UNIVERSITÀ DEGLI STUDI DI PADOVA
Dipartimento Territorio e Sistemi Agro-forestali
Department of Land, Environment Agriculture and Forestry

MSc. in Mediterranean Forestry and Natural Resources Management

Certification of forest reproductive materials and its role in climate-smart forestry: A case study of identification of origin of beech forest reproductive materials using molecular methods in South-Eastern Europe

Supervisor:
Dr. Mauro Masiero

Co-supervisors:
Prof. Dr. Hojka Kraigher
Dr. Marjana Westergren

Submitted by:
Sujata Shrestha

Student no.
2047593

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Abbreviation and acronyms

CFRI	Croatian Forest Research Institute
CSF	Climate-smart forestry
DNA	Deoxyribonucleic acid
EC	European Commission
EU	European Union
EUFORGEN	European Forest Genetic Resources Programme
FAO	Food and Agricultural Organization
FBiH	Federation of Bosnia and Herzegovina
FGR	Forest Genetic Resources
FRM	Forest Reproductive Material
OECD	Organization for Economic Co-operation and Development
OG	Official Gazette
PAFF	Plants, Animals, Food and Feed
N/A	Not Available
SFI	Slovenian Forestry Institute
SFRY	Socialist Federal Republic of Yugoslavia
SWOT	Strength, Weaknesses, Opportunities and Threats
YUS	Yugoslavian Standards

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Summary

The quality and origin of forest reproductive material (FRM) shapes the genetic diversity, structural composition, survival dynamics, and growth of seedlings, coupled with the prospective resilience of future forests to stressors and disturbances. Certified FRM assists in ensuring the quality, authenticity, and adherence to specified standards in the production and distribution phase of FRM. This study explores the status of FRM certification, the laws and regulations based on it, in four Southeast European countries: Bosnia and Herzegovina, Croatia, Slovenia and Serbia. There are two FRM certification schemes in Europe, namely Council Directive 1999/105/EC and Organization for Economic Co-operation and Development (OECD)'s Forest Seed and Plant Scheme. Their aim is to make sure that these materials are collected, processed, grown, labelled, and distributed in a way that ensures they are true to their given names.

Literature review and data mining through secondary sources were employed to compile laws and regulations relating to FRM and conservation of forest genetic resources (FGR) for the countries under study. The case study presented deals with the identification of the origin of beech (*Fagus sylvatica*) using molecular markers. Samples of 32 beech stands were analyzed using microsatellites.

Bosnia and Herzegovina, Croatia and Serbia still follow Yugoslav Standards (YUS) for certification measures. Bosnia and Herzegovina and Serbia heavily rely on it, while Croatia relies on it partly. Only Slovenia has a compulsory system to check and verify the origin of FRM intended for marketing and use. All the target countries have distinct laws pertaining to FRM, except Bosnia and Herzegovina where only its entity Republic of Srpska has law on FRM, but not the state. This highlights the need for Bosnia and Herzegovina to give more importance to conservation of genetic resources.

From a technical perspective, the molecular markers did not consistently prove to be suitable for the identification of the individuals. There were discrepancies in the results, where K=7 was unable to trace the origin of the individuals, whereas with K=3 the samples were correctly matched with their origin. Hence in the case study presented, the applied methods for the molecular identification of the origin of FRM did not prove to be very useful. This result stresses on the need for more technical advancements needed on application of molecular methods on tracing the origin of FRM.

1. Introduction

This chapter introduces the thesis topic and provides the background for the research, as well as the research questions and objectives.

1.1 Background

Suitable origin and high quality of forest reproductive materials (FRM) constitutes the backbone of a good forest regeneration which is a vital part of forest management practices (McNabb, 2004). Among other things, forest management may deal with raising new forests, rejuvenation, and regeneration of existing forests with the goal of maintaining high quality, resilient forests to provide high quality wood while retaining optimal beneficial functions and ecosystem services (Hrvatske šume, n.d.). Forest regeneration can be performed through natural regeneration that can depend on fructification of seeds of mother trees (plus trees) in a particular area (in terms of management and growth of high forest form) or through vegetative regeneration from stump shoots (in terms of management of coppice forests): in both cases the genetic material is preserved on a local base. Alternatively, artificial forest regeneration can be performed, i.e., by sowing seeds or planting seedlings from the same or similar origin within a certain country. The genetic material is preserved and maintained for each country to a certain extent which gives close-to-nature forest regeneration (Duryea and Dougherty, 1991). Both natural and artificial regeneration methods are intricately tied to the availability of forest genetic resources (FGR) (Ivetic *et al.*, 2016). Natural regeneration relies on the existing resources within a specific site, while artificial regeneration often involves the intentional transfer of FRM. (Konnert *et al.*, 2015).

While many forests are regenerated using natural techniques, especially in Europe, increasing annual wood harvests to face the growing demand of wood-based products to address bioeconomy needs will likely depend upon plantation forestry (Hetemäki *et al.*, 2022). Moreover, planting is necessary for afforestation on degraded lands, abandoned agricultural lands, or anywhere that trees are to be reintroduced without a natural seed source (McNabb, 2004). In this perspective targets set by ambitious policies and strategies, such as the Three Billion Trees Pledge by the European Union Biodiversity Strategy to 2030 and the European Union Forest Strategy to 2030, the increasing afforestation and reforestation initiatives under the umbrella of multiple forest carbon projects (Di Sacco *et al.*, 2021) and the recently approved European

Union Nature Restoration Law – just to mention a few – are posing severe challenges in terms of finding appropriate FRMs.

Throughout history, seeds and seedlings were transported within Europe and neighbouring countries, but to a lesser extent in comparison to present since artificial regeneration was not as common and several countries were limiting the use of foreign or unknown seed sources (Konnert *et al.*, 2015). Natural regeneration was the primary method of renewing forests until the 19th century, when extensive imports of diverse seed sources began (Myking *et al.*, 2016). This marked a paradigm shift in forestry, allowing for regeneration beyond local FRM boundaries, thus turning FRM into a substantial commodity crossing European borders and climatic gradients.

1.1.1 Regulation and trade

As part of the ongoing efforts to combat climate change, including through afforestation and reforestation as well as improved forest management activities, the regulation of FRM has gained significant attention (Koskela *et al.*, 2007).

Most European countries have recommendations or guidelines for selecting species and provenances that can be used in a given site or zone. However, these recommendations are mostly based on present or past climatic conditions (Konnert *et al.*, 2015). So, there is an increasing need that a policy framework of FRM is established since it would contribute to control the quality, origin and trade both within the EU (i.e., among EU member states) and between the EU and other countries/regions.

Comprehensive understanding of all processes related to FRM production for artificial regeneration, as well as their interactions, is essential to maximize genetic gain and maintain genetic diversity (Edwards & El-Kassaby, 1996). The transfer of FRM during afforestation/reforestation significantly influences genetic diversity by altering gene frequency or introducing genes previously absent (Edwards & El-Kassaby, 1996). In Central Europe, there have been discussions on genetic diversity in forestry and planted forests, nevertheless, due to the need to increase timber productivity, many planted forests have been created with FRM with untraceable origins (Koski, 2000; Kremer, 2007). Using species from known FRM origin for forest regeneration may help leading to successful adaptation even to changing environments (Hufford & Mazer, 2003).

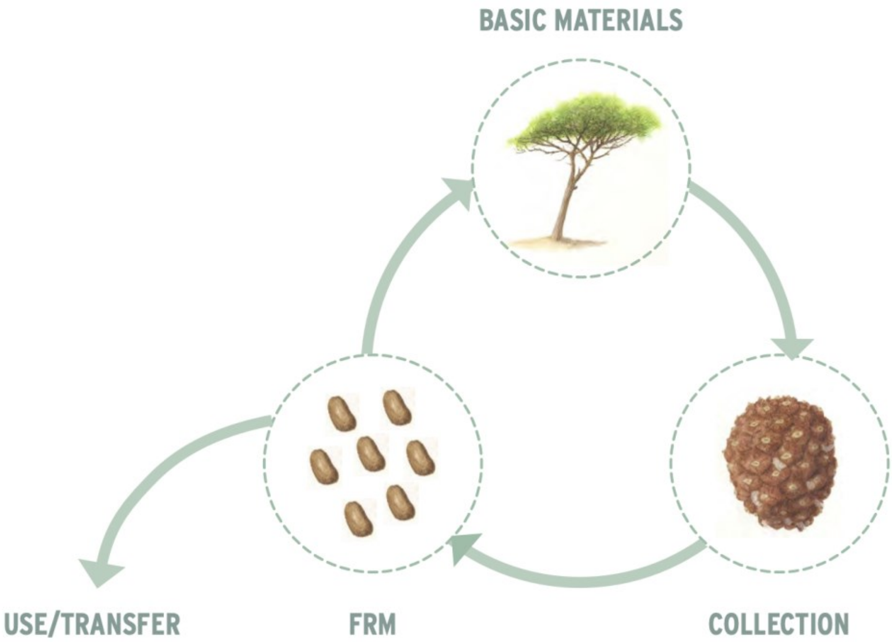

Figure 1: A seed production chain

Tracing FRM involves thorough documentation at both national and regional (e.g., European) scale. Seeds harvested (Figure 1) for forest regeneration purposes are recorded and distinctly marked with a specific certification number. After collection, the FRM is issued a master certificate (Figure 2) by the designated authority, verifying that it is collected from approved basic material (Gömöry *et al.*, 2021). Cross-border transfers of FRM, whether for permanent exports or contract production, necessitate reporting to authorities. While there are regulations for national record-keeping, a standardized EU-wide documentation for FRM is yet to be established (Jansen *et al.*, 2019). Currently, two trading schemes for FRM are implemented within Europe (Ackzell, 2002; Ackzell and Turok, 2005):

1. The Organisation for Economic Cooperation and Development (OECD)'s Forest Seed and Plant Scheme for non-EU member states trading with the EU, and
2. Council Directive 1999/105/EC on the Marketing of Forest Reproductive Material applicable for EU Member States.

On the international stage, OECD Forest Seed and Plant Scheme was established in 1967 to ensure the documentation of origin and promote the production and use of FRM. The scheme was designed to incentivize the safe production and utilization of forest tree seeds or plants. The goal is to ensure that these materials are collected, processed, cultivated, labelled, and distributed in a manner that guarantees their trueness to name. This certified material is intended for various forestry uses, including timber production, soil protection, and adherence to environmental criteria (OECD, 2017). For European Union (EU) member countries, FRM trade is regulated under common EU rules i.e., Council Directive 1999/105/EC. This directive governs approval and marketing, but not the subsequent use of FRM (FAO, 2014). The EU Directive 1999/105, which addresses the marketing of FRM, is synchronized with the OECD scheme and came into effect on January 1st, 2003, across all EU member states (Robbins, 2002). The primary goal is to ensure compliance while providing buyers with necessary information for selecting suitable material. Despite these developments, detailed guidelines on the deployment of available seed sources remain under the responsibility of single national authorities (FAO, 2014).

The OECD Forest Seed and Plant Scheme and the Council Directive 1999/105/EC ensure that only classified FRM is traded for forestry purposes, establishing standards for seed sources. Both trading schemes are harmonized and regulate the production, consistent labelling, and traceability of FRM (Ackzell, 2002; Ackzell and Turok, 2005).

FRM can be imported into EU from non-EU countries, known as third countries, provided their certification scheme is recognized as equivalent to the EU scheme. Special permission and approval are required from a competent authority appointed within each EU member country (e.g., the Slovenian Forest Institute - SFI for Slovenia, the Croatian Forest Research Institute - CFRI for Croatia etc.) for such imports (Gömöry *et al.*, 2021). Under exceptional circumstances of short supply, the EU may authorize the marketing of FRM that doesn't meet Council Directive 1999/105/EC standards: this is termed derogation. Suppliers exporting FRM to another EU member state must notify the concerned authorities (Forest Commission, 2019).

While trade statistics on FRM could offer valuable insights into the genetic patterns of European tree populations and their adaptability to future climatic changes, recent research has largely overlooked such data (Jansen *et al.*, 2019).

PART A

MODEL MASTER CERTIFICATE OF IDENTITY FOR REPRODUCTIVE MATERIAL DERIVED FROM SEED SOURCES AND STANDS

(Certificate must contain all the information outlined below, and in the exact format)

ISSUED IN ACCORDANCE WITH DIRECTIVE 1999/105/EC

MEMBER STATE:	CERTIFICATE No EC:/(MEMBER STATE CODE)/(No) ....

It is certified that the forest reproductive material described below has been produced:

in accordance with the EC Directive ❐
reproductive material under transitional arrangements ❐

1. **Botanical name:** ....

2. **Nature of reproductive material:**
   Seed unit ❐
   Part of plants ❐
   Planting stock ❐

3. **Category of reproductive material**
   Source-identified ❐
   Selected ❐
   Tested ❐

4. **Type of basic material:**
   Seed source ❐
   Stand ❐

5. **Purpose:** ....

6. **Country register reference or identity of basic material in National register:**
   .... / Mixture: ....

7. Autochthonous ❐ Non-autochthonous ❐ Unknown ❐
   Indigenous ❐ Non-indigenous ❐

8. **Origin of basic material (for non-autochthonous/non-indigenous material, if known):** ....
   ....

9. **Country and Region of provenance of basic material:** ....
   Provenance (Short title, if appropriate): ....

10. **Altitude or altitudinal range of site of basic material:** ....
    ....

11. **Year in which seeds ripened:** ....

12. **Quantity of reproductive material:** ....

13. **Is the material covered by this certificate the result of a subdivision of a larger lot covered by a previous EC Certificate?** Yes ❐ No ❐
    Previous certificate number .... Quantity in initial lot ....

14. **Lenght of time in nursery:** ....
    ....

15. **Has there been subsequent vegetative propagation of material derived from seed?** Yes ❐ No ❐
    Method of propagation .... Number of cycles of propagation ....

16. **Other relevant information:** ....

17. **Name and address of supplier**

Name and Address of Official Body:	**Stamp of Official Body:** **Date:**	**Name of Responsible Officer:** **Signature:**

Figure 2: Model master certificate of identity of derived FRM

Source: Council Directive 1999/105/EC

1.1.2 Molecular markers

The application of molecular markers simplifies the monitoring of the origin and genetic quality of FRM (Westergren *et al.*, 2017). There are two distinct classes of molecular markers, as elucidated by Bousquet *et al.* (1992). The first category is comprised of molecular genetic markers, which are derived through the direct analysis of polymorphism in the Deoxyribonucleic acid (DNA) sequences. These markers provide insights into variations in the genetic code. The second category is biochemical markers, which originate from the examination of chemical products resulting from gene expression. This category offers a complementary perspective by focusing on the observable outcomes of gene activity. There are various types of molecular markers like: isozymes, Random Amplified Polymorphic DNA, microsatellites, Amplified Fragment Length Polymorphism, organelle DNA markers and others.

In this study, microsatellites have been used as a molecular method to trace origin. A microsatellite refers to a short segment of DNA, typically ranging from one to several base pairs in length, that undergoes repetitive sequences at a specific genomic location. The number of repeated segments within a microsatellite sequence often varies among individuals, rendering them useful as polymorphic markers. In other terms, microsatellites are short pieces of DNA that repeats and the way the patterns repeat can be unique to each individual, similar to the uniqueness as seen in individual fingerprints. Microsatellites have been widely used in analyzing genetic diversity of European species (Kajba and Andrić, 2015; Westergren *et al.*, 2017; Grdiša *et al.*, 2021 and Ivanović *et al.*, 2021).

1.1. Problem statement

Climate change is one compelling reason that pressurizes countries to review and adjust their policies on FRM. A key challenge is the unprecedented task for forest managers to consider the future climate for new trees, choosing materials that thrive in the current climate and withstand anticipated changes. Unfortunately, many forest owners see FRM as an expense to minimize rather than an investment with potential returns (Konnert *et al.*, 2015).

Forest managers and forest owners are seeking to minimize costs of regeneration by matching the material purchased with ecological site conditions and ensure high genetic and physiological quality of the material. If a manager or an owner makes a mistake by obtaining seeds and seedling that are not acclimated to the sowing/planting area, the end quality in wood mass or any ecosystem services declines drastically and may produce negative economic and ecological impacts (Chizmar and Parajuli, 2022).

Despite the increasing attention being directed towards climate change adaptation, the certification standards does not include measures for incorporating strategies to build resilient forests. Those standards were formed in the past, thus does not have guidelines pertaining to present and future problems (Grossnickle and El-Kassaby, 2016; Andivia *et al.,* 2021).

Negative effects of climate change to the forest ecosystems have increased in recent years, including warming-induced shifts in species distribution, drought-related increases in tree mortality and magnification of local disturbances which challenge traditional silvicultural strategies that have been evolving through centuries (Konnert *et al.*, 2015). This requires developing and adopting a new adaptable forest management framework of climate-smart forestry (CSF). CSF can be defined as forestry that sustainably raises timber productivity, increases resilience, stores carbon and enhances the achievement of development goals while involving monitoring forest functions and anticipating disturbance effects, as well as undertaking resilient actions to avoid the negative consequences on the provision of ecosystem services and forest productivity (Nabuurs *et al.*, 2018). Practising climate-smart forestry directly influences forest health, wildlife risk, water quantity and quality, wildlife, timber production, soil productivity, emerging forest-based bio-products and technology (Chizmar and Parajuli, 2022).

Furthermore, there has been an increase in the mishandling of FRM such that either they are mislabelled, or they come from unidentified origin Such instances pose immense threat of introduction of pests and pathogens into the forests (Westergren et *al.,* 2017).

1.2 Objectives and research questions

1.2.1 Objectives

The general objective of this research is to evaluate the current situation and effectiveness of certification regulations in ensuring the quality of FRM and FGR conservation in Southeast Europe.

The specific objectives are:

a) To assess the current legislation and regulations of FRM and FGR certification schemes in four targeted Southeast European countries (Bosnia and Herzegovina, Croatia, Slovenia, and Serbia).
b) To explore how FRM transfer is conducted among EU, OECD, and third countries.
c) To identify the origin of beech samples using molecular markers (as a case study).
d) To explore if the analysis of FRM by molecular markers should be encouraged while dealing with transfer of FRM among target countries.

1.2.2 Research questions

The research objectives defined according to the problem statement are demanding answers to the following questions:

a) How is the certification of FGR and FRM conducted in Southeast European countries? What is the legislation and regulation like?
b) What challenges and opportunities are involved while adopting the current laws and regulations?
c) What is the importance of FRM and FGR conservation considering climate change?
d) How is the marketing of FRM done among the target countries?
e) Should the use of molecular markers be encouraged to ensure quality of FRM?

1.3 Structure of the thesis

The thesis is organised into five chapters. Chapter 1 sets out a brief introduction and background to the research topic including general explanations, definitions, objectives, research questions and describes the thesis structure itself. Chapter 2 discusses basic definitions and existing literature relevant for the research topic. Chapter 3 describes the methodology used in the research. From data collection to data analysis methods, this section explores the way this research was conducted. In chapter 4 results are presented and then discussed. Chapter 5 draws conclusions based on research results vis-à-vis research objectives and develops implications for certification of FRM as a part of CSF amid climate changes.

2. Theoretical background

This chapter sets the theoretical background of the research and provides key concepts and definitions used throughout the thesis.

2.1 Definitions:

The terms related to FRM as stated by the Council Directive 1999/105/EC are presented below:

Basic material is the plant material from which FRM is obtained. This encompasses seed stands, seed orchards, individual clones, or clonal mixtures.

Clone is a cluster of individuals (ramets) originating from a single individual (ortet) through vegetative propagation methods like cuttings, micropropagation, grafts, layers, or divisions.

Clonal mixture is a combination of identified clones in predetermined ratios.

Seed orchard is a plantation area of selected clones or families, isolated or controlled to prevent or minimize external pollination. The plantation is managed to produce regular, abundant, and easily harvestable seed crops.

Seed source is trees in a specific region from which seeds are gathered.

Stand is a clearly defined group of trees that demonstrates significant uniformity in composition.

Origin is the place from where the seeds were originally introduced or the place where they are currently growing.

Parents of family/families are Trees employed to generate offspring through either controlled or open pollination, where one specified parent serves as the female, fertilized by the pollen of one parent (full-sibling) or several identified or unidentified parents (half-sibling).

Provenance region is the region or collection of regions characterized by consistently uniform ecological conditions where stands or seed sources with similar phenotypic or genetic traits are located, considering relevant altitudinal boundaries.

2.2 Theoretical approach

According to the study to control the origin of FRM by using DNA-fingerprints made by Degen et al (2010, p. 272): "*highly variable nuclear microsatellites and genotype assignment are feasible for controlling of the origin of FRM on a large scale. The group-wise assignment approach is completely feasible in the trade of FRM.*" By giving significance to genetic diversity and testing, it is possible to establish a firm framework on FRM certification. The genetic diversity within forest ecosystems forms a fundamental basis for their adaptability in the face of climatic disruptions (Anderson & Green, 2021). Climate change introduces various challenges, including temperature and precipitation shifts, along with the emergence of new pests and diseases (Freer-Smith *et al.,* 2019). Forests possessing genetic diversity are better equipped to evolve and withstand these environmental stressors (Johnson, 2017). The conservation and sustainable use of FGR play a crucial role in sustainable forest management. The diversity in genetic resources ensures the survival, adaptation, and evolution of forest trees in the face of changing environmental conditions. Genetic diversity is essential for maintaining forest vitality and resilience against pests and diseases. Forest management practices in Europe predominantly revolve around the management of wild and semi-wild tree populations. Whether through artificial or natural regeneration, the establishment of new forests entails the deployment of genetic material (State of Europe's Forest, 2020). The origin of reproductive material holds significance in forest regeneration, influencing the growth and development of trees (Myking *et al.,* 2016).

2.3 Forest reproductive materials

According to the United Nations' Food and Agricultural Organisation (FAO), FRM are defined as seeds, seedlings, cuttings, and any other propagating materials that are used for forestry and reforestation purposes (FAO, n.d.). Additionally, certification of FRM refers to the on-site inspections of the areas where FRM was produced (EC, 2023). The certification process verifies and ensures the quality and identity of tree seeds, seedlings, and other propagating materials used in forestry. It further involves evaluating the genetic characteristics of the plant material, as well as the conditions under which it was produced and handled (Wang & Sziklai 1969). The success of any tree-planting initiative, be it land restoration, wood fibre production, conservation, or other goals, hinges on the use of high-quality FRM (FAO, n.d.).

2.4 Climate-smart forestry

CSF uses certain common forest management practises that include thinning and harvesting methods which control light and limit overcrowding to promote sustainable growth, reduction of wildfire risk with prescribing different fire fuel management, area preparation like bedding and herbicide usage against current and new biotic threats (pest and competing vegetation control) and above everything to sow/plant a diverse mixture of species with genetic traits to decrease forest loss in case of a disturbance occurrence (Chizmar and Parajuli, 2022). CSF strategy differs from the common forest management practises since it targets to increase climate benefits from forests and the forest sector itself by reducing and/or removing greenhouse gas (GHG) emissions, adapting the forest sector to build more resilient forests and to sustainably increase productivity and provide all benefits that forests can provide through active forest management (EFI, 2018).

Climate change sets new climatic conditions that local species lack the resistance and resiliency of its natural provenance, so there is a steady increase to transfer genetic material across countries borders (especially regarding EU) since different phenotype characteristics are made of genotype and environmental factors which then decide the quality and cost-effectiveness of forest regeneration (Chizmar and Parajuli, 2022).

2.5 Forest reproductive material certification

Certification of FRM is aimed to promote the production and use of seeds, parts of plants and seedlings that were collected, transported, processed, raised, and distributed so that there is insurance about the accuracy in handling of plant material along the production line, that proper reproductive techniques are applied and that proper selection procedures were used (Botta *et al.,* 2004).

According to OECD forest seed and plant scheme from 2019 and the EU Council Directive 1999/105/EC, the FRM can be certified through categories like source-identified, selected, qualified, and tested (Figure 2). The definitions of FRM categories displayed in Figure 3 are presented in Table 1.

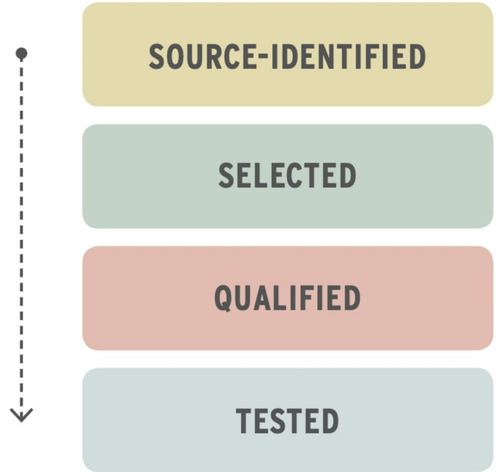

Figure 3: FRM categories with the direction of arrow indicating the selection level

Source: adapted from Gömöry et al., (2021)

Table 1: Comparison of FRM categories between Council Directive 1999/105/EC and OECD forest seed and plant scheme

Categories	Council Directive 1999/105/EC	OECD Forest Seed and Plant Scheme
Source-identified	It includes the reproductive material obtained from the basic material which could be a seed origin, or a stand situated within a single provenance region and fulfils the criteria outlined in Annex II.	This denotes the minimum acceptable criterion, mandating the documentation of the location and altitude of the site(s) where reproductive material is collected, with little to no emphasis on phenotypic selection.
Selected	It is the reproductive material obtained from basic material, specifically a stand situated within a singular provenance region. This stand has undergone phenotypical selection at the population level and satisfies the criteria outlined in Annex III.	Under this category, phenotypic selection at the population level is required for the basic material.

Qualified	This category includes reproductive material obtained from basic material, namely seed orchards, parent individuals of families, clones, or combinations of clones. These components have undergone phenotypical selection at the individual level and comply with the criteria specified in Annex IV. It is not mandatory for testing to have been carried out or concluded.	It states that the constituents of the basic material have been chosen at the individual level; nevertheless, an evaluation may not have been conducted or finalized.
Tested	It incorporates the reproductive material sourced from basic material, comprising stands, seed orchards, parents of families, clones, or clonal mixtures. The excellence of the reproductive material should be proven through either comparative testing or an assessment of its superiority derived from the genetic evaluation of the foundational material components. This material must conform to the stipulated criteria in Annex V.	The superiority of the reproductive material should be proven through comparative testing, or an assessment of its superiority should be calculated from the genetic evaluation of the constituents of the basic material.

(*Source: Own elaboration from Council Directive 1999/105/EC and OECD Forest Seed and Plant Scheme*)

2.6 Importance of correct choice of forest reproductive material considering climate change

Choosing appropriate FRM has gained increased significance due to the long lifespan of trees and the anticipated influence of rapid climate change on the environmental conditions during their growth and maturation (Koskela *et al.*, 2007). The suitability of a specific location for FRM is assessed based on its capacity to endure, thrive, and propagate in a specific site. An individual's unique genetic composition, known as its genotype, dictates its performance or phenotype at a specific location. The successful implementation of FRM can be achieved by examining genetic variations and understanding their organization at the individual, population, and species levels, both currently and in the future (Konnert *et al.*, 2015).

The presence of genetically diverse tree populations is vital for climate change mitigation and forest adaptation to changing conditions. Planted forest areas have been expanding globally, with expectations of continued growth in response to climate change mitigation and increasing demands for forest products (Palombi and Sessa, 2013). Despite global commitments to large-scale tree planting initiatives, many countries face challenges related to the quantity and quality of FRM, often due to deficient national tree seed systems (Gömöry *et al.*, 2021).

In the context of climate change, there is a renewed focus on the debate surrounding the transfer of FRM. This attention arises from the belief that the natural adaptive processes of tree populations may not occur quickly enough to prevent adverse effects on forest functions, as noted by Kremer (2010). Consequently, to address the risk of genetic maladaptation induced by climate change and meet future socio-economic and industrial demands, there is a discussion about increasing the frequency of pre-adapted genes. This involves the transfer of pre-adapted FRM, which is seen as a promising strategy to enhance forest stability and improve the efficiency of wood production. Various studies, including those by Broadmeadow *et al.* (2005), Koskela *et al.* (2010) and Dumroese *et al.* (2015), support this notion.

Currently, the primary source of improved FRM is seed orchards. These orchards consist of either clones obtained from vegetatively propagated plus trees selected in the wild (first-generation seed orchards) or their offspring. Alternatively, they may include elite genotypes (progeny tested) in seed orchards of advanced generations (Kavaliauskas *et al.*, 2018).

The standards for FRM in "selected" category originating from stands, are higher than those for "Source-identified", and they are based on phenotypic traits. Nevertheless, both categories exhibit similar levels of genetic variation. FRM with genetic improvements (in the "qualified" and "tested" categories) is primarily cultivated in seed orchards. The number and origin of clones/genotypes in a seed orchard, along with their low relatedness levels and the experimental designs employed during establishment, ensure high levels of genetic variation in the produced seed (GenTree, 2020) (Table 2).

Table 2: Categories of FRM and origin of breeding material.

Origin of breeding material	Categories of FRM			
	Source identified	**Selected**	**Qualified**	**Tested**
Seed source	X			
Stand	X	X		X
Seed orchard			X	X
Parents of families			X	X
Clone			X	X
Clonal mixture			X	X

Source: adapted from GenTree (2020)

The categories of FRM also further reflect the breeding improvement (Table 2). The standards for FRM in "selected" category originating from stands, are higher than those for "Source-identified", and they are based on phenotypic traits. Nevertheless, both categories exhibit similar levels of genetic variation. FRM with genetic improvements (in the "qualified" and "tested" categories) is primarily cultivated in seed orchards. The number and origin of clones/genotypes in a seed orchard, along with their low relatedness levels and the experimental designs employed during establishment, ensure high levels of genetic variation in the produced seed (GenTree, 2020).

Furthermore, Kelleher et al. (2015) highlighted the significance of incorporating the conservation of FRM and FGR into national strategies for climate change adaptation. Their purposeful utilization not only has the potential to reduce risk but also facilitates their adaptation to climate change.

3. Research methodology

This research was conducted in collaboration with the SFI (Slovenia). This study aimed to explore the current role and potential role of FRM and FGR within the South-East European context, given the challenges posed by climate change. As a specific example, a case study has been presented to identify the origin of FRM of Fagus sylvatica using molecular markers based on the dataset provided by SFI.

3.1 Study area

The study area comprised four countries located in the Balkan peninsula (Southeast Europe): Bosnia and Herzegovina, Republic of Croatia, Republic of Slovenia, and Republic of Serbia (Figure 4). have a common historical connection stemming from their former affiliation with Yugoslavia, which left a mark on their cultural, social, and political landscape (Table 3). Further, the countries share flora and fauna, and there is potential to transfer/import or export FRM from warmer to cooler regions in the face of warming climate.

Figure 4: Map of targeted countries

As of now, Croatia and Slovenia are members of the EU, while Serbia is a candidate country and Bosnia and Herzegovina is a potential candidate country. Hence Croatia and Slovenia are subject to Council Directive 1999/105/EC. Moreover, Croatia, Serbia and Slovenia are members of the OECD Forest Seed and Plant Scheme.

Being EU and OECD members means to be subject to specific guidelines for the collection, testing, and certification of forest seeds and plants. Thus, marketing, import and transfer of FRM within members of EU and members of OECD is facilitated. However, when it comes to third countries like Bosnia and Herzegovina, import/export of FRM becomes more difficult. This brings an interesting aspect to how in such situations, the matters relating to import and export of FRM from such third countries are dealt with. Hence this study has been focused on the current status, legislation and regulation of FRM in the targeted countries.

Table 3: General information of target countries (Source: References from tables)

Country	**Bosnia and Herzegovina**	**Croatia**	**Serbia**	**Slovenia**
Country size	51.197 km2	56.561 km2	77.592 km2	20.273 km2
Population	3.531.158 (2013)	3.871.833 (2021)	6.647.003 (2022)	2.116.972 (2023)
Official languages	Bosnian, Serbian, Croatian	Croatian	Serbian	Slovenian
Climate	Continental and Mediterranean climate	Continental, Mediterranean, and snowy forested climate	Continental climate	Continental, sub-Mediterranean, and Alpine climate
Forest area	53% total country area (out of which 81% public, 19% private)	49,3% total country area (out of which 76% public, 24% private owned)	30,7% total country area including Kosovo (43% public, private 57%)	58,2% total country area (21% in public, 79% private)
% of natural forests	N/A	95%	92,2%	97%
% of artificial forests	N/A	5%	9,8%	3%

3.2 Data collection

This research builds on both qualitative and quantitative data collected via secondary sources only. The data collection methodology included a comprehensive review of scientific and grey literature, specifically focusing on the certification status of FRM and its substantive significance within the context of Southeast European countries. This involved using Google Scholar's extensive search features as well as scientific databases like Scopus. Furthermore, books, and research publications were also studied and reviewed from the library of SFI. Whenever relevant, specific information were also gathered from online sources. This preliminary investigation served as a foundational step to gather insights into the current state of certification practices and their implications in the region.

The laws and regulations dealing with FRM, and conservation of genetic diversity (Table 4) were collected from the Official Gazettes of the targeted countries. Publication/reports made available by the concerned bodies in charge of checking the quality of FRM in each country (e.g., SFI for Slovenia) were thoroughly studied to analyze how quality of imported FRM is assured. With the aim to obtain current data and access the latest literature on FRM certification and the status of FGR, the publications catalogue of the European Forest Genetic Resources Programme (EUFORGEN) was utilized.

As for the specific case study on the use of molecular markers, SFI provided a molecular marker datasets of *Fagus sylvatica* stands across South-Eastern Europe. Molecular markers, together with wood anatomy and isotopes, are a promising tool for identifying the origin of wood and FRM (e.g., Global Timber Tracking Network) and could potentially be used to identify the origin of unknown FRM by comparing unknown FRM with a reference database. The dataset contained genetic information of beech analyzed by using microsatellites. Based on the dataset provided, the genetic structure and source of the populations of the unknown European beech stands were examined (see 3.3 for details) to find out if molecular markers can correctly trace the origin of samples.

Table 4: List of secondary data sources

S.N.	Title	Overview	Reference
1	Law on nature protection., OG FBiH No. 66/13.	This law outlines the responsibilities for nature protection, measures for conservation, habitat protection, species preservation, and biodiversity, encompassing forest ecosystems.	https://faolex.fao.org/docs/pdf/bih143206.pdf
2	Law on forests, OG FBiH, No. 20/02, 20/03, 37/04	This law governs the preservation, protection, and sustainable management of forests and addresses ecological, economic, and legal aspects, including supervision, and criminal matters related to forest	https://fbihvlada.gov.ba/bosanski/zakoni/2002/zakoni/13%20boszakon%20o%20sumama.htm
3	Law on forests, OG Republic of Srpska, No. 75/08, 60/13	It governs the holistic management of forests, encompassing policy, planning, protection, financing, valuation, cadastre, and information systems.	https://faolex.fao.org/docs/pdf/bih143406.pdf
4	Law on forest Reproductive Material, OG Republic of Srpska, No. 60/09	It covers criteria for evaluating and approving starting material, production, control, and processing of reproductive material.	https://faolex.fao.org/docs/pdf/bih144966.pdf
5	Law on forests, OG Brčko District, No. 14/10	It outlines the conservation, protection, and enhancement of overall forest functions in the Brčko District of Bosnia and Herzegovina.	https://www.skupstinabd.ba/index.php/hr/zakon.html?lang=hr&id=/Zakon%20o%20s--umama%20Brc--ko%20distrikta%20BiH
6	Law on forests, OG Republic of Croatia, No. 68/18, 115/18, 98/19, 32/20, 145/20, 101/23	This act guides the sustainable management, use, and disposal of forests and forest land, emphasizing economic viability, social acceptance, and social responsibility.	https://www.zakon.hr/z/294/Zakon-o-%C5%A1umama

S.N.	Title	Overview	Reference
7	Law on forest reproduction material, OG Republic of Croatia, No. 75/09, 61/11, 56/13, 14/14, 32/19, 98/19	It establishes guidelines governing the production, promotion, and importing of FRM.	https://zakon.hr/z/597/Zakon-o-%C5%A1umskom-reprodukcijskom-materijalu
8	Law on environment protection, OG Republic of Croatia, No. 80/13, 153/13, 78/15, 12/18, 118/18	It outlines the principles of environmental protection within the context of sustainable development, safeguarding environmental elements and overall environmental protection.	https://www.zakon.hr/z/194/Zakon-o-zaštiti-okoliša
9	Law on seeds, planting material and recognition of agricultural plant variety OG Republic of Croatia, No. 110/21	It regulates the production, market placement, and importation of reproductive material for specific plant groups.	https://www.zakon.hr/z/774/Zakon-o-sjemenu%2C-sadnom-materijalu-i-priznavanju-sorti-poljoprivrednog-bilja
10	Law on reproductive Material of Forest Tree Species, OG Republic of Serbia, No. 135/04, 8/05,1/09	It handles the production, control, processing, quality, transportation, and use of FRM, including the maintenance of registers for forest plant varieties.	https://faolex.fao.org/docs/pdf/bih144966.pdf
11	Law on environmental protection, OG Republic of Serbia, No. 14/2016	It promotes the right to a healthy environment while balancing economic development and environmental concerns.	https://www.zzps.rs/wp/pdf/zakoni/LAW%20ON%20ENVIRONMENTAL%20PROTECTION.pdf
12	Law on forests, OG Republic of Serbia No. 30/10	It governs the management, protection, and use of forests, including planning, cultivation, and disposal of forest land	https://faolex.fao.org/docs/pdf/srb143404.pdf

S.N.	Title	Overview	Reference
13	Law on nature protection, OG Republic of Serbia No. 36/2009, 88/2010 and 91/2010 – corr. and 14/2016	This law regulates the protection and preservation of nature, encompassing biological, geological, and landscape diversity.	https://www.pregovarackagrupa27.gov.rs/wp-content/uploads/2021/06/LAW-ON-NATURE-PROTECTION-2016.pdf
14	Forest reproductive material act, OG Republic of Slovenia, No. 58/02, 85/02 - corr. , 45/04 - ZdZPKG and 77/11	This law sets the conditions for the production, marketing, and utilization of FRM.	http://www.pisrs.si/Pis.web/pregledPredpisa?id=ZAKO1342
15	Regulations on the determination of provenance areas, OG Republic of Slovenia, no. 72/03 , 58/12 , 69/17 and 92/23	The law defines the necessary provenance areas for the production and marketing of FRM and provides usage guidelines.	http://www.pisrs.si/Pis.web/pregledPredpisa?id=PRAV3788
16	Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material	It is designed to govern the commercial marketing and production for marketing purposes of forest reproductive material within EU.	https://faolex.fao.org/docs/pdf/eur34525.pdf
17	OECD Forest Seed and Plant Scheme	It is a certification system that promotes international trade in forest seeds and plants, ensuring the accuracy of their labeling.	https://www.oecd.org/agriculture/forest/documents/forest-scheme-rules-and-regulations.pdf

3.3 Data sampling

The molecular markers dataset (Annex 1) consists of simple sequence repeat data, or so-called microsatellite data, for 32 European beech stands across SE Europe. Different cohorts (seeds, natural regeneration, adult trees) were genotyped with molecular markers. Different cohorts sampled in a single stand provide an opportunity to test whether molecular marker signal is consistent across development stages of a forest and therefore robust enough to use as a discriminating tool. Theoretically, all cohorts from the same stand should always belong to the same gene pool or origin. If this is not the case, the set of molecular markers is not appropriate for studying the origin of FRM.

SFI prepared the molecular markers dataset in a way that stands labeled one to 26 had an origin associated to them, mocking a reference samples database. The origin of stands 27 to 31 remained hidden from the investigator in order to determine the origin of these five stands by comparison with the database of reference samples. The aim of the study was to check whether the particular set of molecular markers was an appropriate tool to correctly determine the origin of those unknown-labelled samples.

16 nuclear single sequence repeat markers, also called microsatellites were chosen for the study. These markers are codominant, highly variable and provide enough discriminating power to identify individuals for applications such as paternity analysis (also used in human paternity analysis). The set of 16 markers is routinely used by SFI for beech samples, Briefly, DNA was isolated from tissue using a commercially available extraction kit, then three separate Multiplex Polymerase chain reaction (M-PCR) were performed to amplify DNA on all 16 genetic loci. This amplified DNA was then loaded on a sequencer to perform fragment analysis with which peaks of different sizes were revealed. The sizes of the peaks corresponded to a value that is later analysed with different methods to reveal parameters of genetic diversity and structure of a single stand. From the parameters of genetic structure, the origin of the source material, i.e., forest stands, can potentially be determined.

3.4 Data analysis

Collected data (see 3.2) was thoroughly comprehended and its summaries were presented in the results (see 4.1). Based on these summaries as exploratory data and with use of thematic data analysis, certain themes were established which are presented in the comparative assessment (see Table 4).

For the case study, the genetic structure or the origin of the populations was analyzed with the algorithm for classifying individual specimens based on Bayesian probability in the program Structure 2.3.4 (Pritchard et *al.,* 2000; Falush *et al.,* 2003; Hubisz *et al.,* 2009). Upon analysis, sample 6 was concluded to be *Fagus moesiaca,* since this study is based on *Fagus sylvatica*, sample 6 was later removed.

A mixed model in combination with a model of correlated allele frequencies was used in the Structure software (default option with default parameters). This analysis was done to identify the genetic structure with prior information and pre-identified origin of the sample. For the calculations, the number of groups K was varied between 1 and 10 and 5 repetitions were carried out for each K. Here, K is the number of gene pools or genetic clusters that the software identifies within a dataset. Evanno's method (Evanno *et al*., 2005) with Structure Harvester (Earl and von Holdt, 2012) was used to detect the true value of K as it indicates the most probable number of genetic clusters in the population. Then, the average of repetitions was calculated, and the outcomes were visualized using the CLUMPAK software (Kopelman *et al.,* 2015).

Furthermore, a Strengths, Weaknesses, Opportunities and Threats (SWOT) analysis (Namugenyi *et al*., 2019) was done on the use of molecular markers focusing to evaluate if the application of molecular markers should be encouraged.

4. Results and discussion

In this chapter, the results of the data analysis are presented. The governing laws and regulations in Bosnia and Herzegovina, Croatia, Serbia, and Slovenia are briefly discussed, where a comparative analysis based on the framework of those FRM laws among the target countries is also depicted. Furthermore, this chapter comprises a case study on tracing the origin of beech FRM, followed by a SWOT analysis of the use of molecular markers.

4.1 Current laws and regulations in target countries

In this section, the status of regulatory frameworks of the targeted countries such as laws, regulations, rules etc are described separately for each country.

4.1.1 Bosnia and Herzegovina

State of Bosnia and Herzegovina is administratively comprised out of 2 constituent entities (Federation of Bosnia and Herzegovina (FBiH) and Republic of Srpska) and the Brčko District autonomous province Both federal entities and the Brčko District have their own laws and regulations. Due to this administrative setting and considering that there is no common law or regulation regarding FRM, for the aims of this study we will include all relevant laws and regulations on FRM from both federal entities and the Brčko District.

4.1.1.1 Federation of Bosnia and Herzegovina

The laws and regulations based on FRM and conservation of genetic diversity in the Federation of Bosnia and Herzegovina are mentioned below:

4.1.1.1.1 Law on Nature Protection

The law on Nature protection under articles from 120 to 124 explains genetic diversity of autochthonous domestic species/subspecies and defines genetic material as “part of plant, fungi, animal or microorganism which contains parts of deoxyribonucleic acid” and taking genetic material cannot endanger the survival of an ecosystem or a population in their habitats. It also says that a gene bank must be established which contains controlled or grown populations or parts of animals, fungi or plants as biological materials which are defined as microorganisms, molecules and fragments of DNA, viruses, and active cell-cell cultures. Furthermore, it is stated that it is forbidden to patent a part of genetic diversity of FBiH, and no one can become a patent owner of genetic material made on a basis of genetic material of wild animal species/sub species.

4.1.1.1.2 Law on Forests

Under the Law on Forests, it is stated under Article 3 subsection 15 that FRM is:

Seeds: cones, fruits and seeds intended for the production of plants.

Parts of plants: twigs and bundles of cut branches intended to produce plants.

Young plants: plants grown from seeds or parts of plants, including young plants from natural young-sapling stage.

According to Article 32, all surfaces after clear cut (clear cuts are forbidden in FBiH except in special cases) or forest fire with natural or artificial regeneration within 2 years. In case of artificial regeneration, it must be regenerated only with healthy seeds or seedlings adapted to the habitat and material of confirmed origin.

FRM is stated within Article 33 where the federal and cantonal administrations will determine the regions of provenance, select forest seed facilities, and stands from which reproductive material will be provided, keep a register of forest seed facilities and stands and issue certificates of origin of FRM in terms of public authority.

Industrial seed and cone facilities and nurseries will monitor the origin of FRM and will inform customers about the categories of FRM as well as its origin. The competent forestry inspector will supervise the circulation of forest seeds and forest planting material. The import of FRM is possible only on the basis of a permit issued by the Federal Ministry based on the opinion of the Federal Administration. A permit will only be issued if the FRM is of certified origin. Cantonal administrations will keep records of facilities for the production and processing of forest seeds and producers and processors of forest seeds and forest and ornamental trees and shrubs.

Article 57 is stating who performs professional tasks and issues all required documentation about health status and quality of FRM (Federal administration) and Article 66 is authorizing the forest inspector to supervise all regulation implementation regarding production and traffic of FRM. Furthermore, if a legal imports FRM without a licence, penalties up to 5000KM (equivalent to 2500€) will be enforced.

There are articles regarding biological reproduction of forests where forestry companies (at least 15% of the total income from sale of wood assortments and the value of wood used for own needs at the average annual price of the assortments and as well as income from sale of secondary forest products) and private forest owners (at least 15% of the amount of wood mass approved for felling at market prices of wood mass per stump before putting wood in circulation) are obliged to allocate funds for simple and extended biological reproduction of forests which include among other things, also production of FRM and preparation of land for natural regeneration. Penalties for non-compliance to allocate funds is ranging between 5000 to 25000 KM (equivalent to 2500 to 12500 €).

4.1.1.2 Republic of Srpska

Laws and regulations relating to FRM and conservation of FGR in the Republic of Srpska have been briefly reported below.

4.1.1.2.1 Law on forests

Production of FRM is comprised within Article 66 where seed facilities, except seed facilities of known origin, is maintained by the user of forests and forest soil so that it secures the maximum production of quality forest seed and enables easier seed gathering.

Article 67 is allowing cuttings of trees for management methods to achieve the optimal structure in stands which are recognised as starting material for production of forest seed with the exception of starting material for production of seeds of known origin. If a company cuts trees in seed stands which are recognised as starting material for production of forest seed against this article, he will be fined with a penalty fine of 3000 to 9000 KM (equivalent to 1500 to 4500€).

Article 68 defines FRM as forest seed and planting material. Forest seed production is realised in seed facilities through activities of its extraction or founding, managing, up keeping, picking, finishing, and keeping of seeds and fruits, methods of quality assessment and organisation of transport. Forest planting material represents plants reproduced and raised in a nursery as a special land area where with use of technological-technical system of procedures seedlings are produced for appropriate purpose.

According to Article 34, User of forests and forest soils is obligated to do different tasks, one of which is the production of FRM.

4.1.1.2.2 Law on Forest reproductive material

Official Gazette of the Republic of Srpska, No. 60/09 states that the law on FRM governs the essential features of the distribution, utilization, production, and trade of FRM, encompassing standards and conditions. It outlines the methods for validating and recognizing initial materials (classification) and further delineates the control and processing procedures for FRM. The legislation also establishes rules for ensuring the quality and inspection of such material, along with provisions for maintaining related registers.

Article 5 specifies that the Ministry of Agriculture, Forestry, and Water Management is responsible for issuing a regulation outlining the roster of forest tree species, hybrids, and varieties eligible for FRM extraction.

In accordance with Article 9, imported FRM may be utilized if it adheres to the standards of the EU and is necessary due to the absence of a seed crop, insufficient seed crop, or insufficient quantities of FRM in stock for the purposes of afforestation and forest renewal in that particular year. The decision regarding the fulfilment of these conditions' rests with the Ministry.

Article 19 stipulates that the mandatory control of reproductive material production must occur once during the growing season. However, there is an exception to this rule for poplar and willow, for which the control of reproductive material production is conducted twice during the growing season. It further states that Reproductive material production is obligated to undergo a compulsory health examination, regulated by specific provisions. The production control is conducted by the Ministry and hold the authority to delegate the professional responsibilities of production control to an economic company or another legal entity outlined.

Article 21 mandates that a certificate of origin for reproductive material is issued by a business company or another legally authorized entity, with the approval of the Ministry and in adherence to the law and relevant regulations.

In Article 26, the examination and assessment of the quality of imported FRM are conducted by a business company or another legal entity possessing certificates from the international association responsible for assessing reproductive material quality.

Article 41 outlines the authority and obligation of forestry inspectors to prohibit the use and market placement of imported reproductive material lacking approval by the Ministry.

Article 46. An entrepreneur shall be fined from 6,000 to 18,000 KM for a misdemeanour, if failed to inform the Ministry about the type and quantity of the produced, i.e., imported FRM.

4.1.1.3 Brčko District

This section comprises of the laws and regulations pertaining to FRM and conservation of genetic diversity in Brčko District.

4.1.1.3.1 Law on forests

As published on the Official Gazette of the Brčko District of BiH, number 2/10, the law on forests mandates the conservation, safeguarding, and enhancement of the overall functions of forests, as well as the planning, management, and economic aspects of forestry in the Brčko District of Bosnia and Herzegovina.

There is no separate law on FRM issued by Brčko District however the provisions for FRM are included in law on forests under two articles. According to Article 36, the Department for Agriculture, Forestry, and Water Management of the District Government (herein after: the Department) identifies forest seed facilities and locations that provide FRM, maintains a registry of such facilities and locations, and issues certificates verifying the origin of FRM.

The department also grants permits for the import of FRM of confirmed origins. Furthermore, the Department is also responsible for maintaining records of facilities engaged in the production and processing of forest seeds, as well as producers involved in the production and processing of forest seeds, forest, and ornamental trees and shrubs.

Moreover, under Article 63, there is provision for a business company or other legal entity to be fined if the FRM has been imported without consent and used for reproduction contrary to Article 36 of this law.

4.1.1.4 Forest reproductive material issues in Bosnia and Herzegovina

Bosnia and Herzegovina continue to employ traditional "know-how" in the cultivation of seedlings and in establishing the standards for high-quality seedlings. Typically, they have not modernized their approaches in accordance with updated knowledge (still follows YUS standards) and changes in paradigms. There are illegal activities taking place in the forestry sector which includes illegal obtaining of seeds (Mataruga *et al.,* 2023). There is inadequate regulation over the utilization and export of different products derived from the exploitation of local genetic resources. There is a lack of a singular state-level agency responsible for nature protection issues and the financing mechanisms are not well-developed. (Drešković *et al.,* 2011).

4.1.2 Croatia

Laws and regulations relating to FRM and conservation of FGR in Croatia have been briefly reported below.

4.1.2.1 Law on forests Official Gazette 68/18, 115/18, 98/19, 32/20, 145/20, 101/23

Croatia's Law of Forests defines the system and methods of management, usage and disposal of forests and forest lands based on principles of sustainable management, economic and ecological acceptability, and social responsibility. The stated articles in the law are discussed below:

Article 4 is defined within the economic functions of forests along with production of wood and non-wood forest products.

In Article 8 subsection 23c, FRM is defined as seed material, parts of plants and planting material of forest taxa and hybrids which primarily serves for the establishment and restoration of forests.

Within Article 22 subsection 11, where forest seed objects include seed stands, seed plantations, parent trees, clones and clone mixtures and their primary purpose is the production of FRM.

Later, under use of forests, forests lands and forest products, in article 37 subsection 2, it is stated that FRM can be transported only if a regulated consignment note (Figure 5) has been issued. The consignment note is regulated according to the "Regulation on tree marking, forest product characteristics, consignment note and forest order NN71/19" where it is stated that this is a unique numbered document which is issued before the transportation of wood and non-wood forest products and wood cut outside the forest and forest land and that it can be only issued by a person with a forestry profession.

Prilog 10
Obrazac "TL - NŠP"

TERETNI LIST (POPRATNICA) ZA NEDRVNE ŠUMSKE PROIZVODE

Nº

Licencirani izvođač:..

MJESTO UTOVARA

Skladište (g.j., odjel/odsjek, k.č.):
....

Prijevoznik: ....
Vozač: ....

MJESTO ISTOVARA

Kupac: ....
Skladište: ....

Registarska oznaka:
....

Redni broj	Naziv nedrvnog šumskog proizvoda	Jedinica mjere	Količina
1			
2			
3			
4			
5			
6			
7			
8			
9			

Otpremio:
....

Mjesto i datum:
....

Potpis vozača:
....

Primio
....

Figure 5: Official consignment not for non/wood forest products

Source: Regulation on tree marking, forest product characteristics, consignment note and forest order OG77/19)

4.1.2.2 Law on Forest reproduction material, Official Gazette 75/09, 61/11, 56/13, 14/14, 32/19, 98/19

The legislation regarding FRM governs the production, market placement, and importation of FRM, as well as the production and importation of Christmas trees. It establishes the conditions that FRM suppliers and Christmas tree suppliers must adhere to during enrolment processes in registers. The law also outlines procedures for enrolling forest seed objects in the Register of Forest Seed Objects, creating and utilizing reserves of seed material, genetic and seed banks for forest tree taxa, and forming a committee for FRM. Additionally, it covers the collection, utilization, and exchange of data and information, the costs associated with implementing the measures outlined in the law, the competent bodies responsible for enforcing the law, and the supervision of its implementation.

This Law transposes into the legal system of Croatia directive 1999/105/EC of the Council of the EU of December 22, 1999, on placing FRM on the market. Its aim is to ensure the production, placing on the market and import of high-quality and habitat adapted FRM which enables sustainable and optimal management of forest ecosystems and their restoration in accordance with the protection of FGR. For non-compliance of this law, certain penal provisions are set in place in Article 64 of this law.

FRM according to this Law includes seed material, plant parts and planting material of forest taxa intended for use in forestry and scientific research work in forestry where “use in forestry” specifically means biological restoration in accordance with forest regulations and establishment and maintenance of cultures and plantations of forest trees. The list of forest taxa is determined by the minister responsible for forestry affairs.

According to Article 5 of this Law the provisions of this Law do not apply to planting material or plant parts of forest taxa that are not used for the purposes of use in forestry, but it is subject to provisions of a special regulation where such material must be accompanied by a label where it is stated “not for use in forestry” and it does not apply to FRM intended for export or re-export.

4.1.2.3 Law on environment protection Official Gazette 80/13, 153/13, 78/15, 12/18, 118/18

The Law on environmental protection includes protection of forest areas of natural and partially natural stands, forest complexes, as well as the protection of forest soil, plant, and animal life in the forest area, and important for FRM genetic seed stands of autochthonous tree species.

4.1.2.4 Law on seeds, planting material and recognition of agricultural plant variety Official Gazette 110/21

This law only mentions FRM in defining what is planting material within Article 3. Subsection 4 where it is stated that "planting material includes reproduction planting material, fruit seedlings, vine cuttings, fruit seedlings, vegetable seedlings and reproduction material of decorative plants, except forest planting material". It is because defined like this since forest planting material and consequently FRM has its own regulation and laws to which it needs to abide.

4.1.2.5 Forest reproductive material issues in Croatia

Republic of Croatia's FRM issues were mostly regulated after the implementation of the EU Directive 1999/105/EU which supplied the framework of use of FRM. Main issues of FRM in Croatia are accompanied with production of FRM due to climate change.

4.1.3 Serbia

Here is a brief overview of the laws and regulations regarding FRM and conservation of FGR in Serbia.

4.1.3.1 Law on Reproductive Material of Forest Tree Species, 2004

The Official gazette of Republic of Serbia, No. 135/04, 8/05 and 41/09 has stated that Law on Reproductive Material of Forest Tree Species regulates the approval of basic materials for production of forest trees, production, production control, processing, quality, marketing as well as the use of reproductive material of forest trees. It was published in the official gazette in 2004.

Even though Serbia is not a member of the EU, it's law on FRM is on full concordance with Council Directive 1999/105/EC. Furthermore, the Commission Implementing

Decision (EU) 2021/773 of 10 May 2021 has now authorized the member states, in accordance with Council Directive 1999/105/EC to temporarily decide on the equivalency of FRM of certain categories produced in certain third countries. Here the third countries are the member countries of the OECD Scheme for the certification of FRM moving in international trade (OECD Forest Seed and Plant Scheme). Hence, the import of FRM from Serbia to EU countries is considered as equivalent to the transfer of FRM between other EU countries (Kraigher *et al.,* 2021).

4.1.3.2 Law on environmental protection, 2004

It states that natural values must be utilized in a manner and under conditions that guarantee the preservation of geodiversity, biodiversity, protected natural goods, and the landscape. Renewable natural resources should be employed under conditions that ensure their continual and efficient renewal and ongoing quality improvement. Non-renewable natural resources should be utilized with a focus on long-term, economical, and sensible usage, including the limited exploitation of strategic or rare natural resources. This should also involve substitution by other available resources, composite materials, or artificial alternatives. Following provisions are made under these articles:

Article 25 - To safeguard and enhance forest ecosystems, forests should be managed in a way that ensures rational forest management, preservation of the genetic fund, improvement of the structure, and fulfilment of forests' priority functions.

Article 27 - To safeguard biodiversity and biological resources, including indigenous plant and animal species and their distribution, the Ministry, other competent bodies, and organizations shall monitor the import and cultivation of plant and animal species of foreign origin (Source: Official Gazette of the Republic of Serbia No. 14/2016 of 22/02/2016)

4.1.3.3 Law on nature protection, 2009

Under this law, only Article 22 relates to the conservation of FGR. It mentions "A gene bank is set up with the primary goal of preserving the genetic diversity of wild plants, animals, and fungi. This involves storing biological materials from monitored or selectively bred populations, including components such as plant and animal parts, seeds, spores, reproductive cells, and other biological elements. The purpose of maintaining these materials in gene banks is to actively contribute to the conservation of species, safeguarding their genetic makeup and potential. (Source: Official Gazette of Republic of Serbia No. 36/2009, 88/2010 and 91/2010 – corr. and 14/2016)

4.1.3.4 Law on forests, 2010

The law outlines the concept of forests, emphasizing their role in both preserving and utilizing the gene pool of forest tree species. Furthermore, it designates forests as crucial for conserving the biodiversity of genes, species, ecosystems, and landscapes (Nonić et. al, 2019). As per Article 13, forest management plans must incorporate actions aimed at safeguarding, responsibly utilizing, and expanding the range of protected species of forest trees to ensure the conservation and sustainable utilization of their gene pool (Source: Official Gazette of Republic of Serbia No. 30/10).

4.1.3.5 Transfer of forest reproductive materials to Serbia

The Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia is responsible for granting authorization for the import of seeds. Additionally, it is essential that the imported FRM must comply with the requirements as mentioned in the Law on Plants Health of the Republic of Serbia (Kraigher *et al.,* 2021).

Article 8 of the Law on FRM states that the imported FRM of trees under Article 3 can be used for afforestation and regeneration of forests if:

a) It falls under the category qualified or tested,
b) Seed production is either non-existent, insufficient, or, if available, the quantities are inadequate for the purpose of afforestation and forest regeneration in the given year (Official gazette of Republic of Serbia, No. 135/04, 8/05 and 41/09).

Furthermore, Article 19 proclaims that the importer of FRM must notify the Ministry regarding the species and quantity of imported reproductive material within a maximum of 15 days from the date of import. Additionally, the transfer of FRM from EU to Serbia is further governed by the Law on Plants Health as published on the Official Gazette of Republic of Serbia No., 41/2009 and 17/2019.

4.1.3.6 Conditions to abide by to import forest reproductive material into Serbia

The import of FRM into Serbia is allowed only if it meets specific conditions:

i) It must not be infected with harmful organisms listed in List IA part I, List IA part II, List IIA part I, and List IIA part II, or regulated harmful organisms.
ii) A valid phytocertificate issued in accordance with the International Plant Protection Convention is required (Article 67).
iii) It must not include plants, plant products, or prescribed objects from List IIIA (Article 35).
iv) Compliance with specific phytosanitary conditions outlined in List IVA part I and List IVA part II is necessary (Article 35). *(Source: Official gazette of Republic of Serbia, No. 135/04, 8/05 and 41/09)*

4.1.3.7 Forest reproductive material related issues in Serbia

The Law on FRM clearly obligates the monitoring of the origin of FRM, however its practical implementation is not always carried out (Mataruga *et al.,* 2023). It is common that all countries in the world have updated, quality standards and guidelines mainly based on seedling size and age, to regulate proper growth (Šijačić-Nikolić *et al.,* 2019). However, that is not the case in Serbia. It still has old guidelines that have not been updated for a long time. For the most common tree species, Serbia still follows YUS that were established in 1964 within the former Socialist Federal Republic of Yugoslavia (SFRY). Even after the dissolution of SFRY in the early 90's Serbia still heavily relies on YUS that aims to ensure uniformity, quality, and compatibility within the Yugoslav countries (Mataruga *et al.,* 2023). Nevertheless, initiatives have been proposed to change the reigning standards (Ivetić *et al.,* 2016).

4.1.4 Slovenia

In the following sections, laws and regulations adhering to FRM and conservation of FGR in Slovenia are presented:

4.1.4.1 Forest reproductive material act, 2002

As published on the Official Gazette of the Republic of Slovenia, No. 58/02, 85/02 - corr., 45/04 - ZdZPKG and 77/11, FRM act outlines rules for producing, marketing, and using FRM. It specifies obligations for those involved in its production, marketing, and import. The law covers tasks and procedures related to the origin, quality, and identity of reproductive material, as well as data management, seed reserves, and forest gene banks. It designates authorities for implementation and inspection control. It further states that only seed facilities approved by the SFI and listed in the register of forest seed facilities can be used for producing FRM intended for marketing. FRM eligible for marketing must adhere to the conditions set by this law and plant health regulations.

According to Article 7, only registered suppliers are authorized to market FRM. To be eligible for marketing, FRM must meet the following criteria:

- Align with the categories source-identified, qualified, selected and tested.
- Possess the main certificate as stipulated in Article 13 of this Act.
- Demonstrate sufficient quality, including size, health condition, and other general characteristics.
- Be accompanied by the supplier's document, issued in compliance with Article 48.
- Be appropriately labelled and packaged to facilitate identity verification.

The criteria for determining adequate quality and the methods of labelling and packaging will be specified by the Minister.

Article 13 states that SFI issues a main certificate for each batch of seed material, plant parts, or sprouts obtained in the seed facility. This main certificate extends to all reproduction material produced from the same seed material, plant parts, or sprouts. The Minister outlines the detailed content and format of main certificates for reproduction material from different types of seed facilities, as well as the procedure and costs for obtaining them.

Furthermore, as per Article 19, if it is found that certain reproductive material poses potential harm to the forest, environment, genetic resources, or biodiversity due to its physical or hereditary traits, its marketing to end-users will be prohibited. To determine or demonstrate these harmful effects, the Minister will appoint a contractor and define the procedure. The Minister has the authority to enforce the ban on the marketing of such reproductive material. Consequently, the use of this material is prohibited within the territory of the Republic of Slovenia.

To guarantee the identity of reproductive material throughout its production and marketing stages, an official control system is established, covering the entire process from obtaining reproductive material in the seed facility to delivering it to end-users.

SFI oversees the acquisition of reproductive material in stands and groups of seedlings, while the institute manages control for reproductive material in seed plantations, parent families, clones, and clone mixtures. Forestry inspectors supervise suppliers in the production and marketing processes, excluding cases where the supplier obtains reproductive material in a seed facility, and oversee the use of reproductive material by end-users. Import of reproductive material is monitored by phytosanitary inspectors. All authorities involved in supervising the production, marketing, import, or use of reproductive material collaborate, exchange data and information, and provide reports on their activities to the Ministry (Article 61).

Moreover, Article 62 mentions that Forestry and phytosanitary inspectors are responsible for overseeing the enforcement of the regulations outlined in this law, its associated directives, and Community regulations on FRM. Individuals or entities involved in the production, marketing, or import of reproductive material, and those utilizing it as per the specified section of Article 3 in this Act, must facilitate and not impede forestry or phytosanitary inspectors in supervising their activities. They are required to provide inspectors with necessary documents, information, explanations, or items during inspection supervision. Both legal entities and individuals are obliged to adhere to enforceable decisions made by forestry or phytosanitary inspectors, which mandate the implementation of measures outlined in this law within a specified timeframe.

4.1.4.2 Regulations on the determination of provenance areas, 2003

Official Gazette of the Republic of Slovenia, no. 72/03, 58/12, 69/17 and 92/23 states that these regulations outline the specific provenance areas that must be considered in the production and marketing of FRM along with providing guidelines for its utilization.

To guide the utilization of FRM, the following suitability scale is used:

- Most suitable: FRM from a seed facility within the same provenance sub-area and altitude zone.
- Very suitable: FRM from a seed facility within the same provenance area and altitude zone.
- Suitable: FRM from a seed facility in the adjacent area of origin and the same altitude zone.

- Less suitable: FRM from the same provenance area and altitude zone, but from different seed facilities.
- Exceptionally suitable: FRM from other provenance areas and the adjacent altitude zone in the same provenance area and altitude zone.

If most suitable or very suitable FRM is unavailable in the seed facilities of a specific provenance and altitude zone, and it's not in the seed store, FRM for suitable or less suitable use may be employed for a maximum of one year. If FRM for less suitable use is unavailable for more than 10 years, FRM for exceptionally suitable use may be utilized for a maximum of one year. If FRM for exceptionally suitable use is unavailable for more than 10 years and there's an increased need for FRM due to large-scale area rehabilitation after damage, with a positive expert opinion from the SFI, FRM from other countries may be used, but for a maximum of one year. Regardless of the rules mentioned before, in order to preserve FGR in the Šavrin sub-area of provenance, only FRM from this sub-area is allowed.

4.1.4.3 Forest reproductive material issues in Slovenia

To assess the long-term effectiveness of FRM it is essential to maintain records of its origin. However, these records are stored in archives specific to forestry districts and are not easily accessible (Gömöry *et al.,* 2021. Currently, there is minimal, or no knowledge transfer aimed at enhancing seedling production and improving the quality attributes (Mataruga *et al.,* 2023).

4.2 Comparative analysis of target countries

Table 5: Comparative assessment of target countries' laws and regulations

Country	**Bosnia and Herzegovina**	**Croatia**	**Slovenia**	**Serbia**
Laws based on FRM	No state law for FRM, only Republic of Srpska has Law on FRM	Law on forest reproduction material	Forest reproductive material act	Law on Reproductive Material of Forest Tree Species
Year of adoption of FRM law	2009	2009	2002	2004
Other laws and regulations for FRM and FGR conservation	Law on forests; FBiH has another law for nature protection as well	Law on Forests; Law on environment protection; Law on seeds planting material and recognition of agricultural plant	Regulations on the determination of provenance areas; Act on Forests	Law on environmental protection; Law on nature protection, law on forests, Law on Plant Health
Responsible body for use of FRM	Ministry of Agriculture, Forestry and Water Management	Croatian Forest Research Institute and Croatian Forestry Institute	Slovenian Forestry Institute and Slovenia Forest Service	Ministry competent for forestry
Regions of provenances	Not delineated	5 regions, 13 zones and 57 seed units.	7 regions for *Quercus robur, Quercus petraea, Fagus sylvatica, Picea abies* and *Abies alba*; For other tree species, Slovenia is a single region of provenance.	Specific provenance for *Quercus robur* and *Fraxinus angustifolia*; For all other tree species, Serbia is one provenance region

Country	Bosnia and Herzegovina	Croatia	Slovenia	Serbia
Member of EU/OECD	No/No	Yes/Yes	Yes/Yes	No/Yes
Quality check of FRM	Only for Republic of Srpska: conducted by a commercial company holding certificate for quality testing with international standards	Conducted by CFRI	Conducted by SFI	Not necessarily conducted
Phytosanitary checks	Handled by forestry inspector	Handled by phytosanitary inspector at entry point	Handled by phytosanitary inspector at entry point	Handled by phytosanitary inspector at entry point
Importing seeds from third country	Ministry of Agriculture, Forestry and Water Management	Ministry responsible for forest affairs makes decisions	Ministry responsible for forest affairs makes decisions	Ministry competent for forestry
Additional rules other than mentioned in Council directives and OECD	N/A	Law on FRM also includes production and import of Christmas trees	Compulsory checking of origin of FRM in case of suspicion	Quality check of FRM is not included under Law on FRM but under Law on Plant health and it is not compulsorily done.
Penal provision for non-compliance to laws	700-12750€	4500-13000€	400-2000€	85-8500€

Source: own elaboration

Table 5 showcases the comparative analysis of the FRM and FGR laws and regulations in the target countries. It can be interpreted that all countries have a definite law on FRM, except Bosnia and Herzegovina. Only the entity Republic of Srpska in Bosnia and Herzegovina has a definite law on FRM. However, Bosnia and Herzegovina encompass regulations relating to FRM under separate articles in Law on Forests. Similarly, no regions of provenances have been delineated in Bosnia and Herzegovina, while the other countries have it defined. Serbia does not have provisions for checking the quality of the FRM, while Croatia, Slovenia and only Republic of Srpska from Bosnia and Herzegovina have that provision.

All countries are subjected to perform phytosanitary checks on the imported FRM by the designated inspector. The records and control of the importing of FRM from third countries is handled by their respective Ministry for forest affairs. In addition to the guidelines formulated by the Council Directives and OECD Forest Seed and Plant Scheme, the target countries have added few things to their national regulations which is unique to other countries. Law on FRM of Croatia also comprises the production and import of Christmas trees. Only Slovenia has adopted the measures of checking the origin of FRM in case of doubt on the documents provided by the supplier. Moreover, Serbia has included the checking the quality of FRM when required, under the Law on Plant Health.

Additional, there is penal provisions for non-compliance to the adhering laws (like importing FRM illegally and breaking any law). In Bosnia and Herzegovina, one can be fined from 700€ to 12750€ (amount of penalty differs between the entities). Similarly, in Croatia, Slovenia, and Serbia, one would have to pay 4500€ to 13000€, 400€ to 2000€ and 85-8500€ respectively. It can be interpreted that Croatia is stricter than other countries when it comes to following FRM regulations.

4.3 Transfer of forest reproductive material among target countries

To preserve FGR within the national borders, it is recommended to market only a limited number of regions of provenance for a specific forest tree species to end users in that region (Forestry Commission, 2019). The marketing of FRM across national borders (even within EU) may be restricted unless approval is granted by a specified state authority based on professional opinion. Importing to EU and OECD members call for additional phytosanitary checks (Konnert *et al.,* 2015).

When moving seeds and seedlings within Croatia and Slovenia, both EU member states, it is required that for each trade unit, whether it's a subset or the entire lot, it should be accompanied by an EU plant passport. In case of Croatia, the plant passport is provided by a designated specialized entity registered in the Ministry of Agriculture, while in Slovenia by the supplying authorized nursery. The plant passport serves as an official confirmation that the FRM is free from both quarantine and non-quarantine pests (EC, n.d.; Kraigher *et al.,* 2021)

In case of Serbia, when importing FRM from Serbia to Croatia and Slovenia, additional phytosanitary regulations come into play. In these situations, the official national authorities must issue a phytosanitary certificate, as the plant passport is applicable only within EU member states. The phytosanitary certificate is issued by the phytosanitary inspector after fulfilling the inspection at the border (Kraigher *et al.,* 2021).

Since Bosnia and Herzegovina isn't an EU nor OECD member, importing FRM from there is subjected to further rules. The other countries must request to the European Commission for the approval. Upon receiving the request of member states with valid reasons, the EC in consultation with the Standing Committee on Plants, Animals, Food and Feed (PAFF) and its Working Group on FRM, makes decisions about importing FRM from third countries like Bosnia and Herzegovina (Kraigher *et al.,* 2021).

To import and market FRM from Bosnia and Herzegovina, these countries must demonstrate that their control procedures align with those specified in Council Directive 1999/105/EC. After a review of their production, marketing, and control systems, if they are deemed to be in accordance with the EC Directive (and implicitly with the OECD Forest Seed and Plant Scheme), the other countries can seek approval from the PAFF Working Group on FRM. Upon approval, the EC then issues a Decree of approval for the eligibility of FRM from these countries within their national borders. Furthermore, additional national laws may apply as per countries which may require a professional opinion. For example, the imported FRM is subjected to quality checks in Croatia and Slovenia at the point of entry; however such procedure is not required in Serbia (OG Republic of Croatia, No. 75/09, 61/11, 56/13, 14/14, 32/19, 98/19; OG Republic of

Serbia, No. 135/04, 8/05,1/09; OG Republic of Slovenia, no. 72/03 , 58/12 , 69/17 and 92/23, Council directive 1999/105/EC; OECD Forest Seed and Plant Scheme).

4.4 Case study

In this section, the results of an attempt to trace the putatively unknown samples of FRM of *Fagus sylvatica* (samples 28 to 32) back to their origin (i.e., reference samples 1 to 27) are presented.

4.4.1 Identification of sample origin

Upon analyzing the genetic information of beech seeds in the program Structure, clusters of genetic groups based on population averages are obtained. In the following figures, each vertical line is a single sample (seed, seedling, adult tree). The black lines divide one forest stand from another. The colours represent the genetic contribution of each individual to a genetic cluster (number of colours is equal to K). Similar patterns of genetic contributions to individual gene pools should signalize the same origin of the stand or in our case, FRM.

Figures 6 and 7 presented below are the two most likely solutions according to Evanno's method. Here, K refers to the number of gene pools that represents the genetic diversity of a group, allowing for the inheritance of different traits and facilitating the potentials to adapt to varying environmental conditions.

4.4.1.1 Seven genetic clusters

Figure 6 shows results for K=7 that was identified via Evanno's method as the best value.

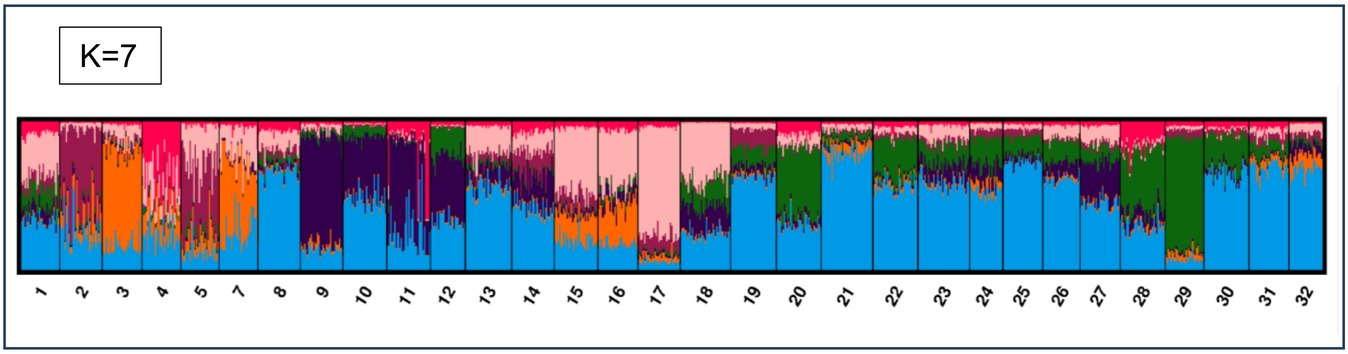

Figure 6: Results of the sample assessment for K=7

According to Evanno's method, the most likely number of genetic clusters in the analysed dataset K is 7. Therefore, we first analysed the data according to this option by visual interpretation. We first looked at the "known" samples, i.e., samples 1 to 27.

We noticed that K=7 is probably not the best option for our dataset and that the true number of genetic clusters is lower. We noticed that samples sample no. 8 and 13 look to have similar genetic features but come from Hungary and Bosnia and Herzegovina respectively, two countries with dissimilar ecological conditions and beech migration history, which largely determines the genetic structure of nowadays beech stands at single sequence repeats molecular loci. This makes the result unlikely correct.

Furthermore, looking at the "mock unknown" samples, the unknown sample 28 looked similar to "mock known" sample 20. It was revealed that both samples are from Slovenia but not from the same forest stand. It was difficult to match "mock unknown" sample 29 as none of the other samples looked similar. "Mock unknown" sample 30 shared the patterns with "mock known" sample 22 and even though they were both from Slovenia, they were not from the same stand. Likewise, "mock unknown" samples 31 and 32 were also from Slovenia, but it was difficult to match them with their origin.

4.4.1.2 Three genetic clusters

Figure 7 reports results for K=3. When using three genetic clusters, the most likely solution based on biology of beech and information on glacial refugia for beech, which in the proximity of the study area were located in nowadays Slovenia and Istria, Rodopi, Rila and Balkan Mountains and tentatively in the Carpathians and Bosnia (Magri *et al.,* 2006), the matching of origin of "mock" known and unknown samples is closer to the truth.

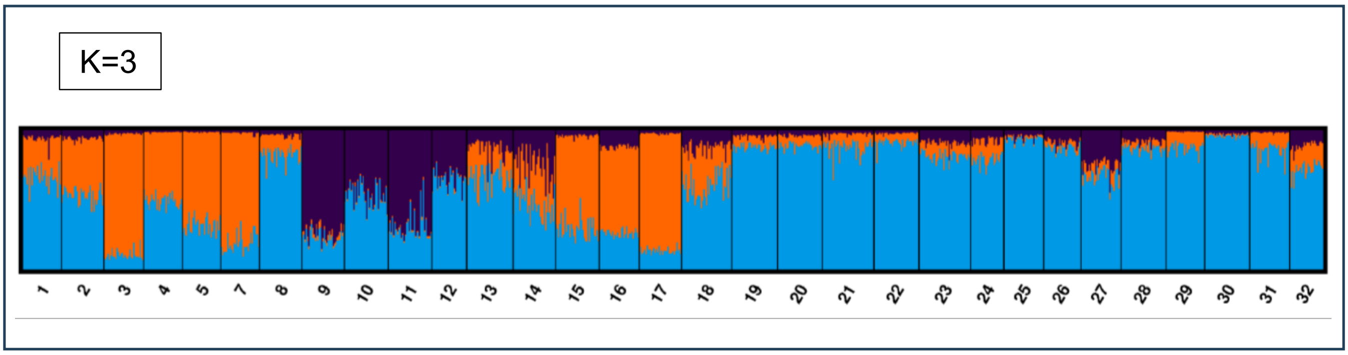

Figure 7: Results of the sample assessment for K=3

Upon trying to match "mock unknown" sample 28, it is observed that it shares pattern with sample 20, which is not a correct match. For sample 29, it exhibits patterns similar to sample 20 and when compared to the dataset, that is a correct match. Sample 30 can be matched correctly to 24 as they both have similar colour distribution in the histogram. However, the same cannot be said for sample 31, despite originating from the area of sample 24, sample 31 does not look similar to 24. Also, sample 32 shares similar patterns with sample 23, but it was found to be an incorrect match. From these observed, it can be interpreted that maybe, this is not the best method to correctly trace origin of FRM.

Further analysing the clusters, here, sample no. 17 (comes from Serbia) shows similar genetic structure as samples number 7, 15 and 16. When compared with Annex 1, it can be observed that the samples 7, 15, 16 are correctly matched to Serbia pointing to the set of markers in question and using three genetic clusters potentially being a useful tool in this situation.

4.5 SWOT analysis for the use of molecular markers

After a comprehensive study of existing literatures, the following SWOT analysis (Table 6) was conducted. This analysis was done to compare the strengths, weaknesses, opportunities, weaknesses, and threats that come together with the application of molecular markers. It has been a hot topic of discussion recently (Salgotra et al., 2020; Westergren et al., 2017), especially to identify the origin of FRM in terms of suspicion of mislabelling, however in this study it did not prove to be very accurate. Hence, this analysis has been conducted and it is presented:

Table 6: SWOT analysis regarding the use of molecular markers

Strengths	**Weaknesses**
- Helps to identify basic materials, individuals, populations and provenances. - Improves traceability by precise identification of origin. - Confirms genuineness of FRM, hence enables its import and export among countries. - Assesses genetic diversity which provides valuable information for conservation.	- Extremely expensive, requires special equipment and skilled personnel. - Time consuming - Success is not guaranteed, as it depends on the marker type (as seen in 4.4.1.1) and number of markers, availability of reference material and the quality of collected samples. - Incorrect choice of marker and incorrect interpretation of results may lead to inaccurate conclusions.
Opportunities	**Threats**
- Holds the capacity to identify and clone genes that may be economically important. - Can be applied to analyze quantitative traits such as growth rate, wood quality and other traits related to environmental adaptability. - Ongoing advancements in genetic technologies may lead to more cost-effective and efficient molecular marker techniques.	- Might be concerns over the application of molecular markers in countries with limited resources. - Potential risks to genetic data security and privacy. - Inconsistent or unclear regulations regarding the use of molecular markers to trace origin of FRM may create legal problems.

(*Source: Acquaah, 2009; Edwards and Batley, 2010; Finkeldey et al., 2010; Jw, 2011; Pandey and PANDEY, 2018; Qureshi et al., 2019)*

5. Discussion

Within this chapter, we will explore the theoretical implications of the results, offering plausible explanations for the observed findings.

5.1 Forest reproductive material and climate change

The positive association between origin and genetic quality of FRM not only influences the survival, growth, productivity, and adaptive capacity of tree populations but also holds significance for broader ecosystem functioning and resilience (Thomas *et al.,* 2017). This dual impact gains increasing importance in the face of climate change, as emphasized by studies conducted by Bozzano *et al.* (2014); Havens *et al.* (2015).

The importance of FRM has been well established in the previous chapters. Here, the implications of FRM on combatting climate change, based on other studies are briefly discussed.

i) Future projections hint that the Mediterranean southern and continental south-eastern parts of Europe are expected to experience significant rise in temperature decline in summer precipitation. This is projected to lead to a gradual decrease in productivity and, particularly in more vulnerable areas, a decline in fitness, along with an elevated susceptibility to pests and diseases. As populations approach their genetically determined tolerance thresholds for severe drought, higher mortality rates are expected, resulting in a subsequent reduction in adaptability. Consequently, the implementation of suitable FRM is deemed advisable and helps promote CSF (Kramer, 2016).

ii) Another fundamental reason to prioritize the selection of suitable FRM is the need for adaptation to local conditions. In the face of climate change, where shifts in temperature and precipitation patterns are occurring, the adaptive capacity of tree populations becomes crucial for long-term forest health. Trees that are well-matched to their environment exhibit higher rates of survival, growth, and successful reproduction. (White *et al.,* 2007).

iii) Selecting the most suitable forest reproductive material is essential for preserving biodiversity and maintaining the ecological functions of forests

(Freer-Smith *et al.,* 2019). Forests are composed of various tree species, each adapted to specific environmental conditions. The use of appropriate reproductive material ensures that the genetic diversity within a forest is maintained, enabling trees to adapt to changing environmental conditions, resist pests and diseases, and enhance overall ecosystem resilience (Young *et al.,* 1996).

iv) Trees grown from well-selected seeds or planting stock are more likely to exhibit desirable traits such as rapid growth, high timber quality, and resistance to pests and diseases (Bozzano *et al*., 2014). These traits contribute to increased wood production, improved timber quality, and reduced susceptibility to environmental stresses. As a result, the forestry sector benefits from higher yields, reduced management costs, and increased overall profitability (Ray *et al.,* 2022).

The outcome of the choice of FRM is dependent upon what is accessible to forest managers, and this accessibility is determined by the origin of the seed used and the quantity of seed collected from mother trees (Kavaliauskas *et al.,* 2018).

5.2 Challenges associated with certification schemes for forest reproductive materials

In this section, the challenges that comes along the adoption of Council Directive 1999/105/EC and OECD Forest Seed and Plant Scheme are briefly discussed.

5.2.1 Challenges:

The most notable challenges presented by the certification schemes are:

i) Restricted use of non-native FRM

Certification policies have a downside in the fact that they set constraints on the selection of genetic material and tree species, particularly restricting the use of non-native FRM. The implementation of the EU directives and OECD guidelines has led to the adoption of local seed sources of native trees species for forestry practices, guided by the principle that "local is the best" (MCPFE, 1993; Gömöry *et al.,* 2021; Dimitrova *et al*., 2022). This principle disregards the potential advantages of genetic diversity and the use of non-native species as a measure of climate change adaptation. Since climate change is expected to occur more rapidly than the natural ability of tree species to adapt and migrate (Savolainen *et al.,* 2007; Aitken *et al.,* 2008), there is a risk that the connection between site-specific climate conditions and local adaptations may be disrupted (Hamann and Aitken, 2019; Polechová and Barton, 2015).

ii) Lack of provision knowledge dissipation among end users

The Council Directive mandates member states to compile a list of national basic material, providing brief descriptions of seed stands and seed orchards, including information on the number and origin of genotypes. However, it undermines the fact that forest owners typically acquire FRM rather than basic material. Many foresters may not be fully aware that a particular basic material can yield FRM with varying genetic quality. There lies a serious gap of knowledge transfer to the forest managers and owners about the positive side of genetic diversity (Laakkonen *et al.,* 2018).

iii) No criteria delineation for high-quality seedlings

Although the importance of seedling quality is widely acknowledged, there are disagreements on what defines a high-quality seedling (Ivetić *et al*., 2016). Neither the Council Directive nor the OECD scheme has clearly defined methods, laws or protocols in seedling production explicitly aimed at improving their quality. This is further backed by Hazarika *et al.* (2021) which highlights uncertainties in the awareness of existing national and European-level policies and their potential impact on the use of improved FRM.

iv) Limited use of the available guidelines

The current standards are not very useful and seriously require redefinition by incorporating the latest research and updated reviews of published scientific literature (Grossnickle and El-Kassaby, 2016; Andivia *et al.,* 2021). For instance, in numerous countries, regulations specify that seedlings with a significant imbalance in size, where the shoot is disproportionately larger than the root system, should not be utilized. However, both Council Directives and OECD Scheme failed to delineate criteria for defining such imbalances, accounting for species differences, and measuring them. As a result, these end-users might interpret this standard subjectively which means by relying on their individual background or personal opinion. Diseases and pests can have devastating effects on forest ecosystems and genetic resources, and the risk of introducing or spreading pathogens through FRM is a concern, but it is not talked about enough (Mataruga *et al.,* 2019).

v) Lacks provision to determine areas for the use of specific FRM

The current state of knowledge on matters related to FRM still contains gaps and uncertainties. Key areas of concern include the determination of which specific FRM is most suitable for a given site and understanding the adaptive potential of forest tree populations (Alia *et al.,* 2021). There is still a lack of improved communication between policymakers, the private sector, and practitioners concerning issues related to this issue. The formulation of policies related to FGR is hindered by inadequate support from policymakers. However, the ongoing research initiatives are actively working to address these gaps, aiming to provide valuable insights and scientific evidence (GenTree, 2020).

Moreover, recommendations are also proposed in order to fill those gaps.

5.2.2 Recommendations:

In order to address those challenges mentioned in the previous section, these recommendations have been proposed:

i) Establish new guidelines focused on forest adaptation to climate change

There should be a discussion to add or revise guidelines focusing on better adaptation of forests to climate change. Since the present state of standards pose obstacles to adaptation management (Andivia *et al.,* 2021).

ii) Recognition of the need of non-native species

Trees do not recognize borders is a point that should be integrated into current guidelines for the transfer of FRM. There is a need of increasing awareness regarding the potential benefits associated with adopting non-native FRM than solely relying on natural regeneration in response to climate change (Gömöry *et al.,* 2021).

iii) Prioritize phytosanitary regulations

Efficient phytosanitary regulations are necessary to prevent the introduction and transmission of forest pests and pathogens through transportation and trade. It is vital for international phytosanitary regulations related to transport, travel, and trade to align with each other, aiming to minimize or eradicate the risk of spreading severe pests and diseases (Eschen *et al.,* 2015). Additionally, there is a crucial need to enhance awareness among professionals, forest owners, and policymakers in various countries regarding the importance of adhering to phytosanitary measures (GenTree, 2020).

iv) Delineation of breeding zones

It's important to define EU-wide breeding zones and provide recommendations for transferring Forest Reproductive Material (FRM). Considering the predicted climate change, there is a crucial need to prioritize studying the adaptability of forest tree species, especially those at their drier and lower limits (Mátyás, 2007).

v) Establish communication regarding choice of suitable FRM

It is crucial for authorities to engage in effective communication with end users, aiming to enhance their ability to choose suitable FRM. This means to consider where it comes from, following provenance recommendations. Also, there is an additional need to increase the understanding of assisted gene flow and assisted migration (Alia *et al.,* 2021).

5.3 Certification and traceability of forest reproductive material

The existing framework for FRM traceability involves generating an audit trail that spans from the collection to the end user in trade. This process relies on compiling several documents for each commercial harvest of FRM. After that, a main/master certificate is provided (Figure 2). This certificate travels with the FRM material throughout the trade chain until it reaches the final end-user. The legal control regulations mandate consistent monitoring of the master certificate. However, this process is resource-intensive in terms of both staffing and costs. Relying solely on plausibility controls through the monitoring of such documents is considered not secure enough (Gömöry *et al.,* 2021).

Occasionally, there have been reports of deliberate mislabelling and false declarations of the origin of FRM, most likely for illicit gains. Such cases are getting more common along the years (Finkeldey *et al.,* 2010; Westergren *et al.,* 2017). A fine example could be taken from France where the use of geographical markers to trace the geographical origin of wood was exemplified in the cooperage industry. Chloroplast DNA extracted from oak (Quercus spp.) wood was utilized to identify false declarations about the origin of wood used in making winery barrels in France (Deguilloux *et al*., 2004; Finkeldey *et al*., 2010).

Another interesting instance of mislabelled origin of seeds can be taken from Slovenia where in 2016, SFI had suspicions over one Slovenian seed facility. The suspicion was based on inconsistent statements that the seed provider had submitted seeds that were not collected from the place as declared in the documentation. To verify the suspicion, molecular markers were used to trace the origin of seeds by comparing to samples stored in Slovenian Forest Gene Bank. Upon analyzing it was indeed concluded that the seeds were not collected in the declared stand. More so, those seeds were likely to have come from outside Slovenia (Westergren *et al.,* 2017). In this case study as well, the application of molecular methods to identify the FRM origin proved to be crucial that prevented the introduction of inappropriate FRM to Slovenian forests.

Keeping these false declarations in mind, let's take a hypothetical example of Serbia and Bosnia and Herzegovina. Serbia is an OECD country while Bosnia and Herzegovina is not, so it is considered as a third country. A Serbian private nursery owner with just plainly financial prosperity in mind, can illegally buy seeds in Bosnia and Herzegovina at a much cheaper price. They then can label those illegal seeds as originating from approved Serbian provenances and sell them to EU and OECD member countries. If only paper trail is to be trusted, some countries may really buy those seeds and use them at sites which are not compatible to their growth. Some of those seeds may bore good results for the first few years but along the years, trees planted in unpreferred sites may decline. This particular action will result in huge economic losses. That's a huge loss of time, effort and money which can be avoiding by the application of molecular markers to verify the origin of FRM before undergoing major plantation programs.

These situations emphasize the need for additional control measures, going beyond the existing national and international regulations. These situations emphasize the need for additional control measures, going beyond the existing national and international regulations. Stakeholders from various European countries, including representatives from forest owners, private enterprises, and inter-trade organizations, along with members of research institutes and universities, have explicitly expressed the necessity for advancing marker-based verification of FRM origin (Gömöry *et al.,* 2021).

5.4 Credibility of use of molecular markers

From the obtained results (see 4.4.2 and 4.4.3), it was observed that a lot of stands exhibited similar colours and patterns, thus it highlights that it might be extremely difficult for an interpreter to correctly match the samples to its origin. While we were able to match some samples correctly to their origin, it was difficult to match some samples. If an interpreter without the prior knowledge of the source of origin of the samples, there is a possibility of mistakes while trying to trace the origin. So, in this study, the use of molecular markers (microsatellites) did not prove to be a suitable method to identify the origin of FRM. This result contradicts Westergren *et al.* (2017), where molecular markers were proved to be suitable for tracing of FRM origin. Hence, maybe the markers can be an appropriate tool, however the choice of the most suitable marker specific research approach undertaken and the extent of resolution and polymorphism necessary for the particular study (Amom and Nongdam, 2017).

Markers are sometimes useful if they have correct discriminating power but not in other cases. However, with technological advances, there are more markers and more potential for their usefulness (Hasanaj *et al.,* 2022). Also, there are tree species that are more differentiated between different countries/regions, populations and it is easier to use markers for those species than beech (Finkeldey *et al.,* 2010). Hence, this study states that molecular markers are still undergoing technical advances, maybe there will be a time where there will be no mistake while assigning the sample of FRM to its origin. When so happens, then maybe it can be argued that identifying the origin of FRM by using molecular markers should be made compulsory in the future, but not at the present.

5.5 Future use of molecular markers to trace origin

There have been newer developments with significant applications in forensics, particularly in the realm of identifying timber species and tracing the origin of wood and wood products using DNA markers. Currently, molecular genetic tools allow for the accurate detection of false claims about the source of illegally harvested timber, although this capability is limited to a small subset of relevant species and issues

(Finkeldey *et al.,* 2010). Still, additional research is required to adapt existing molecular methods.

With regard to the targeted countries by this study, they are also familiar with the topic of using molecular markers. It has been found that not every country under study has performed research to trace the origin of FRM by using molecular methods. Only Slovenia has a mandatory system in place regarding verifying the origin of FRM as in cases of doubt as part of national regulations. This requires that a sample is to be submitted to SFI for every tree from which seeds have been collected. This task is carried out by the on-the-field control personnel. The sample can include a specific quantity of seeds/cones or dormant buds per tree and undergoes immediate freeze-drying, or DNA extraction is performed for storage in a DNA library (Gömöry *et al.,* 2021). And on accounts of suspicion, suspected samples are verified using molecular markers to check their credibility.

In Croatia, there has been research conducted with the use of microsatellites to analyze genetic diversity and genetic differentiation within orchards (Kajba and Andrić, 2015; Grdiša *et al.,* 2021). In a similar manner, research have also been conducted in Bosnia and Herzegovina and Serbia with the use of molecular markers (microsatellites in particular) to analyze genetic diversity and genetic characterization for few species (Mataruga *et al.,* 2019; Arsenijevic *et al*., 2021; Ivanović *et al*., 2021). This hints that there is good potential in Bosnia and Herzegovina, Croatia and Slovenia to infer the use of molecular markers to identify the origin of FRM when required.

Furthermore, there have been instances of voluntary systems where owners are willing to pay a premium for seedlings, ensuring both their origin and quality. That system relies on a neutral individual collecting samples of trees, seeds, and seedlings at different stages, spanning from seed facility approval to the sale/planting in the forest. Molecular and biochemical markers are then used to compare these samples (Westergren *et al.,* 2017). This marks as a beginning of verification of FRM origin being a compulsion in other countries, just like in Slovenia.

Moving forward there is a need for more and improved markers to obtain more reliable results, which includes advancements in technology for quicker outcomes (Salgotra *et al.,* 2020). However, the biggest challenge lies in the associated costs. The implementation of such schemes could only be made obligatory if there is substantial support from the official control body in each country. It is also essential to educate end-users about the risks associated with using inappropriate FRM and inform them about the possibilities for certification and tracking (Gömöry *et al.*, 2021).

5.6 Limitations of the study:

There were few challenges that came along the way while conducting this research. The first and foremost limitation was the availability of the country specific laws and regulations mostly in their own official language only. Some were available in English, while some had to be translated hence, they might not have been an accurate translation of the information as depicted in the regulations. This may have led to potential misinterpretations of the certification standards. There was absence of updated data and only limited data was available, that made it difficult to make direct comparisons among the four countries. While secondary data analysis offers an efficient means of exploring datasets, interviews with experts could have offered more valuable insights and profound understanding of the subject. Moreover, the dataset provided by SFI may be subject to human or instrumental error.

6. Conclusion

"*All the flowers of all the tomorrows are in the seeds of today*" is an old, famous Chinese proverb and after a thorough study on the topic of FRM, this research profoundly stands by that statement. The certification of FRM plays a pivotal role in ensuring the sustainability, biodiversity, and resilience of forest ecosystems. In Europe, Council Directive 1999/105/EC and OECD Forest Seed and Plant Scheme are the two certification schemes that develops guidelines and standards regarding production, use and transfer of FRM.

Bosnia and Herzegovina, Croatia, Slovenia and Serbia are neighbouring South-Eastern European countries that were represented in this study. Croatia, Slovenia and Serbia have their guidelines regarding certification of FRM, based on the EU Directive 1999/105/EC. This makes it easier for the transfer of FRM between these countries, whereas it takes special permission from concerned authority to manage the transfer of FRM from/to Bosnia and Herzegovina.

As explored throughout this study, the strategic selection and improved choice of FRM, guided by certification frameworks, are instrumental to enhancing the adaptive capacity of forests. When a tree is planted in the environment, a long-term production cycle is initiated, with a span of 50 to 100 years until the tree reaches harvest maturity. It is crucial that the planted tree aligns precisely with the intended specifications. So, having a good certification scheme provides authenticity regarding the whole production cycle right from collecting the seed to planting the tree in the forest. However, the prevalent certification guidelines (Council Directives and OECD Scheme) are not enough to deal with the challenges related to FRM faced by the end users. Hence, it is now time to revise them.

Globally more forest and more trees are being planted. So, the demand for having access to the certified planting material throughout the globe is going to get higher. In specific scenarios, natural regeneration alone may not be satisfactory to ensure forest adaptation, necessitating the movement of forest reproductive material guided by scientific principles. When required, the implementation of artificial regeneration should not be limited by normative frameworks.

The case study presented in this research depicted that molecular markers are not always accurate to trace the origin of FRM. It may be due to the influence of the chosen marker. From that perspective, it is reported that the use of molecular markers is not necessarily the perfect tool for identity verification. Nevertheless, the importance of identifying the origin of FRM cannot be overstated in the context of conservation and sustainable forestry.

Generally, molecular markers provide a powerful tool for confirming the authenticity of seed sources and ensuring the preservation of genetic diversity. This level of precision is particularly crucial in the face of global challenges such as climate change, where the adaptability and resilience of forest ecosystems depend on the careful selection and deployment of suitable reproductive materials. Hence, there is a dire need of technological advancements in that field as its application has the potential to enhance the ability to monitor and manage reforestation and afforestation efforts. By precisely identifying the geographic origin of reproductive materials, foresters can make informed decisions to promote the resilience of ecosystems, mitigate the impact of pests and diseases, and foster the long-term health of forested landscapes.

As new challenges arise, such as new pests, diseases, or unforeseen environmental changes, the versatility of molecular markers provides a dynamic tool for adaptation. However, it takes extensive hours to use molecular methods and is very expensive, that prevents countries with less resources from reaping its benefits. Nevertheless, with the ongoing technological advancements, there may be a time when it will be easier to gain access for using these molecular markers.

In a nutshell, the certification process of FRM serves as a gatekeeper, ensuring that FRM meet stringent standards for authenticity, quality, and adaptability. Incorporating molecular markers into this certification framework elevates its efficacy, offering an unprecedented level of precision in verifying the origin of these materials.

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Annex I

S.N.	Site code	Country	Number of samples	Latitude	Longitude
1	P24	Croatia	30	45,28333	18,01667
2	P25	Croatia	33	45,61667	17,31667
3	P30	Bosnia	31	44,38333	18,05
4	P32	Bosnia	30	44,55	44,98333
5	P33	Bosnia	30	44,76667	16,26667
6	P36	Serbia	30	45,16667	19,91667
7	P38	Serbia	30	43,16667	20,83333
8	P42	Hungary	33	46,5	16,75
9	P47	Germany	33	48	10
10	P48	Germany	34	49,01667	13,23333
11	P49	Germany	34	53,13333	8,433333
12	P56	Austria	27	47,9	13,96667
13	P61	Bosnia	36	44,75	16,23333
14	P63	Romania	33	47,18333	22,25
15	P66	Serbia	34	44,38333	20,75
16	P67	Serbia	31	44	19,75
17	P69	Serbia	33	44,2	19,83333
18	Abi	Slovenia	39	45,44	13,83
19	KH	Slovenia	35	45,79	15,07
20	OS-S	Slovenia	35	46,448	15,42
21	POK	Slovenia	40	45,76	15,31

22	RR-S	Slovenia	35	45,65	15,03
23	TUR	Slovenia	40	46,13	15,16
24	STD	Slovenia	26	45,77537	14,94903
25	KOra	Slovenia	31	45,833	15,417
26	STra	Slovenia	29	45,8	15,37
27	KAra	Slovenia	31	46,21	14,62
28	RajhMla	Unknown	35	-	-
29	OsanMla	Unknown	35	-	-
30	PriStudMla	Unknown	26	-	-
31	PrtStudSeme	Unknown	31	-	-
32	OsanSeme	Unknown	30	-	-