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Genetics and Plant Breeding

Estimation of Genetic Variability, Heritability and Genetic Gain for Wood Density and Fibre Length in 36 Clones of White Willow (*Salix Alba* L.)

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Abstract

Variability of wood density and fibre length was determined in 36 genotypes of *Salix alba* L. procured from seven different European countries namely Italy, Hungary, U.K, Netherlands, Turkey, Yugoslavia and Croatia. Genetic parameters were worked out with regards to estimate of heritability (broad sense), genetic advance, genetic gain as per cent of mean and correlation coefficient among them. Wood density was recorded in the range of 0.30-0.53 with mean value 0.40gcm^{-3} whereas fibre length ranged from 0.45-0.65 with mean 0.55mm. High heritability values show that the genetic control is stronger for wood density (h^2=90.30) than for fibre length (h^2=78.20). Both the characters were having high heritability with good genetic gain. Clone 84/22 from Turkey had given best performance in view of both the character. Further control crossing is underway to produce ideotype with regard to different end users.

Highlight

1. Variability of wood density and fibre length was determined in 36 genotypes of *Salix alba* L.
2. Significant differences existed among the clones for fibre length
3. High heritability value (90.30) for wood density and fibre length (78.20) coupled with high genetic gain (33.33%) for wood density was recorded.

Keywords: *Salix alba* L., wood density, fibre length, heritability, genetic gain.

Salix species, commonly known as willows belong to family *Salicaceae*. The word *Salix* is derived from *Celtic*, 'Sal' meaning near and 'lis' meaning water. The genus has about 300 species all over the world except in Australia and New Zealand. The haploid chromosome number of genus *Salix* (Willow) is n = 19, but many species are tetraploid and higher ploidy levels are common. The genus is very heterogeneous and shows considerable variation in size, growth form, and crown architecture, ranging from small dwarf shrubs in the subgenus *Chamaetia*, middle-size shrubs in the subgenus *Vetrix*, to the tree willows of the subgenus *Salix* (Lindegaard and Barker, 1997).

It has a wide natural distribution over the whole of Europe except in extreme north, and occurs also in western Asia and in small part of north Africa. *S. alba* belongs to the boreal-Mediterranean type of habitat. Its natural distribution

in the British Isles (Without Scotland), in the west, to western Siberia in the East, from Southern Scandinavia in the North to the Near East, Palestine, Morocco and Algiers in the South. In the South it reaches altitudes of 2400 m and in the North to 600m above sea level (Weber, 1974). Soil suitable for the growth of *Salix alba* are alluvial, pseudo-gleyed, field-swamp soil, marsh black soil, swamp-gleyed, peat-gleyed and halomarphic soils. The pH range is 6.5-8.0 and humus content ranges from 0.55-1.90% (Skorio, 1973). Within and among the central European white willow populations are very noticeable variations in the intensity of growth, straightness of bole, colour, thickness and furrows of bark, branch thickness, morphological leaf characteristics, seed production and length of growing season (Skvortsov, 1968).

Salix alba consists of three varieties v*ar. abla, var. vitellina* and *var. caerulea. Salix alba var. vitellina* is commonly known as Golden willow is particularly used for decorative purposes whereas commercially important is *Salix alba var. caerulea* commonly known as cricket-bat willow. It is a special timber crop in U.K. mainly for the production of cricket bat and also for other uses where toughness, lightweight characters are required. It is a female cultivar of hybrid origin between *S. alba and S. fragilis* (Stott, 1984).

There are about 33 species present in India. Out of these most of them are categorized as shrubs except *S. alba, S. babylonica, S. daphnoides, S. fragilis, S. elegans* and *S. tetrasperma*. *Salix alba* L. commonly known as white willow is a moderate to large sized tree. In India, poplar wood constitutes 80% of plywood and particle-board followed by paper and pulp. Poplar is not suitable for integration with rice crop or water and salt affected soils whereas willow has its inherent qualities to cope up with such challenged situations. Therefore, willow cultivation as farmer's friendly species is being taken up vigorously to diversify the short rotation forestry through clonal means of propagation.

Species of the genus *Salix* (willows) have among their advantages two remarkable characteristics that promote their cultivation. One of them is that they are adapted to thrive in flooding environments and the other is their easy vegetative propagation. Wood basic density is considered to be one of the most important features in genetic improvement programmes (Zobel and Talbert, 1988) and is one of the most often-studied quality traits (Bonavia De Guth, 1981; Carrizo, *et al.*, 1997; Peszlen 1998). For willow biomass crops using unimproved genetic material with planting densities of 14,000 to 18,000 plants ha^{-1}, the peak mean annual increment is reached in three to five years (KOPP, *et al.*, 1997; Willebrand and Verwijst, 1993; Willebrand, *et al.*, 1993). It is a complex feature influenced by cell wall thickness, the proportion of the different kind of tissues, and the percentages of lignin, cellulose and extractives (Valente, *et al.*, 1992). Wood density and fibre length are strongly inherited and variable, thus enabling good gains from genetic manipulations. The bulk of studies had been done on the genetics of diffuse porous hardwoods especially in *Populus* and *Eucalyptus* with very little effort in Willows. Moreover juvenile to mature correlations in wood density for fast growing species such as poplar had been already established (Nepveu, *et al.*, 1978). Interest in the genetics of wood density in the hard wood is increasing and with inheritance of wood density is moderate to strong be good for energy uses of wood (Goldstein, 1980). Fibre length is considered to be the most important character for making high quality groundwood in Eucalyptus (Campinhos and Claudio-DA-Silva, 1990). Both wood density and fibre length determine whether the quality of raw material is suitable for a specific use in the paper and plywood industry. Fibre length also has impacts on paper characteristics, such as strength, optical properties and surface quality.

Significant clonal differences were observed in many qualities of wood, bark and foliage of willows. The feasibility of simultaneous selections for several traits such as growth rate, specific gravity, high biomass productivity was also studied (ZSUFFA, *et al.*, 1993). The present investigation was undertaken with the objective of estimation of variability and genetic parameter estimation in wood basic density and fibre thirty six clones of *Salix alba* L. growing in nursery conditions.

Materials and Methods

36 clones of Salix alba L. were selected from seven different European countries namely UK, Italy, Netherlands, Hungary, Yugoslavia, Croatia and Turkey (Table 1). Cutting material was collected and multiplied at an experimental nursery at Shilly, Solan.

An experiment was established at the Shilly forest nursery with 36 clones of *Salix alba* L. including one local Kashmir willow [India (UK)] for the comparison. The experimental site is located at an elevation of 1465m above mean sea level in north-west of Himalaya and lies between 32Ú 41'

N latitude and 77° 07' E longitude. The area experiences a wide range of temperature with a minimum of 1°C in winters to a maximum of 33°C in the summers with occasional snowfall in January and February. The soil is loamy with pH of 7.20. Five cuttings of each clone of standard size were planted in randomized complete block design at 40 cm X 30 cm. spacing in three replications.

Wood density (gcm^{-3})

Specific gravity also known as relative density (expressed as gcm^{-3}) is merely its density in comparison with a standard density, usually that of pure water at 4°C. For, measuring the wood density the blocks of wood were first weighed and then coated with paraffin wax to make them impervious. Then the beaker of water (approximately at 4°C) kept on digital electronic balance and the reading were tarred to zero then the block of wood, suspended by a needle clamped in a stand was gently lowered into the beakers and completely immersed in the water without touching it either the sides of the beaker and not allowing any of the water to run over the top of the beaker. The new mass displayed was then read off. Since the density of water is 1.00 gcm^{-3} at 4°C, the new mass reading is equal to the volume of the test block in cubic centimeters. The wood density was calculated by using the formula given by Desch and Dinwoodie (1996):

$$\text{Wood density} = \text{Specific gravity} = \frac{\text{Mass of block (g)}}{\text{New reading of mass after immersing the block (g)}}$$

Fibre length (mm)

For fibre length measurements, small segments (slivers) were removed from the wedges. Fibres were macerated (Franklin, 1938) in a mixture having equal parts of 10 per cent chromic acid and 10 per cent nitric acid for 12-24 hours. Pulp was thoroughly washed with water, stained with 2 per cent aqueous safranin, teased in 10 per cent glycerine and mounted in glycerine jelly with coverslip. The length of minimum 30 randomly selected fibres was measured with the help of ocular micrometer scale standardized with the help of stage micrometer.

Statistical analysis

The data obtained for natural variation were subjected to statistical analysis using RBD design. The statistical analysis for each parameter was carried out on mean values and the analysis of variance (ANOVA) was worked out as per the procedure suggested by Panes and Sukhatme (1967) and Singh and Chaudhary (1985).

Genetic parameters

Heritability in broad sense was calculated using the formula suggested by Johnson, *et al.*, 1955.

$$h^2_{b.s} = \frac{Vg}{Vp} \times 100$$

Where,

$h^2_{b.s}$ = Heritability (broad sense)

Vg = Genotypic variance

Vp = Phenotypic variance

The expected genetic advance at 5 per cent selection intensity was calculated by the formula suggested by Lush (1940) and further used by Burton and De-Vane (1953) and Johnson *et al.*, (1955).

$$\text{Genetic Advance (GA)} = \left[\frac{Vg}{Vp}\right] \times \left(\sqrt{Vp}\right) \times K$$

Where,

$\frac{Vg}{Vp}$ Vg = Genotypic variance

Vp = Phenotypic variance

K = Selection differential at 5 per cent selection intensity. The value of K = 2.06 (Allard, 1960).

Genetic gain was worked out following the method suggested by Johnson *et al.*, (1955) as under:

$$\text{Genetic Gain (\%)} = \frac{GA}{\bar{X}} \times 100$$

Results and Discussion

Wood density and Fibre length

Wood samples of various clones (Table 1) showed a wide variation in wood density (0.30-0.53gcm^{-3}). Maximum value (53gcm^{-3}) was registered for clone 006/05 (U.K.) followed by 0.5253gcm^{-3} for 84/22 (Turkey). Minimum wood density (0.38 53gcm^{-3}) was reported for Bedai egynas (Hungary). The range of variation between clones agrees with that observed in previous studies. Earlier large clonal differences 0.35 to 0.40 g/ml were reported in *Populus tremuloides* (Yanchuk *at al.*, 1983).

Table 1: Mean values of wood density and fibre length of *Salix alba* L. clones collected from different countries

Code	Original identity	Scientific Name	Source Country	Wood density (g cm^{-3})	Fibre length (mm)
T_1	SI- 64-009	*Salix alba*	Italy	0.40	0.52
T_2	878 (Caerulea)	*Salix alba*	Netherlands	0.41	0.50
T_3	1987-3674 (Vitellina)	*Salix alba*	U.K.	0.42	0.55
T_4	SI-64-005	*Salix alba*	Italy	0.41	0.57
T_5	668 (Lockinge)	*Salix alba*	Netherlands	0.48	0.45
T_6	SI-62-096	*Salix alba*	Italy	0.34	0.50
T_7	SI-63-007	*Salix alba*	Italy	0.33	0.57
T_8	SI-62-104	*Salix alba*	Italy	0.41	0.50
T_9	SI-62-096	*Salix alba*	Italy	0.31	0.59
T_{10}	SI-64-036	*Salix alba*	Italy	0.35	0.51
T_{11}	SI-63-012	*Salix alba*	Italy	0.36	0.48
T_{12}	SI-63-011	*Salix alba*	Italy	0.32	0.54
T_{13}	Bedai egynes	*Salix alba*	Hungary	0.30	0.58
T_{14}	C. Sertai	*Salix alba*	Hungary	0.31	0.59
T_{15}	I-1/59	*Salix alba*	Hungary	0.37	0.51
T_{16}	I-4/59	*Salix alba*	Hungary	0.33	0.54
T_{17}	Veliki bajar	*Salix alba*	Hungary	0.35	0.60
T_{18}	SI-2/61	*Salix alba*	Hungary	0.31	0.58
T_{19}	Sarvar-I	*Salix alba*	Hungary	0.33	0.53
T_{20}	55/182	*Salix alba*	Hungary	0.43	0.52
T_{21}	20	*Salix alba*	Hungary	0.36	0.53
T_{22}	MB 368	*Salix alba*	Croatia	0.39	0.55
T_{23}	664 (Ellemore)	*Salix alba*	Netherlands	0.38	0.54
T_{24}	665 (Foreman)	*Salix alba*	Netherlands	0.40	0.57
T_{25}	Gvidale 13 (Vitellina)	*Salix alba*	Italy	0.35	0.55
T_{26}	Lignaro PD (Vitellina)	*Salix alba*	Italy	0.35	0.51
T_{27}	Lignaro PD* (Vitellina)	*Salix alba*	Italy	0.39	0.59
T_{28}	79/64/2	*Salix alba*	Yugoslavia	0.48	0.65
T_{29}	380	*Salix alba*	Yugoslavia	0.51	0.54
T_{30}	006/05	*Salix alba* cv. *Caerulea*	U.K.	0.53	0.49
T_{31}	006/06	*Salix alba* cv. *Caerulea*	U.K.	0.51	0.51
T_{32}	82/11	*Salix alba*	Turkey	0.48	0.50
T_{33}	84/21	*Salix alba*	Turkey	0.47	0.56
T_{34}	84/22	*Salix alba*	Turkey	0.52	0.58
T_{35}	84/03	*Salix alba*	Turkey	0.43	0.56
T_{36}	Kashmiri Willow	*Salix alba* cv. *Caerulea*	India (U.K.)	0.43	0.58
	Mean			0.39	0.55
	CD (5%)			0.035	0.034
	CD (1%)			0.05	0.045
	CV (%)			5.37	3.77

* Different genotypes

Significant differences existed among the clones for fibre length that ranged between 0.45-0.65mm. Clone 79/64/2 exhibited maximum fibre length (0.65) whereas minimum value (0.45) was recorded for clone 668 (Netherland). The results are in conformity with findings of mean values of wood density and fibre length for all the clones in Argentina (Bonavia De GUTH 1982; Piussan *et al.*, 1990), Deka *et al.*, (1992), Deka *et al.*, (1994), Sennerby-Forse (1985), Pan *et al.*, (1998), Monteoliva *et al.*, (2005) and Huse, *et al.*, (2008) obtained on willow clones.

Estimation of genetic Parameters

Success of any breeding programme depends on the variability present in the plant material. Sufficient variability in the material provides liberty to the breeder for the selection as per his objectives. Therefore to start the improvement work in willows, the assessment of genetic variation on the material under study becomes imperative. There may be various parameters used for assessing the inherent genetic variability. Hence, information on variation

Table 2: Estimates of variability and genetic parameter of wood characteristics of *S. alba* L. clones

Characters	Mean	Range	C.V. (%)	Coefficient of variance		Heritability	Genetic advance (K= 2.06)	Genetic gain
				Genotypic	Phenotypic			
Density (g cm^{-3})	0.40	0.30-0.53	5.37	16.43	17.28	90.30	0.13	33.33
Fibre length (mm)	0.55	0.45-0.65	3.77	7.14	8.08	78.20	0.07	12.72

among the desirable parameters and their correlation is important for selection and breeding programme (Johnson *et al.,* 1955).

The analysis of variance for individual willow clone showed significant differences among different clones for both the characters (Table 2). Regarding coefficient of variation, it was as 5.37% for wood density as compared with 3.77% for fibre length. Wood density and fibre length recorded 16.43% and 7.14% genotypic coefficient of variation for both the wood characteristics respectively.

High heritability value (90.30) for wood density and fibre length (78.20) coupled with high genetic gain (33.33%) for wood density and low genetic gain (12.72) for fibre length was recorded. These were very well in line with the studies of Moteoliva *et al.,* (2005) on *Salix* clones. Earlier Wang *et al.,* 1984 reported high heritability (0.83) at family level in one year old *Eucalyprus grandis*. Olsen *et al.,* 1985 reported that differences in *Populus deltoides* clones were large and highly heritable wood density suggesting strong genetic control of the character. MC- Cutchan (1982) reported that gains of 29-37% in dry weight would occur from selection for wood density in *Platanus occidentalis.* Similarly high heritability (0.90) at clonal level was reported in six-year-old *E. grandis (*Bertolucci, *et al* 1992*).* Wood density showed negative and non-significantly correlation with fibre length. Similar types of results were reported earlier by Huse (2004) on willow clones.

Conclusion

Clonal forestry based on willow is utilitarian if clones used in planting are highly productive with good quality of wood density and fibre length. Selection criteria used and the effectiveness of nursery testing followed by field evaluation is the basis for obtaining meaningful results under different agroforestry models and monoblock plantations. In view of the multipurpose role of *Salix* species, possibilities of increasing the cultivation area to provide raw material for the paper, pulp and plywood industries, cricket bat and artificial limbs manufacturing and energy uses requires a deeper knowledge of their genetic variability of wood traits. Clone 006/05 from UK had shown maximum value of wood density (0.53gcm^{-3}) followed by clone 84/22 (0.52gcm^{-3}) from Turkey, clone 380 and 006/06 (0.51gcm^{-3}) from Yugoslavia and U.K. respectively. The highest value for fibre length (0.65mm) was expressed by the clone 76/64/2 from Yugolavia closely followed by Veliki bajar (0.60 mm) from Hungary and SI-62-098, Lingaro P D and C. Sertai (0.59mm) from Italy and Hungary respectively. Clone 84/22 from Turkey is the winner clone for both the traits. Wood density and fibre length are strongly inherited and variable, thus enabling good gains from genetic manipulations particularly for wood based industries and cricket bat manufacturing in short rotation forestry coupled with coppice system of regeneration. Wood density and fibre length both exhibited high heritability along with good genetic gain which indicated that these characters are more under genetic control. Hence, to make an effective improvement in these characters, one has to go for heterosis breeding based on the performance of clones followed by clonal propagation in order to capture additive and non-additive genetic variances that can be easily achieved by clones of *Salix*..

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