Diversity of the Willow Complex *Salix alba – S.* x *rubens – S. fragilis*

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(Received 18th March 2003)

Abstract

A comparative phenotypic and genetic analysis was performed to investigate the variation within the *S. alba – S.* x *rubens – S. fragilis* complex in Flanders (Belgium). Multivariate analyses revealed the division of this willow complex into two main groups containing (i) *S. alba* and *S.* x *rubens* and (ii) *S. fragilis* and *S.* x *rubens* var. *basfordiana.* Moreover, the genetic similarity between *S.* x *rubens* and *S.* x *rubens* var. *basfordiana* was lower than between either taxon and *S. alba* or *S. fragilis*, respectively. These findings were unexpected as *S.* x *rubens* and *S.* x *rubens* var. *basfordiana* are classified as hybrids of *S. alba* and *S. fragilis.* The phenotypically distinguishable varieties of *S. alba* and *S. fragilis* did not cluster separately, indicating that they are not genetically differentiated. All varieties displayed a significant level of genetic distance between the individuals, contradicting the clonal nature of some varieties, as has been suggested before.

Key words: Salix alba, Salix x *rubens*, *Salix fragilis*, White willow, Crack willow, morphological variation, genetic distance, hybrids, introgression, AFLP.

Introduction

The tree forming willows *Salix alba* L. (White willow), *Salix fragilis* L. (Crack willow) and the hybrid *Salix* x *rubens* Schrank, characterised by a fast growth, flexible twigs and soft wood are keystone species of the riverbank vegetation in Europe. At the beginning of the previous century, the wood and twigs were widely used in basketwork, in clogmaking and for all kinds of small utensils. Because of their economic importance, willows were bred and cultivated by humans. Present day vegetation in the main river basins in Flanders still shows the rich cultural history of willows. Old osier varieties can be found intermixed with native willows (Maes and Rövekamp, 1998; Rövekamp et al., 2000; Opstaele, 2001; Zwaenepoel, 2000).

"Willows and Poplars of Great Britain and Ireland" (Meikle, 1984) is one of the more complete works on willows in Northwestern Europe and forms the starting point for this study. Although the name Crack willow is popularly attached to a complex group of willows, the morphological differences between the taxa are so consistent that four varieties of *S. fragilis* (var. *fragilis*, var. *russelliana*, var. *furcata* and var. *decipiens*) are recognised by Meikle (1984). Both the varieties *russelliana* and *furcata* are supposed to be clonal, represented by one sex only and evidently planted where they occur. *S. alba* consists of three varieties: var. *alba*, var. *caerulea* and var. *vitellina*. Under the name *S.* x *rubens* one finds a wide range of morphological variation, with *S. fragilis* and *S. alba* at both extremes. Of *S.* x *rubens* var. *basfordiana* two forms are recognised: forma *basfordiana* and forma *sanguinea* (Meikle, 1984).

The different varieties of *S. alba* and *S. fragilis* are supposed to hybridise frequently in nature (Meikle, 1984; Zwaenepoel, 2000). The overlapping morphological characters increase the complexity, resulting in conflicting classifications. Thiébaut (2000), who carried out a morphometric study on the leaves of *S. alba* and *S. fragilis*, and Zwaenepoel (2000) both stated that *S.* x *rubens* is more similar to *S. alba* than to *S. fragilis.* In contrast, Beismann et al. (1997) and Lautenschlager-Fleury et al. (1994) concluded on the basis of an AFLP analysis and on a morphological study, respectively, that *S.* x *rubens* is more similar to *S. fragilis* than to *S. alba.* Based on isozyme and RAPD analyses the complex was divided into a "*S. alba*-like" and a "*S. fragilis*-like" group (Triest et al., 1998, 2000). These latter articles did not discuss the position of *S.* x *rubens.*

So far, no molecular-genetic study aimed at distinguishing the varieties within the complex as defined by Meikle (1984). Furthermore, the conflicting results from the previous studies urged for a detailed and integrated morphological and molecular analysis of the *S. alba – S.* x *rubens – S. fragilis* complex. We sampled individuals of this complex in the catchment areas of three main rivers in Flanders to unravel the hybrid complex by searching the most distinguishing phenotypic characters and by applying highly informative molecular AFLP markers. Furthermore, we wanted to enlarge the core collection of willows of the Institute for Forestry and Game Management (Belgium), used for a breeding program to enhance the knowledge about this willow complex.

Material and Methods

In this paper, we use „*S. alba*" and „*S. fragilis*" when all the varieties are included. The term „*S.* x *rubens*" excludes the variety *basfordiana*. When relevant, a specific variety is indicated with its full name.

Plant material

In this study 103 samples, including both autochthonous trees and man-made osiers of the *S. alba – S.* x *rubens – S. fragilis* complex were collected (*Table 1*). The sampling was based on inventories of autochthonous trees and shrubs in the catchment areas of the rivers Schelde, Maas and IJzer in Flanders (Belgium) (Maes and Rövekamp, 1998; Rövekamp et al., 2000; Opstaele, 2001). The sample sites are shown in *Figure 1*. Man-made osiers were chosen so as to include as many varieties as can be observed in the river catchments. Also 20 reference samples from the collection of the Institute for Forestry and Game Management (Belgium) and from the collection of IACR-Long Ashton Research Station (Great Britain) were included (*Table 1*).

As a control, samples of 12 willow taxa not belonging to the complex were collected in Flanders (Belgium) and included in the genetic analysis. These are *S.* x *sericans, S.* x *dasyclados*,

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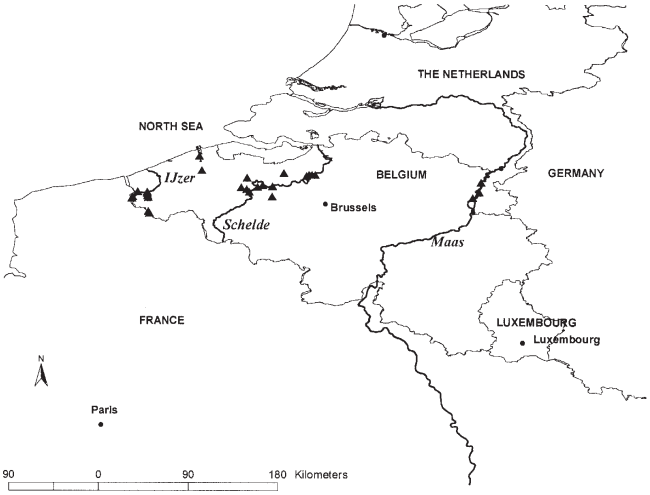

Figure 1. – The geographic distribution of the sample sites in the catchment areas of the three main rivers Schelde, Maas and IJzer in Flanders (Belgium, Europe).

Table 1. – Number of individuals that are determined to the level of variety according to MEIKLE (1984) for each of the sampled catchment areas in Flanders and reference samples from collections.

Variety	Schelde	Maas	IJzer	Reference samples
S. alba var. *alba*	9	6	3	9
S. alba var. *caerulea*	2	1	-	1
S. alba var. *vitellina*	-	-	-	2
S. x *rubens*	13	6	5	2
S. x *rubens* var. *basfordiana* forma *basfordiana*	7	5	1	-
S. x *rubens* var. *basfordiana* forma *sanguinea*	3	1	-	-
S. fragilis var. *fragilis*	11	-	1	3
S. fragilis var. *furcata*	-	1	2	2
S. fragilis var. *decipiens*	-	-	-	1
S. fragilis var. *russelliana*	6	-	-	-
Sum	51	20	12	20

S. triandra, *S.* x *reichardtii*, *S. caprea*, *S. cinerea*, *S. triandra* var. *discolor*, *S. purpurea*, *S. viminalis*, *S. repens*, *S. aurita* and *S.* x *multinervis*.

Morphological analysis

Mature leaves, twigs and flowers were collected from the lower part of the crown of 103 sampled trees. The willows were identified according to MEIKLE (1984) and the leaves and flowers were dried and stored in the herbarium GENT (Ghent, Belgium). The material was studied with a binocular stereoscope (Wild, M5). Measurements were performed with a measuring rod (accuracy 0.5 mm) or an Eschenbach Achromat 10 x loupe with accuracy 0.1 mm. Colours were determined with RHS colour charts (1998). *Table 2* gives a list of the studied characters (based on MEIKLE, 1984; NEWSHOLME, 1992; RULKENS, 1983, 1987a, b; UPOV, 1985). LAUTENSCHLAGER-FLEURY et al. (1993) stated that *S. alba*, *S. fragilis* and their hybrid can be discriminated on the bud scales. However, a difference between the bud scales was not observed in this study.

Table 2. – List of the studied morphological characters and abbreviations used in *Figure 2*.

Structure	Character	Abb.	Structure	Character	Abb.
Twig	colour		Stipule	width	SW
	pubescence			length	SL
	fragility			shape	SS
Bud	length			presence of glands and hairs	SH
	colour			scar size	
	pubescence		Female flower	flowering period	
Leaf	width	LW		length of the bract	
	length	LL		width of the bract	
	pubescence upper surface	PU		pubescence of the bract	
	pubescence lower surface	PL		pistil sessile or pedicellate	
	colour upper surface	CU		pistil length	
	colour lower surface	CL		pistil shape	
	glaucousity	GI		nectaries: number	
	margin	M		nectaries: form	
	venation	V	Male flower	flowering period	
Gland	subfoliaceous?	GS		length of the bract	
	surrounded by hairs?	GH		width of the bract	
Petiole	length	PtL		pubescence of the bract	
	groove	PtT		number of stamens	
	pubescence upperside – lowerside	PtPL		nectaries: form	
				nectaries: number	

AFLP analysis

Young leaves from cuttings or trees in the field were collected for AFLP fingerprinting. The fresh plant material was immediately frozen in liquid nitrogen, freeze-dried and stored under vacuum conditions.

DNA was isolated using Nucleon Phytopure kits (Amersham Pharmacia Biotech) according to the manufacturers instructions, with minor alterations. First, 10 mM 2-Mercapto-ethanol was added to reagent one. Second, 1 µl RNase (10 mg/ml) was added in the final step of the extraction and the mixture was incubated at 37 °C for 30 min. The AFLP reactions were performed according to VOS et al. (1995), with several adaptations. Three hundred ng of DNA was cut with the restriction enzymes *Pst*I and *Mse*I (Life Technologies). Digestion and ligation of the adapters was performed in a single reaction for 4 hours at 37 °C.

The primer combinations used (*Table 3*) are based on BARKER et al. (1999). The preamplification step was performed with a PstI primer (5'-GACTGCGTACATGCAG-3') and a *Mse*I primer (5'-GATGAGTCCTGAGTAA-3') containing one additional selective nucleotide. Final amplifications were performed with primers carrying two or three selective nucleotides. Fragment separation was performed on the ABI Prism 310 Genetic Analyzer (Perkin Elmer) using labelled *Mse*I primers.

Table 3. – Primer combinations used for AFLP analysis.

Preselective primers	Selective primers
*Pst*I + A & *Mse*I + G	*Pst*I + AC & FAM-*Mse*I + GAA
*Pst*I + A & *Mse*I + A	*Pst*I + AC & HEX-*Mse*I + ACA
*Pst*I + C & *Mse*I + G	*Pst*I + CA & FAM-*Mse*I + GAA
*Pst*I + C & *Mse*I + A	*Pst*I + CA & HEX-*Mse*I + ACA

Morphological data analysis

Frequency tables and boxplots (SPSS 11.0) estimated the power of the morphological parameters for species and variety discrimination. In addition, a Principal Components Analysis (PCA) (S-PLUS 2000, MATHSOFT, 1999) and a phylogenetic analysis (PAUP, SWOFFORD, 1993) were performed. As outgroup a fictitious species was used. The strict consensus tree was determined. In MacClade (MADDISON and MADDISON, 2000) the discriminating characters were visualised.

Molecular-genetic data analysis

AFLP patterns were analysed using Genotyper 2.5 (Perkin Elmer) with presence or absence of each marker coded 1 and 0, respectively. Monomorphic bands and bands appearing in only 1% of the samples were excluded from the data set. Paired similarities were calculated using the Simple Matching Index in S-PLUS 2000 (MATHSOFT, 1999). The resulting similarity matrix was further analysed by Principal COordinates analysis (PCO) using S-PLUS 2000 (MATHSOFT, 1999) and UPGMA clustering using TREECON (VAN DE PEER and DE WACHTER, 1994). Bootstrap values were calculated based on 100 replicates.

For the calculation of the genetic distance between (NEI, 1972) and within (NEI, 1973) the morphologically determined taxa, POPGENE (YEH et al., 1997) was used.

Results

Phenotypic diversity between S. alba – S. x *rubens – S. fragilis*

First, both frequency tables and boxplots were used to estimate the power of the morphological parameters for species and variety discrimination. It was concluded that a character discriminates between *S. alba* and *S. fragilis* if the frequency of the character was clearly higher in one species, or if the boxes did not overlap (*Table 4*). The hybrids *S.* x *rubens* display an intermediate position between both parents. As there are no characters which unambiguously discriminate hybrids, a combination of different intermediate characters (e.g. leaf pubescence, leaf margin) had to be used to determine a tree as *S.* x *rubens*.

Second, a phenetic analysis based on distinguishing leaf characters (*Table 4*) was performed. The flower characters were omitted from this data set because willows are dioecious. The PCA analysis (*Figure 2*) explains 66.3% of the variance. Two main groups can be distinguished: 1) *S. alba* clusters with *S.* x *rubens* and 2) *S. fragilis* intermixes with *S.* x *rubens* var. *basfordiana*. The different varieties do not form separate clusters. The distance between the 33 *S. alba* specimens is visibly smaller than between the 27 *S. fragilis* specimens, indicating that the morphological variation is larger in *S. fragilis*. The strong grouping of *S. alba* and *S.* x *rubens* can partly be explained by the similar stipules. Namely, a PCA analysis based on the stipule characters (not shown) displays a very strong grouping of *S. alba* and *S.* x *rubens*. The two *S.* x *rubens* specimens clustering together with S. *fragilis*, have stipules that resemble the typical *S. fragilis* stipules.

Finally, a phylogenetic study confirmes the grouping of *S. alba* with *S.* x *rubens* and of *S. fragilis* with *S.* x *rubens* var. *basfordiana* and does not discriminate between varieties.

Morphological description of the varieties

The results of the multivariate morphological analyses show the difficulty to recognise the hybrids *S.* x *rubens* or the vari-

Table 4. – Discriminating morphological characters between *S. alba* and *S. fragilis*.

Character	*S. alba*	*S. fragilis*
Twig pubescence	dense	sparse or glabrous
Leaf size	smaller than *S. fragilis*	larger than *S. alba*
Leaf pubescence	dense	sparse
Leaf colour	grey-green	shining green
Leaf margin	minutely serrate	coarsely serrate
Leaf venation	obscure	more conspicuous
Petiole length	shorter than *S. fragilis*	longer than *S. alba*
Stipule	smaller than *S. fragilis*, upper surface densely covered with hair, lower surface with many dark glands	larger than *S. alba*, upper surface glabrous, lower surface only with glands at stipule attachment
Stipule gland	small, dark	distinct, often subfoliaceous
Female flower	ovary sessile	ovary stipitate

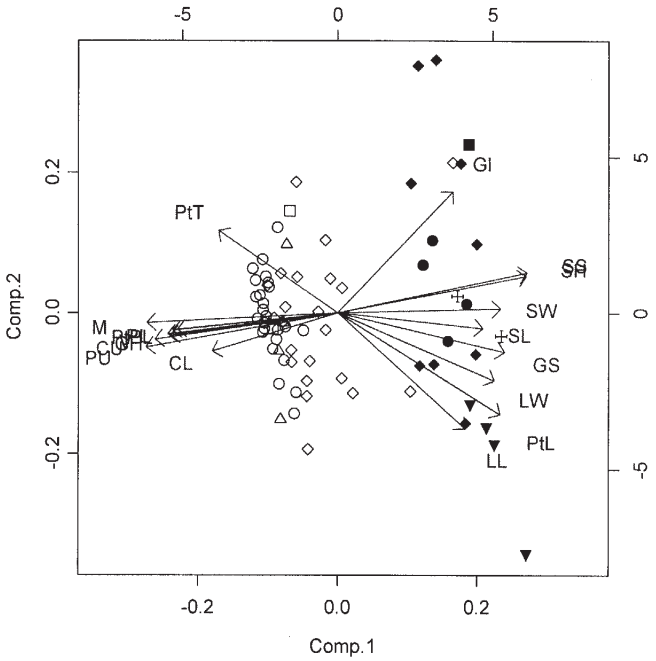

Figure 2. – PCA plot of the most important leaf characters (abbreviations see *Table 2*). The first two axes account for 66.3 % of the variance.

Symbol	Species	Symbol	Species
○	*S. alba* var. *alba*	♦	*S. fragilis* var. *fragilis*
△	*S. alba* var. *caerulea*	▼	*S. fragilis* var. *furcata*
□	*S. alba* var. *vitellina*	■	*S. fragilis* var. *decipiens*
◇	*S.* x *rubens*	✠	*S. fragilis* var. *russelliana*
●	*S.* x *rubens* var. *basfordiana*		

The morphological characters and the first and second component values.

Morphological characters*	Comp. 1	Comp. 2
LW	0.228	-0.277
LL	0.185	-0.474
PU	-0.279	-0.141
PL	-0.233	
CU	-0.265	-0.109
CL	-0 182	-0.158
Gl	0.168	0.497
M	-0.277	
V	-0.247	
GS	0.243	-0.160
GH	-0.243	
PtL	0.236	-0.417
PtT	-0.173	0.337
PtPL	-0.239	
SW	0.237	
SL	0.211	
SS	0.275	0.165
SH	0.274	0.151

*: Full names are given in *Table 2*

eties of the species *S. alba* and *S. fragilis* in Flanders. Nevertheless, some varieties can be distinguished on the basis of one or more persistent and visual characters. *S. alba* var. *caerulea* can not be discriminated based on our data set. The other variety *S. alba* var. *vitellina* (not yet found in Flanders) is recognised by its bright yellow-orange coloured twigs (RHS codes: 163b, 164a-b, 165b-c, 167b). *S.* x *rubens* var. *basfordiana* forma *basfordiana* can easily be distinguished from *S. fragilis* by its yellow – orange coloured twigs (RHS codes: 163b, 164a-b, 165b, 166a-b). *S.* x *rubens* var. *basfordiana* forma *sanguinea* can be determined by its dark red twigs and smaller leaves. *S. fragilis* var. *russelliana* has less fragile twig bases and more conspicuous subfoliaceous glands than var. *fragilis,* but is rather difficult to identify. *S. fragilis* var. *furcata* has larger leaves and bifurcate male catkins. Moreover along the Maas (a main river in Belgium) two specimens very similar to *S. fragilis* var. *furcata* were observed. However, they are different from the latter in having curved male catkins and a conspicuous pink main vein. *S. fragilis* var. *decipiens* (not yet found in Flanders) is recognisable by its glabrous, pale ochre-coloured (RHS codes: 161b-c) twigs and glabrous leaves.

Genetic structure of the willow complex

For the genetic analysis, the same plants as in the morphological study were used. In addition, a sample of 12 willow taxa not belonging to the *S. alba – S.* x *rubens – S. fragilis* complex were included as controls. The four AFLP primer combinations used (*Table 3*), rendered 237 informative bands with a unique banding pattern for all individuals.

In a PCO analysis, the first three axes account for 23%, 19% and 12% of the variation, respectively (*Figure 3*). The samples are visually separated into three main groups. The 12 control individuals are clustered separately, while the other two groups contain all the individuals of the studied complex. One group mainly consists of individuals morphologically identified as *S. fragilis* varieties or *S.* x *rubens* var. *basfordiana,* while the other group is formed by *S. alba* and *S.* x *rubens*. Compared to the morphological classification, some incongruencies are observed. Two samples, morphologically identified as *S.* x *rubens* var. *basfordiana*, group together with the *S. alba* and *S.* x *rubens* specimens and three individuals morphologically identified as *S.* x *rubens*, are found in the *S. fragilis* and *S.* x *rubens* var. *basfordiana* cluster.

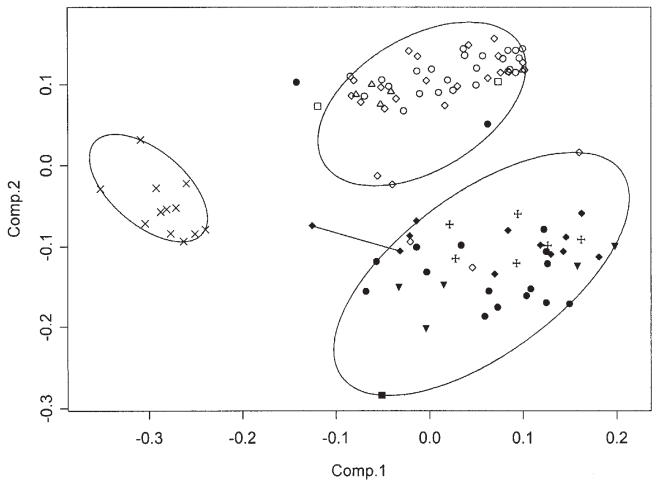

Figure 3. – Plot of the first two Principal COordinates calculated with the Simple Matching coefficient based on the AFLP fingerprinting. The first two axes account for 23% (axis 1) and 19% (axis 2) of the variation. The control willow taxa not belonging to the complex are indicated with an x, further symbols see *Figure 2.*

Because of the low differentiation between the different species, no diagnostic or taxon specific markers could be identified. Nevertheless, eight of the 237 polymorphic bands show significant differences in frequency. These eight markers are all absent in *S. alba* and very rare in *S.* x *rubens* (less than 14%). The frequency of occurrence is high in *S. fragilis* (between 52% and 96%) and *S.* x *rubens* var. *basfordiana* (between 40% and 100%).

The different varieties of the complex, according to the classification of MEIKLE (1984), do not cluster separately in the PCO.

The genetic distance and identity among the Flemish samples, omitting the deviating ones in the PCO plot (*Figure 3*), were calculated according to NEI (1972, 1973). The genetic similarity between the control taxa and the taxa of the *S. alba – S.* x *rubens – S. fragilis* complex is lower than the similarities between the four different taxa of the complex (*Table 5*). Within this complex, the genetic similarity between *S. alba* and *S.* x *rubens* is almost 99%, while it is a little lower (95%) between *S. fragilis* and *S.* x *rubens* var. *basfordiana.* This grouping is similar to the outcome of the PCO. Remarkably, *S.* x *rubens* var. *basfordiana* and *S. alba* individuals are only 87% identical, what is lower than *S. alba* compared to *S. fragilis*, the putative parental species of *S. rubens* var. *basfordiana.*

The genetic diversity within *S. fragilis* equals 34%, which is the highest of all investigated taxa. For *S. alba* it is 30%, whereas for the two hybrid groups *S.* x *rubens* and *S.* x *rubens* var. *basfordiana* it equals 28%.

Table 5. – Genetic identity (above diagonal) and genetic distance (under diagonal) between the investigated species and varieties.

	Control[1]	*S. alba*	*S. fragilis*	*S.* x *rubens*	*S.* x *rubens* var. *basfordiana*
Control[1]	****	0.8572	0.8521	0.8546	0.8046
S. alba	0.1541	****	0.9078	0.9895	0.8724
S. fragilis	0.1600	0.0967	****	0.9053	0.9528
S.* x *rubens	0.1572	0.0106	0.0995	****	0.8782
S.* x *rubens* var. *basfordiana	0.2174	0.1365	0.0483	0.1299	****

[1] Control willow taxa not belonging to the *S. alba – S.* x *rubens – S. fragilis* complex.

Discussion

Both the morphological and the molecular genetic investigation resulted in a division of the *S. alba – S.* x *rubens – S. fragilis* complex into two main groups. One consists of *S. alba* and *S.* x *rubens*, whereas the other contains *S. fragilis* and *S.* x *rubens* var. *basfordiana.* This partitioning is in agreement with previous studies using RAPD and isozyme analyses (TRIEST et al., 1998, 2000). In contrast, the study of BEISMANN et al. (1997) using AFLP markers revealed a division of the complex into three groups with the parental species at both extremes and the hybrids in an intermediate position. In the genetic investigation, the 12 different willow taxa used as control appear to cluster together in a separate group. This is most probably due to the fact that the search for informative markers focussed on identifying polymorphisms within the *S. alba – S.* x *rubens – S. fragilis* complex rather then between the control taxa. Doing so, several of the polymorphisms between the control taxa were not taken into account in our analysis.

Although *S.* x *rubens* and *S.* x *rubens* var. *basfordiana* both belong to the same hybrid taxon, they each display a higher similarity with one of the two parental species than with each

other. Similar to our results, THIÉBAUT (2000) and ZWAENEPOEL (2000) reported a high resemblance between *S.* x *rubens* and *S. alba*. In contrast, BEISMANN et al. (1997) and LAUTENSCHLAGER-FLEURY et al. (1994) both concluded that *S.* x *rubens* is more closely related to *S. fragilis.* None of the available studies on the willow complex *S. alba – S.* x *rubens – S. fragilis* (LAUTENSCHLAGER-FLEURY et al., 1994; BEISMANN et al., 1997; TRIEST et al., 1998, 2000; THIÉBAUT, 2000) distinguished *S.* x *rubens* and *S.* x *rubens* var. *basfordiana*. Perhaps LAUTENSCHLAGER-FLEURY et al. (1994) examined *S.* x *rubens* var. *basfordiana* specimens instead of *S.* x *rubens* specimens what might explain the high similarity of their "*S.* x *rubens*" with *S. fragilis*. Until now, only RULKENS (1983) took notice of *S.* x *rubens* var. *basfordiana* and concluded, similar to our results, that this variety shows a high resemblance to *S. fragilis.*

The observed subdivision and structuring of the complex can be explained by mechanisms such as matroclinal inheritance and preferential backcrossings that further differentiate the hybrid populations. Controlled hybridisation experiments between *S. alba* and *S. fragilis* showed that the offspring resembles the female parent (KRSTINIC, 1966). If the female parents of *S.* x *rubens* and *S.* x *rubens* var. *basfordiana* are *S. alba* and *S. fragilis*, respectively, matroclinal inheritance may cause the resemblance of *S.* x *rubens* with *S. alba* and of *S.* x *rubens* var. *basfordiana* with *S. fragilis*. If, subsequently, *S.* x *rubens* preferentially backcrosses with *S. alba* and *S.* x *rubens* var. *basfordiana* with *S. fragilis*, the offspring will become indistinguishable from the mother parent. Although there is no hard evidence, the overlap in flowering period and the similar frequencies of the eight genetic markers of *S. alba* and *S.* x *rubens* and of *S. fragilis* and *S.* x *rubens* var. *basfordiana* support this hypothesis. The deviating position of three *S.* x *rubens* and two *S.* x *rubens* var. *basfordiana* in the genetic analyses is in agreement with their phenotypic appearance. The three *S.* x *rubens* individuals are more similar to *S. fragilis* and the two specimens of var. *basfordiana* resemble *S. alba.* This is most likely caused by the backcrossing of *S.* x *rubens* with *S. fragilis* and *S.* x *rubens* var. *basfordiana* with *S. alba.* This shows that the boundaries between the taxa are not strictly defined.

The higher genetic similarity between *S. fragilis* and *S. alba* than between *S.* x *rubens* var. *basfordiana* and *S. alba* was unexpected because a descendant and either of its parents should be more similar than the two parents are. Several independent crossing experiments showed that *S.* x *rubens* var. *basfordiana* is a descendant of *S. fragilis* x *S. alba* var. *vitellina* (MEIKLE, 1984). The low similarity between *S.* x *rubens* var. *basfordiana* and *S. alba* might be explained by a difference in genetic constitution of *S. alba* in Flanders compared with *S. alba* var. *vitellina*, which is not present in Flanders. The morphological and genetic variation in the *S. fragilis* group is higher than in the *S. alba* group. This could be due to the higher number of *S. fragilis* varieties in Flanders and thus in our data set. The *S. alba* varieties *vitellina* and *caerulea* are respectively absent and rare in Flanders.

It appears difficult to distinguish between the several varieties within the studied willow complex because of the low discriminating value of the morphological characters and the lack of variety specific markers. MEIKLE (1984) defined the different varieties based on one or two persistent characters that are most likely encoded by only few different genes. Because the varieties *russelliana* and *furcata* are only represented by one sex, MEIKLE (1984) postulated that they are clonal. The outcome of our investigation however shows that they are genetically too diverse to represent clones. The most acceptable hypothesis about the origin of these varieties is that they have developed several times independently from different individuals of the typical *S. fragilis* var. *fragilis*. Remarkably, the more wide-leafed *S. alba* variety of Flanders does not cluster together with the reference *S. alba* var. *caerulea* from Great Britain. An explanation is that *S. alba* var. *caerulea* is not present in Flanders and that the broad-leafed tree is a natural variety within the species *S. alba.*

The unexpected grouping of the taxa belonging to the *S. alba – S.* x r*ubens – S. fragilis* complex and the low similarity between *S.* x *rubens* and *S.* x *rubens* var. *basfordiana* are the most remarkable results of this investigation. Experimental hybridisation analyses might be useful to reconstruct the genesis of the taxa belonging to the complex.

Acknowledgements

We are grateful to STEFAAN MOREELS for skilful technical assistance. This study has been carried out with financial support from the Ministry of the Flemish Community (AMINAL, Forests and Green Areas Division and Nature Division).

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Genetic Variation in Wood Specific Gravity of Half-sib Families of *Pinus nigra* subsp. *pallasiana* Tested at the Juvenile Stage: Implications for Early Selection

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(Received 18th March 2003)

Summary

Seeds from 7 populations (total of 281 half-sib families, *progeny test*) and 35 seed stands (*provenance test*) representing natural range of Anatolian black pine (*Pinus nigra* subsp. *pallasiana*) were sown in a forest nursery in Ankara in 1990 and raised until age 3. Stem wood specific gravity (WSG) of all seedlings was determined at age of 3. The results of this study indicated that WSG did not vary significantly neither among the 7 populations (ranging from 0.41 to 0.42) nor among 35 seed stands (ranging from 0.37 to 0.46). Differences between half sib families for WSG were, however, statistically significant. Estimated family heritablity was moderately high (0.38). Genetic correlations between seedling growth traits and WSG were low, but consistently negative. The families with better height and diameter growth had lower WSG values. Also families with late budset and budburst dates in 1991 had lower WSG values. Seedlings originating from northern latitudes had lower WSG than those from southern latitudes. From the results of the study, it seems that early selection of families for WSG (indirect selection for WSG at mature age) would be possible and substantial genetic gain in WSG could be achieved if the selection based on a multi-trait index selection by giving appropriate weights to WSG and other traits. Further implications of early selection for WSG in Anatolian black pine are also discussed in the paper.

Key words: Pinus nigra subsp. *pallasiana,* wood specific gravity, genetic variation, genetic correlation.

Introduction

The Anatolian black pine (*Pinus nigra* Arnold subspecies *pallasiana* (Lamb.) Holmb.) is an important timber species, occurring naturally as a widespread mid-elevation species (ranging from 250 to 1550 m) in Toros, western and northern Anatolian Mountains of Turkey (*Figure 1*). Anatolian black pine forests cover more than 2 million hectares. It is the first species for afforestation of the high Anatolian steppes (KAYA and TEMERIT, 1994). Anatolian black pine has, therefore, great importance in Turkish forestry (KAYA and TEMERIT, 1994; KOSKI and ANTOLA, 1993).

To date, 88 seed stands and 55 clonal seed orchards have been established by Turkish Ministry of Forestry for Anatolian black pine. Large areas in Turkey are also reforested or afforested with the species, but there is lack of genetic studies which will help to manage and improve the genetic resources of the species. Tree improvement program concerning the species is still in its beginning. Clonal seed orchards and seed stands have been established to provide needed seeds for reforestation and afforestation activities. The first generation progeny tests were established to allow the selection of best parents and roguing of clonal seed orchards.

Since long rotations are used in forestry, early evaluation of genotypes for adaptive and volume traits are an important component of tree improvement strategy (BRIDGEWATER and MCKEAND, 1997; LAMBETH *et al.,* 1982; LOWE and VAN BUIJTENEN, 1989).

Wood density in pines shows considerable genetic, environmental and age related variation (ZOBEL and VAN BUIJTENEN, 1989). Wood density is strongly influenced by the amount of early and latewood produced late in the season. Generally early wood has low density while late wood has higher density (ZOBEL and VAN BUIJTENEN, 1989). Significant genetic variation in early and late wood proportions has been reported in Douglas-fir families (VARGAS-HERNANDEZ and ADAMS, 1994) and in Norway Spruce and Douglas-fir provenances (VARGAS-HERNANDEZ and ADAMS, 1994; SKROPPA and DIETRICHSON, 1999). Also, strong genetic correlations between growth traits in juvenile ages (ages 2–7) and wood density in mature age (age 29) were reported in Norway spruce (SKROPPA and DIETRICHSON, 1999). These correlations were negative and good predictors of wood density at age 29. Diameter growth in pines in juvenile ages is mainly made up of early wood and very little late wood. Thus, overall wood density which is not affected as much as late wood by environmental conditions and assuming that it is made up mainly by early wood could be used to select families

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