

# CONSERVATION OF GENETIC RESOURCES OF FIELD ELM (*Ulmus minor* Mill.) AND EUROPEAN WHITE ELM (*Ulmus laevis* Pall.)

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**Abstract:** *It is considered that the main cause of erosion of field elm (*Ulmus minor* Mill.) genetic diversity is the Dutch elm disease, and in case of European white elm (*Ulmus laevis* Pall.) it is the destruction of natural habitats. Aiming at the conservation of genetic resources of the above species, one population of each species was selected for sampling. The selected trees were located by GPS (Global Position System) and the data were entered in GIS (Geographic Information Systems) database. The position of individual trees was presented on a topographic map, and in case of European white elm also on aerial photograph of the region. The plant material (branchlets with buds) was taken from eight field elms and from ten white elms for clone production through in vitro vegetative propagation. The produced clones of two genotypes of field elm and eight genotypes of white elm were planted in beds in the nursery of the Institute of Lowland Forestry and Environment, and during the next year, they will be used for the establishment of field gene banks on the Institute's experimental estate at Petrovaradin. In this way, the process of ex situ conservation of genetic resources of the above elm species begins.*

A mezei szil (*Ulmus minor* Mill.) esetében a szilfavész nevű betegséget, míg a vénicszil (*Ulmus laevis* Pall.) esetében a pusztulásuk fő okának a termőhelyek tönkretételét tekintik. A fent említett fajok genetikai megőrzését kitűzve célként, kijelöltünk mindkét fajhoz egy-egy mintaterületet. A kiválasztott fák helyét GPS-szel (Global Position System) jelöltük meg, aminek az adatait GIS (Geographic Information System) adatbázisba vittük be. A mintafák helyzetét térképen ábrázoltuk, és a vénic szil esetében még légifényképen is ábrázoltuk. Nyolc mezei szilről és tíz vénic szilről gyűjtöttünk növényi szaporítóanyagot az *in vitro* szaporításhoz. Két mezei szil klónt és nyolc vénic szil klónt sikerült előállítani, amelyek csemetéit az Alföldi és Környezetvédelmi Intézet csemetekrti ágyásaiba ültettünk ki. A következő év folyamán ezekből gén bankot létesítünk az intézet péterváradai kísérleti telepén. Ezzel kezdetét vette ezen fafajok *ex situ* génmegőrzése.

**Key words:** *Ulmus*, *ex situ* conservation, *in vitro*, GIS

## INTRODUCTION

Elms (*Ulmus* spp.) are greatly valued across Northern Hemisphere for their landscape, amenity and timber quality. However, the past century brought the erosion of genetic diversity and the disappearance of elms from natural populations and urban environments, predominantly due to Dutch elm disease (Collin, 2002). The disease is caused by two related species of Ascomycetes: *Ophiostoma ulmi* (Buisman) Nannf. (syn.: *Ceratocystis ulmi* (Buisman) Moreau) and *Ophiostoma novo-ulmi* Brasier (Brasier, 1990), and it is transmitted by elm bark beetles (*Scolytus* sp.) as the vectors or from the diseased to the healthy trees by ingrown roots (Peace, 1962). Dutch elm disease is considered as the main cause of reduction of field elm genetic diversity, which is especially susceptible to the disease. European white elm is less endangered by Dutch elm disease and the main cause of reduction of its genetic resources is thought to be the destruction of its natural habitats (Collin, 2002).

Many international projects have been initiated to conserve the genetic resources of elms. Efficient conservation can be realised by the application of *in situ* or *ex situ* methods of conservation of genetic resources, as well as by their combination (IPGRI, 2000). The usual method of *ex situ* conservation of forest genetic resources is the establishment of field gene banks, by planting the clones obtained by cuttings or grafting (IPGRI, 2000; Collin, 2002). However, it has been shown that some elm species are amenable to tissue culture and that is possible to produce numerous clones in relatively short time (Fenning et al., 1996; Gartland et al., 2000).

Also, modern computer methods (such as GIS) and GPS have been increasingly applied in the conservation of genetic resources and in the assessment of the genetic diversity distribution. The application of the above methods and techniques can show the distribution of a species in a selected region, and based on the correlation between biophysical properties of the sites and edaphic, climatic and other demands of the species, it is possible to monitor the changes and predict the distribution of the species in a selected period (IPGRI, 2000).

The aim of our research is *ex situ* conservation of genetic resources of two elm species (field elm and European white elm) by the establishment of field gene banks. The selected trees were located by GPS and presented on a topographic map of the region. Their clones obtained by *in vitro* vegetative propagation (micropropagation and organogenesis) will be planted in low hedges on the

experimental estate of the Institute of Lowland Forestry and Environment at Petrovaradin. The justification of the application of *ex situ* method of conservation of genetic resources of the above populations will be discussed.

## **MATERIAL AND METHODS**

A natural population of field elm is situated in Forest Estate (F.E.) Sremska Mitrovica, Forest Directorate (F.D.) Morović, Management Unit (M.U.) Vinična-Žeravinac-Puk. This population is the only group of field elm trees on the territory of the entire Management Unit (total area 3,552.8 ha), in addition to some individual, randomly distributed trees. The population consists of 11 trees distributed on the area of 0.08 ha. It is thought that the trees are cca 115 years old. The trees were previously marked, their height and diameter at breast height were measured. The coordinates of individual trees were positioned by GPS and entered in GIS program. The exact position of trees was presented on a topographic map of the region.

A natural population of European white elm is situated in F.E. Novi Sad, F.D. Bačka Palanka, M.U. Palanačke Ade-Čipski Poloj. This M.U. occupies the area of 1,283.03 ha, and the white elm population is situated on a small river island (no name for the time being), area 5.84 ha. Nine white elm plus trees were left after the felling during 2000. The tenth solitary white elm tree grows on the other river island (no name) 2,712 m far, and tree groups and solitary, randomly distributed white elm trees grow on the territory of the above M.U. The trees are cca 40 years old and distributed on the area of 1.86 ha. All trees were marked, their height and diameter at breast height were measured. The coordinates of individual trees were positioned by GPS and entered in GIS program. The exact position of trees was presented on a topographic map and on aerial photograph of the region.

Plant material (branchlets with buds) was taken from 8 field elm trees (the material could not be taken from 3 trees because it was impossible to reach the crowns), as well as from all 10 European white elm trees. The material was collected in March 2003, after which the buds were introduced into tissue culture for *in vitro* vegetative propagation. The optimal protocols for micropropagation and organogenesis of the selected species were applied. *In vitro* methods will not be discussed.

Ten clones for two genotypes of field elm and 30 clones for 8 genotypes of European white elm were planted in beds, spacing 30x30 cm, in the nursery of the Institute of Lowland Forestry and Environment during the summer 2004.

The plants were shaded during the first 30 days, watered when necessary and treated with insecticides. Next year, the clones will be used for the establishment of field gene banks on the Institute's experimental estate at Petrovaradin. Planting will be in the form of low hedges, with spacing between rows 70 cm and spacing between plants in rows 30 cm.

## **RESULTS AND DISCUSSION**

### **Sampling from natural elm populations**

The main problem in conservation of forest genetic resources is the sampling from natural populations in the aim of conservation of the species evolution potentials, which is extremely difficult in the absence of knowledge on distribution and patterns of genetic variability (Eriksson, 1998). According to Eriksson (1995, after Collin, 2002), the alleles which occur with intermediary frequencies (10-90%) are the most interesting ones from the evolution aspect and the first ten randomly selected individuals from one natural population contain a sufficient number of alleles for the conservation of the evolution potentials of the species. The general guidelines for sampling in natural populations for the application of *in situ* and *ex situ* methods of genetic resources conservation of common Noble Hardwoods were reported by Jensen et al. (1999, after Collin, 2002). The additional procedures which should be applied in the conservation of genetic resources of elms were given by Collin (2002).

The sampling in natural populations in the aim of conservation of the greatest possible adaptive value in field elm is extremely difficult. According to Armstrong and Sell (1996), Collin (2002) and other authors, field elm has a great diversity of forms and a complex distribution of genetic variability, which is the consequence of the anthropogenic cultivation during the past 2000 years, the introgression of *Ulmus glabra* Huds. and the coexistence of sexual and asexual reproduction. Also, field elm is extremely susceptible to Dutch elm disease, which led to the destruction of susceptible species throughout the North Hemisphere in two epidemics during the last century (Brasier, 1990). The presence of Dutch elm disease additionally complicates the sampling for the conservation of genetic resources (Collin, 2002).

European white elm is also susceptible to Dutch elm disease, but as it is less attractive to elm bark beetles, which are the vectors of the fungal pathogen, it is not thought to be greatly endangered by the disease. The main cause of reduction of European white elm genetic resources is the destruction of natural

habitats (Collin, 2002). Namely, European white elm inhabits the flooded forests along large rivers which were cleared and substituted by poplar monoplantations, or the land was converted to agricultural land use by the construction of embankments (Tomić, 1992). In this way, natural populations of European white elm were reduced to small size fragmented populations, consisting of not more than 20 adult trees distributed in small areas of several hectares each (Collin, 2002).

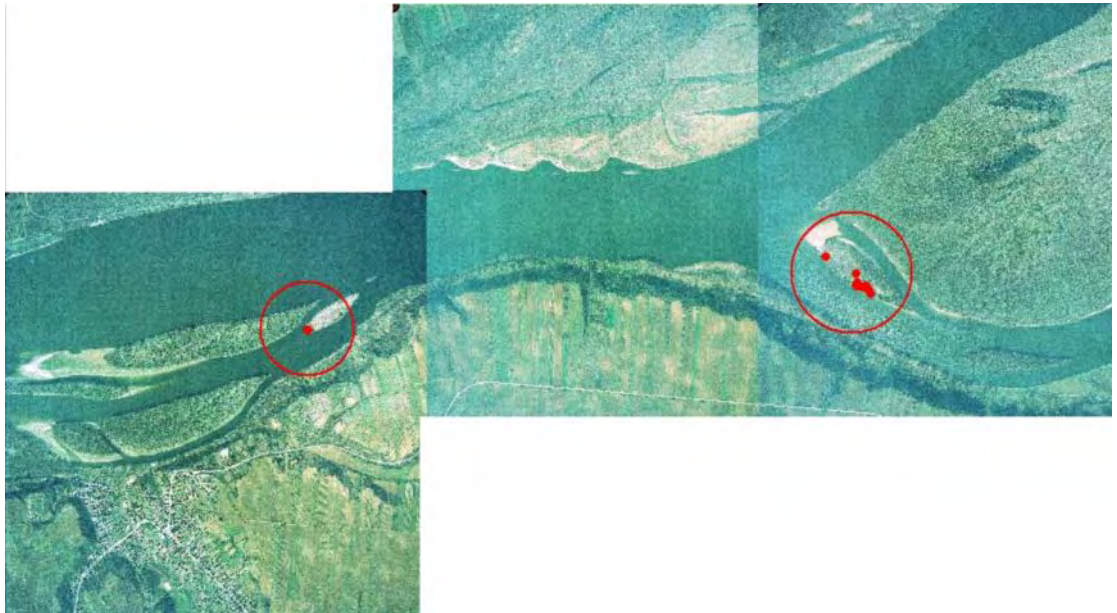
### **Application of *ex situ* method of conservation of elm genetic resources**

Taking into account the specific characteristics and the different causes of reduction of genetic variability of the above elm species, according to Collin (2002), the adequate method of conservation of genetic resources in case of field elm is *ex situ*, and in case of European white elm, *in situ* conservation of genetic resources. Also, *ex situ* method of conservation is recommended for both elm species in case when the populations are too small for the application of *in situ* method and in case of endangered, marginal populations.

In agreement with Collin's study (2002), it is considered that the application of *in situ* method of conservation in the field elm population in M.U. Morović is not an adequate method of conservation. Namely, the population consists of only 11 genotypes. Such a small population does not have a sufficient genetic variability and there is a risk of genetic drift and inbreeding. Also, as it was already noted, the number of individuals for sampling in natural populations should be minimum 10, and for field elm the minimal recommended distance between individual trees is 50 m because of the possibility of the occurrence of clonal patches which do not have genetic variability, as the consequence of an intensive coppicing capacity of field elm (Collin, 2002). In the study population such sampling was not possible, because the trees were spaced between 2 and 30 m and all trees were distributed in the area of only 0.08 ha. On the other hand, thanks to intensive field elm coppicing vigour and narrow spacing between the trees, it is possible that all trees present only one or several genotypes. However, as the trees are cca 115 years old and as they survived both epidemics of Dutch elm disease, they are considered as resistant or tolerant to the disease. According to Collin (2002), the genotypes with some type of resistance or tolerance to Dutch elm disease should be conserved. Therefore, it would be beneficial to conserve all trees, i.e. the entire genotypes, by the application of *ex situ* method of conservation, and by subsequent analyses to identify if it is one or more genotypes. However, it would be useless if the trees survived the Dutch elm disease epidemics by chance (Collin, 2002). During the collection of branchlets with buds for the *in vitro* vegetative propagation, on cross sections of all 8

genotypes of field elm, there were characteristic dark rings which indicate the presence of Dutch elm disease. It was concluded that the trees were not resistant to the disease and that they survived both epidemics accidentally because they were not perceived by the vectors of fungal pathogen or because they were not attractive, due to unfavourable smell, bark taste, etc. (Pajares, 2003; Webber, 2003; Piou et al., 2003; Martin et al., 2003). However, by monitoring during 4 years it was observed that the trees were still vital. Therefore, it was presumed that the genotypes possessed the properties (such as the small vessel diameter) which enabled a certain degree of tolerance and a moderate infection (McNabb et al., 1970, after Gartland et al., 2001).

In agreement with Collin's study (2002), although European white elm is recommended for *in situ* method of genetic conservation, it is considered that the adequate method for European white elm population in M.U. Palanačka Ada-Čipski Poloj is *ex situ* conservation, because the population is endangered. Namely, after felling during 2000, the population was reduced to 9 trees distributed on the area of 1.86 ha. The presence of fragmented populations of European white elm along the banks of large rivers, which are small size and distributed on the areas of several hectares is, according to Collin (2002), a common occurrence resulting from the conversion of natural European white elm habitats into other forms of land use. Small white elm populations can be at risk of genetic drift and inbreeding (Collin, 2002). However, as very often such populations are not very far (1-10 km) from other populations and solitary trees of the same species in the region, it is thought that the effect of genetic drift and inbreeding in European white elm natural populations can be counterbalanced if there is a gene flow (pollen and seed dispersion) between the populations (Collin, 2002). This means that the application of *in situ* method of conservation is in this case justified (Collin, 2002). However, it was presumed that white elm trees in the above population would not survive the changed environmental conditions after felling of the remaining part of the population (direct sun, wind, storms, etc.), because it is known that European white elm is a moderate shade bearer which do well under forest cover (Collin, 2001). This was confirmed in the field, a year after taking the samples for *in vitro* vegetative propagation, when it was recorded that one tree died (tree number 4), most probably because of the weather (thunderstorm), and that the other tree (tree number 10) situated on the very bank of the river island, was endangered by water erosion which undermines the roots. Consequently, the application of *ex situ* method of conservation of the above European white elm population is justified, as an emergency measure for the conservation of genetic resources of the endangered population. The European white elm trees did not show the symptoms of presence of Dutch elm disease.



**Figure 1: Position of individual trees of European white elm on an aerial photograph of the region, scale 1:15.000.**

Average height of field elm trees is  $33.45 \pm 1.92\text{m}$ , and European white elm  $25.10 \pm 1.76\text{m}$ . The average diameter at breast height of field elms is  $40.64 \pm 4.71\text{ cm}$ , European white elms  $62.70 \pm 13.51\text{ cm}$ . All trees were located by GPS and the coordinates were entered in GIS program and transferred into coordinates in LAT/LONG system. The position of individual field elm trees is presented on a topographic map of the region. European white elm is presented on an aerial photograph of the region (Fig. 1). The spacing between individual trees within each population were calculated (the data were not presented), the distance of the solitary European white elm tree (tree number 10) from the study population (2,712 m), as well as the areas occupied by the study populations (0.08 ha - field elm population, 1.86 ha - European white elm population). It was shown that the application of modern methods (GPS and GIS) is suitable for the presentation of spatial distribution of genetic resources (position of individual trees, i.e. genotypes), as well as for the identification of the parameters significant in the conservation of genetic resources.

### ***In vitro* vegetative propagation of elms**

By the application of *in vitro* methods of vegetative propagation (micropropagation and organogenesis) a great number of clones of 2 field elm genotypes (trees 11 and 16) and of 8 European white elm genotypes (trees number 1, 2, 4, 6, 7, 8, 9 and 10) were produced during a time period of 8 months (Fig. 2a). Optimal protocols were applied for *in vitro* vegetative propagation of the study species (Aleksić, in print).



**Figure 2: a) Plantlets of European white elm obtained *in vitro*; b) Clones of European white elm in the nursery.**

### **Establishment of field gene banks of elms**

Ten clones for 2 genotypes of field elm and 30 clones for 8 genotypes of European white elm were planted in beds, spacing 30x30 cm, in the nursery of the Institute of Lowland Forestry and Environment during the summer 2004. The growth of the clones was rapid (Fig. 2b).

In the spring 2005, the clones will be planted on the Institute's experimental estate at Petrovaradin. Planting will be in the form of low hedges, because it was shown that thus planted trees were not attractive to the vectors of the fungal pathogen (Collin, 2002). This will prevent the possibility of the subsequent

infection by Dutch elm disease, and the material will be readily available for further research and improvement (IPRGI, 2000).

In this way, *ex situ* conservation of genetic resources of the study populations of field elm and European white elm, will be realised as the initial activity in the aim of conservation of genetic resources of the study elm species in Vojvodina.

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