Ecotype-Level Genetic Biodiversity of Five Italian Traditional Crops

Francesco Guarino, Stefano Castiglione, Giovanni Improta, Maria Triassi, and Angela Cicatelli

1Dipartimento di Chimica e Biologia "Adolfo Zambelli", Università degli Studi di Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano (SA), Italy
2Dipartimento di Sanità Pubblica, Università degli Studi di Napoli Federico II, Via pansini, 5, 80125 Napoli (NA), Italy

Correspondence should be addressed to Angela Cicatelli; acicatelli@unisa.it

Received 31 January 2019; Revised 5 April 2019; Accepted 22 May 2019; Published 1 July 2019

Guest Editor: Graciela Sobierajski

Copyright © 2019 Francesco Guarino et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Italy displays a high level of agrobiodiversity due to its diversified pedoclimatic zones. The Administrative Region of Campania includes several and divergent biomes, occurring close to each other. In fact, the distance between a sea level environment and that of high mountains can be less than 20 km. These environmental conditions allow the cultivation of many different crops and vegetables, represented by diverse ecotypes and varieties that are well adapted to the distribution range where they have been selected and grown. Efforts to maintain and further increase biodiversity in farming systems require a better understanding of the existing diversity created by traditional farming practices. The aim of our study was to identify and molecularly characterize several ecotypes belonging to five horticultural species commonly cultivated in Campania. In particular, we analysed five ecotypes of maize, two of garlic, four of onion, one of escarole, and two of courgette by means of simple sequence repeat (SSR) markers in order to evaluate their level of genetic biodiversity. The results reveal, for the first time, the high genetic biodiversity of horticultural ecotypes of the Campania Region. This feature is very important to improve the quality and productivity of agroecosystems.

1. Introduction

In agroecosystems, genetic diversity is widely regarded as pivotal in sustaining and preserving ecosystem functioning and services [1]. Traditional crops, in particular, constitute the largest reservoir of genetic diversity, and apart from their importance from an ecological point of view, their conservation has great social, economical, and cultural implications [2, 3]. Indeed, traditional crops, and especially landraces, have a history of cultivation and adaptation to local environmental conditions, which translates into unique tastes or nutritional properties as well as higher resistances to biotic and abiotic stresses [4]. From the “Green Revolution” onwards, however, humans focused their attention only on some species that were considered the most interesting, and within these species, they exerted a selective pressure toward specific phenotypes, thus causing a reduction in allelic diversity. The consequence has, therefore, been the replacement of species and/or traditional and local varieties by highly selected and specialised ones, leading to an overall loss in biodiversity [5] since only a subset of the diversity of the wild species remains after repeated selection for desired traits.

Crop biodiversity has been influenced by a number of genetic bottlenecks occurring during domestication and through modern plant breeding [6]. Nevertheless, existing plant genetic resources can still provide a biological and fundamental basis for world food security and support the livelihoods of human populations. These resources are regarded by breeders and farmers as the most important raw material. Thus, traditional agriculture and local knowledge of agricultural biodiversity, cultural factors, and participatory
processes, as well as tourism associated with agricultural landscapes, are the new challenges for sustainable development [7]. Many traditional crops and landraces survive primarily, thanks to local farmers, who are the keepers of an invaluable biodiversity heritage. Although, in general, the greatest loss of diversity occurred from the landraces than the wild plants, the older farmers, in particular, have become the principal depositories of traditional knowledge, mostly coming from family traditions and handed down through the generations [8]. They have identified and propagated by means of simple selection procedures and specific landraces deemed of particular interest. In this case, domestication causes a net increase in diversity [9]. In fact, behind the rediscovered interest in traditional crops and landraces, there is also the recognition of their important role as sources of genetic diversity, which is potentially useful for crop improvement and sustainable agriculture. Today, biodiversity meets and stimulates the various requirements of the market (in terms of quality standard, rediscovery of traditions, etc.) of the production sector (in terms of plants more tolerant to climate change, new cultivation methods, or biotic stresses) as well as the needs of the processing industry and of modern distribution.

Italy, and in particular southern Italy, displays a broad crop biodiversity, related to its heterogeneity at both the landscape and social/cultural level [10]. However, the current conservation landscape does not reflect the Mediterranean biome's rarity and its importance for plant endemism [11]. Habitat conversion will clearly outpace the expansion of formal protected-area networks, and conservationists will have to replace traditional strategies with new approaches to sustain the Mediterranean biota. Using regional-scale datasets, it is possible to determine the area of land that is protected, converted (e.g., urban or industrial), impacted (e.g., intensive and cultivated agriculture), or lands that can be subjected to conservation potential. The natural and seminatural lands that are unprotected (e.g., private range lands) can sustain numerous native species and associated habitats.

Campania stands out for its extreme landscape heterogeneity and exceptional geomorphological and pedogenetic traits. Thanks to its highly fertile soils, it was called “Campania Felix” during the Roman Empire and crops cultivated on the volcanic soils near Vesuvio were regarded by Goethe as being unparalleled [12]. Landscape heterogeneity is reflected in the presence of numerous ecotypes, which are historically adapted to the areas where they are grown.

In order to protect its local horticultural ecotypes, the Administrative Region of Campania has launched a project called “Agrigenet,” a network for the protection and management of agrofood genetic resources. The project includes a biodiversity study of the regional ecotypes and their conservation both in situ, by keeper farmers, and ex situ through the establishment of a germplasm bank.

Several studies have been conducted to evaluate the biodiversity in horticultural products in different countries and regions of the world [13–16], but much of the existing biodiversity is still unknown. Our study was aimed at genetically characterizing several ecotypes belonging to five horticultural species from the Campania Region, in order to estimate their biodiversity and contribute to their conservation in the framework of the Agrigenet project. For this purpose, five ecotypes of maize (Zea mays L.), four of onion (Allium cepa L.), two of garlic (Allium sativum L.), one of escarole (Cichorium endivia L.), and two ecotypes of courgette (Cucurbita pepo L.) were molecularly characterized using a set of highly polymorphic microsatellite (Simple Sequence Repeat, SSR) markers.

2. Materials and Methods

2.1. Plant Material. The different accessions of diverse ecotypes of maize, onion, garlic, escarole, and courgette were provided by the Agricultural Research Council (CRA-SCS, Battipaglia, SA, Italy) and by the National Research Institute for Food and Nutrition (INRAN, Pontecagnano, SA, Italy). They were selected on the basis of the farmers’ local knowledge. In origin, the institution obtained the ecotypes from thekeeper farmers, but no information is available regarding the contribution of the farmers to these collections. The details of the furnished accessions are given in Table 1.

2.2. SSR Analysis. Leaf samples for DNA isolation were collected from seedlings of each accession. Total DNA was extracted from leaves using DNeasy Plant Mini Kit (Qiagen, Milano, Italia). Quality and quantity were assessed by DNA electrophoresis in 1.0% agarose gel with reference standards and by evaluating the 260/280 ratio with a Picodrop Microcriter UV/Vis Spectrophotometer (Picodrop, UK). A total of 35 SSR primer pairs (seven for maize, six for garlic, seven for onion, eight for escarole, and seven for courgette) were used for PCR amplifications. The primer sequences are given in Table SM1. PCR reactions were performed in a volume of 10 μl containing 20 ng DNA, 1.2–4.0 pmol of each primer, 10X PCR buffer, 1.25 mM dNTPs, and 0.5 units of Taq polymerase. Amplifications were performed using the following PCR amplification conditions: 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 50°C for 1 minute (X indicates the annealing temperature reported in Table SM1), and 72°C for 1 minute followed by final extension at 72°C for 5 minutes. PCR products were separated on ABI Prism 310 Genotype Analyzer (Applied Biosystems, Milano, IT), adding 500 ROX (Applied Biosystems, Milano, IT) as internal size standard. SSR fragments were analysed using Gene Mapper V 3.7. The SSR fragments were finally scored as length of the electrophoresed alleles for each investigated locus.

2.3. Data Analysis. Polymorphism Information Content (PIC) values or expected heterozygosity scores for SSR (polyallelic) markers were calculated using the formula: Hj = 1 – Σpi2, where pi is the frequency for the ith allele [17].

The most used and informative indices for population genetics studies are the observed and expected heterozygosity.
It can also refer to the fraction of loci within individuals that are heterozygous. Typically, the observed ($H_o$) and expected ($H_e$) heterozygosity are considered.

$$ F_{ST}, \Phi_{ST}, \Theta_{ST}, \text{and } D_{est} \text{ are the primary indices used }$$

$$ \text{for estimating and testing the level of genetic divergence among populations. } F_{ST} \text{ is the best choice for datasets consisting of neutral polymorphism datasets involving two alleles per locus. } D_{est} \text{ and } F^2 \text{ tend to be the suitable index for analyses where more than two alleles are considered.}$$

The Shannon Index ($I$) was calculated on a single-locus basis, where $ln$ is the natural logarithm and $p_i$ is the frequency of the $i$th allele. Unlike $H_o$, $I$ is not bounded by 1 and may, therefore, be a better measure of allelic and genetic diversity, though largely overlooked in genetic studies:

$$ I = \sum p_i \ln p_i, \quad (1) $$

The genetic similarity matrices of all accessions were elaborated using Jaccard’s similarity coefficient (JSC) [18]. A cluster analysis was constructed on the similarity matrix by means of the unweighted pair group mean with arithmetical averages (UPGMA) method using NTSYSpc software (Numerical Taxonomy System, version 2.1) based on the JSC.

Structure version 2.2 software [19] was used to define the optimal number of clusters and to infer the population structure using the genetic profiles of each ecotype. The number of populations ($K$) was estimated by performing 10 runs for each population, from $K=1$ to $K=10$. Each run consisted of 100,000 MCMC (Markov Chain Monte Carlo) permutations with a burn-period of 10,000, assuming no $a$ priori information on population affiliation, the admixture, and correlated allele frequencies methods. The optimal population structure was estimated using the method of Evanno et al. [20] with 20 independent runs for each $K$ value.

3. Results

3.1. Maize. Accessions of six maize ecotypes were analysed to estimate genetic biodiversity and population structure. The SSR matrix was elaborated to perform a cluster analysis and obtain the dendrogram, as shown in Figure 1.

The dendrogram displays two macrogroups (1 and 2) connected with a node at JSC = 0.25. Each macrogroup is subdivided in two clusters (A and B) including samples of different ecotypes. In fact, in this analysis, samples of the same ecotype do not fall in well-separated groups. Each subgroup (1A, 1B, 2A, and 2B) includes not only many samples of the same ecotype but also individuals belonging to different ecotypes (e.g., subgroup 1A includes the MBA samples and even some SNB and SB individuals).

The $H_o$ and $H_e$ values are similar for most of the analysed loci; moreover, in many cases, the Fixation Index ($F$) is close to zero, suggesting a random recombination among ecotypes and samples. The $F$ value is greater than zero in the case of locus 6 or 5 relative to ecotypes MBA and SR. The high biodiversity is also confirmed by the PIC (min 0.3, max 0.4) and by the Shannon Index ($I$). In fact, the $I$ values range from 0.00 to 1.28; the highest $I$ value is observed for locus Phi029 in the case of MBA, the lowest for loci Nc130 and Phi014 in the case of SNR, and for locus Bnlg118 in the case of SR (Table 2).

Population structure was estimated by analysing the SSR dataset using structure software. The distribution of $\Delta K$, estimated by the Evanno method, identified a peak corresponding to $K=2$. To investigate the substructure of the maize collection, the proportion of genotype membership $s$ for $K$ equal to 2 was computed (Figure 2).

At $K=2$, the first cluster (red) includes the ecotypes Mais Bianco di Accera (MBA), Spiga Napoletana Rossa (SNR), and Spiga Bianca (SB), whilst the samples belonging to Spiga Rossa (SR) and Spiga Napoletana Bianca (SNB) were grouped in the green cluster. However, the red cluster includes some admixed ecotypes, with a probability of belonging to the green cluster ranging from 20% to 80% as in the case of samples SB3 and SB9, respectively.

3.2. Onion. Four onion ecotypes, Febbrarese (F), Marzatica (M), Ramata di Montoro (R), and Vatolla (V), were analysed using seven microsatellite loci. The $H_o$, $F$, and PIC values suggest that the studied onion ecotypes are not genetically stable. In fact, the positive values of $F$ close to 1 indicate phenomena of intersection or null alleles, while negative values tending to $-1$ suggest an excess of heterozygosity (Table 3), probably due to the selection processes, or to an isolate-breaking effect (i.e., the mixing of two previously isolated populations) in this region.

To estimate the genetic similarity among samples, an UPGMA analysis was performed on molecular data (Figure 3).

The JSC value is very low among different ecotypes, and thus the genetic biodiversity is very high; moreover, the ecotypes are distributed in admixed clusters. Clusters 1, 2, and 3 comprise many samples belonging to three different ecotypes (Febbrarese, Marzatica, and Ramata di Montoro). A similar result was obtained using a Bayesian statistical approach. The most probable $K$ values are 6 and 8. At both $K$ values, the obtained clusters appear admixed, and none of the samples can be clearly attributed to a specific cluster. In

Table 1: List of the analysed ecotypes.

| Species | Ecotype | Abbreviation |
|---------|---------|--------------|
| Allium sativum L. | “Tondo di Torella” | a9-a15 |
| Allium sativum L. | “Schiacciato” | a1-a8 |
| Allium cepa L. | “Febbrarese” | F |
| Allium cepa L. | “Cipolla Marzatica” | M |
| Allium cepa L. | “Ramata di Montoro” | R |
| Allium cepa L. | “Vatolla” | V |
| Zea mays L. | “Bianco di Accera” | MBA |
| Zea mays L. | “Spiga Bianca” | SB |
| Zea mays L. | “Spiga Napoletana Bianca” | SNB |
| Zea mays L. | “Spiga Napoletana Rossa” | SNR |
| Zea mays L. | “Spiga Rossa” | SR |
| Cichorium endivia L. | “Riccia Schiana” | RS |
| Cucurbita pepo L. | “Cilentano” | C |
| Cucurbita pepo L. | “San Pasquale” | S |

3.3. Cucumber. Four cucumber ecotypes, L. “Spiga Rossa” (SR), L. “Spiga Napoletana Rossa” (SNR), L. “Spiga Napoletana Bianca” (SNB), and L. “San Pasquale” (S), were analysed using seven microsatellite loci. The $H_o$, $F$, and PIC values suggest that the studied cucumber ecotypes are not genetically stable. In fact, the positive values of $F$ close to 1 indicate phenomena of intersection or null alleles, while negative values tending to $-1$ suggest an excess of heterozygosity (Table 4), probably due to the selection processes, or to an isolate-breaking effect (i.e., the mixing of two previously isolated populations) in this region.

To estimate the genetic similarity among samples, an UPGMA analysis was performed on molecular data (Figure 4).

The JSC value is very low among different ecotypes, and thus the genetic biodiversity is very high; moreover, the ecotypes are distributed in admixed clusters. Clusters 1, 2, and 3 comprise many samples belonging to three different ecotypes (Febbrarese, Marzatica, and Ramata di Montoro). A similar result was obtained using a Bayesian statistical approach. The most probable $K$ values are 6 and 8. At both $K$ values, the obtained clusters appear admixed, and none of the samples can be clearly attributed to a specific cluster. In
fact, they show the same probability of belonging to diverse clusters (data not shown).

3.3. Garlic. For the two investigated ecotypes of garlic, *Schiacciato* and *Tondodi Torella*, the *H*₀ and *F* values suggest that, for all eight loci, there is an excess of heterozygosity. It is highly probable that the ecotypes were obtained by cross-breeding between each other (Table 4).

The estimated JSC confirms that all analysed samples are identical, with the exception of a4 (0.87; Figure 4).

The molecular data are identical for all analysed samples, and therefore, the two ecotypes are not distinguishable using these SSR loci. The Bayesian bioinformatics elaboration was uninformative, and therefore, these results are not shown.

3.4. Escarole. The SSR analysis for the escarole ecotype revealed that A149, EU03H001, and B214 are homozygotic, and thus, the *F* index cannot be estimated for these loci because both *H*₀ and *H*ₑ are equal to zero (Table 5).

The *F* values for loci A149 and EU0030 are very high (close to 1) due to the fact that *H*ₑ is close to zero; *F* values indicate inbreeding events. *F* values for locus 4 are close to 0, as usually observed in the case of random reproduction; on the contrary, negative values for *F* due to extreme heterozygosity and then to gene flow, are observed for loci B42 and EU01H08 (Table 5). Genetic biodiversity was estimated by calculating the probability of finding the same allele size in the population. This value is very low, the highest being that of loci B42 and EU01H08 (0.57 and 0.50, respectively). Moreover, the mean value of *I* is equal to 0.47, suggesting a limited genetic biodiversity (Table 5).

The cluster analysis dendrogram (Figure 5) reveals that the escarole ecotypes are characterised by a high genetic biodiversity; in particular, all samples, with the exception of 1, 9, and 10, show a genetic similarity >85%.

3.5. Courgette. The biodiversity indices for the two ecotypes of courgette are shown in Table 6. The mean values of *I* are 0.54 and 0.57 for *Cilentano* (C) and *San Pasquale* (S), respectively. However, this is not valid for some loci. For instance, in the case of *Cilentano*, loci 4991 and 5800 show higher values as do loci 1906, 4991, and 5800 for *San Pasquale* (Table 6). In agreement with these results, the *F* values varied from negative (*San Pasquale*, loci 4991 and 5800) to positive (*Cilentano*, loci 1906 and 4782).

The UPGMA dendrogram reveals three clusters (Figure 6): cluster A includes samples z1 to z8; cluster B comprises sample z9, samples z10 to z18, and sample z20; cluster C includes samples z10 to z12 and sample z19 and was dissimilar from others analysed samples (0.37).

The population structure analysis performed on these molecular data shows that the optimal population numbers (K) are 2 and 3 (Figure 7).

For *K* = 2, samples are well separated into two clusters (green and red). However, samples C9 and C10, belonging to *Cilentano*, fall within the cluster including all accessions of *San Pasquale*. For *K* = 3 (data not shown), the resulting clusters are well separated. Samples C1 to C8 of *Cilentano* are allocated to the red cluster, while all
other samples are distributed in two groups: green and blue. The San Pasquale samples C10, S1, S1, and S9 are separate from the others (blue cluster). Moreover, some samples belonging to San Pasquale (green cluster) show a 20% probability of being assigned to the blue cluster (e.g., S4 and S6).

4. Discussion

The Mediterranean Basin is one of the rarest terrestrial biomes, covering a mere 2% of Earth’s land surface [21]. Its small size is compensated by a high biodiversity since over 20% of known vascular plant taxa are present in this biome.
and many of which are exceedingly rare and considered as endemics [23]. Moreover, the Mediterranean region, due to its mild climate, is inhabited by millions of people. Because of its peculiar pedoclimatic conditions, the Mediterranean region has also been the cradle of the most important horticultural crops. In fact, many endemic species persist only on small remnants of their natural habitat separated by intensively cultivated agricultural land, thereby contributing to ecotype development. These ecotypes have an economical, ecological, and biological role. In this work,
we estimated the genetic biodiversity of several ecotypes belonging to five different horticultural species: maize, onion, garlic, escarole, and courgette, occurring in the Campania Region (southern Italy). The final objective is to preserve the horticultural Campania ecotypes by means of in situ and/or ex situ conservation strategies. When the project started, the investigated ecotypes were not genetically characterized, although some of that, and in particular onion, was of interest from organoleptic and economic point of view. Therefore, no program of conservation in situ or ex situ was in progress. For this reason, the data reported in this manuscript are very useful to begin a program of conservation in situ, through the keeper farmers, and, ex situ, maintaining the genetic resources in the Universities and/or Research Centres.

4.1. Maize. Maize has a strategic importance for agricultural and food of all industrialized countries. In addition to human consumption, its grain is also used for raising cattle [24]. In this context, the biodiversity study and germplasm characterization of local ecotypes is needed to adopt

---

**Table 4: Genetic indices calculated for the SSR pattern.**

| Ecotype   | Locus | N  | N_a | N_e | I   | H_o | H_e | UH_e | F     | Gene diversity | PIC  |
|-----------|-------|----|-----|-----|-----|-----|-----|------|-------|----------------|------|
| Schiacciato | 35    | 10 | 2   | 1.98| 0.69| 0.9 | 0.5 | 0.52 | −0.82 | 0.5            | 0.375|
|           | 40    | 10 | 3   | 2.2 | 0.86| 1   | 0.55| 0.57 | −0.83 | 0.5            | 0.375|
|           | 53    | 10 | 2   | 2   | 0.69| 1   | 0.5 | 0.53 | −1    | 0.5            | 0.375|
|           | 59    | 10 | 2   | 2   | 0.69| 1   | 0.5 | 0.53 | −1    | 0.5            | 0.375|
|           | 72    | 10 | 2   | 2   | 0.69| 1   | 0.5 | 0.53 | −1    | 0.5            | 0.375|
|           | 80    | 10 | 2   | 2   | 0.69| 1   | 0.5 | 0.53 | −1    | 0.5            | 0.375|
| Mean value|       | 10 | 2.17| 2.03| 0.72| 0.98| 0.51| 0.54 | −0.94 | 0.50           | 0.38 |
| Tondo di Torella | 35    | 5  | 2   | 1.92| 0.67| 0.8 | 0.48| 0.53 | −0.67 | 0.5            | 0.375|
|           | 40    | 5  | 2   | 2   | 0.69| 1   | 0.5 | 0.56 | −1    | 0.5            | 0.375|
|           | 53    | 5  | 2   | 2   | 0.69| 1   | 0.5 | 0.56 | −1    | 0.5            | 0.375|
|           | 59    | 5  | 2   | 2   | 0.69| 1   | 0.5 | 0.56 | −1    | 0.5            | 0.375|
|           | 72    | 5  | 2   | 2   | 0.69| 1   | 0.5 | 0.56 | −1    | 0.5            | 0.375|
|           | 80    | 5  | 2   | 2   | 0.69| 1   | 0.5 | 0.56 | −1    | 0.5            | 0.375|
| Mean value|       | 5  | 2   | 1.99| 0.69| 0.97| 0.50| 0.56 | −0.95 | 0.5            | 0.375|

| N = number of alleles; N_e = number of effective alleles; I = Shannon Index; H_o = observed heterozygosity; H_e = expected heterozygosity; UH_e = unbiased expected heterozygosity; F = Fixation Index; PIC = polymorphic index content. |
conservation and protection strategies as well as the exploitation of local genetic resources, which are essential to improve the quality, durability, and sustainability of horticulture [25]. This randomly selects half the genes from a given plant to propagate to the next generation, meaning that desirable traits found in the crop (like high yield or good nutrition quality) might be lost in subsequent generations unless certain breeding techniques are used to keep and maintain these superior characters. Maize reproduces sexually each year and has endosperms that ranged in the ploidy level from diploid (2x) to nonaploid (9x). In crosses with diploid males, only kernels of the triploid endosperm class developed normally.

In this study, we investigated six ecotypes of maize through seven SSR markers. The $H_o$ and $H_e$ mean values of the analysed loci were similar; moreover, in many cases, the $F$ value is close to zero, suggesting a random recombination among different ecotypes. An average of six alleles for each locus were found, in agreement with the data reported for the same analysed loci by Ranatunga et al. [26] and Pejic et al. [27]; on the contrary, Molin et al. [28] observed an average of about three alleles per locus. The PIC varied in relation to the locus; however, these values were similar to those reported by Adeyemo et al. [29]. In that case, the authors observed a high genetic diversity among maize plants, as highlighted by the genetic distance, and the intracotype molecular variance was about twice the interecotype one. Our results are in agreement with those obtained by Adeyemo et al. [29] although some differences were detected. Smith et al. [30] analysed loci phi014, phi024, and phi029 and found PIC values equal to 0.20, 0.55, and 0.67, respectively. In the case of phi014, our data are similar

**Figure 4**: UPGMA dendrogram based on SSR profiles obtained from onion ecotypes. The Jaccard similarity index is indicated on the X axis. The sample names with their previously assigned (or not) genotype are reported on the Y axis (a1–a8 = Aglio Schiacciato; a9–a15 = Aglio Tondo di Torella).

**Table 5**: Genetic indices calculated for the SSR pattern.

| Ecotype          | Locus  | $N$ | $N_e$ | $I$ | $H_o$ | $H_e$ | $UH_e$ | $F$   | Gene diversity | PIC  |
|------------------|--------|-----|-------|-----|-------|-------|--------|------|---------------|------|
| Riccia Schiana   | A149   | 10  | 2     | 1.47| 0.5   | 0     | 0.32   | 0.34 | 1             | 0.32 | 0.27 |
|                  | EU03H01| 8   | 1     | 1    | 0     | 0     | 0      | NA   | 0             | 0    |
|                  | EU0030 | 9   | 3     | 1.74| 0.73  | 0.11  | 0.43   | 0.45 | 0.74          | 0.43 | 0.37 |
|                  | EU03D01| 10  | 2     | 1.11| 0.2   | 0.1   | 0.1    | −0.05| 0.1           | 0.1  |
|                  | Sw2h09.2| 9   | 3     | 1.59| 0.68  | 0.22  | 0.37   | 0.39 | 0.4           | 0.37 |
|                  | B214   | 10  | 1     | 1    | 0     | 0     | 0      | NA   | 0             | 0    |
|                  | B42    | 10  | 4     | 2.33| 1     | 0.8   | 0.57   | 0.6  | −0.4          | 0.57 |
|                  | EU01H08| 10  | 2     | 1.98| 0.69  | 0.9   | 0.5    | 0.52 | −0.82         | 0.5  |
| **Mean value**   | 9.5    | 2.25| 1.53  | 0.47| 0.27  | 0.28  | 0.3    | NA   | 0.28          | 0.24 |

$N$ = number of alleles; $N_e$ = number of effective alleles; $I$ = Shannon Index; $H_o$ = observed heterozygosity; $H_e$ = expected heterozygosity; $UH_e$ = unbiased expected heterozygosity; $F$ = Fixation Index; PIC = polymorphic index content.
to those reported by Smith et al. [30] for ecotypes *Spiga Rossa* and *Spogna Bianca*, while in the case of ecotypes *Bianco di Acerra*, *Spiga Bianca*, and *Spiga Napoletana Bianca*, the PIC we obtained is higher. In the case of loci phi024 and phi029, the PIC values are lower than those reported by the same authors for inbred lines, suggesting a lower genetic variability in these loci relative to the inbred lines analysed in other studies. Although the genetic biodiversity of maize has been the object of several studies, this is the first one addressing the Campania Region ecotypes. A biodiversity study was conducted by Legesse et al. [14] to improve the knowledge on genetic diversity and relationships among maize inbred lines to sustain a breeding program. In that case, 56 high land and midaltitude maize inbred lines were genotyped using SSR loci and these genotypes were clearly distinguishable. On the contrary, maize ecotypes analysed by us were admixed and not clearly distinguishable. Despite the high molecular variance existing among the ecotypes, the population structure analysis grouped the samples into two broad clusters. Structure results showed that the best clustering of all samples was when they were split into two groups because of their high genetic biodiversity, which does not allow a clear separation of populations for $K$ values higher than two. This result was confirmed by the graphical representation of the genetic similarity. In the similarity dendrogram, in fact, the samples were separated in two main groups, suggesting that the six maize ecotypes were not stabilized lines. The interecotype genetic biodiversity was greater than the interecotype one. Some ecotypes, which might seem
synonymies because of their names, were, on the contrary, clearly separated. In particular, samples belonging to ecotype *Mais Bianco di Acerra* were grouped in different clusters from those formed by the ecotypes *Spiga Napolletana Bianca* and *Spiga Bianca*, which in turn were divided into two subgroups. However, a basal cluster can be observed, in which samples belonging to different ecotypes were allocated. Our results confirm that the maize ecotypes are not stabilized lines and represent an important source of genetic biodiversity useful for further breeding programmes.

4.2. Onion. Onions are the second most produced horticulture crop after tomatoes, with about 3'642'000 ha grown annually worldwide and a production of 53.6 Mt. (2017). Their large distribution is probably due to the versatile culinary uses as the raw food or in different manners of cooking (baked, boiled, braised, grilled, fried, and roasted). In addition, onions are among the healthier vegetables due to the high levels of bioactive compounds, such as phenolic acids, flavonoids, thiolsulfonates, and anthocyanins.

The flowers are protandrous, the male flower matures before the female one; moreover, fertilization is allogamous, favoured by pollinating insects.

The *H_c*, Fixation Index, and PIC values highlighted that the onion ecotypes analysed were not genetically stabilized. In fact, the *F* values suggest phenomena of out-crossings, null alleles, or an excess of heterozygosity probably due to the selection techniques designed to enhance the heterosis phenomenon [31]. Selection techniques are commonly exploited by genetic hybridisation programs and consist in an event where two distinct genomes undergo a phase of “genetic turbulence” before entering a phase of homeostasis. This stage of “Genomic Shock,” proposed by McClintock [32], may be in relation to gene expression and with the activation of transposons [32]. Onion reproduces exclusively by sexual fertilization, favoured by insect pollinators. This feature could explain the fact that *H_c* values are greater than those of *H_e*. However, present results are in line with those reported by Baldwin et al. [33]. For most of the investigated loci, the gene flow was compatible with natural and random gene flow phenomena (loci 1, 3, and 5 of *Febbrarese*). The *D_e* values also support this hypothesis; in fact, they range from a minimum of 0 at locus 2, to a maximum 0.55 at locus 4. Onion often undergoes self-pollination phenomena; in fact, the ecotypes showed a significant inbreeding depression, even higher than that reported by Vigouroux et al. [34] for corn, which is considered one of the most tolerant species with respect to this phenomenon. The level of...
pollination due to out-crossing phenomena was assayed by Van Der Meer and van Bennekom [35] using two onion varieties, a yellow bulb (recessive trait), against a red bulb (dominant character), and the results showed that, over the four years of study, it could vary from 29 to 92% in relation to climatic and environmental changes that occur seasonally. The bioinformatics analyses performed on the onion ecotypes of Campania confirm not only the remarkable genetic variability but also a probable high genetic rearrangement [36].

4.3. Garlic. In the case of garlic, all the biodiversity indices and the population structure analyses of the two ecotypes, Schiacciato and Tondo di Torella, suggest that they are genetically identical. Therefore, a case of synonymy is possible; moreover, garlic flowers as well as seeds are sterile. Its propagation and production is obtained by means of vegetative reproduction by division and planting of cloves [37]. Garlic genotypes can adapt to different agroclimatic regions [38] and although it is cultivated and propagated worldwide, it probably originated in Central Asia [39]. Our study is the first to investigate the Italian and, in particular, Campania ecotypes of garlic and shows that these do not exhibit molecular variance. Our data, unlike those obtained by Zhao et al. [16], who analysed the same SSR loci in different garlic varieties, show no hypervariability; the mean PIC value is about half of that obtained by Zhao et al. [16], confirming the small genetic biodiversity existing between these two ecotypes of garlic. The similarity analysis also revealed that the JSC was equal to one, with the exception of sample a4, which showed a similarity of 0.83. This lower similarity might be related to a single allele present at SSR locus 40, probably caused by somatic mutation, a common phenomenon for vegetatively propagated crops.

4.4. Escarole. Escarole is diploid (2n = 18) and allogamous [40]; its floral morphology favours genetic exchange. This species has long been used as a medicinal plant [41], and it is now cultivated for several different purposes, including food. Molecular analyses, performed on 10 plants of the Riccia Schiana ecotype, revealed that there is a discrete genetic variability among the investigated specimens, probably favoured by its reproductive mechanism. The mean value was very low, reflecting the high homozygosity of the investigated loci; the Shannon Index suggested that the intraecotype biodiversity was limited. The similarity dendrogram showed that the analysed specimens constituted a fairly homogeneous group with high similarity values (about 85%), with the exception of samples RS1, RS9, and RS10. Genetic variability is affected by a number of factors including out-crossing mechanisms, environment, and natural or artificial selection [42]. In fact, Azevedo et al. [43] reported that the genetic variation distribution of escarole is not random within populations and is determined by the reproductive system, geographic distribution, effective scale of populations, pattern of reproduction, gene flow through the spread of pollen, and evolutionary factors [44]. Italy is probably one of the sites of origin of the genus Cichorium [45]. Therefore, it is necessary to protect the biodiversity of this germplasm and introduce new genotypes from regions with a high genetic biodiversity for further breeding programmes. However, a broader genetic basis and potential gene resources with resistances to biotic and abiotic stresses can be obtained through breeding of wild varieties [46].

4.5. Courgette. Courgette is cultivated all over the world as different varieties and ecotypes well adapted to diverse climates and environments [47]. Zucchini requires plentiful bees for pollination. In areas where pollinator decline or high amount of pesticides is used, such as mosquito-spray districts, gardeners often experience fruit abortion, where the fruit begins to grow and then dries or rots. This is due to an insufficient number of pollen grains delivered to the pistil. It can be corrected by hand pollination or by increasing the bee population. The organoleptic characteristics of courgette are related to the environment in which they are grown and to agricultural practices. Ecotypes Cilentano and San Pasquale have low Hs values for all analysed loci, in line with the Hs, although San Pasquale showed the highest heterozygosity values in the case of loci 1 and 7. The I and PIC values confirmed that the two ecotypes are not characterized by a high genetic biodiversity, probably because of an ancestral selection aimed at the genetic and phenotypic stabilization of the lines [48]. In fact, the PIC mean values were approximately half of those observed by Barzegar et al. [49], while I was about one-third. The dendrogram revealed that courgette ecotypes had a high genetic similarity, especially in the case of Cilentano. Barzegar et al. [49], estimating the genetic biodiversity of 26 accessions of C. pepo by means of microsatellites, reported similarity values with a maximum equal to 0.5 on a scale from 0 to 1. The number of alleles per locus obtained in our study varies from a minimum of one to a maximum of five, in agreement with the data obtained by Paris et al. [50]. Yu et al. [51] reported that the number of alleles, detectable through SSR markers, is directly related to the number of repetitions because microsatellite sequences are usually hypervariable, and it is reasonable to expect that they should occur more frequently in noncoding regions than in coding ones, especially for those with di- or tetranucleotide motifs. In our study, the length of the repetitions varies from six to eight nucleotide bases, but locus 2 was detected despite being monomorphic. The remarks made by Yu et al. [51] about the SSR marker and above reported are contradicted to some extent by Barzegar et al. [49] who showed that a greater number of polymorphic alleles are dinucleotide repeat (AG)8 in C. pepo.

Breeding of compatible, multidisease-resistant ecotypes with tolerance to abiotic stresses is crucial for the sustainable production of courgette. Knowledge on the genetic biodiversity present in ecotypes is of fundamental importance, not only for the genetic improvement but also for cultivar identification and protection. SSR markers have been extensively used for commercial cultivar discrimination and assessment of genetic biodiversity of commercial cultivars [52]. Kong et al. [53] reported that SSR markers were used to determine the genetic diversity and relationships of 35 Cucurbita rootstock accessions.
In conclusion, the molecular analyses conducted on courgette ecotypes of Campania highlight a good stabilization aimed at obtaining homogeneous lines [54].

5. Conclusions

The results reported here reveal, for the first time, that a high genetic biodiversity is present in Italian horticultural crops, in particular those grown in the Campania Region. The ancient traditions of this region have allowed maintaining several agricultural ecotypes with a high genetic biodiversity. This feature is very important in view of climate change and for sustaining improved quality and yield in order to produce agricultural products of excellence with a very important social and economic role. At the present, also on the basis of these results, the Campania Region has planned a series of initiatives to preserve the agrobiodiversity, and some of them are carrying out. The main aim of the study is to ensure that biodiversity can contribute to the development and enhancement of rural areas, especially those where a high crop biodiversity is present and has to be maintained. In this sense, the regional programs foresee the recovery and enhancement of the ecotypes of autochthonous species, as well as safeguard the environment, in the broader perspective of protecting the typical local agriproducts. Starting from these and other results, the Campania region has adopted a model based mainly on the Regional repertoire of the main endangered genetic resources, by means of keeper farmers, germplasm banks, conservation, and safety network.

Data Availability

The binary matrices data used to support the findings of this study have been deposited in the GitHub repository, and they will be made public after publication at https://github.com/fragua1804/agrigenet.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are grateful to Prof. Stefania Biondi. This study was funded by 7th Framework Programme and Regione Campania “AGRIGENET” project.

Supplementary Materials

Table SM1: primers used for the SSR amplifications. (Supplementary Materials)

References

[1] J. R. Miller, "Biodiversity conservation and the extinction of experience," Trends in Ecology & Evolution, vol. 20, no. 8, pp. 430–434, 2005.
[2] V. De Feo, R. Aquino, A. Menghini, E. Ramundo, and F. Senatore, “Traditional phytotherapy in the peninsula sorrentina, Campania, southern Italy,” Journal of Ethnopharmacology, vol. 56, no. 2, pp. 113–125, 1992.
[3] M. M. Lippi, C. Bellini, M. M. Secci, T. Gonnelli, and P. Pallecchi, “Archaeobotany in florence (Italy): landscape and urban development from the late roman to the middle ages,” Plant Biosystems, vol. 149, no. 1, pp. 216–227, 2015.
[4] A. K. Campbell, “Save those molecules! Molecular biodiversity and life,” Journal of Applied Ecology, vol. 40, no. 2, pp. 193–203, 2003.
[5] A. Elia and P. Santamaria, “Biodiversity in vegetable crops, a heritage to save: the case of Puglia region,” Italian Journal of Agronomy, vol. 8, pp. 21–34, 2013.
[6] S. D. Tanksley and S. R. McCouch, “Seed banks and molecular maps: unlocking genetic potential from the wild,” Science, vol. 277, no. 5329, pp. 1063–1066, 1997.
[7] R. Ortiz, S. Tabo, V. H. C. Tovar et al., “Conserving and enhancing maize genetic resources as global public goods—a perspective from CIMMYT,” Crop Science, vol. 50, no. 1, pp. 13–28, 2010.
[8] G. Schneiderman and M. Barrera, “Family traditions and generations,” Family & Community Health, vol. 32, no. 4, pp. 354–357, 2009.
[9] Y.-J. Park, J. K. Lee, and N.-S. Kim, “Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and population structure of a selected core set in garlic and its relatives using novel SSR markers,” Hereditas, vol. 144, pp. 10–17, 2007.
[10] N. Myers, R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent, “Biodiversity hotspots for conservation priorities,” Nature, vol. 403, no. 6772, pp. 853–858, 2000.
[11] L. A. Croce, R. Nazzaro, and V. La Valva, “Evidences of dramatic biodiversity loss in a wet biotope calls for urgent conservation strategies,” Plant Biosystems, vol. 146, no. 4, pp. 827–834, 2012.
[12] J. W. Goethe, Viaggio in Italia (1786–1788, BUR Biblioteca Universale Rizzoli, Bologna, Italy, 1991.
[13] G. S. C. Buso, M. R. Paiva, A. C. Torres et al., “Genetic diversity studies of Brazilian garlic cultivars and quality control of garlic-clover production,” Genetics and Molecular Research, vol. 7, no. 2, pp. 204–211, 2008.
[14] B. W. Legesse, A. A. Myburg, K. V. Pixley, and A. M. Botha, “Genetic diversity of African maize inbred lines revealed by SSR markers,” Hereditas, vol. 144, pp. 10–17, 2007.
[15] Y.-J. Park, J. K. Lee, and N.-S. Kim, “Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops,” Molecules, vol. 14, no. 11, pp. 4546–4569, 2009.
[16] W.-G. Zhao, J.-W. Chung, G.-A. Lee et al., “Molecular genetic diversity and population structure of a selected core set in garlic and its relatives using novel SSR markers,” Plant Breeding, vol. 130, no. 1, pp. 46–54, 2011.
[17] M. Nei, “Analysis of gene diversity in subdivided populations,” Proceedings of the National Academy of Sciences, vol. 70, no. 12, pp. 3321–3323, 1973.
[18] J. P. Jaccard, Nouvelles Recherches sur la Distribution Florale, Société Vaudoise des Sciences Naturelles, Lausanne, Switzerland, 1908.
[19] J. K. Pritchard, M. Stephens, and P. Donnelly, “Inference of population structure using multilocus genotype data,” Genetics, vol. 155, pp. 945–959, 2000.
[20] G. Evanno, S. Regnaut, and J. Goudet, “Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study,” Molecular Ecology, vol. 14, no. 8, pp. 2611–2620, 2005.
[21] R. L. Cox and E. C. Underwood, "The importance of conserving biodiversity outside of protected areas in Mediterranean ecosystems," *PloS One*, vol. 6, Article ID e14508, 2011.

[22] J. S. L. H. Mutke, H. Kreft, G. Kier, and W. Barthlott, "Biodiversity hotspots," in *Biodiversity Hotspots*, Springer, Berlin, Germany, 2011.

[23] R. M. Cowling, P. W. Rundel, B. B. Lamont, M. K. Arroyo, and M. Ariasoustou, "Plant diversity in Mediterranean-climate regions," *Trends in Ecology & Evolution*, vol. 11, no. 9, pp. 362–366, 1996.

[24] J. Kearney, "Food consumption trends and drivers," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 365, no. 1554, pp. 2793–2807, 2010.

[25] D. Tilman, K. G. Cassman, P. A. Matson, R. Naylor, and M. A. B. Ranatunga, P. Meenakshisundaram, S. Arumugachamy, I. Pejic, P. Ajmone-Marsan, M. Morgante et al., "Comparative analysis of genetic similarity among maize inbred lines detected by RFLP markers and AFLPs," *Theoretical and Applied Genetics*, vol. 97, no. 8, pp. 1248–1255, 1998.

[26] D. Molin, C. J. Coelho, D. S. Máximo, F. S. Ferreira, J. R. Gardino, and R. R. Mattiello, "Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers," *Genetics and Molecular Research*, vol. 12, no. 1, pp. 99–114, 2013.

[27] O. Adevyemo, A. Menkir, G. Melaku, and O. Omidiji, "Genetic diversity assessment and relationship among tropical-yellow endosperm maize inbred lines using SSR markers," *Maydica*, vol. 56, pp. 43–49, 2011.

[28] J. S. C. Smith, E. G. L. Chin, H. Shu et al., "An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigrees," *Theoretical and Applied Genetics*, vol. 95, no. 1-2, pp. 163–173, 1997.

[29] D. Fischer and K. Bachmann, "Onion microsatellites for germplasm analysis and their use in assessing intra- and interspecific relatedness within the subgenus *Rhzizideum*," *Theoretical and Applied Genetics*, vol. 101, no. 1-2, pp. 153–164, 2000.

[30] B. McClintock, "The significance of responses of the genome to challenge," *Science*, vol. 226, no. 4676, pp. 792–801, 1984.

[31] S. Baldwin, M. Pitner-Joyce, K. Wright, L. Chen, and J. McCallum, "Development of robust genomic simple sequence repeat markers for estimation of genetic diversity within and among bulb onion (*Allium cepa* L.) populations," *Molecular Breeding*, vol. 30, no. 3, pp. 1401–1411, 2012.

[32] Y. Vignouroux, J. C. Glaubits, Y. Matsuoka, M. M. Goodman, J. G. Sanchez, and J. Doebely, "Population structure and genetic diversity of new world maize races assessed by DNA microsatellites," *American Journal of Botany*, vol. 95, no. 10, pp. 1240–1253, 2008.

[33] Q. P. Van Der Meer and J. L. van Bennekom, "Influence of the environment on the percentage of self-fertilisation in onions and some consequences for breeding," *Euphytica*, vol. 21, no. 3, pp. 450–453, 1972.

[34] J. Jakse, W. Martin, J. McCallum, and M. J. H Levy, "Single nucleotide polymorphisms, indels, and simple sequence repeats for onion cultivar identification," *Journal of the American Society for Horticultural Science*, vol. 130, no. 6, pp. 912–917, 2005.

[35] E. R. Keller and A. Senula, "Micropropagation and cryopreservation of garlic (*Allium sativum* L.)," *Methods in Molecular Biology*, vol. 11013, pp. 353–368, 2013.

[36] G. D. McCollum, "Orion and allies," in *Evolution of Crop Plants*, Chapter 53, N. N. Simmonds, Ed., pp. 186–190, Longman, London, UK, 1976.

[37] A. M. Opatic, M. Nečemer, D. Kocman, and S. Lojen, "Geographical origin characterization of Slovenian garlic using stable isotope and elemental composition analyses," *Acta Chimica Slovenica*, vol. 64, no. 4, pp. 1048–1055, 2017.

[38] P. Baes and P. Catsen, "Electrophoretic analysis of eleven isozyme systems and their possible use as biochemical markers in breeding of chicory (*Cichorium intybus* L.)," *Plant Breeding*, vol. 110, no. 1, pp. 16–23, 1993.

[39] E. E. Hafez, E. Badr, Y. Mabrouk, M. El-Seehy, and S. Aggag, "Molecular genetic evaluation of *Cichorium endivia* L. as an anticaancer agent against colorectal cancer," *International Journal of Phytomedicine*, vol. 8, no. 4, pp. 551–557, 2017.

[40] X. Y. Liang, X. Q. Zhang, S. Q. Bai, et al., "Genetic diversity and relationship of chicory (*Cichorium intybus* L.) using sequence-related amplified polymorphism markers," *Genetics and Molecular Research*, vol. 13, pp. 7736–7746, 2014.

[41] A. L. S. Azevedo, P. P. Costa, M. A. Machado, C. M. P. de Paula, and F. S. Sobrinho, "High degree of genetic diversity among genotypes of the forage grass *Brachiaaria ruziziensis* (Poaceae) detected with ISSR markers," *Genetics and Molecular Research*, vol. 10, no. 4, pp. 3530–3538, 2011.

[42] T. Cadalen, M. Mörchen, C. Blasius et al., "Development of SSR markers and construction of a consensus genetic map for chicory (*Cichorium intybus* L.)," *Molecular Breeding*, vol. 25, no. 4, pp. 699–722, 2010.

[43] A. M. Kiers, T. H. Mes, R. van der Meijden, and K. Bachmann, "A search for diagnostic AFLP markers in *Cichorium* species with emphasis on endive and chicory cultivar groups," *Genome*, vol. 43, pp. 470–476, 2000.

[44] R. Singh, P. Sharma, R. K. Varshney, S. K. Sharma, and N. K. Singh, "Chickpea improvement: role of wild species and genetic markers," *Biotechnology and Genetic Engineering Reviews*, vol. 25, pp. 267–313, 2008.

[45] N. Gerodetti and S. Foster, "Growing foods from home": food production, migrants and the changing cultural landscapes of gardens and allotments," *Landscape Research*, vol. 41, pp. 808–819, 2016.

[46] J. Blanca, J. Cañizares, C. Roig, P. Ziarsolo, F. Nuez, and B. Picó, "Transcriptome characterization and high throughput SSRs and SNPs discovery in *Cucurbita pepo* (*Cucurbitaceae*)," * BMC Genomics*, vol. 12, p. 104, 2011.

[47] R. Barzegar, G. Peyvast, A. M. Ahadi, B. Rabiei, A. A. Ebadi, and A. Babagholzadeh, "Biochemical systematic, population structure and genetic variability studies among Iranian *Cucurbita pepo* (*Cucurbitaceae*) accessions, using genomic SSRs and implications for their breeding potential," *Biochemical Systematics and Ecology*, vol. 50, pp. 187–198, 2013.

[48] H. S. Paris, N. Yonash, V. Portnoy, N. Mozes-Daube, G. Tzuri, and N. Katzir, "Assessment of genetic relationships in *Cucurbita pepo* (*Cucurbitaceae*) using DNA markers," *Theoretical and Applied Genetics*, vol. 106, no. 6, pp. 971–979, 2003.

[49] K. Yu, S. J. Park, and V. Poyaa, "Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus and Vigna*)," *Genome*, vol. 42, no. 1, pp. 27–34, 1999.

[50] N. K. Izzah, J. Lee, S. Perumal et al., "Microsatellite-based analysis of genetic diversity in 91 commercial *Brassica oleracea* L. cultivars belonging to six varietal groups," *Genetic
[53] Q. Kong, J. Chen, Y. Liu et al., “Genetic diversity of Cucurbita rootstock germplasm as assessed using simple sequence repeat markers,” *Scientia Horticulturae*, vol. 175, pp. 150–155, 2014.

[54] S. Tang, Y. Zhang, L. Zeng, L. Luo, Y. Zhong, and Y. Geng, “Assessment of genetic diversity and relationships of upland rice accessions from southwest China using microsatellite markers,” *Plant Biosystems—An International Journal Dealing with All Aspects of Plant Biology*, vol. 144, no. 1, pp. 85–92, 2010.