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CHESTNUT R&D CENTRE: THE STRATEGIC PROJECT OF PIEMONTE TO SUPPORT THE WHOLE CHESTNUT SUPPLY CHAIN ([1])

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To improve the R&D strategies in chestnut cultivation and to further develop the chestnut industry, the University of Torino, Dept. of Agriculture, Forestry and Food, with the financial support of the Piemonte Region, established in 2005 the Chestnut Regional Centre, located in Chiusa Pesio (Cuneo Province, North Western Italy). The Piemonte Region supported the institution of the Centre with human resources and structures (nurseries, land to realize the arboretum). The main activity of the Centre is the research, on several fields, including chestnut germplasm, advanced propagation techniques, optimization of cultural practices, pest and diseases management, extension service activities. In the Centre, a germplasm collection of chestnut genetic variability has been established on a 3 ha surface. Main local and national cultivars are included, with several European varieties from Portugal, Spain, France, Switzerland and other accessions from U.S.A., China, and Japan. The collection is in progress and will be completed in the next years. A newsletter published in English and Italian informs researchers, technicians and growers periodically. The Centre aims to become a national conservatory of the chestnut biodiversity and a reference center for research activities on Castanea *species.*

Key words: *Castanea* spp.; high-density plantations; sustainable orchard management; germplasm conservation.
Parole chiave: *Castanea* spp.; biodiversità; castanicoltura ad alta densità; gestione agronomica sostenibile; *Castanetum*.

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1. Introduction

Castanea sativa grows in Europe in hilly and mountainous areas, but many factors, such as social changes, the collapse of a system based on cattle and sheep farming, the lack of agronomic management, pests and diseases determined the evolution, which is currently ongoing, of chestnut stands from orchards to wild forest (Beccaro *et al.*, 2012). Moreover, today, even chestnut coppices are ever more afflicted by the effect of abiotic and biotic factors, including crop abandonment, drought stress and global warming, intense rainfall events, pests and diseases, including the Asian chestnut gall wasp, although this pest is now in regression due to the adopted successful biological control. On the other hand, in recent years in Italy, the nut market growing value has renewed the interest for chestnut cultivation and today many new traditional and high-density chestnut orchards, in particular in the province of Cuneo, are established. In this paper, the main activities conduced in this context by the Chestnut R&D Centre (CR&DC) of Piemonte are summarized.

2. Chestut R&D Centre activities

With the financial support of Piemonte Region in 2005, the Chestnut R&D Centre of Piemonte (CR&DC) was established in Chiusa Pesio (Cuneo Province, North Western Italy). The Region supports the Centre with human resources and structures (nurseries, land to realize the arboretum); the University of Torino, Dept. of Agricultural, Forest and Food Sciences, ensures the provision of scientific research and innovation. The main activities of the Centre include chestnut germplasm description and conservation, advanced propagation techniques, optimization of agronomic practices, innovation in pests and diseases management, nursery and extension services (Mellano & Beccaro, 2014; Torello Marinoni *et al.*, 2014).

3. Germplasm repository

In the CR&DC, a germplasm collection of chestnut genetic variability has been established on a 3 ha surface. The Centre represents nowadays a National conservatory of the chestnut biodiversity and reference center for research activities on Castanea species. The collection is in progress and includes main local and national cultivars, with several European varieties grown in Portugal, Spain,

France, Switzerland and other accessions coming from the U.S.A., China, and Japan (Mellano *et al.*, 2012). About 140 Castanea cultivars grafted on clonal rootstocks (Marlhac clone) and planted according to a landscaping module (8 for 11 m) make the germplasm collection an open-field laboratory where testing different management techniques.

4. R&D IN HIGH-DENSITY PLANTATIONS AND CLONAL ROOTSTOCKS

In the last 10 years of CR&DC activities, many resources and efforts have been devoted to the study of the best agrotechniques in high chestnut density plantations (HDP), and to study and improve commercial protocols to propagate chestnut clonal rootstocks. The starting consideration in 2005 was that Chinese chestnut (*C. mollissima*), a medium-sized tree, and the Japanese chestnut (*C. crenata*) that normally does not exceed 8–10 m in height, for their habit, are grown in China, Japan and South Korea in very high density plantations (2x3 m; 4x4 m) thinned in the following few years to 4x8 m and 8x8 m, respectively (Bounous, 2014). The European chestnut (*C. sativa*), a tall tree, is grown mainly in forests or plantations and spacing ranges from 10-12 m apart in rows and 12 m or more between rows (Beccaro *et al.*, 2004).

In the last 20-30 years, the trend to increase plant density to develop maximum bearing per unit area in a minimum of time is interesting also the Eurojapanese chestnut hybrids and *C. sativa* cultivars. HDP have been successfully established in many European countries (Italy, France and Spain) and in Australia, Chile, U.S.A, but still many constraints limit their diffusion. High or semi high-density schemes (8x8 m, 7x8 m) implement the productivity and the economics returns and can be an opportunity of conversion for many chestnut countries, mainly in the Mediterranean areas that are still producing according to traditional methods.

The majority of such plantations are established with eurojapanese chestnut hybrids (*C. crenata x C. sativa*) cultivars (resistant to gall wasp, such as Bouche de Betizac, and producing large size nuts), sometimes grafted onto clonal rootstocks tolerant to *Phytophthora* spp. In more recent years many high density plantations are also more or less successfully established with the European chestnut (*C. sativa*) cultivars too (Bounous, 2014; Mellano *et al.*, 2012).

The growing criteria to manage these trees are similar to those adopted in modern orcharding: irrigation, fertilizing, pruning, pest and disease management follow precise schemes in a frame of sustainability.

Starting from the consideration that the chestnut cultivation development needs the support of economic data, in the CR&DC a specific study was carried out in order to better define economical & social strengths and weaknesses of HDP. From this study, chestnut HDP most relevant features are:

– high skills requested to producers, as the management of an intensive chestnut orchard is completely different from a traditional chestnut plantation;

- particular attention needed to management and care of phytosanitary and agronomic critical issues that might decrease yield and life of the orchards;
- high quality land needed for cultivation, in relation to agronomic requirements, structure, position, generating competition with other crops;
- market issues: i) eurojapanese hybrids market will saturate sooner or later; ii) the chestnut and marrons market does not seem to be saturated, but it must be taken into consideration that the supply increase might bring to a reduction of producer price in a long term perspective (Pirazzoli *et al.*, 2017).

However chestnut HDP present important overall strengths: i) possibility to increase the chestnut production and decrease imported products (e.g. for Italy); ii) greater stability and standardization of the productions for the fresh market and processing industry; iii) reduction of production costs (assuming the maintenance of high yields); iv) excellent economical results at actual prices. On the contrary, it's important to evaluate also the weakness: i) high economical and long-term investment; ii) competition with other fruit crops; iii) impact of potential offer increase on product prices (currently not perceived); iv) risk of "trivialization" of national chestnut offer, historically connected to the territory and to high quality standards (Bellat *et al.*, 2017).

Despite the above strengths and weaknesses of HDP, the hybrid rootstocks clones, still lacking on the market, can contribute to the renewal of chestnut culture, allowing to adopt modern agrotechniques and to realize orchards with trees standardized for strength, habit and productivity, resulting in a facilitation of crop and agronomic field operations. Despite technical complexity of the process of their production, this material is useful for the chestnut orcharding upgrading process and presents many benefits: i) homogeneous HD crop and consequently high nut standardized quality; ii) dwarfing effect resulting in facilitation of agronomic management and high nut quantity; iii) *Phytophthora* spp. tolerance/resistance and *Cryphonectria parasitica* tolerance. On the other hand, the orchards realized on clonal rootstocks require more water and grafting compatibility problems can occur. For this reason in the CR&DC cultivar/rootstock compatibility evaluations are performed by visual observations (Francescatto *et al.*, 2010).

A consistent research line of the CR&DC concerns the study of clonal rootstocks propagation techniques: in vitro, by cutting and in C-boxes. The research on in vitro rootstocks propagation aims to individuate the best performing protocol using different for genotype/medium/hormones combination. An MS - Murashige & Skoog's medium (Moorashige and Skoog, 1962) and a CLP - Chalupa's medium (Ewald *et al.*, 1997) with cytokinin (BA benzyladenine) or auxin (NAA naphthaleneacetic acid) are on trial. After a preliminary 30-day screening of the explants, the results, however not yet supported by statistical evidence, show that CLP+BA+NAA protocol led to better results (Rossi *et al.*, 2016). Moreover, the age of the mother plants (between 1 and 3 years) showed no significant effect on in vitro rooting (Figure 1).

To identify the best cutting propagation protocols for rootstocks, two clones of the hybrids Maraval and Marsol (*C. crenata* Sieb et Zucc. x *C. sativa* Mill.) were

used for the trials conducted in the CR&DC. By installing lifted up shelves (mini tunnel) and environmental conditioning systems (fog system and heating carpets) the propagation environment was adequately controlled. The rooting average percentage observed in 2013-2017 varied between 40% and 70%. Two different substrates (perlite based and pine bark based) were identified as the best rooting media with no significant differences in the rooting between them. The tree-juvenility and environmental conditions were, among those examined, very important parameters for a good rooting (Mellano & Donno, 2015).

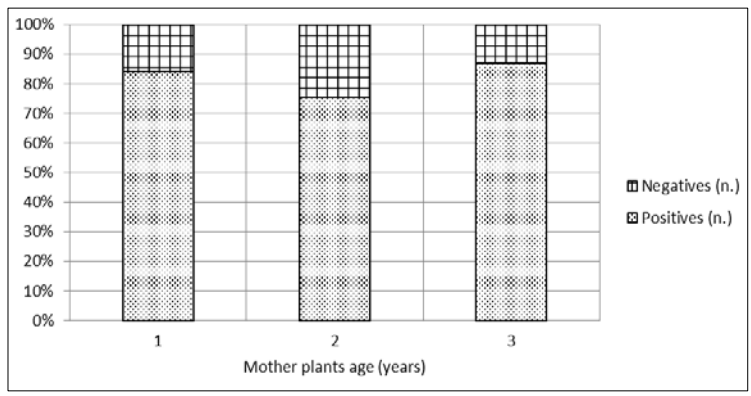

Figure 1 - Viability of Marsol CA07 rootstock explants after 30 days of in vitro cultivation.

Figure 2 - A Marsol CA07 cutting: roots regeneration (good number and polarity) and leafs development.

In order to better conditioning the rooting environment, as well as in the nursery, the C-box technique was developed in the CR&DC. The technique consists in a clonal propagation via climatic chamber, using nursery alveolus closed in plastic bags. Starting from the experience of micro propagation and cutting, the researchers of the CR&DC began to design this technique in 2016. The control of temperature, humidity and photoperiod is easier than in the greenhouse, and a very good rootstocks rooting can reach easily more than 40% (Figure 2).

With the aim of supporting the nursery activity, in the CR&DC trials have been conducted on the response of rootstocks cuttings to different substrates and fertilizing treatments. Identifying optimal and alternative substrates for nursery cultivation is a pivotal need of chestnut nursery industry: rootstocks must grow rapidly, be robust and suitable for entering quickly into the marketing channels once grafted. Six different combinations of substrates and fertilizer treatments were examined. The substrate most suitable for the development of rootstocks consisted of acid peat and compost. A substrate composed of sour peat + pumice + dolomite and a substrate composed of perlite + coconut fiber were the less suitable for growing chestnut rootstocks. The effect of some bio-stimulants products (vegetal glutamic acid and glycinbetaine; activated phosphorus with magnesium; seaweed brown sea *Ecklonia maxima*) was studied. The presence of these bio-stimulants do not seem to have an effect on rootstocks growth (Saggese, 2017).

5. Advances in pest and diseases management

In the last decades the number of invasive alien pest species in Europe has increased significantly and is considered as a major cause of serious economic and biodiversity loss. At the beginning of the XXI century the Asian chestnut gall wasp, *D. kuriphilus,* was reported for the first time in Italy, and rapidly spread in many other European countries, being responsible for a severe reduction in fruiting with a heavy economic impact on chestnut production. A mass rearing of the biocontrol agent *Torymus sinensis* was carried out at the CR&DC in order to contain the outbreaks of the pest. The exotic parasitoid, native to China as its host, was released, proving to settle successfully in the chestnut-growing areas in north-western Italy. Its introduction represents one of the most successful examples of recent European classical biocontrol program, allowing a significantly reduction of the gall wasp population after 7-8 years from its first release (Ferracini *et al.*, 2015; Ferracini *et al.*, 2017; Paparella *et al.*, 2016).

A new disease of chestnut nuts was reported for the first time in Europe right in Piedmont in 2005. The disease was caused by the newly described fungal species *Gnomoniopsis castaneae*, which is both a nut rot agent and an endophyte in green tissues, including thin bark, of chestnut trees (Visentin *et al.*, 2012). The disease subsequently spread, or at least was subsequently reported, in other areas of Italy and in other European countries, including France and Switzerland, making it the most serious chestnut nut rot disease in Europe. Within the activities of the CR&DC, in the last 5 years a number of ecological and epidemiological issues related to *G. castaneae* have been clarified, including a correlation between levels of infection and climatic parameters (Lione *et al.*, 2015), a lack of influence of spatial distribution of host trees on the occurrence of the disease (Lione and Gonthier, 2016), and, based on a population genetics approach, a key role played by sexual rather than asexual spores in the infection biology of

the pathogen (Sillo *et al.*, 2017). Furthermore, a synergic interaction between *G. castaneae* and the Asian gall wasp has been experimentally documented (Lione *et al.*, 2016). Based on the above epidemiological information, some management recommendations have been defined. The level of susceptibility to the disease of chestnut cultivars grown in the CR&DC is currently under investigation, as well as the effect of inoculation of hypovirulent strains of *C. parasitica* on the severity of chestnut blight in the CR&DC.

6. Chestnut composition and quality

Chemical and sensory studies are performed on chestnut productions of the CR&DC repository. Phytochemical fingerprint and polyphenolic pattern (Figure 3) of 80 chestnut cultivars were analyzed and compared and sensory profiles of 15 *C. sativa* and hybrids cultivars were described up to today. The statistical analysis of these preliminary data encourages to try to develop a traceability tool based on secondary metabolites quantification (Rapalino *et al.*, 2017; Mellano *et al.*, 2017).

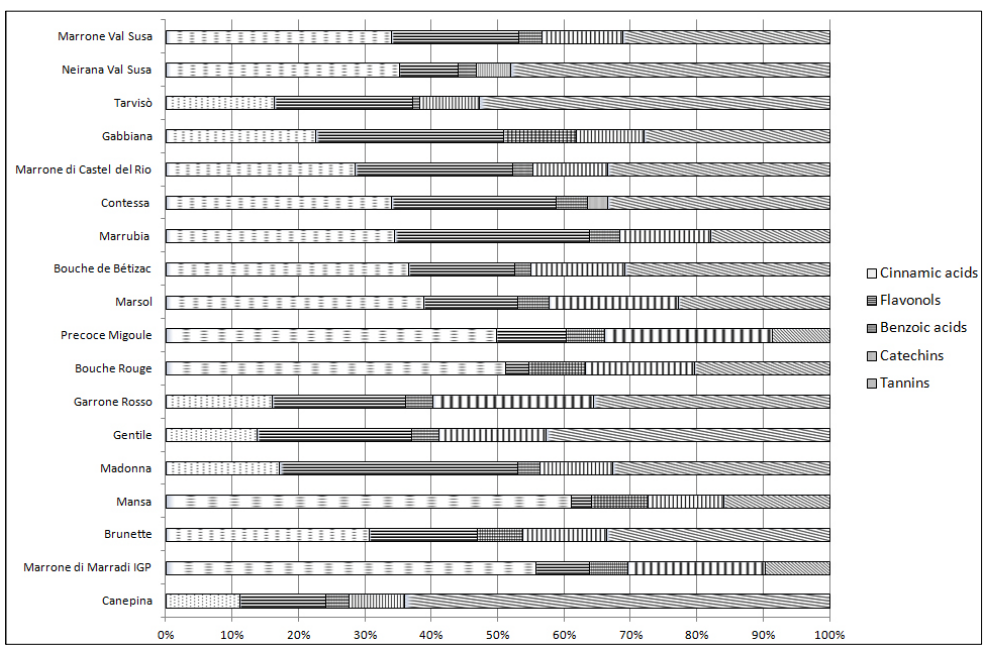

Figure 3 - Polyphenolic pattern of chestnut cultivars grown in the CR&DC repository.

7. Chestnut landscape certification

The analysis, with an historical and participatory approach, of chestnut traditional cultivation models, in order to obtain useful information for a chestnut landscape certification, is a recent and relevant object of study in the frame of

the CR&DC (Gullino *et al.*, 2010). In Piemonte Region (as in many other European traditional areas) chestnut orchards and coppices represent an invaluable bridge between cultivation and historical heritage; they represent an ecosystem that should be protected and improved as it provides great benefits in terms of social welfare and ecosystem services. Starting from the consideration that chestnut agroecosystems are witnesses of the liaison among cultivation, memory and heritage, a relevant effort to obtain a national certification of their landscape is in course.

8. Extension service

The Centre works in synergy with the local and national processing industry in order to increase the quantity and quality of the product put on the market. The Centre nursery service sells to growers trees grafted with local germplasm and to private nurseries seedlings for grafting, clonal rootstocks, seeds, scions. A newsletter (*Castanea*) is published in both English and Italian and periodically informs researchers, technicians and growers (www.centrocastanicoltura.unito.it/newsletter.html) about recent advances in the field. Finally, many training and information sessions are organized for local technicians and growers.

9. Conclusion

The CR&DC of Piemonte provides many research and development activities in support to traditional and modern chestnut orcharding. Currently, these two cultivation models are present at the same time in Italy. Although they are deeply different, they are not necessarily in competition, but rather they are potentially complementary. The research and extension activities of the Centre, summarized in this paper, are the starting point of a strategic project that, in the next years, plan to rethinking the whole chestnut supply chain creating a modern and competitive chestnut productive system.

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RIASSUNTO

Centro Regionale di Castanicoltura: dieci anni di attività in Piemonte

Il rilancio e la valorizzazione del settore produttivo del castagno rappresentano l'impegno, ormai decennale, del Centro Regionale di Castanicoltura, istituito nel 2009 (L.R. n. 4/2009 art. 24) a seguito della collaborazione fra Regione Piemonte, Università di Torino, IPLA ed enti

locali. Le tematiche trattate riguardano frutto e legno e sono orientate a: tutela del germoplasma castanicolo regionale, miglioramento delle tecniche di propagazione, all'ottimizzazione delle pratiche culturali, gestione dei parassiti e delle malattie, nuovi impieghi e possibilità di valorizzazione delle produzioni. Presso la sede di Chiusa Pesio (CN) su una superficie di 3 ettari è presente una collezione in corso di mantenimento e completamento che attualmente è composta da circa 130 cultivar provenienti da diverse regioni italiane nonché Francia, Spagna, Portogallo e Giappone. Il Centro di Castanicoltura, possedendo questo importante conservatorio della biodiversità del castagno, rappresenta un centro di riferimento per le attività di ricerca e sviluppo sulle specie di *Castanea*. Inoltre, grazie alle attività di *extension service* (sportello *counselling* e *newsletter*) si favoriscono formazione, informazione e divulgazione in un contesto di sempre maggiore comunicazione e sinergia fra ricercatori, tecnici e coltivatori.

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