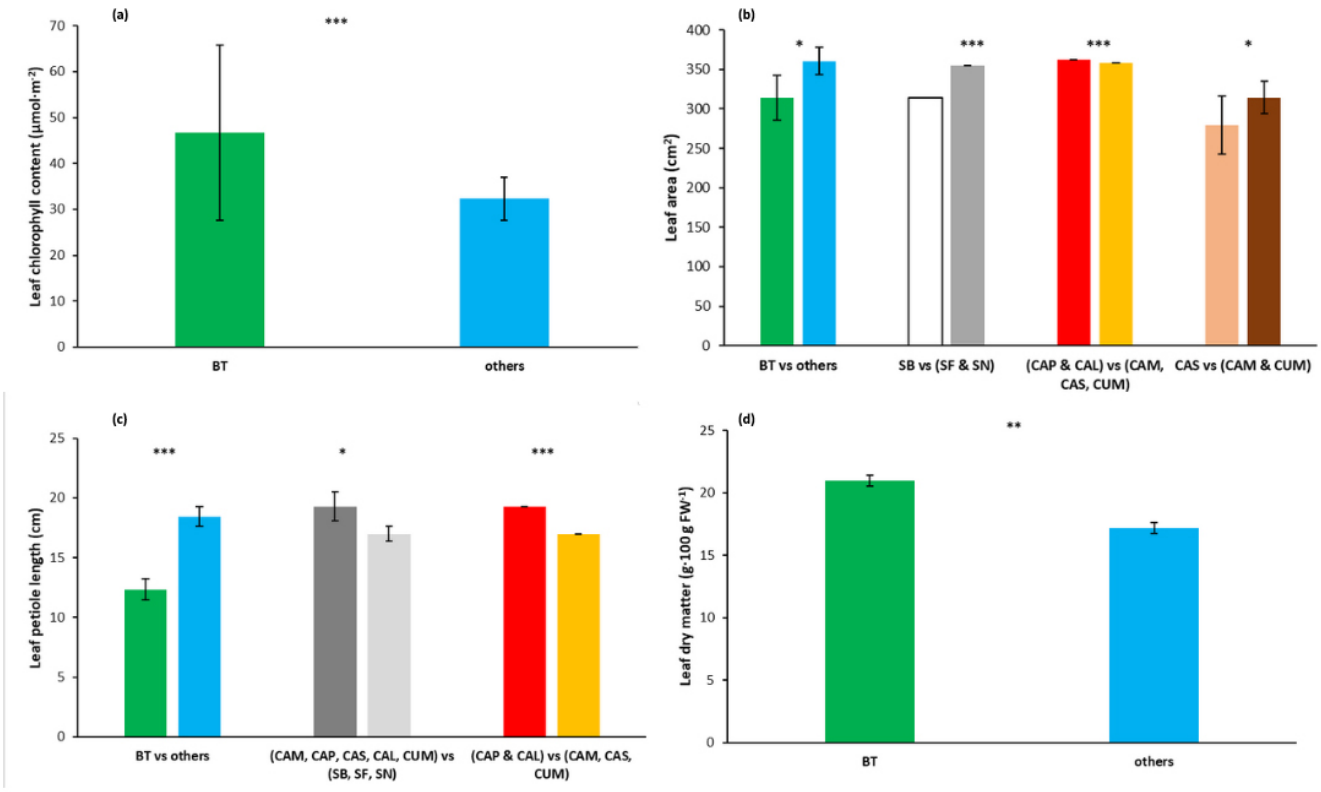

Figure 4. Visual representation of significant contrasts between *C. melo* landraces: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca' (CUM); SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*', with respect to the following parameters: (**a**) leaf chlorophyll content (µmol·m^{-2}), (**b**) leaf area (cm^2), (**c**) leaf petiole length (cm), and (**d**) leaf dry matter (g·100 g FW^{-1}). The term "others" refers to the group comprising the landraces CAL, CAM, CAP,

CAS, CUM, SB, SF, and SN. Significance of contrasts: ***, ** and * for $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, respectively. Full data are available in Table S2.

These results highlight the fact that leaf descriptors make it possible to discriminate BT from the other two macro-groups ("*Caroselli*" and "*Spuredde*"). In contrast, distinguishing the leaves of "*Caroselli*" from those of "*Spuredde*" was more challenging. However, within the macro-groups, some significant differences were identified, particularly in terms of leaf area and petiole length (Figure 4b,c).

Nevertheless, on the basis of common shape and morphological descriptors of the leaves, the nine landraces analyzed may be divided into three hypothetical groups: group 1 (BT, CAM, CAP, and CUM), group 2 (CAL, SB, SF, and SN), and group 3 (CAS) (Figure 5).

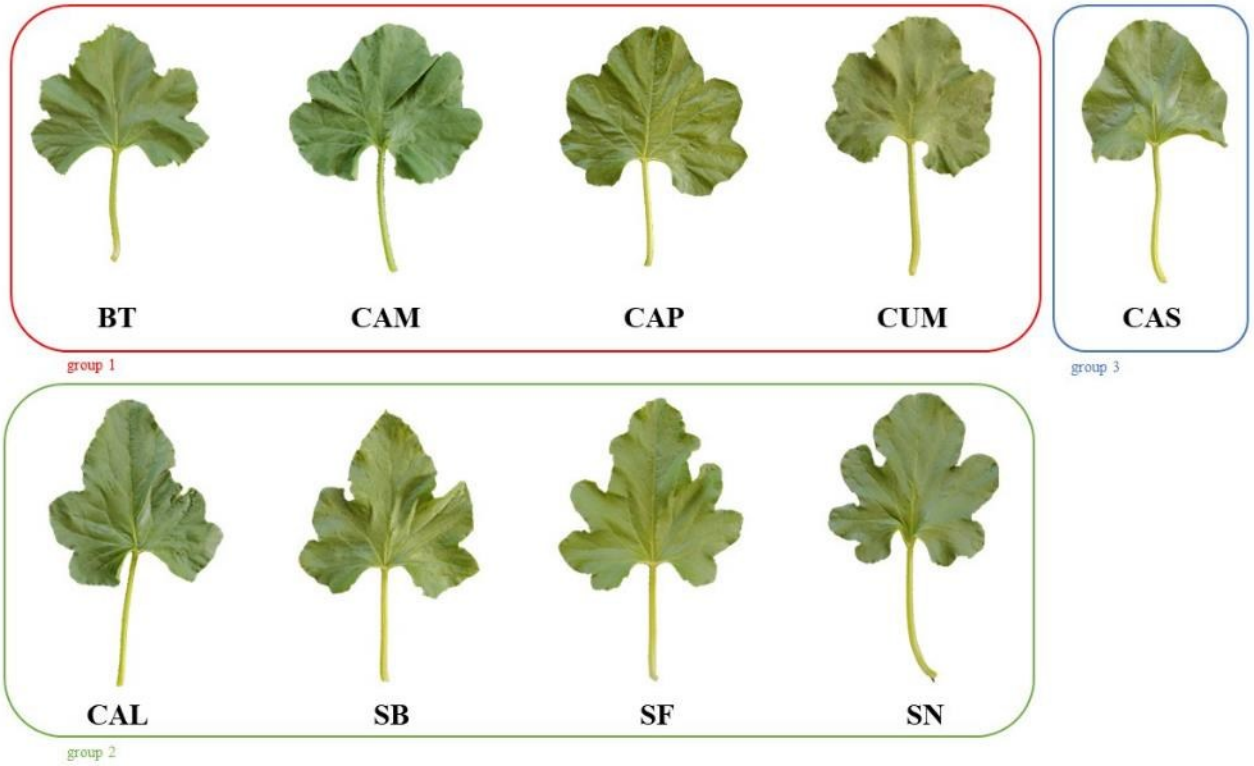

Figure 5. Comparison of representative leaves of nine unripe melon (*Cucumis melo* L.) landraces: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca'; SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. According to the morphological characterization, they can be grouped in three groups. The leaves sizes are not in proportion.

Group 1 shows an intermediate lobe development, the presence of a poorly pronounced terminal lobe, and generally penta-lobed leaves (Figure 5). All the landraces of this group shared the characteristic of a blistered leaf. The lamina colour can vary between green (tending to be darker green in BT) and light green (CAM, CAP, CUM; Figure 5). BT presented a dentate margin and pronounced dentation, while CAM, CAP, and CUM—although presenting a dentate and uneven margin—have weak or almost no margin dentation (Figure 5). On the other hand, group 2 includes the landraces characterized by a leaf with more marked/pronounced and distinguishable lobes, of which there are five (Figure 5). The terminal lobe is more elongated compared to the other groups. The leaves appeared fairly blistered, except for SN, which showed a smoother

leaf than the other landraces of group 2 (Figure 5). The lamina colour was between green and light green. The margin dentation was more pronounced for CAL, SB, and SF than for SN (Figure 5). CAS can be considered a group apart from the other varieties due to the unique characteristics of the leaves (Figure 5). Specifically, the leaves were uniform, trilobed with weak or almost no lobe development and a poorly developed terminal lobe, albeit of medium size; the margin did not present dentation, the lamina was slightly blistered and light green (Figure 5). In this empirical observation of leaf shape and morphology, it is useful to note that the groups 1, 2, and 3 were composed differently among the three macro-groups "*Barattiere*", "*Caroselli*" and "*Spuredde*", despite previously analyzed data. This highlights the critical role of data in distinguishing between the landraces, which may appear to share similar leaf shapes and characteristics upon visual and empirical evaluation.

3.2.4. Fruits

BT differs from the "*Caroselli*" and "*Spuredde*" macro-groups in peduncle length and fruit size (Table 2). In particular, BT had peduncles 24% longer than "*Caroselli*" and "*Spuredde*" and its fruits were 20% wider (equatorial diameter) than the other two macro-groups (Table 2). However, BT had lower fruit and endosperm length measurements compared to the other landraces, by 20% and 26%, respectively (Table 2). These differences were reflected in BT's lower fruit length/equatorial diameter ratio (L/Eq) (37%) and endosperm length/width ratio ($L/W_{(end.)}$) (34%) in comparison to the other landraces. Furthermore, BT presented the highest mesocarp thickness (Table 2).

Similarly, the fruits of the "*Caroselli*" group had 27% longer peduncles than the fruits of the "*Spuredde*" group (Table 2). The "*Caroselli*" group presented wider (equatorial diameter, polar diameter, and endosperm width greater by 11%, 9%, and 23%, respectively) but shorter fruits (fruit and endosperm length 18% and 14% shorter, respectively) compared to the "*Spuredde*" group. Finally, L/Eq and $L/W_{(end.)}$ were both 22% lower for "*Caroselli*" (Table 2).

Within the "*Spuredde*" macro-group, SB showed lower values for four descriptors (fruit length, endosperm length, L/eq, and $L/W_{(end.)}$) compared to SF and SN (20%, 17%, 26%, and 28%, respectively; Table 2). For the same descriptors, SF had lower values than SN (29, 16, 37, and 25%, respectively; Table 2). Other differences, all <20%, were found in endosperm width when comparing SB vs. (SF and SN) and between the latter for equatorial diameter and mesocarp thickness (Table 2). These differences underline that increasingly elongated and narrow fruits were produced in the order SB, SF, and SN.

In the comparison (CAP and CAL) vs. (CAM, CAS, CUM), a double L/Eq and a 2.5 times higher $L/W_{(end.)}$ of the first group over the second one emerged, with CAP and CAL having more elongated fruits than the more roundish fruits of CAM, CAS, and CUM (Table 2). The difference between CAP and CAL and the one between the CAM, CAS, and CUM group were reflected in all length and width descriptors considered: equatorial (5.1 vs. 6.9 cm, respectively) and polar (3.7 vs. 4.7 cm) diameter, fruit length (13.3 vs. 9.2 cm), endosperm length (10.4 vs. 6.5 cm), and endosperm width (2.6 vs. 3.9 cm; Table 2). In addition, CAP and CAL presented 61% longer peduncles and 18% less thick mesocarps than CAM, CAS, and CUM (Table 2). Similar differences—including a longer peduncle length and less mesocarp thickness—were found in the comparison between CAP (more elongated fruit) and CAL (wider fruit; Table 2).

Finally, values for CAS were 13% smaller in endosperm width and 19% greater in mesocarp thickness than CAM and CUM (Table 2). Among the latter, endocarp length and width were 27% and 12% greater in CAM, respectively (Table 2). In contrast, a 22% lower mesocarp thickness was calculated for CAM than for CUM (Table 2).

Table 2. Morphological and biometric descriptors of fruit of nine unripe melon (*Cucumis melo* L.) landraces: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca'; SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. The term "others" refers to the group comprising the landraces CAL, CAM, CAP, CAS, CUM, SB, SF, and SN.

	Peduncle length	**Equatorial diameter**	**Polar diameter**	**Length**	**Endosperm length**	**Endosperm width**	**Mesocarp thickness**	**Length/ equatorial diameter ratio**	**Endosperm length/ width ratio**
Landraces	cm	cm	cm	cm	cm	cm	cm		
BT	2.5 ± 0.9	7.1 ± 0.5	4.5 ± 0.5	9.4 ± 0.6	6.3 ± 0.4	3.2 ± 0.1	1.9 ± 0.1	1.3 ± 0.1	2.0 ± 0.1
CAL	2.3 ± 0.8	5.7 ± 0.6	4.2 ± 0.5	11.5 ± 1.1	8.0 ± 0.9	3.0 ± 0.4	1.5 ± 0.1	2.0 ± 0.1	2.7 ± 0.4
CAM	1.8 ± 0.6	6.9 ± 0.7	4.7 ± 0.7	10.0 ± 1.1	7.4 ± 1.0	4.3 ± 0.4	1.3 ± 0.2	1.5 ± 0.3	1.7 ± 0.4
CAP	3.3 ± 0.5	4.5 ± 0.5	3.2 ± 0.6	15.0 ± 1.4	12.7 ± 1.3	2.3 ± 0.4	1.2 ± 0.2	3.4 ± 0.4	5.7 ± 0.9
CAS	2.0 ± 0.3	7.0 ± 0.7	4.5 ± 0.4	9.3 ± 0.4	6.4 ± 0.6	3.5 ± 0.3	1.8 ± 0.3	1.3 ± 0.1	1.8 ± 0.2
CUM	1.4 ± 0.4	6.8 ± 0.2	4.7 ± 0.2	8.3 ± 0.5	5.8 ± 0.4	3.8 ± 0.2	1.7 ± 0.2	1.2 ± 0.1	1.5 ± 0.1
SB	1.8 ± 0.4	5.8 ± 0.3	4.0 ± 0.2	11.4 ± 1.0	8.2 ± 0.9	3.0 ± 0.4	1.4 ± 0.2	2.0 ± 0.2	2.7 ± 0.3
SF	1.8 ± 0.6	5.8 ± 0.4	4.0 ± 0.6	11.8 ± 0.9	9.1 ± 0.5	2.8 ± 0.3	1.6 ± 0.1	2.0 ± 0.2	3.3 ± 0.5
SN	1.5 ± 0.7	5.12 ± 0.2	3.7 ± 0.3	16.6 ± 7.8	10.8 ± 0.9	2.5 ± 0.2	1.4 ± 0.2	3.2 ± 1.5	4.4 ± 0.4
Significance									
BT vs others	*	***	ns	*	***	ns	***	***	***
(CAM, CAP, CAS, CAL, CUM) vs (SB, SF, SN)	*	***	**	***	***	***	ns	***	***
SB vs (SF and SN)	ns	ns	ns	*	***	*	ns	**	***
SF vs SN	ns	*	ns	***	***	ns	*	***	***
(CAP & CAL) vs (CAM, CAS, CUM)	***	***	***	***	***	***	***	***	***
CAP vs CAL	**	***	***	*	***	***	***	***	***
CAS vs (CAM and CUM)	ns	ns	ns	ns	ns	***	***	ns	ns
CAM vs CUM	ns	ns	ns	ns	***	*	***	ns	ns

Significance of contrasts: ***, ** and *, for p≤0.001, p≤0.01 and p≤0.05, respectively; ns = not significant.

All these results highlight that fruit descriptors make it possible to discriminate the three macro-groups ("*Barattiere*", "*Caroselli*", and "*Spuredde*"). However, within these macro-groups, there are further differences that do not allow the detection of fully homogeneous characteristics within the groups. For example, an analysis of other fruit traits, such as colour and exocarp descriptors, reveals further intragroup differences.

In fact, concerning an empirical observation of fruit exocarp colour (Figure 6; a complete analysis can be found in Section 3.3.2), BT, CAL, CAP, CAS, and SB were uniformly yellow-green in colour, while SN fruits had a uniform dark-olive green skin. The skin of the other landraces showed mixed colour traits (Figure 6). CAM had a ground colouration similar to SN but with yellow-green vertical stripes and patches (Figure 6); CUM showed a predominant yellow-green colouration and dark-olive green patches, which are smaller and reduced compared to CAM (Figure 6); SF showed alternating vertical streaks of olive

green (ground colour, slightly lighter than the other populations) and yellow-green (Figure 6). In terms of rind hairiness, the fruits were generally glabrous or lightly hairy, which is typical of *C. melo* populations [6]; the only exception was CAP, which revealed a very pronounced and characteristic hairiness. Finally, grooves were clearly present in CAP and less pronounced in SF and CAM (Figure 6). Furthermore, BT differed from the other eight landraces in the presence of wrinkles on the fruit-skin, a characteristic that is not found in the other landraces.

These differences are displayed in Figure 6 for fruits harvested before commercial maturity ("young fruits") (Figure 6a) and fruits harvested at commercial maturity (Figure 6b).

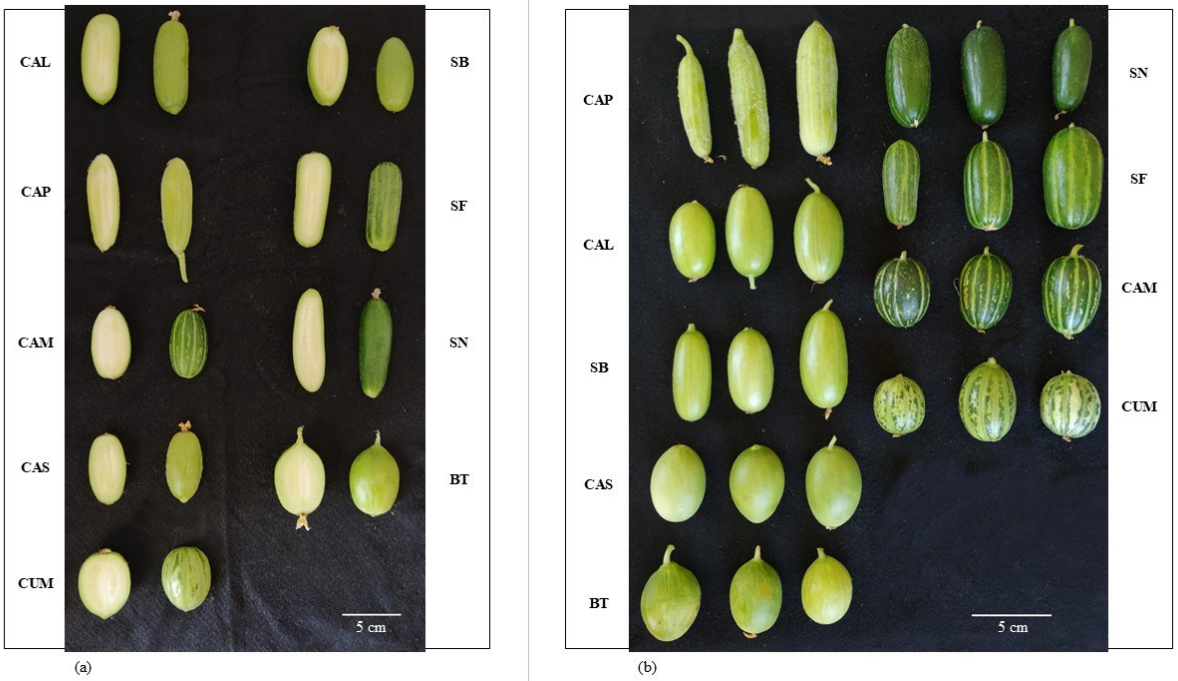

Figure 6. Fruits of nine unripe melon (*Cucumis melo* L.) landraces: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca'; SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. On the left (**a**), photos of the "young fruits" (harvested at an average weight of 80–120 g); on the right (**b**), photos of the commercially mature fruits (harvested at an average weight of 200–350 g).

3.3. Yield and Quality Descriptors

3.3.1. Yield

The beginning of the harvest period varied according to the landraces considered. The first landraces to enter production were CAL, CAM, CAS, CUM, SB, SF, and SN (33 days after transplanting); CAP and BT needed more days to enter in production. Both started to produce after seven days compared to the previous landraces (40 days after transplanting); for BT, this was already observed in previous research [6,37].

BT produced 25% less than the other landraces (Figure 7a) and 33% fewer fruits compared to the other macro-groups (Figure 7b).

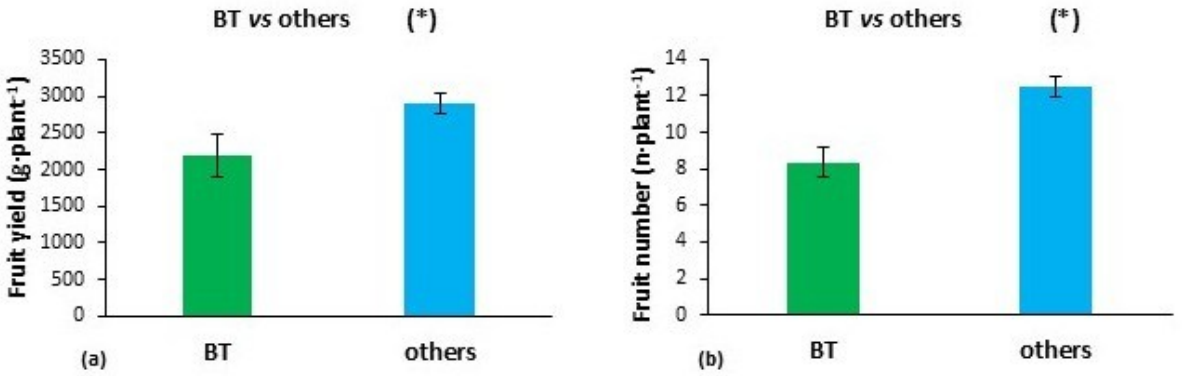

Figure 7. Visual representation of significant contrasts between "*Barattiere*" (BT) and the "others", i.e., C. *melo* landraces: CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca' (CUM); SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; SN, '*Spuredda nera*', with respect to the following parameters: (**a**) fruit yield (g/plant) and (**b**) fruit number (n/plant). The term "*others*" refers to the group comprising the landraces CAL, CAM, CAP, CAS, CUM, SB, SF, and SN. Significance of contrasts: * for $p \leq 0.05$. Full data are available in Table S3.

As reported in Somma et al. (2021), these results are explained by the biology of the BT reproductive apparatus, resulting in a retarded germination and, consequently, fruit growth compared to the other landraces [6,11,28]. This delay in production, combined with the limited duration of the growing cycle of this experiment can explain the lower yield recorded for BT. No differences were detected between the "*Caroselli*" and "*Spuredde*" groups.

Regarding fruit FW, it was 11% more for BT compared to the "*Caroselli*" and "*Spuredde*" groups (Table S3); the "*Caroselli*" group had a 7% lower fruit FW than the "*Spuredde*" group. Within these macro-groups, SB reported a 10% lower fruit FW than SB and SF, the same difference was found in the comparison (CAP and CAL) vs. (CAM, CAS, CUM; Table S3). Finally, CAM presented 27% heavier fruits than CUM (Table S3).

3.3.2. Quality Analysis

BT fruit differed from the other landraces across all the descriptors assessed (Figures 8 and 9). This landrace reached the highest content of TSS, DW, and DM. It was 14, 27, and 37% more than the "*Caroselli*" and "*Spuredde*" groups, respectively (Figure 8a–c). In contrast, the FF was 11% lower in BT (Figure 8d). Further differences were found in the "*Caroselli*" group: CAP registered lower TSS and FF (6 and 17%, respectively) than CAL (Figure 8d). Considering CAS vs. (CAM and CUM), the first contained 26, 28, and 35% more DW, DM, and TSS, respectively (Figure 8a–c). The last difference was finally found in the "*Caroselli*" group, where CAM presented a 10% higher FF than CUM (Figure 8d). The complete data are available in Table S4.

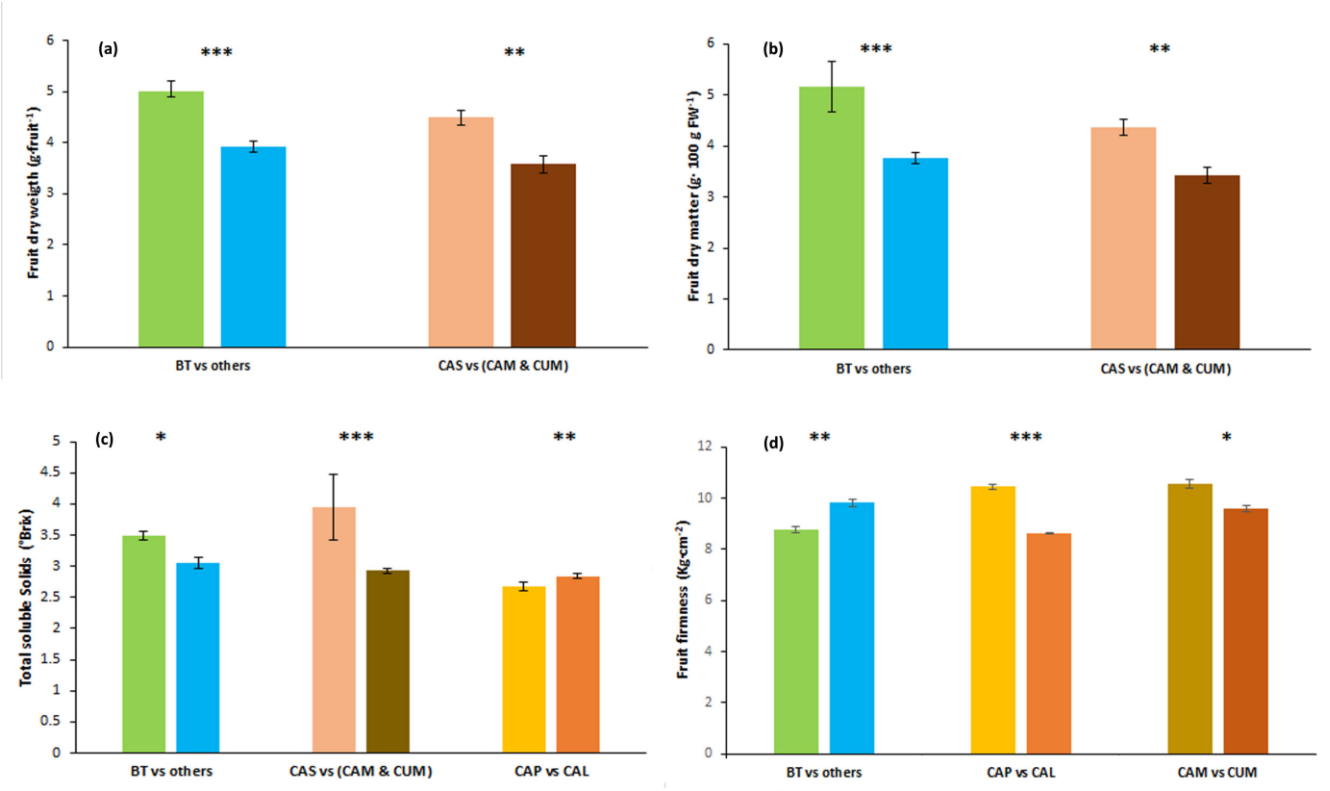

Figure 8. Visual representation of significant contrasts between landraces for the qualitative descriptors: (**a**) fruit dry weight (FW) (g/fruit); (**b**) fruit dry matter (DM) (g/100 g FW); (**c**) total soluble solids (°Brix); (**d**) epicarp hardness (kg/cm²). CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca' (CUM); SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. The term "others" refers to the group comprising the landraces CAL, CAM, CAP, CAS, CUM, SB, SF, and SN. Significance of contrasts: ***, ** and * for $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, respectively. Full data are reported in Table S4.

Regarding colour analysis, it is important to highlight that the colour in vegetables is one of the most important quality descriptor for consumers, as the first impression provided by the colour may influence the acceptance of the product [43]. Especially for unripe melon landraces, the exocarp colour is an important quality indicator that can greatly influence consumer choice [5,43]. In this regard, some studies seem to suggest that consumers prefer fruit vegetables with a higher chroma, which indicates a more vivid colour (saturation, "C") [44–46]. BT registered almost 34% more colour saturation ("C") of the exocarp than the "*Caroselli*" and "*Spuredde*" groups, making it the most appealing landrace in terms of the commercial aesthetic of the colour (Figure 9). In addition, SB registered 41% more of exocarp "C" compared to the other "*Spuredde*", while CAS had 53% higher than CAM and CUM (Figure 9). The chroma of the mesocarp was similar in all landraces (Table S5).

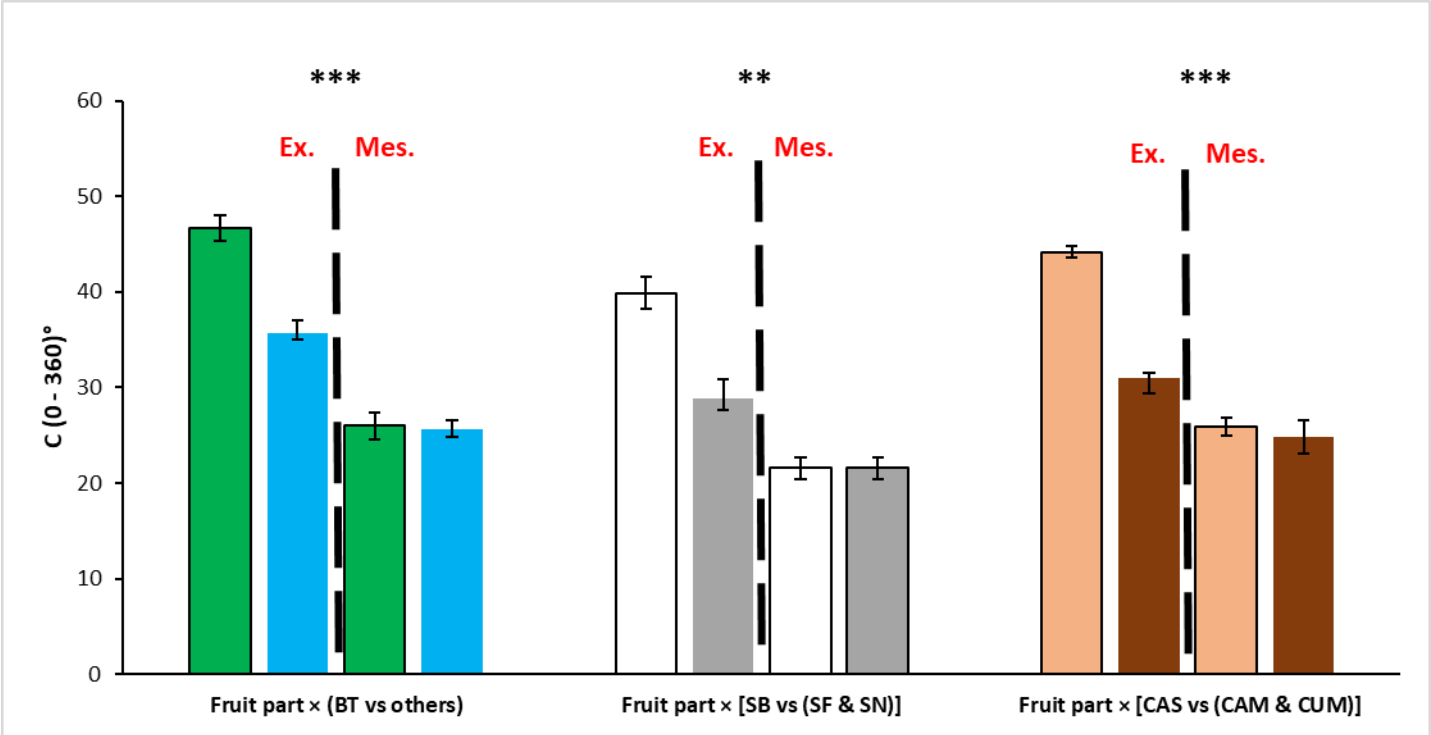

Figure 9. Visual representation of significant contrasts between *Cucumis melo* L. landraces for the descriptor "C" (saturation). "Ex." indicates the C values of the exocarp; "Mes." indicates the C values of the mesocarp. CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca' (CUM); SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. The term "*others*" refers to the group comprising the landraces CAL, CAM, CAP, CAS, CUM, SB, SF, and SN. Significance of contrasts: *** and ** for $p \leq 0.001$ and $p \leq 0.01$, respectively. Full data are reported in Table S5.

Concerning L and h° values, our findings indicate that the overall flesh colour can be described as a light green-yellow, with minimal variation among different landraces. It is well established that melon development involves a reduction in chlorophyll content and a concurrent increase in carotenoids as the fruit matures [47]. Consequently, the pale green-yellow colour of the flesh in the landraces studied can be attributed to their unique characteristic of being harvested and consumed before reaching full ripeness. Furthermore, it is important to note that, beyond the changes in chlorophyll and carotenoid levels during fruit development and ripening, the genotype also plays a significant role in determining flesh colour. For example, wild melons typically have light green flesh, while cultivated varieties may exhibit green (due to a recessive gf allele), white (from a recessive wf allele) or orange flesh (associated with β-carotene, influenced by the dominant gf + and/or wf + alleles) [48]. Although there is limited literature on the CIELab colour attributes of these landraces, Elia and Santamaria [1] described the flesh of BT as light green, even in fully ripe fruits. Thus, our study suggests that the light green-yellow colouration of these landraces represents a distinctive trait, shaped by centuries of selective cultivation by farmers.

3.4. PCA

To better summarize the results of the ANOVA analysis, the biplot in Figure 10 was developed with PCA. In the graph, it is possible to visualize the influence of each variable analyzed with reference to the nine unripe melon landraces. The biplot between the first two principal components (PC1 and

PC2) was considered representative, explaining 62.42% of the total variability of the data examined.

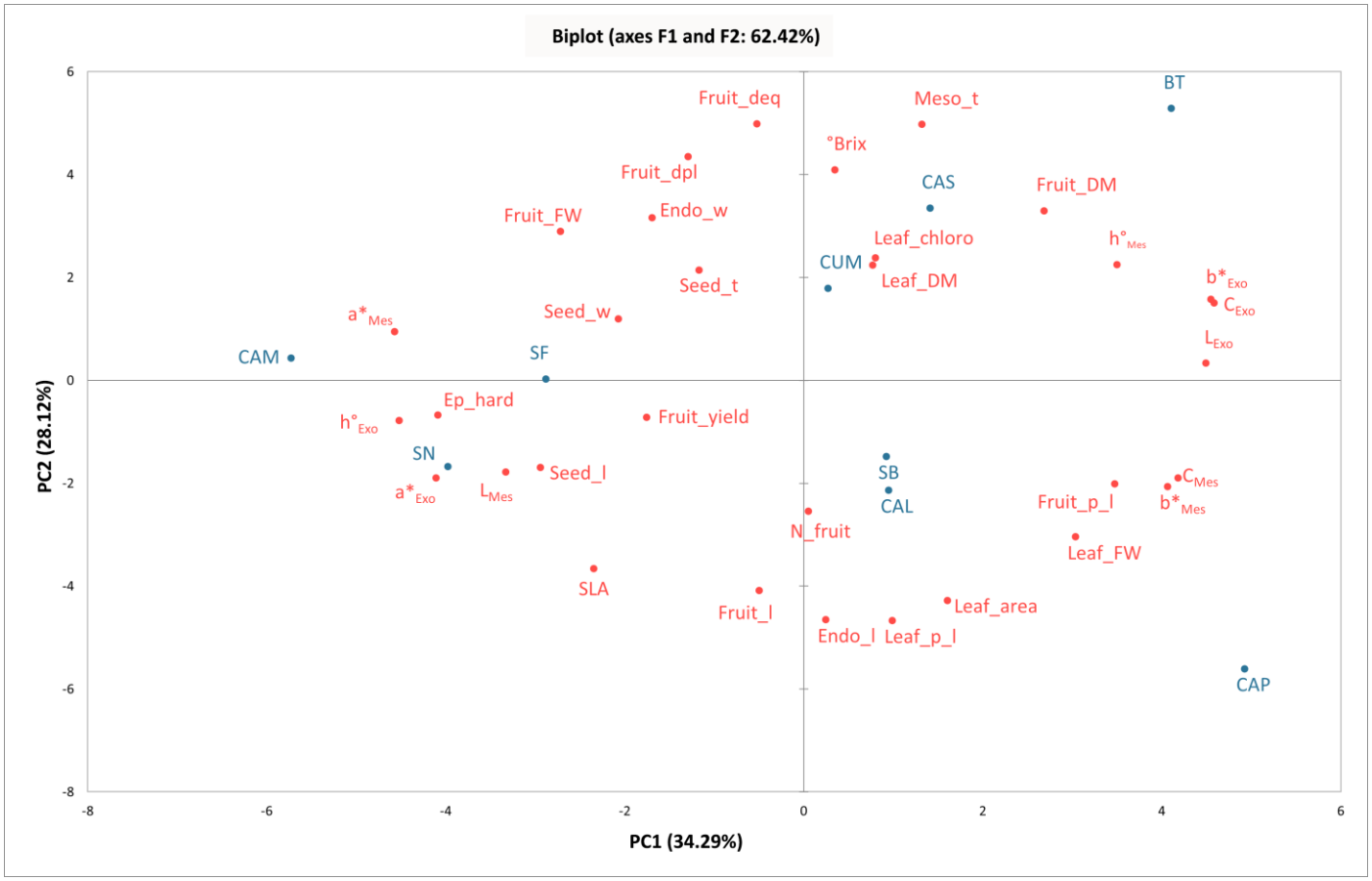

Figure 10. PCA biplot (PC1 vs. PC2) describing the distribution of the morphological and agronomic parameters of nine unripe melon (*Cucumis melo* L.) landraces: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca'; SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. Fruit_yield, fruit yield per plant; N_fruit, number of fruits per plant; Fruit_FW, fruit fresh weight; Fruit_DM, fruit dry matter; Fruit_l, fruit length; Fruit_deq, fruit equatorial diameter; Fruit_dpl, fruit polar diameter; Endo_l, endosperm length; Endo_w, endosperm width; Meso_t, mesocarp thickness; Fruit_p_l, fruit peduncle length; L_{Mes}, mesocarp lightness; a^*_{Mes}, mesocarp redness; b^*_{Mes}, mesocarp yellowness; $h°_{Mes}$, mesocarp hue angle; C_{Mes}, mesocarp saturation; L_{Exo}, exocarp lightness; a^*_{Exo}, exocarp redness; b^*_{Exo}, exocarp yellowness; $h°_{Exo}$, exocarp hue angle; C_{Exo}, exocarp saturation; Leaf_area, leaf area; Leaf_p_l, leaf petiole length; Leaf_FW, leaf fresh weight; Leaf_DM, leaf dry matter; SLA, specific leaf area; Leaf_chloro, leaf chlorophyll content; Seed_l, seed length; Seed_w, seed width; Seed_t, seed thickness; °Brix, soluble solids content; Ep_hard, epicarp hardness.

The PCA allows a unique graphic representation of the variables previously considered (loadings—in red) and their effect on *C. melo* landraces (scores—in blue). The first representation that the graph reports is a clear distribution of the landraces in four groups (Figure 10). The first group—on the left side, on the negative side of PC1—includes SN, SF, and CAM; the latter is located further away from the other two points, but all three share a relatively similar position on the left side of the biplot, associated with descriptors such as seed size and colour indices (a^*_{Mes} and a^*_{Exo}, mesocarp and endocarp redness). Another descriptor associated with this area of the biplot is the hardness of the epicarp, influencing SN and SF equally (Figure 10). The second group, composed of CUM, SB, and CAL, is located on the right-hand side of the graph,

around the origin of the axes (Figure 10). In particular, CAL and SB are moderately influenced by the chlorophyll content in leaf (Leaf_chloro) and leaf dry matter (Leaf_DM) and are very close together, almost overlapping (Figure 10). CUM is slightly displaced to the left in relation to CAL and SB but is also in a central position (Figure 10). It is influenced by variables such as number of fruits produced per plant (N_fruit) and Leaf_DM. In general, the group represents landraces with balanced descriptors, given their biplot position away from the influence of more extreme variables. The third group is composed of the "outlier" landraces; CAP and BT, indeed, are placed in outer areas of the biplot, suggesting very specific and distinctive descriptors (Figure 10). In particular, CAP is located in the bottom right corner, and it is strongly influenced by variables such as the length of peduncle (Fruit_p_I), leaf fresh weight (Leaf_FW), and saturation of mesocarp (C_{Mes}), while BT is in the upper right corner, far from CAP, but also quite isolated (Figure 10). It is influenced by mesocarp thickness (Meso_t) and soluble solids content (°Brix). The last group exclusively comprises CAS; this is very close to the second group but is influenced by other variables such as Meso_t, which bring it closer to BT than the other varieties (Figure 10). CAS, therefore, could either be included in the second group or form a separate group with BT.

To better understand and summarize the outcomes of the PCA, it is useful to consider the dendrogram in Figure 11—obtained with the Agglomerative Hierarchical Clustering (AHC) method—that reports, on the horizontal scale, the landraces divided into groups according to their similarity and, on the vertical scale, the level of dissimilarity between landraces and groups.

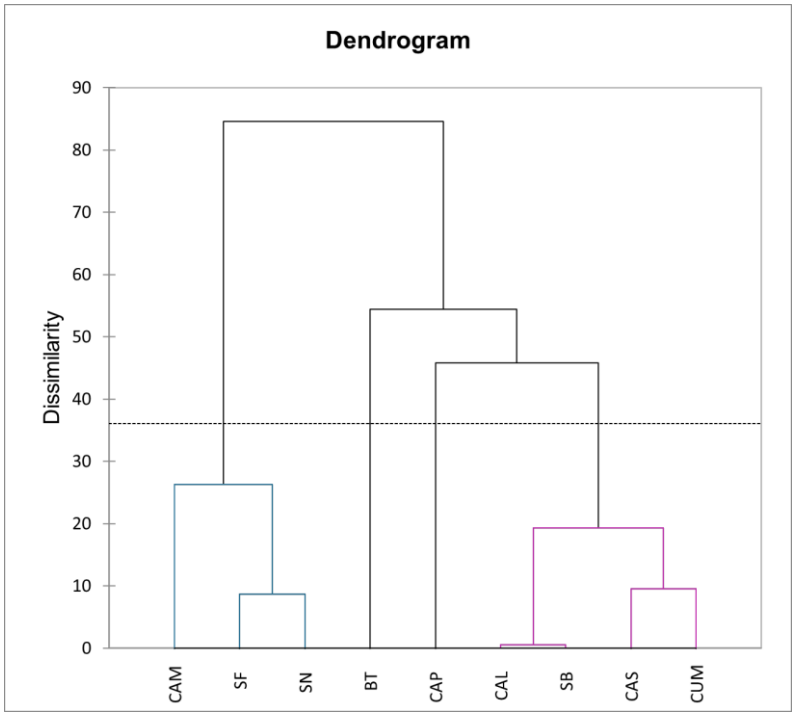

Figure 11. Dendrogram reporting the degree of dissimilarity of nine unripe melon (*Cucumis melo* L.) landraces, grouped according to PCA: BT, '*Barattiere*'; CAL, '*Carosello leccese*'; CAM, '*Carosello* striato tondo di Massafra'; CAP, '*Carosello* di Polignano'; CAS, '*Carosello scopatizzo*'; CUM, '*Cucumbr* di Martina Franca'; SB, '*Spuredda bianca*'; SF, '*Spuredda fasciata*'; and SN, '*Spuredda nera*'. The graph is the result of a classification obtained with the Agglomerative Hierarchical Clustering (AHC) method.

The dendrogram suggests that CAS belongs to the same group as CUM, CAL, and SB and confirms the other groups formed in the biplot (SF, SN, and CAM in the first group; CAP and BT as outliers). Also, it is worth mentioning the high similarity between CAL and SB, for which the level of dissimilarity is around zero (Figure 10). The results suggest that these two landraces are probably synonyms used in the provinces of Bari ('*Carosello leccese*', CAL) and Lecce ('*Spuredda bianca*', SB) for the same landrace. Finally, it should be noted that BT is the variety with the highest level of dissimilarity among those considered, followed by CAP (Figure 10).

In light of these results, the traditional subdivision into the groups "*Barattiere*", "*Caroselli*", and "*Spuredde*" was only partially confirmed by the analysis of the data. As stated above, only the "*Barattiere*" group was found to be fully distinct from the others, a result perfectly in line with the genetic characterisations developed in Pavan et al. [13]. Conversely, the "*Caroselli*" and "*Spuredde*" groups lack sufficiently uniform descriptors to allow them to be aggregated into single statistical groups. On the contrary, the results suggest similarities between mixed groups of "*Caroselli*" and "*Spuredde*". In the first group, this mixed aggregation is mainly determined by the high similarity between SB and CAL—with SB being aggregated with the "*Caroselli*" group. In the "*Spuredde*" group, however, it is CAM that shows similarities with SF and SN, probably due to similar seed size and exocarp colours. These results confirm how the division between "*Caroselli*" and "*Spuredde*" is not so clearly defined from a morphological-productive point of view, but rather derives from factors such as traditions, territories, and local dialects. Accogli et al. [12] emphasize that in the Salento region, "*Spuredde*" are often also called "C*arosidd"*—a dialectal name peculiar to southern Puglia, very similar to the word "*Caroselli*"—confirming, again, that the landraces of these macro-groups could be evolutions of the same landraces, adapted to different territories.

4. Conclusions

The characterization of the nine landraces of *Cucumis melo* L. of the Puglia region provided a comprehensive morphological and agronomic description. The data revealed clear differences between BT and the landraces belonging to the "*Caroselli*" and "*Spuredde*" groups across all analyzed descriptors. However, differentiating between the "*Caroselli*" and "*Spuredde*" macro-groups was more challenging, as the most significant differences were limited to fruit descriptors: CAM, CUM, and CAS fruits can be distinguished from the fruits of other landraces of "*Caroselli*" and "*Spuredde*" by their rounded shape and, specifically for CAM and CUM, the presence of patches on the exocarp. In contrast, CAP is easily identifiable by its elongated, narrow shape and a very pronounced hairiness. The "*Spuredde*" fruit type is characterized by an elliptical to elongated shape and a predominantly glabrous exocarp, whereas SN and SF are notable for their predominantly dark olive-green colouration.

The dissimilarity analysis uncovered new groupings of these landraces compared to the traditional "*Spuredde*" and "*Caroselli*" macro-groups. Notably, the analysis revealed a high degree of dissimilarity for CAP compared to the other "*Caroselli*" landraces. Conversely, the dissimilarity index calculated for SB and CAL was nearly zero, indicating a clear case of synonymy due to the geographical dispersion of the same local variety. Further analysis is warranted for the CAM, SB, and SN groups to determine whether the observed

similarities are attributable to shared genetic and/or evolutionary factors in addition to the previously identified morphological traits.

In light of these considerations, a molecular analysis of the varieties analyzed is suggested in order to better understand the differences between "*Caroselli*" and "*Spuredde*" and identify any common evolutionary lines. Similarly, a more in-depth analysis of these landraces from a qualitative and nutritional point of view could support the analysis presented.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae11040344/s1, Table S1: Morpho-physiological characterization of nine landraces of unripe melon (*Cucumis melo* L.); Table S2: Chlorophyll content, biometric, and biomass parameters of leaf of nine unripe melon (*Cucumis melo* L.) landraces; Table S3: Yield parameters of nine unripe melon (*Cucumis melo* L.) landraces; Table S4: Qualitative parameters of commercially mature fruits (immature stage) of nine unripe melon (*Cucumis melo* L.) landraces; Table S5: Colour analysis (CIELab scale) of exocarp and mesocarp of the fruits of nine unripe melon (*Cucumis melo* L.) landraces.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Supplementary Materials

Table S1. Morpho-physiological characterization of nine landraces of unripe melon (*Cucumis melo* L.). Traits that differed between landraces are highlighted in **bold** in the "Descriptor" column. The descriptors are adapted form UPOV and IPGR. Dash symbolizes not available data.

Part of Plant	Descriptor	BT	CAL	CAM	CAP	CAS	CUM	SB	SF	SN
SEEDS[1]	Shape	Pine-nut shape	Pine-nut shape	Pine-nut shape	Pine-nut shape	Pine-nut shape	Pine-nut shape	Pine-nut shape	Pine-nut shape	Pine-nut shape
	Length	Medium	Medium	Long	Medium	Short	Medium	Medium	Medium	Medium
	Width	Medium	Narrow	Large	Narrow	Narrow	Narrow	Medium	Narrow	Narrow
	Coat colour, colour intensity	Creamy-yellow	Creamy-white	Creamy-white	Creamy-white	Creamy-yellow	Creamy-white	Creamy-white	Creamy-white	Creamy-white
	Coat surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Coat pattern	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Number of seeds·fruit^{-1}	High (>100)	High (>100)	High (>100)	High (>100)	High (>100)	High (>100)	High (>100)	High (>100)	High (>100)
SEEDLINGS	Epicotyl colour	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green
	Hypocotyl colour	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green
	Hypocotyl pubescence	Present	Present	Present	Present	Present	Present	Present	Present	Present
	Cotyledon size	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
	Cotyledon bitterness	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
STEM[2]	Plant growth habit	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate
	Basal rosette	Absent	Present	Present	Present	Present	Present	Present	Present	Present
	Internode length[3]	Intermediate	Short	Short	Intermediate	Short	Short	Short	Short	Intermediate
	Primary colour	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green
	Pubescence	Pilose	Pilose	Pilose	Pilose	Pilose	Pilose	Pilose	Pilose	Pilose
	Tendrills	Simple, one each node	Simple, one each node	Simple, one each node	Simple, one each node	Simple, one each node	Simple, one each node	Simple, one each node	Simple, one each node	Simple, one each node
LEAVES	**Shape**	Entire	Intermediate	Entire	Entire	Entire	Entire	Intermediate	Intermediate	Intermediate
	Base shape	Cordate	Cordate	Cordate	Cordate	Reniform	Cordate	Cordate	Cordate	Cordate
	Development of lobes	Medium	Medium	Medium	Medium	Weak	Medium	Strong	Strong	Strong
	Length of terminal lobe	Short	Long	Short	Short	Short	Short	Long	Long	Long
	Secondary lobation	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Margin dentation	Intermediate	Weak	Weak	Weak	Very weak	Weak	Weak	Weak	Very weak
	Blade blistering	Medium	Weak	Medium	Medium	Weak	Medium	Weak	Weak	Very weak
	Leaf colour	Green	Green/Light-green	Green/Light-green	Green/Light-green	Green/Light-green	Green/Light-green	Green/Light-green	Green/Light-green	Green/Light-green

Table S1. *Cont.*

Part of Plant	Descriptor	BT	CAL	CAM	CAP	CAS	CUM	SB	SF	SN
LEAVES	Prominence of leaf vein	Present	Present	Present	Present	Present	Present	Present	Present	Present
	Colour of leaf vein	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green
	Size (leaf area; cm²)[4]	308.9	434.5	303.7	432.5	257	321.4	441.6	299.2	335.1
	Petiole length (cm)	12.3	22.0	16.7	26.0	16.8	15.0	15.3	17.3	18.3
	Petiole colour	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green
FLOWERS	Sex expression	Andromonics	Andromonics	Andromonics	Andromonics	Andromonics	Andromonics	Andromonics	Andromonics	Andromonics
	Time of male flowering	Early/Medium	Early	Early	Early/Medium	Early	Early	Early	Early	Early
	Time of female flowering	Late	Medium	Medium	Medium/Late	Medium	Medium	Medium	Medium	Medium
YOUNG FRUITS	**Basic colouration**	Light green/ Yellow-green	Light green/ Yellow-green	Dark-olive-green	Light green/ Yellow-green	Light green/ Yellow-green	Light green/ Yellow-green	Light green/ Yellow-green	Dark-olive-green	Dark-olive-green
	Conspicuousness of groove colouring	Absent	Absent	Weak	Weak/ Medium	Absent	Absent	Absent	Weak	Absent
	Intensity of groove colouring	-	-	Light	Light	-	-	-	Very light	-
	Peduncle length	Short	Short	Short	Medium	Short	Short	Short	Short	Short
	Extension of darker area around peduncle	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
FRUITS[5]	**Position of maximum diameter**	At middle	Variable	At middle (variable)	Variable	At middle (variable)	At middle (variable)	Variable	Variable	Variable
	Shape in longitudinal section	Broad elliptic, circular	Ovate, medium elliptic	Ovate, circular, obovate	Elongated	Ovate, broad elliptic, obovate	Broad elliptic circular	Medium elliptic, elongated	Medium elliptic, elongated	Medium elliptic, elongated
	Shape of base	Rounded	Rounded, pointed	Rounded	Pointed	Rounded	Rounded	Rounded	Rounded	Rounded
	Shape of apex	Rounded	Rounded	Rounded	Pointed	Rounded	Rounded	Rounded	Rounded	Rounded
	Ground colour of exocarp	Light green/ Yellow-green	Light green/ Yellow-green	Dark-olive-green	Light green/ Yellow-green	Light green/ Yellow-green	Light green/ Yellow-green	Light green/ Yellow-green	Dark-olive-green	Dark-olive-green
	Intensity of ground colour of exocarp	Light	Light	Medium	Light	Light	Light	Light	Medium	Dark
	Hue of ground colour of exocarp	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish
	Rind hairiness	Absent	Absent	Absent	Long	Very short	Absent	Absent	Absent	Absent

Table S1. *Cont.*

Part of Plant	Descriptor	BT	CAL	CAM	CAP	CAS	CUM	SB	SF	SN
FRUITS[5]	**Dots, patches, warts, cork formation**	Absent	Absent	Present	Absent	Absent	Present	Absent	Present	Absent
	Grooves	Absent	Absent	Weak	Weak/ Medium	Absent	Absent	Absent	Weak	Absent
	Depth of grooves	-	-	Shallow	Medium	-	-	-	Shallow	-
	Colour of groves	-	-	Light-green	Light-green	-	-	-	Dark-green	-
	Creasing of surface	Weak	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Main colour of mesocarp	Light green-yellow	Light green-yellow	Light green-yellow	Light green-yellow	Light green-yellow	Light green-yellow	Light green-yellow	Light green-yellow	Light green-yellow
	Secondary salmon colouring of mesocarp	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Firmness of mesocarp	Firm	Firm	Firm	Firm	Firm	Firm	Firm	Firm	Firm
	Size of pistillar scar	Variable	Variable	Variable	Variable	Variable	Variable	Variable	Variable	Variable
	Blossom end shape	Flattened	Flattened	Flattened	Flattened	Flattened	Flattened	Flattened	Flattened	Flattened

[1]Mature and dry seeds. [2]Main stem was considered for CAL, CAM, CAP, CAS, CUM, SB, SF and SN ("Caroselli" and "Spuredde"); primary side shoot was considered for BT ('Barattiere'). [3]Average internode length of the 10–15th node of the grown stem; intermediate: approximately 10 cm. [4]Average of the fully expanded true leaf (10–15th nodes of grown stem). [5]Descriptors applied to fruit at commercial maturity stage.

Table S2. Chlorophyll content, biometric, and biomass parameters of leaf of nine unripe melon (*Cucumis melo* L.) landraces: BT, 'Barattiere'; CAL, 'Carosello leccese'; CAM, 'Carosello striato tondo di Massafra'; CAP, 'Carosello di Polignano'; CAS, 'Carosello scopatizzo'; CUM, '*Cucumbr* di Martina Franca'; SB, 'Spuredda bianca'; SF, 'Spuredda fasciata'; SN, 'Spuredda nera'. FW = fresh weight; DW = dry weight; DM = dry matter.

Landraces	**Chlorophyll content**	**Leaf Area**	**Petiole length**	**Leaf FW**	**Leaf DM**	**Specific Leaf Area**
	μmol/m²	cm²	cm	g	g/100g FW	cm²/g DW
BT	318.3 ± 46.7	314.1 ± 49.6	12.33 ± 1.53	15.68 ± 1.68	20.97 ± 0.74	96.4 ± 19.3
CAL	261.1 ± 30.3	446.1 ± 39.6	22.00 ± 3.46	18.68 ± 1.68	17.09 ± 0.97	140.6 ± 16.3
CAM	273.8 ± 46.4	339.0 ± 46.8	16.67 ± 1.53	14.03 ± 3.58	15.92 ± 1.11	163.9 ± 71.3
CAP	262.4 ± 20.6	454.7 ± 91.4	26.00 ± 3.00	19.53 ± 4.79	16.14 ± 0.93	153.4 ± 61.9
CAS	254.1 ± 17.2	279.6 ± 63.5	16.83 ± 2.57	13.50 ± 3.90	16.88 ± 0.94	123.6 ± 7.8
CUM	257.4 ± 29.3	289.8 ± 48.0	15.00 ± 2.00	13.69 ± 3.81	17.31 ± 1.05	134.5 ± 62.8
SB	248.5 ± 45.4	437.1 ± 66.5	15.33 ± 1.53	19.18 ± 3.85	17.62 ± 2.58	132.4 ± 22.8
SF	246.2 ± 26.4	288.9 ± 25.2	17.33 ± 1.53	12.21 ± 0.62	16.34 ± 2.39	145.9 ± 9.0
SN	282.4 ± 33.6	348.9 ± 23.3	18.33 ± 1.15	13.41 ± 1.39	20.21 ± 3.36	130.8 ± 15.2
Significance						
BT *vs* others	***	*	***	ns	**	ns
(CAM, CAP, CAS, CAL, CUM) *vs* (SB, SF, SN)	ns	ns	*	ns	ns	ns
SB *vs* (SF and SN)	ns	***	ns	ns	ns	ns
SF *vs* SN	ns	ns	ns	ns	*	ns
(CAP and CAL) *vs* (CAM, CAS, CUM)	ns	***	***	**	ns	ns
CAP *vs* CAL	ns	ns	*	ns	ns	ns
CAS *vs* (CAM and CUM)	ns	*	ns	ns	ns	ns
CAM *vs* CUM	ns	ns	ns	ns	ns	ns

Significance of contrasts: ***, ** and * for p≤0.001, p≤0.01 and p≤0.05, respectively; ns = not significant.

Table S3. Yield parameters of nine unripe melon (*Cucumis melo* L.) landraces: BT, 'Barattiere'; CAL, 'Carosello leccese'; CAM, 'Carosello striato tondo di Massafra'; CAP, 'Carosello di Polignano'; CAS, 'Carosello scopatizzo'; CUM, '*Cucumbr* di Martina Franca' (CUM); SB, 'Spuredda bianca'; SF, 'Spuredda fasciata'; SN, 'Spuredda nera'. FW = fresh weight.

Landraces	**Fruit yield**	**Fruits number**	**Fruit FW**
	g/plant	n/plant	g/fruit
BT	2186.6 ± 636.0	8.4 ± 2.0	259.1 ± 30.0
CAL	2914.4 ± 759.3	13.4 ± 3.3	217.2 ± 16.4
CAM	2965.2 ± 612.3	11.4 ± 2.2	260.5 ± 23.5
CAP	2591.2 ± 956.3	12.8 ± 5.0	203.9 ± 10.7
CAS	3398.9 ± 1132.6	14.1 ± 5.0	244.1 ± 19.8
CUM	2442.3 ± 811.8	11.7 ± 3.3	205.8 ± 19.1
SB	3155.7 ± 1512.7	13.5 ± 4.5	226.0 ± 36.0
SF	3076.7 ± 497.3	12.3 ± 2.4	251.4 ± 17.9
SN	2735.5 ± 575.0	11.1 ± 3.0	252.2 ± 34.5
Significance			
BT *vs* others	*	*	*
(CAM, CAP, CAS, CAL, CUM) *vs* (SB, SF, SN)	ns	ns	*
SB *vs* (SF and SN)	ns	ns	*
SF *vs* SN	ns	ns	ns
(CAP and CAL) *vs* (CAM, CAS, CUM)	ns	ns	**
CAP *vs* CAL	ns	ns	ns
CAS *vs* (CAM and CUM)	ns	ns	ns
CAM *vs* CUM	ns	ns	***

Significance of contrasts: ***, ** and * for p≤0.001, p≤0.01 and p≤0.05, respectively; ns = not significant.

Table S4. Qualitative parameters of commercially mature fruits (immature stage) of nine unripe melon (*Cucumis melo* L.) landraces: BT, 'Barattiere'; CAL, 'Carosello leccese'; CAM, 'Carosello striato tondo di Massafra'; CAP, 'Carosello di Polignano'; CAS, 'Carosello scopatizzo'; CUM, '*Cucumbr* di Martina Franca'; SB, 'Spuredda bianca'; SF, 'Spuredda fasciata'; SN, 'Spuredda nera'. FW = fresh weight, DW = dry weight, DM = dry matter.

Landraces	**Fruit DW**	**Fruit DM**	**Total Soluble Solids**	**Fruit Firmness**
	g/fruit	g/100 g FW	°Brix	kg/cm^2
BT	5.00 ± 0.49	5.17 ± 1.23	3.5 ± 0.2	8.77 ± 0.84
CAL	4.01 ± 0.71	3.76 ± 0.57	2.8 ± 0.1	10.47 ± 0.18
CAM	3.39 ± 0.39	3.25 ± 0.40	3.0 ± 0.1	10.56 ± 1.13
CAP	4.04 ± 0.50	3.85 ± 0.57	2.7 ± 0.2	8.65 ± 0.63
CAS	4.51 ± 0.29	4.38 ± 0.39	3.9 ± 1.3	9.52 ± 0.99
CUM	3.77 ± 0.71	3.60 ± 0.70	2.9 ± 0.1	9.59 ± 0.72
SB	3.59 ± 0.65	3.48 ± 0.65	2.8 ± 0.2	9.73 ± 0.50
SF	4.14 ± 0.70	4.03 ± 0.78	3.4 ± 0.1	9.80 ± 0.70
SN	3.97 ± 1.01	3.79 ± 1.12	2.9 ± 0.1	10.32 ± 0.70
Significance				
BT *vs* others	***	***	*	**
(CAM, CAP, CAS, CAL, CUM) *vs* (SB SF, SN)	ns	ns	ns	ns
SB *vs* (SF and SN)	ns	ns	ns	ns
SF *vs* SN	ns	ns	ns	ns
(CAP and CAL) *vs* (CAM, CAS, CUM)	ns	ns	ns	ns
CAP *vs* CAL	ns	ns	**	***
CAS *vs* (CAM and CUM)	**	**	***	ns
CAM *vs* CUM	ns	ns	ns	*

Significance of contrasts: ***, ** and * for p≤0.001, p≤0.01 and p≤0.05, respectively; ns = not significant.

Table S5. Colour analysis (CIELab scale) of exocarp and mesocarp of the fruits of nine unripe melon (*Cucumis melo* L.) landraces: BT, 'Barattiere'; CAL, 'Carosello leccese'; CAM, 'Carosello striato tondo di Massafra'; CAP, 'Carosello di Polignano'; CAS, 'Carosello scopatizzo'; CUM, '*Cucumbr* di Martina Franca'; SB, 'Spuredda bianca'; SF, 'Spuredda fasciata'; SN, 'Spuredda nera'.

Landraces_fruit part	L*	a*	b*	h°	C
BT_mesocarp	80.84 ± 2.32	-10.51 ± 1.69	23.80 ± 2.79	113.74 ± 1.22	26.02 ± 3.21
BT_exocarp	70.24 ± 5.04	-19.97 ± 1.06	42.19 ± 2.67	115.40 ± 2.33	46.71 ± 2.15
CAL_mesocarp	88.49 ± 1.59	-9.75 ± 1.69	25.84 ± 5.39	110.86 ± 2.40	27.64 ± 5.52
CAL_exocarp	69.03 ± 6.10	-17.77 ± 1.73	34.45 ± 2.09	117.26 ± 1.61	38.78 ± 2.48
CAM_mesocarp	87.86 ± 5.05	-7.62 ± 1.07	19.66 ± 3.51	111.35 ± 2.20	21.10 ± 3.58
CAM_exocarp	45.01 ± 3.11	-13.45 ± 1.18	20.00 ± 2.04	123.97 ± 0.89	24.10 ± 2.33
CAP_mesocarp	83.19 ± 1.48	-12.92 ± 1.42	30.90 ± 3.97	112.72 ± 1.01	33.49 ± 4.17
CAP_exocarp	71.96 ± 1.27	-17.79 ± 0.64	36.69 ± 1.27	115.87 ± 0.64	40.77 ± 1.35
CAS_mesocarp	86.70 ± 3.74	-10.18 ± 0.46	23.56 ± 1.81	113.40 ± 1.04	25.67 ± 1.81
CAS_exocarp	67.96 ± 2.72	-20.40 ± 0.43	39.50 ± 1.28	117.33 ± 0.72	44.46 ± 1.23
CUM_mesocarp	84.18 ± 2.90	-10.38 ± 1.97	26.56 ± 3.64	111.28 ± 2.17	28.54 ± 4.0
CUM_exocarp	64.82 ± 11.34	-15.99 ± 1.70	29.94 ± 6.84	118.84 ± 5.09	34.03 ± 6.56
SB_mesocarp	88.32 ± 1.82	-9.69 ± 1.57	23.62 ± 3.42	112.44 ± 3.03	25.57 ± 3.48
SB_exocarp	70.10 ± 5.83	-18.30 ± 1.87	35.45 ± 4.15	117.35 ± 1.27	39.90 ± 4.15
SF_mesocarp	87.87 ± 2.95	-8.12 ± 3.01	19.95 ± 4.93	111.45 ± 3.80	21.57 ± 5.65
SF_exocarp	50.19 ± 3.47	-16.36 ± 1.33	25.68 ± 3.08	122.63 ± 2.29	30.47 ± 3.08
SN_mesocarp	87.63 ± 2.19	-7.24 ± 0.58	20.29 ± 0.46	109.63 ± 1.69	21.55 ± 0.37
SN_exocarp	44.01 ± 3.28	-15.12 ± 1.70	21.15 ± 3.22	125.67 ± 1.28	26.00 ± 3.22
Significance					
Fruit part	***	***	***	***	***
BT *vs* others	ns	**	***	*	***
(CAM, CAP, CAS, CAL, CUM) *vs* (SB, SF, SN)	***	**	***	**	***
SB *vs* (SF and SN)	***	**	***	**	***
SF *vs* SN	ns	ns	ns	ns	ns
(CAP and CAL) *vs* (CAM, CAS, CUM)	***	**	***	**	***
CAP *vs* CAL	ns	ns	*	ns	*
CAS *vs* (CAM and CUM)	***	***	***	ns	***
CAM *vs* CUM	***	**	***	**	***
Fruit part × (BT vs others)	***	ns	***	***	***
Fruit part × [(CAM, CAP, CAS, CAL, CUM) *vs* (SB, SF, SN)]	***	ns	ns	***	ns
Fruit part × [SB *vs* (SF and SN)]	***	ns	**	***	**
Fruit part × (SF *vs* SN)	ns	ns	ns	ns	ns
Fruit part × (CAP and CAL) *vs* (CAM, CAS, CUM)	***	ns	ns	ns	ns
Fruit part × (CAP *vs* CAL)	**	ns	ns	ns	ns
Fruit part × [CAS *vs* (CAM and CUM)]	**	***	***	**	***
Fruit part × (CAM *vs* CUM)	***	ns	ns	**	ns

Significance of contrasts: ***, ** and * for p≤0.001, p≤0.01 and p≤0.05, respectively; ns = not significant.

Chapter 2

First Report on the Occurrence of Cucurbitacins in an Italian Melon Landrace (*Cucumis melo* L.)

horticulturae

MDPI

Communication

First Report on the Occurrence of Cucurbitacins in an Italian Melon Landrace (*Cucumis melo* L.)

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Abstract: 'Scopatizzo', belonging to the *Cucumis melo* L., is a local variety of Apulia (Southern Italy), which is consumed as unripe melon as an alternative of cucumber due to its better-quality profile and for the absence of cucurbitacins. The latter are tetracyclic triterpenes synthesized by some Cucurbitaceae species, known to confer an unpleasant taste to fruits and cause health problems. Following the discovery of 'Scopatizzo' fruits with bitter taste, cucurbitacins were searched for in their ethanolic extract. Flow injection analysis with detection performed by atmospheric pressure chemical ionization-high resolution mass spectrometry provided evidence for the presence of at least four cucurbitacins, which were absent in typical, sweet-tasting fruits. Further insight into this discovery will be required in the near future to assess if the detection of cucurbitacins may mark the appearance of genotypes whose fruits have features not compatible with commercialization.

Keywords: bitterness; (–)APCI/FTMS; cucurbitacins; Mediterranean biodiversity; melon landraces; Scopatizzo

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1. Introduction

Cucurbitacins constitute a group of plant secondary metabolites corresponding to tetracyclic triterpenoids found in various species of the Cucurbitaceae family. They are known for their bitterness and toxicity, and their presence in several species of vegetables is generally considered undesirable firstly for their unpleasant taste [1].

The consumption of food products containing high amounts of cucurbitacins can lead to gastrointestinal distress and other health issues [1]. For this reason, commercial Cucurbitaceae varieties are carefully bred to minimize cucurbitacin content [2]. Despite their toxicity, cucurbitacins have also been studied for their potential medicinal properties: some studies suggested that certain cucurbitacins may have anticancer and anti-inflammatory properties, but their use in this context is still in the early stages of research [3].

The Cucurbitaceae family stands out as one of the most genetically diverse plant families globally. Among its members, there are numerous significant vegetables and medicinal plants with a wide global distribution. The swift progress in sequencing technologies and bioinformatics algorithms has empowered the creation of genome sequences for many crucial Cucurbitaceae species [4].

This advancement has significantly eased research in areas like gene discovery, genome evolution, genetic diversity, and the molecular breeding of cucurbit crops. So far, genome sequences of 18 different cucurbit species belonging to tribes Benincaseae, Cucurbiteae, Sicyoeae, Momordiceae, and Siraitieae have been deciphered [4]. Understanding the genome information plays a vital role in aiding breeders to improve the quality of Cucurbitaceae vegetables through the application of biotechnological methods.

In this regards, nonconventional breeding approaches can complement conventional breeding methods leading to savings in time, expenses, and improving selection efficiency [5]. Variability in breeding techniques and approaches is essential in both methods to develop new and improved Cucurbitaceae varieties with enhanced nutritional profiles [5]. Breeders have focused on developing Cucurbitaceae varieties with low or negligible cucurbitacin levels [4]. These efforts aim to remove the bitterness and toxicity associated with cucurbitacins. Research has been conducted to understand the genetic basis of cucurbitacin content in Cucurbitaceae fruits [2]. Genes associated with cucurbitacin pro- duction have been identified, and efforts have been made to develop markers for selective breeding [2]. Apulia region (Southern Italy) is an important secondary centre of diversity for *C. melo* L. [6]. Several landraces of this species are still grown there including the so-called unripe melons, such as 'Barattiere', 'Carosello', 'Scopatizzo' (Figure 1), etc. [7–10].

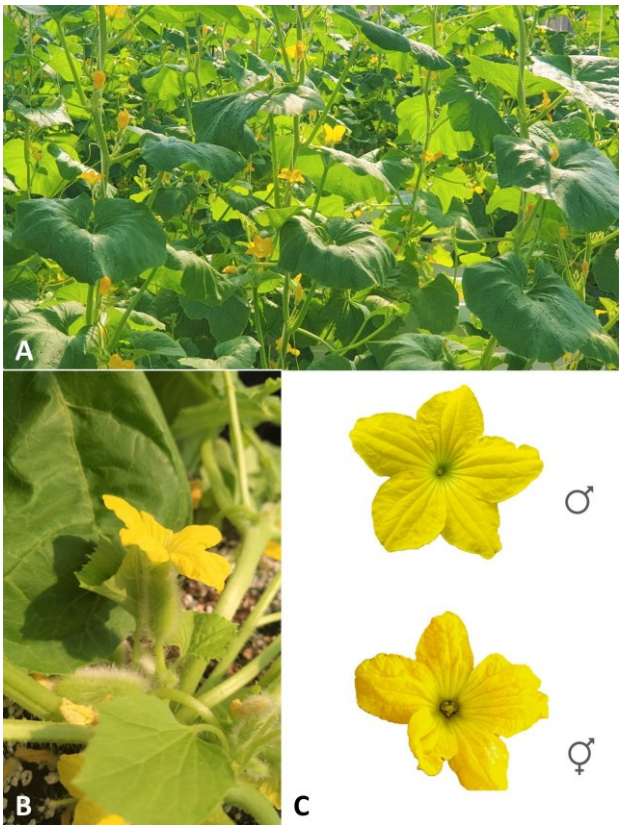

Figure 1. Plants of 'Scopatizzo' with main stem vertically trained at full flowering stage (**A**). Side view of hermaphrodite flower of 'Scopatizzo' (**B**). Top view of male (top) and hermaphrodite (bottom) flower of 'Scopatizzo' (**C**).

'Scopatizzo' is characterized by its relatively small-sized fruits, which have a light and sparse fuzziness. They are light green in colour, have an elliptical shape and an oval-shaped longitudinal section (Figure 2) [10,11]. At the commercial harvest, the 'Scopatizzo' fruits do not have well-formed seeds. In fact, in addition to the flesh (mesocarp), the central part (placenta) of the fruit can be eaten like other genotypes of Apulian unripe melons [9–11]. The leaves are of medium size and have a pentalobed shape with slight leaf margin serration. The flowers, typically yellow in colour for the species, exhibit an andromonoecious-male expression, with a predominance of male flowers [10,11]. Each 'Scopatizzo' plant can produce more than 10 fruits with an average yield of around 2,600 g plant^{-1} [10].

Figure 2. Fruits of 'Scopatizzo' melon.

Although unripe melons, including 'Scopatizzo', are taxonomically *C. melo*, their fruits are harvested at the immature stage to be consumed fresh and raw, in salads or without dressings and are appreciated as an alternative to cucumber (*C. sativus*) due to their better- quality profile and for the absence of cucurbitacins [8,11]. Effectively, to our best knowledge, the literature lacks evidence with regards to cucurbitacins presence in these Apulian landraces of *C. melo*. Nevertheless, in the summer of 2022, the presence of bitter tasting 'Scopatizzo' fruits (Figure 2) was recognized during a genotype comparison trial in an experimental farm, thus suggesting the first-time presence of cucurbitacins in an Apulian landrace of *C. melo*. Notably, other warnings about bitter tasting 'Scopatizzo' fruits were received from local markets in 2022.

To further support the occurrence of cucurbitacins in bitter fruits, flow-injection analysis (FIA) with detection based on high-resolution Fourier Transform mass spectrometry with Atmospheric Pressure Chemical Ionization (APCI-FTMS) was exploited in this study for the characterization of the moderately

polar fraction of the metabolome of sweet and bitter 'Scopatizzo' fruits. As a result, the main difference observed between the corresponding APCI-FTMS spectra could be tentatively recognized as the remarkably higher content of cucurbitacin molecules in bitter-tasting fruits. The accurate *m*/*z* values were exploited for the tentative identification of cucurbitacins, and data were compared with those reported in the literature on cucurbitacins in *C. melo* fruits.

2. Materials and Methods

2.1 Cropping Details

The experimental trial from which the first bitter 'Scopatizzo' fruits emerged was carried out from May to July 2022 at the Experimental farm "La Noria" of the Institute of Sciences of Food Production, National Research Council [Mola di Bari, Italy (41.062156° N, 17.066914° E)], in open field conditions. Nine local varieties of *C. melo* L. were cultivated including 'Scopatizzo'. Seedlings were purchased from an Apulian plant nursery, and they were transplanted at a plant density of 2.5 plants·m^{-2}, with a plant distance on the raw and between the raw respectively of 0.40 m and 1 m.

Plants were daily fertigated according to "Specification for integrated production of field cucumber, Apulia Region, 2022", with a micro-irrigation system, to optimize water consumption. Plant stems were trained vertically to optimize leaves light interception, air flow through the canopy and to avoid fungal proliferation on leaves. Pollination was guaranteed by introduction of bumblebees (*Bombus terrestris* L.). Starting from 30 days after transplant (DAT) the fruits were harvested twice a week, at unripe stage, when the grooves on the epicarp of the fruits were barely noticeable and the seeds were barely outlined, and the placenta was not divided from the mesocarp.

2.2 Materials

LC-MS grade acetonitrile and water used for FIA, LC-MS grade formic acid used as the additive for the FIA carrier fluid, and ACS reagent grade ethanol used for the extraction of cucurbitacins were all purchased from Sigma Aldrich (Merck, Milan, Italy).

2.3 Sample Preparation

Once harvested, the 'Scopatizzo' unripe melons were subjected to an organoleptic essay to separate bitter from sweet-tasting fruits. Thereafter, the plant material was stored at −20 °C for 24 h prior to the freeze-drying process to occur in a ScanVac CoolSafe 55-9 Pro freeze dryer (LaboGene ApS, Lynge, Denmark).

2.4 Extraction of Cucurbitacins from Lyophilized Plant Material

A slightly modified version of the protocol proposed by Chen et al. [12] was adopted for the extraction of cucurbitacins. Here, the lyophilized plant material was powdered in a ceramic mortar. The resulting powder was suspended into an ethanol/water 95:5 *v*/*v* mixture to a nominal concentration of 100 mg/mL. The suspension was subjected to ultrasound-assisted extraction in a DU-32 ultrasonic bath (Argo Lab, Carpi, Italy), operating at 40 kHz at maximum power (120

W). The liquid phase was clarified after centrifugation (4500 rpm) for 10 min. Once withdrawn, the supernatant was filtered using 0.2 μm nylon filters and diluted to a suitable volume by a 1:10 factor in pure ethanol prior to the FIA- APCI-FTMS analysis.

2.5 Instrumentation and Operating Conditions for FIA-APCI-FTMS Analysis

FIA-APCI(−)-FTMS analysis was performed using a LC-MS platform implementing an Ultimate 3000 HPLC quaternary chromatographic system and a Q Exactive high-resolution quadrupole-Orbitrap mass spectrometer (Thermo Fisher, West Palm Beach, CA, USA). Here, the chromatographic pump module was set to deliver the carrier phase at a 150 μL/min flow rate. The carrier was obtained by mixing acetonitrile (60% *v*/*v*) and water (40% *v*/*v*), both containing formic acid (0.1% *v*/*v*). The diluted ethanolic plant extract (see Section 2.4) was introduced into the flowing carrier phase at a 5 μL/min flow by using a Fusion 100T syringe pump (Chemyx, Stafford, TX, USA). A T-valve was used for the hydraulic connections. The resulting mixture was transferred into the Atmospheric Pressure Chemical Ionization (APCI) interface (Thermo Fisher, West Palm Beach, CA, USA) mounted on the Q Exactive mass spectrometer.

The operating parameters of the APCI interface and of the ion optics of the Q-Exactive spectrometer were set as follows:

- sheath gas flow rate: 40 a.u.;
- auxiliary gas flow rate: 20 a.u.;
- discharge current: 5 μA;
- capillary temperature: 320 °C;
- S-lens RF level: 55%;
- vaporizer temperature: 300 °C.

The mass spectrometer was calibrated on alternate days by infusing, at a flow rate of 5 μL/min, calibration solutions provided by the instrument manufacturer for negative polarity acquisitions. As a result, a mass accuracy always better than 5 ppm was achieved.

The orbitrap mass analyser was operated at its maximum resolving power (140,000 at *m*/*z* 200) for full scan MS experiments. FTMS spectra were acquired in a 300–800 *m*/*z* interval. Here, the Automatic Gain Control (AGC) level was set to 1×10^6, with a maximum ion injection time of 100 ms. The total ion current (TIC) signal was constantly monitored and the FTMS spectra acquisition started when the TIC variation was stable below the 20% threshold.

3. Results

The presence of cucurbitacins is notoriously responsible for the unpleasant bitter taste of fruits produced by cucurbitaceous plants [1,13,14]. Hence, a rapid screening method based on FIA-APCI-FTMS was adopted to assess the presence of cucurbitacins in unexpectedly bitter 'Scopatizzo' melons. Cucurbitacins, along with their glycosylated forms, can be detected as negatively charged adduct ions when both APCI and electrospray ionization (ESI) ion

sources are employed [12,13]. Both formic acid and trifluoroacetic acid have been commonly used as adjuvants for the ionization process [14–16]. Notably, when compared to ESI, the use of APCI results in a more sensitive detection of cucurbitacins, although a more extensive in-source fragmentation of the analyte ion is expected [15].

Figure 3 shows a comparison between the APCI(−)-FTMS spectra acquired for the ethanolic extracts of sweet (plot A) and bitter-tasting (plot B) 'Scopatizzo' melons. Apart from the different intensity ratio of the dominating MS signals (i.e., those detected at nominal *m*/*z* 341 and *m*/*z* 387), the main discrepancy between the two FTMS spectra was represented by the presence of peaks at *m*/*z* 561.3095 and 603.3205. The latter were detected only in the case of bitter fruits. As reported in Table 1, these accurate *m*/*z* values are consistent, respectively, with the theoretical *m*/*z* values of $[M–H+HCOOH]^-$ adduct ions of cucurbitacins D and B (see structures reported in Figure 4), and, hypothetically, also of the respective isomeric forms reported so far in the literature, like cucurbitacin L and iso-cucurbitacin D, in the first case, and isocucurbitacin B and 23,24-dihydrocucurbitacin E in the second one.

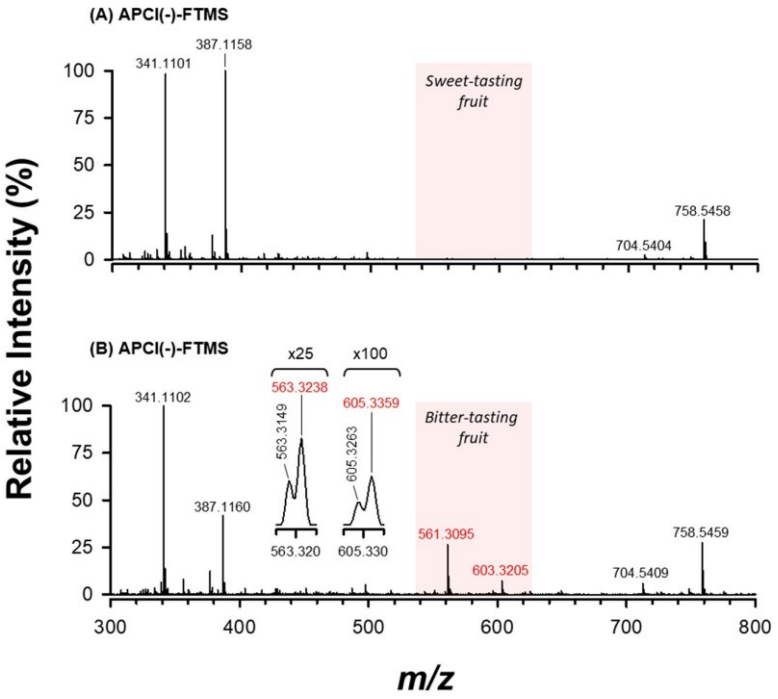

Figure 3. APCI(−)-FTMS spectra of the ethanolic extracts of sweet (**A**) and bitter-tasting (**B**) 'Scopatizzo' melons (see Section 2.3 and 2.4 for additional information on sample preparation). The red-shaded *m*/*z* interval refers to the spectral region containing MS signals that discriminate the two samples due to the presence of cucurbitacins in bitter fruits. Highlighted in red are the accurate *m*/*z* values used for the tentative identification of cucurbitacins (see Table 1).

Table 1. Cucurbitacins tentatively identified in bitter 'Scopatizzo' melons. The identification was based on accurate m/z values (see the main text for details).

Identified Cucurbitacins	Theoretical m/z of the $[M-H+HCOOH]^-$ Adduct Ion	Experimental m/z	Mass Error (ppm)
Cucurbitacin D Iso-cucurbitacin D Cucurbitacin L	561.3069	561.3095	4.6
Cucurbitacin R Cucurbitacin F Cucurbitacin O	563.3226	563.3238	2.1
Cucurbitacin B Iso Cucurbitacin B 23,24-dihydrocucurbitacin E	603.3175	603.3205	5.0
Cucurbitacin C 23,24-dihydrocucurbitacin B Cucurbitacin Q	605.3331	605.3359	4.6

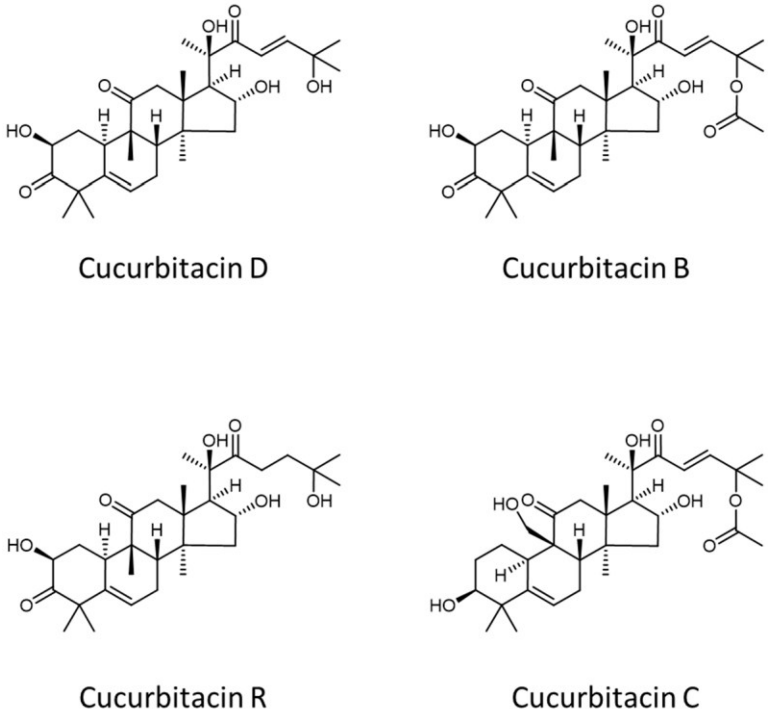

Figure 4. Molecular structures of some of the cucurbitacins whose occurrence was tentatively assessed in the ethanolic extract of a bitter-tasting 'Scopatizzo' melon.

As shown in the insets of Figure 3B, a closer inspection of the isotope pattern of the two ions revealed the presence of two further signals, at m/z 563.3238 and 605.3359, that could be tentatively ascribed to $[M-H+HCOOH]^-$ ions of cucurbitacins R and C (see Figure 4), respectively, and their respective isomers (see Table 1 for details).

Notably, cucurbitacin D differs from the R one for the presence of an additional C=C bond in the side chain linked to the tetracyclic backbone (see Figure 4), thus its nominal molecular mass is 2 Da lower. As it can be inferred from Figure 4, this is the same difference observed between the molecular masses of

cucurbitacins B and C. As a result, the M + 2 isotopic peaks of cucurbitacins D and B ions are expected to exhibit the same nominal *m*/*z* values than those of the monoisotopic (M + 0) peaks of cucurbitacins R and C, respectively. However, the available mass resolution was sufficiently high to observe the partial separation between those couples of peaks in mass spectra, thus enabling the distinct recognition of the corresponding compounds.

4. Discussion

In the present report we described for the first time the detection of cucurbitacins in an Apulian landrace of C. melo, an expression of the Mediterranean agrobiodiversity. 'Scopatizzo' is one of the several landraces of unripe Apulian melon that is establishing itself on the market for its peculiar quality traits, high fruitfulness, and good income for farmers. Moreover, it is listed as an item in the 'List of Traditional Agri-Food Products' of the Italian Department for Agriculture, since its processing, preservation and ageing methods are consolidated in time, harmonious for all the region involved, according to traditional rules, for a period not less than 25 years [11,17]. The discovery of 'Scopatizzo' fruits with a bitter taste represents a negative point even in the case of a low percentage of fruits with this characteristic, because consumers could associate this sporadic feature with a distinctive trait of the 'Scopatizzo', translating in a setback of its market rise.

Results of the present work show that bitter tasting 'Scopatizzo' fruits discovered during the genotypes comparison trial contained different types of cucurbitacins. As discussed in Section 3, FIA-APCI-FTMS analysis allowed the clear distinction between the ethanolic extracts of sweet and bitter 'Scopatizzo' melons. Indeed, four monoisotopic peaks, detected at m/z 561.3095, 563.3238, 603.3205, 605.3359, were considered as diagnostic for bitter-tasting fruits (see Figure 3). The corresponding accurate m/z values were compared with the theoretical m/z values calculated for the [M−H+HCOOH]− adduct ions of the main cucurbitacins reviewed by Cai et al. [18]. Here, only those matches showing a mass error lower than 5 ppm were considered to be reliable, as summarized in Table 1. Three isomeric species were detected among those reported by Cai et al. [18] for each of the matching theoretical m/z values. Notably, the presence of cucurbitacin D and B was previously reported in C. melo plants. Cucurbitacin B was found to be prevailing in the radicles and the cotyledons of the muskmelon vine [1].

The fruits of cultivated Cucurbitaceae have been selectively bred to eliminate the presence of cucurbitacins. It is generally believed that they possess a suppressor gene or a mutation responsible for the absence of cucurbitacins. However, back mutations can occur randomly, potentially resulting in plants with fruits containing cucurbitacins [1]. For examples, back-mutated watermelons and squash can produce between 930 and 3100 mg cucurbitacin E per kg fresh fruit and the offspring of such plants may also produce cucurbitacins [1]. Therefore, it is possible that even in the case of 'Scopatizzo', mutation

phenomena have occurred in some plants. However, it must be considered that 'Scopatizzo' is a local variety, that is, a crop propagated through self-production of seeds by farmers without any organized program of genetic improvement. Therefore, it is also possible that cross-pollination phenomena have occurred between 'Scopatizzo' plants and other wild species belonging to the Cucurbitaceae family. Indeed, within the 'Scopatizzo' cultivation region, squirting cucumber (*Ecballium elaterium* L.) plants are abundantly distributed. This particular wild species is renowned for its elevated levels of cucurbitacins [19].

A more extensive analytical investigation based on high-performance liquid chromatography coupled with high-resolution/multi-stage mass spectrometry will be per- formed in a future work to assess the effective presence of isomeric species, along with a more confident identification of cucurbitacins occurring in the ethanolic extracts of bitter 'Scopatizzo' melons.

5. Conclusions

Evidence for the occurrence of Cucurbitacins D, B, R, and C and/or their isomeric forms in a bitter-tasting fruit of 'Scopatizzo' melon, a landrace of *C. melo* typical of the Apulia region in Southern Italy, was obtained using flow-injection analysis with detection based on atmospheric pressure chemical ionization-Fourier transform mass spectrometry.

To the best of our knowledge, this is the first report of the occurrence of such compounds, known for their potential toxicity, along with the related unpleasant organoleptic features, in a fruit of a *C. melo* Apulian landrace. An extended study will be performed in the near future on fruits harvested from *C. melo* plants grown in Puglia, to assess if the detection of cucurbitacins described in the present work may mark the appearance of genotypes whose fruits have features clearly not compatible with commercialization. Future research will also be conducted to identify genes associated with cucurbitacin production in 'Scopatizzo' fruits and develop markers for selective breeding, considering the possibility of back-mutations as well as cross-pollination between different genotypes of the Cucurbitaceae family.

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Chapter 3

The Conservation Varieties Regime: Its Past, Present and Future in the Protection and Commercialisation of Vegetable Landraces in Europe

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Review

The Conservation Varieties Regime: Its Past, Present and Future in the Protection and Commercialisation of Vegetable Landraces in Europe

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Abstract: In 1998, the European Union aimed to make the official Common European Catalogue of Plant Varieties more flexible and to mitigate the decline of agricultural biodiversity in European rural areas by introducing the Conservation Varieties Regime (CVR): a set of rules pertaining to local and/or traditional varieties cultivated in specific regions that are at risk of genetic erosion. This initiative was intended to permit the sale of those varieties that do not fully meet the distinctness, uniformity and stability (DUS) criteria required for the registration of standard varieties in the Common European Catalogue. In this review, we examine the impact of establishing the CVR 25 years after its first definition. As of the date of data collection, 191 conservation varieties were registered throughout Europe, representing only 0.88% of the total number of varieties included in the Common Catalogue. The most important countries are Spain, Italy and Croatia, which have, respectively, 57, 43 and 26 conservation varieties. The case study from Italy highlights that the CVR is poorly structured and is characterised by the initiatives of individual entities that take it upon themselves to protect and/or promote specific vegetable varieties. In this review, we discuss such data in relation to the protection and commercialisation of vegetable landraces in Europe. Overall, the CVR has failed to promote and enforce a dedicated market for all those varieties excluded from registration in the Common Catalogue due to DUS requirements.

Keywords: seed legislation; Common European Catalogue; conservation varieties; landraces; vegetable species

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1. Introduction

For centuries, the breeding work that farmers undertook resulted in numerous, valuable crops and landraces that were useful for agriculture and human nutrition. Such work favoured the development of an increasingly rich system of agricultural biodiversity, locally adapted and closely linked to socio-environmental conditions [1]. Since the second half of the last century, this system has been increasingly challenged by intensive farming practices and the use of varieties—so-called 'modern' varieties, e.g., F1 hybrids—specifically selected for their high productivity [2–4]. On the one hand, this has caused the abandonment and—in many cases—the loss of numerous varieties that had resulted from farmers' centuries-old selection work. On the other hand, it has resulted in an informal division of the agricultural world into two models: (i) the peasant system, which is mainly based on the adaptation and selection of varieties that evolve and change over time and in which genetic diversity is still managed by farmers and (ii) the industrialised system, in which farmers are clients of seed companies, which create new stable and uniform varieties that require the use of chemical inputs, irrigation and mechanisation [5–8].

The need to counter the erosion of Plant Genetic Resources for Food and Agriculture (PGRFA), protect farmers' rights and promote farmers' breeding efforts since farmers' varieties are a useful resource for breeding in the formal seed system as well has led, over the years, to the creation of numerous international conventions and treaties. These include, for example, the Convention on Biological Biodiversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Such agreements define the rules for Access to Biodiversity and Sharing the Benefits (ABS) arising from their use, recognising an important role for farmers in the conservation, sustainable use and improvement of genetic resources [9–11].

In this international landscape, one of the most influential tools in terms of diversity management and conservation is seed-marketing regulations. In fact, such regulations—i.e., the set of laws and acts that regulate the commercial production and marketing of seeds and/or, in general, propagation materials—have a major impact on agricultural biodiversity since they impact breeding policies and programmes and, therefore, affect what farmers will cultivate or abandon [8,11,12]. In Europe, the two pillars of this legislation are registration and certification. This means that a variety must be registered in a catalogue for its seeds to be marketed and that the seeds should be certified by public authorities. The main criteria for registration are distinctness, uniformity and stability (DUS)—the three fundamental characteristics without which it is impossible to register varieties in the Common European Catalogue (CC) [13]. The adoption of these requirements has led to two different seed systems: a formal one promoted by current seed legislation and based on modern varieties resulting from specific breeding programmes conducted by public or private breeders and an informal one characterised by diversified varieties that are formally called "farmers' varieties". This term includes not only landraces but also all those new varieties resulting from farmers' breeding activities or from participatory programmes [7,14–16]. Indeed, the adoption of DUS has proven to be a real barrier to the marketing of many landraces because they are by definition "variable populations, however well identifiable, characterised by a specific adaptation to the environmental and cultivation conditions of a specific territory" [8,17–19].

To allow the marketing of these excluded varieties, the European Union introduced two exceptions to the existing seed regulations with Directive 95/98/EC: (i) varieties with no intrinsic value for commercial crop production, only for vegetables (modified as "Varieties Developed for growing under Particular Conditions" in successive Directives—VDPC) and (ii) conservation varieties, which include both agricultural species and vegetables. After ten years of negotiations, EU Directive 95/98/EC was implemented for the latter category via a dedicated directive on agricultural species (2008/62/EC), one on vegetable species (2009/145/EC), and one on mixture and fodder species (2010/60/EU). The complete definitions of conservation varieties and VDPC are presented in Table A1 [20].

For the CVR, three key elements can be identified in the definition that distinguish the varieties covered by this exception: (i) agricultural landraces and varieties with some degree of diversity, (ii) region of origin and (iii) risk of genetic erosion [17,21].

The concept of region of origin was implemented differently in different Member States. For some countries, such as Austria, the reference region may include the entire nation; for others, such as Belgium, it is not unusual to find very restricted areas of origin. In some particular cases, such as for the Italian conservation variety wheat 'Frassineto' and 'Sieve' (*Triticum aestivum* L. subsp. *aestivum*), it was also observed that the area of origin was modified after the registration of those varieties in the CVR [22]. Another critical point that emerged during implementation was the definition of the risk of genetic erosion [23], which was subject to different readings depending on the authority responsible for the evaluation of the application [21]. In Italy, for example, the Italian National Plan on Biodiversity of Agricultural Interest dedicated a chapter to the different interpretations at a regional level [17,18].

In addition to the critical issues just listed, some authors have pointed out that the definition of conservation varieties and the requirements for their registration are quite "static" and do not take into account the evolution of these resources in farmers' fields to adapt to specific environments [22,24]. Furthermore, this definition cannot include "new population varieties" or "new farmers' varieties" bred by farmers or farming communities which, instead, could be useful to market as seeds [25].

Therefore, although the CVR allowed the marketing of some varieties beyond the commercial market, it has also presented critical issues and limitations, which also include the number of registered varieties, especially in the case of vegetable species [26–28].

In this regard, the Proposal for Regulation 2023/0227 (COD) of the production and marketing of Plant Reproductive Material (PRM) in the European Union, which was promulgated in July 2023 by the European Commission and updated by subsequent Legislative Resolution of the European Parliament, which was adopted on 24 April 2024, is significant. Its aim is to rethink the European Union's PRM legislation in light of the European Green Deal policies and related strategies: the Farm to Fork Strategy, the Biodiversity Strategy, and the EU Strategy on adaptation to climate change [27,29]. The Proposal identified CVR as "important for the conservation and sustainable use of plant genetic resources" [27]; for these reasons, the Proposal places a lot of emphasis on improving the CVR and proposes including new varieties bred for local conditions.

Given the objective of implementing the tools for the protection of agrobiodiversity defined by the European Union and the importance of the European Commission to CVR in this sense, the aim of this work is to analyse the impact of CVR 25 years after its creation. In particular, (i) an analysis of the vegetable varieties registered in CC was conducted, starting with an overview at the European level; (ii) a statistical and regulatory analysis was carried out in order to identify the strengths, weaknesses and applicability of the CVR with regard to its purpose of conserving vegetable landraces; (iii) Italy was considered as a case study in order to better analyse the effects of the CVR nationally; (iv) the reference legislation, including the European Commission's 2023 Proposal and its updates, was analysed to identify its limitations and advantages and better understand the current CVR and its possible future evolutions.

2. Methodology

The European Commission has a public database called the "Commission's Common Catalogue Information system where, as a public user (e.g., breeder, maintainer, farmer, title holder or citizen), it is possible to consult and read the list of agricultural plant and vegetable species that are registered in the CC and that can, therefore, be marketed throughout the EU [30]. Varieties are registered in the database after a technical examination by EU Member States and notification of the Commission. The database was consulted on 15 June 2023. The list of agricultural and vegetable varieties was extracted, including all information offered by the database. The following information was considered for data analysis: country, register subtype, UPOV species, denomination, variety status, registration date, end date, registration under the CVR, rootstock name and hybrid name. Only the plant species ("vegetables") data of registered varieties ("registered") were considered in the analysis performed, as dismissed varieties ("surrendered") were discarded. In particular, the varieties entered in the CC—also including varieties registered under the CVR—were analysed by connecting them first to the species they belonged to and second to the EU country of registration. Considering country and species as two different subsets of data, the data obtained for CC were finally compared with those obtained for the CVR.

To complete the analysis, a comparison was made between the varieties included in the CC or registered under the CVR in 2018—as derived from the 37th edition of the CC and presented in previous work [31]—and the dataset used for this article. To develop this comparison, the 2018 data had to be reworked because, in this edition, varieties were organised by common name and not by scientific name, contrary to their organisation in the 2023 dataset. This difference required an adaptation of the data to allow their analysis; in particular, the varieties that were identified as "Curly kale" in 2018 were subdivided into the two species *Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L. and *Brassica oleracea* L. var. *palmifolia* DC in 2023. All the corrections are shown in Table S1.

After this first set of analyses had been carried out, the Italian case was considered in detail. For this analysis, the Italian CVR data from the reference dataset included in the Commission's Common Catalogue Information system were considered. These data are slightly different from the data included in the Italian database of the Ministry of Agri- culture, Food Sovereignty and Forestry (MASAF), which is responsible for the list. More specifically, the CC does not include—as of 15 June 2023—the following varieties, which are instead listed in the Italian register under the CVR: 'Borlotto di Gambolò' (*Phaseolus vulgaris* L.), 'Fiaschello battipagliese' (*Solanum lycopersicum* L.) and 'Rossina di Pescia' (*Lactuca sativa* L.). On the other hand, 'Riccio di Parma' (*Solanum lycopersicum* L.) is not listed within the CVR in the Italian Register, contrary to its listing status in the CC.

Furthermore, the Italian production data were analysed. In that context, in the absence of a dataset showing the production (expressed in hectares) of Italian conservation varieties, in order to evaluate the potential application of the CVR for commercialization of Italian vegetable landraces, it was decided that the maximum number of cultivable hectares allotted to each conservation variety—derived from Annex X of Legislative Decree 20/2021 [32]—would be compared with the number of hectares allotted to the same species in the reference area. To do this, the public-access database of the Italian National Institute of Statistics (ISTAT) was

consulted, considering the 2022 production data for open-field vegetable species in Italy as a whole and by region. These data are collected by ISTAT following an estimation-type methodology. Estimates are made based on evaluations by local experts in the field who are located throughout the area, and the crops surveyed are different for each month and consider the phenological stage of cultivation. For this reason, when using ISTAT data, it is always preferable to use data from the year prior to the research. These data—the maximum number of cultivable hectares per conservation variety and the hectares cultivated in each Italian region and in all of Italy—were compared, approximating the region of origin of each conservation variety to the corresponding regional territory as there were no specific cultivation data for each province and/or municipality associated with the specific conservation variety registered.

3. Results

3.1. The European CC and CVR Scenario

As of the date of data collection, 21,593 varieties were registered in the CC, representing 66 vegetable species and 25 European countries. Concerning the CVR, 191 varieties were registered throughout Europe, representing 31 vegetable species and 18 European countries. The conservation varieties represent only 0.88% of the total number of the varieties included in the CC (Figure 1).

Considering the EU27 Countries, the only European Countries in the CC that did not have any registered varieties were Finland, Ireland and Malta; in contrast, Norway—not a member of the EU27 Countries but belonging to the European Free Trade Association (EFTA)—had 17 varieties registered in the CC. In terms of the CVR, the countries just listed were joined by Austria, Cyprus, the Czech Republic, Denmark, Luxembourg, Poland and Slovakia in the list of EU27 Countries with no registered varieties.

Regarding the varieties recorded in the CC according to the 2018 data [31], the most- represent species was tomato (*Solanum lycopersicum* L.), for which 4202 varieties have been registered (19.46% of the varieties registered in the CC), followed by pepper (*Capsicum annuum* L.), with 2517 varieties (11.66%), and lettuce (*Lactuca sativa* L.), with 2243 varieties (10.39%). Compared to the 2018 data, the species for which the greatest increase in absolute terms was recorded was *S. lycopersicum*, with 527 new varieties registered (+14.34% since 2018), *C. annuum* (301, +13.58%) and bean (*Phaseolus vulgaris* L.) (97, +8.61%). In percentage terms, there were increases in less-represented species: the number of gourd (*Cucurbita maxima* Duchesne) varieties increased from 75 to 130, for an increase of 73.33%; that of curly kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L.) varieties increased from 38 to 61—also considering the varieties that, after 2018, were reclassified as *Brassica oleracea* L. var. *palmifolia* DC.—for a percentage increase of 60.53%; and that of black radish (*Raphanus sativus* L. var. *niger* (Mill.) S. Kerner) varieties increased from 61 to 93 (+52.46%). However, no species were reported for which there were significant reductions in the number of varieties registered in the CC. Overall, in 2023, 17 out of 66 vegetable species accounted for more than 80% of the varieties registered in the CC—exactly 84.42%—with the remaining varieties representing only 15.56% of the varieties registered in the CC (Figure 2). Table S1—available in the Supplementary Materials—shows the varieties registered

in the CC by species.

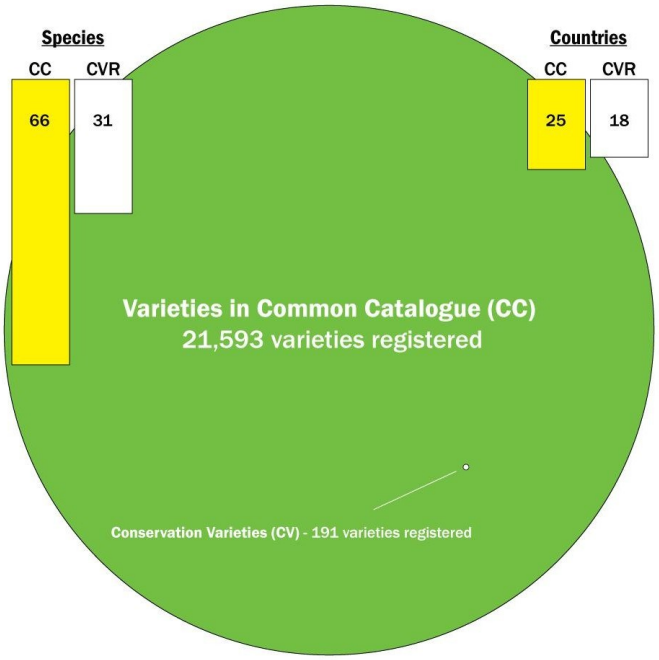

Figure 1. Graphic comparison of Common Catalogue varieties and conservation varieties registered in Europe. CVR = Conservation Varieties Regime.

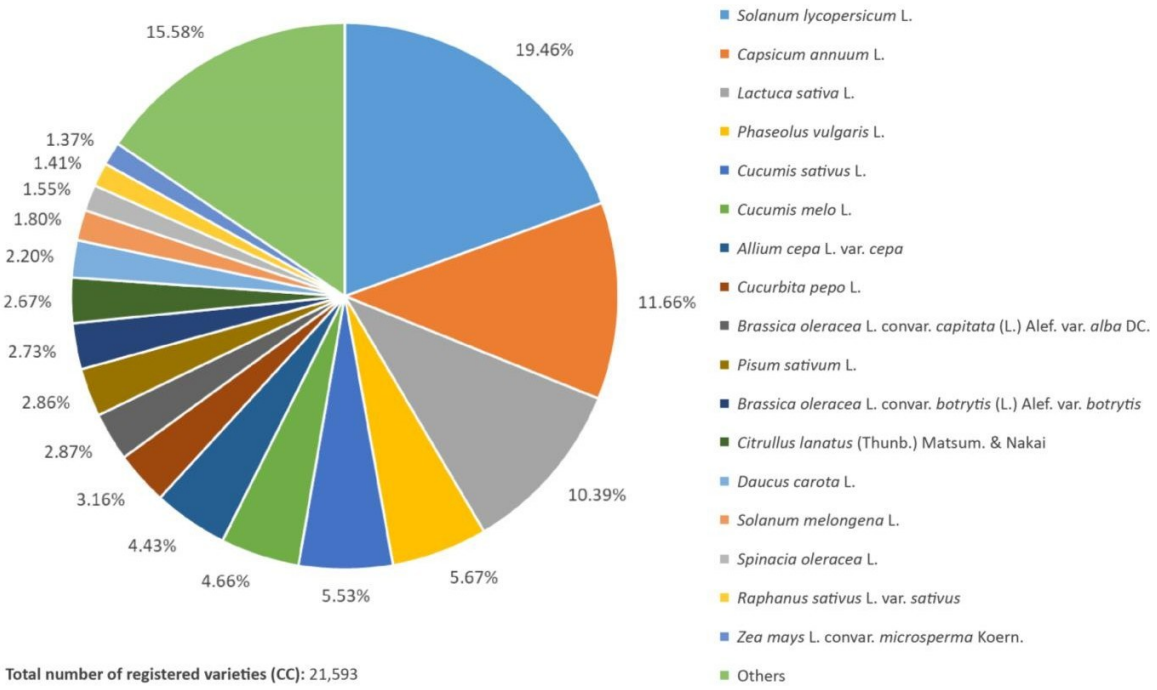

Figure 2. Varieties registered in the Common European Catalogue (CC) by species.

Regarding the 191 conservation varieties registered in Europe, the most-represented species was *P. vulgaris*, with 36 registered varieties (18.85%), followed by *S. lycopersicum* and *C. annuum*, with 35 (18.32%) and 28 (14.46%) registered varieties, respectively. The only other species with more than ten registered varieties was onion (*Allium cepa* L. var. *cepa*), with 15 varieties (7.85%) (Figure 3).

No relevant data could be found to enable a comparison of the numbers of conservation varieties registered in 2018 with the numbers of conservation varieties registered in 2023. The only interesting consideration was that, from 2018 to 2023, four new species were added to the CVR register, for a total of seven new varieties: specifically, varieties of melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), shallot (*Allium cepa* L. var. *aggregatum* G. Don) and savoy cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L.) were added. The whole list of registered conservation varieties, classified by species, is available in Table S2 in the Supplementary Materials.

Notably, the CVR register did not include varieties belonging to so-called "hybrid" species or "rootstock" species such as, for example, the species identified with the UPOV code "CUCUM_MEL_MEF", which was defined in the CC as "hybrids between *Cucumis melo* L. subsp. *melo* var. *flexuosus* (L.) Naudin and *Cucumis melo* L. subsp. *melo*", or the "SOLAN_LHA" species, which was defined as "hybrids between *Solanum lycopersicum* L. and *Solanum habrochaites* S. Knapp & D.M. Spooner". The only exception was the Spanish conservation variety 'De Guernica' (*Cucurbita moschata* L.), which was listed in the 37th edition in the section dedicated to varieties belonging to "species and interspecific crossings for use as rootstocks" in the CC. This indication was difficult to confute in the 2023 dataset because the variety in question—as well as most of the varieties indicated in the 37th edition as "rootstock"—was neither indicated as a "hybrid" nor listed as "rootstock".

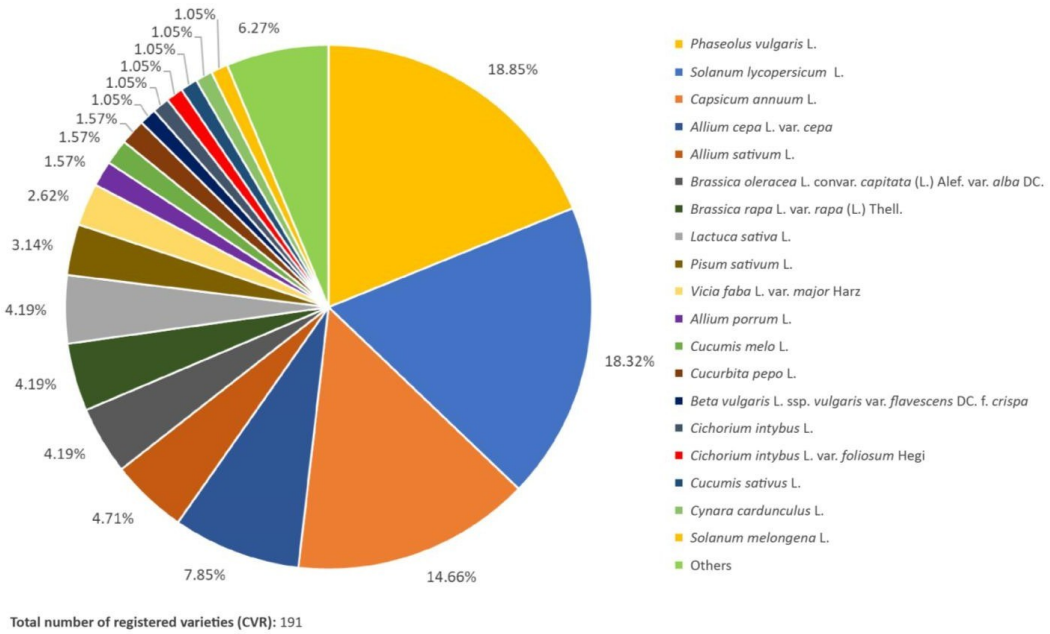

Figure 3. Conservation varieties registered in the Common European Catalogue by species. CVR = Conservation Varieties Regime.

Finally, Table 1 compares the numbers of varieties entered in the CC to the number listed as conservation varieties, broken down by species, considering only those species with a percentage of registered conservation varieties higher than 1%.

Table 1. Comparison of varieties registered as conservation varieties and in the Common European Catalogue (CC), broken down by species.

Species List (UPOV Name)	No. of Conservation Varieties (CVR)	No. of Conservation Varieties (CVR) (%)	No. of Varieties (CC)	No. of Varieties (CC) (%)
Phaseolus vulgaris L.	36	18.85%	1224	5.67%
Solanum lycopersicum L.	35	18.32%	4202	19.46%
Capsicum annuum L.	28	14.66%	2517	11.66%
Allium cepa L. var. *cepa*	15	7.85%	956	4.43%
Allium sativum L.	9	4.71%	144	0.67%
Brassica oleracea L. convar. *capitata* (L.) Alef. var. *alba* DC.	8	4.19%	620	2.87%
Brassica rapa L. var. *rapa* (L.) Thell.	8	4.19%	122	0.56%
Lactuca sativa L.	8	4.19%	2243	10.39%
Pisum sativum L.	6	3.14%	618	2.86%
Vicia faba L. var. *major* Harz	5	2.62%	117	0.54%
Allium porrum L.	3	1.57%	161	0.75%
Cucumis melo L.	3	1.57%	1007	4.66%
Cucurbita pepo L.	3	1.57%	682	3.16%
Beta vulgaris L. ssp. *vulgaris* var. *flavescens* DC. f. *crispa*	2	1.05%	77	0.36%
Cichorium intybus L.	2	1.05%	60	0.28%
Cichorium intybus L. var. *foliosum* Hegi	2	1.05%	110	0.51%
Cucumis sativus L.	2	1.05%	1195	5.53%
Cynara cardunculus L.	2	1.05%	83	0.38%
Solanum melongena L.	2	1.05%	389	1.80%
Others	12	6.00%	5066	23.46%

CVR = Conservation Varieties Regime.

In percentage terms, some species such as *P. vulgaris*, *C. annuum*, *A. cepa* var. *cepa*, *Allium sativum* L. (garlic), *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC. (white cabbage) and other minor species were represented more often as conservation varieties than as entries in the CC. On the other hand, other species, such as *S. lycopersicum*, *L. sativa*, and *C. melo*, were more represented in the CC than they were as conservation varieties.

Observing the data concerning the country distribution of the varieties registered in the CC in Europe, the Netherlands' predominance was unequivocal, with 8552 registered varieties, that being more than one third (39.60%) of the registered varieties in the whole of Europe. The Netherlands was followed by France, with 2880 registered varieties (13.34%), Spain (1992; 9.22%) and Italy (1909; 8.84%), as already observed in previous work [2,31]. The group of countries with more than 1000 registered varieties also included Hungary (1298; 6.01%) and the Czech Republic (1181; 5.47%).

In terms of comparison between the 2023 data and the data reported in Santamaria and Signore (2021), the results obtained from countries such as Sweden, which almost tripled the number of varieties previously registered in the CC (+250%), Slovenia (+221%) and Austria (+168) were reported as significant. The Netherlands remained almost stable in terms of varieties registered (+1.58%), while the country that registered the most varieties in the period under consideration was France, with 412 new varieties (+16.69%). In contrast, the case of Denmark, the representation of which decreased from 74 varieties registered in the CC in 2018 to only 18 varieties registered in 2023 (−75.68%), should be noted. Lastly, the entry of Luxembourg into the CC, which recorded its first variety within the CC during the enrolment period, and the exit of the United Kingdom from the CC because of Brexit (2020), should be noted. The complete list is available in Table S3 in the Supplementary Materials. The situation was different when only varieties registered under the CVR were considered. In this category, Spain was the European country with the greatest number of registered varieties (57; 29.84%), followed by Italy (43; 22.51%) and Croatia (26; 13.61%). The Netherlands, which occupied 1st place in the previous classification regarding numbers of varieties recorded in the CC, was in 15th place (along with three other countries), with only one registered conservation variety. France also dropped in the ranking—from 2nd to 6th place—with eight registered conservation varieties. From 2018 to 2023, two new countries, the Netherlands and Lithuania, registered their first conservation variety, while the country with the greatest number of conservation varieties was Spain, with 19 new CVR registrations. There was a decrease in the number of CVR registrations for Romania and Portugal, with the loss of one variety each. The complete list can be found in Table S4 in the Supplementary Materials.

Table 2 shows a comparison of the varieties registered under the CVR and those in the CC, broken down by country, with the aim of highlighting the contribution of each country to the constituents of those two categories. The table includes the data from European countries with a percentage of CVR registrations over 2% and data from the Netherlands because this country is so highly represented among varieties registered in the CC.

Table 2. Comparison of varieties registered as conservation varieties and in the Common European Catalogue (CC), broken down by country.

Country	No. of Conservation Varieties (CVR)	No. of Conservation Varieties (CVR) (%)	No. of Varieties (CC)	No. of Varieties (CC) (%)
Spain	57	29.84%	1992	9.23%
Italy	43	22.51%	1909	8.84%
Croatia	26	13.61%	62	0.29%
Hungary	13	6.81%	1298	6.01%
Slovenia	10	5.24%	135	0.63%
France	8	4.19%	2880	13.34%
Germany	6	3.14%	621	2.88%
Sweden	6	3.14%	63	0.29%
Belgium	4	2.09%	299	1.38%

Table 2. *Cont.*

Country	No. of Conservation Varieties (CVR)	No. of Conservation Varieties (CVR) (%)	No. of Varieties (CC)	No. of Varieties (CC) (%)
…	…	…	…	…
Netherlands	1	0.52%	8552	39.61%
…	…	…	…	…
Others	14	7.33%	3782	17.51%

CVR = Conservation Varieties Regime.

As might be expected, Spain and Italy were more strongly represented, by percentage, in varieties registered under the CVR than in varieties registered in the CC; this was truest of countries such as Croatia, Slovenia and Sweden, which recorded—as a percentage—more conservation varieties than varieties registered in the CC. The opposite situation applied to France and, above all, to the Netherlands, for which the percentages of varieties registered in the CC—calculated out of the total number of registered varieties—were much higher than the percentages of varieties registered under the CVR.

3.2. The Evolution of the CVR in Europe

Considering the date of registration of the European CVR, it can be shown that this scheme was not used until the definition of registration rules with Directive 2009/145/EC; in fact, almost all conservation varieties were registered after 2009, except for the varieties 'Nora' (*C. annuum*) and 'Carico Montañés' (*P. vulgaris*), both of which were registered in Spain.

According to Table 3, 2015 was the year in which the greatest number of varieties was registered (31, 16.23%), followed by 2021 (23, 12.04%) and 2017 (21, 10.99%). Following the same type of analysis as above, conservation varieties were listed by species and year of registration (Figure 4).

Table 3. Year of registration of European conservation varieties.

Registration Date	N° of CVR Registrations	N° of CVR Registrations (Progressive)
2006	1	1
2007	0	1
2008	1	2
2009	0	2
2010	10	12
2011	7	19
2012	7	26
2013	11	37
2014	15	52
2015	31	83
2016	19	102
2017	21	123
2018	14	137
2019	12	149
2020	10	159
2021	23	182
2022	9	191

CVR = Conservation Varieties Regime.

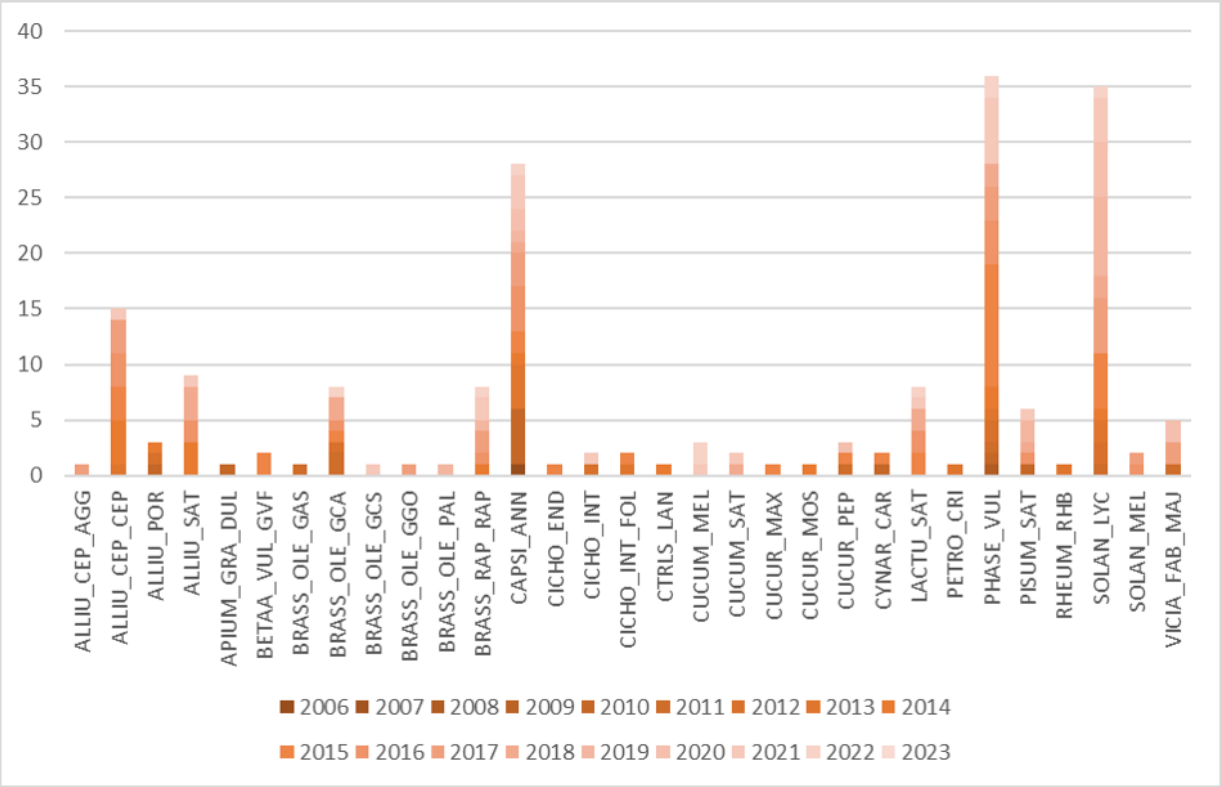

Figure 4. Conservation Varieties Regime: registrations by year and species.

The greatest numbers of years with associated entries occurred in the three most- represented species (*P. vulgaris*, *S. lycopersicum*, and *C. annuum*), for which conservation varieties were recorded for twelve, eleven and twelve years, respectively. Registrations of varieties of onion, the 4th-most-CVR-registered species, were instead all concentrated in the years from 2013 to 2017, with only one later entry, in 2021.

The cases of white cabbage (*B. oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC.) and turnip (*B. rapa* L. var. *rapa* (L.) Thell.), which, despite having only eight varieties entered, distributed these entries over a period of six years, were interesting.

Finally, the trend in CVR registrations by year and by European country, considering only European countries with a percentage of CVR registrations equalling at least 2% of the total CVR registrations, is shown in Figure 5.

Considering the historical evolution of the CVR from 2006—the year of the first CVR registration—to the present, until 2010, Spain was the only European country with CVR registrations. In 2010, Italy produced eight of the 43 current CVR registrations, becoming the top European country for registered varieties. This supremacy endured for only three years because in 2013, Croatia, with a total of 11 registered varieties, established itself as the top country in Europe. Thereafter, the leading position alternated between Italy (from 2015 to 2019) and Spain (in 2014 and from 2020 to present days).

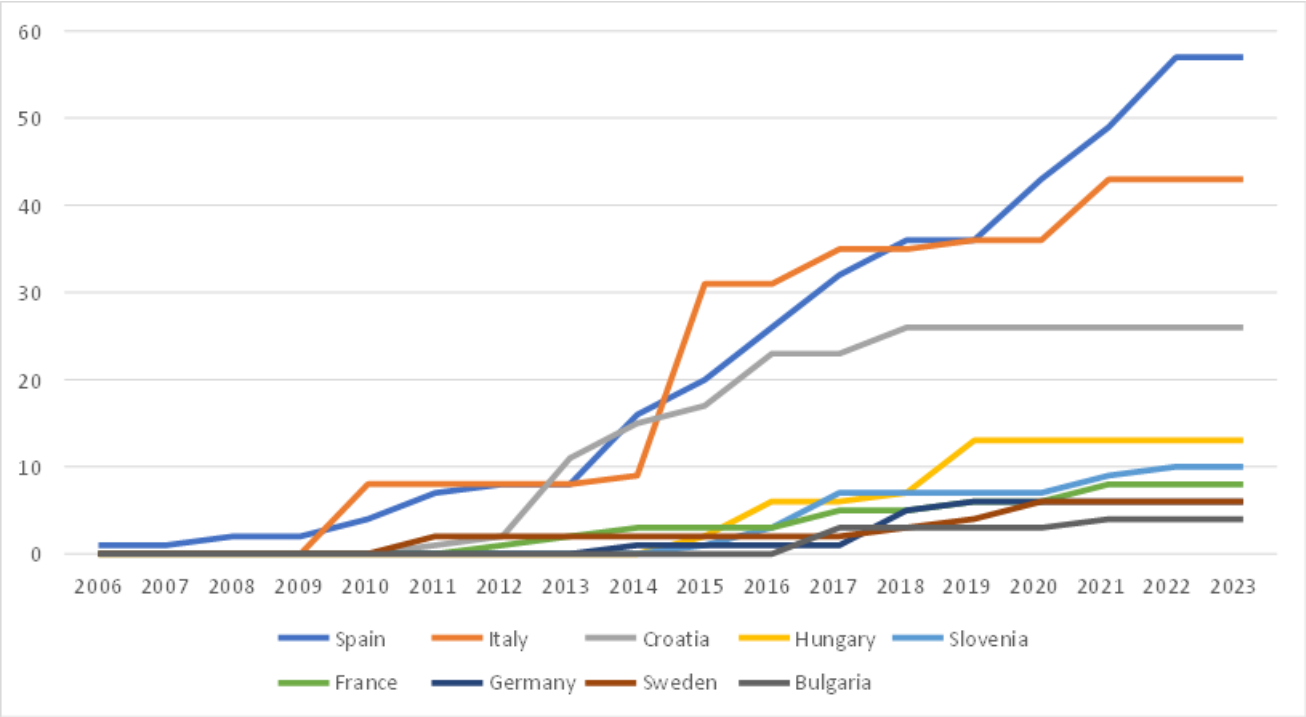

Figure 5. Conservation Varieties Regime registrations by year and country.

3.3. Case Study: Analysis of Italian CVR Registrations

In Italy, seed marketing is regulated by Legislative Decree No 20 of 2 February 2021: "Standards for the production for marketing purposes and the marketing of seed products in implementation of Article 11 of Law No 117 of 4 October 2019 for the adaptation of national legislation to the provisions of Regulation (EU) 2016/2031 and Regulation (EU) 2017/625". In this Decree, the regulatory provisions derived from the different European directives and national regulations have been summarised, including rules concerning the CVR.

Referring to the regulatory indications of Decree 20/2021, Table 4 was developed to summarise derogations and limits identified for the CVR under the current regulations [32–35].

Table 4. Exceptions and restrictions for Conservation Varieties Regime vegetables in Italian legislation.

Exceptions	Restrictions
Free registration in the National Registers and not subject to any official examination if the information submitted in the application is sufficient	Conservation-variety seeds may only be marketed if they are produced and sold in the area of origin (with exemptions)
Adoption of specific DUS criteria	The number of seed marketed must not exceed the limits set by the regulations
Exceptions to the varietal denomination scheme governed by Regulation 637/2009/EC	Obligation for producers to notify the relevant administration (region or autonomous province) of the quantity of seeds marketed
Exceptions to the minimum requirements for varietal purity	Requirement to demonstrate historical connection to the area of origin and degree of genetic erosion to apply for registration
Official inspections carried out retrospectively and through surveys	
Exemption from demonstration of possession of the requirements and professional knowledge of (i) mechanical production/selection techniques and (ii) seed- and plant-health regulations concerning the seed categories for which authorisation to produce is requested	
Right to direct local sales and right to free trade within the "National network of biodiversity of agricultural and food interest"	

Italy has 43 CVR registrations. The first varieties—the 'Dorato di Rissone' (*A. graveolens* L. var. *dulce* (Mill.) Pers.) and the 'Quarantin di Casalborgone' (*Pisum sativum* L.)—were registered on 5 January 2010; the last varieties, all of which belong to the species *P. vulgaris* L., were registered on 23 July 2021. In terms of year, most Italian conservation varieties were registered in 2010, when eight varieties were registered, in 2015, when 22 varieties were registered and in 2021 (seven varieties).

In total, Italy has fifteen species represented among the CVR registrations; the most- represented species is *P. vulgaris* L., with 18 registered varieties, followed by *A. cepa* var. *cepa* (five CVR registrations) and *S. lycopersicum* L., with four CVR registrations (Table 5).

Table 5. Italian Conservation Varieties Regime (CVR) registrations by species.

Species	Number of CVR Registrations	Number of *CVR Registrations* (%)
Allium cepa L. var. *cepa*	5	11.63
Allium porrum L.	1	2.33
Apium graveolens L. var. *dulce* (Mill.) Pers.	1	2.33
Beta vulgaris L. ssp. *vulgaris* var. *flavescens* DC. f. *crispa*	2	4.65
Brassica oleracea L. var. *gongylodes* L.	1	2.33
Brassica oleracea L. var. *palmifolia* DC.	1	2.33
Capsicum annuum L.	3	6.98
Cichorium endivia L.	1	2.33
Cichorium intybus L. var. *foliosum* Hegi	1	2.33
Cucurbita maxima Duchesne	1	2.33
Cucurbita pepo L.	1	2.33
Cynara cardunculus L.	2	4.65
Phaseolus vulgaris L.	18	41.86
Pisum sativum L.	1	2.33
Solanum lycopersicum L.	4	9.30

Although Italy is the second-largest country in Europe in terms of CVR registrations, the numbers are not representative of the whole country. In fact, only seven Italian regions—Emilia Romagna, Lombardy, Piedmont, Sardinia, Sicily, Tuscany, and the Veneto—have CVR registrations. Of these, Tuscany is the most-represented territory, with 22 CVR registrations, which is more than half of the registered varieties in Italy (51.26%). Tuscany is followed by Piedmont (eight CVR registrations), Veneto (five CVR registrations), Lombardy and Sardinia (three CVR registrations each). Sicily and Emilia Romagna round out the ranking, with one CVR registration each. Figure 6 shows the map showing the Italian CVR registrations, categorised by area of reference and by species.

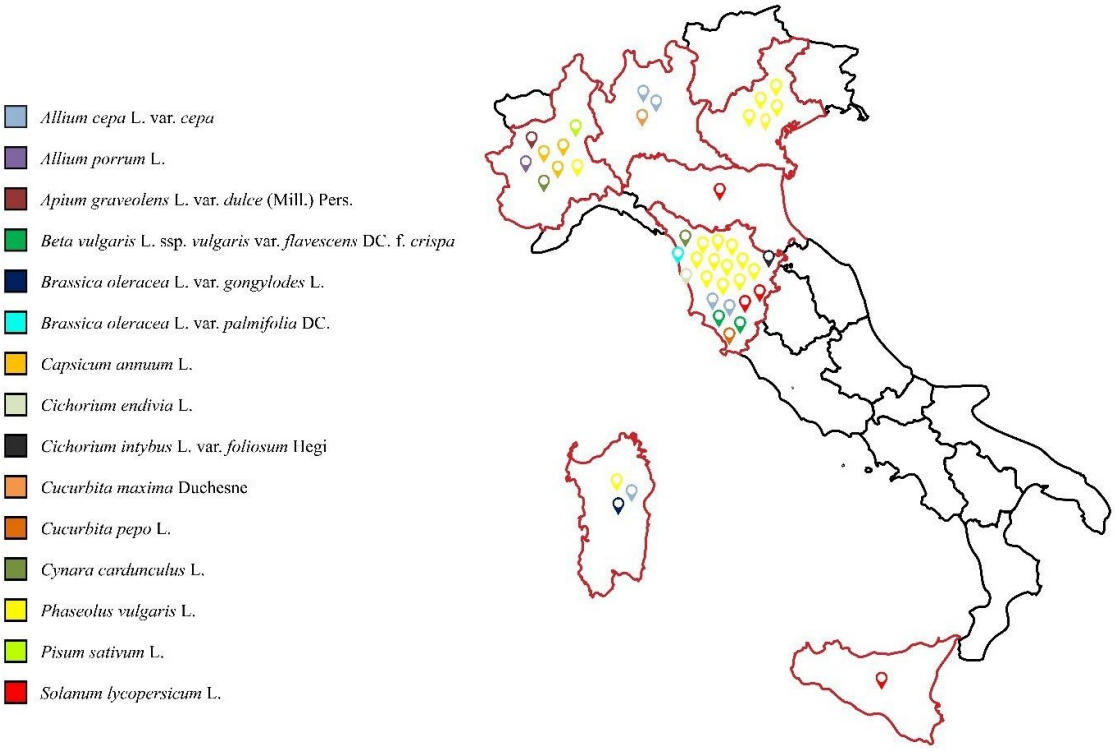

Figure 6. Italian Conservation Varieties Regime registrations categorised by area of reference and by species.

With reference to the variety maintainers identified for each CVR registration, the Italian situation is relatively varied. In fact, for all the Italian conservation varieties, seed companies or public or collective bodies (consortia, committees, associations, etc.) are identified as maintainers. Tuscany has a single maintainer for all the varieties for which a maintainer is indicated: a seed company called "Gargini Sementi di Alessandro Gargini & Giulio Godi s.n.c.". A similar situation applies in the Veneto, which, however, identifies a local consortium as the unique maintainer for the five conservation varieties. The situation is more varied in Piedmont, where public bodies are identified as maintainers—one conservation variety has a research body traceable to the Province of Turin, and the other is maintained by the Department of Agricultural, Forestry and Environmental Sciences of the University of Turin—and a research consortium. The situation in Emilia Romagna is also unique: here, the registered conservation variety is conserved by a private company, which, however, collaborates with research bodies as an experimental farm. Finally, in Sardinia, CVR registrations are conserved by a local committee aimed at safeguarding local biodiversity, while in Lombardy, the variety 'Dorata di Voghera' (*A. cepa* var. *cepa*) is conserved by a consortium called "*Consorzio produttori cipolla di Voghera*" (Voghera onion producers' consortium). This consortium was set up in 1990 and was the promoter of the variety's registration under the CVR in 2015 (Table 6).

Table 6. Italian Conservation Varieties Regime maintainers list.

Name	Type of maintainer	N° of varieties being maintained	Region
AZIENDA AGRARIA SPERIMENTALE STUARD S.C.R.L.	Private (with connection to public research programme)	1	Emilia-Romagna
COMITATO CUSTODI DELLA BIODIVERSITA DI ALGHERO (CCBA) "LO REVELLI"	Committee, consortium or association	2	Sardinia
CONSORZIO PER LA TUTELA DEL FAGIOLO DI LAMON	Committee, consortium or association	5	Veneto
CONSORZIO PRODUTTORI CIPOLLA DI VOGHERA	Committee, consortium or association	1	Lombardy
CRAB - CENTRO DI RIFERIMENTO PER L'AGRICOLTURA BIOLOGICA	Public	1	Piedmont
CRESO - CONSORZIO RICERCA E SVILUPPO PER L'ORTI-FRUTTICOLTURA PIEMONTESE	Committee, consortium or association	6	Piedmont
DIPARTIMENTO DI SCIENZE AGRARIE, FORESTALI E ALIMENTARI UNIVERSITA DEGLI STUDI DI TORINO	Public	1	Piedmont
GARGINI SEMENTI DI ALESSANDRO GARGINI & GIULIO GODI SNC	Seed company	21	Tuscany
Not available	Not available	5	Lombardy (2), Tuscany (1), Sardinia (1), Sicily (1)

To conclude the analysis of the Italian case, the area (ha) available in each Italian region for each conservation variety was calculated, following the indications given by the legislation [32] (Table 7).

Table 7. Hectares potentially available for the cultivation for each conservation variety in Italy.

Species	Conservation Variety	IT Region (1)	Available area for Conservation Variety (ha) (2)	Cultivated area for species (ha) (3)	Available area for conservation variety (%) (4)
Allium cepa L. var. *cepa*	Dorata di Voghera	Lombardy	40	333	12.01%
Allium cepa L. var. *cepa*	Rossa di Breme	Lombardy	40	333	12.01%
Allium cepa L. var. *cepa*	Mitja Valmella	Sardinia	40	122	32.79%
Allium cepa L. var. *cepa*	Rossa a fiasco	Tuscany	40	187	21.39%
Allium cepa L. var. *cepa*	Rossa massese	Tuscany	40	187	21.39%
Allium porrum L.	Di Cervere	Piedmont	20	47	42.55%
Apium graveolens L. var. *dulce* (Mill.) Pers.	Dorato Rissone	Piedmont	10	18	55.56%
Beta vulgaris L. ssp. *Vulgaris* var. *flavescens* DC. f. *crispa*	Livornese da taglio	Tuscany	20	186	10.75%
Beta vulgaris L. ssp. *Vulgaris* var. *flavescens* DC. f. *crispa*	Verde da taglio lucchese	Tuscany	20	186	10.75%
Brassica oleracea L. var. *gongylodes* L.	Colatronxo	Sardinia	40	24	>100,00%
Brassica oleracea L. var. *palmifolia* DC.	Braschetta	Tuscany	40	74	54.05%
Capsicum annuum L.	Corno di Carmagnola	Piedmont	40	119	33.61%
Capsicum annuum L.	Cuneo	Piedmont	40	119	33.61%
Capsicum annuum L.	Quadrato di Carmagnola	Piedmont	40	119	33.61%
Cichorium endivia L.	Tardiva lucchese	Tuscany	10	39	25.64%
Cichorium intybus L. var. *foliosum* Hegi	Del Marzocco	Tuscany	40	178	22.47%

Table 7. *Cont.*

Species	Conservation Variety	IT Region (1)	Available area for Conservation Variety (ha) (2)	Cultivated area for species (ha) (3)	Available area for conservation variety (%) (4)
Cucurbita maxima Duchesne	Cappello da prete mantovana	Lombardy	40	n.a.	n.a.
Cucurbita pepo L.	Mora pisana	Tuscany	20	386	5.18%
Cynara cardunculus subsp. *scolymus* (L.) Hayek	Gobbo di Nizza Monferrato	Piedmont	40	n.a.	n.a.
Cynara cardunculus subsp. *scolymus* (L.) Hayek	Pieno inerme lucchese	Tuscany	40	657	6.09%
Phaseolus vulgaris L.	Bianco di Bagnasco	Piedmont	40	984	4.07%
Phaseolus vulgaris L.	Gioghedda	Sardinia	40	87	45.98%
Phaseolus vulgaris L.	Aquila	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Diecimino	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Fico di Gallicano	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Garfagnino	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Giallorino della Garfagnana	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Malato	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Mascherino	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Rosso di Lucca	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Schiaccione di Pietrasanta	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Stortino di Lucca	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Zolfino	Tuscany	40	306	13.07%
Phaseolus vulgaris L.	Calonega	Veneto	40	958	4.18%
Phaseolus vulgaris L.	Canalino	Veneto	40	958	4.18%
Phaseolus vulgaris L.	Spagnolit	Veneto	40	958	4.18%
Phaseolus vulgaris L.	Spagnolit nano	Veneto	40	958	4.18%
Phaseolus vulgaris L.	Spagnolo	Veneto	40	958	4.18%
Pisum sativum L.	Quarantin di Casalborgone	Piedmont	40	444	9.01%
Solanum lycopersicum L.	Riccio di Parma	Emilia Romagna	40	25,505	0.16%
Solanum lycopersicum L.	Pizzutello dell'Agro Ericino	Sicily	40	12,525	0.32%
Solanum lycopersicum L.	Canestrino di Lucca	Tuscany	40	2,714	1.47%
Solanum lycopersicum L.	Pisanello	Tuscany	40	2,714	1.47%

(1) Italian region in which the Conservation Varieties Regime "area of origin" is located; (2) maximum hectares available for the cultivation of the conservation variety, by regulation (source: Annex XI, Legislative Decree 20/2021); (3) cultivated hectares in each region for each species in 2022 (source: ISTAT); (4) percentage of hectares available for each conservation variety in the region of origin.

4. Discussion

This section provides an overview of the discussions and prospects regarding the CVR's possible evolution.

There is general agreement that the CVR scheme, introduced 25 years ago within the EU seed system in order to provide a means of protection and commercialization for so- called "old and traditional varieties", has not, to date, achieved the desired results [26,36,37]. This is reflected particularly in the small number of CVR registrations in the Community Register in recent years, which, when compared to the number of vegetable varieties registered within the CC, offers an indication of the limited impact that this scheme has had and still has in Europe.

In quantitative terms, the number of varieties registered under the CVR both by species and by European country matches what has been reported in

previous works [31,38]. For example, regarding the country distribution of varieties registered in the CC and under the CVR, it is confirmed that the Netherlands, despite being only the fourth-ranked country in Europe for vegetable production—5.6 × 10^6 t, 7.00% of total production in Europe in 2021 [39]—is the most-represented country in terms of the number of registered vegetable varieties in the CC. This derives from the type of production system that has developed in the Netherlands, which consists of large infrastructure that strongly connects the activities of sorting, breeding and retailing [31,40]. In contrast, Spain and Italy, first in terms of horticultural production in Europe—13.5 × 10^6 t (18.00%) and 11.4 × 10^6 t (15.00%), respectively [39]—together account for slightly more than one fifth (22.67%) of the registered varieties in the CC. This is because both Mediterranean countries are characterised by an entrepreneurial structure mainly made up of small and medium-sized enterprises (SME), where farmers prefer using local and traditional varieties, in part thanks to the great richness of agrobiodiversity in these countries [2,31].

Nevertheless, the comparison between the CC and the varieties registered under the CVR offers some insights. In the latter, there is more space—in percentage terms—for many varieties that play a marginal role in the CC, such as garlic (*A. sativum*), turnip (*B. rapa* var. *rapa*.), chard (*Beta vulgaris* L.) and artichoke (*Cynara cardunculus* subsp. *scolymus* (L.) Hayek). The CVR could therefore be a tool for protecting and promoting not only landraces at risk of erosion but also "minor" varieties, i.e., those that have been less attractive for breeding programmes either because of the difficulties in improving these varieties with

Conventional Breeding Techniques (CBT) or because of a lack of market interest in them. In addition, the possibility of registering certain vegetable conservation varieties as rootstocks, as in the case of the Spanish variety 'De Guernica', has garnered considerable interest in terms of PRGFA conservation and protection plans. In fact, the genetic variability inherent to local varieties is a highly favourable factor, allowing numerous diseases and pests to be managed naturally when such varieties are used as rootstock. For example, the Italian varieties 'Barattiere' (*C. melo*) and 'Pomodoro di Manduria' (*S. lycopersicum*) are reported to be varieties that are resistant to, respectively, tomato leaf curl New Delhi virus (ToLCNDV) and tomato spotted wilt virus (RB-TSWV), two of the most problematic pathogens for their respective species [41–43]. Despite their important roles, however, neither of these two local varieties is present in the CC or registered under the CVR: registering them as useful rootstock conservation varieties could not only better protect these varieties but, above all, further promote good natural pathogen-management practices related to them.

However, what are the reasons for the CVR's low impact on the European seed market? Among the main reasons, some authors point out that the requirements for registration are still too stringent, although some exemptions from the DUS characteristics are recognised in the legislation [21,44]. Others consider the retrieval of historical information attesting to the historical and traditional link of the varieties with their region [17] as an obstacle to registration. In general, there was a lack of information among farmers,

who often saw the CVR more as a threat than an opportunity because of the possible restrictions, including limitations on self-production of seed, quantity limits on marketing and the prohibition on the sale of propagation material outside the reference region [45].

Regarding the Italian case study, the national regulatory provisions are largely aligned with the provisions of European legislation. In reference Law 20/2021, the provisions of Directive 2009/145/EC are applied. Among these, the most significant in terms of promoting conservation are the adoption of specific DUS criteria and the exceptions to the minimum requirements for the marketing of conservation varieties, as well as the simplifications in terms of official monitoring. In addition to these, there are the regulatory provisions of Law 1096/71, which pertains to the right to the direct sale of plant propagative materials at the local level, and Law 194/2015, which pertains to the free marketing of conservation variety seeds within the "National Network of Biodiversity of Agricultural and Food Interest". Finally, a further exemption is contained in Art. 4 of the Ministerial Decree of 12 November 2009, according to which producers of conservation variety seeds are exempted from the obligation to demonstrate possession of the requirements and professional knowledge inherent to (i) mechanical production and breeding techniques and (ii) seed and phytosanitary standards relating to the categories of seeds for which production authorisation is requested.

In terms of potential restrictions, in addition to the previously identified critical issues regarding the registration of new varieties (Table 4), another potential problem identified by farmers is the quantitative limits imposed on the marketing of seeds of each variety. To confute this claim, the data in Table 7 were reported and analysed. If for some varieties, such as 'Riccio di Parma' (*S. lycopersicum*), the maximum quantities imposed by the regulations seem to be restrictive in terms of cultivation and marketing (a maximum quantity of seeds to cover only 0.16% of the hectares currently cultivated with tomatoes in Emilia-Romagna could be marketed), for other varieties, this limit does not seem to exist. For example, the varieties 'Dorato di Rissone' (*A. graveolens* var. *dulce*.) and 'Braschetta' (*Brassica oleracea* L. var. *palmifolia* DC.), in Piedmont and Tuscany, respectively, could be sufficient to cover 55.56% and 54.05% of the hectares currently dedicated to the cultivation of the reference species in these regions. Additionally, for the 'Colatronxo' variety (*Brassica oleracea* L. var. *gongylodes* L.), the maximum number of hectares provided for in the regulations (40 ha) is even greater than the number of hectares currently used to cultivate turnip cabbage in Sardinia (24 ha). This shows that, in some territories and for certain species, these quantitative limits do not really represent limits, contrary to the perception of some farmers [45].

Moreover, other critical issues encountered for registration—such as the need to demonstrate the connection of the variety with the reference territory—could also be overcome by other regulatory instruments. For example, in Italy, it is possible to use the information contained in the National Register of Biodiversity of Agricultural and Food Interest, the Regional Registers of Autochthonous Genetic Resources—such as the one established in Apulia by Regional Law n. 39 of December 2013—or the list of Traditional Agrifood Products (TAP) [34,46] to demonstrate the traditional nature of certain

varieties in terms of their historical link with the reference region. More specifically, to obtain recognition as a TAP, the product must be demonstrated to have been linked with the region for at least 25 years [47,48]. Therefore, if a product is recognised as a TAP, the link with the region has already been demonstrated; therefore, this information can be consulted and utilised for the CVR registration procedure.

Nevertheless, the use of the CVR in Italy has been and still is limited. In fact, out of the twenty regions in Italy, only seven have registered conservation varieties. Basilicata, Apulia and Lazio, which are rich in landraces and traditional vegetables [47], have never used this scheme to create a market for the seeds of endangered vegetable varieties.

In addition, Italy has only eight entities registered as maintainers for the 43 conservation varieties, of which one—the only registered seed company, operating in Tuscany—is responsible for the maintenance of almost half (48.83%) of the registered conservation varieties. The remaining conservation varieties are conserved—a maximum of 5–6 CVR registrations per entity—by consortia, associations or public bodies scattered across the country. Moreover, most Italian conservation varieties have been registered within a few limited periods; registrations are mainly distributed over four different years—2010 (8 varieties), 2015 (22), 2017 (4) and 2021 (7)—with two single registrations in 2014 and 2019.

Although Italy is the second-largest country in Europe in terms of CVR registrations, overall, it is true that its case study represents a context in which the CVR is poorly structured and characterised by the initiatives of individual entities that take it upon themselves to protect and/or promote specific vegetable varieties. Fifteen years after Directive 145/2009/CE on vegetable species, these results confirm that the CVR has not achieved the desired results and that, therefore, a revision, at least, of the legislation is necessary.

Proposal 2023/0227 (COD), which pertains to the production and marketing of plant and reproductive material in the European Union [27], takes action in this regard. As drafted by the EU Commission, this proposal introduces a clear contrast between the varieties eligible for registration in the CC—identified as varieties subject to the DUS criteria—and the conservation varieties, identified as varieties for which the DUS criteria are no longer required. So, for the CVR, the Proposal simplifies the registration procedure by requesting less documentation to proceed with the application for registration. Indeed, article 53 of the Proposal indicates as requirements for registration (i) an official recognised description, specifying the characteristics of the variety for the CVR; (ii) an indication of the initial region of origin; (iii) a denomination reflecting current regulatory requirements and (iv) the identification of a European maintainer. The official description shall be based, particularly, on the results of unofficial tests and knowledge gained from practical experience during cultivation. In addition, further simplifications derive from the Legislative Resolution of the European Parliament of 24 April 2024, in which point (ii) of Art. 53 present in the Proposal was changed to "[. . .] an indication of its initial region of origin, when known, or the local conditions under which it has been newly bred". Basically, the new legislative text

simplifies the work of registration promoters, requiring them to simply identify the specific local conditions where the variety is bred for cases in which a region of origin cannot be unequivocally demonstrated. The most important indication is contained in the new Article 53, par. 1, sub. 1a, which specifically states that the CVR registration must be free of charge for the proposer. Thirdly, it is stipulated that, if an application for registration is refused, the competent authority must notify the applicant of its decision, stating the reasons for the refusal (Article 53, par. 2, sub. 2).

Another change that the Proposal identifies for the CVR is the substitution of the new definition of conservation variety for the definition presented in the current regulation [20]. In fact, in the proposal, under letter 29 of art. 3, "a variety that is (a) traditionally grown or locally newly bred under specific local conditions in the Union and adapted to those conditions; and (b) characterised by a high level (changed to "satisfactory level" by the 2024 Parliamentary Resolution) of genetic and phenotypical diversity between individual reproductive units" is defined as a conservation variety. The term "newly bred" is better explained in the Parliamentary Resolution of 2024. Here, the indication that "newly bred varieties" means "[modern landraces] derived from on-farm selection or bred for adaptation to local conditions in the context of the sustainable use of plant genetic resources for food and agriculture" is added to the text present in the Proposal. In the Resolution, the new points "aa" and "ba" are also added to Art. 3, par. 1, point 29, specifying that a conservation variety cannot be an F1 hybrid variety and that a conservation variety, to be so defined, must not be "subject, as a whole or in genetic components, to intellectual property rights that limit its use for conservation, research, breeding, education, including on a farm by a farmer who uses the PRM grown on the farm, of that variety for those objectives".

Comparing this new definition with the definition contained in the Directive 2009/145/EC and its three key concepts—agricultural landraces and varieties, region of origin and genetic erosion risk [13,17,20,21]—it is possible to see that (i) the definition now includes either traditional landraces and new breeding varieties and (ii) the risk of genetic erosion is no longer present in the definition. According to the Proposal, a satisfactory grade of genetic diversity—typical of the landraces—and the ability to adapt to a specific territory is sufficient to identify a conservation variety.

Although this new definition includes more varieties than did the previous definition, it still has flaws. For example, there is the problem for species not included in the CC, species such as broccoli rabe (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort) [31].

In commercial terms, this proposal contains an important step forward in article 26, which says that conservation-variety seeds should be produced and marketed in the European Union as standard seed, accompanied by an operator's label with the indication "conservation variety". Therefore, the burden of certification in the field has been removed, facilitating seed production and marketing. Furthermore, there is no direct reference in the Proposal to the obligation to produce and market PRM in the so-called "area of origin". This obligation was contained in previous legislation [20] that severely restricted the marketing of conservation varieties. Indeed, the article

indicates how conservation varieties can be produced and marketed in the European Union as standard seeds.

Finally, it should be noted that, on 24 June 2024, the Belgian Council Presidency presented the interim status of the talks on various legislative proposals, based on what was discussed and published by the European Council on 18 June 2024 [49], to the meeting of EU Agriculture Ministers. Concerning the CVR, the document reported the results of a discussion centred around the addition of the definition of "newly bred conservation variety" under the definition of "conservation variety". Some EU Countries fear that this new definition would open a backdoor for the inclusion of commercial varieties that had not passed the DUS requirement. For this reason, the Council suggested not including the "newly bred conservation variety" under the definition of "conservation variety".

In conclusion, regarding biodiversity protection and farmer's rights, the Commission's Proposal and subsequent amendments seem to promote a clear direction toward sustainably using PRM and protecting the work of farmers who, for decades, have been developing breeding activities based on these resources. In fact, the introduction of the concept of "dynamic conservation" as the "preservation of genetic diversity within and between cultivated plant species, [including] both in situ conservation and ex situ conservation, with the aim of a sustainable use of plant genetic resources and agrobiodiversity" (art. 3, par. 1, point 35 of the Proposal as modified by the Parliament's Resolution) moves in this direction. So do the new important exceptions to the exchange of seeds, even for payment, between farmers (art. 30)—now allowed for small quantities—as well as the official, legislative recognition of the role of organisations and bodies (non-profit) that deal with dynamic conservation and seed marketing (art. 29). The European Council's advice published on 18 June, however, seems to apply some pressure to the brakes on this major opening of the seed market in Europe. On the one hand—as also written above—it suggests excluding the "newly bred conservation variety" from the "conservation variety" regime, and, on the other hand, it also proposes to remove Article 30, which concerns the free exchange of small quantities of seed between farmers, from the Proposal. The more conservative stance of the European Council and some delegations is based on the fear that the new regulations approved by the Commission on 24 April 2024 could undermine the European seed system, opening the way to "informal channels for marketing of PRM with no scrutiny whatsoever regarding its identity, quality, health" [50]. Other stakeholders, however, point out how, if the regulatory proposals made by the European Commission in April were confirmed, there would be more tools to promote, market and protect local varieties; these aims are objectives of the CVR [51–53].

5. Conclusions

More than twenty-five years after its introduction into European legislation, the CVR has failed to promote and enforce a dedicated market for all

those varieties (landraces, new farmers' varieties, etc.) excluded from registration in the CC because of DUS requirements. The few CVR registrations in Europe and the improper use of this scheme—often used not by seed companies or other commercial entities but by public, research and associative organisations—required the intervention of the European Commission and, subsequently, of the Parliament. In line with the European Green Deal policies, Farm to Fork Strategy, Biodiversity Strategy, etc., these bodies proposed major changes to the commercialization system—and therefore to the protection system—for varieties that are representative of Europe's national and regional biodiversity.

At the moment, it is unclear what direction these reforms will take in the future, as there are different and opposing positions; on the one hand, there is the proposal to expand the definition and the market of conservation varieties, and on the other hand, there is a more conservative strategy, motivated by the desire to maintain a certain level of safety for the seed market.

In this regard, this study, analysing the CVR, has shown that such exceptions do not actually represent a threat to the European seed market, as they have a non-significant impact on the structured system defined by European seed legislation. Nevertheless, the exceptions defined within the CVR—like the VDPC regime, whose impact also deserves to be analysed—offer unique tools for the protection and promotion of European plant biodiversity. To confirm these assertions, it would be necessary to study more deeply the commercial impact that conservation varieties or VDPCs have on the European market, as well as the rate of farmers' use of seed of F1 hybrid varieties—or of varieties otherwise registered in the CC—and of seed falling under to the above-mentioned exemption schemes.

However, as stated above, it seems to be clear that the seed system should be improved and implemented to facilitate the achievement of the objectives defined for CVR and, more generally, for the protection and valorisation of European agrobiodiversity.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Definition of conservation varieties and VDPC (EU Directive 2009/145/EC).

Waiver	Legislative Indication
Conservation Varieties (CVR)	i. Definition: landraces and varieties that have been traditionally grown in particular places and regions and that are threatened by genetic erosion. **ii.** Requirements: To be accepted under the CVR, a landrace or variety shall be interesting in terms of conserving plant genetic resources. To demonstrate distinctiveness and stability, candidate varieties should comply with the test protocols of the Community Plant Variety Office (CPVO) or with the technical questionnaires of the Guidelines of the International Union for the Protection of New Varieties of Plants (UPOV). In addition, in a departure from the standard used for other vegetable seeds, a minimum uniformity level of 90% is set (maximum off-type level = 10%). An official examination is not required. The following information is enough for the application: (a) a description of the conservation variety and its denomination; (b) the results of unofficial tests; (c) knowledge gained from practical experience during cultivation, reproduction and use by the applicant; (d) the indication of a region of origin in which the variety has historically been grown and to which it is naturally adapted. **iii.** Production and marketing: A conservation variety may be produced and marketed only in its region of origin. The regulations in this regard provide two exceptions: (i) if it is not possible to produce the conservation variety in the region of origin, production may be carried out in another designated location; (ii) at the time of registration of the conservation variety, areas other than the region of origin that have a similar natural habitat may be designated as permitted regions in which to market the seed. The two exceptions are alternatives and are not complementary. CVR seeds may be marketed either as "certified seed of a conservation variety" or as "standard seed of a conservation variety", according to the fulfilment of the minimum conditions required by the tests. Annex I of the Directive indicates the quantitative restrictions for production and marketing of CVR seeds. For horticultural species, the restrictions were calculated as a percentage of the production provided for each species in the region of origin. iv. Official tests: CVR seeds are subjected to official, ex post checks to assess the varietal identity and varietal purity via random inspections.
Varieties developed for growing under particular conditions (VDPC)	**i.** Definition: varieties with no intrinsic value for commercial crop production but that were developed for growing under particular conditions. **ii.** Requirements: to be accepted as a VDPC, a variety shall have no intrinsic value for commercial production but must have been developed for growing under particular agrotechnical, climatic or pedological conditions. To demonstrate distinctiveness and stability, candidate varieties should comply with the test protocols of the Community Plant Variety Office (CPVO) or with the technical questionnaires of the Guidelines of the International Union for the Protection of New Varieties of Plants (UPOV). In addition, in a departure from the standard used for other vegetable seeds, a minimum uniformity level of 90% is set (maximum off-type level = 10%). An official examination is not required. The following information is enough for the application: (a) a description of the conservation variety and its denomination; (b) the results of unofficial tests; (c) knowledge gained from practical experience during cultivation, reproduction and use by the applicant. **iii.** Production and marketing: VDPC can be marketed only as "standard seed", according to the fulfilment of the minimum conditions required. Annex II of the Directive indicates the quantitative restrictions for the production and marketing of VDPC seeds as maximum net weight per package expressed in grams (250, 25 or 5 g, depending on the species). **iv.** Official tests: VDPC seeds are subjected to official, ex post checks to assess the varietal identity and varietal purity via random inspections.

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Supplementary Materials

Supplementary materials associated with this article can be found, in the online version, at doi.org/10.3390/horticulturae10080877.

Table S1. Registered varieties in CC for species.

Species list (UPOV code)	*Species list (UPOV name)*	*37th edition name*	*Number of registered varieties for species (2018)*	*Number of registered varieties for species (%)(2018)*	*Number of registered varieties for species (2023)*	*Number of registered varieties for species (%) (2023)*	*Variation between 2018 and 2023 data*	*Variation between 2018 and 2023 data (%)*
ALLIU_CEP_AGG	*Allium cepa* L. var. *aggregatum* G. Don	Shallot	53	0.27%	65	0.30%	12	22.64%
ALLIU_CEP_CEP	*Allium cepa* L. var. *cepa*	Onion	906	4.55%	956	4.43%	50	5.52%
ALLIU_FIS	*Allium fistulosum* L.	Welsh onion	52	0.26%	64	0.30%	12	23.08%
ALLIU_POR	*Allium porrum* L.	Leek	163	0.82%	161	0.75%	-2	-1.23%
ALLIU_SAT	*Allium sativum* L.	Garlic	112	0.56%	144	0.67%	32	28.57%
ALLIU_SCH	*Allium schoenoprasum* L.	Chives	41	0.21%	38	0.18%	-3	-7.32%
ANTHR_CER	*Anthriscus cerefolium* (L.) Hoffm.	Chervil	4	0.02%	4	0.02%	0	0.00%
APIUM_GRA_DUL	*Apium graveolens* L. var. *dulce* (Mill.) Pers.	Celery	98	0.49%	114	0.53%	16	16.33%
APIUM_GRA_RAP	*Apium graveolens* L. var. *rapaceum* (Mill.) Gaud.	Celeriac	52	0.26%	70	0.32%	18	34.62%
ASPAR_OFF	*Asparagus officinalis* L.	Asparagus	83	0.42%	98	0.45%	15	18.07%
BETAA_VUL_GVC	*Beta vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef.	Beetroot	134	0.67%	150	0.69%	16	11.94%
BETAA_VUL_GVF	*Beta vulgaris* L. ssp. vulgaris var. *flavescens* DC. f. *crispa*	Spinach beet or Chard	59	0.30%	77	0.36%	18	30.51%
BRASS_OLE_COS	*Brassica oleracea* L. var. *costata* DC.	n.d.	0	0.00%	4	0.02%	4	n.a.
BRASS_OLE_GAS	*Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L.	Curly kale	38	0.19%	61	0.22%	23[1]	60.53%

[1] In 2018, the *Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L. varieties include also the varieties belonging to *Brassica oleracea* L. var. *palmifolia* DC. For the calculation of the variation between the 2018 and 2023 data, the transfer of 14 varieties from the first species to the second was considered.

Table S1. *Cont.*

Species list (UPOV code)	*Species list (UPOV name)*	*37th edition name*	*Number of registered varieties for species (2018)*	*Number of registered varieties for species (%)(2018)*	*Number of registered varieties for species (2023)*	*Number of registered varieties for species (%)(2023)*	*Variation between 2018 and 2023 data*	*Variation between 2018 and 2023 data (%)*
BRASS_OLE_GBB	*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *botrytis*	Cauliflower	604	3.03%	590	2.73%	-14	-2.32%
BRASS_OLE_GBC	*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.	Sprouting broccoli	174	0.87%	181	0.84%	7	4.02%
BRASS_OLE_GCA	*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC.	White cabbage	626	3.14%	620	2.87%	-6	-0.96%
BRASS_OLE_GCR	*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *rubra* (L.) Thell.	Red cabbage	102	0.51%	94	0.44%	-8	-7.84%
BRASS_OLE_GCS	*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L.	Savoy cabbage	149	0.75%	156	0.72%	7	4.70%
BRASS_OLE_GGM	*Brassica oleracea* L. var. *gemmifera* Zenker	Brussels sprouts	113	0.57%	99	0.46%	-14	-12.39%
BRASS_OLE_GGO	*Brassica oleracea* L. var. *gongylodes* L.	Kohlrabi	109	0.55%	117	0.54%	8	7.34%
BRASS_OLE_PAL	*Brassica oleracea* L. var. *palmifolia* DC.	n.d.	0	0.00%	14	0.06%	14	n.a.
BRASS_RAP_PEK	*Brassica rapa* L. Emend. Metzg. ssp. *pekinensis* (Lour.) Hanelt	Chinese cabbage	61	0.31%	59	0.27%	-2	-3.28%
BRASS_RAP_RAP	*Brassica rapa* L. var. *rapa* (L.) Thell.	Turnip	123	0.62%	122	0.56%	-1	-0.81%
CAPSI_ANN	*Capsicum annuum* L.	Chili, pepper	2,216	1.12%	2,517	11.66%	301	13.58%
CICHO_END	*Cichorium endivia* L.	Curled leaved endive + Plain leaved endive	214	1.07%	200	0.93%	-14	-6.54%
CICHO_INT	*Cichorium intybus* L.	Witloof chicory	51	0.26%	60	0.28%	9	17.65%
CICHO_INT_FOL	*Cichorium intybus* L. var. *foliosum* Hegi	Large-leaved chicory / Italian chicory	100	0.50%	110	0.51%	10	10.00%

Table S1. *Cont.*

Species list (UPOV code)	*Species list (UPOV name)*	*37th edition name*	*Number of registered varieties for species (2018)*	*Number of registered varieties for species (%)(2018)*	*Number of registered varieties for species (2023)*	*Number of registered varieties for species (%)(2023)*	*Variation between 2018 and 2023 data*	*Variation between 2018 and 2023 data (%)*
CICHO_INT_SAT	*Cichorium intybus* L. var. *sativum* DC.	Industrial chicory	45	0.23%	67	0.31%	22	48.89%
CTRLS_LAN	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Watermelon	517	2.59%	576	2.67%	59	11.41%
CUCUM_MEL	*Cucumis melo* L.	Melon	942	4.73%	1,007	4.66%	65	6.90%
CUCUM_MEL_MEF	hybrids between *Cucumis melo* L. subsp. *melo* var. *flexuosus* (L.) Naudin and *Cucumis melo* L. subsp. *melo*	n.d.	1	0.01%	2	0.01%	1	100.00%
CUCUM_SAT	*Cucumis sativus* L.	Cucumber + Gherkin	1,180	5.92%	1,195	5.53%	15	1.27%
CUCUR_MAX	*Cucurbita maxima* Duchesne	Gourd	75	0.38%	130	0.60%	55	73.33%
CUCUR_MMO	*Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne	n.d.	1	0.01%	45	0.21%	-1	-100.00%
CUCUR_MOS	*Cucurbita moschata* Duchesne	n.d.	43	0.22%	13	0.06%	2	4.65%
CUCUR_PEP	*Cucurbita pepo* L.	n.d.	10	0.05%	682	3.16%	3	30.00%
CYNAR_CAR	*Cynara cardunculus* L.	Marrow or courgette	603	3.03%	83	0.38%	79	13.10%
DAUCU_CAR	*Daucus carota* L.	Globe artichoke + Cardoon	68	0.34%	475	2.20%	15	22.06%
FOENI_VUL	*Foeniculum vulgare* Mill.	Carrot + Fodder carrot	459	2.30%	97	0.45%	16	3.49%
LACTU_SAT	*Lactuca sativa* L.	Fennel	96	0.48%	2,243	10.39%	1	1.04%
LAGEN_SIC	*Lagenaria siceraria* (Molina) Standl.	Lettuce	2,214	11.11%	17	0.08%	29	1.31%
PETRO_CRI	*Petroselinum crispum* (Mill.) Nyman ex A. W. Hill	n.d.	17	0.09%	108	0.50%	0	0.00%
PHASE_COC	*Phaseolus coccineus* L.	Parlsey	97	0.49%	53	0.25%	11	11.34%
PHASE_VUL	*Phaseolus vulgaris* L.	Runner bean	76	0.38%	1,224	5.67%	-23	-30.26%
PISUM_SAT	*Pisum sativum* L.	Dwarf french bean + Climbing french bean	1,127	5.65%	618	2.86%	97	8.61%

Table S1. *Cont.*

Species list (UPOV code)	*Species list (UPOV name)*	*37th edition name*	*Number of registered varieties for species (2018)*	*Number of registered varieties for species (%)(2018)*	*Number of registered varieties for species (2023)*	*Number of registered varieties for species (%)(2023)*	*Variation between 2018 and 2023 data*	*Variation between 2018 and 2023 data (%)*
RAPHA_SAT_NIG	*Raphanus sativus* L. var. *niger* (Mill.) S. Kerner	Wrinkled pea + Round pea + Sugar pea	637	3.20%	93	0.43%	-19	-2.98%
RAPHA_SAT_SAT	*Raphanus sativus* L. var. *sativus*	Black radish	61	0.31%	304	1.41%	32	52.46%
RHEUM_RHB	*Rheum rhabarbarum* L.	Radish	297	1.49%	24	0.11%	7	2.36%
SCORZ_HIS	*Scorzonera hispanica* L.	Rhubarb	22	0.11%	7	0.03%	2	9.09%
SICYO_ANG	*Sicyos angulatus* L.	Scorzonera, Black salsify	6	0.03%	1	0.00%	1	16.67%
SOLAN_AET	*Solanum aethiopicum* L.	n.d.	1	0.01%	2	0.01%	0	0.00%
SOLAN_LCH	*Solanum lycopersicum* L. x *Solanum cheesmaniae* (L. Ridley) Fosberg	n.d.	1	0.01%	3	0.01%	1	100.00%
SOLAN_LHA	hybrids between *Solanum lycopersicum* L. and *Solanum habrochaites* S. Knapp & D.M. Spooner	n.d.	0	0.00%	70	0.32%	3	n.a.
SOLAN_LPE	*Solanum lycopersicum* L. x *Solanum peruvianum* (L.) Mill.	n.d.	52	0.26%	3	0.01%	18	34.62%
SOLAN_LPI	*Solanum lycopersicum* x *Solanum pimpinellifolium*	n.d.	3	0.02%	9	0.04%	0	0.00%
SOLAN_LYC	*Solanum lycopersicum* L.	n.d.	4	0.02%	4,202	19.46%	5	125.00%
SOLAN_MEL	*Solanum melongena* L.	Tomato	3,675	18.44%	389	1.80%	527	14.34%
SOLAN_MET	hybrids between *Solanum melongena* L. and *Solanum aethiopicum* L.	Aubergine, eggplant	326	1.64%	1	0.00%	63	19.33%
SOLAN_MTO	*Solanum melongena* × *Solanum torvum*	n.d.	1	0.01%	2	0.01%	0	0.00%
SOLAN_TOR	*Solanum torvum* Sw.	n.d.	3	0.02%	14	0.06%	-1	-33.33%
SPINA_OLE	*Spinacia oleracea* L.	n.d.	14	0.07%	335	1.55%	0	0.00%
VICIA_FAB_MAJ	*Vicia faba* L. var. *major* Harz	Spinach	327	1.64%	117	0.54%	8	2.45%
VLRNL_LOC	*Valerianella locusta* (L.) Laterr.	Broad bean	115	0.58%	69	0.32%	2	1.74%
ZEAAA_MAY_MIC	*Zea mays* L. *saccharata* Koern.	Corn salad, lamb's lettuce	55	0.28%	86	0.40%	14	25.45%
ZEAAA_MAY_SAC	*Zea mays* L. convar. *microsperma* Koern.	Popcorn	78	0.39%	296	1.37%	8	10.26%

Table S2. Conservation varieties registered, classified for species.

Species list (UPOV code)	*Species list (UPOV name)*	*37th edition name*	*Number of registered varieties for species (2018)*	*Number of registered varieties for species (%) (2018)*	*Number of registered varieties for species (2023)*	*Number of registered varieties for species (%) (2023)*	*Variation between 2018 and 2023 data*	*Variation between 2018 and 2023 data (%)*
ALLIU_CEP_AGG	*Allium cepa* L. var. *aggregatum* G. Don	Shallot	0	0%	1	0.52%	1	n.a.
ALLIU_CEP_CEP	*Allium cepa* L. var. *cepa*	Onion	15	11.11%	15	7.85%	0	0%
ALLIU_POR	*Allium porrum* L.	Leek	3	2.22%	3	1.57%	0	0%
ALLIU_SAT	*Allium sativum* L.	Garlic	8	5.93%	9	4.71%	1	12.50%
APIUM_GRA_DUL	*Apium graveolens* L. var. *dulce* (Mill.) Pers.	Celery	1	0.74%	1	0.52%	0	0%
BETAA_VUL_GVF	*Beta vulgaris* L. ssp. *vulgaris* var. *flavescens* DC. f. *crispa*	Spinach beet or Chard	2	1.48%	2	1.05%	0	0%
BRASS_OLE_GAS	*Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L.	Curly kale	2	1.48%	1	0.52%	-1	-50.00%
BRASS_OLE_GCA	*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC.	White cabbage	5	3.70%	8	4.19%	3	60.00%
BRASS_OLE_GCS	*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L.	Savoy cabbage	0	0%	1	0.52%	1	n.a.
BRASS_OLE_GGO	*Brassica oleracea* L. var. *gongylodes* L.	Kohlrabi	1	0.74%	1	0.52%	0	0%
BRASS_OLE_PAL	*Brassica oleracea* L. var. *palmifolia* DC.	n.d.	1	0.74%	1	0.52%	0	0%
BRASS_RAP_RAP	*Brassica rapa* L. var. *rapa* (L.) Thell.	Turnip	4	2.96%	8	4.19%	4	100%
CAPSI_ANN	*Capsicum annuum* L.	Chili, pepper	22	16.30%	28	14.66%	6	27.27%
CICHO_END	*Cichorium endivia* L.	Curled leaved endive + Plain leaved endive	1	0.74%	1	0.52%	0	0%
CICHO_INT	*Cichorium intybus* L.	Witloof chicory	1	0.74%	2	1.05%	1	100%
CICHO_INT_FOL	*Cichorium intybus* L. var. *foliosum* Hegi	Large-leaved chicory / Italian chicory	2	1.48%	2	1.05%	0	0%

Table S2. *Cont.*

Species list (UPOV code)	***Species list (UPOV name)***	***37th edition name***	***Number of registered varieties for species (2018)***	***Number of registered varieties for species (%) (2018)***	***Number of registered varieties for species (2023)***	***Number of registered varieties for species (%) (2023)***	***Variation between 2018 and 2023 data***	***Variation between 2018 and 2023 data (%)***
CTRLS_LAN	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Water-melon	1	0.74%	1	0.52%	0	0%
CUCUM_MEL	*Cucumis melo* L.	Melon	0	0%	3	1.57%	3	n.a.
CUCUM_SAT	*Cucumis sativus* L.	Cucum-ber + Gherkin	0	0%	2	1.05%	2	n.a.
CUCUR_MAX	*Cucurbita maxima* Duchesne	Gourd	1	0.74%	1	0.52%	0	0%
CUCUR_MOS	*Cucurbita moschata* Duchesne	n.d.	1	0.74%	1	0.52%	0	n.a.
CUCUR_PEP	*Cucurbita pepo* L.	Marrow or cour-gette	2	1.48%	3	1.57%	1	50.00%
CYNAR_CAR	*Cynara cardunculus* L.	Globe ar-tichoke + Cardoon	2	1.48%	2	1.05%	0	0%
LACTU_SAT	*Lactuca sativa* L.	Lettuce	4	2.96%	8	4.19%	4	100%
PETRO_CRI	*Petroselinum crispum* (Mill.) Nyman ex A. W. Hill	Parlsey	1	0.74%	1	0.52%	0	0%
PHASE_VUL	*Phaseolus vulgaris* L.	Dwarf french bean + Climbing french bean	26	19.26%	36	18.85%	10	38.46%
PISUM_SAT	*Pisum sativum* L.	Wrinkled pea + Round pea + Sugar pea	3	2.22%	6	3.14%	3	100%
RHEUM_RHB	*Rheum rhabarbarum* L.	Rhubarb	1	0.74%	1	0.52%	0	0%
SOLAN_LYC	*Solanum lycopersicum* L.	Tomato	20	14.81%	35	18.32%	15	75.00%
SOLAN_MEL	*Solanum melongena* L.	Auber-gine, eggplant	2	1.48%	2	1.05%	0	0%
VICIA_FAB_MAJ	*Vicia faba* L. var. *major* Harz	Broad bean	3	2.22%	5	2.62%	2	66.67%

Table S3. Vegetable varieties registered in CC for countries.

Country list	Country list (complete name)	Number of registered varieties for Country (2018)	Number of registered varieties for Country (%) (2018)	Number of registered varieties for Country (2023)	Number of registered varieties for Country (%) (2023)	Variation between 2018 and 2023 data	Variation between 2018 and 2023 data (%)
AT	Austria	115	0.58%	283	1.311%	168	146.09%
BE	Belgium	31	0.16%	28	0.130%	-3	-9.68%
BG	Bulgaria	263	1.33%	299	1.385%	36	13.69%
CY	Cyprus	11	0.06%	28	0.130%	17	154.55%
CZ	Czechia	936	4.72%	1,181	5.469%	245	26.18%
DE	Germany	454	2.29%	621	2.876%	167	36.78%
DK	Denmark	74	0.37%	18	0.083%	-56	-75.68%
EE	Estonia	25	0.13%	27	0.125%	2	8.00%
EL	Greece	239	1.21%	224	1.037%	-15	-6.28%
ES	Spain	1,793	9.04%	1,992	9.225%	199	11.10%
FR	France	2,468	12.44%	2,880	13.338%	412	16.69%
HR	Croatia	29	0.15%	62	0.287%	33	113.79%
HU	Hungary	1,042	5.25%	1,298	6.011%	256	24.57%
IT	Italy	1,639	8.26%	1,909	8.841%	270	16.47%
LT	Lithuania	31	0.16%	22	0.102%	-9	-29.03%
LU	Luxembourg	0	0%	1	0.005%	1	n.a.
LV	Latvia	6	0.03%	6	0.028%	0	0%
NL	Netherlands	8,419	42.45%	8,552	39.605%	133	1.58%
NO	Norway	10	0.05%	17	0.079%	7	70.00%
PL	Poland	794	4.00%	846	3.918%	52	6.55%
PT	Portugal	443	2.23%	462	2.140%	19	4.29%
RO	Romania	330	1.66%	431	1.996%	101	30.61%
SE	Sweden	18	0.09%	63	0.292%	45	250.00%
SI	Slovenia	42	0.21%	135	0.625%	93	221.43%
SK	Slovakia	170	0.86%	208	0.963%	38	22.35%

Table S4. Vegetable conservation varieties registered, classified for countries.

Country list	**Country list (complete name)**	***Number of registered varieties for country (2018)***	***Number of registered varieties for country (%) (2018)***	***Number of registered varieties for country (2023)***	***Number of registered varieties for country (%) (2023)***	***Variation between 2018 and 2023 data***	***Variation between 2018 and 2023 data (%)***
BE	Belgium	2	1.48%	2	1.05%	0	0%
BG	Bulgaria	3	2.22%	4	2.09%	1	33.33%
DE	Germany	1	0.74%	6	3.14%	5	500.00%
EE	Estonia	1	0.74%	3	1.57%	2	200.00%
EL	Greece	1	0.74%	3	1.57%	2	200.00%
ES	Spain	38	28.15%	57	29.84%	19	50.00%
FR	France	3	2.22%	8	4.19%	5	166.67%
HR	Croatia	25	18.52%	26	13.61%	1	4.00%
HU	Hungary	7	5.19%	13	6.81%	6	85.71%
IT	Italy	36	26.67%	43	22.51%	7	19.44%
LT	Lithuania	0	0%	1	0.52%	1	n.a.
LV	Latvia	1	0.74%	1	0.52%	0	0%
NL	Netherlands	0	0%	1	0.52%	1	n.a.
NO	Norway	2	1.48%	3	1.57%	1	50.00%
PT	Portugal	4	2.96%	3	1.57%	-1	-25.00%
RO	Romania	2	1.48%	1	0.52%	-1	-50.00%
SE	Sweden	2	1.48%	6	3.14%	4	200.00%
SI	Slovenia	7	5.19%	10	5.24%	3	42.86%

Chapter 4

Traditional Italian Agri-Food Products: A Unique Tool with Untapped Potential

agriculture

MDPI

Article

Traditional Italian Agri-Food Products: A Unique Tool with Untapped Potential

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Abstract: In the agri-food market, there is an increasing interest in local and traditional food products. In a context characterised by private labels and European Geographical Indications (GIs), the Italian Traditional Agri-food Product (TAP) denomination seems to be a particularly interesting tool for the promotion of agri-food products. This work analysed the effectiveness of this denomination in promoting local and traditional Italian products with a particular focus on vegetable products and landraces, which is the most represented category in the TAP list. The analysis included literature and bureaucratic reviews, a questionnaire administered to consumers and a comparison of the TAP denomination with European GI schemes in order to identify the opportunities, strengths and weaknesses of the TAP denomination. True to the SWOT analysis, the TAP denomination appears not to very incisive in terms of commercial protection and promotion, although it can represent a useful first step for the designation of traditional Italian products to the European GI schemes (56.94% conversion rate) and the unique recognition of Italian cultural heritage. In conclusion, the suggestion is to improve the TAP denomination by developing activities to increase consumer awareness, by allocating more financial resources for TAP productions (local products) and by proposing better integration with regional and private labels to protect the unique characteristics of Italian traditional agri-food products.

Keywords: common agricultural policy; cultural heritage; marketing; promotion; rural development; SWOT analysis; traditional agri-food products; vegetables

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1. Introduction

Local and traditional agri-food products have seen increasing interest from European consumers in recent years [1,2]. Already in 2017, Italian consumers expressed a marked preference for buying products with the following features: products produced predominantly in Italy—also defined as "made in Italy"—(74.1%), with the origin certified by the European schemes of Geographical Indication (GI) and Traditional Specialities (TSG) (53.1%), whose origin is close to the point of purchase ("km 0" products) (59.3%) and sea- sonal (80.4%) [3]. At the same time, the COVID-19 pandemic has accentuated the need to encourage sustainable, resilient and environmentally balanced production typical of local production [4–6]. Moreover, the growing interest of consumers towards the healthiness of products—expressed in terms of food safety and nutritional aspects of the product—and the spread of new purchasing channels (e.g., online market), favoured by the pandemic situation, have allowed the greater commercial development of local and traditional food products [2,7–10].

Despite these trends, the characteristics and definitions of a "local product" and "traditional product" are still not unambiguously defined. A

product is generally defined as local if the area in which it is marketed coincides with the production area, although there is no unique definition of the so-called local area [1,11]. For example, in the United States, this distance can be hundreds of kilometres, including the entire provincial territory and beyond; in France, instead, the so-called "short circuit" ("*circuit court*") is defined as 150 km from the production area; in Italy, "kilometre zero products" are defined as "agricultural and livestock products, including aquaculture . . . originating from places of production and processing of the raw material or primary agricultural raw materials used at a distance of not more than 70 km from the place of production or processing sale, or coming from the same province of the place of sale" [12].

Regarding traditional products—the subject of this work—the concept has been de- fined in the literature as "a product frequently consumed or associated with particular celebrations or seasons, normally transmitted from one generation to the next, produced precisely according to the gastronomic heritage, with little or no processing or handling, distinguished and known for its sensory properties and associated with a certain local area, region or country" [13]. This definition is based on the opinions collected among consumers during the TRUEFOOD Project (Traditional United Europe Food), an integrated project funded by the European Commission in 2009, whose aim was to improve quality and safety and introduce innovation into traditional European food production systems through research, demonstration, dissemination and training activities [14].

In Italy, the legislature has chosen to protect traditional products by giving them an explicit definition and a specific method of identification, defining traditional agri-food products (TAPs) as those products "whose methods of processing, storage and maturing are consolidated over time". For the identification of these products, "the regions and autonomous provinces […] ensure that these methods of processing are carried out on their territory in a homogeneous manner and according to traditional rules and protracted over time; however, for a period not less than twenty-five years" (article 1, paragraph 1, D.M. 8 September 1999, n. 350) [15]. This denomination was introduced in the Italian legislation with the adoption of article 8, paragraph 1, Legislative Decree (D. Lgs.) n. 173/1998 [16]. The Italian legislator has included this rule in a program of enhancement of the national gastronomic heritage whose purpose, among others, is to "promote and disseminate typical Italian quality food products […] as part of an integrated programme to enhance the national cultural, craft and tourist heritage" (article 8, paragraph 3, D.Lgs. n. 173/1998) [16]. The objective of this recognition is, therefore, to promote and valorise the products that are recognised as traditional in each Italian region, in order to promote the national gastronomic heritage and strengthen small- and medium-sized farms structurally. Previously, the decree provided for an identifying mark for TAPs and an atlas of TAPs, integrated with references to the cultural, artisanal and artistic heritage that these products intrinsically present; provisions that were never entirely fulfilled.

The one mentioned in the Italian legislation is the only formal definition that identifies this category of products [13,17,18]; the term "traditional agri-food products", in fact, is not found in any other national European legislation.

The only other use of the term "traditional" referring to agri-food products is offered in jurisprudence in Regulation (EU) n. 1151/2012, in which "traditional" is defined as "the proven use on the national market for a period that allows to pass on knowledge from one generation to another; this period must be at least thirty years" (article 3, paragraph 1, pt. 3) [18,19], a definition that is applied only in European Traditional Speciality Guaranteed (TSG) scheme.

The aim of this work was to analyse the current effectiveness of TAP recognition in relation to the objectives set by Italian legislation, considering the hypothesis that despite the recognised status of more than 5000 traditional food products in Italy, actually this recognition is not sufficient to promote these products on the Italian and international markets. Furthermore, we considered the assumption that TAP recognition is currently not extensively known in the national territory and has not been the subject of particular attention by the scientific community in the past. Finally, we considered the assumption that this recognition is, despite these drawbacks, a useful promotional lever for Italian TAPs, which could be used to support other trademarks or commercial initiatives. Therefore, an overall assessment of this recognition was made, considering: (a) the state of the art in the academic research; (b) the bureaucratic procedure and the current state of the art; (c) market perception and knowledge of the TAP denomination by the consumers; (d) the current and potential commercial effectiveness of TAP recognition. The results of these analyses were summarised and presented in a SWOT analysis, developed with the aim of assessing the efficiency of the TAP denomination in terms of the promotion and valorisation of traditional Italian agri-food products. Finally, considering the results of the developed analyses, the future opportunities for this instrument were explored, with particular attention paid to traditional vegetable products. This category of products is in fact deserving of special attention, as it is representative not only of food traditions but also of Italy's rich horticultural agrobiodiversity, composed of a great number of cultivated plants and wild species that often are enhanced historically in traditional preparations. The list of TAPs includes numerous local varieties (also called "landraces"), wild herbs and traditional preparations involving their use, which are representative of the agrobiodiversity that exists in the different Italian regions. These categories of products represent one of the expressions of agrobiodiversity to be safeguarded for various aspects, including historical and cultural ones. Therefore, enhancing and promoting such preparations means protecting indigenous varietal genetic resources (VGR) and especially local varieties, defined as follows: "a local variety of a crop that reproduces by seed or vegetative propagation is a variable population, however well identifiable, and usually has a local name. It has not been the subject of an organised program of genetic improvement, is characterised by specific adaptation to the environmental and growing conditions of a given area, and is closely associated with the customs, knowledge, habits, dialects, and recurrences of a human population that has developed it or continues its cultivation" [20].

2. Materials and Methods

In order to better illustrate the methodological procedures used in the

research, a flowchart summarising the performed activities is presented below (Figure 1).

To our best knowledge, the literature lacks information with regard to a comprehensive analysis of TAP recognition. For this reason, the first activity was to analyse the state of the art of the academic research on TAP recognition, with the aim of identifying benchmark scientific papers analysing TAP recognition comprehensively or in one of its applications. In order to do this, we chose the search terms "TITLE-ABS-KEY (("traditional agri-food products") OR ("traditional food products") AND (LIMIT-TO (DOCTYPE, "ar") OR LIMIT- TO (DOCTYPE, "re") OR LIMIT-TO (DOCTYPE, "cp"))" as key terms for searching within the article title, abstract and keywords using the Scopus database. For the literature review, we considered only articles, conference papers and reviews, excluding book chapters. Quality criteria, such as journal rankings, were not used for exclusion purposes because this research aimed to give a comprehensive academic overview of the TAP denomination. Subsequently, a review protocol for the content analysis of the publications was determined. The review protocol encompassed two sections: (i) bibliographic data for each publication, such as the author(s), year and title of the publication; authors' affiliations; and type of publication (and if it was a journal the journal's name); (ii) the content of the publication. Finally, a manual selection of articles was carried out, identifying those articles that placed the main focus on TAP recognition and excluding bibliographies that only mentioned TAPs but did not analyse any aspect of this recognition. For example, we discarded an article that was concerned to highlight the distinctive characteristics of four TAPs, "Caprino", "Pecorino", "Vaccino" and "Cacioricotta", with the objective to compare the microbiological and biochemical characteristics of these cheeses [21]. In this article, TAP recognition was only used to emphasise the traditional character of the products considered and was not in line with our research objectives.

After the literature review, the Italian legislation was perused to evaluate both the TAP definition and characteristics. To better characterise what this denomination has achieved over the years, the current composition of the national list of TAPs, defined by the Ministerial Decree (D.M.) of 25 February 2022 [22], was also analysed through a statistical analysis. The distribution of TAPs in the different Italian regions and among the different product categories were then defined with reference to vegetable products, as well as the impact that the latter category has on the total number of TAPs for each region.

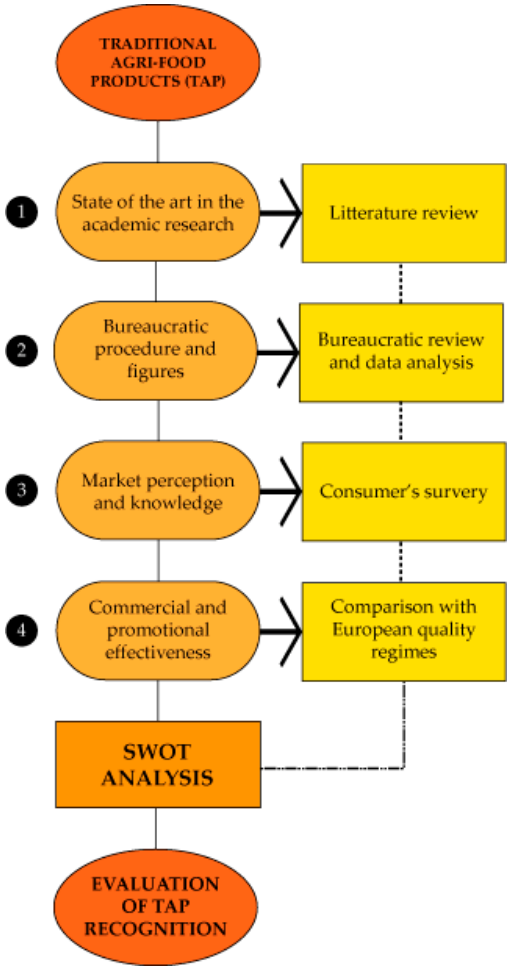

Figure 1. Flowchart of the research activities.

To assess the impact that the TAP recognition has commercially on consumers, with reference to the objective of the diffusion and promotion of Italian agri-food products, a consumer questionnaire was developed and disseminated online between 20 and 30 September 2022. The questionnaire was prepared to understand the consumers' knowledge of TAP recognition, whether TAP recognition provides added value in the purchasing process of agri-food products and what the perception is of consumers about traditional products. The questionnaire participants' eating habits were also assessed with a focus on vegetable products to validate the hypothesis that these products are indeed important and widespread in the Mediterranean diet and traditional cuisine, supporting the focus that this work places on this category of products. In proposing the questionnaire to the public, the aim was to collect the most objective data possible by administering it in a random manner to consumers other than stakeholders, avoiding sector researchers or manufacturing companies. For this reason, the final questionnaire was developed and disseminated online with a Google Form; the distribution was achieved by attempting to involve the average consumers from all Italian provinces through the sharing of the questionnaire in relevant Facebook groups of the main provincial capitals. Previously, a first version of the questionnaire in paper form was disseminated among consumers for a pilot test carried out during two local events

linked to the promotion of Apulian TAP, organised in Zollino (LE) (14 July 2022) and Ostuni (BR) (21 July 2022). As part of these events, 50 test questionnaires were collected, which provided important feedback that made it possible to rework the questionnaire so that it would be clearer to respondents and enhance it with more specific questions on traditional and local vegetable products.

The final questionnaire (available in the Supplementary Materials) comprised three sections: a first biographical section; a second section dedicated to the consumption habits of vegetable products, composed of seven questions (Q1–Q7) (Supplementary Materials); and a final section dedicated to assessing the consumer awareness of TAP recognition and its current and potential impact on the Italian agri-food market, also composed of seven questions (Q8–Q14) (Supplementary Materials). The questions in which respondents were asked to give a score to certain parameters (Q4–Q7, Q12, Q13) (Supplementary Materials) were developed according to a 5-point Likert scale, assigning a value from 1 (lowest score) to 5 (highest score) to the different answers in the analysis phase.

Regarding the data processing, a descriptive statistical analysis was developed. To assess the correlation between the answers offered by the respondents regarding the different perceptions between local and traditional products (Q6, Q7) (Supplementary Materials), a Wilcoxon test was instead developed for non-parametric variables—with the data not being distributed as a normal, due to the use of the Likert scale—using the statistical software R-Studio. Regarding Q13 (Supplementary Materials), which asked participants to list at least three regional TAPs of which they were aware, only answers with three or more valid TAPs were considered valid. In any case, all TAPs indicated were divided by category and Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) or TSG products were excluded. The responses indicating generic product categories (e.g., courgettes, aubergines, onions) were also considered invalid, unless these products were included among the regional TAP with this name (e.g., table grapes, Apulia cherries, mozzarella).

Finally, TAP recognition was compared with the main instruments for the valorisation and promotion of agri-food products recognised in Europe: the PDO, PGI and TSG marks. The analysis was developed both from a regulatory aspect, by comparing the laws governing these instruments, and from a statistical aspect, by comparing the products registered in the different lists. In this last regard, the D.M. of 25 February 2022, containing the 22nd revision of the national list of TAPs, and the European GI Register updated on 8 September 2022 were considered as data sources. The descriptive statistical processing of the data was subsequently performed. The average registration time required to register Italian agri-food products in one of the indicated registers was also calculated; to do this, the average was calculated on the days of difference between the date of submission of the registration application for PDO, PGI and TSG products and the effective registration of the agri-food product in the lists.

A subsequent analysis concerned the calculation of the conversion rate of TAPs into PDO, PGI and TSG products to assess the potential leverage effect that the registration of a product among the TAPs could have for greater

valorisation through European trademarks. This analysis was developed considering that agri-food products cannot be simultaneously recognised as a TAP and as a PDO or PGI [23], as better explained in Section 3.2. To develop this analysis, the number of Italian PDO, PGI and TSG products (n. 216) whose registration date was after the publication of the first national list of TAPs, which took place with the

D.M. of 18 July 2000 [24], was considered as the basis for calculation, compared with the number of TAPs that subsequently became PDO, PGI or TSG products (n. 123):

$$Tap\ convertion\ rate\ (\%) = \frac{Tot.Converted\ TAP}{Tot.PDO,PGI,TSG\ (registered\ after\ D.M.18\ July\ 2000)} \quad (1)$$

In addition, the distribution of converted TAPs in the different categories was calculated by relating the number of converted TAPs in each category to the total number of converted TAPs:

$$Converted\ TAP\ distribution\ by\ category\ (\%) = \frac{Tot.Converted\ TAP\ for\ each\ category}{Tot.Converted\ TAP} \quad (2)$$

Finally, the impact that each product category has on the TAP conversion rate was calculated, comparing the number of TAPs converted per category with the number of TAPs that subsequently became PDO, PGI or TSG products:

$$TAP\ convertion\ rate\ by\ category\ (\%) = \frac{Tot.Converted\ TAP\ for\ each\ category}{Tot.\ PDO,PGI,TSG\ (registered\ after\ D.M.18\ July\ 2000)} \quad (3)$$

At the end of the work, a SWOT analysis was developed to assesses the internal strengths and weaknesses of the analysed element and also external opportunities and threats, following the model of previous work performed to investigate the effectiveness of other types of marks or awards for agri-food product promotion [17,25,26]. The SWOT analysis assesses the internal strengths and weaknesses of the analysed element and also external opportunities and threats. The distinction between strengths and opportunities and between weaknesses and risks is not always clear, so we tend to refer to two strategic dimensions to distinguish them: external and internal. The former refers to the reference environment and influences that are not dependent on the analysed element but which affect it; the latter, on the other hand, looks at the intrinsic and controllable characteristics of the element itself [27]. With this premise, all characteristics of TAP denomination that are inherent to the legislation or the data analysed were considered as strengths and weaknesses (internal factors); on the other hand, all commercial aspects or those collected as consumer opinions, which influence or may influence the efficiency of TAP denomination for the purposes of enhancing and promoting traditional Italian agri-food products, were considered as risks and opportunities (external factors).

3. Results

3.1. TAP Recognition: The Literature Research Results

The literature research developed using the Scopus database returned a total of 250 results. Of these, three articles were immediately discarded because they were published before 1998, the year of the introduction of TAPs into Italian regulations. Regarding the remaining articles, a manual selection process was developed to search out those articles that developed an analysis concerning TAPs or their applications. A total of 16 articles mentioning or analysing TAP recognition were found—all written by Italian research groups—of which only two articles considered TAPs as their main focus (Table 1).

Table 1. Results of the TAP literature review.

Author	Year	Title	Aim of study	Main Findings
Renna et al. [1]	2018	Traditional Agrifood Products: An Expression of Italian Cultural Heritage	Analysing the numbers and uses of TAP recognition in relation to vegetable landraces.	The article proposes the TAP denomination as a useful leverage for the promotion of "Made in Italy". Calling for the formulation of a model of enhancement, including a simple labelling regime and the creation of a national atlas of TAPs
Cafiero et al. [28]	2020	Traditional Agri-Food Products as a Leverage to Motivate Tourists: A Meta-Analysis of Tourism Information Websites	Provide evidence on the extent to which Traditional Agri-food Products (TAPs) constitute leverage to promote tourism in the province of Reggio Calabria, Italy	The database on the TAPs of the province of Reggio Calabria permits easy reading of the geographical distribution of the different categories of products, useful as a resource for further studies and as a local development policy support tool

3.2. TAP in Italy: Bureaucratic Procedures and Figures

TAPs were introduced by the Italian legislator in 1998 with D.Lgs. n. 173/1998. This legislative decree was followed by numerous rules in the years that have better defined the registration procedure, the purpose and the effects of this denomination. In addition to the already mentioned D.Lgs. n. 173/1998 and D.M. n. 350/1999, the regulatory framework is complemented by the Ministerial Circular (C.M.) n. 10 of 21 December 1999, in which the elements characterising the TAP identification board and the requirements for requesting a health derogation for registered TAPs have been defined [23]. In the same ministry circular, it is also specified that in the regional lists of TAPs it is not possible to insert products registered in PDO and PGI schemes; in the case of products previously registered in the regional lists of TAPs, it is necessary to remove TAPs from the regional lists at the moment of products' registration in the EU schemes. With the C.M. of 3 July 2000, protocol n. 62359 [29], three

important aspects of the TAP scheme have been defined: (a) the name identifying the traditional product cannot be registered as a trademark; (b) the geographical name under which the traditional product is identified cannot assume the value of proof of origin or provenance; (c) at the time of release for consumption, a product registered as TAPs may not qualify as "traditional" but may contain references to that denomination in the labelling. These indications are also present in the Italian legislation in articles 3 and 5 of the D.M. of 18 July 2000, with the addition that "the inclusion of a product in the aforementioned list is not constitutive of rights resulting from publication" (article 3). Finally, the D.M. 9 April 2008, recognised TAP as an expression of Italian cultural heritage [30].

Annually, the Ministry of Agricultural Food and Forestry Policies publishes with a special D.M. a revision of the national list of TAPs. The 22nd revision of the national list, published with D.M. 25 February 2022, counts 5450 TAPs, divided by region and into twelve product categories. The regions with the highest numbers of TAPs are Campania (580), Tuscany (464) and Lazio (456) (Table 2); the most represented product category is 'fresh pasta and bakery products, biscuits, pastries and confectionery' (29.65%), followed by 'natural or processed plant products' (28.94%) and 'fresh meat (and offal) and preparations' (15.08%) (Figure 2). As for the vegetable TAPs, in 2022 there were 911 vegetable products detected considering exclusively the category 'natural or processed plant products', which represented about 17% of the total number of TAPs. The region with the highest number of vegetable TAPs is Campania (126), followed by Tuscany (122), and Apulia (100) (Table 2). Comparing the number of vegetable TAPs with the total number of TAPs recorded for each region, the most performing regions are Puglia (30.40%), Tuscany (26.29%) and Basilicata (24.64%) (Table 2).

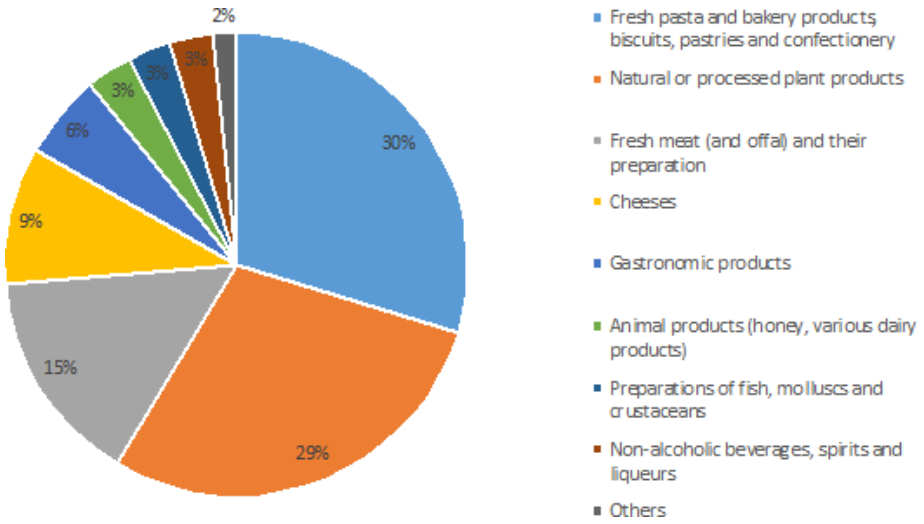

Figure 2. Distribution of TAPs for each product category (2022).

Table 2. TAP distribution in Italy, showing the total number of TAPs registered in each Italian region, number of TAPs registered in the 'natural or processed plant products' category for each region, number of vegetable TAPs in the 'natural or processed plant products' category for each region, and percentage of vegetable TAPs for each region in relation to the total number of TAPs.

Region	(A)	(B)	(C)	(D)
Abruzzo	149	30	17	11.41%
Aosta Valley	36	2	0	0.00%
Apulia	329	127	100	30.40%
Basilicata	211	81	52	24.64%
Bolzano Aut.Pr.	102	18	6	5.88%
Calabria	269	73	37	13.75%
Campania	580	240	126	21.72%
Emilia-Romagna	398	58	19	4.77%
Friuli-Venezia Giulia	181	49	32	17.68%
Lazio	456	110	73	16.01%
Liguria	300	105	45	15.00%
Lombardy	268	34	18	6.72%
Marche	154	42	13	8.44%
Molise	159	30	22	13.84%
Piedmont	342	94	70	20.47%
Sardinia	222	58	23	10.36%
Sicily	269	81	35	13.01%
Trento Aut.Pr.	105	16	11	10.48%
Tuscany	464	194	122	26.29%
Umbria	69	12	11	15.94%
Veneto	387	123	79	20.41%
Total	**5450**	**1577**	**911**	**16.72%**

3.3. Consumer Knowledge and Awareness of TAPs

Following the participatory research described in paragraph 2, a total of 317 questionnaires were collected in the survey conducted among consumers throughout Italy. The demographic composition of the respondents is shown in Table 3.

In the second section of the questionnaire, concerning consumer consumption habits of vegetable products, 304 answers were validated, corresponding to those consumers who declared themselves as in charge of food purchases in their household (Q1). Of these, the majority (80.26%) stated that they consume vegetable products in at least five to eight meals per week, with one in three consumers (31.91%) claiming to consume vegetables every day (Q2). The favourite purchasing locations of the interviewed consumers were 'small traditional shop' (36.72%) and 'supermarket, discount or hypermarket (medium and large size)' (36.72%), followed by 'local market or directly from the producer (also through group purchasing organisations)' (20.33%) and 'food shops within a shopping centre' (1.87%); among those who indicated other sources of purchase (4.36%), half of them declared that they grow vegetables in their own personal garden (Q3). The parameter that most influences the purchase of vegetable products (Q4) is the seasonality of the products—a character that almost all the interviewees (93.75%) declared to follow during the purchasing process (Q5)—followed, in order, by the nutritional aspects and the regional origin of the product. On the other hand, the traditionality of the product (expressed by the 'historical and cultural link with the territory of

origin' parameter), the price and the belonging of the vegetable product to a certified origin scheme (e.g., PDO, PGI) or organic regime were rated as less important in the purchasing process (Table 4).

Table 3. Sociodemographic distribution of the collected samples.

Variable	Levels	Frequency	(%)
Gender	Female	216	68.1%
	Male	101	31.9%
Age (in years)	< 18	1	0.3%
	18-35	73	23.0%
	35-64	217	68.5%
	> 64	26	8.2%
Education	Primary school qualification	0	0.0%
	Junior high school qualification	21	6.6%
	High school qualification	116	36.6%
	Bachelor's degree	32	10.1%
	Master's degree	105	33.1%
	Post graduate training/PhD	43	13.6%
Geographical Distribution	North-West	14	4.5%
	North-East	39	12.4%
	Centre	14	4.5%
	South	246	77.6%
	Islands	3	1.0%
Area of origin	Rural	43	13.6%
	Urban	274	86.4%
Economic status	Very difficult	25	7.9%
	Difficult	22	6.9%
	Stable	175	55.2%
	Satisfactory	80	25.2%
	Very satisfactory	15	4.7%
Occupation	Employee (public or private)	155	48.9%
	Entrepreneur	14	4.4%
	Freelance	39	12.3%
	Housewife	15	4.8%
	Retired	31	9.8%
	Student	23	7.3%
	Unemployed	23	7.3%
	Others	17	5.2%

Table 4. Average scores of influence parameters on the purchase of vegetable products, calculated from consumer responses.

Parameter	Mean	Standard Deviation
Historical and cultural link with the territory of origin	2.76	1.11
Nutritional aspects	3.28	1.09
Organic product	2.48	1.06
Price	2.65	0.78
Product of certified origin (PDO, PGI, etc.)	2.57	1.08
Regional origin of the product	3.14	1.08
Seasonality	3.59	1.04

At the end of the second section, the consumer perceptions of the characteristics of traditionality and locality, applied to a vegetable product, were calculated (Q6, Q7). For each of the parameters identified (e.g., qualitative,

environmental, social), an average score was calculated from the responses offered by the consumers in order to compare the two types of product characteristics (Table 5), similarly to what has been done in other studies comparing local and organic products [31].

Table 5. Average scores for characteristics of local and traditional food vegetable products, calculated based on responses of the questionnaire (Q6, Q7). LVP = average scores for 'local vegetable product' parameters; TVP = average scores for 'traditional vegetable products'; SD = standard deviation; S = significance test results.

Parameter	(LVP)	(SD)	(TVP)	(SD)	(S)
Healthier product	3.17	1.00	3.14	0.98	
Higher-quality product	3.18	0.95	3.07	0.92	*
Higher level of food safety	2.99	0.98	2.97	0.93	
Improved nutritional values	3.06	1.04	3.05	0.99	
Increased respect for local farmers' rights	3.17	1.02	2.99	1.05	***
More expensive product	2.64	1.04	2.67	1.01	
More sustainable product	3.46	0.99	3.18	1.04	***

Level of significance: '***' 0.001, '*' 0.05.

Finally, in the third section of the questionnaire it was calculated that of the 317 respondents, 190 (59.94%) stated that they were aware of the term "Traditional Agri-Food Product (TAP)" designation prior to the administration of the questionnaire (Q8). The main communication channels through which consumers learnt about TAPs were 'online (articles, websites, social networks, etc.)' (24.92%) and 'territorial promotion initiatives (meetings, events, fairs, festivals, etc.)' (24.60%), followed by 'mass media (television, radio, newspapers, etc.)' (22.01%) and 'books, academic publications and trade magazines' (20.71%). Among those who indicated other options (7.77%), work and university were the most reported sources (Q9). To verify these responses, we asked participants (Q10) to indicate among four different definitions (TAP, PDO, PGI and TSG) which was the correct definition of TAPs. In total, 119 respondents (37.54%) selected the correct option, which was followed by the PDO (30.28%), TSG (13.88%) and PGI (8.52%) definitions. The remaining 9.78% of participants stated that they did not know the correct answer. Furthermore, we asked the interviewees to list at least three TAPs from their region of origin or residence or from other Italian regions (Q14); only 72 participants (22.71%) were able to answer by listing at least three traditional products. Of the 317 participants, about half (49.84%) were not able to correctly list at least one TAP on the appropriate list. Considering all answers, the TAP category most known and mentioned by the participants was 'natural or processed plant products' (58.93%), followed by 'fresh meat (and offal) and preparations' (12.00%) and 'fresh pasta and bakery products, biscuits, pastries and confectionery' (11.20%) (Figure 3).

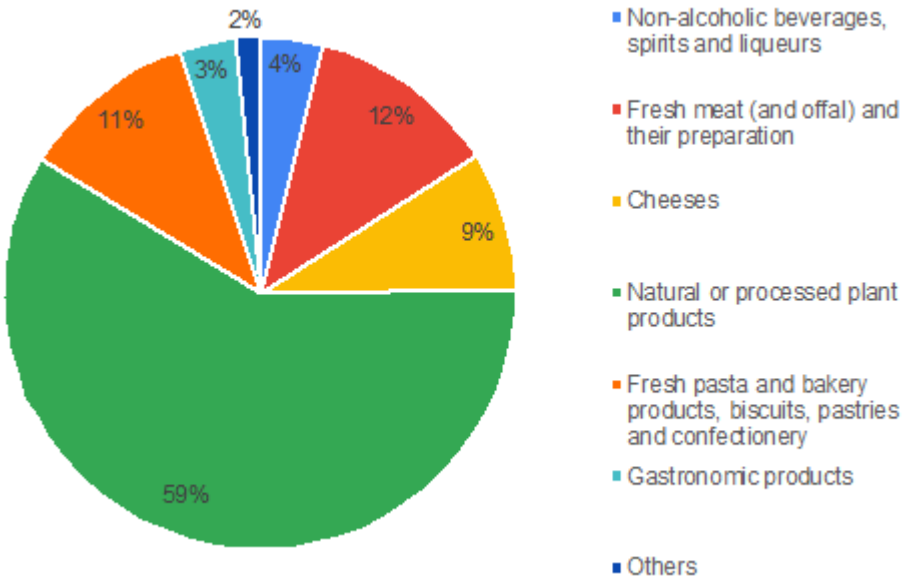

Figure 3. Consumer awareness of TAPs by category, calculated based on responses to the questionnaire (Q14).

In conclusion, we asked participants to indicate how easy it was for them to identify an agri-food product as a traditional product and whether their consumption choice was influenced by agri-food products belonging to the TAP list (Q12, Q13). For these parameters, calculated considering only the answers of the interviewees in charge of the purchase of agri-food products in their household, two similar values were found, with a higher average value calculated for recognisability (μ: 3.09; σ: 1.03) rather than for purchasing influence (μ: 2.99; σ: 0.96).

3.4. TAP and European Quality Regimes: An Analysis and Comparison

In their September 1996 session, the European Committee of the Regions (CoR) issued an opinion, published in Official Journal C/034 of 3 February 1997, expressing the need to "significantly improve the framework conditions in favour of typical products of the European Union" [32]. This opinion followed European Council Regulation n. 2081/92, which had already introduced into community legislation the definitions of designations of origin and geographical indications, as well as n. 2082/92 about the certificates of specific character for agricultural products and foodstuffs, which was subsequently channelled into the TSG regulations [33,34]. The legislation was then amended by Regulation (EC) n. 509/2006 and Regulation (EC) n. 510/2006 of the European Council, until reaching its final form with Regulation (EU) n. 1151/2012 of the European Parliament and of the council, supplemented and amended by Delegated Regulation (EU) n. 664-665/2014 and Implementing Regulation (EU) n. 668/2014 of the European Commission [35–37]. Regulation (EU) n. 1151/2012 defines:

- 'Protected Designation of Origin' (PDO): A name that identifies a product: (a) originating in a specific place, region, or in exceptional cases, a country; (b) whose quality or characteristics are essentially or

exclusively due to a particular geographical environment with its inherent natural and human factors; and (c) the production steps of which all take place in the defined geographical area.

- Protected Geographical Indication' (PGI): A name that identifies a product: (a) originating in a specific place, region or country; (b) whose given quality, reputation or other characteristic is essentially attributable to its geographical origin; and (c) at least one of the production steps of which takes place in the defined geographical area.
- 'Traditional Specialty Guaranteed' (TSG): A name that describes a specific product or foodstuff (a) that results from a mode of production, processing or composition corresponding to traditional practices for that product or foodstuff or (b) is produced from raw materials or ingredients that are traditionally used (article 18, paragraph 1). Furthermore, for a name to be registered as a traditional specialty guaranteed, it shall: (a) have been traditionally used to refer to the specific product; or (b) identify the traditional character or specific character of the product (article 18, paragraph 2).

For PDO and PGI products (also identified as GI products), therefore, the intrinsic link between the product's characteristics and its geographical origin—understood as the place of production—which must be precisely and unambiguously delimited, is relevant. This link is justified by the peculiar characteristics of the geographical environment (e.g., climate, soil, humidity) or human factors (e.g., cultivation techniques, processing, storage). For GI products, the character of traditionality is not expressly required in the European legislation, defined as "proven use on the national market for a period of time […] of at least thirty years" (article 3, Regulation (EU) n. 1151/2012). This character is instead expressly required for the TSG scheme. In the Italian regulations, on the contrary, the traditional link with the territory is also required for PDO and PGI products; as part of the application for registration, in fact, a "historical report, accompanied by bibliographical references, proving the production for at least twenty-five years, even if not continuous, of the product in question, as well as the consolidated use, in commerce or in common language, of the name for which registration is requested" is required (article 6, paragraph 1, D.M. of 14 October 2013) [38].

In 2022, 1592 GIs were registered in Europe, including 676 PDOs (42.46%) and 916 PGIs (57.54%). Among these, the most represented product class was 'fruit, vegetables and cereals fresh or processed' with 468 products (29.40%), followed by 'cheeses' (254 products, 15.95%) and 'meat products (cooked, salted, smoked, etc.)' (187 products, 11.75%). The countries with the highest numbers of registered GIs were Italy (315 products, 19.79% of registered European GI)s, France (259 GIs, 16.27%) and Spain (202 GIs, 12.69%). Considering only the category 'fruit, vegetables and cereals fresh or processed', Italy remained at the top of the ranking with 120 products (25.64% of the total GIs of the category) registered, of which 53 were vegetable products, compared to 56 Italian vegetable products in total, calculated considering all product classes, followed by Spain (63 vegetable GIs, 13.46%) and France (60 vegetable GIs, 12.82%).

As far as TSGs were concerned, a total of 68 traditional specialties had been registered in Europe by 2022. The European country with the largest number of TSG products was Poland with 10 registered products (14.71%), followed by Slovakia (7 TSGs, 10.29%), Belgium, Bulgaria and the Czech Republic (5 TSGs, 7.35%). The most represented product classes were 'meat products (cooked, salted, smoked, etc.)' with 17 TSGs (25.00%); 'bread, pastry, cakes, confectionery, biscuits and other baker's wares' (15 TSGs, 22.06%); and 'cheeses' (7 TSG, 10.29%). Only one product was registered in the category 'fruit, vegetables and cereals fresh or processed'. Italy had only 4 TSGs (5.88%) registered in 2022, none of which was in the category 'fruit, vegetables and cereals fresh or processed' or considered as vegetables.

By comparing Italian PDO, PGI and TSG products with TAPs included in the ministerial list since the first publication, the conversion rate of agri-food products recognised as traditional into products protected by IG designation or registered as a TSG was calculated. The conversion rate, considering products registered as a PDO, PGI and TSG after the publication of the first national list of TAP, was equal to 56.94%. The most converted categories were 'fruit, vegetables and cereals fresh or processed' (52.85%), of which the majority (55.36%) were vegetables; 'cheeses' (14.63%); and 'meat products (cooked, salted, smoked, etc.)' (10.57%). This ranking was also stable when considering the number of PDO, PGI and TSG products registered after the first publication of TAPs list, for which 30.09% of the products belonging to the category 'fruit, vegetables and cereals fresh or processed' were previously registered in the national list of TAP. Lower percentages were calculated for the categories 'cheeses' (8.33%) and 'meat products (cooked, salted, smoked, etc.)' (6.02%) (Table 6).

Having identified the reference legislation and reported the descriptive statistics, it was, therefore, possible to compare the TAP denomination's characteristics with those found in European PDO, PGI and TSG schemes. The comparison was aimed at identifying the weaknesses and strengths of the TAP denomination compared to the main tools for the valorisation and promotion of agri-food products active in Europe (Table A1).

Table 6. Numbers of PDO, PGI and TSG products registered; numbers of PDO, PGI and TSG product registered after the first TAPs list published on 21 August 2000; numbers of products registered in the past as TAPs and now registered as PDO, PGI or TSG products; rankings of TAP products converted to PGI, PDO or TSG products by product class; conversion rates for each category; and conversion rates by category.

Class of Product	(A)	(B)	(C)	(D)	(E)	(F)
Fruit, vegetables and cereals fresh or processed	120	96	65	52.85%	67.71%	30.09%
Cheeses	56	25	18	14.63%	72.00%	8.33%
Meat products (cooked, salted, smoked, etc.)	43	19	13	10.57%	68.42%	6.02%
Bread, pastry, cakes, confectionery, etc.	16	15	9	7.32%	60.00%	4.17%
Fresh fish, mollusks and crustaceans	6	6	5	4.07%	83.33%	2.31%
Pasta	5	5	4	3.25%	80.00%	1.85%
Oils and fats (butter, margarine, oil, etc.)	49	28	3	2.44%	10.71%	1.39%
Other products of Annex I of the Treaty	7	7	2	1.63%	28.57%	0.93%

Table 6. *Cont.*

Class of Product	(A)	(B)	(C)	(D)	(E)	(F)
Other products of animal origin	5	5	1	0.81%	20.00%	0.46%
Fresh meat (and offal)	6	5	1	0.81%	20.00%	0.46%
Chocolate and derived products	1	1	1	0.81%	100.00%	0.46%
Aromatised wines	1	0	0	0.00%	n.c.	0.00%
Prepared dishes	2	2	1	0.81%	50.00%	0.46%
Essential oils	1	1	0	0.00%	0.00%	0.00%
Salt	1	1	0	0.00%	0.00%	0.00%
Total	**319**	**216**	**123**	**100%**		**56.94%**

4. Discussion

In this study, for the first time, an analysis of TAP recognition and its applications for the purpose of enhancing and promoting traditional Italian agri-food products was carried out. We firstly discussed the research activities summarised in a SWOT analysis. Then, a focus on the protection and valorisation of traditional agri-food vegetable products was reported. Finally, we concluded the discussion section with an analysis of the future prospects and possible applications of TAP recognition for the promotion, protection and valorisation of traditional Italian agri-food products.

4.1. TAP Denomination for Protection and Valorisation of Traditional Agri-Food Products: SWOT Analysis

Compared to the PDO, PGI and TSG marks, the TAP denomination appears to have numerous shortcomings in terms of traditional agri-food products' valorisation and promotion, the objective for which it was introduced into the legislation. The lack of a production specification, protection of the registered name, a single trademark and a control body appear to be the most significant weaknesses for this denomination, which is not sufficient to protect and promote registered products on the market. The regulatory framework, there- fore, does not seem to recognise any specific commercial advantages to the Italian TAP [39], framing an instrument aimed at a mere census of traditional Italian agri-food products, susceptible to the risk of commercial fraud to the detriment of the consumer. Another threat noted is the possibility of the same product being present in different regional lists, registered under different names or with the same name but for different products. Nonetheless, the possibility of registering products by regions, autonomous provinces and other public bodies (e.g., universities, research centres), as well as the quick timeframes and the simplicity of the registration procedure, represent advantages that have allowed the registration of a much higher number of traditional products (′1700%) than the number recognised as PDO, PGI and TSG products. Moreover, the similarity between some of the requirements for TAP denomination and the application for PDO, PGI, and TSG products (description; years of production in the territory, bibliographic and historical references; area of origin, etc.) represents an advantage for the TAP denomination, whose descriptive sheets could serve as a basis for the drafting of PDO, PGI and TSG production specifications—as also demonstrated by the high conversion rate calculated (56.94%)—thereby overcoming some of the difficulties often encountered by producer associations during this phase [40].

Considering the opinions collected through the questionnaire, a further criticality found for the TAP denomination is the lack of correct information among consumers, who are not fully aware of the TAP definition and not able to correctly associate national food products with this denomination. Despite this, most of the interviewees admitted that they recognise the term 'traditional food product', demonstrating the high level of attention paid to this type of product. From a commercial point of view, the product's belonging to the TAP list was not considered an influential characteristic in the interviewees' choice of consumption; moreover, products defined as 'local' seem to perform better in terms of perceived quality than 'traditional' products. Finally, among the opportunities that this recognition would appear to offer as a result of the analysis conducted there is the lack of use of the recognition of TAPs as an expression of Italy's cultural heritage—which could be better utilised to promote these products—as well as the possibility of improving consumer awareness of these products, considering the high level of interest shown, including in the questionnaire responses.

By comparing these points, it was possible to develop a SWOT analysis, as shown in Figure 4.

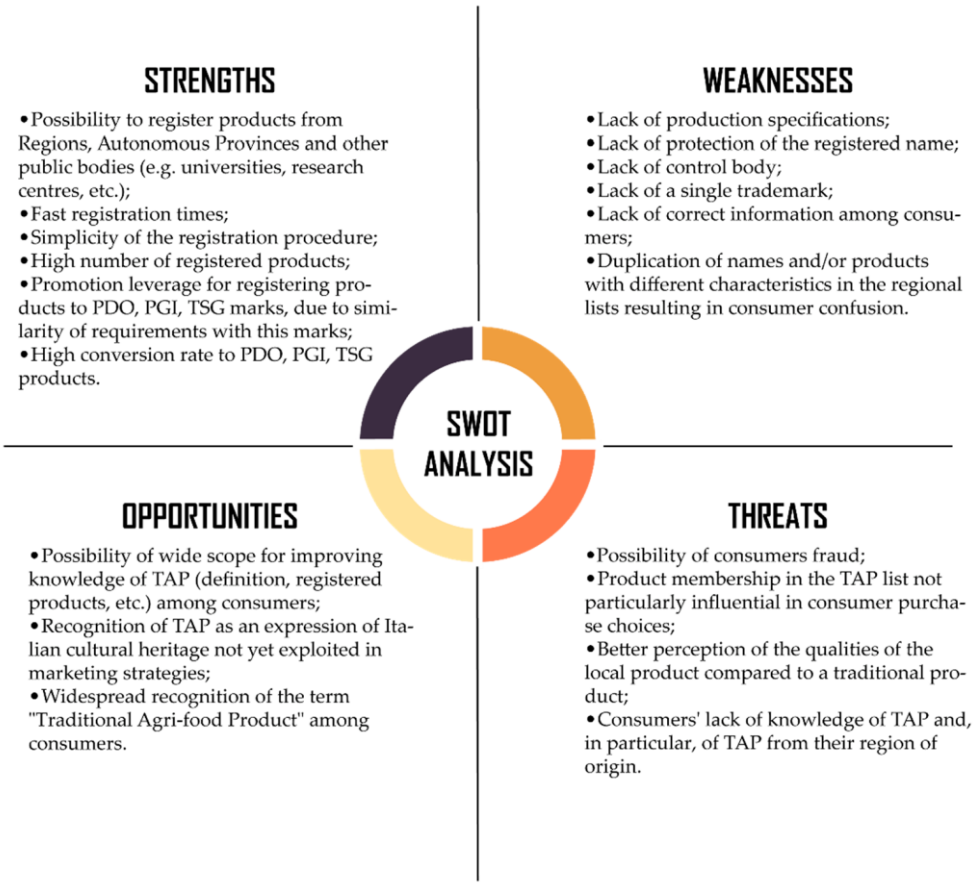

Figure 4. A SWOT analysis of the TAP denomination for the marketing and promotion of traditional Italian agri-food products.

4.2. A Focus on the Protection and Valorisation of Traditional Agri-Food Vegetable Products

Plant-based products, especially vegetables, represent an important sector of Italian agri-food production [41,42]; in fact, they are widely consumed products, as demonstrated by the results of the questionnaire administered to consumers, and a fundamental component of the Mediterranean diet [43,44]. Additionally, in terms of trademarks and awards, it has been calculated in the previous sections how plant-based products, and especially vegetables, are always among the most represented, protected and promoted categories; this is true both for products recognised among the GI and for products on the TAP list. Moreover, a strong correlation was found between these products and environmental sustainability, a topic of growing consumer interest, thanks to the increased use of traditional and more sustainable cultivation techniques in these production areas [40]. Considering these factors, it is easy to see that there is an ample margin for the improved promotion and protection of traditional vegetable agri-food products. However, producers often encounter numerous difficulties in the promotion of vegetable products, and in particular in the registration of vegetable products to European GI marks. On the one hand, there is a fragmented reality, in which most traditional crops are developed in small production areas or family gardens [45], a problem that is also accentuated by the limited ability of producers to come together in associations and consortia [40]; on the other hand, producers often find the procedures for registering and modifying PDO and PGI products, both at the national and European levels, excessively long and complex [46].

Thus, in terms of the protection and valorisation of this class of products, the TAP recognition may be useful specifically for the local landraces; for these small production areas, obtaining TAP recognition is a first step for the protection and promotion process. It is sufficient to demonstrate a link with the territory concerned for a period no shorter than 25 years and the request can be submitted by any public or private entity.

Nevertheless, as also shown in previous sections, TAP recognition alone cannot pro- vide sufficient protection and valorisation for local products and landraces; therefore, it is necessary to improve this model or supplement it with other instruments. In terms of valorisation, the European GI denomination offers a valid promotion and marketing solution, while in terms of protection, the Italian legislation and especially Italian regions in recent years have moved to develop plans to protect local varieties at high risk of genetic erosion. The Apulia region, for example, established Regional Law n. 39/2013 on the Regional Register of Autochthonous Genetic Resources, within which Apulian varieties and breeds at risk of genetic erosion are registered [47,48]; these genetic resources are subsequently also included within the National Register of Biodiversity of Agricultural and Food Interest, established by Law n. 194/2015 [49]. Some of these resources, such as the 'Carota di Polignano' [50–52], the 'Pomodoro Regina' [53,54], 'Carciofo locale di Mola' and 'Cavolo broccolo *mugnulu*' (Figure 5), are varieties also included within the TAP list, confirming the strong link that exists between agrobiodiversity and local traditions.

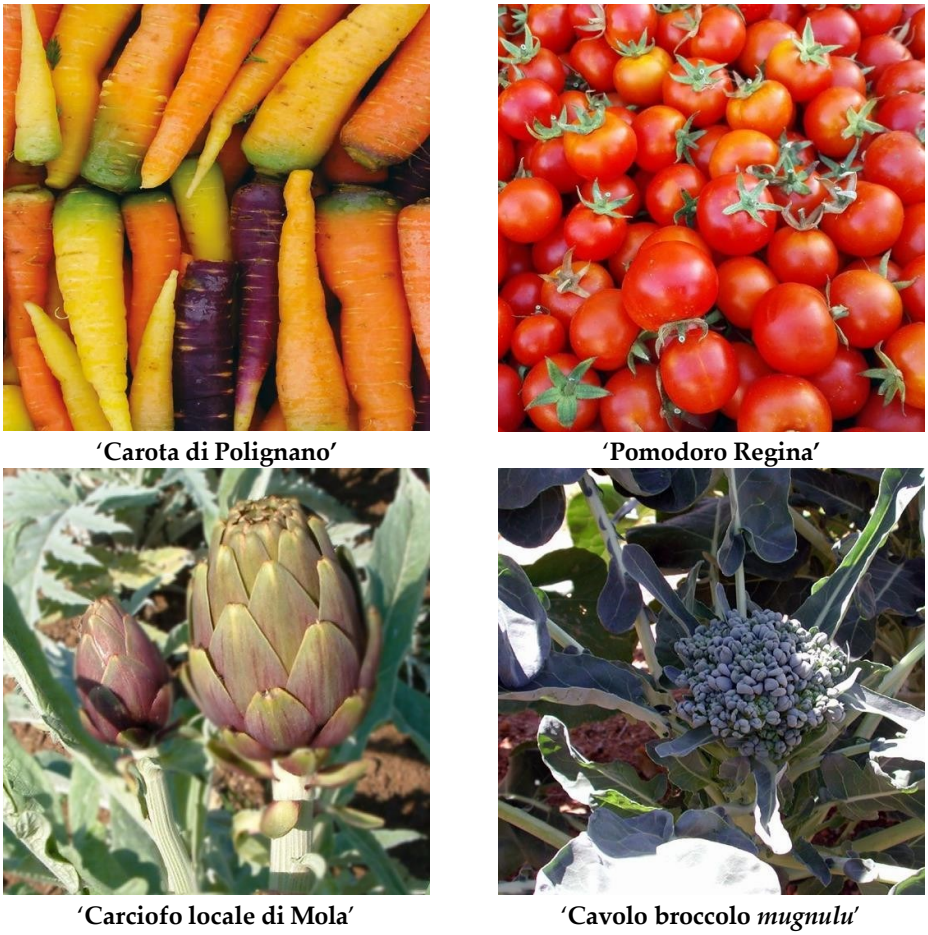

Figure 5. Some examples of Apulian TAPs also included in the Regional Register of Autochthonous Genetic Resources of Apulia Region and in the Italian Register of Biodiversity of Agricultural and Food Interest.

4.3. Italian Traditional Agri-Food Products: Future Evolutions of TAP Recognition

The TAP denomination, introduced more than twenty years ago in the Italian legislation, is a useful tool for the census of traditional Italian food products. In particular, products belonging to the categories 'fresh pasta and bakery products, biscuits, pastries and confectionery'; 'natural or processed plant products'; and 'fresh meat (and offal) and preparations' benefit from this tool. In addition, the TAP denomination appears to be familiar to average consumers, although they are not always able to correctly associate the term TAP with their region, neither do they have a clear definition of the Traditional Agri-Food Product denomination. Moreover, this denomination appears to be lacking in terms of promotion and commercial enhancement because it lacks its own unambiguous brand, production specifications and an established control system.

In view of this, the following actions could also be taken to improve the effectiveness of the TAP denomination: (1) The development of activities aimed at improving consumer awareness, including through the development of editorial products (e-books, web-sites, etc.) that are easy for consumers to consult, along the lines of what the Apulia region has done with the drafting

of the "Atlante dei Prodotti Agroalimentari Tradizionali di Puglia" and the website www.patpuglia.it (accessed on 2 May 2023) [55]. (2) The allocation of more financial resources to producers, associations and entities that promote and enhance the value of traditional Italian agri-food products through targeted operations closely linked to the territory of origin. Encouraging, in this regard, is an amendment in the 2022 budget law that allocated for the first time one million euros for the promotion of Italian TAPs [56]. In addition, the Lazio region (Central Italy), during the COVID-19 emergency in 2020, allocated measures to support restaurant operators and producers of PDO, PGI and TAP products in the Lazio region, in order to boost the recovery of the area, focusing on issues such as the enhancement of regional biodiversity and food safety [57]. (3) The development of a new European trademark; a further possibility could be to envisage a protection and promotion scheme developed along the lines of the "mountain products" label, established by Regulation (EU) n. 1151/2012 as part of the Optional Quality Terms. This label, whose registration procedures are far simpler than the GI labels, although it does not provide for production specifications, helps to protect and promote mountain products through the use of a single commercial trademark, obtainable through the compilation of a simple self- declaration by producers and a regional control system. (4) Greater coordination between the TAP recognition and the others promotion tools as regional trademarks (e.g., Apulian "Prodotti di Qualità" trademark—even though it has run into numerous problems related to incompatibility with European GI marks [58]) or private labels or initiatives (e.g., slow food movement promotion initiatives) [59].

Moreover, the still untapped potential of the TAP denomination is extensive. On the one hand, the attribution of the TAP denomination as a representative element of Italy's cultural heritage has never yet been valorised in marketing terms; on the other hand, the high number of products registered in the TAP list shows how much attention is given to this kind of product and how many products are still waiting to be commercially valorised, despite registration to European GI labels. In connection with the European GI labels, it is also necessary to consider that the European Commission, in its proposal for a Regulation of the European Parliament and of the Council of 31 March 2022, proposed a substantial modification of the TSG scheme, which was considered insufficient to protect and promote traditional European products [46]. For TSGs, one of the document's proposals states that "the TSG scheme is replaced by an official recognition of traditional agricultural and food products by member state authorities, with a limited list of criteria to be defined at the union level, while member states would notify the commission of the names of traditional products in order to make them public". A scheme similar to the Italian TAP, which could be a model to be followed throughout Europe, not only as a baseline for new regulatory provisions by the European Union's central administration, but also as a model for individual countries where the attention—scientific and commercial—for local and traditional products, is increasing. For example, there are case studies in the literature regarding national initiatives for protecting local products, with the establishment of national or regional commercial labels, and initiatives directed at promoting these products [26,60]; however, neither of these takes into consideration the

traditional character of these products, a trait that could be an added value that can be extrapolated from the Italian TAP model.

5. Conclusion

This work evaluated the TAP denomination from different points in order to give an overall view of its effectiveness in promoting local and traditional Italian products. The analyses developed allowed for an overall assessment of this recognition, highlighting its characteristics, weaknesses and potential, for the first time in a scientific study. More specifically, the results reported in the SWOT analysis highlighted the lower effectiveness of this denomination in comparison with European GI labels; at the same time, the TAP de- nomination may be considered a useful first step for the designation of local and traditional Italian products to the European GI schemes, also representing a unique recognition of Italian cultural heritage. With a particular focus on vegetables products, the category most represented among the TAPs, these could benefit more than other types of products with a greater effectiveness of the TAP denomination, also given the possibility of enhancing many local varieties that are an important expression of agrobiodiversity. With reference to this, it would be interesting in the future to analyse how these landraces could be protected and promoted by leveraging the TAP recognition. If from a commercial point of view the link with the PDO, PGI and TSG labels has been analysed in part by this paper, from a protection perspective, it might be interesting to link this recognition to the conservation variety scheme. In the final part of this paper, some future development prospects concerning TAP recognition were discussed, with a focus on the possibility of using TAP recognition as a model for future European brands, a topic left for possible future research.

Considering that this is the first sector research study entirely dedicated to TAP recognition and its applications in terms of the valorisation and promotion of traditional Italian agri-food products, it was not possible to have a benchmark to compare with the results obtained here, especially for the SWOT analysis results and for the consumer questionnaire. It would be useful in the future to replicate and expand the analysis among consumers, considering a wider catchment area, as well as to improve the section dedicated to the future prospects of Italian TAPs and vegetable products by referring to Italian or European success stories, with an analysis of the possible alternative promotion tools that are alternatives to the TAP and European GI schemes.

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Appendix A

Table A1. Comparison of the main characteristics of TAP, PDO, PGI and TSG denominations.

Characteristics	TAP	PDO	PGI	TSG
Traditionality and connection to the territory (years) *	Yes (25 years)	Yes (25 years—also non-continuous)	Yes (25 years—also non-continuous)	Yes (30 years)
Guaranteed local origin	No	Yes (all stages of production)	Yes (at least one production stage)	No
Subject entitled to register	Autonomous region or province, public and private entities	Association consisting mainly of producers or processors involved in production (c.d. «group»)	Association consisting mainly of producers or processors involved in production (c.d. «group»)	Association consisting mainly of producers or processors involved in production (c.d. «group»)
Requirements for registration*	(a) Name of the product and any other names (b) Brief description of the product (c) Area of origin of the product and territory concerned (d) Nutritional aspects (c) Description of the processing, storage and maturing methods (f) Materials, specific equipment used for preparation and conditioning (g) Description of the processing,	(a) Constitutive act and/or articles of association, resolution of the assembly referring to the application (b) Name to be protected (c) Description of the product, including main physical, chemical, microbiological and organoleptic characteristics (d) Definition of the defined geographical area (e) Evidence that the product originates in the	(a) Constitutive act and/or articles of association, resolution of the assembly referring to the application (b) Name to be protected (c) Description of the product, including main physical, chemical, microbiological and organoleptic characteristics (d) Definition of the defined geographical area (e) Evidence that the product originates in the	(a) Constitutive act and/or articles of association, resolution of the assembly referring to the application (b) Name to be protected (c) Description of the product, including the main physical, chemical, microbiological and organoleptic characteristics and demonstration of the product's specificity d) Description of the production method to be

	storage and maturing rooms (h) Evidence that the methods have been applied uniformly and according to traditional rules for a period of not less than 25 years (i) Production holdings (l) Promotion initiatives	defined geographical area (f) Description of the method of production and, where applicable, local, fair and consistent methods, as well as information on packaging (g) Elements establishing the link between the quality or characteristics of the product and the geographical environment (f) Historical report, accompanied by bibliographical references, proving the production for at least twenty-five years, even if not continuous, of the product, as well as the established use, in trade or in common parlance, of the name for which registration is sought * (g) Socio-economic report containing the quantity produced with reference to the last three years of production and the number of companies involved (current and potential) (h) Cartography on a scale	defined geographical area (f) Description of the method of production and, where applicable, local, fair and consistent methods, as well as information on packaging (g) Elements establishing the link between a given quality, reputation or other characteristic of the product and the geographical environment (f) Historical report, accompanied by bibliographical references, proving the production for at least twenty-five years, even if not continuous, of the product, as well as the established use, in trade or in common parlance, of the name for which registration is sought * (g) Socio-economic report containing the quantity produced with reference to the last three years of production and the number of companies involved (current and potential)	followed by the producers comply with, including, where applicable, the nature and characteristics of the raw materials or ingredients used and the method of production of the product (e) Key elements attesting to the traditional character of the product (f) Historical report, accompanied by bibliographical references, proving that the product is obtained by a method of production, processing or composition corresponding to traditional practice for that product or foodstuff or obtained from raw materials or ingredients used traditionally, as well as the use established in the trade or in common parlance of the name for which registration is sought (g) Socio-economic report containing the quantity produced with reference to the last three years of production and

		sufficient to permit identification of the production area and its boundaries (i) Name and address of the inspection authority or body (l) Possible specific labelling rules	h) Cartography on a scale sufficient to permit identification of the production area and its boundaries (i) Name and address of the inspection authority or body (l) Possible specific labelling rules	the number of companies involved (current and potential) (l) Possible specific labelling rules
Product Specification	No	Yes	Yes	Yes
Protection of the registered name	No	Yes	Yes	Yes
Monitoring organisation	Absent	Ministry of Agricultural, Food and Forestry Policies—Department of the Central Inspectorate for the protection of quality and repression of frauds of agri-food products; monitoring organisations authorised according to article 14, paragraph 6, L.526/99; protection consortium according to article 14, paragraph 15, L.526/99, in compliance with article 34–40, Regulation (EU) n.1151/2012.	Ministry of Agricultural, Food and Forestry Policies—Department of the Central Inspectorate for the protection of quality and repression of frauds of agri-food products; monitoring organisations authorised according to article 14, paragraph 6, L.526/99; protection consortium according to article 14, paragraph 15, L.526/99, in compliance with article 34–40, Regulation (EU) n.1151/2012.	Ministry of Agricultural, Food and Forestry Policies—Department of the Central Inspectorate for the protection of quality and repression of frauds of agri-food products; monitoring organisations authorised according to article 14, paragraph 6, L.526/99; protection consortium according to article 14, paragraph 15, L.526/99, in compliance with article 34–40, Regulation (EU) n.1151/2012.
Labelling indications	No trademark. Is possible to insert in the label the indication 'Product on the List of Traditional Agri-Food Products'.	Name of the product; indication of protection (PDO); EU PDO protection symbol; control information; logo of the product or of	Name of the product; indication of protection (PGI); EU PGI protection symbol; control information; logo of the product or of	Name of the product; indication of protection (TSG); EU TSG protection symbol; control information; logo of the product or of

		the Protection Consortium (where present), ex-article 12, Regulation (EU) n.1151/2012 and article 13, Regulation (EU) n.668/2014.	the Protection Consortium (where present), ex-article, 12 Regulation (EU) n.1151/2012 and article 13, Regulation (EU) n.668/2014.	the Protection Consortium (where present), ex-article 12, Regulation (EU) n.1151/2012 and article 13, Regulation (EU) n.668/2014.
Number of registered products (Italy)	5450	173	142	4
Number of registered vegetable products (Italy)	911	19	37	0
Average time for registration in days (Italy)	365 **	1107	621	N.R. ***

* With reference to Italian legislation, implementing European provisions; ** considering the annual update of the TAPs list; *** not relevant because of the registration of only 4 products and the registration time of 1822 days for 'Pizza Napoletana TSG' and the lack of data for 'mozzarella TSG'.

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Supplementary Materials

Supplementary materials associated with this article can be found, in the online version, at doi.org/10.3390/agriculture13071313.

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
DIPARTIMENTO DI SCIENZE DEL SUOLO, DELLA PIANTA E DEGLI ALIMENTI

Traditional Agri-food Products

Questionnaire for consumers

PERSONAL DATA SECTION	
Sex ☐ Male ☐ Female ☐ Others	**Age** ☐ < 18 ☐ 18-35 ☐ 35-64 ☐ > 64
Education level ☐ Primary school qualification ☐ Junior high school qualification ☐ High school qualification ☐ Bachelor's degree ☐ Master's degree ☐ Post graduate training/PhD	**Geographical Distribution (Italy)** ☐ North-West ☐ North-East ☐ Centre ☐ South ☐ Islands
Area di residenza ☐ Urban ☐ Rural	**Economic status** ☐ Very difficult ☐ Difficult ☐ Stable ☐ Satisfactory ☐ Very satisfactory
Occupation ☐ Employee (public or private) ☐ Entrepreneur ☐ Freelance ☐ Housewife ☐ Retired ☐ Student ☐ Unemployed ☐ Others	

KARPOS BiodiverSO
ON FOODS
VEG BiodiverSO

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
DIPARTIMENTO DI SCIENZE DEL SUOLO, DELLA PIANTA E DEGLI ALIMENTI

CONSUMER'S CONSUMPTION HABITS OF VEGETABLE PRODUCTS

QUESTION NO.1 – Do you habitually buy (for yourself or those living with you) food products?

☐ Yes

☐ No

QUESTION NO.2 – How often do you consume vegetables in your daily diet? [maximum one answer].

☐ Rarely (≤ 1 meal per week)

☐ Occasionally (2-4 meals per week)

☐ Sometimes (5-8 meals per week)

☐ Usually (9-12 meals per week)

☐ Every day

QUESTION NO.3 – Which types of shops do you usually go to in order to buy vegetables? [maximum 2 answers].

☐ Small traditional shop

☐ Supermarket, Discount or Hypermarket (medium and large size)

☐ Food shops within a shopping centre

☐ Local market or directly from the producer (also through group purchasing organisations)

☐ Online shops or home delivery

☐ Others (please specify) _____

QUESTION NO.4 – When you buy vegetables, how much do the following parameters influence your choice? [please give a value from 1 (not at all) to 5 (very much)].

	1	2	3	4	5
A. Price	☐	☐	☐	☐	☐
B. Regional origin of the product	☐	☐	☐	☐	☐
C. Historical and cultural link with the territory of origin	☐	☐	☐	☐	☐
D. Seasonality	☐	☐	☐	☐	☐
E. Organic product	☐	☐	☐	☐	☐
F. Product of certified origin (PDO, PGI, etc.)	☐	☐	☐	☐	☐
G. Nutritional aspects	☐	☐	☐	☐	☐

KARPOS BiodiverSO — BIODIVERSITÀ DELLE SPECIE ORTICOLE PUGLIESI DA FRUTTO
ON FOODS
VEG BiodiverSO — BIODIVERSITÀ DELLE SPECIE ORTICOLE PUGLIESI NON DA FRUTTO

QUESTION NO.5 – Do you know and follow the seasonality of the vegetables you buy and consume?

☐ Extremely unlikely

☐ Unlikely

☐ Neutral

☐ Likely

☐ Extremely likely

QUESTION NO.6 – With reference to a TRADITIONAL fruit and vegetable product, please indicate how much you agree with the following statements. [please give a value from 1 (not at all) to 5 (very much)].

	1	2	3	4	5
A. Healthier product	☐	☐	☐	☐	☐
B. Higher-quality product	☐	☐	☐	☐	☐
C. Higher level of food safety	☐	☐	☐	☐	☐
D. Improved nutritional values	☐	☐	☐	☐	☐
E. Increased respect for local farmers' rights	☐	☐	☐	☐	☐
F. More expensive product	☐	☐	☐	☐	☐
G. More sustainable product	☐	☐	☐	☐	☐

QUESTION NO.7 – With reference to a LOCAL fruit and vegetable product, please indicate how much you agree with the following statements. [please give a value from 1 (not at all) to 5 (very much)].

	1	2	3	4	5
A. Healthier product	☐	☐	☐	☐	☐
B. Higher-quality product	☐	☐	☐	☐	☐
C. Higher level of food safety	☐	☐	☐	☐	☐
D. Improved nutritional values	☐	☐	☐	☐	☐
E. Increased respect for local farmers' rights	☐	☐	☐	☐	☐
F. More expensive product	☐	☐	☐	☐	☐
G. More sustainable product	☐	☐	☐	☐	☐

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TAP SECTION

QUESTION NO.8 – Before today, were you familiar with the term 'Traditional Agri-food Product'?

☐ Yes

☐ No

QUESTION NO.9 – If yes, how did you learn about it? [multiple answer].

☐ Books, academic publications, trade magazines

☐ Territorial promotion initiatives (meetings, events, fairs, festivals, etc.)

☐ Online (articles, websites, social networks, etc.)

☐ Mass media (television, radio, newspapers, etc.)

☐ Others (please specify) _____

QUESTION NO.10 – What do you think is the correct definition of 'Traditional Agri-food Product'? [maximum one answer].

☐ Product originating from a certain region, the characteristics of which are essentially or exclusively due to a particular geographical environment (and its inherent natural and human factors). Furthermore, all stages of production take place in the defined geographical area

☐ Product whose processing, preservation and ageing methods have been consolidated over time and practised in its territory in a homogenous manner and according to traditional rules and protracted over time, in any case for a period of not less than twenty-five years

☐ Product originating in the region or country, the characteristics of which can be traced back to the geographical origin. At least one stage of production must take place in the identified area

☐ Product or food obtained by a method of production, processing or composition corresponding to a traditional practice for that product, or obtained from raw materials or ingredients used traditionally

☐ I don't know

QUESTION NO.11 – In your opinion, the terms 'traditional' or 'typical' applied to an agri-food product are:

☐ Synonyms

☐ Different

☐ I don't know

QUESTION NO.12 – When buying, do you find it easy to identify a food product as a traditional product of its region of origin?

☐ Extremely unlikely

☐ Unlikely

☐ Neutral

☐ Likely

☐ Extremely likely

QUESTION NO.13 – At the time of purchase, is/would your choice of consumption be influenced by whether the food product belongs to the list of Traditional Food Products?

☐ Extremely unlikely

☐ Unlikely

☐ Neutral

☐ Likely

☐ Extremely likely

QUESTION NO.14 – Could you list <u>at least</u> three products recognised as PAT in your region of origin/residence and/or other regions?

☐ No

☐ Yes (please specify):

_____

_____

_____

Chapter 5

Biodiversity of vegetable species: issues and opportunities through environmental policies and research

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Biodiversity of vegetable species: issues and opportunities through environmental policies and research

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ABSTRACT

In Europe, the protection of the environment and biodiversity is provided in numerous national Constitutions and the European Union's policies. However, vegetable species are becoming too uniform to meet the challenges threatening crops.

KEYWORDS

Agrobiodiversity; landraces; European common policies; Agenda 2030; farmers

Biodiversity and agrobiodiversity

Biodiversity is the variability of life forms. It is the planet's natural capital and one of the key factors in sustainable development.

Before 1986, this term did not exist. Walter G. Rosen coined the neologism during the organization of the "National Forum on BioDiversity" held from 21 to 24 September in Washington (Takacs, 1996). The new term was used to shorten the term "biological diversity." Takacs (1996) reports that, as a keyword, "biodiversity" was not present in Biological Abstracts in 1988, while the first paper with "biodiversity" in its title, "Canadian Biodiversity," was published in 1991. Nowadays, Scopus lists more than 150 journals with "biodiversity" in the title.

Agrobiodiversity, a subset of biodiversity, refers to the variety within cultivated agricultural systems. It encompasses three key components: (i) species, (ii) genes and combinations of genes within each species, and (iii) combinations of biotic and abiotic elements that define different agro-ecosystems.

Biodiversity and agrobiodiversity are relatively "new" concepts that are not immediately understood by the public. The survey "Attitudes of Europeans toward the issue of biodiversity" (European Commission, 2010) revealed that the majority of European citizens did not feel adequately informed on biodiversity: only 38% of European citizens knew the meaning of the term "biodiversity." Nine years later, the same source found that most of the 27,643 European citizens interviewed had either not heard of biodiversity or did not know what it means; in fact, only two out of five Europeans said they had heard of the term and knew what it meant (European Commission, 2019).

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Biodiversity in the constitutional charters

In Europe and worldwide, Constitutional Charters of Countries show particular attention to the environment and biodiversity. Regarding environmental protection, it is present in a large number of constitutions. In the EU, as many as 22 Member States provide for it (unlike Austria, Cyprus, Denmark, Ireland, and the Czech Republic). By contrast, the reference to biodiversity is far less frequent, considering that worldwide the term "biodiversity" is present only in the Constitutional Charters of 22 Countries (Bangladesh, Bolivia, Bhutan, Ecuador, Chile, Colombia, Dominican Republic, Finland, Gambia, Hungary, Italy, Kenya, Kosovo, Maldives, Nepal, Somalia, South Sudan, Sudan, Thailand, Vietnam, Yemen, Zambia) out of 196, according to the constituteproject.org website.

Furthermore, most of these references are very recent. For example, the Constitutional Charter of Italy (in force since 1948) did not mention the words "environment" or "biodiversity" until 2022. Constitutional Law, No. 1 of 11 February 2022, amended Articles 9 and 41 of the Italian Constitution by emphasizing environmental protection. More specifically, the new Article 9 of the Italian Constitution – "protects the environment, biodiversity, and eco- systems, also in the interest of future generations" – establishes a general criterion that directs the action of public powers toward environmental protection. Article 41, on the other hand, increases the perspective of the role of private enterprises that "may not be carried out in conflict with social utility or in a way that is detrimental" not only "to security, freedom, and human dignity" but (now) also to "health" and "environment".

The 2030 agenda for sustainable development

The European Union is committed to the 17 Sustainable Development Goals (SDGs) of the 2030 Agenda for Sustainable Development action program, adopted in 2015 by the United Nations (UN) Assembly and signed by the governments of the 193 UN member states.

Among the SDGs, is the so-called "Zero Hunger" target (SDG 2), which aims to "end hunger, achieve food security and improve nutrition and promote sustainable agriculture". However, the world is still far from achieving "Zero Hunger" with the global prevalence of undernourishment. Between 713 and 757 million people may have faced hunger in 2023: 1 out of 11 people worldwide and one of every five in Africa (FAO, IFAD, UNICEF, WFP, and WHO, 2024).

SDG 15, entitled "Life on Earth," is to "protect, restore and promote the sustainable use of the terrestrial ecosystems, sustainably manage forests, com- bat desertification, and halt and reverse land degradation and halt biodiversity loss." Over the past 50 years, the diversity of cultivated species has declined by 36%; 97% of nations feed on wheat, 90% feed on rice, and 74% use once "exotic" products such as soya or palm oil (Khoury et al., 2014).

SDGs 2 and 15 are still a long way off and can be achieved by reconsidering how we grow, share, and consume food. Not least, agriculture has to deal with climate change, competition for water, loss of productive land, and continued migration from rural to urban areas. These challenges are compounded by the need to feed an ever-growing population – estimated that some 9–10 billion people will crowd the planet by 2050 – offering them a more diversified diet. The diets of different countries are becoming increasingly similar, gaining in calories, proteins, and fats (Khoury et al., 2014); this uniformity makes pro- duction more vulnerable to epidemics and climate change, and increasingly unhealthy diets that, in the poorest segments, are based on these cheap, calorie-rich, but nutrient-poor foods.

Therefore, it becomes necessary to produce diversely and sustainably to ensure the availability of resources for future generations. In this, agrobiodiversity plays a central role: landraces offer a supply of valuable genes for cross-breeding resistant to hostile climates and pathogens and make it possible to differentiate the diet and produce with reduced agronomic inputs, even in marginal areas.

Landraces and modern varieties

For the most important vegetable species cultivated in Europe, since 1972, the European Community has adopted the "Common Catalogue of Varieties of Vegetable Species," which incorporates the relevant national registers, where varieties that have demonstrated valuable characteristics in particular experimental centres are registered. This registration is a prerequisite for marketing seeds of varieties of vegetable species. The main criteria for registration are distinctness, uniformity, and stability (DUS), the three fundamental characteristics without which it is impossible to register varieties in the Common European Catalogue (CC) (Didonna et al., 2024).

In the last five years (2018–2023), the number of varieties of vegetable species registered in the CC increased from 19,686 (Santamaria and Signore, 2021) to 21,593 (Didonna et al., 2024). These are varieties that have been improved for fruit characteristics as, for example, uniformity of shape and size, intensity and colour variation, strong flavour and high shelf-life, and/or plant characteristics such as hardiness or multiple resistance to pathogens and pests. The aim is to have modern ("hybrids") varieties selected for large-scale adaptation and more productive in high-input conditions. This production system was preferred to less productive systems but adapted to specific conditions (e.g., for marginal areas, with low agronomic inputs, for organic farming systems), typical of the pre-Green Revolution era when local varieties with a high capacity to tolerate biotic and abiotic stresses were used.

In the new agricultural model, therefore, there is no longer any place for varieties selected over time by farmers, the so-called "local varieties" or "farmer's varieties," which are mostly abandoned and forgotten. A phenomenon that also affects modern varieties. Considering the case of tomato (*Solanum lycopersicum* L.), the species with the highest number of registered varieties in the CC (19.46%), just think that over the last ten years – precisely from 2013 to 2023 – 2,440 varieties have been deleted from the CC.

In addition to the loss of agrobiodiversity and the abandonment of landraces, it is today particularly difficult to find seeds of local varieties. Indeed, as stated above, in the EU, only seeds of varieties registered in the CC can be marketed. Landraces, on the other hand, are excluded. The seeds of these varieties are preserved only thanks to farmers' selection work and seed exchange. With all the problems – poor germinability, confusion between varieties, relations with nurseries, etc. – that can arise from this.

The Netherlands registered 8,552 varieties, or more than one-third (39.60%) of the varieties included in the latest CC of varieties of vegetable species, although the area under vegetable cultivation in the Netherlands represents only 2.96% of the total vegetable area in the EU (87.00×10^3 ha). The most important countries in Europe for the cultivation and production of vegetables (FAOSTAT, 2023) are Italy (316.00×10^3 ha, 10.74%) and Spain (313.00×10^3 ha, 10.64%), which have registered, respectively, 1,909 (8.84%) and 1,992 (9.22%) vegetable varieties. Emblematic is the situation of Italy, which cultivates tens of thousands of hectares of artichoke (*Cynara cardunculus*

subsp. *scolymus* (L.) Hayek), chicory (*Cichorium intybus* L.), broccoli rabe (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort), and rocket (*Diplotaxis tenuifolia* (L.) DC.) using local varieties not registered in the CC – also considering that both rocket and broccoli rabe species are not present in the CC – often maintained by the farmers themselves or by plant nurseries. Finally, it should be considered that many Italian regions have very few, or even none, varieties registered in the CC; Apulia, for example, despite being one of the most regions in Italy for vegetable crops, has only 17 varieties registered in the CC of varieties of vegetable species, less than 0.01% of the national varieties. In 1998, the European Union aimed to make the CC more flexible and to mitigate the decline of agricultural biodiversity by introducing the Conservation Varieties Regime (CVR): a set of rules for local and/or traditional varieties at risk of genetic erosion and cultivated in specific areas. This initiative was intended to allow the sale of those varieties that do not fully meet the DUS criteria. In one of our reviews (Didonna et al., 2024), we examine the impact of establishing the CVR 25 years after its first definition. Across the European Union, 191 conservation varieties of vegetable species were registered at the data collection date (2023), representing only 0.88% of the total number of varieties included in the CC. The most important Countries are Spain and Italy, which have 57 and 43 conservation varieties, respectively. Nevertheless, the CVR has failed to promote and enforce a dedicated market for all those varieties excluded from registration in the CC (Didonna et al., 2024).

The role of (bio)research

The analysis conducted so far shows the need for new biodiversity and "new" research, especially in the most important countries and territories for vegetable crops and marginal areas.

Taking Italy as a reference, and Apulia in particular, the region where we operate, with the "BiodiverSO" project we have recovered, characterized, con- served, and (also) valorised some local varieties that were at risk of erosion or extinction (Renna et al., 2018). To give just two examples, we have made a significant contribution to the knowledge and diffusion of the 'Carota di Polignano' (*Daucus carota* L.), a local variety that produces yellow, purple, and orange roots in the same plot of land, and of the 'Scopatizzo' (*Cucumis melo* L.). This immature melon is used in the same way as the cucumber, and in hydroponic cultivation, in 71 days from transplanting, it produced 5.3 kg/plant (26.2 fruits/ plant; Palmitessa et al., 2024). Furthermore, for this last local variety, we have reported that bitter fruits have recently emerged, as was the case for some local cucumber varieties (Palmitessa et al., 2023). These examples show that some local varieties have been unable to express their full potential before being replaced by modern varieties; this is without prejudice to the fact that the local varieties are useful for genetic improvement work. Furthermore, especially in vegetable crops, there is a need for new expressions of biodiversity: organic vegetable farming, protected crops, growing plants in pots, grafting, special climatic conditions (climate change), simplifying the production process (e.g., for processing vegetables), consumer demands (tailored to tastes, eating habits, age, and lifestyles), for "special" products (microgreens, for example), etc.

Therefore, it is time to increase biodiversity and agrobiodiversity on farms. But there is also a need for further training of farmers for vegetable breeding that involves them and increases the biodiversity of vegetable species with, for example, "decentralised participatory breeding" and "evolutionary breeding" (Ceccarelli and Grando, 2024), to promote, in addition, knowledge about the pollination of species (often allogamous) and the value of ecosystem services provided by biodiversity.

We researchers, who perform public functions and private activities, must follow the fundamental constitutional principle of protecting the environment and biodiversity, working in the interests of future generations. Even with activities that allow us to interact directly with society and be in direct contact with farmers.

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Conclusions

The final aim of the PhD thesis was to identify strategies and tools to protect and promote Apulian vegetable landraces, with a particular focus on fruit vegetable varieties. To achieve this goal, a specific methodology was developed, starting with the (re)discovery of ancient landraces and the recovery of their seeds from custodian farmers. This process culminated in the analysis of legislative and commercial instruments suitable for enhancing and promoting these landraces in both regional and national markets.

The case study of the landrace 'Carosello Scopatizzo' (*C. melo*) was representative of this methodology, developed as part of the "BiodiverSO Karpos" project. For this landrace, the first step in the protection and valorisation pathway involved morphological characterization, as reported in Chapter 1. This activity satisfied two main purposes: firstly, the characterization was essential for identifying the unique traits of this landrace, particularly regarding its production and nutrition profiles. Additionally, the characterization activities aimed to discover a potential issue associated with this landraces, namely the presence of some fruits with an elevated bitter taste. To investigate the cause of this bitterness, it was therefore necessary to analyse the chemical profile of some samples of 'Carosello Scopatizzo' fruits. The analysis made it possible to discover – for the first time in literature – the presence of Cucurbitacins, the compounds responsible for bitter taste in *Cucumis* species, in certain fruits of this landrace. Identifying the molecular basis of the fruit's bitterness, a problem which would have had a very negative impact on the Apulian unripe melon market, made it possible to start a process of recovery and valorisation of this landrace – one of the most interesting varieties of Apulian *C. melo* in terms of potential production – aimed at limiting the presence of plants with bitter fruit. The second aim satisfied with the morphological characterization of 'Carosello Scopatizzo' was its registration in the Apulian Regional Register of Autochthonous Genetic Resources (Regional Law of Apulian Region of 8 April 2013, no. 39), in the National Register of Biodiversity of Agricultural and Alimentary Interest (National Law 1 December 2015, no. 194) and in the National List of Traditional Agrifood Products (TAP). In the PhD thesis, the latter was identified as a valuable normative instrument, usable both for the protection and valorisation of Apulian landraces. The registration of a landrace, a traditional product associated with the landrace, or a culinary recipe prepared using the landrace in the TAP list, serves as an important mechanism to safeguard the cultural knowledge related to these landraces. Although the TAP recognition does not provide for the possibility of using a commercial trademark, it is an exclusive Italian instrument useful for preserving and protecting ancient varieties and traditions. As discussed in Chapter 4, TAP recognition represents an initial step towards the commercial

valorisation of landraces, facilitating their promotion and marketing. In the same Chapter, the European Geographical Indications were identified as the final stage of this valorisation scheme, aimed at expanding the presence of landraces on local, national and international markets.

To complement this market valorisation process, another critical activity was required: consumer education. This effort is fundamental for the implementation of the virtuous cycle, "if you eat me, you preserve me." With this aim, as part of PhD research work, two editorial products were developed for consumers: the website www.patpuglia.it and the book "Atlas of Traditional Agrifood Products of Apulia" (Didonna A., Colonna M.A., Renna M., Signore A., Santamaria P., 2022. University of Bari Aldo Moro, Bari. ISBN 978-88-6629-038-4. Available online, in Italian: patpuglia.it/book). Both products aim to narrate the qualities, traditions, and histories of Apulian TAP, many of which involve vegetable landraces used in traditional recipes. Additionally, as a result of the PhD research and as part of the "BiodiverSO Karpos" project, the book "Vegetables in traditional Apulian cuisine. 1. Fruit vegetables" (Didonna A., Renna M., Santamaria P., 2024. University of Bari Aldo Moro, Bari. ISBN 978-88-6629-082-7. Available online, in Italian: karpos.biodiversitapuglia.it/pubblicazioni) was published, telling the traditions and stories of Apulian fruit vegetables.

In conclusion, as a result of the protection and valorisation strategy of Apulian fruit vegetables, the PhD thesis identified two possible solutions: the use of specific trademark and recognition for the commercial promotion of landraces and the consumer education activities.

However, during the PhD research a further problem was identified in the early stages of the protection and valorisation of landraces: the supply of seeds by farmers. As a potential solution, the conservation variety regime was identified and analysed, which revealed numerous limitations at the end of the analysis. In this sense, work is underway to revise the regulation of this derogation scheme by the EU. Nevertheless, it is necessary to consider that, at present, the conservation variety regime - together with the regime of varieties with no intrinsic value, so-called "amateur varieties" - remains the only legal framework for the commercialisation of landrace seeds. As part of the PhD work, this instrument was analysed, highlighting its limitations and future opportunities. Expanding the authorised market for conservation varieties (currently restricted to their zone of origin) and simplifying the registration process could provide significant improvements to this regime, offering new opportunities for the conservation and valorisation of European vegetable landraces.

The research presented in this PhD thesis was developed in alignment with Italian and European programmes aimed at preserving biodiversity and agrobiodiversity. However, the majority of these programmes approach agrobiodiversity conservation primarily through the recovery and storage of landrace seeds in gene banks or similar

conservation structures. This approach, often referred to in the literature as "static conservation," has certain limitations. Specifically, it fails to capitalise on one of the most distinctive features of landraces: their natural adaptability to external changes, such as varying cultivation conditions or climate challenges. This approach goes beyond the sole focus on preserving seeds in Gene Banks by emphasising the cultivation of landraces on farms, allowing them to evolve over time under natural conditions. Such a paradigm shift requires transitioning from merely preserving biodiversity to actively valorising landraces, with the ultimate goal of enhancing biodiversity.

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