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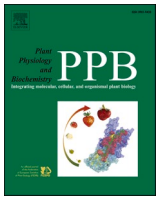

A roadmap of tissue culture and biotechnology in European hazelnut (*Corylus avellana* L.)

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ABSTRACT

The increasing interest in European hazelnut (*Corylus avellana* L.) cultivation registered in the last years has led to a significant increase in worldwide hazelnut growing areas, also involving regions characterized by a marginal presence of hazelnut orchards. Despite this increasement, world production still relies on the cultivation of few varieties, most of which are particularly suitable to the environment where they have been selected. Therefore, it is necessary to develop new cultivars with high environmental plasticity capable of providing constant and high-quality productions in the new environments and under the climatic change conditions of traditional growing areas. Over the years, many molecular markers for genetic breeding programs have been developed and *omics* sciences also provided further information about the genetics of this species. These data could be of support to the application of new plant breeding techniques (NPBTs), which would allow the development of cultivars with the desired characteristics in a shorter time than traditional techniques. However, the application of these methodologies is subordinated to the development of effective regeneration protocols which, to date, have been set up exclusively for seed-derived explants. A further aspect to be exploited is represented by the possibility of cultivating hazelnut cells and tissues *in vitro* to produce secondary metabolites of therapeutic interest. This review aims to consolidate the state of the art on biotechnologies and *in vitro* culture techniques applied on this species, also describing the various studies that over time allowed the identification of genomic regions that control traits of interest.

TDZ: thidiazuron.
WPM: woody plant medium.
ZEA: zeatin.

1. Introduction

European hazelnut (*Corylus avellana* L.) is a plant shrub belonging to the Betulaceae family, Fagales order. It is a deciduous, monoecious, dichogamous, diploid species (2n = 2x = 22) characterized by self-incompatibility controlled by a single locus with multiple alleles. This plant species has been cultivated for its nuts, rich in fatty acids (Cristofori et al., 2008), particularly requested by chocolate, confectionery and bakery industries, since 90% of hazelnuts are destined for processing while raw consumption is less than 10% (Romero-Aroca et al., 2021). Turkey is the major producer followed by Italy, Azerbaijan, Iran, Georgia, United States of America, Chile, Spain and China. Although its cultivation area has soared in the last few years from 632,955 ha (FAOSTAT, 2014) to 1,039,147 ha (+64%) (FAOSTAT, 2021), world production still relies on about twenty varieties, which are particularly suitable for cultivation in the environments where they were selected (Mehlenbacher, 1994; Mehlenbacher and Molnar, 2021). The cultivation area expansion led to the hazelnut planting in areas that are not particularly suitable for the pool of cultivars currently available, so the development of new cultivars with adaptability traits is needed. Genetic improvement of hazelnut started in the 1960s, with many programs in different countries around the world, aimed to develop varieties with interesting traits such as: high yield, early ripening, pests and pathogens resistance, cold hardiness (Mehlenbacher, 1994, 2018; Parnia and Botu, 1994; Weijiang et al., 1994). The breeding activity led by the OSU was particularly prolific of results: since 1990 OSU has released 11 cultivars for production and 12 cultivars to be used as pollinizers, with particular focus on developing varieties resistant to EFB, a destructive disease common in the American orchards caused by the fungal pathogen *Anisogramma anomala* (Mehlenbacher, 2018). Although breeding methods

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
2-iP	N^6-(2-isopentenyl)adenine
ABA	abscisic acid
ANFIS	adaptive neuro-fuzzy inference system
ApMV	*Apple Mosaic Virus*
BAC	bacterial artificial chromosome
BAP	6-benzilaminopurina
CHAID	chi-squared automatic interaction detection
CPW	cell and protoplast washing
CRISPR/Cas	clustered regularly interspaced short palindromic repeats/CRISPR associated protein
ddRADseq	double digest restriction-site associated DNA sequencing
DKW	Driver and Kuniyuky walnut
EDDHA	ethylenediamine-N,N′-bis((2-hydroxyphenyl)acetic acid)
EDTA	ethylenediaminetetraacetic acid
EFB	easter filbert blight
GA_3	giberellic acid
GBS	genotyping by sequencing
HM	hazelnut medium
HMGCR	3-hydroxy-3-methylglutaryl Coenzyme A reductase
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
INIA	Instituto de Investigaciones Agropecuarias
IRTA	Institute of Agrifood Research and Technology
KIN	kinetin
LG	linkage group
LOD	logarithm of odds
MAS	marker-assisted selection
MLO	mildew locus O
MS	Murashige and Skoog
mT	meta-topolin
NAA	1-naphthaleneacetic acid
NBS-LRR	nucleotide-binding site and leucine-rich repeats
NB-ARC	nucleotide-binding-ARC
OSU	Oregon State University
PVP	plant vitrification solution
QTL:	quantitative trait locus
RAPD	randomly amplified polymorphic DNA
RLK	receptor-like kinases
RLP	receptor-like proteins
RNP	ribonucleoprotein
RSM	response surface methodology
RT-PCR	real time-polymerase chain reaction
SH	Schenk and Hildebrandt
S-locus	self-incompatibility locus
SNP	single nucleotide polymorphism
SSR	simple sequence repeat

have been standardized, it still takes 17 years from the time a cross is made until a new cultivar is released (Mehlenbacher, 2018). Many markers have been developed for mapping traits of interest, which are often set under polygenic control, enabling their employment in MAS (Beltramo et al., 2016; Ozturk et al., 2017; Torello Marinoni et al., 2018). In the last few years, the drop in the costs of sequencing technologies has favoured the application of *omics* sciences in hazelnut molecular characterization, with the sequencing of valuable cultivars, providing a huge amount of data for gene mining (Lucas et al., 2021; Pavese et al., 2021a; Rowley et al., 2018). In this context, biotechnological methods such as genetic transformation and genome editing, could represent valid alternatives to speed up the process of new cultivar development, although an efficient regeneration protocol is essential when these methods are used on woody species, often recalcitrant to *in vitro* morphogenesis (Pavese et al., 2021b, 2022). As the cultivation area is increasing, efficient propagation methods should be also considered. European hazelnut is routinely multiplied through rooting suckers since it is one of the fruit tree species most prone to suckering. However, this practice presents many drawbacks as suckers production at the base of the trunk must be regularly controlled with negative effects on production costs, on environment and on the disease spread (Fideghelli and De Salvador, 2009; Pacchiarelli et al., 2022). For this purpose, micropropagation is an attractive method that could enable the production of pathogen-free, high-quality cultivars at a commercial scale (Bacchetta et al., 2008; Damiano et al., 2005; Díaz-Sala et al., 1990). Furthermore, *in vitro* culture of cells and tissues could also be exploited to produce valuable secondary metabolites such as paclitaxel, since hazelnut has been recognized as a natural source of this effective anti-neoplastic compound (Hoffman and Shahidi, 2009). The aim of this review is to discuss the biotechnological tools which have been already used in European hazelnut, paving the way for the development of new strategies, never applied up to date, which can accelerate the development of new varieties or improve the adaptability of the already established ones, for a species whose commercial interest is growing year by year.

2. Tissue culture of hazelnut

2.1. Micropropagation

Although several protocols suitable for hazelnut micropropagation have been proposed, most have been tested or resulted to be effective for just one or two varieties (Bacchetta et al., 2008; Damiano et al., 2005; Díaz-Sala et al., 1990; Mardani et al., 2020), while very few have been proved to perform well on a wider range of cultivars, as different genotypes usually need different combinations and concentrations of salts and growth regulators. Indeed, many basal media have been employed for micropropagation of this species. For example, Yu and Reed (1993) compared the effect of DKW (Driver and Kuniyuki, 1984), WPM (Lloyd and McCown, 1980) and Anderson medium (Anderson, 1984) on shoot cultures of Italian round-shaped nut 'Tonda Gentile Romana' and the local American variety 'Nonpareil', founding out that DKW medium outperformed the others in terms of shoot multiplication, elongation and appearance. They also studied the effect of different carbon sources, reporting that explants grown on 3% glucose or fructose medium produced more and longer shoots than those grown on sucrose. On these bases, they effectively multiplicated 10 cultivars on a DKW medium supplemented with 3% glucose, 1.5–3 mg/L BAP and 0.01 mg/L IBA, with subculture at 4-week intervals. Considering the mineral composition of hazelnut and almond nuts, as this last one is a species particularly adaptable to *in vitro* environment, Bacchetta et al. (2008) developed a modified version of MS medium (Murashige and Skoog, 1962), named HM, which has been employed to effectively propagate six traditional Italian cultivars, although rooting phase needed some improvements. Silvestri et al. (2020) tested the effect of different concentrations of NH_4^+/NO_3^- in both proliferation and rooting phases of 'Tonda Gentile Romana' nodal segments and concluded that a reduction to half concentration of NH_4NO_3 in MS basal medium produced shoots with a greater internode number and higher chlorophyll *a and b* contents. The lack of ammonium nitrogen, coupled both with the reduction to half concentration of the nitrate nitrogen or leaving that unchanged in half strength MS medium, resulted in a higher percentage of rooting. They also reported that an iron supply in the form of Fe-EDDHA at the

concentrations of 100 and 200 mg/L increased the shoot height and node number and produced better quality shoots respect to explants grown in the presence of Fe-EDTA, as demonstrated by the higher total chlorophyll, chlorophyll *a* and chlorophyll *b* contents. Furthermore, this study showed that adding 2.5 mg/L $CuSO_4 5H_2O$ to the establishment medium was a practical and effective way to reduce contamination rate, maintaining high bud sprouting in the explants. Several statistical approaches have been used to model a medium whose composition was suitable for *in vitro* cultivation of a wide range of cultivars. A genotype-specific mineral requirement was highlighted by Hand et al. (2014) and Hand and Reed (2014) who used a RSM to determine the most appropriate mineral nutrient concentrations for shoot growth in five and three cultivars, respectively, testing different concentrations of DKW medium salts. Hand et al. (2014) concluded that new medium formulations will require optimization for higher $Ca(NO_3)_2$, NH_4NO_3, meso- and micronutrients. Hand and Reed (2014) focused on micronutrients requirement of three cultivars and concluded that improved growth and shoot quality in 'Dorris' and 'Jefferson' required greatly increased B, Mo and Zn combined with low Mn and Cu, while 'Sacajawea' required a higher content of B, Mn, Zn and Ni with low Mo for the best growth. Akin et al. (2017b) employed a CHAID data mining algorithm to analyse the growth of 'Dorris', 'Wepster' and 'Zeta' shoots varying DKW macronutrient salts concentration from 0.5 × to 3 × , reporting that an improved version of that medium should contain 0.5 × NH_4NO_3, 3 × KH_2PO_4, 1.5 × $Ca(NO_3)_2$, while other macronutrient salts could be set at the standard DKW concentrations (1 ×). The same algorithm was employed using ions instead of salts as independent variables, in order to prevent ion confounding, for modelling the best ion concentrations for shoot growth responses of 'Barcelona', 'Jefferson' and 'Wepster' in a DKW-based medium, concluding that it should include $NO^{3-} \leq 88$ mM, $NH^{4+} \leq 20$ mM, $Ca^{2+} \leq 5$ mM, $Mg^{2+} > 5$ mM and $K^{+} \leq 46$ mM (Akin et al., 2017a). Since shoot length and multiplication rate are influenced by concentration of cytokinins (Bassil et al., 1990), many attempts have been carried out to define which of these growth regulators are the most effective for hazelnut micropropagation and in which concentration. Thomson and Deering (2011) reported BAP to perform better than KIN, ZEA and 2-iP in promoting new shoots formation and elongation in cultivar 'Daviana' when present at a concentration of 5 mg/L. Prando et al. (2014) reported that coconut water, which is particularly rich in nutritious substances and cytokinins, promoted shoot elongation and proliferation in the Italian cultivar 'Tonda Gentile delle Langhe' shoots when applied at a 20% concentration in presence of 2 mg/L BAP, 0.01 mg/L IAA and 0.5 mg/L GA_3 on a DKW medium with an 80% reduction of macronutrients. Díaz-Sala et al. (1990) employed a double-phase system for efficient proliferation and elongation of 'Tonda Gentile delle Langhe' explants cultured on a modified MS medium supplemented with 5 mg/L BAP, 0.01 mg/L IAA and 0.1 mg/L GA_3. They showed that a 3-month cold storage of field collected branches prior to forced outgrowth of axillary buds greatly increases morphogenic capacity of nodal segments excised from newly formed shoots. Plant rooting was achieved by immersing the single micro-shoot basal end in 0.1–1 mg/L IBA solution for 10 s followed by a 20-day culture on a modified MS medium. An efficient protocol was developed by Sgueglia et al. (2019) for micropropagation of four traditional Sicilian cultivars: 'Carrello', 'Ghirara', 'Minnulara', and 'Panottara'. They reported a concentration of 17.6 μM IBA to be effective for obtaining more than 85% of rooted and acclimatized shoots in all tested genotypes. It has been suggested that rooting is positively affected by polyamines which could act synergistically with auxins. Rey et al. (1994) reported an enhanced rooting ability in 'Gironell' micro-shoots both when these explants were stimulated for 15 s with a solution containing 5 μM IBA alone or in combination with 1 μM of putrescine, spermine or spermidine, and when the same amount of one of these polyamines was present in the basal medium and not in the stimulating solution. A beneficial effect of putrescine (1 mg/L), coupled with 1 mg/L IBA, has been reported also by Ellena et al. (2018) on rooting of 'Tonda Gentile delle Langhe' and 'Barcelona' after multiplication stage, which has been shown to be significantly enhanced when a temporary immersion system on liquid DKW medium was employed respect to cultivation on the same solid medium. Micropropagation has the potential to become an effective technique for rapid multiplication of valuable cultivars, representing an alternative to traditional methods of propagation and allowing the establishment of this culture also in countries where the presence of hazelnut orchards is limited by lack of plant material.

2.2. *In vitro* virus eradication and germplasm conservation

Different techniques based on *in vitro* culture have been proposed for virus eradication from hazelnut infected explants, some of which could also be effectively employed for germplasm conservation. The less laborious way to carry out germplasm conservation probably consists in slowing down shoot growth rate acting on *in vitro* environmental conditions. Sgueglia (2015) evaluated the effect of two types of sugar, sucrose and sorbitol, at two concentrations, 87.5 mM and 131.2 mM, on growth rate of 'Tonda Gentile Romana' shoots under dark conditions at 4 °C. After a period of 10 months, with bimonthly observations starting from the fourth month of cultivation, plant grown in presence of 131.2 mM sucrose had a lower percentage of survival and a higher chlorosis and necrosis symptoms than plants grown in the other conditions, with statistically lower levels of chlorophylls *a and b* and carotenoids. Control plants, which were grown in the same media, but at standard growth temperature, did not survive more than four months, confirming that keeping the explants at low temperature is an effective way to slow down the growth rate, reducing the frequency of subcultures. Kaya (2021) compared the efficacy of meristem culture, thermotherapy and cryotherapy for eradication of ApMV from infected *C. avellana* cultivar 'Palaz' plantlets. Meristems from thermotherapy-treated shoots failed to regenerate as they rapidly turned brown and died, probably because the temperature employed were too high (40 °C) for hazelnut growth. Although meristem culture resulted in 100% shoot regeneration, shoots still presented slight symptoms of the disease, and the presence of the virus was confirmed by RT-PCR analysis. Cryotherapy, performed through chemical vitrification treating meristems excised from 2-week cold hardened (4 °C) shoots with 3 μL of PVS 2 (Sakai et al., 1990) on an aluminium strip plate for 60 min and then plunging these directly into liquid nitrogen for at least 24 h, resulted to be the most effective method for virus eradication among the three tested, with a 46.7% of regenerated shoots and no detectable ApMV infection. A similar approach was employed by Sgueglia et al., 2021a for cryopreservation of 'Tonda Gentile Romana' axillary buds collected from *in vitro* grown shoots. They tested the effects of two different vitrification solutions, PVS 2 and PVS 3 (Nishizawa et al., 1993), and two application times, 60 or 90 min, on shoots regrowth rate, reporting the highest value (56.7%) for explants treated with PVS 3 for 60 min. The effect of a cold pre-treatment at 4 °C for 3 months on axillary buds was also evaluated, finding out that this treatment did not affect the regrowth rate. Cryopreservation of hazelnut was firstly reported by using embryo axis as explant for long term storage (Gonzalez-Benito and Perez, 1994; Reed and Hummer, 2001; Reed et al., 1994). Anyway, to preserve the genotype of a certain cultivar, the cryopreservation of explants with a clonal origin such as shoot tips and axillary buds is necessary. Sgueglia et al., 2021b tested different conditions for an optimal cryopreservation of axillary buds excised from *in vitro* grown shoots of 'Tonda Gentile Romana' and 'Montebello', through the encapsulation-dehydration method. They obtained the best regrowth rate treating 3% alginate beads of both cultivars for 1 day in 0.75 M sucrose MS medium, followed by an 8 h desiccation stage with silica gel. Concerning the type of cytokinin employed in regrowth phase, 'Tonda Gentile Romana' did not show significant differences between the two phytohormones tested, whereas 'Montebello' responded better when mT was applied instead of BAP. The encapsulation of meristems was reported by Yahyaoui et al. (2021) to be an effective technique for virus eradication in *vitro* grown

plants of seven Sicilian cultivars, previously assessed to be ApMV infected. Of 128 regenerated plants, with at least 10 individuals per genotype tested, a 96.9% resulted to be virus-free as checked with RT-PCR analysis. The difference between the results obtained in this study and those obtained by Kaya (2021) is probably due to the explant size, as meristems in the order of 0.2–0.3 mm are less prone to be infected than the larger ones. Despite some valuable methods have been reported for hazelnut germplasm conservation, these have been tested for only one or two cultivars. It would be interesting to compare the response in terms of regrowth rate extending the number of cultivars evaluated, as different genotypes usually give different responses to *in vitro* cultivation. Some of these techniques have also been employed for effective eradication of ApMV, the most threatening virus affecting hazelnut culture, which could lead to severe yield losses in particular environments such as Spain and Southern Italy (Rovira and Aramburu, 2001; Yahyaoui et al., 2021).

2.3. De novo organogenesis and somatic embryogenesis

The first report of somatic embryogenesis in hazelnut has been made by Radojević et al. (1975), who obtained somatic embryos from callus culture induced from zygotic embryos isolated from immature seeds and cultured on a modified MS solid medium in presence of 1 mg/L of both KIN and 2,4-D. Regarding this kind of auxin, they found out that it did not inhibit the embryoid induction, whilst it arrested further development into plantlets. Pérez et al. (1983) have been the first to obtain somatic embryos starting from a vegetative tissue, cultivating cotyledonary nodes excised from 5-week-old *in vitro* grown seedlings. They achieved embryogenesis in 60% of the explants cultivated for 20 days on a K(h) medium (Cheng and Smith, 1975) in the presence of either 5 μM IBA plus 0.5 μM BAP or 5 μM BAP plus 0.5 μM IBA. Subsequent proliferation was successfully maintained during 5 subcultures on K(h) basal medium and in an unlimited number of subcultures in K(h) supplemented with 0.5 μM BAP. They were also the first to achieve plant regeneration *via* somatic embryogenesis from 50 to 55% of the embryoids formed after 10–20 days of cultivation into a basal K(h) medium. Centeno et al. (1997) have evaluated the correlation of specific plant growth regulators with embryogenic competence of cotyledons from free-open pollinated hazelnut fruits of two Spanish cultivars, 'Casina' and 'Negret'. The first one were tested in two different ontogenetic stages, as immature and mature fruits, respectively, while for the second one, only mature fruits were tested. Of these three kinds of explants, only the ones obtained from 'Casina' were able to produce somatic embryos. They did not find any difference associated with the ABA content of cotyledons and their developmental stage, while the IAA/ABA ratio found in cotyledons of the embryogenic genotype of hazelnut ('Casina') was nearly twice as high as that in the non-embryogenic one ('Negret'), despite the small differences observed in the levels of each individual phytohormone. Furthermore, although the three cotyledonary systems studied had a similar content of total cytokinins, they showed a very different 2-iP-type/ZEA-type cytokinin ratio, which was higher in the embryogenic explants, and they also reported that an excess of ZEA-type cytokinins relative to endogenous IAA reduced or inhibited the competence of hazelnut cotyledons to respond to exogenous hormonal stimuli. All these parameters could represent reliable indexes of cotyledons embryogenic competence. Aygün et al. (2009) tested the effect of different auxins, 2,4-D and NAA, in combination with BAP on somatic embryogenesis from cotyledons of open-pollinated immature seeds of most relevant Turkish cultivar 'Tombul', sampled in two different dates, approximately 45 and 30 days before the harvest date, respectively. They found out that 1.0 mg/L BAP alone was able to induce somatic embryogenesis in 52.5% of the explants from the first sampling date; the ratio increased to 66.7% when 0.5 mg/L NAA were used in addition, after 4 weeks of cultivation on MS medium. They were the first to test the effect of NAA in hazelnut somatic embryogenesis, reporting that, in general, it induced more somatic embryos respect to 2,4-D. Silvestri et al. (2016) were able to regenerate for the first time plantlets from adventitious shoots obtained using several *in vitro* rejuvenated mature tissues of 'Tonda Gentile Romana' as explants. They also evaluated the effect of an antibiotic pre-treatment on the cultures from which the explants have been taken, as many antibiotics are thought to exert an auxin-like effect. In particular, 15 mg of carbenicillin, vancomycin and cefotaxime were individually supplemented to a 2-year-old axenic culture grown on HM (Bacchetta et al., 2008) in the presence of 1 mg/L BAP, 0.5 mg/L ZEA and 0.2 mg/L GA_3. Antibiotic pre-treatments with carbenicillin and vancomycin resulted in an average shoot height and internode number significantly higher than the control. Leaves, petioles, internodes and stipules were collected for carrying out regeneration experiments. Callus with nodules formation was observed after a 3-week cultivation of the explants on induction medium based on MS basal salts supplemented with 30 g/L sucrose, 0.55% plant agar, 1 mg/L BAP, 2 mg/L IBA, 2 mg/L KIN. Then, calli were transferred on half-strength MS medium, supplemented with 30 g/L sucrose, 0.55% plant agar and 0.5 mg/L BAP, resulting in the appearance of spots of red pigments on the callus surface. Furthermore, many tracheary elements, particularly tracheids with annular or helical secondary wall thickenings, differentiated in calli obtained from different kinds of explants. The regenerated shoots were placed on proliferation medium and after that, single shoots were put on half-strength MS medium supplemented with 2% sucrose and 1 mg/L IBA for rooting, which took place after 20 days of culture; a 60% of plant survival was reported. Plantlet regeneration represents the main bottleneck to the application of genetic engineering on hazelnut, so major efforts for the establishment of effective protocols for somatic embryogenesis or adventitious organogenesis induction from mature tissues should be pursued.

2.4. Haploid culture, polyploids and protoplast technology

Individuals with different ploidy levels could enrich the genetic variability pool of a species, representing a benefit for breeding programs. Gametic embryogenesis allows the rapid development of a completely homozygous line in a single generation, which is particularly attracting in a self-incompatible species like hazelnut, characterized by high heterozygosity and long juvenile phase. Haploid and doubled haploid plants are useful for a series of applications such as parental line fixing, production of F1 hybrids, introgression of new traits through backcrossing, genomic studies and genome sequencing. A first attempt to perform gametic embryogenesis in *C. avellana* was made by Chiancone et al. (2013) who carried out anther culture of six cultivars, evaluating the effect of two different thermal stresses exposing anthers to 60 min at 35 °C and 30 min at −20 °C. Although a sporophytic pathway was initiated, as bicellular (with symmetrical nucleus division), tricellular and multicellular structures were observed, and a strong interaction between the cultivar and the type of thermal stress was observed, no embryo was obtained. Gniech Karasawa et al. (2016) were the first to perform hazelnut microspore culture. They tested the effect of two media, P and N6, and four thermal stresses (35 °C for 30 min, 40 °C for 60 min, −20 °C for 30 min and −20 °C for 60 min) on microspores isolated from anthers of five cultivars, previously stored at 4 °C for 2 weeks. Also in this study, different responses among genotypes tested were reported. After 20 months of culture, microspores development was assessed revealing a sporadic presence (less than 3%) of binucleated microspores with asymmetrical division of the nucleus, indicating a high number of microspores which underwent the sporophytic pathway. For the first time, microspore-derived embryos were detected, as assessed through SSR marker analysis, and average number of callus and embryo production was also evaluated. Although thermal stress has been reported as an enhancer of gametic embryogenesis onset, four out of five of the cultivars evaluated produced a higher number of embryos when cultured on both media without stress treatment. Silvestri et al. (2018) tested the effect of two basal media, N6 and BN, and three

phytohormones combinations (1 mg/L 2,4-D, 0.5 mg/L IAA, 1 mg/L ZEA; 2.5 mg/L BAP, 0.1 mg/L NAA and 1 mg/L 2,4-D, 1 mg/L KIN) on Italian cultivars 'Nocchione', 'Tonda Gentile Romana' and 'Tonda di Giffoni' anther culture. Explants were grown for 21 days in dark conditions and then subcultured in half-strength MS supplemented with 3% sucrose, 0.5 mg/L IAA, 1 mg/L TDZ and placed under light. They observed that hormone combination has a strong interaction with both cultivars and basal salts and reported the combination of 1 mg/L 2,4-D, 0.5 mg/L IAA and 1 mg/L ZEA as the most effective for swollen and callus induction on anthers, although no haploid callus was detected in any of the conditions tested. Developing plants with a higher level of ploidy also represents an important achievement for breeders, as polyploidy often results in individuals which show enhanced agronomical performances compared to the relative diploids, as gene duplication leads to alteration of gene expression, which has an impact on plant morphology and physiology. To date, only one study has reported polyploid induction in hazelnut: callus treated for 5 and 6 days with 0.2% colchicine on solid medium and callus-derived cells treated for 3 and 4 days with 0.3% colchicine in liquid medium resulted in the obtainment of tetraploid cells as assessed through flow cytometry (Rahpeyma et al., 2017). Tetraploid cell suspensions showed a 1.7-fold increase in paclitaxel production compared to diploid cells. NPBTs such as CRISPR/Cas systems allow the editing of specific genomic regions, without the drawback of DNA integration in the host genome when these systems are delivered as a RNP complex. For this application, protoplasts seem to be the most suitable explant due to their high permeability to exogenous macromolecules. Up to now, only one protocol for hazelnut protoplast isolation has been published (Revilla et al., 1987). Briefly, the most recently fully expanded leaves were harvested from 1-4-month-old greenhouse grown seedlings, chopped into narrow strips and then plasmolyzed for 1 h in 13 M CPW medium. Then, about 200 mg of the plasmolyzed leaf tissue were incubated in 5 mL aliquots of a cell wall degrading enzymatic solution at 25 °C with a continuous diffuse illumination, for 18 h, in agitation. After digestion, the tissue was filtered through a 64 μm nylon sieve and the filtrate centrifuged for 10 min at 100×*g*. The supernatant was discarded, and the protoplasts were purified through a 20 μm nylon sieve. The protoplasts were resuspended in 9 M CPW medium, and the absence of cell walls was confirmed microscopically with Calcofluor White and protoplast viability was measured with fluorescein diacetate. This protocol allowed the recovery of 21×10^6 protoplasts/g fresh weight, with a 99% viability. Although these are valuable approaches for hazelnut genetic variability pool enlargement, a particularly intriguing aspect for genetic improvement programs, research still needs to make steps forward to determine the most suitable experimental conditions for the obtainment of the desired responses from different hazelnut explants.

2.5. Genetic engineering

To date, genetic engineering experiments on hazelnut genome have not been reported yet, nevertheless, recombinant DNA technology has been successfully employed for molecular cloning and heterologous expression of several hazelnut genes codifying for allergens (Akkerdaas et al., 2006; Beyer et al., 2002; Garino et al., 2010; Lauer et al., 2008; Lüttkopf et al., 2002) or involved in the biosynthesis of taxanes (Qaderi et al., 2013; Wang et al., 2007, 2010). The HMGCR gene, which acts as a key regulator in taxanes biosynthesis catalysing the first committed step in mevalonate pathway, has been cloned in a binary vector and transiently expressed *via* agroinfiltration in hazelnut leaves and calli, resulting in a higher amount of recovered paclitaxel in both tissues respect to the controls (Qaderi et al., 2013). The amenability of hazelnut to *Agrobacterium*-mediated transformation has been also reported by Jalalipour Parizi et al. (2020) and Vaedi et al. (2020), who induced hairy roots in *C. avellana* explants incubated with *Agrobacterium rhizogenes* with the aim of establishing a new source for paclitaxel production. The positive effect of *A. rhizogenes* on hazelnut rooting induction has been also reported by Bassil et al. (1991), although T-DNA integration has not been assessed. Genetic engineering could represent a reliable method for the rapid obtainment of new varieties or the improvement of the traditional ones, as it enables the rapid introduction of desired traits, with the possibility to perform site-specific modifications when genome editing techniques are employed. The most limiting factor in the application of these methodologies on hazelnut results in the lack of a protocol for regeneration starting from somatic tissues, which would allow the genetic base of an established cultivar to be maintained.

3. Genome sequencing, mapping and QTL

Several SSRs have been detected and characterized in hazelnut, allowing their employment as markers in germplasm characterization, phylogenetic studies and gene mapping (Bassil et al., 2005; Boccacci et al., 2005; Gürcan et al., 2010). A particular effort has been made through the years by the OSU to identify markers tightly linked to EFB resistance (Mehlenbacher, 2018). At first, many RAPD markers linked to a dominant locus derived from 'Gasaway', an obsolete pollinizer resistant to EFB, have been identified studying the segregation pattern in 138 individuals of an F1 generation derived from a cross between 'OSU 252.146' X 'OSU 414.062', the maternal susceptible and the paternal resistant parent, respectively (Mehlenbacher et al., 2004). The information obtained from this study, coupled with the use of SSR markers, allowed the construction of the first genetic map for hazelnut aimed to unravel the presence of markers linked to this qualitative resistance trait and to the S-locus as well, enabling their localization on LG 6 and 5, respectively (Mehlenbacher et al., 2006). From this F1 generation, through the years, breeders have selected the cultivar 'Jefferson' which is resistant to EFB as it carries the 'Gasaway' gene. To better characterize the region responsible for resistance, Sathuvalli et al. (2017) used a BAC library of 'Jefferson' to developed a high-resolution genetic map for that resistance trait from 1488 individuals from the F1 generation obtained by Mehlenbacher et al. (2004) and their reciprocal crossings. Furthermore, they constructed a high-density physical map of the resistance region and detected five candidate genes, two of which were recognized as part of super-families known to have disease-resistance properties. EFB resistance has been studied in several cultivars (Mehlenbacher and Molnar, 2021). Although analysis through SSR markers has mapped the resistance to LG 6 in 'Culplà', 'Crvenje', 'OSU 495.072' and 'Uebov', the absence of two RAPD markers tightly linked to 'Gasaway' resistance and irregular segregation ratios probably indicate that the loci involved in EFB resistance in these accessions are different respect to 'Gasaway' (Bhattarai et al., 2017; Colburn et al., 2015). Another source of qualitative resistance was recognized in 'Ratoli', a Spanish cultivar for which segregation ratios indicated the presence of a dominant allele at a single locus mapping on LG 7 (Sathuvalli et al., 2011a). The construction of a SNP marker–based ddRADseq genetic linkage map revealed that EFB resistance source from 'Rutgers H3R07P25' resides on LG 2 (Honig et al., 2019), similarly to what has been observed in Georgian accession 'OSU 759.010' (Sathuvalli et al., 2011b). Other than qualitative traits, also quantitative traits have been investigated. Beltramo et al. (2016) was the first to generate a QTL linkage map aimed to study the genetic basis of variation in vegetative traits like vigour, sucker habit, and time of bud burst, crossing 'Tonda Gentile delle Langhe' X 'Merveille de Bollwiller'. They genotyped 163 plants of an F1 population with 152 SSR markers, obtaining a map of the 11 hazelnut LGs from which they have identified 15 QTLs. Ten of these explained more than 10% of the phenotypical variance and were representative of all the three traits investigated. Particularly interesting was the detection of a stably expressed region on LG 2 controlling leaf bud burst which explained around 50% of the phenotypical variance with a LOD score higher than 20. Over time, this map has been further saturated through a GBS approach by Torello Marinoni et al. (2018) who discovered 9999 SNP markers enabling the detection of 11 and 18 QTLs for leaf budburst on the 'Merveille de Bollwiller' and on the 'Tonda Gentile delle Langhe' map, respectively.

On LG 2 of this last one, they confirmed the presence of a stable QTL responsible for 31.4–54.6% of the phenotypical variance over a 5-year period, and relatively to that genomic region, they reported the presence of three genes which are potentially involved in leaf bud burst control. These genetic maps were also employed by Valentini et al. (2021) to map phenology related traits, discovering an overall of 71 QTLs. They investigated the genomic regions surrounding these QTLs, detecting five and 16 candidate genes for time of male and female flowering, respectively, 14 candidate genes for dichogamy and five genes potentially involved in seed development. Employing for the first time on hazelnut an association mapping approach, which takes advantage of the historical recombination in a population without relying on biparental crossings, Ozturk et al. (2017) performed QTL analysis for 17 nut and kernel related traits on a diversity panel comprising 24 cultivars and 40 wild Slovenian accessions genotyped with 49 SSR primer pairs. They reported the general linear model corrected with the population structure Q-matrix as the one with the highest proportion of significant results among those tested, which enabled the identification of 49 SSR markers associated with nine of the 17 traits studied. Based on a similar approach, the largest scale QTL analysis performed on hazelnut has been carried out by Frary et al. (2019a) who have screened 30 SSR primers in a diversity panel of 390 accessions composed of 16 cultivars, 232 landraces and 142 wild individuals from nine provinces in Turkey with the aim of unravel the association of these markers with 44 agro-morphological traits. They detected 145 QTLs with the largest proportions identified for involucre (26%) and inflorescence (14%) morphology. Several markers co-localized with more than one trait, such as markers for male catkin abundance which were shared with plant vigour and height, whereas markers for female flower abundance co-localized with suckering and alternate bearing. The same diversity panel has also been characterized for 13 nut and 12 kernel traits allowing the detection of 78 loci, the most of which were related to kernel (26%) and nut (24%) morphology followed by quality (19%), shell thickness (16%) and yield-related (15%) traits (Frary et al., 2019b). The era of high-throughput sequencing technologies has enabled the application of *omics* sciences also on hazelnut, allowing the generation of a huge amount of data. Rowley et al. (2012) have provided the first *de novo* assembled transcriptome of this species retrieved from cultivar 'Jefferson' young leaves, catkins, bark, and whole young seedlings, employing an Illumina-based technology. They obtained a transcriptome assembly comprising 28,255 transcript contigs, about the 93.3% of which encode proteins as recognized by OrfPredictor tool (Min et al., 2005), with about the 75% of these which aligned to sequences already deposited in NCBI database as shown by BLASTX analysis, resulting in 16,488 transcript contigs functionally annotated by Gene Ontology analysis through Blast2GO program (Conesa et al., 2005). A differential expression analysis was also performed, allowing the identification of genes whose expression was found to be different among the four tissues analysed. The first hazelnut genome assembly was reported by Rowley et al. (2018) who sequenced the cultivar 'Jefferson' using two paired-end and one mate-pair Illumina libraries, collectively representing 93x genome coverage, obtaining 36,641 contigs and scaffolds with an N50 of 21.5 Kbps and total sequence length of 345 Mbps, which is about 91% of the estimated hazelnut genome size. With AUGUSTUS tool (Stanke et al., 2004), a total of 36,090 putative coding loci were identified, 22,474 of which have been functionally characterized *via* alignment through BLASTP tool on NCBI database, then, Blast2GO program (Conesa et al., 2005) functionally classified 11,221 of these protein coding loci. Emphasis was placed on the identification of resistance and self-incompatibility determining genes, since 115 putative NBS-LRRs and 17 candidate genes annotated as encoding self-incompatibility or S-locus-linked proteins have reported. Lastly, they re-sequenced seven cultivars representatives of four different regions of the world, detecting several variations among these accessions and the 'Jefferson' reference assembly, probably involved in different responses against pathogens and different sexual compatibility. Since it was highly fragmented, 'Jefferson' genome was also sequenced using the long-read technology Pacific Biosciences (PacBio), providing a backbone scaffold with 49× coverage, which was error-corrected using the previously obtained Illumina reads and subsequently assembled into chromosome-level scaffolds using HI–C proximity ligation method (Dovetail™), obtaining 11 pseudomolecules corresponding to the haploid karyotype of hazelnut (Hill et al., 2021; Snelling et al., 2018). Hill et al. (2021) have sequenced both 'Jefferson' parents using an Illumina platform and the sequences retrieved were aligned to the reference 'Jefferson' genome. Those sequences were used for the development of SSR and HRM markers close to the S-locus, narrowing that region to 193.5 Kbps and detecting 18 genes within it. Lucas et al. (2021) produced a 370 Mbps chromosome-level genome assembly for the most highly valued Turkish cultivar 'Tombul', representative of the 97.8% of the estimated *C. avellana* genome size, combining three different sequencing technologies which provided data with different size ranges: short reads (0.1–1 Kbp; Illumina paired-end), long reads (1–10 Kbps; NanoPore) and proximity ligation (10 Kbps–10 Mbps; Dovetail™). They identified 27, 270 protein-coding genes, 20,000 of which have been functionally annotated and emphasis was placed on the detection of genes involved in susceptibility to powdery mildew-causing pathogens, identifying 12 full-length MLO genes, and on genes encoding for allergens, detecting all known hazelnut allergens-encoding genes and several others previously unreported which could encode for potential allergenic proteins. 'Tombul' genome was used as a guide by Pavese et al. (2021a) for scaffolding the sequence retrieved from the linked-reads sequencing (10× Genomics) of the Italian reference quality cultivar 'Tonda Gentile delle Langhe', obtaining 11 pseudomolecules. The Maker-P tool (Campbell et al., 2014) identified a total of 27,791 genes, whose function has been annotated using BLASTP tool on SwissProt database and the InterProScan domain inspection tool (Jones et al., 2014). Particular attention was given to the identification of genes involved in resistance response as 810 putative resistance genes were detected, with the chromosome 2 being the richest one, followed by 5, 3, and 4. They reported most resistant genes to be RLKs, followed by RLPs, while only few of resistant genes contain at least one NB-ARC domain. *Omics* sciences have shown that a big amount of information could be retrieved relating to the genetic basis of traits of interest, enabling their employment in MAS or genetic engineering for the obtaining of high value cultivars in less time.

4. Secondary metabolite production

European hazelnut contains many important phytochemicals like phenolic acids, flavonoids, tannins, proanthocyanidins, diarylheptanoids, lignans, taxanes and volatile compounds (Bottone et al., 2019). Special attention has been given to taxanes: among these molecules, paclitaxel or Taxol™, is known for its antimitotic effect in G2-M phase of the cell cycle, leading to an important antitumor activity (Jennewein and Croteau, 2001). This molecule is already used as a drug, but it sees the strong limit in the short supply, due to the non-easiness of the synthetic production (Croteau et al., 2006). At first, paclitaxel was found in *Taxus* genus but its extraction from the *in vivo* growing plants resulted ecologically unsustainable and *Taxus* spp. Is known to be recalcitrant to *in vitro* culture (Jennewein and Croteau, 2001). Once it was individuated in hazelnut, many studies were carried out to establish efficient protocols for using hazelnut plant tissue culture to produce paclitaxel, or at least its precursors.

4.1. Cell suspensions

First detection of paclitaxel in hazelnut cell suspension was reported by Bestoso et al. (2006) when they succeeded to produce this compound at 17 μg/mL concentration. With the aim to increase taxanes content, chemical elicitation was carried out using 200 μM methyljasmonate plus

182 μg/mL chitosan resulting in a doubling of taxol production, with respect to the control. This first result was encouraging and not so far from the results obtained with *Taxus baccata* L. cell suspensions (Malik et al., 2011). The second report on hazelnut cell as biofactory of paclitaxel was given by Bemani et al. (2013): they proposed to elicitate cells with 6 mM phenylalanine, obtaining an increment of paclitaxel respect to the control, even though the concentration was still at a low level (48 μg/L for both intracellular and extracellular paclitaxel). Moreover, the combination of 3 μM phenylalanine with 0.1 mM vanadyl sulfate led to the production of 4.2 μg/g dry weight of paclitaxel (Rahpeyma et al., 2015). The first physical elicitation treatment of hazelnut cells was carried out using ultrasounds at 455 mW for 8 and 20 min, obtaining the production of major taxanes: paclitaxel, 10-deacetylbaccatin, and baccatin III at a concentration of 0.46, 0.26, and 0.07 mg/L, respectively (Safari et al., 2012). Sonication with 29 KHz continuous ultrasound at 4 mW/cm^2 intensity for 20 min also provided the enhancement of lipoxygenase, phenylalanine ammonia-lyase, and 1-deoxy-D-xylulose-5--phosphate reductoisomerase activities (Ghanati et al., 2015). Given the extraordinary variety of possible cultivation and elicitation techniques, a model for forecasting paclitaxel biosynthesis in hazelnut cell culture based on ANFIS was proposed. Using this technology, Farhadi et al. (2020) set up a protocol employing three fungal elicitors, derived from an endophytic fungus isolated in *Taxus baccata* L., resulting in a final paclitaxel concentration of 0.40 mg/L. Among fungal elicitors, also *Camarosporomyces flavigenus* isolated from *C. avellana* was tested, obtaining a concentration of total paclitaxel of 351.4 μg/L (Salehi et al., 2020). One of the crucial steps in cell culture establishment for an industrial production is the transition from a laboratory scale to a bioreactor scale. For hazelnut cells, a protocol based on UniVessel®SU bioreactor resulted efficient with a total taxanes production of 6.24 mg/L (Gallego et al., 2015).

4.2. Callus culture

A fast-growing and optimized callus culture is the basis for an efficient cell suspension for the accumulation of metabolites. Salehi et al. (2017) investigated several components of the culture medium for cotyledon-derived callus growth optimization. First, they focused on the basal salts, starting from a modified MS medium. They also evaluated the effect of several concentrations of casein hydrolysate, spirulina powder and various amino acids. The modified MS medium developed, named M10, with 1000 mg/L algae powder, 1000 mg/L casein hydrolysate and 3 g/L gelrite (pH 6.0), resulted in an increasement of callus biomass of *C. avellana*. Using this medium, a cell suspension producing 106.6 μg/L of paclitaxel was set up (Salehi et al., 2017). One of the major problems concerning hazelnut callus is the browning effect in the callus induction phase. To overcome this problem, Shirazi et al. (2020) proposed two different systems: first, the addition in the medium of antioxidant molecules such as PVP, acetic acid and citric acid; second, a change in the subculture system, where the induced calli were transferred to a liquid medium with the same composition and then the resulting cells were immobilized on a solid medium. The latter resulted in a 10-fold growth rate increasing respect to the routine cultivation methods. To evaluate the effect of different medium composition on callus growth and metabolite production, Hazrati et al. (2022) set up an experiment combining different basal salts, hormones and ultrasound exposures, carrying out subsequent physiological and metabolic analysis. They found out that combining 2,4-D (2 mg/L) and KIN (0.2 mg/L) with the sonication of explants for 1 min resulted in an optimized condition for callus induction and growth. Moreover, WPM and MS basal salts allowed the highest accumulation of baccatin III (147.98 and 147.85 mg/L, respectively), while the highest paclitaxel content (44.89 mg/L) was obtained in WPM medium.

4.3. Hairy roots culture

Hairy roots technique is one of the most appreciated for the bioaccumulation of molecules because it consists in the transformation of the plant matrix with *A. rhizogenes* that, thanks to *Ri* plasmid, leads to a neoplastic production of adventitious roots without using hormones and with the possibility to scale-up to bioreactor scale (Pistelli et al., 2010; Wawrosch and Zotchev, 2021). Hazelnut hairy root induction was firstly investigated by Jalalipour Parizi et al. (2020), testing three different *A. rhizogenes* strains and six culture media, using hazelnut seedlings as starting material. Hazelnut leafstalk inoculated with c58c1pRiA4 strain in quarter-strength WPM medium resulted to be the most suitable medium for hairy roots induction, while half-strength SH (Schenk and Hildebrandt, 1972) was the best culture medium for their growth in liquid medium. These hairy roots contained 3.2 μg/g dry weight of paclitaxel. Similar results were obtained by Vaedi et al. (2020), who suggested quarter-strength WPM medium supplemented with B5 vitamins (Gamborg et al., 1968) for the induction phase and a quarter-strength SH medium supplemented with B5 vitamins for the development in liquid media. Ascorbic acid was also tested with positive results to reduce the browning effect. Paclitaxel content was assessed at 4.02 μg/g dry weight, very close to what other authors have previously reported (Jalalipour Parizi et al., 2020; Vaedi et al., 2020).

5. A hazelnut ideotype: which are the most important traits?

According to the most relevant hazelnut breeding program carried out in Corvallis (Oregon – United States of America) at the OSU (Botta et al., 2019), and to some other minor breeding activities such as those carried out in Turkey at the Hazelnut Research Institute of Giresun, in Spain at the IRTA, in Chile at the INIA and at other few Italian research centres, as also detailed by Silvestri et al. (2021), the European hazelnut ideotype can be set as follow.

- develop new cultivars for the blanched kernel market, according to the high request of the confectionery industry (Romero-Aroca et al., 2021), for which the round-shaped nut Italian cultivars 'Tonda Gentile delle Langhe' and 'Tonda di Giffoni' set the standard for quality;
- spread the resistance to EFB, which remains the main concern for the Oregon's hazelnut growers, as well as the resistance to the most relevant diseases in other countries, such as nut rot caused by *Fusarium* spp. (Turco et al., 2021), *Diaporthe* spp. (Arciuolo et al., 2022), *Gnomoniopsis castaneae* (Lione et al., 2019), the emerging powdery mildew by *Erisiphe corylacearum* (Mazzaglia et al., 2021; Sezer et al., 2017), and the anthracnose by *Piggotia coryli/Cryptosporiopsis tarraconensis* (Altin and Gulcu, 2023; Drais et al., 2023) and pests, such as the marmorated stink bug, *Halyomorpha halys* (Bosco et al., 2018) and the bud mites, primarily *Phytoptus avellanae* (Contarini et al., 2022);
- shortcomings and undesirable traits in the new releases include long nut shape, thick shells, low nut yield per tree, poor pellicle removal after roasting, and a high frequency of nut and kernel defects.

The minimum standard of the main OSU breeding program objectives is summarized below as a reference, since it targets the main outputs expected from breeding programs. Seedlings and selections must meet or exceed all the following minimum standards for each objective to be further evaluated for a possible release as new cultivars; the minimum standard for bud mite resistance is cultivar 'Clark' that has an intermediate susceptibility; 'Tonda Gentile delle Langhe' is the reference for round-shaped nut; the minimum percent kernel (kernel/nut ratio) is fixed at 48%; precocity of fruit-set is also highly desired trait (at least 35 nuts in 5th leaf on ground). High yield, easy pellicle removal, few defects and early maturity are other requested traits, and for all these traits the minimum standard is referred to cultivar 'Barcelona'. Finally, a high

free-falling ripening nuts from the husks is requested, at least of 85%, to promote the mechanical harvesting.

Furthermore, European hazelnut is self-incompatibility species of the sporophytic type under the control of a single locus with multiple alleles (Mehlenbacher, 1997), where the stigmatic surface is the site of the incompatibility reaction (Hampson et al., 1993). In incompatible pollinations, pollen germination is delayed, and pollen tubes are distorted and fail to penetrate the stigma. Its allelic variants are grouped according to a dominance hierarchy of S-alleles in hazelnut pollen, structured in eight different levels. Alleles are dominant to alleles below them in the hierarchy, and codominant with those at the same level. Thus, according to its floral biology European hazelnut in commercial orchards requires to be mixed with compatible pollinizers for the allele expressions and characterized by male blooming overlapping with the female blooming of the main cultivar. To this effect, universal hazelnut pollinizers are researched and specifically through controlled crosses, attempts are made to fix rare allele expressions for the S-locus, favouring seedlings characterized by alleles located in lower dominance hierarchical levels, and with abundant release of viable pollen (Ascari et al., 2020).

6. Future and perspectives

The rapid increase registered in the last years for hazelnut cultivated area has posed the need for the development of new varieties which show adaptability to a wide range of environments, providing high quality yields. Molecular markers developed for MAS and the data retrieved from *omics* studies represent a solid base for leading genetic improvement programs relying on advanced biotechnological approaches. Genetic engineering could represent an effective way to speed up the process of new European hazelnut varieties development, allowing the rapid introduction of desired traits. Despite the application of these techniques on hazelnut is feasible, their employment must rely on effective protocols for *in vitro* tissue culture. Although hazelnut response to *in vitro* conditions has been reported to be genotype specific, many effective protocols have been published for hazelnut micropropagation and research is still ongoing for the development of media suitable for many cultivars. On the contrary, few works dealt with hazelnut plantlet regeneration, none of which reported satisfactory results when mature tissues were used. Major efforts should be also made to define the best culture conditions for the induction and regeneration of individuals which show different levels of ploidy, as those could enlarge the genetic pool of this species and be useful for genomic studies. Regeneration through protoplasts isolation and cultivation is also relevant as they represent the eligible type of explant to obtain transgene-free edited plants. *In vitro* tissue culture also provides the opportunity to produce valuable secondary metabolites such as paclitaxel, one of the most important anticancer compounds, which is naturally produced by hazelnut. Anyway, tissue culture conditions need to be optimized to scale up the process from laboratory bench to industrial scale. Biotechnologies applied to plant sciences could represent a sustainable way to increase the production level of a species in a suboptimal environment, with less inputs with respect to traditional agriculture.

Author contribution

AF and CS conceptualized and designed the outline of the manuscript. AF, ML, VC and AM wrote the first draft of the manuscript. AF, ST, VP, DTM, RB, AM and CS revised the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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