



Challenges in the micropropagation of economically important fruit species in Europe

Valbona Sota¹ · Hannes Wilms² · Buhara Yücesan³ · Yeşim Yalçın Mendi⁴ · Bruce Christie⁵ · Jaroslav Nisler⁶ · Şafak Esra Aslan⁷ · Liva Purmale-Trasune⁸ · Cristian Silvestri⁹ · Stefaan P. O. Werbrouck¹⁰ · Lucie Fischerová⁶ · Tuija Aronen¹¹ · Branislav Cvjetković¹² · Maurizio Lambardi¹³

Received: 18 June 2025 / Accepted: 25 July 2025
© The Author(s) 2025

Abstract

Commercial micropropagation plays a crucial role in increasing the production of many economically important fruit crops worldwide, including Europe. The focus has been on many woody horticultural species, such as stone and pome fruits, berries, nuts, and palms. This paper presents an updated overview of the European commercial micropropagation landscape, highlighting the countries currently operating in this sector, crop targets, and the distribution of micropropagation companies based on recent survey data. In Europe and the broader Mediterranean basin, Italy, Spain, Türkiye, and Germany emerge as the leading contributors to the large-scale in vitro production of woody fruit species, based on the number of operating commercial companies and efforts to adopt new technology. Despite this progress, several challenges limit the scalability and sustainability of in vitro propagation systems, and their expansion in underrepresented countries. These main issues include species recalcitrance, high labour and infrastructure costs, further improvement of stock maintenance capacity, logistical issues in plantlet shipment, and barriers related to intellectual property rights. To address these challenges, this paper discusses solutions such as utilizing automation and robotics, exploring new compounds to enhance micropropagation rates, and using European funding opportunities. Furthermore, being involved in pan-European cooperation and technology transfer networks is also encouraged. The COST Action 21157 “CopyTree: European Network for Innovative Woody Plant Cloning” is discussed as a practical example of collaboration in harmonizing protocols for woody plant cloning and facilitating knowledge sharing among research groups with varying levels of experience in the field. Future advancements in the micropropagation sector will depend on the investment in automation and innovative technologies, combined with policy alignment with EU standards on a broader international level, and regional development strategies.

Key message

Addressing technological disparities through innovation, automation, and collaborative networks is crucial for expanding fruit crop micropropagation in Europe and bridging the gap between advanced producers and under-represented regions.

Keywords Micropropagation · Woody fruit crops · Commercial companies · In vitro propagation

Overview of commercial micropropagation in Europe

Micropropagation is a well-established sector of the nursery industry for many plant species, with a long history in Europe. Initial developments date back to the early 1950s

and 1960s, following the discovery of totipotency in specific plant cells. At the beginning of the 1970s, commercialization began, especially in the Netherlands, Germany, Italy, and the UK, with a primary focus on ornamental plants and some fruit species, such as the strawberry. In the 1980s and 1990s, a significant expansion in forestry and horticulture

Communicated by Sergio J. Ochatt.

Extended author information available on the last page of the article

was observed (Debergh and Read 1991). Applications on such a large scale demonstrate and reinforce the advantages of this technique for cloning woody plant species, including the rapid mass production of genetically uniform plants, continuous year-round propagation, and the production of disease-free plant material (Dobrąnszki and Teixeira da Silva 2010). Improved micropropagation protocols for woody plant species (Lambardi et al. 2013), the application of innovative propagation systems like temporary immersion bioreactors (Etienne and Berthouly 2002; Méndez-Hernández and Loyola-Vargas 2024), and the development of low-cost protocols (Castro-Camba et al. 2023) are effective practices that may ensure achieving a fast and high rate of clonal production throughout the year.

By optimizing growth conditions, micropropagation ensures the production of true-to-type plants (Wójcik et al. 2021; Martins et al. 2004). However, because the ability of micropropagated plants to maintain genetic stability is genotype-based, it is crucial to develop adequate propagation protocols that minimize the occurrence of somaclonal variation, limit the onset of epigenetic alterations which may persist for long periods after the transfer to the field, and validate the results using molecular diagnostic tools (Mirzaei et al. 2021; Martins et al. 2004).

A major problem in crop production is maintaining plant health, as it affects both food production and public health. Pathogen-free plants (i.e., free from virus, viroids, bacteria, and phytoplasma that can affect the morphology of plants and fruits) can be produced through the application of suitable *in vitro* techniques, which involve virus eradication protocols, such as meristem culture, thermotherapy, chemotherapy, cryotherapy, and electrotherapy, and then multiplied by micropropagation (Knapp et al. 1995; Panattoni et al. 2013), thus facilitating international germplasm exchange (Hussain et al. 2012). To ensure the safe use and exchange of plant genetic resources simplify and optimize international trade activities, and reduce pesticide use, several international organizations, including the Food and Agriculture Organization (FAO), the World Trade Organization (WTO), and The Alliance of Bioversity International and CIAT, have established guidelines to minimize phytosanitary risks. In this regard, many certification schemes and phytosanitary regulations incorporate *in vitro* techniques as essential tools to produce virus-free plants and ensure alignment with food security standards (IPPC Secretariat 2022; EPPO 2008, 2012).

It is observed that the resilience of the horticulture industry is enhanced by academia-industry cooperation and the integration of biotechnological applications, which ensures the reliable application of scientific research in practical production systems. While Universities and Government Research Centers are the leading producers of scientific

knowledge and technological innovation, industry turns this knowledge into commercially viable products. Strengthening their collaboration helps in developing the horticultural industry sector and increasing the number of well-established micropropagation companies specializing in propagating and exploiting woody plants. Unlike forest species, clonal commercial micropropagation of horticultural plants is today widely accepted by the public and is widely available on the consumer market (Ríordáin 2003). This positive attitude towards clonal micropropagation is due to the clear evidence, matured over time, that this technique is reliable, repeatable, and free from risks of genetic alterations. Indeed, it can guarantee the production of millions of high-quality plants that meet certification requirements for standardized nursery production. Considering the advantages of micropropagation and the financial benefits derived from these practices, micropropagation is well established in many European countries. A wide range of economically important plant species is commercially propagated through this method (Lambardi et al. 2013). In this regard, the present review aims to identify the current distribution of commercial laboratories across Europe with a particular focus on major woody fruit crops. Additionally, this sector also faces several challenges, which are also discussed in this paper, and solutions have been proposed to overcome them.

Major producing countries

Over the past thirty years, two extensive surveys have been conducted to compile a list of all commercial tissue culture labs in COST member countries (Ríordáin 2000, 2003). However, the last survey dates from 2002 and is thus more than 20 years old (Table 1). Therefore, a new survey was necessary to create an updated overview of each country's micropropagation companies. This helps visualize changes in the commercial sector over the past twenty years. The previous survey results were reevaluated to compile this new list, and additional companies were identified through various web searches and country registers, where available (Table 1).

Given the absence of centralized or official registries in most countries, the survey methodology varied across regions. It involved a combination of desk research, expert consultation, and direct communication with colleagues and national stakeholders active in the field. A list of the identified companies is provided in the Supplementary File.

It is essential to note that the survey encompassed micropropagation companies currently operating in Europe. Most of them do not specialize in woody horticulture alone, as they work based on contracts and market demand. Furthermore, some of them have not yet worked on the clonal

Table 1 The number of micropropagation companies in COST member countries

	1996 (Ríordáin 2000)	2002 (Ríordáin 2003)	2025
Austria	1	5	1
Belgium	14	8	8
Bulgaria	0	0	3
Croatia	Na	Na	3
Cyprus	Na	0	1
Czech Republic	3	3	4
Denmark	8	Na	1
Finland	6	2	2
France	12	4	4
Germany	31	25	30
Greece	8	4	4
Hungary	8	6	1
Ireland	3	2	3
Italy	14	6	26
Latvia	Na	Na	3
Moldova	Na	Na	1
Netherlands	36	30	16
North Macedonia	Na	Na	3
Norway	6	1	0
Poland	7	7	8
Portugal	0	1	2
Romania	Na	Na	2
Serbia	Na	Na	4
Slovakia	4	4	0
Slovenia	1	Na	1
Spain	12	6	20
Sweden	3	2	2
Switzerland	4	3	0
Türkiye	Na	Na	22
United Kingdom	18	10	9
Total	199	129	184

Only countries with companies in at least one of the three surveys are displayed. Na means this country was not included in that specific survey

propagation of woody horticultural plants, instead focusing on herbaceous or forest species. However, this inclusiveness helps to highlight the potential of *in vitro* commercialization and the infrastructural capacities currently available to implement large-scale *in vitro* production of woody horticultural plants.

Although the total number of commercial producers identified in the 2025 survey is higher than in 2002, this increase is primarily due to the inclusion of new COST countries, such as Serbia, Türkiye, and Croatia, which were not covered in earlier assessments. Additionally, Italy has experienced a significant increase in the number of micropropagation companies over the past twenty years.

A closer examination of the 129 companies identified in 2002 reveals that only 39 (30.2%) are still active in the micropropagation business as of today. However, the

reasons behind this drop are diverse. The first reason is that some of the companies from the previous survey are no longer in business. The second reason is that many have ceased their micropropagation activities but continue to operate as breeders or sellers. The third reason is that some companies have relocated their micropropagation activities to subsidiaries or laboratories abroad. Although 90 companies have stopped their micropropagation activities in Europe since the previous survey, 145 new companies have emerged. It is noteworthy to mention that, while these are referred to as “new” companies, some have already been in operation for over a decade.

Significantly, these companies engaged in producing micropropagated plantlets or offering micropropagation services do not operate in isolation; rather, they can contribute to broader economic benefits by generating demand for supporting industries. Their presence encourages the development of other businesses that supply specialized equipment and consumables needed for tissue culture. In this context, many European companies are actively involved in producing and marketing such products, including plastic consumables, culture media chemicals, energy-efficient lighting systems, ergonomic tools such as tissue culture scalpels and forceps, and hardware like laminar flow hoods, sterilizers, incubators, or growth chambers. Innovation within these supplier companies can play a valuable role in enhancing the effectiveness and efficiency of micropropagation operations.

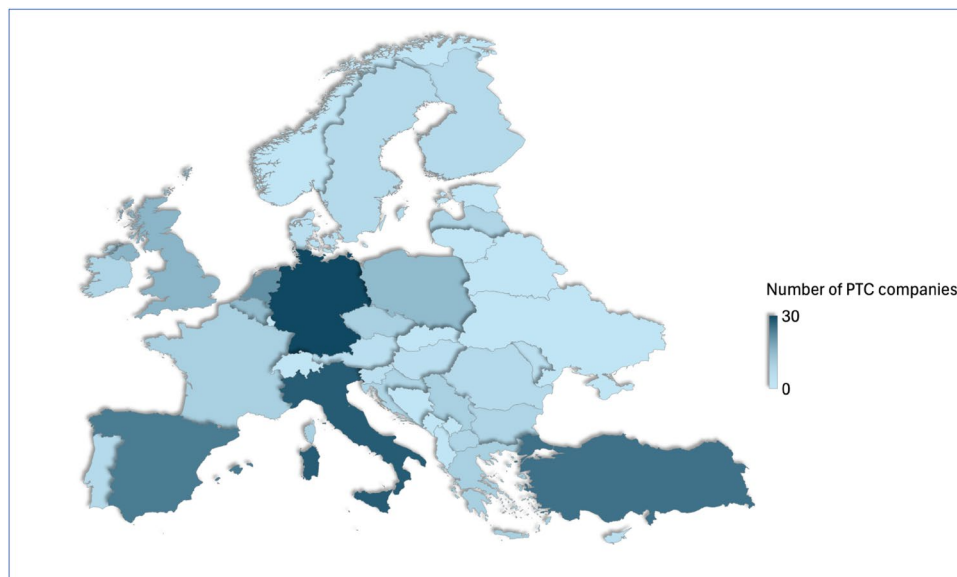
To better illustrate the geographic distribution and concentration of micropropagation companies across Europe, a visual representation was developed (Fig. 1). This map provides a comparative overview of company presence by country, highlighting regional hubs of activity and identifying areas with limited commercial engagement in the sector.

As previously mentioned, not all commercial companies listed in Table 1 focus on fruit tree species; many primarily produce ornamental or other herbaceous plant species. Among the countries presented, Italy, Spain, Türkiye, and Germany stand out as the leading producers of fruit plants in Europe through micropropagation, with the highest number of micropropagation companies specialized in this sector (Fig. 1). Further information on the leading European producers of fruit species via micropropagation is provided below.

Italy

Micropropagation has long been an essential sector of the Italian nursery industry, with 26 micropropagation companies currently operating in the country. However, the commercial micropropagation cycles remain highly labor-intensive across various stages of production, making them

Fig. 1 Geographical distribution of micropropagation companies across Europe based on 2025 data presented in Table 1. Color intensity (from pale blue to dark blue) indicates the number of companies per country, ranging from low to high



vulnerable to competition from *in vitro* culture plants coming from Mediterranean countries with lower labour costs.

To better understand this important commercial reality, the Working Group on "Micropropagation and In Vitro Technologies" affiliated to the SOI-Italian Society of Horticulture periodically conducts specific surveys on the consistency and characteristics of Italian companies, as well as on the varietal assortment of micropropagated plants. Since the first survey 27 years ago, the four surveys (conducted in 1998, 2006, 2010, and 2022) have highlighted a constant increase in production, from nearly 19 million in 1998 to the current 52 million together with a continuous growth trend in the last decade of almost 2 million plants annually (Lambardi and Previati 2012; Lambardi et al. 2024). Six of the currently active companies were already operating before the 1990s, but it is during the last decade that the sector, finding new strength, has seen the start-up of 13 new micropropagation companies. Over one-third of the companies have between 6 and 30 workers employed in the laboratory, while the two largest companies employ more than 50 workers in the entire micropropagation cycle.

According to the 2022 survey on production, fruit species (rootstocks and cultivars) continue to dominate micropropagation, ensuring the highest value of production (77% of the total), characterized by a doubling in output since 2011. Rootstocks prevail among fruit trees (over 29 million). Of these, the most requested remains the 'GF677' (peach × almond), which accounts for nearly 50% of the total *in vitro* rootstock production, followed by rootstocks of apricot, cherry, and plum. Noteworthy is the entry into production of rootstocks of pistachio ('UCB1'), citrus, and hazelnut. As for cultivars, over 10 million units are produced; almost triple the quantity recorded 12 years ago. Among the most popular species, we still find kiwifruit, represented

in micropropagation mainly by the historical cv. Hayward. Great interest has recently been paid to hazelnuts, whose cultivars represent over 28% of the total plants obtained *in vitro*, and berry fruits, especially blueberries, which account for 21% of the fruit cultivars via *in vitro* culture. Olive, fig, and walnut cultivars share a minor production.

An increase was also recorded in the case of vegetables (artichoke), with almost 3 million more plants, compared to 2010. In contrast, the trend for ornamentals has been one of continuous decline in production from 2006 to the present.

Türkiye

Türkiye's involvement in plant tissue culture began in the 1970s through academic initiatives, primarily led by Ankara and Ege Universities (FAO 2001). These early efforts focused on establishing protocols for the *in vitro* propagation of herbaceous crops, including potatoes, bananas, and strawberries. Through the 1980s and mid-1990s, tissue culture technologies gradually transitioned into the private sector, marking the beginning of commercial-scale applications. According to Gözen et al. (1995), most micropropagation companies were financed by the Turkish Ministry of Agriculture, under the supervision of universities and research institutes. During this period, the focus remained on high-throughput crops, such as ornamentals and tuberous species, while woody crops remained largely underdeveloped.

The 2000s marked a period of institutional growth and increasing commercialization in the micropropagation sector. Numerous micropropagation companies were established with support from the Ministry of Agriculture and Forestry and TÜBİTAK (the Scientific and Technological Council of Türkiye)-funded programs. Acclimatization processes were integrated into modern greenhouse systems,

and quality control frameworks and certification mechanisms began to take shape (Çetiner 2015). On the other side, the private sector maintained its production cycles with rootstocks such as ‘GF677’ (peach × almond), ‘M9’ and ‘MM106’ (apple), and ‘Myrobalan 29C’ (plum), which were widely adopted, indicating a strategic shift toward woody crops.

However, this trajectory was not without its limitations. Despite the rise in institutional infrastructure and research expenditure, which reached 55.3 million EUR between 2010 and 2012 for biotechnology R&D, many Biotechnology Research Centers failed to yield commercially viable outcomes. In this respect, Çetiner (2015) highlighted that these centers largely replicated low-impact international studies and struggled to translate findings into patents or industrial applications. The strategy documents were often vague and impractical, and the number of patents successfully converted into products was nearly negligible. In addition, the proliferation of biotech startups within technoparks—originally designed to foster collaboration between universities and industry—often led to the misuse of R&D incentives. Many such ventures engaged in clonal propagation of patented fruit varieties without due licensing, effectively undermining Intellectual Property (IP) regulations and creating unfair competition for established labs outside these zones (Çetiner 2015).

From 2016 onward, the sector experienced significant contraction, particularly following the global COVID-19 pandemic. Economic volatility, high inflation, labour costs, and the lack of automation reduced the production capacity. In the absence of comprehensive sectoral reports, field-based observations and informal consultations with stakeholders suggest that numerous small and medium-sized companies either ceased operations or significantly scaled down their activities. Some certified facilities remained legally active but functionally dormant. This downturn underscored the fragility of a sector heavily reliant on public funds and disconnected from sustainable commercial models.

By 2023, a phase of strategic realignment had become increasingly evident. Instead of a broad-based recovery, the sector consolidated around a small number of high-capacity companies, primarily located in the Mediterranean region that shifted their focus to export-oriented production strategies. These companies concentrated on the clonal propagation of woody crops, including apple, peach, pear, pistachio, and walnut, and expanded their operations into international markets across Central Asia, the Far East, and the Middle East and the North Africa (MENA) region. As a result of these strategies, the country has become one of the major rootstock producers in Europe (Table 1). In recent years, olive and palm trees, as well as ornamental woody species for indoor and outdoor use, have gained popularity within

this export-driven framework. While these developments are not fully documented in official publications, they align with observed trends in company portfolios, participation in trade exhibitions, and anecdotal accounts from industry professionals.

Although recent records from the Ministry (BÜGEM 2024) indicate that only 16 certified micropropagation companies remain operational in the country—11 of which are primarily dedicated to woody crop propagation—informal market tracking suggests that the actual number of active firms providing on-demand services for woody crops may be closer to 22 (see Table 1). While this trend may reflect a degree of specialization and value addition, it also highlights a reduction in crop diversity and a growing reliance on a limited set of high-value species for commercial survival.

In conclusion, the plant tissue culture industry in Türkiye has undergone three developmental stages over the past 30 years: initial academic consolidation (1970–1990 s), commercial expansion (2000–2015), and economic contraction coupled with regional specialization (2016–2025). While the country’s fruit rootstock production is technically sophisticated and internationally competitive, the sector continues to face significant challenges.

Germany

The story of the commercial application of micropropagation techniques in Germany has seen a significant transformation since the early 1980s. The foundation of the Association of German In Vitro Culture Laboratories (ADIVK) in 1985 marked the beginning of a coordinated effort to collect statistical data on micropropagation laboratories and plant production across the country. According to Winkelmann et al. (2006), the number of micropropagation companies increased from 12 in 1984 to approximately 27–30 by 2004. This growth was associated with a remarkable increase in production output, rising from 5 million plants per year in the mid-1980s to nearly 49 million by 2004.

Hutter and Schneider (2019) extended this analysis using data collected from ADIVK members up to 2017. Their findings confirm that the number of active commercial laboratories remained relatively stable at 27, indicating structural stability within the sector. This stability is attributed to the predominance of family-owned businesses, conservative growth strategies, and the outsourcing of labor-intensive production steps to partner micropropagation companies in lower-cost regions. However, plant production volumes experienced a notable decline, dropping to 28 million plants—a nearly 40% decrease—in 2017. This reduction can primarily be attributed to the outsourcing of labor-intensive production stages to countries with lower labour costs, particularly in Eastern Europe and Asia. Despite these

challenges, the micropropagated plants, such as orchids, strawberries, and selected woody plants, continue to play a central role in Germany's plant production industry (Hutter and Schneider 2019).

The number of micropropagated woody plants in Germany decreased from approximately 2.6 million in 2004 to 1.44 million in 2017, which represents a decline of about 44.6%. While genera such as *Rhododendron*, *Syringa*, and *Prunus* remain prominent, overall production has decreased due to shifting economic priorities and technical limitations, such as difficulties with rooting and genotype-specific responses. Notably, *Syringa* was micropropagated in 2017, resulting in 426,000 units, reflecting growing interest in ornamental landscaping (Hutter and Schneider 2019). In contrast, the micropropagation of forest tree species, such as *Populus*, *Robinia*, and *Juglans*, remains limited to fewer than 250,000 units annually, primarily due to insufficient commercial demand and the need for improved somatic embryogenesis protocols.

Although woody plants account for only around 5% of total production (1.44 million units in 2017), they represent a niche segment where customers are often willing to pay a premium for high-quality, pathogen-free material. Winkelmann et al. (2006) suggested that innovations such as temporary immersion systems (TIS) and automated culture management could reduce costs and support the wider adoption of tissue culture for forest trees. However, these innovations have not yet been widely adopted in commercial practice due to technical constraints, such as hyperhydricity and contamination risks in large-scale liquid culture systems (Hutter and Schneider 2019).

Consequently, the German micropropagation sector has demonstrated remarkable continuity in the number of micropropagation companies, despite facing economic pressures and evolving market conditions. The sector's ability to adapt to new challenges, combined with ongoing innovations in plant propagation and breeding, positions it well for future sustainability and growth.

Spain

Plant tissue culture in Spain has seen significant development, particularly in the areas of micropropagation, germplasm conservation, and genetic improvement. Specialized research groups and institutions that were developing in vitro studies independently have been more unified by the formation of the Spanish Society of In Vitro Cultivation of Plant Tissues (SECIVTV) in 1992, by the Spanish branch of the International Association of Plant Tissue Culture (IAPTC). Since then the SECIVTV (<https://secivtv.org>) has

very ably achieved its primary aim to encourage interest in the development of in vitro culture of plant cells, organs, and tissues, as well as organizing scientific conferences and other meetings in Spain that serve to educate and promote contact between people interested in this rapidly expanding area of plant science with general interest groups, more than 100 species specialist groups, a host of nearly 40 groups to share specialist aspects biotechnology.

The SECIVTV has more than 120 members, including researchers and students from universities, public research organizations and private companies. The association provides a forum for sharing basic and applied knowledge of in vitro culture and contributes significantly to industry innovation and application of ideas such as widespread LED use (Barceló-Muñoz et al. 2022) or confronting issues related to efficiency and automation or recalcitrance and bioreactor use in plant tissue culture laboratories (Georgiev et al. 2014; Vidal and Sanchez 2019) plus in vitro application to problem solving in the diverse sectors of agriculture, horticulture, forestry and germplasm conservation.

Spain has an extended history of integrating in vitro procedures for research, conservation, plus commercial propagation of indigenous and exotic forest and fruit species. Recalcitrance issues are common in many woody plants of commercial and research interest. To overcome these issues, various methods have been explored to enhance propagation efficiency using improved protocols and culture systems. Ornamental nursery plant production in vitro is typically carried out on a smaller scale by relatively small companies and it remains limited in comparison to the large-scale propagation of diverse fruit and multipurpose crops. Within the industry, there is a strong focus on high-quality and high-health plants at competitive prices. Specialist in vitro research services are also available to support problem-solving, particularly for newly introduced species or cultivars or economic interest.

Several of the larger laboratory and nursery operations also have international links to related businesses acting as global distributors or producers of their in vitro plant materials. These businesses often employ over 1000 staff and collectively produce more than 10 million plants annually, both for their own use and custom orders from clients. The primary fruit crops propagated in vitro include rootstocks for stone fruits (such as cherry, peach, and almond), pome fruits (such as pear and apple), and berry crops, particularly blueberries, raspberries, and blackberries. Additionally, other specialty woody plants, these include chestnut, white oak, walnut, pistachio, avocado, pineapple, and guava, with further growth opportunities in banana and date palm, are gaining commercial interest in expanding micropropagation

efforts. The Supplementary Information includes a list of Spanish tissue culture companies and plants produced, sourced from SECIVTV (<https://secivtv.org>). The list of plants in production at any time under in vitro conditions is constantly changing as commercial opportunities arise and decline.

Major woody crops produced by micropropagation

This section outlines the primary woody plant species currently propagated in micropropagation companies across Europe. The focus is placed on high-value crops such as stone fruits, berries, nuts, and palms, which are selected based on market demand, propagation challenges, and the benefits of clonal fidelity. Each crop group is discussed in terms of commercial importance, technological readiness, and, where possible, geographic distribution of production based on literature and expert consultation.

Stone and pome fruits—rootstocks and cultivars

Stone and pome fruits represent a cornerstone of Mediterranean horticulture, encompassing peaches, apricot, cherry, plum, almond, olive, apple, and pear. Since most pome fruit production is based on grafting cultivars onto selected rootstocks, the production of micropropagated plants is strongly focused on producing the rootstocks most requested by the nursery market. Among the most produced selections, we find ‘GF677’ (peach × almond rootstock), ‘Garnem’ (peach rootstock), the ‘Gisela[®]’ series (cherry rootstock), ‘M29C’ (apricot rootstock), ‘Marianna’ (plum rootstock), and ‘UCB1’ (pistachio rootstock). The production of stone fruit cultivars is limited to those species for which there is still a good demand for self-rooted plants, such as olive. In this sense, olive represents a special case in the scope of commercial micropropagation. The market today requires large quantities of plants for modern intensive cultivation systems, and efficient micropropagation protocols have been developed for a long time and recently optimized (Lambardi et al. 2023). Nevertheless, the production of olive micropropagated plants remains limited due to several persistent constraints. First, significant genetic heterogeneity among cultivars affects propagation efficiency, while some varieties respond well to in vitro culture, others remain recalcitrant. Second, most multiplication protocols rely on zeatin, an expensive cytokinin, which raises production costs and often makes micropropagated plants less competitive than those obtained through traditional cutting techniques. Third,

as with conventional propagation, the rooting phase remains inefficient in a high number of cultivars (Fabbri et al. 2004). On the other hand, micropropagation is increasingly regarded as a strategic tool for producing certified, pathogen-free olive plants, especially in response to the outbreak of *Xylella fastidiosa*, a quarantine bacterium responsible for devastating losses in olive orchards across south-eastern Italy. In vitro propagation offers a controlled system for the multiplication of elite, disease-free genotypes, supporting long-term replanting programmes and efforts to contain the spread of this pathogen to other Mediterranean olive-growing regions (Catalano et al. 2022).

Much more limited is the production of pome fruits by micropropagation, primarily restricted to a narrow range of apple and pear rootstocks. This reality reflects the greater difficulty of developing highly efficient protocols for pome fruit rootstocks, such as making their production by micropropagation economically advantageous, as an alternative to traditional vegetative propagation techniques (mainly cuttings).

Berry fruits, kiwi, and fig

Berry fruits, including blueberries (*Vaccinium* spp.), raspberries (*Rubus idaeus*), blackberries (*Rubus fruticosus*), and currants (*Ribes* spp.), have shown notable growth in cultivation and micropropagation throughout Europe. In recent years, berry crops have attracted substantial commercial interest, with the production of micropropagated plants more than doubling over the past decade. This trend reflects the increasing market demand for virus-free, uniform planting material, alongside the expanding use of in vitro techniques for the major *Vaccinium* and *Rubus* species.

The kiwifruit (*Actinidia* spp.) is considered relatively easy to propagate in vitro, with efficient protocols and low costs associated with the final acclimatized plants. However, unrestricted production is only available for the green cv. ‘Hayward’, while the new selections of yellow kiwi (such as the ‘Zespri Sungold’, and the Italian selections ‘Jintao’ and ‘Soreli’) are protected by plant breeders’ rights, thus their in vitro propagation is restricted to micropropagation companies authorised by the respective breeders.

The situation differs in the case of fig (*Ficus carica*), a member of the Moraceae family, which is also considered easy to micropropagate. However, in this case, market demand is quite limited. Consequently, some micropropagation companies produce only the low quantities the market requires from the laboratory.

Nuts—hazelnut, walnut, pecan, pistachio

In recent years, there has been growing interest in expanding the use of micropropagation techniques to a broader range of woody crops, particularly in response to rising demand for high-quality planting material.

Among nut trees, *Corylus avellana* (hazelnut) stands out as a major species targeted by commercial tissue culture operations, followed, to a much lesser extent, by walnut (*Juglans regia*) and pecan (*Carya illinoensis*) (Sharma et al. 2025; Korkmaz et al. 2022; Vahdati et al. 2020; Ferrucci et al. 2023). For pistachio, there is an important and yearly increasing commercial production through the micropropagation of the rootstock ‘UCB1’ (*Pistacia atlantica* x *Pistacia integerrima*). These species are favored due to long juvenile periods and difficulties in conventional propagation, high market demand for nuts in both local and global markets, and the ability to produce clonally uniform and elite genotypes, especially for rootstock development or disease resistance.

Commercial-scale micropropagation of woody trees, including nut trees, is increasingly performed in Europe in countries such as Italy, Spain, Türkiye, and Germany (Table 1), where industry-driven laboratories produce thousands of plantlets annually. Notably, efforts in Italy have focused on developing hazelnut trees with a single-stemmed growth habit, making them suitable for mechanical harvesting (Ferrucci et al. 2023).

Palms

Palms are a unique group of woody plants; in contrast to the other fruit crops, they are monocotyledons. Monocotyledons cannot form adventitious (de novo) meristems (Tomlinson 1973), which limits the propagation techniques that can be used. Among the broad diversity of palm species, only three species—the coconut, oil, and date palms—are cultivated on a larger scale (Christenhusz and Byng 2016; FAO 2025). While micropropagation protocols exist for all three species (Amine Mazri and Meziani 2015; Romyanon et al. 2015; Wilms et al. 2021), to date, only the date palm has been effectively micropropagated in Europe, according to our survey data (Table 1). It is commercially micropropagated in Europe by companies in the UK and Spain, supplying micropropagated clones of elite palms to European markets. However, on the global stage, these producers face strong competition from other producers. Whilst no other palms are yet widely propagated within Europe, several European companies are collaborating with universities to develop scalable protocols and overcome propagation obstacles in different palm species (Weckx et al. 2019).

Constraints and pathways forward for the large-scale propagation of woody horticultural species

Significant advancements have occurred in tissue culture propagation systems; however, the widespread adoption of in vitro techniques in woody horticultural species remains constrained by limitations that affect the scalability and sustainability of commercial operations. Understanding such limitations is essential for optimizing micropropagation systems, thereby enabling broader implementation across diverse woody horticultural crops and further expansion, including underrepresented countries. The following section outlines the major limiting factors currently impeding commercial success and proposes some solutions to overcome these issues.

Key limitations in commercial micropropagation

Limitations related to botanical and physiological aspects in certain species, as well as methodological and infrastructural aspects, are still observed (Fig. 2). These limitations constitute real challenges that must be minimized first within the general framework of commercial micropropagation and then, on a case-by-case basis, for the genotype-based ones.

Recalcitrance is a limitation that prevents the efficient in vitro propagation of some commercially important woody species, such as chestnut, walnut, and avocado, thereby limiting their commercialization or use in ex situ conservation programs (Benson 2000). The inability to stabilize shoot cultures is a phenomenon related not only to the genotype and its physiological characteristics, but also to the possible stress that can be caused during physicochemical manipulations under culture conditions (Hazarika 2006; Bairu and Kane 2011; Ruffoni and Savona 2013). Recalcitrance can occur at different stages of micropropagation and is also manifested by various symptoms which compromise the stabilization ability and regeneration potential of shoot cultures (McCown 2000; Choudhary et al. 2020; Rathore et al. 2007). Typical symptoms of recalcitrance include: (i) Shoot tip necrosis that causes death of the shoot tip, which limits further proliferation in culture (Bairu et al. 2009; Teixeira da Silva et al. 2020); (ii) Tissue browning that results from oxidative stress and the release of phenolic compounds at the base of the explant cut, which causes tissue death (Cassells and Curry 2001; Thorpe et al. 2008); (iii) Poor lateral shoot growth, producing a low and uneconomic micropropagation rate (Benson 2000; Abdalla et al. 2022;); (iv) Excessive callus formation at the base of the shoots without further differentiation into organized structures (Choudhary et al. 2020; Moyo et al. 2011); (v) Leaf chlorosis and deformation accompanied by abnormal and reduced growth of explants

Limiting factors	Problem-Solving Approach	Timeline	Stakeholders
RECALCITRANCE	Use of novel cytokinins and CKX inhibitors; Identify stage-specific failures; Improving protocols for limiting vitropathologies	Short to Long Term	R&D Labs, Private Companies
LABOR & ENERGY COST	Introduce automation, robotics and LED systems; Use efficient HVAC, multi-tier systems and AI-assisted platforms	Short Term	Private Companies, Nurseries
STOCK MANAGEMENT	Optimize subculture, and regeneration cycles; Improve slow growth storage; Use digital inventory and barcoding systems	Mid Term	R&D Labs, Private Companies
SHIPMENT & VIABILITY	Maintain a sufficient quantity of stock plants per variety; Optimize packaging; Use molecular tools to monitor genetic fidelity	Short Term	Nurseries, Distributors
IP & REGULATION	Balanced intellectual property policy; Facilitate SMEs access to IPRs; Promote equitable licensing policies	Long Term	Governments, Policy Makers, NGOs

Fig. 2 Strategic roadmap addressing limiting factors in the commercialization of in vitro production of woody horticultural plants

in culture (Lebedev et al. 2019; Nurtaza et al. 2024) (vi) Reduced rooting competence causing difficulty or complete failure in the formation of adventitious roots even in the presence of applied exogenous auxins (George 1993; Bunn 2009) (vii) Precocious senescence observed with premature leaf drop and significant reduction in the regenerative potential of explants (Choudhary et al. 2020; Mahmoud et al. 2020) (viii) Hyperhydricity, the phenomenon of accumulation of excess amount of water in plant tissues, causing reduction of metabolic processes and physiological functions (Kevers et al. 2004; Polivanova and Bedarev 2022). The severity and combination of these symptoms can vary depending on the plant species, genotype, composition of the culture medium, physical incubation conditions, and culture duration (George et al. 2008; Fang and Jao 2000; Ruffoni and Savona 2013). From a commercial perspective, the occurrence of one or more of these factors frequently affects the micropropagation cycle in a way that renders that specific production line to be economical.

Labour costs remain one of the most substantial expenses in plant micropropagation, alongside culture materials and reagents. According to Tomar et al. (2007), 35% of expenses during plant micropropagation in the early 2000s could be attributed to labour costs. This percentage, of course, is region-dependent, as in some countries, such as Italy, it can increase up to 60–70% (De Paoli and Masini

2009). This cost remains high, as more recent reports from Poland indicate that labour costs contribute up to 48% of the total cost for *Paulownia tomentosa* × *P. fortunei* production (Pożoga et al. 2019). It has been suggested (Tomar et al. 2007) that labour costs can be reduced if a plant tissue culture lab is established in a country with lower daily wages. Labour costs, net income per year, and gross salary between European countries vary, thus resulting in competition between countries. According to Eurostat, in 2023, the annual net average earnings (NAE) in Europe ranged from €4,677 in Bulgaria to €45,481 in Switzerland, with an average of €16,410 across the European area (Eurostat 2025a). Salary is affected by taxes. One way to measure the extent to which labour tax has a negative impact on employment is by calculating the average tax wedge. The tax wedge (TW) comprises legal deductions from gross salary (Efecan Aktaş 2023). In the EU, the average tax wedge in 2023 was 39.3%, with the highest value at 46.1% in Belgium and the lowest at 20.4% in Switzerland (Eurostat 2025b). If we look at annual NAE and TW at major in vitro plantlet producing countries in Europe, we find Italy (NAE 14852 EUR, TW 38.4%), Spain (NAE 14106 EUR, TW 36%), Türkiye (NAE 5133 EUR, TW 33.7%), The Netherlands (NAE 27537 EUR, TW 27.2%), Poland (NAE 7648 EUR, TW 32.4%), Germany (NAE 21660 EUR, TW 43.7%). Four of the six countries have below-average NAE and TW (the Netherlands has

NAE above average but TW below, and only Germany has both parameters above average). If we examine other European countries with below-average NAE and TW, Bulgaria, Romania, Croatia, Latvia, Lithuania, Czechia, Portugal, Greece, Estonia, and Malta are the countries that have this potential.

Infrastructure costs are an essential factor in determining the financial viability of micropropagation companies. Investing in infrastructure requires careful calculations to ensure the necessary space and facilities for a well-functioning micropropagation laboratory on an industrial scale. Setting up such an infrastructure requires significant capital investments, which can act as a barrier to market entry for small enterprises, particularly in regions with limited funding support or access to grants. The initial investment is in the building's surface area, with numerous investments in various facilities within it that ensure the effective implementation of each work process while maintaining high hygiene standards. However, in subsequent years, equipment-related costs—excluding those associated with infrastructure—may account for as little as 9% of the plant's total annual production costs (Tomar et al. 2007). In this respect, the spatial arrangement of equipment and work areas should be optimized to minimize personnel traffic within the system. Clonal propagation laboratories are designed to produce a large number of plants annually, often reaching into the millions. The size of the growth rooms, the preparation and sterilization rooms for the nutrient media, the number of laminar flow hoods and other equipment, as well as the size of the conservation rooms and greenhouses, must be significant. Such an initial investment requires a substantial amount of capital, which, of course, must be invested with a well-developed business plan in advance.

Managing stock size, conservation, and shipment challenges is critical to ensuring sustainable and profitable in vitro woody crop production. A significant limitation for many commercial companies is maintaining in vitro a sufficient number of high-quality stock plants to meet fluctuating market demands and ensure production continuity (Tigrel et al. 2022). However, sustaining such large stock inventories places a considerable workload, space, and resources, particularly when regeneration rates are not precisely controlled. Adjusting cytokinin concentrations during subsequent subcultures may help to stabilize regeneration and prevent labour overload, supporting efficient production cycles (Bayhan and Yücesan 2024; Chugh and Kumar 2022).

Another critical issue is the long-term conservation of stock lines, as an extended number of subcultures increases the risk of somaclonal variation and physiological decline. In this sense, although the optimized commercial protocols minimize the risk of genetic alterations even for very

long periods of subculturing, as a precautionary principle the Italian legislation for the production of certified plants by micropropagation imposes a limit of 18 subcultures of exploitation of the shoot line before the obligation to renew the culture, divided into six subcultures in the introduction in vitro and stabilization phase and 12 in multiplication phase. The inability to optimize regeneration rates, physical and chemical conditions, and the failure to apply molecular monitoring techniques to verify the genetic fidelity and vigor of source material across multiple cycles present a serious threat to the uniformity and commercial reliability of propagated plants (Wibowo et al. 2022).

Shipment management presents its own set of challenges, particularly in maintaining plant viability during transportation due to the sensitivity of in vitro materials and the need for sterile, controlled packaging. Plantlets are often shipped in ex-agar conditions to reduce costs, especially when competing with low-cost markets such as those in Asia (Yücesan and Tigrel 2024), a process that may cause desiccation, contamination, and mechanical damage. In addition, the lack of synchronization between production cycles and shipment schedules can lead to overproduction, underutilization, or delays, all of which undermine profitability and customer satisfaction. Currently, the development of new micropropagation techniques has become less critical than optimizing logistics and fostering customer interactions. For example, the use of barcoding for culture vessels serves as a safety system, facilitating the calculation of propagation cycles, tracking of vessels, and online stock analysis, thereby ensuring the highest level of production reliability (Hutter and Schneider 2019).

Intellectual property rights (IPRs) can be of vital importance for agriculture. The main IPRs relevant for agricultural innovation are: (i) patents, which protect inventions; (ii) plant variety rights, which protect the cultivation of new and different plant varieties; and (iii) trademarks and geographical indications, which facilitate the marketing of products by protecting symbols of production or geographical origin (Blakeney 2022). Espacenet, the database of the European Patent Office (2025), contains the entire collection of European patent applications published by the EPO, offering access to more than 150 million patent documents worldwide, including information on inventions and technical developments from 1782 to the present day. According to searches conducted in the Espacenet database using the keywords 'plant tissue culture' and 'micropropagation', respectively, 981 and 284 invention applications were identified (European Patent Office 2025). The Community Plant Variety Office (CPVO, n.d.), the European Union agency responsible for managing the Community Plant Variety Rights system, recognizes intellectual property rights to new plant varieties in EU countries, and provides policy

guidance and assistance to stakeholders on how to exercise these rights. They help to protect new plant varieties across the EU, protect the interests of breeders, and ensure that their investments in research and innovation are profitable.

However, in addition to their advantages, IPRs can have adverse effects, particularly for small and medium enterprises (SMEs) and individual farmers, as they can restrict competition, increase production costs, and limit access to essential goods and services (Scaria et al. 2024; LexDogma 2022). The limitations imposed by patents are more pronounced in developing countries, as the number of small enterprises and individuals affected by these restrictions is high, and the impact of corporate control on plant biotechnology and agricultural control is significant (Solanki and Rathi 2021; Scaria et al. 2024).

In this context, it is essential to strike a balance between protecting IPRs and patents, and facilitating access to innovative methods and new inventions (Lesofé 2017). Achieving this balance requires the active involvement of multiple stakeholders. Intellectual property holders must be assured adequate protection for their innovations, while users (such as those engaged with biotechnological plant products, seeds, and newly developed plant varieties) must be granted fair access to these innovations. Governments, policymakers, and non-governmental organizations (NGOs) play a critical role in mediating this balance, ensuring that the protection of IPRs does not compromise broader public interest.

Strategic solutions for advancing commercial micropropagation

To ensure the sustainable advancement of commercial micropropagation, innovative solutions must be implemented at multiple levels (Fig. 2). This section presents strategic directions, highlighting promising practices and tools that can help overcome existing barriers and promote sector-wide resilience and growth.

Developing automation, robotization, and innovative culture systems

Bioreactor technology is an advancement that has provided effective alternatives to traditional solid media cultures, increasing efficiency for promoting uniform growth by alternating exposure to liquid media and air (Etienne and Berthouly 2002). Over time, different TIS bioreactors have been proposed, such as RITA[®] (Alvard et al. 1993), SETIS[™] (Lotfi and Werbrouck 2020), PLANTFORM[™] (Welander et al. 2014), Twin-Flask TIS (Escalona et al. 1999), and ElecTIS (Sota et al. 2021; Elazab et al. 2023), which have proven to be more cost-effective than conventional agar-based

micropropagation systems. A challenge remains to integrate bioreactors with sensor arrays capable of real-time monitoring of pH, atmospheric gases, nutrient levels, and biomass density, allowing dynamic control of the growth environment. Nevertheless, applying bioreactors across a wide range of species continues to be difficult, as species-specific adjustments to culture conditions are often necessary.

Automation and robotization can be effectively used since both production costs and the time required for in vitro manipulation can be reduced. Such efforts began in the early 1990s (Tisserat 1991; Peleg et al. 1993; Aitken-Christie et al. 1995; Fári et al. 1996) and have continued to advance to date (Yang et al. 2011; Lee et al. 2019; Rajan et al. 2025). In recent years, numerous advances have been applied in commercial companies to activities that precede and follow the subculture phase, such as preparing culture jars and plastic boxes, organizing the growth chamber, and transplanting operations from in vitro to in vivo for acclimatization. The situation is different with regard to the subculturing activity, which is one of the most important and costly steps of the micropropagation cycle. So far, the prototypes of robotization for this procedure are always developed on species characterized by a certain “simplicity” of cutting, while the development of subculturing prototypes suitable for “complex” species, that multiply in vitro through the division of crowded clusters of shoots, remains problematic. However, some recent experimental platforms, focusing on designing robots to assist operators by automating manual procedures, are worthy of mention. Examples of such innovations include the Tissue Culture Planter (*Viscon Group*, The Netherlands), the RoBo[®]Cut (*Robo[®]Tec, Brema, Germany*), and the SmartClone[™] (*NuPlant, Queensland, Australia*). However, there are still limitations to their widespread adoption in large-scale production, as many systems are specifically designed to optimize the manipulation of specific genotypes (Lee et al. 2019; Sluis 2008) rather than supporting broad application across diverse plant species, because the biological heterogeneity in explants complicates precise cutting, as variations in tissue texture and structure often require human judgment. One promising direction involves integrating robotic arms with artificial intelligence and machine learning algorithms trained on image recognition and adaptive decision-making. Hamm et al. (2024) reported that machine learning-enhanced robotic systems could progressively learn to mimic expert-level interventions, potentially improving tissue identification and precision handling in plant micropropagation tasks. In this model, the use of multivariable and multiple data sets can enhance the quality of exposure to physical-chemical factors, as well as more effectively predict potential expected events (Hesami et al. 2019, 2022; Ibrahim et al. 2023). The application of machine learning models has also been reported to

be effective for the automated detection of hyperhydricity (Bethge et al. 2023a) or an automated phenotyping system for in vitro plants (Bethge et al. 2023b; Mestre et al. 2017).

Energy-efficient systems are essential for reducing operational costs in micropropagation systems, especially within capital-intensive micropropagation facilities that operate under controlled environmental conditions. The primary energy demands arise from artificial lighting and Heating, Ventilation, and Air Conditioning (HVAC) systems, which are essential for maintaining optimal growth parameters. According to Tomar et al. (2007), electricity consumption alone can account for up to 20% of total production costs, particularly due to continuous shelf lighting and the thermal load generated by air conditioning. These insights highlight the critical need for energy-efficient strategies to enhance the economic sustainability of micropropagation operations. Compared to other plant factory models, such as hydroponic systems (Kozai 2018a, b), micropropagation systems generally exhibit lower overall energy requirements. This is largely due to their use of small culture vessels, such as jars, rather than large-scale, fully enclosed cultivation chambers, which significantly reduces energy demand. One of the most effective methods for improving energy efficiency in micropropagation environments is the adoption of LED lighting technologies. LEDs operating at approximately 11 watts can replace 18 W fluorescent tubes, yielding energy savings of 30–50% (Akiyama and Kozai 2018). In a comparative study, Miler et al. (2019) further demonstrated that LED systems reduced electricity use by 40% compared to traditional high-pressure sodium (HPS) lighting, while maintaining or improving biomass yield and lowering carbon emissions. These results support the broader application of LEDs in in vitro systems, where control over spectral output and thermal load is crucial.

In addition to lighting innovations, optimizing temperature and humidity control, while reducing dependence on HVAC systems, offers another avenue for cost savings. Although strategies from large-scale plant factories are informative, they must be adapted to account for the inherently lower energy intensity and structural characteristics of micropropagation facilities. For instance, controlled enrichment of CO₂ levels, commonly used to boost photosynthesis in plant factories, can be selectively integrated into photoautotrophic micropropagation systems to improve growth while minimizing climate control costs (Graamans et al. 2018). Moreover, the implementation of multi-tier cultivation systems can enhance space and energy efficiency, reducing the fixed energy cost per propagated unit. Effective thermal management of LED-generated heat, combined with the use of low-capacity, energy-saving equipment, can further reduce the operational energy burden. While research in this area remains limited, adapting principles

from plant factory models holds promise for maintaining energy expenditure below 20% of total production costs in in vitro micropropagation systems. Such advancements are essential for building a more economically viable and environmentally sustainable infrastructure for commercial micropropagation.

Developing new compounds to promote advances in the composition of culture media

The discovery and application of new compounds can help overcome recalcitrance in micropropagation, thereby enhancing plant growth and regeneration at various stages of in vitro propagation. In addition to genotype and physical incubation conditions, the chemical composition of the nutrient medium is a fundamental factor that determines the success of the culture and the stabilization and effective in vitro regeneration of plantlets. The presence of plant hormones, especially cytokinins and auxins, is particularly important. The most commonly used naturally occurring cytokinins are zeatin, 2-isopentenyladenine (2-iP), and zeatin riboside. However, these cytokinins can be naturally degraded or inactivated by plant enzymes, which decreases the concentration of active cytokinin and also its efficiency. For this reason, cytokinins (mainly synthetic) with reduced susceptibility to metabolic transformations were employed. This group includes kinetin, 6-benzyladenine (BAP), thidiazuron, meta-topolin, and *meta*-methoxy-topolin cytokinin family (Nisler 2021). Studies have shown that the addition of these compounds to the nutritional medium can reduce the rate of hyperhydration and shoot tip necrosis, while increasing biomass production under controlled in vitro conditions (Werbrouck 2021; Ahmad and Anis 2019).

Recently, a new class of compounds has emerged that appears promising for certain species and applications. These compounds are inhibitors of cytokinin oxidase/dehydrogenase (CKX inhibitors), which prevent the degradation of cytokinins in plants. An extensive study on the development, synthesis, and biological activity of over 100 CKX inhibitors was published by Nisler et al. (2021; 2022; 2024) (see also 4.2.4.). Most of the compounds are substituted diphenylurea derivatives, but unlike thidiazuron (TDZ), these compounds do not exhibit their own cytokinin activity. This is their main advantage, because the CKX inhibitors only prolong the life-time and activity of natural cytokinins, whether intrinsic or exogenously applied. Such CKX inhibitors thus provide a new tool to gently balance and prolong the level of active cytokinins in the tissue culture. For example, it was demonstrated that during 14 days in culture, CKX inhibitor number 52 reduced the degradation of 2-iP in tobacco leaf disks by ~25% (Nisler et al. 2022). Using CKX inhibitors in combination with natural

cytokinins allows the use of lower cytokinin concentrations and keeps this concentration more stable for a longer period. In some species, this may result in positive changes in cell and tissue development. Thus, CKX inhibitors 19 and 21 (from Nisler et al. 2021) stimulated the 2iP-induced direct somatic embryogenesis in *Coffea arabica* (Murvanidze et al. 2021). The CKX inhibitors numbers 19 and 77 were shown to improve shoot regeneration in many species, including *Populus* sp., *Lobelia* sp., *Lavandula* sp., *Drosera* sp. and *Plectranthus* sp. (Nisler et al. 2024).

Financial opportunities—the role of Horizon Europe grants

One of the challenges facing micropropagation in the 21st Century is the substantial costs associated with driving Research and Development forward (Cardoso et al. 2018). Although the high cost of research is a challenge, grants and government incentives offer financial opportunities for researchers and companies. The European Commission has been supporting collaborative research projects since 1987, when the first Research and Development Framework Programme was launched. Since then, the relevant European budget envelope has grown steadily (Wedekind and Philbin 2018). Horizon Europe Program is the European Union's largest source of public funding for research, development and innovation (RDI) (European CAP Network, 2023). This programme is a research and innovation framework programme run by the European Union from 2021 to 2027. With a budget of €95.5 billion, Horizon Europe is based on three pillars (Comte et al. 2024; European Commission 2021). These pillars are the (i) Excellent Science, (ii) Global Challenges and European Industrial Competitiveness, and (iii) Innovative Europe.

The Excellent Science pillar enables the European Union to enhance its global competitiveness. The Global Challenges & European Industrial Competitiveness pillar supports research relating to societal challenges and reinforces technological and industrial capacities through clusters. Through the European Innovation Council, the Innovative Europe pillar aims to make Europe a leader in innovation by creating the market (European Commission 2021). The pillars have a vast target audience and are intended for specific purposes to help overcome the financial impossibilities sometimes encountered in realizing research projects. Therefore, these grants offer a unique opportunity, especially for research and development studies with good commercialization potential and high added value.

Furthermore, the Horizon Europe Strategic Plan (2025–2027) is directly related to Cluster 6 (Food, Bioeconomy, Natural Resources, Agriculture and Environment), especially regarding the micropropagation of economically significant European fruits. Through grant applications under

Cluster 6, grant topics such as the development of in vitro techniques for scaling up production, the effective use of digital technologies to increase production efficiency are in line with Horizon Europe's objective of transition to sustainable agricultural systems and such grants offer unique opportunities for those with financial constraints (European Commission 2024). Although it is essential to emphasize the important role played by European projects and government funding in promoting research in the sector, the contribution that micropropagation companies can and must make remains fundamental, as they finance studies aimed at solving the various problems of the micropropagation cycle through direct collaborations with Institutes and Research Centers. It would be desirable for the voice 'R&D' to be a constant presence in the budget of each micropropagation company, especially those of medium to large size.

Promoting networking—the case of 'CopyTree - COST action 21157'

Facing the challenges mentioned above and effectively resolving them requires multidisciplinary cooperation, which can only be achieved by creating effective international networks that implement a strategic approach to contribute to the EU's priorities.

Within this framework, the COST Action 21157 - CopyTree (www.copytreetree.eu), launched in 2022, has clearly defined its mission to take concrete steps towards achieving its intended vision of providing sustainable, long-term solutions. This would facilitate the identification of priorities, current situation, and solutions or measures that should be taken to achieve long-term sustainable benefits in the service of the wider community and business. To address these complex challenges, CopyTree has structured its activities into five dedicated Working Groups (WGs), each focusing on a specific aspect of the micropropagation process. These include overcoming plant recalcitrance, improving conservation of virus-free plants, advancing automation technologies, commercialization, and promoting communication and technology transfer (Fig. 3). Through the synergy of these WGs, CopyTree is establishing a coordinated group of stakeholders that cooperate to facilitate the transfer of the propagation process from the laboratory to the industry.

A significant initiative within this Action is the implementation of a collaborative experimental platform known as the *Ring Test*, designed to evaluate the role of cytokinin oxidase/dehydrogenase (CKX) inhibitors in enhancing micropropagation of woody plants.

CopyTree members from European countries are conducting collaborations with each other, particularly when they share a focus on the same plant species, with the goal of harmonizing protocols and methodologies. The Ring Test

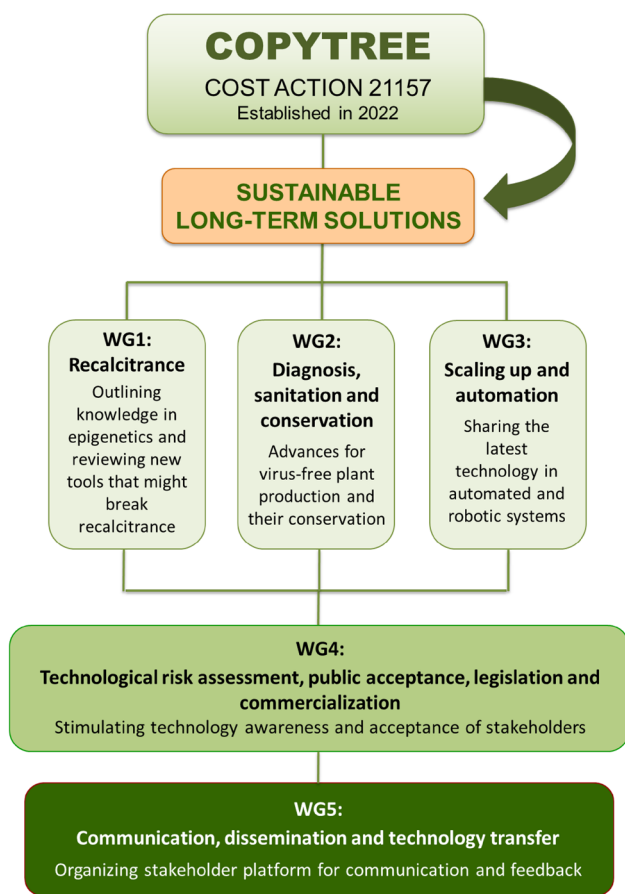


Fig. 3 Organization of COST Action 21157—CopyTree, highlighting its working groups addressing key challenges in woody plant propagation

includes the application of four recently synthesized CKX inhibitor compounds, which belong to three chemically different groups:

- I. Diphenylurea with ortho-hydroxyethyl group
 - 1-(2-(2-Hydroxyethyl)phenyl)-3-(3-(trifluoromethoxy)phenyl)urea (compound no. 19).
 - 1-(3-Chloro-5-(trifluoromethoxy)phenyl)-3-(2-(2-hydroxyethyl)phenyl)urea (compound no.20).
- II. Halogenated diphenylureas
 - 1-(3,5-Dichlorophenyl)-3-(3-(trifluoromethoxy)phenyl)urea (compound no. 52).
- III. Diphenylurea with ortho-amide group
 - 2-(3-(3,5-Dichlorophenyl)ureido)-4-methoxybenzamide (compound no. 77).

The compounds are currently provided for research purposes by Dr. Nisler upon request. They are not yet commercially available. The patent rights (patents EP4120837A1 and EP4121415A1) for their production, sale, and use are held by Czech Advanced Technology and Research Institute (Catrin) at Palacký University in Olomouc. The compounds have attracted interest from agrochemical companies as well as businesses involved in plant propagation. Licenses for use in plant tissue culture are available through the patent holder. Commercial application of selected compounds in agriculture is expected within 2–3 years.

During Ring Test, these compounds will be tested across various woody species, such as bilberry, raspberry, chestnut, grape, hazelnut, olive, and plum, in many cases using both conventional and TIS micropropagation platforms.

Through coordinated collaboration, the Action aims not only to standardize protocols but also to promote innovation, reduce duplication of efforts, and accelerate the adoption of advanced technologies across Europe. Beyond its scientific objectives, the Ring Test as a coordinated platform reinforces international collaboration and promotes knowledge sharing, thereby helping to establish a network model and engaging community members in woody plant cloning.

Future perspectives

The further expansion of commercial micropropagation in Europe depends not only on technological advancements and improved protocols in countries that already show significant production in the sector, but also on the development and implementation of similar initiatives in under-represented and emerging countries.

Many countries in Northern Europe, including Denmark, Sweden, Norway, and Finland, are lagging in effectively implementing large-scale production systems for woody horticultural plants compared to countries in the Mediterranean area, such as Italy, Spain, Türkiye, and Greece (Table 1). One of the primary reasons is the absence or small number of well-established commercial micropropagation companies that relate to, for example, smaller horticultural industries and high labour costs. Furthermore, recent RDI efforts in these countries have primarily focused on forest trees, such as the commercial propagation of conifers through somatic embryogenesis (SE). Recent developments in tissue culture applications for the forestry sector could provide novel business opportunities also for existing laboratories specialized in horticultural woody plants. This, however, would require the acquisition of SE techniques and an increase in production capacities, as the number of forest plants needed is much higher than for horticultural markets.



Fig. 4 Geographical focus areas for micropropagation companies in Europe, highlighting the distinct sectors in Northern Europe (green), which focuses on forest tree studies, Central Europe (blue) on ornamental species, and Southern Europe (red) on plant production of fruit rootstocks and cultivars

Moreover, even across Eastern and Southeastern Europe, countries such as Albania, Bosnia and Herzegovina, Moldova, Latvia, Kosovo, and Montenegro have either very low or no activities related to the commercial tissue culture sector. Reasons might be associated with the lack of funding, inadequate public awareness, and limited partnerships among academia, industry, and government, which are necessary for establishing and maintaining the essential infrastructure that ensures large-scale plant production (Sota et al. 2025). A key barrier that can make it challenging to produce on a large scale in these countries is that many of them are not EU countries. In this regard, it is necessary to align all steps and procedures, such as production, conservation, and germplasm transfer, with the EU guidelines.

An interesting case to study the dynamics of such investments and developments is the Western Balkan countries (Albania, Bosnia and Herzegovina, Kosovo, Montenegro, North Macedonia, and Serbia). Besides being characterized by a rich biodiversity, lower labour costs, and growing research capacity, most of them have not implemented large-scale production systems. A perfect example of the cost-effectiveness of production in such countries is the Republic of North Macedonia, where the two largest companies involved in commercial propagation are branches of Dutch enterprises operating in the sector. Serbia shows great potential in advancing in this regard, with four new emerging companies; however, they still rely on the local market and face difficulties competing in the international market (Sota et al. 2025).

Another strategy that may help underrepresented or emerging countries advance in commercial propagation is focusing on the propagation of regionally adapted plant

species rather than conventional horticultural crops. This approach could help not only in producing high-quality autochthonous plants but also in preserving biodiversity and promoting agritourism. Additionally, diversifying plant targets may help small commercial companies become competitive first in a regional market and later in broader international markets.

Concerning countries that already have significant fruit plant production from micropropagation (such as Italy, Spain, Türkiye and Germany), here efforts will have to be increasingly concentrated on developing technological innovation that can minimize production costs, through automation and robotization interventions of the different phases of the production cycle in the laboratory and the greenhouse, and aimed at reducing the labour cost component, currently at values still around 60% of the final price of the micropropagated plant. In addition to implementing new production systems (e.g., liquid culture in TIS) and developing new plant growth regulators, efforts are being directed toward enhancing production quality, meeting the requirements for certified plant material, and addressing the persistent recalcitrance observed in many economically important fruit species.

Given this, Fig. 4 illustrates the varying focus areas for micropropagation across Europe, highlighting the distinct regions involved in large-scale plant propagation.

The green circle represents Northern Europe, where forest tree studies are most prevalent, particularly those related to somatic embryogenesis in conifer species. The blue circle signifies Central Europe, with an emphasis on ornamental crops, while the red circle represents Southern Europe, where the production of fruit plants is most prevalent. This geographical differentiation in focus areas reflects the varying economic conditions, research priorities, and market demands across the continent. The further expansion of commercial micropropagation in Europe will necessitate addressing regional imbalances, with emerging countries requiring strategic investments to develop their micropropagation sectors in line with EU standards and guidelines.

In this strategic vision for the future of the sector, networks such as the COST Action 21157—CopyTree are key platforms for knowledge exchange, protocol standardization, and joint experimentation. These outcomes are consistent with the mission and vision of EU initiatives to integrate and encourage the active participation of underrepresented countries, thereby establishing a more inclusive, resilient, and innovative micropropagation sector across Europe.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11240-025-03165-5>.

Acknowledgements This article is based upon work from COST

Action CA21157 “European Network for Innovative Woody Plant Cloning” www.copytree.eu, supported by COST (European Cooperation in Science and Technology) www.cost.eu.

Author contributions VS—Supervising; Conceptualization; Writing original draft; Review and Editing; HW - Conceptualization; Writing original draft; Review and Editing; BY—Conceptualization; Writing original draft; Review and Editing; YYM—Writing original draft; BC—Conceptualization; Writing original draft; Review; JN—Writing original draft; ŞEA—Writing original draft; Review; LPT—Writing original draft; Review; CS—Writing original draft; SPOW—Review; LF—Review; TA—Review; BC—Review; ML—Supervising; Conceptualization; Writing original draft; Review and Editing.

Funding This publication is funded by COST Action CA21157 “European Network for Innovative Woody Plant Cloning” www.copytree.eu, supported by COST (European Cooperation in Science and Technology) www.cost.eu.

Data availability The commercial companies identified in the 2025 survey are included in the Supplementary File.

Declarations

Competing interests Maurizio Lambardi and Cristian Silvestri were among the Associate Editors of the journal at the time of submission. This had no impact on the peer review process and the final decision.

Ethical approval Not applicable.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

- Abdalla N, El-Ramady H, Seliem MK, El-Mahrouk ME, Taha N, Bayoumi Y, Shalaby TA, Dobránszki J (2022) An academic and technical overview on plant micropropagation challenges. *Horticulturae* 8(8):677. <https://doi.org/10.3390/horticulturae8080677>
- Ahmad A, Anis M (2019) Meta-topolin improves in vitro morphogenesis, rhizogenesis and biochemical analysis in *Pterocarpus marsupium* roxb.: a potential drug-yielding tree. *J Plant Growth Regul* 38:1007–1016. <https://doi.org/10.1007/s00344-018-09910-9>
- Aitken-Christie J, Kozai T, Takayama S (1995) Automation in plant tissue culture—general introduction and overview. In: Aitken-Christie J, Kozai T, Smith MAL (eds) Automation and environmental control in plant tissue culture. Springer, Dordrecht, pp 1–18. https://doi.org/10.1007/978-94-015-8461-6_1

- Akiyama T, Kozai T (2018) Light environment in the cultivation space of plant factory with leds. LED lighting for urban agriculture. In: Kozai T, Fujiwara K, Runkle E (eds) LED lighting for urban agriculture. Springer, Singapore, pp 91–109. https://doi.org/10.1007/978-981-10-1848-0_7
- Alvard D, Cote F, Teisson C (1993) Comparison of methods of liquid medium culture for banana micropropagation. *Plant Cell Tissue Organ Cult* 32:55–60. <https://doi.org/10.1007/BF00040116>
- Amine Mazri M, Meziani R (2015) Micropropagation of date palm: A review. *Cell Dev Biology* 4(3):1–5. <https://doi.org/10.4172/2168-9296.1000160>
- Bairu MW, Kane ME (2011) Physiological and developmental problems encountered by in vitro cultured plants. *Plant Growth Regul* 63:101–103. <https://doi.org/10.1007/s10725-011-9565-2>
- Bairu MW, Stirks WA, Van Staden J (2009) Factors contributing to in vitro shoot-tip necrosis and their physiological interactions. *Plant Cell Tissue Organ Cult* 98:239–248. <https://doi.org/10.1007/s11240-009-9560-8>
- Barceló-Muñoz A, Barceló-Muñoz M, Gago-Calderon A (2022) Effect of LED lighting on physical environment and microenvironment on in vitro plant growth and morphogenesis: the need to standardize lighting conditions and their description. *Plants* 2022(111):60. <https://doi.org/10.3390/plants11010060>
- Bayhan N, Yücesan B (2024) The impact of sucrose and 6-benzylaminopurine on shoot propagation and vitrification in *Aronia melanocarpa* (black chokeberry). *Plant Cell Tissue Organ Cult* 156:55. <https://doi.org/10.1007/s11240-023-02652-x>
- Benson EE (2000) In vitro plant recalcitrance: an introduction. *In Vitro Cell Dev Biol Plant* 36(3):141–148
- Bethge H, Mohammadi Nakhjiri Z, Rath T et al (2023a) Towards automated detection of hyperhydricity in plant *in vitro* culture. *Plant Cell Tissue Organ Cult* 154:551–573. <https://doi.org/10.1007/s11240-023-02528-0>
- Bethge H, Winkelmann T, Lüdeke P et al (2023b) Low-cost and automated phenotyping system phenomenon for multi-sensor in situ monitoring in plant in vitro culture. *Plant Methods* 19:42. <https://doi.org/10.1186/s13007-023-01018-w>
- Blakeney M (2022) Agricultural innovation and sustainable dDevelopment. *Sustainability* 14(5):2698. <https://doi.org/10.3390/su14052698>
- BÜGEM - Ministry of Agriculture and Forestry, General Directorate of Plant Production (2024) Authorized Organizations for Seed Production via Tissue Culture. Retrieved from <https://www.tarimorman.gov.tr/BUGEM/Belgeler/doku%20k%C3%BCt%C3%BCr%C3%BC.pdf> Accessed May 28, 2025
- Bunn E (2009) Investigations of alternative in vitro rooting methods with rare and recalcitrant plants. *Acta Hort* 829:279–282. <https://doi.org/10.17660/ActaHortic.2009.829.41>
- Cardoso JC, Sheng GLT, Teixeira da Silva JA (2018) Micropropagation in the twenty-first century. *Methods Mol Biol* 1815:17–46. https://doi.org/10.1007/978-1-4939-8594-4_2
- Cassells AC, Curry RF (2001) Oxidative stress and physiologicpigeneic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. *Plant Cell, Tissue and Organ Culture* 64:145–157 (2001). <https://doi.org/10.1023/A:1010692104861>
- Castro-Camba R, Vielba JM, Covelo P, Vidal N, Sánchez C (2023) Reducing costs, improving profits: A low-cost culture media for Woody plants micropropagation. *J Sci* 69(9):377–388. <https://doi.org/10.17221/56/2023-JFS>
- Catalano C, Pucci N, Benvenuti C, Terenzi V, Maresca V, Pietrini F (2022) A roadmap for in vitro propagation of Olive tree (*Olea Europaea* L.): A review of the state of the Art and future challenges. *Antibiotics* 11(7):947. <https://doi.org/10.3390/antibiotics11070947>

- Çetiner S (2015) Biyoteknoloji stratejisi Mi dediniz? Tarlasera Düşünceler, 22–23. <https://www.tarlasera.com/makale-9708-biyoteknoloji-stratejisi-mi-dediniz>
- Choudhary M, Arya ID, Arya S (2020) Problems Encountered during En vitro culture establishment in *Terminalia Arjuna*. Annual Res Rev Biology 35(12):154–160. <https://doi.org/10.9734/arrb/2020/v35i1230320>
- Christenhusz MJM, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa 261(3):201–217. <https://doi.org/10.11646/phytotaxa.261.3.1>
- Chugh P, Kumar A (2022) Novel plant growth regulators in in vitro establishment of horticulture and plantation crops. In: Gupta S, Chaturvedi P (eds) Commercial scale tissue culture for horticulture and plantation crops. Springer, Singapore, pp 65–84. https://doi.org/10.1007/978-981-19-0055-6_4
- Comte A, Juban S, Leblanc L, Volle C (2024) Sustainability science in horizon Europe collaborative projects. In: Dangles O, Sabrié ML, Fréour C (eds) Sustainability science, vol 3. French National Research Institute for Sustainable Development, pp 124–127
- CPVO - Community Plant Variety Office (2025) (n.d.) Community Plant Variety Office. <https://cpvo.europa.eu/en> Accessed May 28
- De Paoli G, Masini M (2009) Commercial micropropagation laboratory: organization and technique to limit production costs. Italus Hortus 16(2):84–88
- Debergh PC, Read PE (1991) Micropropagation. In: Debergh PC, Zimmerman RH (eds) Micropropagation. Springer, Dordrecht, pp 1–13. https://doi.org/10.1007/978-94-009-2075-0_1
- Dobránszki J, da Teixeira Silva JA (2010) Micropropagation of apple—a review. Biotechnol Adv 28(4):462–488. <https://doi.org/10.1016/j.biotechadv.2010.02.008>
- Efecan Aktaş E (2023) How tax wedge of low and upper-income households affects income distribution: findings from OECD countries. Prague Econ Papers 32(3):246–272. <https://doi.org/10.18267/j.pep.831>
- Elazab D, Capuana M, Ozudogru EA, Anichini M, Lambardi M (2023) Use of liquid culture with the electis bioreactor for faster recovery of blackberry (*Rubus fruticosus* L) shoots from conservation at 4°C horticulturæ. 9(6):680. <https://doi.org/10.3390/horticulturæ9060680>
- EPPO - European and Mediterranean Plant Protection Organization (2012) EPPO Technical Document No. 1061: EPPO study on the risk of imports of plants for planting. Paris: European and Mediterranean Plant Protection Organization. Retrieved from https://www.eppo.int/media/uploaded_images/RESOURCES/eppo_publications/td_1061_plants_for_planting.pdf Accessed May 28, 2025
- EPPO - European and Mediterranean Plant Protection Organization (2008) PM 4/17 (2) certification scheme for Olive trees and rootstocks. EPPO Bull 38(3):390–395. <https://doi.org/10.1111/j.1365-2338.2008.01235.x>
- Escalona M, Lorenzo JC, Daquinta M, Desjardins Y, Borroto CG (1999) Pineapple (*Ananas comosus* L Merr) micropropagation in temporary immersion systems. Plant Cell Rep 18:743–748. <https://doi.org/10.1007/s002990050653>
- Etienne H, Berthouly M (2002) Temporary immersion systems in plant micropropagation. Plant Cell Tissue Organ Cult 69:215–231. <http://doi.org/10.1023/A:1015668610465>
- European Commission (2024) Horizon Europe strategic plan 2025–2027. Publications Office of the European Union. <https://op.europa.eu/en/publication-detail/-/publication/6abcc8e7-e685-11ee-8b2b-01aa75ed71a1> Accessed May 4, 2025
- European Patent Office (2025) Espacenet Database <https://worldwide.espacenet.com/> Accessed May 28, 2025
- European CAP Network (2023) Funding opportunities under Horizon Europe (Calls 2024). Accessed April 24, 2025 https://eu-cap-network.ec.europa.eu/sites/default/files/publications/2023-09/EUCAPNetwork_Brochure_Funding-opportunities-under-Horizon-Europe-Calls2024_0.pdf
- European Commission (2021) Directorate-General for Research and Innovation, Horizon Europe, the EU research and innovation programme (2021–27): for a green, healthy, digital and inclusive Europe, Publications Office of the European Union, 2021 <https://data.europa.eu/doi/10.2777/052084n>. Accessed April 12, 2025
- Eurostat (2025b) Tax rate on low wage earners: Tax wedge on labour costs. https://doi.org/10.2908/EARN_NT_TAXWEDGE Accessed April 26, 2025
- Eurostat (2025a) Annual net earnings. https://doi.org/10.2908/EARN_NT_NET Accessed April 26, 2025
- Fabbri A, Bartolini G, Lambardi M, Kailis SG (2004) Olive propagation manual. CSIRO Publishing, Collingwood. <https://doi.org/10.1071/9780643091016>
- Fang W, Jao RC (2000) A review on artificial lighting of tissue cultures and transplants. In: Kubota C, Chun C (eds) Transplant production in the 21st century. Springer, Dordrecht, pp 108–113. https://doi.org/10.1007/978-94-015-9371-7_17
- FAO - Food and Agriculture Organization (2001) Seed policy and programmes for the Central and Eastern European countries, Commonwealth of Independent States and other countries in transition: Proceedings of the Regional Technical Meeting, Budapest, Hungary, 6–10 March 2001. <https://www.fao.org/4/y2722e/y2722e00.html> Accessed May 28, 2025
- FAO - Food and Agriculture Organization (2025) FAOSTAT. <http://www.fao.org/faostat/en/#data/QC/visualize> Accessed May 28, 2025
- Fári M, Kertész T, Franklin de Melo N, Andrásfalvy A (1996) Novel tendencies of automation and the rationalization of in vitro explant manipulation in micropropagation industry. Hortic Sci 28(3/4):12–17
- Ferrucci A, Lupo M, Turco S, Pavese V, Torello Marinoni D, Botta R, Cristofori V, Mazzaglia A, Silvestri C (2023) A roadmap of tissue culture and biotechnology in European hazelnut (*Corylus Avelana* L). Plant Physiol Biochem 205:108167. <https://doi.org/10.1016/j.plaphy.2023.108167>
- George EF (1993) Plant propagation by tissue culture. Part 1. The technology. Exegetics Ltd., Basingstoke, UK. 503 574 pages. ISBN: 978-0-9509-3254-5
- George EF, Hall MA, De Klerk GJ (2008) Plant Propagation by Tissue Culture (3rd ed.). Springer, Dordrecht. 503 pages. ISBN: 978-1-4020-5005-3
- Georgiev V, Schumann A, Pavlov A, Bley T (2014) Temporary immersion systems in plant biotechnology. Eng Life Sci 14:607–621. <https://doi.org/10.1002/elsc.201300166>
- Gözen A, Abak K, Kasnaoğlu H, Çetiner S, Güzel A (1995) Priorities of plant biotechnology in Turkey (TTGV publication no. 2) [In Turkish]. Turkish Technology Development Foundation
- Graamans L, Baeza E, van den Dobbelaars A, Tsafaras I, Stanghellini C (2018) Plant factories versus greenhouses: comparison of resource use efficiency. Agric Syst 160:31–43. <https://doi.org/10.1016/j.agsy.2017.11.003>
- Hamm J, Seonghyeon L, Jiae P, Jiwon K, Injun L, Yoongeun L, Jiseok K et al (2024) A modular robotic platform for biological research: cell culture automation and remote experimentation. Adv Intell Syst 6(5):2300566. <https://doi.org/10.1002/aisy.202300566>
- Hazarika BN (2006) Morpho-physiological disorders in *in vitro* culture of plants. Sci Hortic 108:105–120. <https://doi.org/10.1016/j.scienta.2006.01.038>
- Hesami M, Naderi R, Tohidfar M, Yoosefzadeh-Najafabadi M (2019) Application of adaptive neuro-fuzzy inference system-non-dominated sorting genetic algorithm-II (ANFIS-NSGAI) for modeling and optimizing somatic embryogenesis of chrysanthemum. Front Plant Sci 10:869. <https://doi.org/10.3389/fpls.2019.00869>
- Hesami M, Alizadeh M, Jones AMP et al (2022) Machine learning: its challenges and opportunities in plant system biology. Appl

- Microbiol Biotechnol 106:3507–3530. <https://doi.org/10.1007/s00253-022-11963-6>
- Hussain A, Ahmed I, Nazir H, Ullah I (2012) Plant tissue culture: Current status and opportunities. Recent Adv. Plant Vitro culture. In: Leva A and Rinaldi LMR (ed) Recent Advances in Plant in vitro Culture. InTech, pp 1–28. <https://doi.org/10.5772/50568>
- Hutter I, Schneider C (2019) Commercial micropropagation in Germany. J Appl Bot Food Qual 92:226–231. <https://doi.org/10.5073/JABFQ.2019.092.031>
- Ibrahim N, Adedamola AM, Ibrahim B, Ahmed RT, Raji ID, Bello-Salau H (2023) Survey of machine learning and optimization algorithms in plant tissue culture. Engineering Proceedings, 56(1), 122. <https://doi.org/10.3390/ASEC2023-15259>
- IPPC Secretariat (2022) Phytosanitary certificates. International Standard for Phytosanitary Measures No. 12. Rome. FAO on behalf of the Secretariat of the International Plant Protection Convention. <https://openknowledge.fao.org/server/api/core/bitstreams/4ae0018d-5df7-4b26-b847-592254042548/content> Accessed May 28, 2025
- Kevers C, Franck T, Strasser RJ, Dommes J, Gaspar T (2004) Hyperhydricity of micropropagated shoots: a typically stress-induced change of physiological state. Plant Cell Tissue Organ Cult 77:181–191. <https://doi.org/10.1023/B:TICU.0000016825.18930.e4>
- Knapp E, Hanzer V, Weiss HH, da Câmara Machado A, Weiss B, Wang Q, Katinger H Laimer Da Câmara Machado M (1995) new aspects of virus elimination in fruit trees. Acta Hort 386:409–418. <https://doi.org/10.17660/ActaHortic.1995.386.56>
- Korkmaz E, Yaşar R, Soydan B, Aasım M et al (2022) Optimization of in vitro sterilization for pistachio (*Pistacia Vera* L) rootstocks. Anatol J Bot 6(1):1–6. <https://doi.org/10.30616/ajb.999973>
- Kozai T (2018a) Benefits, problems and challenges of plant factories with artificial lighting (PFALs): a short review. Acta Hort 1227:25–30. <https://doi.org/10.17660/ActaHortic.2018.1227.3>
- Kozai T (2018b) Current status of plant factories with artificial lighting (PFALs) and smart PFALs. In: Kozai T (ed) Smart plant factory. Springer, Singapore, pp 3–13. https://doi.org/10.1007/978-981-13-1065-2_1
- Lambardi M, Previati A (2012) Indagine Sulla micropropagazione in Italia Tra Ricerca e produzione. Acta Italus Hortus 6:13–19. ISBN: 978-88-905628-6-0
- Lambardi M, Ozudogru E, Jain S (2013) Protocols for micropropagation of selected economically-important horticultural plants. Methods in Molecular Biology, vol 994. Humana, Totowa, NJ, p 490. <https://doi.org/10.1007/978-1-62703-074-8>
- Lambardi M, Fabbri A, Micheli M, Vitale A (2023) Olive propagation and nursery. In: Fabbri A. (ed.), The Olive. Botany and production. CABI., Wallingford, UK, pp. 228–256. <https://doi.org/10.1079/9781789247350.0013>
- Lambardi M, Micheli M, Silvestri C (2024) Micropropagazione, un must per chi Ffa ivaismo innovativo. Rivista Di Frutticoltura 10:16–21 ISSN: 0392-954X
- Lebedev V, Arkaev M, Dremova M, Pozdnyakov I, Shestibratov K (2019) Effects of growth regulators and gelling agents on ex vitro rooting of raspberry. Plants 8(1):3. <https://doi.org/10.3390/plant8010003>
- Lee TJ, Zobayed SMA, Firmani F, Park EJ (2019) A novel automated transplanting system for plant tissue culture. Biosyst Eng 181:63–72. <https://doi.org/10.1016/j.biosystemseng.2019.02.012>
- Lesofe I (2017) Finding the right balance between the enforcement of competition law and the protection of intellectual property rights. Competition Commission South Africa. https://www.compcom.co.za/wp-content/uploads/2017/09/Paper_Competition-and-IP-Law_Itumeleng-Lesofe_Final_24082017.pdf Accessed May 25, 2025
- LexDogma (2022) Intellectual property rights and agriculture biotechnology. <https://www.lexdogma.in/post/intellectual-property-right-s-and-agriculture-biotechnology> Accessed May 25, 2025
- Lotfi M, Werbrouck SPO (2020) SETIS™, a novel variant within the temporary immersion bioreactors. Acta Hort 1285:253–258. <https://doi.org/10.17660/ActaHortic.2020.1285.37>
- Mahmoud LM, Grosser JW, Dutt M (2020) Silver compounds regulate leaf drop and improve in vitro regeneration from mature tissues of Australian finger lime (*Citrus australasica*). Plant Cell Tissue Organ Cult 141:455–464. <https://doi.org/10.1007/s11240-020-01803-8>
- Martins M, Sarmiento D, Oliveira MM (2004) Genetic stability of micropropagated almond plantlets, as assessed by RAPD and ISSR markers. Plant Cell Rep 23:492–496. <https://doi.org/10.1007/s00299-004-0870-3>
- McCown BH (2000) Recalcitrance of woody and herbaceous perennial plants: dealing with genetic predeterminism. In Vitro Cell Dev Biol Plant 36(3):149–154
- Méndez-Hernández HA, Loyola-Vargas VM (2024) Plant micropropagation and temporary immersion systems. Methods in molecular biology. (Clifton NJ) 2827:35–50. https://doi.org/10.1007/978-1-0716-3954-2_3
- Mestre D, Fonseca J, Mora A (2017) Monitoring of in vitro plant cultures using digital image processing and random forests. 8th International Conference on Pattern Recognition Systems, ICPRS2017, Madrid 1113 July 2017 IET Digital Library, ISBN 9781785616525. <https://doi.org/10.1049/cp.2017.0137>
- Miler N, Kulus D, Woźny A et al (2019) Application of wide-spectrum light-emitting diodes in micropropagation of popular ornamental plant species: a study on plant quality and cost reduction. In Vitro Cellular & Developmental Biology 55:99–108. <https://doi.org/10.1007/s11627-018-9939-5>
- Mirzaei L, Yadollahi A, Kermani MJ et al (2021) Evaluation of genetic stability in Olive callus-induced and meristem-induced shoots using flow cytometry and amplified fragment length polymorphism techniques. Plant Methods 17:31. <https://doi.org/10.1186/s13007-021-00724-7>
- Moyo M, Finnie JF, Van Staden J (2011) Recalcitrant effects associated with the development of basal callus-like tissue on caulogenesis and rhizogenesis in *Sclerocarya birrea*. Plant Growth Regul 63:187–195. <https://doi.org/10.1007/s10725-011-9562-5>
- Murvanidze N, Nisler J, Leroux O, Werbrouck SPO (2021) Cytokinin oxidase/dehydrogenase inhibitors stimulate 2iP to induce direct somatic embryogenesis in *Coffea arabica*. Plant Growth Regul 94:195–200. <https://doi.org/10.1007/s10725-021-00708-6>
- Nisler J (2021) Cytokinin properties of meta-topolin and related compounds. In: Ahmad N, Strnad M (eds) Meta-topolin: A growth regulator for plant biotechnology and agriculture. Springer, Singapore, pp 23–30. https://doi.org/10.1007/978-981-15-9046-7_3
- Nisler J, Kopečný D, Pěkná Z, Končítíková R, Koprna R, Murvanidze N, Werbrouck SPO, Havlíček L, De Diego N, Kopečná M, Wimmer Z, Briozzo P, Moréra S, Zalabák D, Spíchal L, Strnad M (2021) Diphenylurea-derived cytokinin oxidase/dehydrogenase inhibitors for biotechnology and agriculture. J Exp Bot 72(2):355–370. <https://doi.org/10.1093/jxb/eraa437>
- Nisler J, Pěkná Z, Končítíková R, Klimeš P, Kadlecová A, Murvanidze N, Werbrouck SPO, Plačková L, Kopečný D, Zalabák D, Spíchal L, Strnad M (2022) Cytokinin oxidase/dehydrogenase inhibitors: outlook for selectivity and high efficiency. J Exp Bot 73:4806–4817. <https://doi.org/10.1093/jxb/erac201>
- Nisler J, Klimeš P, Končítíková R, Kadlecová A, Voller J, Chalaki M, Karampelias M, Murvanidze N, Werbrouck SPO, Kopečný D, Havlíček L, De Diego N, Briozzo P, Moréra S, Zalabák D, Spíchal L (2024) Cytokinin oxidase/dehydrogenase inhibitors: progress towards agricultural practice. J Exp Bot 75(16):4873–4890. <https://doi.org/10.1093/jxb/erae239>

- Nurtaza A, Dyussebekova D, Islamova S et al (2024) In vitro conservation and genetic diversity analysis of rare species *Ribes janczewskii*. *Sci Rep* 14:31117. <https://doi.org/10.1038/s41598-024-82320-y>
- Panattoni A, Luvisi A, Triolo E (2013) Review. Elimination of viruses in plants: twenty years of progress. *Span J Agric Res* 11(1):173–188. <https://doi.org/10.5424/sjar/2013111-3201>
- Peleg K, Ziv M, Alchanatis V, Sklansky J, Hoffmann F, Vriesenga M (1993) Automation of tissue culture processes by a conveying system. *IFAC Proc Vol* 26(2):1159–1162. [https://doi.org/10.1016/S1474-6670\(17\)48653-8](https://doi.org/10.1016/S1474-6670(17)48653-8)
- Polivanova OB, Bedarev VA (2022) Hyperhydricity in plant tissue culture. *Plants* 11(23):3313. <https://doi.org/10.3390/plants1123313>
- Požoga M, Olewnicki D, Jabłońska L (2019) In vitro propagation protocols and variable cost comparison in commercial production for *Paulownia tomentosa* × *Paulownia Fortunei* hybrid as a renewable energy source. *Appl Sci* 9(11):2272. <https://doi.org/10.3390/app9112272>
- Rajan P, Kang JH, Natraj P et al (2025) Advances, challenges, and future prospects of in vitro propagation in Papaya (*Carica papaya* L.). *Plant Cell, Tissue and Organ Culture (PCTOC)* 161:36. <https://doi.org/10.1007/s11240-025-03051-0>
- Rathore JS, Rathore MS, Singh M, Singh RP, Shekhawat NS (2007) Micropropagation of mature tree of citrus *Limon*. *Indian J Biotechnol* 6:239–244
- Riordáin FO (2000) COST 822 - The directory of European plant tissue culture — 1996. *Acta Hort* 530:33–38. <https://doi.org/10.17660/ActaHortic.2000.530.2>
- Riordáin FO (2003) EUR 20720 — COST Action 843 — Directory of European plant tissue culture laboratories 2002. Office for Official Publications of the European Communities, Luxembourg, 195 pp. <https://op.europa.eu/en/publication-detail/-/publication/e1c83533-1f78-4ad1-a918-e1049078836a> Accessed May 28, 2025
- Romyanon K, Mosaleyanon K, Kirdmancee C (2015) Direct-shoot organogenesis as an alternative protocol for in vitro regeneration of oil palm (*Elaeis guineensis* Jacq.). *Sci Hortic* 195:1–7. <https://doi.org/10.1016/j.scienta.2015.08.038>
- Ruffoni B, Savona M (2013) Physiological and biochemical analysis of growth abnormalities associated with plant tissue culture. *Hortic Environ Biotechnol* 54:191–205. <https://doi.org/10.1007/s13580-013-0009-y>
- Scaria J, Vithayathil RK, Fariza F (2024) The economic impact of biotechnology and intellectual property rights on agricultural trade and market accessibility in developing nations. *JSS J Leg Stud Res* 10(2):85–103
- Sharma S, Sharma S, Kumar P (2025) In vitro establishment of walnut (*Juglans regia* L) from the embryonic axis with customized nutrient media and synergy of growth regulators. *South Afr J Bot* 177:598–603. <https://doi.org/10.1016/j.sajb.2024.12.034>
- Sluis CJ (2008) Integrating automation technologies with commercial micropropagation. In: Gupta SD, Ibaraki Y (eds) *Plant tissue culture engineering*. Focus on biotechnology, vol 6. Springer, Dordrecht, pp 231–251. https://doi.org/10.1007/978-1-4020-3694-1_13
- Solanki RS, Rathi A (2021) Biotechnology and intellectual property rights: challenges and problems facing by the developing nations. *Int J Creative Res Thoughts* 9(8):b376–b388. <http://www.ijcrt.org/papers/IJCRT2108156.pdf>
- Sota V, Benelli C, Çuko B, Papakosta E, Depaoli C, Lambardi M, Kongjika E (2021) Evaluation of electis bioreactor for the micropropagation of *Malus sylvestris* (L) mill., an important autochthonous species of Albania. *Hort Sci (Prague)* 48(1):12–21. <https://doi.org/10.17221/69/2020-HORTSCI>
- Sota V, Nacheva L, Bošnjak D, Abraham E, Jevremović S, Cvjetković B, Galović V, Jevremović D, Marković Z, Kongjika E, Bogunović S, Zeljković S, Andonovski V, Dančić V, Vujović T (2025) Unveiling balkans' advances: *In vitro* biotechnology of Woody plants in the early 21st century. *Front Plant Sci* 16. <https://doi.org/10.3389/fpls.2025.1586013>
- Teixeira da Silva JA, Nezami-Alanagh E, Barreal ME, Kher MM, Wicaksono A, Gulyás A et al (2020) Shoot tip necrosis of in vitro plant cultures: a reappraisal of possible causes and solutions. *Planta* 252(47):1–35. <https://doi.org/10.1007/s00425-020-03449-4>
- Thorpe TA, Stasolla C, Yeung EC, de Klerk GJ, Roberts A, George EF (2008) The components of plant tissue culture media II: organic additions, osmotic and pH effects, and support systems. In: George EF, Hall MA, De Klerk GJ (eds) *Plant propagation by tissue culture*, 3rd edn. Springer, Dordrecht, pp 115–173. https://doi.org/10.1007/978-1-4020-5005-3_4
- Tigrel A, Arslan M, Arıcı B, Yücesan B (2022) Selection and Preparation of explants for the clonal propagation of horticultural plants in plant factory systems. In: Gupta S, Chaturvedi P (eds) *Commercial scale tissue culture for horticulture and plantation crops*. Springer, Singapore, pp 23–48. https://doi.org/10.1007/978-981-19-0055-6_2
- Tisserat B (1991) Automated systems. In: Bajaj YPS (ed) *High-Tech and micropropagation I. Biotechnology in agriculture and forestry*, vol 17. Springer, Berlin, Heidelberg, pp 419–431. https://doi.org/10.1007/978-3-642-76415-8_23
- Tomar UK, Negi U, Sinha AK, Kumar P (2007) An overview on economic factors influencing micropropagation. *My for* 43:523–534
- Tomlinson PB (1973) Branching in monocotyledons. *Q Rev Biol* 48(3):458–466
- Vahdati K, AjamGard F, Rahemi M, Driver J (2020) Advances in micropropagation of commercial pecan cultivars. *Int J Fruit Sci* 20(sup2):S925–S936. <https://doi.org/10.1080/15538362.2020.1772942>
- Vidal N, Sánchez C (2019) Use of bioreactor systems in the propagation of forest trees. *Eng Life Sci* 19:896–915. <https://doi.org/10.1002/elsc.201900041>
- Weckx S, Inzé D, Maene L (2019) Tissue culture of oil palm: finding the balance between mass propagation and somaclonal variation. *Front Plant Sci* 10:1–17. <https://doi.org/10.3389/fpls.2019.00722>
- Wedekind GK, Philbin SP (2018) Research and grant management: the role of the project management office (PMO) in a European research consortium context. *J Res Adm* 49(1):43–62
- Welander M, Persson J, Asp H, Zhu L (2014) Evaluation of a new vessel system based on temporary immersion system for micropropagation. *Sci Hortic* 179:227–232. <https://doi.org/10.1016/j.scienta.2014.09.035>
- Werbrouck SPO (2021) *Meta*-topolin and related cytokinins as a solution to some in vitro problems. In: Ahmad N, Strnad M (eds) *Meta-topolin: A growth regulator for plant biotechnology and agriculture*. Springer, Singapore, pp 85–91. https://doi.org/10.1007/978-981-15-9046-7_9
- Wibowo AT, Antunez-Sanchez J, Dawson A, Price J, Meehan C, Wrightsman T et al (2022) Predictable and stable epimutations induced during clonal plant propagation with embryonic transcription factor. *PLoS Genet* 18(11):e1010479. <https://doi.org/10.1371/journal.pgen.1010479>
- Wilms H, De Bièvre D, Longin K, Swennen R, Rhee J, Panis B (2021) Development of the first axillary in vitro shoot multiplication protocol for coconut palms. *Sci Rep* 11:1–10. <https://doi.org/10.1038/s41598-021-97718-1>
- Winkelmann T, Geier T, Preil W (2006) Commercial in vitro plant production in Germany in 1985–2004. *Plant Cell, Tissue Organ Cult* 86(2):319–327. <https://doi.org/10.1007/s11240-006-9125-z>
- Wójcik D, Trzewik A, Kucharska D (2021) Field performance and genetic stability of micropropagated gooseberry plants (*Ribes grossularia* L). *Agronomy* 11(1):45. <https://doi.org/10.3390/agronomy11010045>














Yang L, Zhang T, Wang L (2011) Automated cutting and transplanting system for tissue culture seedlings. International Conference on Mechanical Engineering and Technology (ICMET-London 2011). Ed. Lee G. ASME Press, 2011. <https://doi.org/10.1115/1.859896.paper17>

Yücesan B, Tigril A (2024) Strategic innovations in in vitro woody crop production: Balancing technical excellence with market and

stakeholder dynamics. In Proceedings of the 2nd Conference of Cost Action CA21157 CopyTree, Bulduri, Latvia, pp 191–206

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Valbona Sota¹  · Hannes Wilms²  · Buhara Yücesan³  · Yeşim Yalçın Mendi⁴  · Bruce Christie⁵  · Jaroslav Nisler⁶  · Şafak Esra Aslan⁷  · Liva Purmale-Trasune⁸  · Cristian Silvestri⁹  · Stefaan P. O. Werbrouck¹⁰  · Lucie Fischerová⁶  · Tuija Aronen¹¹  · Branislav Cvjetković¹²  · Maurizio Lambardi¹³ 

✉ Valbona Sota
valbona.sota@fshn.edu.al

¹ Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Tirana, Albania

² Research Institute for Nature and Forest (INBO), Team Genetic Diversity, Brussels, Belgium

³ Department of Seed Science and Technology, Faculty of Agriculture, Bolu Abant İzzet Baysal University, Bolu, Türkiye

⁴ Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Türkiye

⁵ The Green Plant Co Ltd, Palmerston North, New Zealand

⁶ Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic

⁷ Technology Transfer Office, Giresun University, Giresun, Türkiye

⁸ Bulduri Biotechnology Center, Bulduri Technical School, Jūrmala, Latvia

⁹ Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy

¹⁰ Department of Plants and Crops, Faculty of Bioscience Engineering, University of Ghent, Ghent, Belgium

¹¹ Natural Resources Institute Finland (Luke), Savonlinna, Finland

¹² Department of Forest Genetics and Forest Establishment, Faculty of Forestry, University of Banja Luka, Bosnia and Herzegovina, Banja Luka, Bosnia and Herzegovina

¹³ IBE-Institute of BioEconomy, National Research Council, Florence, Italy