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Genetic heritage of sweet chestnut genotypes in Montseny Natural Park, Catalonia

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Abstract:

The fruits of sweet chestnut (***Castanea sativa*** Mill.) have traditionally been an important economical income for local people whose lived in the Montseny area (mountain located in the northeast of Catalonia-Spain). At the beginning of the last century, the chestnuts of Montseny were highly appreciated for their high quality and mainly exported to France. However, after several decades of unmanaged chestnut groves combined with the virulent emergency of new diseases such as canker (***Cryphonectria parasitica***), many of these chestnut trees have disappeared or are at risk of extinction. Therefore, the social interest to re-valuate the local and native products is currently growing, and in this area, demand is increasing to replant native cultivars of ***Castanea sativa*** to produce fruit. Within this context, 120 genotypes distributed around Montseny were selected for the study. Then, morphology, phenology and fruit characterization parameters were studied in all genotypes, and 11 molecular markers (SSRs) analysed.

All studied parameters showed a low genetic diversity of the Montseny chestnut tree, indicating that inbreeding is occurring. In addition, the presence of two clonal groups widely distributed throughout the mountain were identified, suggesting that chestnut trees were probably grafted in the past with one or two ancient 'cultivars'. The present study provides information of genetic variability of the Montseny chestnut tree indicating that conservation of genetic heritage must be a priority. In addition, four chestnut genotypes were selected for its registration as cultivars. The selected plant material will ensure to producers to plant chestnut trees with high quality sweet chestnuts well adapted to this area.

Keywords: *Castanea sativa*, Cultivars, Fruit production, Wild populations, Microsatellites

Biocultural diversity of *Castanea sativa* (Mill.) across Eurasia

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Abstract:

A biocultural diversity approach integrates plant biology and germplasm dispersal processes with human cultural diversity. An increasing number of studies have identified cultural factors and ethnolinguistic barriers as the main drivers of the genetic diversity in crop plants. In Eurasia, sweet chestnut has been economically and culturally important crop for millennia. In this study, we integrated archaeological, linguistic, and genetic data to address the role of landscape and ethno-linguistic boundaries, used as a proxy of cultural similarities between human communities, on shaping the genetic structure of chestnut natural populations across Eurasia. Our data indicated that isolation by distance processes, and landscape heterogeneity might have promoted simultaneously human language diversification and chestnut differentiation across the same geographic macro-regions. Hotspots of sweet chestnut genetic diversity were associated with areas of linguistic enrichment in the Trans-Caucasus and Pyrenees Mountains, where sweet chestnuts had sustained ties to human culture since the Early Bronze Age (EBA). Partial geographic coincidence between the distribution of major cognitive sets of word-related terms, and the inferred genetic clusters of sweet chestnut populations across Eurasia supported (*i*) the indirect role of humans in shaping chestnut diversity across Eastern Mediterranean from the EBA and (*ii*) highlighted the differential importance of Romans on its cultivation history in the Eastern and Western Empire, where the Roman impact on the onset of chestnut domestication can be postulated. The predominant denomination of the sweet chestnut with the Latinized form of Greek *kástanon* and its wide spread across Western Europe reflected in fact the growing importance of chestnut for fruit production during the Roman Empire. However, the term *marron*, ultimately from Medieval Latin *marro**, correlated to the concept of "large and tasty edible chestnuts" has been subsequently recorded, since 13th century in Northern Italy and 16th century in France/Spain. Indeed, the first genetic evidence of clonal propagation by grafting of chestnut varieties was attested in Spain from 15th century and in Italy for *Marrone Fiorentino* variety only from 17th-18th centuries.

Keywords: chestnut, Eurasia, archaeological, linguistic, and genetic data

***Castanea sativa* ancient trees across Europe: source of genetic diversity**

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Abstract:

Large old trees are extraordinary organisms. They not only represent an historical, landscape and environmental heritage of inestimable value, but they are also witness to a long history of environmental changes and human interventions. For all this, they constitute a reserve of genetic variability, which can be considered a great resource for management programmes of forest species. The genetic characterization of these trees can be crucial to improve our knowledge of the molecular basis of local adaptation, identify selective drivers, and contribute to explain the contemporary genetic variation of tree species. In this study, we performed a comprehensive field survey to uncover the occurrence of large old chestnut trees in South-Central Italy, Spain and UK. We selected a total of 182 ancient trees that were genotyped using nuclear (SSRs) and adaptive (EST-SSRs) microsatellite markers with the main goals of: (1) to asses their genetic identity; (2) to derive temporal indications on the application of the grafting practice; (3) to compare their diversity with the present chestnut genetic resources; (4) to provide hypotheses on the origin of this ancient germplasm. Using the softwares GeneALex and HPrare, we evaluated the observed (Ho) and expected (He) heterozygosity, allelic richness (Ar) and private allelic richness (pAr). A Bayesian analysis was performed using the software STRUCTURE to identify the different genepools and genotypes. The obtained genetic data were compared with those of natural populations and cultivars collected in the same geographic areas. Higher values of allelic richness were observed in the ancient chestnut trees, a genetic similarity of these individual trees to the natural populations was highlighted.Our final objective is to contribute towards knowledge and valorisation of these large old trees, and to highlight germplasm sources of potential interest for both genetic improvement and conservation of European chestnut.

Keywords: Ancient trees, genetic diversity, microsatellite markers, genetic resources conservation

Location and differentiation of chestnut tree (*Castanea sativa* Mill.) individuals grafted with traditional cultivars using spatio-temporal analysis of remote-sensed imagery, dendro-chronological analysis, and SSR genotyping

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Abstract:

. The objectives of this work are to identify old chestnut varieties in order to improve the knowledge of the species in northwestern Iberia, and to analyse theircontribution to genetic, specific and ecosystem diversity in socio-ecological systems where chestnut orchardspreviously or still occupyan important place in the economic, ecological and cultural aspects.For this, ancient specimens are being located using geographic information systems (GIS) to process remote-sensing and digital cartographical data, coupled with fieldwork for the verification of previously analysed data
and sample collection for laboratory analysis. These analyses will involve a) dendrochronological surveysfor the establishment of specimens' age and the characterization of their temporary resilience to changes in climate, land use and forest dynamics; b) genotyping using SSR markersto genetically identify the localized cultivars.We expect that these results will allow the identification and location of traditional cultivars yielding a high value from genetic, ecological, and cultural perspectives, which will constitute a strong potential for their future use in the regeneration of chestnut orchards by increasing their genetic, specific, and cultural diversity in landscapes, and thus enhancetheir capability to provide ecosystem services

Keywords: Traditional chestnut orchards; cultivar SSR genotyping; Dendro-chronological Analysis; Geographical Information Systems; Remote Sensing; Ecosystem Services

Monitoring and protection of local genetic resources of sweet chestnut (Castanea sativa Mill.) by integrated analysis of cultivated and wild populations: case study of Umbria and Lombardy regions (Italy)

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Abstract:

Genetic analysis by DNA markers is very useful for characterization and traceability of traditional varieties and typical products. The genetic identity of products is generally referred to the cultivars and does not consider the genetic contribution of pollen from external sources. The gene flow between cultivated and wild populations is important in chestnut, because grafted cultivars, selected pollen donors, and wild trees are often located in the same areas. Indeed, the chestnut cultivars “Marrone” produce poor o none pollen.In “Multicast” and “CASTADIVA” research programmes, chestnut groves of Umbria and Lombardy regions, respectively, were characterized by genetic analysis of orchards, fruits, and wild populations. The newly started “CASTANEVAL” programme will continue the study in Lombardy region. Genetic data depict the structure of the regional cultivated chestnut. A conventional management system was found in Umbria region, with chestnut orchards composed of genetically uniform trees grafted with few cultivars. A more heterogeneous situation was found in Lombardy region, where two distinct areas were investigated. Chestnut orchards of Varese area showed a great genetic diversity, with genetically heterogeneous plantations, numerous grafted genotypes and few clonal replicates. On the contrary, in Brescia area, chestnut groves included a relatively high fraction of grafted “Marrone” plants, together with other local cultivars. The integrated analysis of mother plants, fruit embryos, and wild populations showed that the fruits from “Marrone” trees were genetically intermediate between mother plants and local wild populations, which act as pollen donor. On the opposite, the offspring from genetically heterogeneous orchards displayed a genetic profile similar to the respective mother plants. The *in situ* interaction between cultivated and wild chestnut confers a geographic and genetic signature to chestnut fruits. These results open perspectives to improve the characterization and traceability of chestnut productions.

Keywords: Genetic Resources; Chestnut Varieties; Genetic Diversity; Gene Flow

Principal Component Analysis and phytochemical fingerprinting as tools for genotype traceability in *Castanea* spp. fruits

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Abstract:

The phytochemical and morphological characterisation of fruits from *Castanea* spp., one of the most important Italian and European fruit and forest species, is very important for the development of effective strategies for the biodiversity conservation and traceability valorisation of chestnuts. In this study, several cultivars of *C. sativa* (‘marroni’ and ‘chestnut’ type), *C. crenata*, *C. pumila*, and hybrids of *C. sativa* x *C. crenata* were characterised by morphological (UPOV descriptors), spectrophotometric (Folin-Ciocalteu assay for the total polyphenolic content and Ferric Reducing Antioxidant Power test for the antioxidant capacity), and chromatographic (High-Performance Liquid Chromatography coupled to a diode array UV-Vis detector) protocols to define their phytochemical composition, nutraceutical properties, and main morphological traits. Phytochemical results were then used to build a multivariate statistical model (by Principal Component Analysis) and obtain an effective and rapid tool to discriminate unknown cultivars (i.e., no information about their origin) belonging to different species. The multivariate approach showed that the genotype was a significantly discriminating variable ($p < 0.05$) both for phytochemical composition and morphological traits. Polyphenols (in particular, phenolic acids and tannins) have been identified as the main bioactive classes with the highest discriminating power among the different genotypes. The Principal Component Analysis applied to phytochemical variables together with a rapid screening of morphological traits (by UPOV descriptors) has proved to be an excellent and effective tool for genotype differentiation to be used as a preliminary method for identifying the species of *Castanea* spp. fruits with unknown origin. The present study showed that a multivariate approach, based on phytochemical data and preliminary to genetic analysis, may represent a rapid, effective, and low-cost tool for the evaluation of traceability and quality of chestnuts from different species and hybrids with no information on their origin.

Keywords: phytochemical composition, multivariate approach, bioactive classes, chestnut cultivars

CONSERVATION OF EUROPEAN CHESTNUT GERMPLASM USING BIOTECHNOLOGICAL TOOLS

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Abstract:

We describe here the main advances in conserving European chestnut germplasm by using biotechnological methods based on in vitro culture

developed in our research group in the last years. Biotechnological tools based on in vitro culture methods are valuable for ex situ conservation of phytogenetic resources especially in woody species that cannot be conserved by conventional methods. In vitro culture techniques are very effective for conserving the germplasm of woody species that undergo vegetative propagation and of those that produce recalcitrant seeds. It is also effective for conserving plant material derived from biotechnological procedures (axillary cultures, somatic embryos and genetically transformed material). In vitro banking of culture material is an excellent complement to conventional ex situ conservation methods (i.e. seed banks, plant collections and botanical gardens).

In European chestnut, procedures for the short- and medium-term conservation of cultures were developed using reduced growth conditions, while procedures for long-term conservation of the material were developed using cryopreservation techniques (storage in liquid nitrogen at -196ºC). In the first approach, the chestnut plant material (axillary buds and somatic embryos) was maintained by routine culture, in many cases indefinitely, by periodic subculture (4-6 weeks). However, the subculture period can be greatly extended (to 6-12 months or even longer) when the cultures are subjected to reduced growth conditions (4ºC and low illumination). In addition, successful procedures have been developed for the cryopreservation of zygotic embryos, somatic embryos and shoot tips, enabling economic, safe and effective long-term storage of the material.

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Keywords: axillary budding; chestnut; cryopreservation; in vitro conservation; micropropagation; somatic embryos

Genetic diversity of the Italian chestnut accessions

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Abstract:

The *Castanea* includes seven major species: *Castanea mollissima* Bl., *Castanea henryi* (Skam) Rehd. et Wils., *Castanea seguinii* Dode, *Castanea crenata* S. et Z., *Castanea dentata* (Marsh.) Brokh, *Castanea pumila* Mill. and *Castanea sativa* Mill. Among them, the last species is the most present and cultivated in Europe and Italy with different cultivars and ecotypes. Only a few useful tools are available to help breed cultivars more resistant to diseases, climate changes and high nut quality. Moreover, the variants identification can be used for traceability to avoid accession misnomers to conserve and valorize the local varieties. To allow the study of the genetic diversity within *C. sativa* we performed a whole genome sequencing of more than 50 accessions of different origin and we identified more than 10 millions of SNPs and small indels using the genome of *C. mollissima* as reference. These new genomic resources for sweet chestnut provide valuable insights into its genome structure and diversity, and will be powerful tools for future research and breeding on this economically important species. They will also aid in the breeding of new varieties with improved agronomic performance, and contribute to the conservation of genetic resources in *C. sativa.*

Keywords: *Castanea sativa*, whole genome resequencing, SNP discovery, genotyping

Genetic diversity of chestnut trees of Martaínha variety assayed by iPBS and ISSR markers

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Abstract:

Chestnut (*Castanea sativa* Mill.) culture has high economic importance and its increase may reduce the genetic variation. Also as a consequence of climate change, the chestnut tree became more prone to abiotic and biotic stresses. Therefore, it is urgent to evaluate the European chestnut tree's genetic diversity in order to maintain its conservation and better understand its adaptive potential.

The chestnut culture is currently considered a strategic activity in the Portuguese economy, especially in the interior mountain regions. Among the several chestnut varieties existent in Portugal, Martaínha variety is one of the first to be harvested, giving it a huge strategic advantage. Martaínha has an excellent quality, with an enormous aptitude for fresh consumption.

This work relied on a first genetic diversity evaluation of the chestnut Martaínha variety from "Castanha dos Soutos da Lapa" using the inter-primer binding site (iPBS) and Inter-Simple Sequence Repeats (ISSR) molecular markers. The pooled molecular data revealed 51% of genetic similarity among individuals. The low genetic variability observed among the selected individuals from Martaínha variety can be justified by the fact that they share the same origin. These preliminary results allowed us to confirm the effectiveness of iPBS and ISSR markers for genetic variability assessment of chestnut trees and future studies with a substantial increase in the sample size and number of primers to achieve a higher number of markers will be performed.

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Keywords: *Castanea sativa*, genetic diversity, Martaínha, molecular markers

Morphological characterization of characters associated with chestnut fruit from Martaínha variety

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Abstract:

In the Portuguese context of the chestnut harvest calendar, the Martainha variety is one of the first to be harvested, which gives it a very large strategic advantage, namely an above-average price, as a result of the great imbalance between supply and demand for the product. This appetite arises from the high quality of this variety, with a great aptitude for fresh consumption, namely by street roasters, but also for the agro-industry. It is essential to select and propagate improved chestnut plant material, adapted to the soil and climate conditions of the production regions, while ensuring its genetic diversity and greater compatibility, aiming to increase the productivity of national orchards, thus bringing them closer to the productivity of orchards in other European countries. In order to increase the production potential and the nuts' quality of the Martaínha variety, it is essential to make available selected material, properly characterized, for carrying out new grafting. In this work, samples collected from 34 trees in different areas were analysed, based on the indication given by producers on trees that produced a high-quality chestnut. For each tree, 30 hedgehogs were collected, and the chestnuts were analysed for several traits. The average results obtained were of 38.2% of well-formed chestnuts, a fertility index of 2.8chestnuts/hedgehog, 92% of healthy chestnuts, and a polyspermy of 2.6. This work was carried out within the scope of the Breedmartainha project, which one of its objectives is to select improved plant material from the Martaínha variety, properly characterized, to carry out new grafts and, in this way, increase the production potential and quality of the chestnuts.

Keywords: *Castanea sativa*, chestnut quality, Martaínha