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EX SITU CONSERVATION OF GENETIC RESOURCES OF FIELD ELM (*ULMUS MINOR* MILL.) AND EUROPEAN WHITE ELM (*ULMUS LAEVIS* PALL.)

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Principles of the conservation of genetic resources of elms (*Ulmus* spp.) do not differ fundamentally from the general principles accepted for the conservation of genetic resources of other common Noble Hardwoods. Efficient conservation can best be achieved through appropriate combination of in situ and ex situ methods, which have distinct advantages. Besides that, ex situ conservation is employed when emergency measures are needed for rare, endangered populations and when populations are too small to be managed in situ (e.g. risks of genetic drift and inbreeding). The aim of our research is ex situ conservation of genetic resources of field elm (*Ulmus minor* Mill.) and European white elm (*Ulmus laevis* Pall.) through establishment of field genebanks. Sampling was conducted in one population of field elm and one population of white elm. Plant material (buds) from 8 trees of field elm and 10 trees of white elm was used for in vitro production of clones. Obtained clones will be used for establishment of field genebanks on the experimental estate of the Institute of Lowland Forestry and Environment.

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

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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 (DED). 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). DED 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 DED 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. The usual method of *ex situ* conservation of forest genetic resources is the establishment of field genebanks, 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).

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 genebanks. 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.

MATERIALS 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.

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, 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 (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.

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. *In vitro* techniques will not be discussed.

Obtained clones 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 genebanks on the Institute's experimental estate. 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

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).

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 studied field elm population 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, be-

cause 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 DED, they are considered as resistant or tolerant to the disease. According to COLLIN (2002), the genotypes with some type of resistance or tolerance to DED should be conserved. Therefore, it would be beneficial to conserve all trees, i.e. the entire genotypes, through *ex situ* method of conservation. However, it would be useless if the trees survived the DED 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 DED. 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. (COLLIN, 2002). 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 the studied white elm population 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 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 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 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. 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 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 most probably because of the weather (thunderstorm), and that the other tree, 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 white elm

population is justified, as an emergency measure for the conservation of genetic resources of the endangered population. The white elm trees did not show the symptoms of presence of DED.

Optimal protocols for *in vitro* vegetative propagation (micropropagation and organogenesis) were applied for production of clones (ALEKSIĆ, in print). Numerous clones of 2 field elm genotypes and of 8 European white elm genotypes were produced during a time period of 8 months (Fig. 1a). Ten clones for 2 genotypes of field elm and 30 clones for 8 genotypes of European white elm were planted in beds in the nursery of the Institute of Lowland Forestry and Environment during the summer 2004. The growth of the clones was rapid (Fig. 1b).

Fig. 1. a) *Ulmus laevis* Pall. plantlets obtained via micropropagation; b) Clones of Ulmus laevis Pall. in the nursery.

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 DED, and the material will be readily available for further research and breeding.

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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EX SITU KONZERVACIJA GENETIČKIH RESURSA POLJSKOG BRESTA (*ULMUS MINOR* MILL.) I VEZA (*U. LAEVIS* PALL.)

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Izvod

Osnovni principi očuvanja genetičkih resursa brestova (*Ulmus* spp.) se ne razlikuju značajno od principa koji su prihvaćeni i koji se primenjuju u očuvanju genetičkih resursa ostalih tvrdih lišćara. Efikasna konzervacija genetičkih resursa se može ostvariti adekvatnim kombinovanjem *in situ* i *ex situ* metoda konzervacije, koji poseduju različite prednosti. Pored toga, *ex situ* metod konzervacije se primenjuje i u slučajevima kada je potrebno preduzeti odgovarajuće mere radi očuvanja ugroženih populacija ili kada su populacije suviše male da bi mogle biti očuvane *in situ* (odnosno, kada postoji mogućnost za pojavu genetičkog drifta i inbreedinga u populaciji). Cilj našeg istraživanja je *ex situ* konzervacija genetičkih resursa poljskog bresta (*Ulmus minor* Mill.) i veza (*U. laevis* Pall.) putem osnivanja poljskih banaka gena. Uzorkovanje je sprovedeno u po jednoj populaciji poljskog bresta i veza. Biljni materijal (pupoljci) sa 8 stabala poljskog bresta i 10 stabala veza je iskorišćen za vegetativnu *in vitro* propagaciju. Dobijeni klonovi će biti iskorišćeni za osnivanje poljskih banaka gena na oglednom dobru Instituta za nizijsko šumarstvo i životnu sredinu.

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