

**MODEL OF EXPERIMENTAL CLONAL SEED ORCHARD FOR THE  
PRODUCTION OF SERBIAN SPRUCE (*PICEA OMORIKA*  
/PANČ./PURKYNE) INTRASPECIFIC HYBRIDS**

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The presented model for the establishment of an experimental clonal seed orchard of Serbian spruce was designed based on the results of the analysis and assessment of the genetic potential of Serbian spruce seedling seed orchard at Godovik. Based on the results of the analyses, eight superior half-sib lines of Serbian spruce were selected, of which 24 genotypes were selected. Their hybridisation, by the model of incomplete diallel cross, resulted in 21 combinations at the level of half-sib lines, i.e. 48 combinations at the level of parent genotypes. The applied study methods identified the potentially valuable genotypes-cone producers i.e. pollinators, which will be fixed by cloning in the seed orchard of the second generation for the production of the promising hybrids.

*Key words:* Serbian spruce, selection, hybridisation, clonal seed orchard

INTRODUCTION

The quality and regularity of yield in clonal seed orchards is primarily conditioned by the genetic potential of the vegetative genotypes selected by replications and by ecological characteristics of the seed orchard locations. The selection of genotypes whose vegetative copies will be incorporated in the seed orchard

should be preceded by multiannual analyses of the degree of genetic determination and variation of their sexual functionality and the properties which shall be incorporated in the future generation, through the quality of yield (ISAJEV *et al.*, 1999). Starting from the above general criteria of the selection of potentially significant genotypes and from the characteristics of the seedling seed orchard of Serbian spruce at Godovik, by multiannual analyses, the genotypes were selected for the establishment of the clonal seed orchard. The seedling seed orchard of Serbian spruce at Godovik was established from 5956 genotypes from 50 half-sib lines, (TUCOVIĆ *et al.*, 1988). Based on the arrangement of genotypes - trees of the same line, in the separate, more or less isolated blocks, the three types of spontaneous reproduction were ensured for the first time: inbreeding, outbreeding and uniparental (ISAJEV *et al.*, 1989). The selection of genotypes to be cloned was based on multiannual analyses of numerous properties of flowering regularity and abundance, dynamics of tree growth and development, as well as of the yield quantity and quality. The controlled hybridisation and the control of the obtained hybrid seed determined the degree of heritability of properties in the relation parents - hybrids. In this way, the potentially valuable genotypes were identified, which should be fixed by cloning in the seed orchard of the second generation, for the production of the promising hybrids.

#### SITE AND METHOD

The superior lines, i.e. the genotypes within lines, were selected based on intra-line and inter-line variability of the incorporated half-sib lines, by multiannual analyses and the resulting study data on the dynamics of tree growth and development, flowering regularity and abundance, yield, as well as the seedling morphometric characters. Controlled hybridisation was performed by the model of incomplete diallel cross, including 21 combinations at the level of lines, i.e. 48 combinations among different parent genotypes. The analysis of cone morphometric characters of parent individuals and their hybrid combinations revealed the combinations with heterotic effect for all or for only some of the analysed cone characters. Starting from the significance of the application of iso-enzymatic method in the determination of the heterotic effect in the controlled hybridisation (MLADENOVIĆ-DRINIĆ *et al.*, 1997; 1998), based on salt-soluble seed proteins, the coefficients of similarity were calculated, i.e. the genetic structure of each hybrid combination was analysed. In this way were identified the bands - protein fractions inherited from mother; inherited from father; common for both parents; bands identified only in hybrids and bands identified only in parents, but absent in hybrid combinations.

#### RESULTS

Based on the study results, 12 hybrid combinations were selected, distinguished by high mean values of the analysed cone morphometric characters - marked by asterisk (Table 1). Table 1 also shows hybrid combinations with the

recorded heterotic effect – “X”, for one, two or all the three analysed characters. The analysis of parent individuals participating in these hybrid combinations displays that the superior combining ability is shown by mother genotypes: 1B1/1 and 1C2/4, 1C2/2 and 1D1/4, represented each in three i.e. two hybrid combinations. Superior combining ability of the selected fathers is shown by individuals: 1A1, 1F1/1 and 1F7/1, of which the tree 1A1 participates in five hybrid combinations as the pollinator. Based on the analyses in salt soluble proteins of Serbian spruce seeds, parent individuals and their hybrid combinations, it was concluded that hybrids inherit a greater number of bands - protein fractions from mother than from father genotypes.

*Table 1. Hybrid combinations characterised by the highest mean values of cone morphometric characters and heterotic effect*

Hybrid combinations	Mean value of cone length	Mean value of cone width	Mean value of seed number per cone	Recorded heterotic effect	Number of characters with a heterotic effect
1B1/1X1F7/1	*	*	*	X	1
1C2/2X1F7/2	*	*	*	X	1
1C2/2X1A1	*	*	*	X	2
1C2/4X1F1/1	*	*	*		
1B1/1X1A1	*	*	*	X	3
1B1/1X1F1/1	*	*	*		
1C2/4X1F1/4	*	*	*		
1D1/4X1F1/1	*	*	*		
1C2/4XA1	*	*	*	X	2
1C4/1X1A1	*	*	*	X	2
1D1/4X1A1	*	*	*		
1D1/1X1F7/1	*	*	*		

## PROPOSED MODEL

In the design of the model for the establishment of experimental clonal seed orchard for the production of intraspecific hybrids of Serbian spruce, starting from the long-term study results, mass selection, selection of plus trees (ISAJEV, 1987) and testing of the selected genotypes by intraspecific hybridisation, (TUČOVIĆ *et al.*, 1982; ŠIJAČIĆ-NIKOLIĆ, 2001), we decided on the cultivar concept. The cultivar concept includes the selection of parental pairs, aiming at the combining of their positive characters in the progeny, in order to produce hybrid seed in the specialised seed orchards (TUČOVIĆ *et al.*, 1993).

The results of the study of the obtained hybrid characters point to the fact that further improvement by hybridisation should be based on the crossing of carefully selected parent genotypes, with known alternative characters (TUČOVIĆ *et al.*, 1998). The new selection, with improved characters, can be synthesised only by

selecting and using a number of more superior seed trees - genotypes, or by controlled hybridisation. The mixture of the seed of the best half-sib lines, i.e. genotypes ensures the so-called synthetic selections with more or less genetic gain (TUČOVIĆ *et al.*, 1991). It is not sufficient to select the parents only based on the desirable characters. They should also have the good combining abilities in order to obtain more superior progenies than parent plants (TUČOVIĆ *et al.*, 1995). If the created changes are more concordant, the result is a more favourable development of the whole organism, which can result in the heterosis. A good combining ability is conditioned by a divergent genetic base of parents. However, each divergence does not produce favourable results, it must be at a definite level, i.e. within the definite limits (TUČOVIĆ and HERPKA, 1978).

The study results at the level of hybrid seed, based on which we select hybrid combinations distinguished by good cone and seed characteristics, as well as parent genotypes with good combining ability – »promising genotypes«, are the starting material for the establishment of an experimental clonal seed orchard for the production of seed of Serbian spruce intraspecific hybrids. As these parent individuals were subjected to multiple selection based on morphological characters, parameters of flowering, yield and sexuality at the level of half-sib lines, our further interest will be primarily focused on the control of the presented results and on the enhancement of seed and seedling production with more or less desirable characteristics.

The initial criteria in the design of the model of Serbian spruce experimental clonal seed orchard are as follows (ŠIJAČIĆ-NIKOLIĆ, 2001):

- selection of parent trees based on morphological criteria and physiological characteristics, such as sexuality, abundance of flowering and yield, pollen quality and resistance to extreme ecological factors;
- the model of incomplete diallel cross produced 48 different combinations of parent individuals, which enabled the assessment of the general combining ability of parent plants;
- based on the cone morphometric characters of parent individuals and hybrid cones, we determined the combinations with a heterotic effect for some of the analysed characters;
- the resulting data are important for the selection of the best hybrid combinations, i.e. the trees with the best general combining ability, and
- by the analysis of the salt-soluble proteins, it was concluded that hybrids inherit a greater number of bands - protein fractions from mother than from father genotypes.

The proposed model of clonal seed orchard will eliminate the observed functionality disadvantage of the previously established clonal seed orchards of various coniferous species (TUČOVIĆ *et al.*, 1982; ISAJEV *et al.*, 1993). By using the results of the analyses of the causes of non-functionality of the previous generations of clonal seed orchards, we create the preconditions of the successful Serbian spruce experimental clonal seed orchard of the second generation, for the production of intraspecific hybrids.

Depending on the size and form of the area selected for seed orchard establishment, it should be composed of at least six blocks with the spacing not less than 5 m, although the desired spacing between blocks should be 10 m, in order to prevent the unfavourable pollination. Each block should contain one mother clone and six pollinator clones, planted by the honeycomb system (Fig. 1). The main characteristic of the planting scheme is that the ramets of pollinator clones are planted in the vertexes of a regular hexagon, and one mother clone individual is planted in the centre of each hexagon, Table 2. This method ensures the pollination in which each mother individual has equal chances to be pollinated by the pollen of the individuals selected as pollinators. The number of repeated hexagons within each block will depend on the concrete conditions at the moment of seed orchard establishment. The spacing between plants should be 5 x 5 m.

*Table 2: Mother individuals and individual pollinator clones*

Individuals	Symbol on planting scheme
MOTHER CLONES	
1B1/1	A
1C2/2	B
1C2/4	C
1C4/1	D
1D1/1	E
1D1/4	F
POLLINATOR CLONES	
1F1/1	1
1F1/4	2
1F7/1	3
1F7/2	4

Serbian spruce experimental clonal seed orchard, organised in this way, will be significant for:

- testing the selected genotypes;
- selection of mother genotypes with the best combining ability;
- research of vegetative reproduction;
- fixing of plus trees in the clonal archives;
- assessment of interaction genotype x environment; and
- mass production of seed intraspecific hybrids.

The proposed »honeycomb« planting system has 5 m spacing between plants and the area of the hexagon is 75 square meters. Supposing that each block contains 10 hexagons, the area of a block is 750 square metres, i.e. the area of six blocks is 0.45 ha. Taking into account that the spacing between blocks is from 5 to 10 m, the area for the establishment of a Serbian spruce clonal seed orchard by this model should amount to 5 ha, if possible of the regular form.

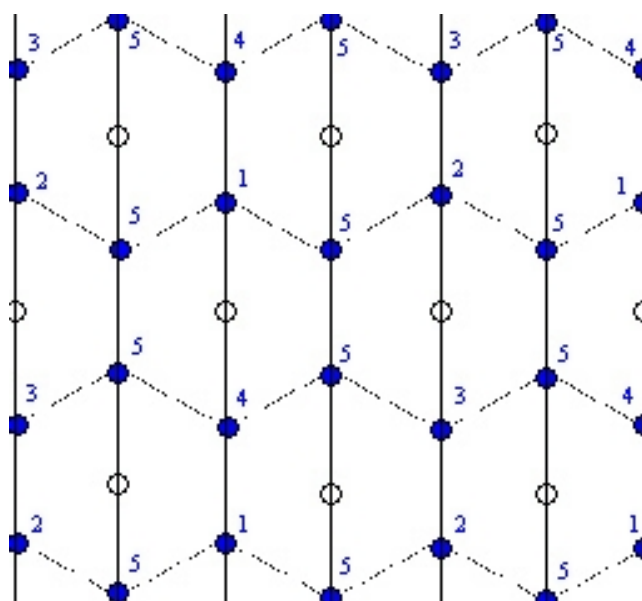


Fig. 1: Planting scheme - honeycomb system

#### CONCLUSION

The proposed model of Serbian spruce clonal seed orchard enables a more reliable study and directed use of the hereditary and combining values of the selected Serbian spruce genotypes. Based on the study results, 12 hybrid combinations were selected, distinguished by high mean values of cone morphometric characters, some of which show the heterotic effect for one, two or three analysed characters. The analysis of parent individuals participating in these hybrid combinations displays that the superior combining ability is shown by mother genotypes: 1B1/1 and 1C2/4, 1C2/2 and 1D1/4, represented each in three i.e. two hybrid combinations. Superior combining ability of the selected fathers is shown by individuals: 1A1, 1F1/1 and 1F7/1, of which the tree 1A1 participates in five hybrid combinations as the pollinator. The selection of parent individuals i.e. their combinations, with a good general combining ability and specific combining ability is the material base for the establishment of experimental clonal seed orchard for the production of Serbian spruce intraspecific hybrids, which will enable the production of genetically improved seeds and seedlings.

The direction of our further interest in the genetic composition of hybrids will depend primarily on the silvi-technical conditions for which the nursery stock is produced, as well as on the actual market demand. All the above factors must be taken into account in the future mass production of seed, so that already in the initial phases, the desired clone genotype must be clearly planned. The new multi-line

clones resulting from the controlled hybridisation and from the composition of the seed orchard should ensure a greater stability of yield and adaptation.

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**MODEL EKSPERIMENTALNE KLONSKE SEMENSKE PLANTAŽE ZA  
PROIZVODNJU UNUTARVRSNIH HIBRIDA OMORIKE (*PICEA*  
*OMORIKA* PANČ./PURKYNE)**

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Izvod

Polazeći od ošptih kriterijuma pri izbor potencijalno značajnih genotipova, kao i od karakteristika generativne semenske plantaže omorike u Godoviku, sa 5959 individua iz 50 linija polusrodnika, sprovedenim višegodišnjim analizama selekcionisani su genotipovi za osnivanje klonske semenske plantaže. Izbor genotipova koji će se klonirati obavljen je na osnovu višegodišnjih analiza većeg broja svojstava koji se odnose na redovnost i obilnost cvetanja, dinamiku rasta i razvića stabala kao i na kvantitet i kvalitet uroda. Primenom kontrolisane hibridizacije i proverom dobijenog hibridnog semena obavljena je, primenom izoenzimskog metoda analize - na osnovu rastvorljivih proteina semena u solima, analiza genetičke strukture svake hibridne kombinacije i utvrđen je stepen nasledljivosti svojstava na relacije roditelji-hibridi. Ovim putem su identifikovani potencijalno vredni genotipovi, koje treba kloniranjem fiksirati u semensku plantažu II generacije za proizvodnju perspektivnih hibrida. Osnovna odlika predloženog modela klonske semenske plantaže omorike je u originalnoj šemi sadnja po kojoj se ramete klonova polinatora sade u temenima pravilnih šestougaonika, a u centru svakog šestougaonika nalazi se jedna individua materinskog klona. Na ovaj način uspostavlja se sigurnost oprašivanja, pri čemu svaka materinska individua ima podjednake šanse da bude oprašena polenom individua koje su izabrane za polinatore. Broj ponovljenih šestougaonika unutar svakog bloka zavisiće od konkretnih uslova u momentu podizanja semenske plantaže. Predloženim modelom klonske semenske plantaže otkloniće se evidentirani nedostaci funkcionalnosti do sada podignutih klonskih semenskih plantaža različitih vrsta četinarara. Koristeći se rezultatima obavljenih analiza uzroka nefunkcionalnosti klonskih semenskih plantaža prethodnih generacija ostvaruju se neophodni preduslovi za uspešno osnivanje eksperimentalne klonske semenske plantaže omorike II generacije sa ciljem proizvodnju unutarvrsnih hibrida koji će posedovati heterotična svojstva.

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