

NURSERY EVALUATION OF WILLOW (*Salix* spp.) CLONES

Thesis

by

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(F-2014-23-M)**

Submitted to



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Partial fulfilment of the requirements for the degree

of

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Willow

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CERTIFICATE - I

This is to certify that the thesis titled **“Nursery evaluation of willow (*Salix spp.*) clones”** submitted in partial fulfilment of the requirements for the award of degree of **Master of Science (Forestry)** in the discipline of **Forest Genetic Resources** of Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP)-173 230 is a bonafide research work carried out by **Ms. Leena Kumari (F-2014-23-M)** daughter of Sh. Parshotam Ram under my supervision and no part of this thesis has been submitted for any other degree or diploma.

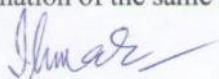
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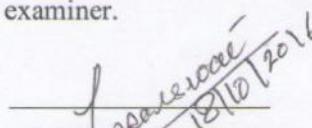
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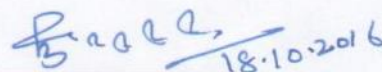


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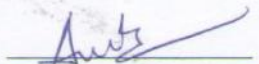
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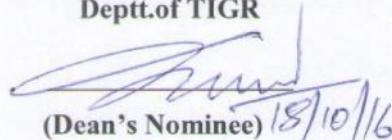
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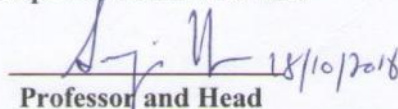
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This is to certify that all the mistakes and errors pointed out by the external examiner have been incorporated in the thesis entitled “**Nursery evaluation of Willow (*Salix* spp.) Clones**” submitted to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni Solan (H.P.) by **Ms. Leena Kumari (F-2014-23-M)** daughter of Sh. Parshotam Ram in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE (FORESTRY)** in the discipline of **FOREST GENETIC RESOURCES**.

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LIST OF ABBREVIATIONS

bp	:	Base pair
cm	:	Centimeter
CTAB	:	Cetyl trimethyl ammonium bromide
CV	:	Coefficient of variation
DNA	:	Deoxyribosenucleic acid
df	:	Degree of freedom
dNTP's	:	Deoxyribonucleotide triphosphates
EDTA	:	Ethylene diamine tetra acetic acid
G	:	Genotypic
g	:	Grams
GCV	:	Genotypic coefficient of variation
M	:	Meter
MgCl ₂	:	Magnesium chloride
mm	:	Millimeter
NaCl	:	Sodium chloride
Ng/μl	:	Nanogram per microlitre
NTSYS-pc	:	Numerical taxonomy and multivariate analysis system
P	:	Phenotypic
PCR	:	Polymeric chain reaction
PCV	:	Phenotypic coefficient of variation
PVP	:	Polyvinyl Pyrolidone
RAPD	:	Randomly amplified polymorphic DNA
Rpm	:	Rotations per minute
S.D	:	Standard deviation
TAE buffer	:	Tris acetate EDTA
Taq	:	Thermus aquaticus
UPGMA	:	Unweighted pair group method with arithmetic averages
UV	:	Ultra violet

Chapter-1

INTRODUCTION

The restricted opportunities of wood production in natural forests and a rapid development of industrial processing of wood have brought about the decrease of timber supply and to the creation of the ever increasing deficit of wood as raw material. This problem has been somewhat mitigated by wood production in the plantations of fast growing tree species primarily poplars and willows (Markovic *et al.*, 2007).

Short rotation coppice (SRC) is a production method that utilizes fast growing and often hybridized tree species such as poplar (*Populus* spp.) and willow (*Salix* spp.). Such plantations are grown within a short rotation time of approximately three to five years but remain viable for 15 to 30 years given a coppice culture, a well-established method of increasing biomass yields while minimising costs (Morhart *et al.*, 2013). Fast growing woody crops are emerging as an attractive source of biomass because they have the capability to meet many of the agronomic, environmental and social parameters associated with successful deployment as a source of energy. Willows have many of the characteristics desired in dedicated energy crops, making them an excellent feed stock choice in a number of settings.

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 (Gupta *et al.*, 2014).

The willows, belonging to the genus *Salix* (Salicaceae), are one of the most important taxonomic entities of the world because of the great number of species and varieties. Willows are commonly found in three groups namely: arborescent willows, osiers or basket willows and sallows or pussy willows *i.e.* low shrubs to small trees with multiple stems (Singh *et al.*, 2012). The area under indigenous *Salix* species in the country is 108,000 hectare and only 28,300 hectare is under agroforestry (NPC, 2012).

Willow or Salicaceae family is a family of flowering plants. The word *Salix* is derived from Celtic 'sal' meaning near and 'lis' meaning water. Willows are largely shrub or forest

margin species rather than forest trees in their natural habitats and only few become ecological dominant. Willows range from prostrate shrubs to large trees over 30 meters high but most are shrubs or small trees. Willows diversity is high at all levels i.e. genetic, species and ecological with 520 species in the world, mainly in North-Temperate Zone, as arborescent willow and few (around 15 species) are tree willows. There are about 33 species present in India. Out of these most are categorized as shrubs except *S. alba*, *S. babylonica*, *S. daphnoides*, *S. fragilis*, *S. elegans* and *S. tetrasperma*.

Willow leaves are simple, generally long slender and oblong or lanceolate with smooth or toothed edges arranged alternatively on the branches and are rarely opposite. Willows are dioecous in nature; flowers are born in early spring, either before or after emergence of leaves. Both type of flowers are borne in catkins (a long cluster of flowers), which are carried erectly on the branch. The male flowers are more noticeable than the female flowers, the former being a yellow reddish when mature, very rarely male and female flowers are on the same tree. The pollination is mainly entomophilous and seeds ripen generally during May-June and are whitish in colour. Seeds are produced sparingly in cotton fibre.

Willows are typical short rotation species because of early fast growth. Their wood is excellent for pulp and also relatively high mechanical intensity. Moreover, willows have wide ecological adaptability. They have high tolerance to flood and drought, moderate tolerance to salt. Therefore, in willow genetic improvement programmes, on one hand, fast growing clonal selection should be promoted for intensive culture and industrial use. On the other hand, tolerance property against biotic and abiotic stress should be focussed so that they could be cultured extensively for commercial purpose (Mingjian *et al.*, 2004).

Willows are eco-friendly, multipurpose, fast growing and are widely used for plantation world over. *Salix alba* consists of three varieties *var. alba*, *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).

Cricket bat manufacturing industry is one of the most important wood based industries. Cricket bats are mostly manufactured by willow trees. The industry has an important role in foreign exchange and job creation and is expected to play an important part in future economic development. Growing willow of high quality can help this industry to become competitive at global scale and provide a pathway to international markets. Willow wood is also used in the manufacture of boxes, brooms, cradle boards, chairs and other furniture, dolls, flutes, poles, toys, turnery, tool handle, veneer and whistles. In addition tannin, fibre paper, rope and string can be produced from the wood (Monteoliva *et al.*, 2005)

Various species of *Salix* are grown all over the world for fodder, fuel, construction and environmental preservation because of their wide adaptability. The ability to outgrow the sprouting willow shoots after harvesting of aboveground biomass; makes it suitable for phytoremediation purposes of contaminated soils (extract heavy metals Cd, Zn and reduce organic pollution). Stands of willows reduce unfavourable effects of wind erosion and prevent soil erosion and have been traditionally used in protective structures (windbreaks, shelters and living walls) because the stands create a strong root system (Gerger *et al.*, 1999). Willows are comparatively better adapted than the poplar for monoculture as well as in agroforestry systems under short rotation forestry (6-10 years) for veneer, pulp, plywood, laminated wood, reconstituted wood products, artificial limbs, fruit boxes, agriculture implements, furniture, tool handles and sports goods like cricket bat, polo balls etc.

The essential purpose of tree improvement is to develop a suitable clones or variety that eventually brings about economic returns and related benefits to growers. An efficient and practical means of screening the genetic resources is essentially required (Luna and Singh, 2009). Knowledge of the genetic and environmental variation present in forest stands allows the development of adequate strategies for the selection of superior genotypes. A higher variability also permits a higher gain obtained by selection.

Large scale collection, evaluation and distribution of superior clones and interspecific hybrids are necessary for increasing biomass production (Zsuffa and Mosseler, 1986). In such collection evaluation and distribution process, proper identification of species and clones is essential.

The last few decades have witnessed the emergence of wide spread concern to solve the major problem of erosion of genetic resources. The only realistic solution of the

continuing loss of plant germplasm is the genetic conservation; that is the collection and systematic preservation of germplasm in gene resources centres with a wide representation of genetic diversity of the species as practicable.

The assessment of genetic variation is a major concern in plant improvement programmes. This is important for several reasons: first, the ability to distinguish reliably different genotypes important for designing the breeding programmes and population-genetic analysis, second, an estimation of the amount of variation within and between genotypes is useful for predicting potential genetic gain in a breeding programme and in setting up appropriate cross-breeding strategies. Traditionally, morphological and phenotypic characteristics have been used for these purposes. Since such characteristics are often controlled by multiple genes and subject to varying degrees of environmental modification and interaction, the differences between genotypes are not always absolute and reliable (Falconer, 1986).

Molecular markers are considered to provide the best estimates of genetic diversity since they are independent of the confounding effects of environmental factors. A genetic marker can be defined as a measurable character that can detect variation in either the phenotype or the genotype. Genetic markers can be based on visually assessable traits (morphological markers), on gene products (biochemical markers), or rely on a DNA assay (DNA markers). Molecular markers especially RAPD are being applied to a greater extent in forest trees to study genetic diversity. The invention of PCR in 1984 by Mullis (1990) brought the development of new marker technologies that were able to surmount some of the limitations of RFLP (Restriction fragment length polymorphism). The random amplified polymorphic DNA technique (RAPD) is a PCR-based technique. No prior sequence information is required for the design of RAPD primers and they can easily be applied to any organism (Hadrys *et al.*, 1992).

Use of molecular markers therefore provides an objective assessment of genetic diversity in a plant species and enables unequivocal identification of elite genotype. The RAPD technique has a wide range of applications, including genetic similarity analysis (Sulima *et al.*, 2009), fingerprinting (Castiglione *et al.*, 1993), and genetic mapping (Hadrys *et al.*, 1992).

Application of RAPD and SSR markers at population level provides the opportunity to explore the extent and distribution of genetic variation at a wide range of loci within the species.

Keeping this in view, the current investigation entitled **“Nursery evaluation of willow (*Salix spp.*) clones”** was carried out with the following objectives:-

1. To determine the clonal variation for morphometric traits.
2. To workout the wood characteristics of selected clones.

Chapter-2

REVIEW OF LITERATURE

A genetically variable population and a method of selecting superior individuals provide the basis for tree improvement by breeding. The studies pertaining to variation help in analysing the natural pattern of variation and group of organisms into species, genera and families. The more variable a tree species, the more likely it is to respond to its improvement. The prime objective of a breeding programme is the improvement of desirable qualitative and quantitative characters like yield, fodder/ timber quality etc. Willow provide a wide range of genetic diversity and its breeding programme should focus on improving growth, optimizing relevant traits and lowering production costs to ensure long term variability of willow crop systems for producing energy, restoring degraded sites and improving water quality.

In the present investigation, “**Nursery evaluation of willow (*Salix* spp.) clones**”, various parameters were studied regarding the variability among hybrid for growth, wood traits and through molecular characterization. The relevant literature is reviewed on these parameters and presented under following broad heads:

2.1 Growth performance and biomass studies

2.2 Wood characteristics

2.3 Molecular characterization

2.1 GROWTH PERFORMANCE AND BIOMASS STUDIES

Genetic variation in short rotation *Salix* species has been demonstrated for different growth and wood characters by various workers. The results showed large variation between species and also between clones within species (Ager *et al.*, 1986). The existence of genetic variation for dry matter production has also been proven beneficial in improvement programmes (Vihero-Aarino, 1988; Gullberg, 1989). Vihero-Aarino (1987) estimated broad sense heritabilities for different coppicing character and for production in different *Salix* hybrids.

Orlovic *et al.* (1998) studied genetic variability in anatomical, physiological and growth characteristics of hybrid poplar and eastern cottonwood clones. At the end of rotation

period, the main plant growth elements (diameter, height and dry weight biomass) were measured and results showed high interclonal variability for most parameters. The leaf area was strongly correlated with height. Similarly diameter and height were found significant and positively correlated with dry weight biomass, which were found significant and positively correlated with dry weight biomass, which is the indicator of productivity.

Saini *et al.* (2002) studied the effect of stem cutting diameter on growth and survival of *Salix* clones in nursery under shallow water, tarai condition of Pantnagar. Maximum collar diameters (2.07cm, 2.18cm) were recorded in SI clones and D4 diameter class, respectively, whereas, maximum heights (258cm, 233cm) were observed in S3 clone and D4 diameter class, respectively.

De Cock *et al.* (2003) studied the diversity of the willow complex *Salix alba* - *S. rubens* - *S. fragilis*. Multivariate analysis revealed the division of the willow complex into two groups. The genetic analysis showed that all the varieties displayed a significant level of genetic distance between the individuals.

Tharakan *et al.* (2005) studied the variation in 11 morphological traits among 30 willow clones at the end of a 3-years rotation and determined its relationship to biomass production. A mix of tested clones was used with the objective of comparing the productivity of untested clones and evaluating their performance relative to superior performance that had been previously identified. SV1 (*Salix dasyclados*) was used as a reference clone. The 30 clones were grouped into five clusters; these clusters were significantly different based on the pair wise comparison of the distance from centroid. The difference in strategies was apparent at the species level as well and they concluded that the multiple ideotypes needed to be incorporated into willow breeding and selection efforts in the management.

Smaliukas *et al.* (2008) studied three *Salix viminalis* and four *Salix dasyclados* genotypes for the evaluation of morphobiological, biomass and annual energy characteristics of their genotypes in short rotation plantations. The highest bushes reached up to 578 cm (*S.viminalis* 'Tora') and 504 cm (*Salix dasyclados* 9977) in height after four years of growth. There was a tendency of change of the number of sprouts per bush, the maximum number were reached during second year of growth, while it decreased during the third and the fourth years. The thickest stems were characteristics of *S.dasyclados* (4.1cm) and *Salix dasyclados* × *Salix viminalis* 04120 (3.5cm) genotypes. The obtained data showed that *Salix*

dasyclados 04122 and 9977, *Salix dasyclados* × *Salix viminalis* 04120 and *Salix viminalis* ‘Americana’ 9976 were the most valuable biomass producers.

Mleczek *et al.* (2010) studied selected *Salix* clones to determine capacities for biomass production and accumulation of heavy metal ions and found that *S.alba* var. *Chermesina* and, *S. viminalis* ‘1056’ were the taxa characterized by the greatest biomass production (6.8 and 4.3kg of fresh mass per shrub per year respectively). The results revealed significant differences among clones. The greatest cellulose content was observed in *Salix viminalis* ‘Sprint’ (49.69 %) and the lowest in *Salix viminalis* ‘1059’ (42.09 %).

Weger and Bubeník (2011) evaluated experimental plantation with clones of native willows and their natural hybrids (*Salix alba*, *Salix* × *rubens*, *Salix viminalis* and *Salix* × *smithiana*), which were grown in 3-years rotation on locality with suitable growing conditions. Biomass yield in four harvests and tree height, number and diameter of stems and survival rate were measured as well. Yields were statistically evaluated and significant differences were found. Four best willow clones reached yields 13.1–18.9t(DM)/ha/year in average from whole experiment. It was possible to conclude from collected results that native willows can reach very good biomass yields on suitable sites

Slepetyes *et al.* (2012) established the biomass potential of 14 grass species and 3 tree species grown for the bioenergy purpose. Amongst the tree species, the largest amount of dry biomass was produced by *Salix viminalis* 32.7-38.1 t ha⁻¹ and *Populus nigra* 24.0-33.01 t ha⁻¹. When forest plants were grown in arable land, nitrogen fertilization had little effect on biomass yield, a biomass reduction trend was observed at N120 kg/ha fertilization. *Salix viminalis* had also low nitrogen and sulphur contents. *Salix viminalis* was best suited for combustion.

Singh *et al.* (2012) evaluated juvenile growth traits in various clones of *Salix* at UHF Nauni. Significant differences were recorded for morphological trait among test clones and test verses check clones except in leaf length among test clones and volume index, leaf length and petiole length among check verses test clones. The clones SI-63-016, J-799, PN-722, NZ-1002, PN-733, PN-731, SN-2, S×61 194 and 084/03 were found superior to check clones for growth traits namely height, basal diameter and volume index. Heritability in broad sense (91.08%) and genetic gain (75.24%) were found highest for leaf breadth and volume index respectively. Principal component analysis through correlation matrix revealed that three out

of eight components contributed 85.03% of the overall variation out of which the principal explains 38.57% of the variance weighted maximum (0.97) by volume index.

Similarly, Sankanur (2013) also revealed significant differences for all the nursery characters in *Terminalia chebula*. Further, mean values varied between 47.62 to 100.00 for germination percentage, 30.00 to 100.00 for survival percentage, 1.36mm to 3.38mm for collar diameter, 12.80cm to 20.80cm for plant height and numbers of leaves (8.50 to 15.43) for intra-specific hybrids.

Han *et al.* (2013) studied seven clones of one-year-old willow (*Salix caprea* L.) under field conditions and monitored height and diameter growth, extractive content, lignin content, carbohydrate composition and energy content. The highest above ground biomass was represented in clones BH5 and BH11. There were also significant differences among seven clones of *S. caprea* in terms of chemical composition, carbohydrate composition, elementary composition and energy content.

Singh *et al.* (2014) evaluated twenty-five clones from five countries for various nursery growth and wood characteristics. Maximum values for plant height (290.35cm) and collar diameter (17.38mm) were recorded for clone 799 (U.K.). The highest value for shoot fresh weight (243.25gm), shoot dry weight (138.94gm), root fresh weight (184.05gm) and root dry weight (102.87gm) were recorded for Kashmir willow (U.K.). Clone SE-75-001 (Italy) and Kashmir willow (India) showed the highest values in wood density (0.58) and fibre length (0.81), respectively. Heritability (broad-sense) was recorded maximum (99.78%) for leaf area followed by wood density (56.10%), and fibre length (55.29) depicting stronger genetic control of these characteristics. Maximum genetic gain (61.58%) was recorded for leaf area that was closely followed by number of branches (60.39%). Growth characteristics were found to have positive and significant correlation with most of the characters; only wood density was recorded to have negative correlation with other traits.

Demo *et al.* (2014) carried out studies in the fourth year of the first rotation cycle of willow and poplar aimed at evaluation of production parameters. There were four varieties of willow (*Salix* spp.) and four varieties of poplar (*Populus* spp.) each included in the experiment. The studied willow varieties were Inger, Express, Klara and Dimitrios, and the poplar varieties were Monviso, Pegaso, AF-02 and Sirio. The results of the individual production parameters showed that the number of shoots per individuals were higher in

willow varieties. The willow and poplar varieties with the highest average number of shoots had the lowest average shoot lengths. The varieties with the lowest average number of shoots had the highest average shoot diameters. The highest yield of dry matter of poplar was achieved by the variety Monviso (87.16 t ha^{-1}), while in willows it was the variety Express (94.50 t ha^{-1}). All willow and poplar varieties exceeded the economic production threshold of $10\text{--}12 \text{ t ha}^{-1}$ in dry matter. The dry matter of willow varieties ranged from 17.92 to $23.64 \text{ t ha}^{-1} \text{ year}^{-1}$ whereas the dry matter of poplar varieties ranged from 16.85 to $21.79 \text{ t ha}^{-1} \text{ year}^{-1}$.

Sharma *et al.* (2014) evaluated the promising *Salix* species/clones and hybrids. After nursery screening, the selected clones were planted in the field. These selected clones were repeatedly raised in nursery every year from 2011 to 2013 to find out the most adaptive and superior clones along with check clone (Kashmiri willow). Plant survival, height and basal diameter were recorded and volume index was calculated. The data revealed significant differences among clones. Plant survival was at par among clones except in clones J194, NZ1002 and V-311. Maximum plant height (389.77 cm) was recorded by clone J799 followed by clones J795 (355.36 cm) and NZ 1002 (344.95 cm). Basal diameter was recorded at par in the clones SI-64-017 (17.66 mm), J799 (17.52 mm), NZ1002 (16.49 mm), 131/25 (16.25 mm) and NZ1140 (16.11 mm). The clone J799 recorded maximum (1329 cm^3) volume index, which was at par with clone SI-64-017 (1171 cm^3) followed by clone NZ 1002 (1015 cm^3). Check clone Kashmiri willow remained at 5th, 6th, and 7th position for basal diameter, plant height and volume index, respectively.

2.2 WOOD CHARACTERISTICS

Deka *et al.* (1994) studied the variation of specific gravity, fibre length and cell wall thickness in young *Salix* clones. He reported that the clone with longest fibre was 1.34 times, that of clone with shortest fibre and the clone with long fibre were also found to have high specific gravity.

Klansja and Kopitovic (1997) studied structural, physical characteristics and chemical composition of wood of some white willow clones (*S. alba* L.) of age 13 grown at Popular Research Institute, Novisad and found that the average length of wood fibres was above 1 mm except for one clone 79/64/2 whose fibre length was noticed to be 0.9 mm . The nominal wood density was found 340 kg m^{-3} .

Pan *et al.* (1998) studied genetic variation on fibre length in willow stock of different hybrids and clones and found significant differences in fibre length of clones at sapling stage. There were also significant differences in fibre length/diameter among different ages of the same clone.

Singh and Huse (2004) studied wood characteristics viz., moisture content, bark per cent, wood per cent, fibre length and basic density for variability estimates and genetic parameters on 23 clones of 7 species the variation was found maximum (4.89) in bark per cent and minimum (1.80) in wood per cent. The percent genotypic coefficient of variation (GCV) was maximum (11.27) for bark percent and minimum (3.36) for wood per cent. Maximum per cent heritability ($h^2_{bs} = 83.10$) was present for the bark per cent whereas minimum for basic density ($h^2_{bs} = 76.30$). Genetic gain as per cent of mean was recorded maximum (21.33) for bark per cent.

Lieven *et al.* (2004) selected 100 willows clones of *Salix alba*, *Salix fragilis* and their crosses *S. × rubens* at the age of 7 to 8 years for the study of physical properties such as density, moisture content, shrinkage and swelling, amount of tension wood, annual growth and mechanical quality based on static and dynamic bending strength, modules of elasticity and compression strength. They observed that *Salix fragilis* and its hybrids have a significantly higher density, modulus of elasticity and bending strength than the group *Salix alba* and its hybrids. They concluded that the selection of willow clones should be made based on minimum requirements on physical and mechanical properties.

Monteoliva *et al.* (2005) studied the variability of wood basic density and fibre length in six 13 years old clones of willow under two different site conditions. They observed that site influence was significant for basic density which ranged between 0.364 kg/dm^3 and 0.455 kg/dm^3 and for fibre length mean values vary between $837.1 \mu\text{m}$ - $1142.1 \mu\text{m}$. They concluded from heritability values that the clones “Americano” and A-13-44 stand out because of their high density and long fibres.

Zhang *et al.* (2006) selected some clones of Chinese fir (*Cunninghamia lanceolata*) with fast growth and superior timber properties through excellent provenances, population and individual trees. There were significant differences among these clones in terms of wood basal density and fibre length. The average wood basal density and fibre length of these selected clones were 0.3193 gcm^{-3} and $3317 \mu\text{m}$, respectively, in 12 years old trees.

Mani and Parthasarathy (2007) reported the basic wood specific gravity of 41 tree species determined by oven-dry weight by volume which ranged from 0.46 to 0.92 gcm⁻³ for inland sites and 0.47 to 0.89 gcm⁻³ for the coastal sites of tropical dry evergreen forests of peninsular India.

Kilmewa (2010) while analyzing genetic parameters for various wood characteristics found highly significant positive genotypic and phenotypic correlation coefficients between diameter and wood percentage while highly negative genotypic and phenotypic correlation coefficients were noticed between wood percentage and bark percentage.

Wani and Khan (2010) reported significant variation in the wood anatomical features viz. fibre length, fibre diameter, fibre wall thickness from different positions of trees of *Populus nigra* L.

Variation of physical and biometrical properties of willow (*Salix* spp.) wood along the stem were analysed by Kord (2011). Results indicated the considerable difference within the tree for different properties. The fibre length, fibre diameter, fibre wall thickness, dry density, basic density and volumetric shrinkage of *Salix* wood decreased along the stem from the base upwards. However, the lumen diameter and porosity increased with the increase in height of tree.

Pande (2011) studied 10 clones of *Populus deltoides* Bartr. Ex Marsh. raised by WIMCO Plantations Ltd. at Rudrapur (Udhamsingh Nagar), India. He reported that within ramet variation due to radial location was significant for specific gravity with increasing trend from pith to periphery. Variation for the d.b.h. was also significant for the clones. Female parents showed higher specific gravity than the male parents.

Vergas *et al.* (2012) studied the physical and mechanical wood properties of *Salix viminalis*, *S. × rubens*, *S. purpurea* and *S. species* cultivated in the Canoas River Valley, in the Serra Catarinense region of the state of Santa Catarina, Brazil. They found that there are differences in the physical and mechanical properties of the species. *S. viminalis* and *S. purpurea* were similar in density, modulus of elasticity and higher values of tensile and strength modulus than *Salix × rubens* and *S. species*.

Gupta *et al.* (2014) estimated genetic variability, heritability (broad sense), genetic advance, and genetic gain as per cent of mean and correlation coefficient for wood density and fibre length in 36 clones of white willow (*Salix alba* L.). 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 showed 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.

Huda *et al.* 2014 studied the clonal variation of selected physical and mechanical properties of seven hybrid poplar clones grown at three sites in southern Quebec, Canada. They found that all properties of hybrid poplar wood showed significant interclonal variation in wood properties, especially density, flexural modulus of rupture and ultimate crushing strength. They also reported that the high heritability values of these properties were under moderate to high genetic control. The genetic gain for these properties was ranged from 2.0% to 13.5%.

Sharma *et al.* 2014 investigated the variability in growth and wood properties of the willow clones. The growth and wood traits of four years old 21 clones were investigated. Analysis of variance showed that clones differed significantly for all the studied growth and wood traits. Among the growth parameters the maximum heritability value was showed by height (92%) and minimum (59%) was recorded for wood and bark percent. The values for phenotypic and genotypic coefficients of variability, heritability, genetic advance and genetic gain ranged between 2.51-35.63%, 2.09-30.49%, 24-92%, 0.03-31.20 and 3.95-53.73%, respectively.

Raja (2014) while evaluating the variation with respect to wood density and fibre length in 162 clones along with 8 checks of willow reported that the wood density ranged from $0.40\text{--}0.56\text{ gcm}^{-3}$ with mean value of 0.48 gcm^{-3} whereas fibre length ranged from 0.61-0.79 mm with the mean value of 0.70 mm. Heritability value observed for wood density ($h^2=0.38$) was higher in comparison to fibre length ($h^2=0.29$).

2.3 MOLECULAR CHARACTERIZATION

Molecular markers are being applies to a greater extent in forest trees (Forrest, 1994). With the advent of PCR technique (Mullis and Faloona, 1987) a deluge came in molecular

marker studies by its multifarious applications. Soon after Saiki *et al.* (1998) reported novel analysis of DNA on the amplification of DNA sequencing using PCR ; after which many techniques have been developed based on this basic polymerase chain reaction. Out of several approaches developed, the most widely used method is random amplified polymorphic DNA (RAPD) (William *et al.*, 1990).

Barker *et al.* (2003) tested 46 microsatellites isolated from an enriched library of *Salix burjatica* on 20 individuals from national willow collection U.K. Out of 46, 17 microsatellites gave 2-22 alleles/locus. Three microsatellites successfully cross amplified in 31 additional *Salix* species and further they were tested 6 on panel comprising 6-25 individuals from the 31 species. The results suggested that microsatellites isolated were useful for population studies in a wide range of *Salix* species.

Gupta (2005) assessed genetic variation in 36 genotype of *Salix alba* from 7 countries using RAPD-PCR technology. RAPD pattern was studied using 10 primers; 5 showed amplification with 100% polymorphism. All 55 RAPD markers were amplified in the size ranging from 280bp – 165bp. Maximum similarities of 87% was observed between SI-64-036 and Veliki bajar clones.

Fossati *et al.* (2005) fingerprinted a collection of 66 poplar commercial clones widely cultivated in Italy, China, Countries of Southern Europe and belonging to various poplar species and hybrids using both amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) techniques. High level of variation was detected with both molecular techniques. A total of 201 AFLP bands were amplified out of which 96% turned out to be polymorphic. Up to 15 SSR alleles were identified at a single locus with a mean of 9.3 alleles per locus in the case of *Populus × Canadensis*.

Mallikarjuna *et al.* (2005) carried out the genetic diversity studies on 32 accessions of wild species of *Arachis* belonging to 25 species, grouping under six sections of RAPD. They used 29 primers for the study. They observed that the similarity values ranged from 0 to 49 per cent. On the basis of their study they explained the grouping of wild species as per their relationships with each other.

Lin and Smart (2005) reported the use of microsatellites markers to assess genetic diversity of willow species. They tested 26 microsatellites loci that were originally derived

from *Populus* and 19 microsatellites loci that were isolated from diverse *Salix* species. Similarly Rajora and Rahman (2005) reported the use of *Poplar* microsatellites DNA markers in willow.

Kant *et al.* (2006) carried out RAPD analysis to determine genetic diversity of *Pinus gerardiana* in Himachal Pradesh. Twenty five genotypes of *Pinus gerardiana* growing at different locations in the distribution range of species were sampled randomly for analysis. Thirty random primers generated 413 fragments of which 390 (94%) were polymorphic in nature. Eleven of the 24 individual genotypes were distinguish by unique bands specific to them. The similarity coefficient values suggested a wide genetic base of genotypes taken in experiment. An UPGMA dendrogram based on similarity coefficient indices indicate that genotype did not cluster according to their site of collection. This can be attributed to highly cross-pollinating nature of the species and small distribution range in the area.

Meneghetti *et al.* (2007) used AFLP, ISSR and RAPD markers to assess the relationship among genotypes of *S. alba* and *S. fragilis*. Since the two species were differentiated into distinct subgroups on the basis of their genomic DNA fingerprinting, molecular markers could represent a reliable tool for the rapid and precise identification of *S. alba* and *S. fragilis* individuals. Moreover, all marker system showed that the two species possess a number of private alleles so that the two gene pools appear differentiated. Species diagnostic RAPD markers were also identified by Triest *et al.* (1999) in progenies obtained from controlled crosses between *S. alba* and *S. fragilis* parents.

Vijayan *et al.* (2008) studied species differentiation among the genotypes of the genus *Morus*. Attempts were made to elucidate the genetic relationships among 18 mulberry genotypes collected from India and Japan using 15 Inter Sequence Repeats (ISSR) and 15 Random Amplified Polymorphic DNA (RAPD) primers. The ISSR primers generated 81.13% polymorphism while the RAPD generated 71.78% polymorphism.

Kumar *et al.* (2011) analysed genetic diversity of 40 clones of *Dalbergia sissoo* Roxb. using Random Amplified Polymorphic DNA (RAPD) markers by selecting 30 decamer primers, which were later reduced to 10 based on the preliminary PCR amplification. A total of 129 distinct DNA fragments (bands) were amplified of which 104 bands were polymorphic ranging from 200 to 1300 base pairs. The number of amplified bands per clone varied from 11 to 15 and polymorphism percentage ranged from 18.18 to 100 with an average

of 79.23. Jaccard similarity coefficient ranged from 0.66 to 0.95 showing a wide range of variability among the clones. The maximum similarity was found between clone 10 and 12 and the most dissimilar clones were 9032 and 5030 with minimum similarity coefficient of 0.66. In fact clone 5022 was found to be the most divergent which could be used for number of combinations to be established in the seed orchards and as a parent in hybridization programme.

Andleeb *et al.* (2011) studied the genetic diversity in *Salix viminalis*, using AFLP, which revealed that four cultivars taken for the study were highly diverse at genetic level. The four primers used generated a total 240 bands 197 (82%) were polymorphic over all the genotypes. The capability of different primers to generate polymorphic AFLP markers varied significantly ranging from 40-50 polymorphic bands per primers over all the genotypes that confirm the high multiplex ratio expected from AFLP technique.

Singh *et al.* (2013) evaluated 25 promising willow clones from six countries after nursery screening. Molecular diversity among the clones was estimated using 16 RAPD and 14 SSR primers. A total of 363 markers were generated and out of which 351 were showing high (96.7%) rate of polymorphism. Out of 30, 18 primers showed 100% polymorphism. RAPD markers widely scored over SSR markers in polymorphic information content. UPGMA dendrogram based on both RAPD and SSR markers resolved into four major clusters. The similarity coefficient among 25 clones of willow ranged from 0.71 to 0.86. Maximum similarity (86%) was observed between 795 and PN-721 and SI-63-007 and V-99. Thus, these genotypes showed maximum degree of similarity in their genetic makeup. However, minimum values were observed between SE-69-002 and 84/11 (0.71) followed by SE-69-002 and 17-93-A (0.72). RAPD and SSR analysis proved helpful for estimating the magnitude of genetic diversity at molecular level. Clustering further indicated that the geographic distribution may not be the true index of genetic diversity in willow clones. On the basis of banding pattern RAPDs and SSRs were effectively used for molecular characterization of willow clones used in this study.

Sahu and Koche (2013) accessed the genetic diversity within each population, among the populations and also in micro propagated plantlets of *Terminalia bellirica* species (Roxb.) using random amplification amplified polymorphic DNA (RAPD) marker. Twenty primers were used for RAPD analysis. RAPD analysis yielded 168 total bands of which 152 polymorphic bands (90%polymorphism), with an average of 8.4 amplified bands per primer.

The Dendrogram was constructed using UPGMA method. Results revealed very high level of genetic diversity in the species. These results also indicate that RAPD is a good molecular marker to study the genetic diversity of these species.

Mei *et al.* (2014) genetically characterized *Ginkgo biloba*. DNA was extracted from the leaves of these samples for inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) analysis. ISSR analysis showed high genetic variation among the nine varieties of *G. biloba*; the polymorphism and similarity coefficients were 87% and 0.40 - 0.84, respectively. RAPD analysis also showed 93% polymorphism, and the similarity coefficients ranged from 0.44-0.87. Study also suggested that ISSR and the improved RAPD markers are useful molecular tools for the genetic characterization of plants.

Ukwubile *et al.* (2014) studied the genetic characterization of 94 genotypes (4 female, 10 male, and 80 half sibs) of *Salix* collected from reserve forest Enugu, Nigeria were analyzed using 10 SSRs and 15 RAPDs PCR-based molecular markers. RAPD analysis yielded 87 polymorphic fragments (98.9%), with an average of 5.8 polymorphic fragments per primer. Similarly, SSR analysis produced 33 bands, out of which 26 were polymorphic (78.8%) with an average of 2.6 polymorphic fragments per primer. The genetic diversity was high among the genotypes (Nei's genetic diversity = 0.354 and Shannon's information index = 0.536) as measured by combination of both RAPD and SSR markers. The mean coefficient of gene differentiation (G_{st}) was 0.037, indicating 96.6% of the genetic diversity resided within the genotypes. It was found that the genetic diversity among genotypes was broader suggesting the importance and feasibility of introducing elite genotypes from different hybrids for willows germplasm conservation and breeding programs.

Thakur *et al.* (2014) estimated molecular diversity among 23 promising progenies of *Ulmus villosa*, which were raised from the seeds collected from various seed sources in Himachal Pradesh (India), using 10 RAPD primers. A total of 57 markers were generated, all of the 10 primers showed 100% polymorphism. The similarity coefficient among 23 progenies of *U. villosa* ranged from 0.00 to 0.70. Progeny Jugahan-T was found to be the most divergent which separated itself from rest of the progenies at similarity value (0.04) and could be used as a parent in hybridization programme and further improvement programmes. RAPD analysis proved helpful for estimating the magnitude of genetic diversity at molecular level.

Verma *et al.* (2015) used RAPD (random amplified polymorphic DNA) and ISSR (inter simple sequence repeats) markers to study DNA polymorphism of thirty *Grewia optiva* genotypes raised from seeds collected from various districts of Himachal Pradesh (India) and selected on the basis of morphological parameters. Using 25 RAPD and 18 ISSR primers molecular diversity based on Jaccard's similarity coefficients among 30 genotypes ranged from 0.17-0.83 in RAPD assay and 0.30-0.84 in ISSR assay. RAPD primers amplified more loci than ISSR primers. RAPD and ISSR primers amplified 96.31 and 91.72% polymorphic loci, respectively. Genotype SO-12 (Kasauli) was found to be the most divergent genotype which could be used for number of combinations to be established in the seed orchards and as a parent in hybridization programme.

Chapter-3

MATERIALS AND METHODS

The present investigation entitled “Nursery evaluation of willow (*Salix* spp.) clones” was carried out in the Department of Tree Improvement and Genetic Resources, Dr. Y. S. Parmar University of Horticulture and Forestry, Solan (H.P.) during 2015-16. The experimental details relating to clonal material, experimental site, material and methodology adopted for study are described under following headings:

3.1 CLONAL MATERIAL

3.2 EXPERIMENTAL SITE

3.3 EXPERIMENTAL PROCEDURE

3.4 RAPD STUDIES

3.5 STATISTICAL ANALYSIS

3.1 CLONAL MATERIAL

The present investigation included 42 willow clones. The 42 clones were selected based on their performance from initial screening experiment done on the basis of plant height and collar diameter in the departmental nursery (main campus, Nauni).

Ten cuttings of each clone of standard size were planted at 40cm × 40cm spacing in 4 replications in RBD design. The details of clones are given, in Appendix-I.

3.2 EXPERIMENTAL SITE

The experiment was conducted in the main campus of Dr. Y. S. Parmar University of Horticulture and Forestry in the experimental farm near Administration Block and study of wood and molecular characteristics was carried out in the departmental laboratory.

3.3 EXPERIMENTAL PROCEDURE

3.3.1 Study of growth and biomass characters

3.3.1.1 Plant height (cm)

The height of the shoot was recorded from the ground level to the apex leading shoot by measuring scale.

3.3.1.2 Basal diameter (mm)

Shoot diameter of the plant in the nursery was measured with the help of digital calliper at region 5 cm above ground level.

3.3.1.3 Volume index (cm³)

Volume index for each clone was calculated by using following formula (Hatchel, 1985 and Manavalan, 1990).

$$(\text{Diameter})^2 \times \text{Height}$$

3.3.1.4 Number of branches per plant

The number of branches per plant was recorded on the basis of five randomly selected ramets of each clone.

3.3.1.5 Number of nodes

The total numbers of nodes were counted when the shoots were brought to laboratory for anatomical studies.

3.3.1.6 Internodal length (cm)

Internodal length was measured as the distance between two consecutive leaves on the main axis at 30 cm, from the base with the help of measuring scale.

3.3.1.7 Leaf area (cm²)

Five fully expanded leaves per ramet were selected for this purpose. The leaves were randomly detached from different ramets and put in envelopes. These leaves were brought to the laboratory and the leaf area was determined by using portable leaf area meter (CI-203, INC.USA). Each leaf was placed under arm leaf area meter and thereafter scanned to determine the leaf area digitally; procedure was repeated for rest of the samples. Leaf area was expressed in cm².

3.3.1.8 Fresh shoot weight (g)

The fresh shoot weight for each ramet was recorded by using electronic pan balance.

3.3.1.9 Dry shoot weight (g)

The fresh shoots were packed in paper bags and dried at 80⁰C in an oven over 96 hours till constant weight was attained. Then the dry shoot weight was recorded using electronic top pan balance.

3.3.1.10 Fresh root weight (g)

The root fresh weight was recorded in the month of November by using electronic top pan balance as that fresh shoot weight

3.3.1.11 Dry root weight (g)

The dry root weight was recorded similar as to that of shoot dry weight.

3.3.1.12 Total dry biomass (g)

Total dry biomass was calculated by adding dry shoot weight and dry root weight.

3.3.2 Study of wood characters

3.3.2.1 Moisture content (%)

The fresh weight of the samples was recorded just after the samples were removed from the plants. Then after weighing the samples were transferred for drying in oven. For first few hours the oven was run at 60⁰ C then temperature was raised to 105⁰ C and the samples were left in oven for overnight. The samples were then again weighted till the difference between the last two weighing values was less than 0.2 per cent.

The moisture per cent of the samples was calculated by using the formula given by Desch and Dinwoodie (1996).

$$\text{Moisture content (\%)} = \frac{M_{\text{init}} - M_{\text{od}}}{M_{\text{od}}} \times 100$$

Where,

M_{init} = Initial weight of sample (g)

M_{od} = Oven dried weight of sample (g)

3.3.2.2 Specific gravity (gcm⁻³)

Specific gravity of the samples was determining by the maximum moisture content method (Smith, 1954). The green weight of sample was taken at maximum moisture content. Then these samples were kept in oven dried at 105±3⁰C for drying until a constant weight

was attained. The specific gravity is determined for these samples separately as per the formula given below:

$$\text{Specific gravity} = \frac{1}{\frac{M_m - M_o}{M_o} + \frac{1}{GS}}$$

Where,

Mm = Fresh/Green weight of the sample having moisture

Mo = Oven dried constant weight of the sample

GS = Average density of wood substance, a constant, having value 1.53

3.3.2.3 Bark per cent (%)

For determination of bark per cent, the selected wood samples (5 cm in length) were debarked and kept for oven drying by following the same procedure as for moisture content (%) determination. Then oven dry mass of bark and bark plus wood were measured with the help of electronic balance. Bark per cent was then calculated as:

$$\text{Bark per cent} = \frac{\text{Oven dry bark weight}}{\text{Oven dried bark} + \text{oven dried wood weight}} \times 100$$

3.3.2.4 Wood per cent (%)

Wood per cent is determined by using the following formula:

$$\text{Wood per cent} = 100 - \text{Bark per cent}$$

3.3.2.5 Fibre length (mm)

For fibre length measurements, small segments were removed from the wedges. Fibres were macerated in Jeffery's fluid, i.e. 10 per cent chromic acid and 10 per cent nitric acid, for 48 hours. Pulp was thoroughly washed with water, stained with 2 per cent aqueous safranin, teased in 10 per cent glycerine and mounted with cover slip. The length of minimum 10 randomly selected fibres was measured with the help of ocular micrometer scale standardized with the help of stage micrometer.

3.4 RAPD STUDIES

In the present study 25 clones of *Salix* spp. were assessed for their genetic variation using RAPD markers. Thus, to assess the variability the following methodology was followed.

3.4.1 DNA Isolation

Nuclear DNA was extracted from young leaves using the protocol described by Doyle and Doyle (1987) with slight modification.

3.4.1.1 Solution for DNA extraction and purification

10% (w/v) CTAB (N-cetyle N,N,N-trimethyle-ammonium bromide) - 10g of CTAB was dissolved in 70ml of water at 65⁰C then final volume was made to 100ml.

0.5M EDTA (pH 8.0) – 93.05g of ethylene diamine tetra acetate was added to 400ml of water. The pH of solution was adjusted to 8.0 by addition NaOH. The final volume was made to 500ml and sterilized by autoclaving.

5M NaCl – 146.1g of sodium chloride was dissolved in 400ml of water and the final volume was made to 500ml and sterilized by autoclave.

Phenol: Chloroform: Isoamyl alcohol (25:24:1) - 750µl Phenol, 720µl Chloroform and 30µl Isoamyl alcohol were mixed by vortexing. The resulted solution was kept at 4⁰C in coloured container.

IM Tris –60.58g Tris base was dissolved in 400ml of water and the pH was adjusted to 8.0. The final volume was made to 500ml and sterilized by autoclaving.

Phenol: Chloroform (24:1) - 720µl Chloroform and 30µl Isoamyl alcohol were mixed by vortexing. The resulted solution was kept at 4⁰C in coloured Container.

DNA extraction buffer –DNA extraction buffer contained 100mM Tris-HCl, 20mM EDTA, 1.4M NaCl, 2.0(w/v) CTAB and 0.2% (v/v), 2-Mercaptoethanol.

3.4.1.2 DNA extraction and purification

- Leaf material (1g) was grounded to fine powder in liquid Nitrogen (-196⁰C) in a pre-chilled pestle and mortar.
- The powder was transferred to a 15ml centrifuge tube containing 7ml extraction buffer the contents were mixed well by gentle inversion and the tube was incubated at 65⁰C for about 1 hour.
- Following incubation, 7ml of Chloroform: Isoamyl alcohol (24:1) was added and the contents were mixed to form an emulsion.

- The emulsion was centrifuged at 15,000 rpm for 15 minute at 4⁰C temperature.
- The upper aqueous phase was carefully transferred to fresh 15ml centrifuge tubes without disturbing the interface.
- The nucleic acids were precipitated by addition of 4ml of cold (-20⁰C) Isopropanol.
- The DNA pellet was precipitated by centrifugation at 13, 000 rpm for 12 minutes at 4⁰C.
- The pellet was collected by centrifugation at 13, 000 rpm for 5 minutes at 4⁰C and washed with cold (-20⁰C) 70% Ethanol.
- After final centrifugation at 13, 000rpm for 15 minutes at 4⁰C the supernatant was drained and pellet was dried in vacuum drier approximately 20 minutes and resuspended in 400µl of sterile distilled water.
- 5µl of RNase (10mg/ml) was added to each sample, mixed gently and incubated for 1 hour at 37⁰C.
- Added equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) and mixed thoroughly to emulsify.
- The resultant solution was spun at 12, 000 rpm for 10 minutes and upper aqueous phase was transferred to new centrifuge tube.
- Added 1/10th volume of 3M Sodium acetate (pH 5.2) and two volumes of absolute ethanol (95%) were added and mixed by gentle inversion and kept in ice for 30 minutes.
- Spun at 10, 000 rpm for 10 minutes to pellete the DNA.
- The DNA pellet was washed with 70% Ethanol.
- The pellet was dried in vacuum drier and dissolved in sterilized water.

3.4.2 DNA amplification

DNA amplification was carried out for RAPD analysis using decamer random primers. A total of eleven decamer primers were used in the present study out of which nine gave polymorphic amplification products. Others gave no amplification product. The sequences of primer are given in Table 8.

3.4.2.1 Standardization of concentration of template DNA and RAPD studies

There is a necessary knowing of the concentration of DNA that can give us maximum amplification products. For standardizing the concentration of template DNA, PCR amplification was performed with different DNA concentration using previously standardizes

master-mix concentration. Five different concentration of DNA were used which were 5ng, 10ng, 15ng, 20ng and 25ng, for obtaining the maximum number of amplification products.

3.4.2.2 Standardization of PCR amplification conditions

Tests were performed for standardizing polymerase chain reaction amplification conditions mainly the annealing temperature. PCR amplification conducted at different annealing temperatures *i.e.* 36⁰C, 37⁰C, 38⁰C using standard concentrations of various components of reaction mixture.

S.NO.	Reaction		Temperature and Time specifications	Number of cycles
1	Initial Denaturation		94 ⁰ C-for 3 min	1
2	A	Denaturation	92 ⁰ C-for 45 sec	45
	B	Annealing of Primer	36 ⁰ C-for 1 min	
	C	Primer amplification	72 ⁰ C-for 2 min	
3	Final amplification		72 ⁰ C- for 10 min	1
After amplification			4 ⁰ C- till electrophoresis	

3.4.3 Amplification of DNA by PCR

The amplification reactions were performed in a total volume of 25µl containing.

Sterile distilled water	15 µl
Taq buffer (10X)	2.5 µl
dNTPs (2.5mM)	1.25 µl
Primer (10ng)	2.0 µl
Taq DNA polymerase (3U/µl)	0.25 µl
DNA (5ng/ µl)	4.0 µl
Total for 1 reaction	25.0 µl

The reagents were mixed thoroughly in a 2ml Eppendorf tube and vortexed for few seconds. 21 µl of mixture was distributed to each PCR tube and 4.0 µl of template DNA (5ng/µl) was added to each amplification reaction in thermal cycler (Corbett thermal cycler) programmed as above.

3.4.4 Electrophoresis in agarose gel

Amplification products were separated on 2.5% agarose gel using 1X TBE buffer (Tris HCl Ph8.0, Boric acid, Ethylene diamine-tetra acetic acid) on horizontal gel

electrophoresis apparatus (Genei, Bengaluru). Ethidium bromide at a concentration of (5mg/ml) was used as intercalating agent. RAPD amplification products were mixed with 3 μ l (10X orange dye) of loading dye and loaded onto the gel. Gel was run according to 5V/cm of the length of gel till the bands separate. 1kb DNA Mass Ladder Marker (100-10000bp) was used as a standard in the first well of each gel.

3.4.5 Analysis of RAPD data

3.4.5.1 Viewing of amplified DNA

After the run was over, the gel was viewed under the UV light using Gel Documentation system and was photographed.

3.4.5.2 Scoring of bands

The amplified bands after separation were visualized using Gel documentation system. Further the bands were scored for percentage polymorphism for each set of primer amplified product using NTSYS 2.2.

3.4.5.3 Analysis of RAPD data

The second bands were analysed in the form of binary system to prepare the similarity index. The bands with same molecular weight and mobility were treated as identical fragments. Data matrices were prepared in which the presence of a band was coded as 1 whereas the absence as 0. The data matrices were analysed by the SIMQUAL program of NTSYS-PC (Version 2.2) and similarities between genotypes were estimated using Jaccard similarity coefficient, calculated as $J = A/(N-D)$, where A is the number of positive matches (i.e. presence of band in both samples), D is the number of negative matches (i.e. absence of band in both sample) and N is the total sample size including both the number of matched and unmatched. Dendrogram was produced from the resultant similarity matrices using the UPGMA method.

3.5 STATISTICAL ANALYSIS

The data obtained for natural variation were subjected to statistical analysis using Randomized Block Design (RBD). The statistical analysis for each parameter was carried out separately and the analysis of variance (ANOVA) table was set up as under:

3.5.1 ANOVA

Source of variation	Degree of freedom	Mean of squares	F _{Cal}
Replication	(r-1)	M _r	
Treatments	(t-1)	M _t	M _t / M _e
Error	(r-1)(t-1)	M _e	
Total	(rt-1)		

Where,

r	=	No. of replications
t	=	No. of treatments
M _r	=	Mean sum of squares due to replications
M _t	=	Mean sum of squares due to treatments
M _e	=	Mean sum of squares due to error

3.5.2 Variance components

Genotypic, phenotypic and environmental variance were calculated as follows:

Coefficient of variability was worked out by the formula suggest by Burton and De Vane (1953).

PCV (%)	=	$\sqrt{V_p/\bar{X}} \times 100;$	V _p	=	Phenotypic variance
GCV (%)	=	$\sqrt{V_g/\bar{X}} \times 100;$	V _g	=	Genotypic variance
ECV (%)	=	$\sqrt{V_e/\bar{X}} \times 100;$	V _e	=	Environmental variance

where,

PCV	=	Phenotypic coefficient of variability
GCV	=	Genotypic coefficient of variability
ECV	=	Environmental coefficient of variability
$\bar{X}$	=	Population mean of the character

Coefficient of variability was calculated as given by Pillai and Sinha (1968);

$$CV (\%) = \frac{SD}{\bar{X}} \times 100$$

Where,

SD	=	Standard deviation
$\bar{X}$	=	Population mean

3.5.3 Heritability (broad sense)

Heritability (broad sense), was calculated as suggested by Burton and De Vane (1953) and Johnson *et al.* (1955).

$$h^2 (\%) = \frac{V_g}{V_p}$$

3.5.4 Genetic advance

The expected genetic advance at 5% 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).

$$\text{Genetic advance} = h^2 \times \sqrt{V_p} \times K$$

Where,

$$h^2 = \text{Heritability (broad sense)}$$

$$K = \text{Selection differential at 5\% selection intensity.}$$

Value of K = 2.06 (Allard, 1960)

3.5.5 Genetic gain

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

$$\text{Genetic gain (\%)} = \frac{\text{Genetic advance}}{\bar{X}} \times 100$$

3.5.6 Correlation coefficient

The simple correlation coefficients (Karl Pearson's) were worked out by using formula (Panse and Sukhatme, 1967):

$$r(X,Y) = \frac{\text{Cov. XY}}{\sqrt{V(X)V(Y)}}$$

Where,

$$r(X,Y) = \text{Simple correlation between X and Y}$$

$$V_{(X)} = \text{Variance of X character}$$

$$V_{(Y)} = \text{Variance of Y character}$$

The significance of 'r' values was tested against (n-2) degrees of freedom by using Fisher and Yates table.

Chapter-4

RESULTS AND DISCUSSION

The present investigation entitled “Nursery evaluation of willow (*Salix spp.*) clones” was carried out to determine clonal variation for growth, biomass and wood characters as well as at molecular level in order to identify superior clones. In all the experiments conducted, for explanatory convenience the clones were denoted as T₁, T₂, T₃,...T₄₂. The results obtained and discussions are presented separately on different aspects under the following headings:-

4.1 GROWTH AND BIOMASS CHARACTERS

4.2 WOOD CHARACTERS

4.3 VARIABILITY AND GENETIC PARAMETERS

4.4 CORRELATION STUDIES

4.5 MOLECULAR STUDIES

4.1 GROWTH AND BIOMASS CHARACTERS

The growth and biomass characters viz., plant height, basal diameter, volume index, number of branches, number of nodes, Internodal length, leaf area, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight and total dry biomass, depicted highly significant differences among all the clones. The results pertaining to variation among clones for these characters are presented in Table 1.

4.1.1 GROWTH CHARACTERS

4.1.1.1 Height (cm)

Results represented in the Table 1 indicated the maximum value (317.3cm) for T₁, followed by T₂₉ (315.7cm), T₇ (313.58cm), T₃₉ (308.5cm) T₂ (308.50cm), T₁₅ (306.13cm), T₁₉ (305.9cm), T₉(304.28cm), T₂₅(302.45cm), T₄(302.23cm), T₁₄ (301.08cm) and T₅ (298.98cm). The minimum value (260.23cm) was recorded for T₂₈.

4.1.1.2 Basal diameter (mm)

The analysis showed significance difference among the clones. The maximum value (18.60mm) was obtained by T₂₇ which was followed by T₇ (18.47mm), T₃₀ (17.82mm) and T₁₅

(17.00mm), T₂₀ (16.82mm) and T₆ (16.81mm). The minimum value (11.47mm) was recorded for T₂₅.

Table 1: Mean values of growth parameters

Clone	Height (cm)	Basal diameter (mm)	Volume index (cm ³)	Number of branches	Number of nodes	Internodal length (cm)	Leaf area (cm ²)
T ₁	317.30	15.85	725.62	14.00	102.25	3.11	13.65
T ₂	308.50	13.97	602.47	11.75	104.00	2.99	16.50
T ₃	268.75	13.96	525.40	11.00	109.50	2.48	14.78
T ₄	302.23	15.43	720.73	17.75	101.50	3.00	16.60
T ₅	298.98	12.93	501.39	14.25	110.00	2.76	16.98
T ₆	270.70	16.81	767.32	17.00	120.00	2.30	16.58
T ₇	313.58	18.47	1071.92	20.50	100.75	3.13	19.58
T ₈	282.46	16.63	783.84	17.00	97.00	2.94	14.55
T ₉	304.28	13.15	531.26	11.00	113.50	2.69	14.48
T ₁₀	268.63	14.39	556.75	9.25	108.75	2.49	17.88
T ₁₁	287.98	15.38	682.43	11.50	113.50	2.56	13.55
T ₁₂	279.25	13.13	480.84	16.75	96.00	2.92	13.03
T ₁₃	283.35	14.25	574.70	17.25	113.50	2.51	14.78
T ₁₄	301.08	13.82	576.32	9.25	105.25	2.89	21.13
T ₁₅	306.13	17.00	889.95	9.75	108.50	2.85	22.73
T ₁₆	260.98	16.01	671.85	14.25	97.75	2.68	17.15
T ₁₇	278.95	12.21	422.17	14.50	106.75	2.62	18.70
T ₁₈	264.68	16.26	701.83	17.25	119.75	2.22	18.50
T ₁₉	305.90	14.95	685.87	18.50	101.75	3.03	22.03
T ₂₀	274.05	16.82	770.85	11.50	113.25	2.44	21.55
T ₂₁	275.85	12.72	462.20	14.25	105.00	2.71	24.90
T ₂₂	270.28	13.61	501.42	15.75	108.25	2.51	24.13
T ₂₃	279.13	14.16	560.30	19.00	104.50	2.69	25.75
T ₂₄	266.33	14.09	528.61	14.25	106.00	2.54	23.35
T ₂₅	302.45	11.47	405.04	14.50	116.50	2.62	14.85
T ₂₆	290.03	13.75	551.87	16.25	100.50	2.93	19.40
T ₂₇	281.83	18.60	982.12	21.00	110.25	2.58	24.38
T ₂₈	260.23	15.73	645.21	20.50	102.50	2.56	23.35
T ₂₉	315.70	15.62	772.81	16.25	118.50	2.68	25.83
T ₃₀	295.68	17.82	939.36	14.75	115.75	2.57	25.43
T ₃₁	285.03	13.93	558.38	12.75	117.50	2.45	25.78
T ₃₂	295.95	16.10	774.26	8.50	105.75	2.81	23.75
T ₃₃	274.10	12.24	412.18	14.00	117.75	2.34	26.85
T ₃₄	283.05	11.86	396.54	12.00	110.00	2.61	25.50
T ₃₅	263.83	11.64	358.77	15.00	100.75	2.63	25.43
T ₃₆	274.38	13.50	504.68	11.75	106.50	2.60	26.18
T ₃₇	271.88	12.20	403.16	10.00	110.75	2.46	20.38
T ₃₈	267.30	12.64	439.13	11.00	105.00	2.56	20.58
T ₃₉	308.93	14.82	679.15	12.50	94.75	3.27	15.20
T ₄₀	279.28	12.11	416.01	8.75	109.75	2.56	15.70
T ₄₁	296.65	14.23	613.33	12.50	96.00	3.12	15.68
T ₄₂	292.10	15.63	720.44	22.50	112.00	2.63	16.20
CD _{0.05}	19.72	1.85	151.42	5.23	14.31	0.41	2.99
CV (%)	4.94	9.13	17.61	26.13	9.53	10.86	10.78

4.1.1.3 Volume Index (cm³)

Volume index varied significantly with the maximum value (1071.92cm³) observed for T₇ was at par with T₂₇ (982.12cm³) and T₃₀ (939.36cm³) whereas, minimum value (358.77cm³) was recorded for T₃₅.

4.1.1.4 Number of Branches

Results from Table 1 showed the maximum value (22.5) for T₄₂ which was followed by T₂₇ (21.00), T₇ (20.50), T₂₈ (20.50) T₂₃ (19.00), T₁₉ (18.51), T₄ (17.75), T₁₃ (17.25) and T₁₈ (17.25). The minimum value (8.50) was recorded for T₃₂.

4.1.1.5 Number of Nodes

The maximum number (120) was recorded for T₆ which was statistically at par with T₁₈ (119.75), T₂₉ (118.50), T₃₃ (117.75) and T₃₁ (117.50). The minimum value (94.75) was recorded for T₃₉.

4.1.1.6 Internodal Length (cm)

It was evident from the Table1 that maximum Internodal length showed significant difference among clones. The maximum (3.27cm) internodal length was obtained for T₃₉ which was followed by T₇ (3.13cm), T₄₁ (3.12cm), T₁ (3.11cm) and T₁₉ (3.03cm). The minimum value (2.22cm) was recorded for T₁₈.

4.1.1.7 Leaf Area (cm²)

The leaf area varied significantly with the highest value (26.85cm²) observed in T₃₃ which was at par with T₃₆ (26.18cm²), T₂₉ (25.83cm²), T₃₁ (25.78cm²), T₂₃ (25.75cm²), T₃₄ (25.5cm²), T₃₀ (25.43cm²), T₃₅ (25.43cm²), T₂₁ (24.9cm²), T₂₇ (24.38cm²) and T₂₂ (24.13cm²). The minimum value (13.03cm²) was recorded for T₁₂.

4.1.2 BIOMASS CHARACTERS

4.1.2.1 Fresh shoot weight (g)

The analysis revealed that the maximum fresh shoot weight (380.59g) was observed for T₂₅ and it was at par with T₂₈ (359.88g), T₂₆ (357.94g) and T₂₀ (353.42g). The minimum value (129.84g) was found in T₃₃.

Table 2: mean value of biomass characters

CLONE	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Total dry biomass (g)
T₁	262.03	105.96	70.26	34.93	140.88
T₂	202.68	105.85	67.13	31.73	137.57
T₃	212.75	106.64	80.96	46.74	153.38
T₄	300.46	186.16	75.99	35.63	221.79
T₅	196.48	97.12	51.25	33.45	130.57
T₆	184.49	103.36	54.97	31.33	134.69
T₇	344.62	188.14	79.11	32.99	211.13
T₈	230.41	147.65	59.92	27.28	174.93
T₉	255.03	150.44	68.38	31.78	182.22
T₁₀	230.82	129.89	57.35	27.46	157.36
T₁₁	193.65	85.07	43.42	22.46	107.52
T₁₂	196.08	110.44	60.75	30.70	141.13
T₁₃	190.63	100.30	54.63	31.30	131.59
T₁₄	149.89	70.37	60.11	36.34	106.71
T₁₅	205.59	96.71	64.55	40.85	137.56
T₁₆	205.71	104.13	82.66	47.48	151.61
T₁₇	258.48	152.82	65.73	43.42	196.24
T₁₈	195.55	90.59	52.04	26.33	116.92
T₁₉	329.54	168.41	80.50	40.84	209.25
T₂₀	353.42	207.51	102.67	53.63	261.14
T₂₁	207.49	124.45	70.48	35.29	159.74
T₂₂	137.23	130.78	67.40	33.21	183.99
T₂₃	325.70	196.61	97.44	51.70	242.31
T₂₄	215.05	108.54	79.04	43.97	152.51
T₂₅	380.59	208.05	107.01	59.86	267.91
T₂₆	357.94	185.06	78.41	48.74	233.80
T₂₇	321.47	183.36	73.68	42.16	225.52
T₂₈	359.88	180.43	80.67	34.21	214.63
T₂₉	203.58	101.67	74.26	43.05	144.72
T₃₀	218.60	109.66	79.91	44.48	154.13
T₃₁	146.80	69.02	64.30	32.93	101.95
T₃₂	137.23	79.80	69.25	42.75	122.54
T₃₃	129.84	70.17	48.97	27.53	97.70
T₃₄	217.25	106.88	55.77	38.29	145.18
T₃₅	274.20	160.32	91.50	39.00	179.32
T₃₆	289.25	153.07	93.59	51.48	204.55
T₃₇	259.83	138.46	87.62	49.55	188.00
T₃₈	260.04	140.66	105.16	57.35	198.01
T₃₉	331.57	181.77	119.68	62.12	243.89
T₄₀	240.46	128.48	72.21	44.71	173.18
T₄₁	315.65	166.17	122.46	54.79	220.96
T₄₂	255.13	139.64	59.89	30.67	170.30
CD_{0.05}	53.99	23.26	14.53	10.07	26.08
CV (%)	15.36	12.57	13.96	18.27	10.86

4.1.2.2 Dry shoot weight (g)

The data in Table 2 clearly indicated that the maximum dry shoot weight (208.05g) was found in T₂₅. It was followed by T₂₀ (207.51g), T₂₃ (196.61g), T₇ (188.14g) and T₄ (186.16g). The minimum value (69.02g) was recorded for T₃₁.

4.1.2.3 Fresh root weight (g)

Data regarding the variation in fresh root weight (Table 2) clearly depicted that the maximum value was attained (122.46g) by test clone T₄₁ which is at par with T₃₉ (119.68g). The minimum value (43.42g) was observed in T₁₁.

4.1.2.4 Dry root weight (g)

It is evident from the Table 2 that all clones vary significantly among themselves with the maximum (62.12g) recorded for T₃₉ which was followed by T₂₅ (59.86g), T₃₈ (57.35g), T₄₁ (54.79g) and T₂₀ (53.63g). The minimum value (22.46g) was again found in T₁₁.

4.1.2.5 Total dry biomass (g)

It is apparent from the table that T₂₅ (267.91g) attained maximum total dry biomass and was found statistically at par with T₂₀ (261.14g), T₃₉ (243.89g) and T₂₃ (242.31g). The minimum value (97.70g) was recorded for T₃₃.

Analysis of variance revealed highly significant differences among clones for growth and biomass characters thereby indicating existence of variability among different genotypes studied for different characters.

The range of mean values with respect to these characters among different clones were; plant height (260.23-317.30cm), basal diameter (11.47-18.60mm), number of branches (8.5-22.5), internodal length (2.22-3.27cm), leaf area (13.63-26.85cm²), fresh shoot weight (129.84-380.59g), dry shoot weight (69.02-208.05g), fresh root weight (43.42-122.46g), dry root weight (22.46-62.12g) and total dry biomass (97.70-267.91g) (Table 1 & 2). The results of the present studies are in consonance with findings of Gupta (2005), Joshi (2009) and Raja (2014).

In consonance with present study, Tharakan *et al.* (1998) observed significant difference among *Salix* clones in terms of height, diameter, growth, leaf area and biomass

production. Smaliukas *et al.* (2008) found large variation with respect to morphological, biomass and energy characteristics in short rotation plantation of different genotypes of *Salix viminalis* and *Salix dasyclados*. Mleczek *et al.* (2010) also noticed large variation in *Salix* clones with respect to biomass production. Sharma *et al.* (2014) evaluated the *Salix* clones and found significant differences among clones for plant survival, height, basal diameter and volume index.

4.2 WOOD CHARACTERS

4.2.1 Bark per cent (%)

Data pertaining to bark per cent in Table 3 depicted maximum value of bark per cent (27.45%) for T₁₅. Which was at par with T₇ (26.87%), T₃₇ (26.78%), T₁₂ (26.71%), T₃₃ (26.62%), T₆ (26.56%) and T₄ (26.47%). Minimum bark per cent was recorded (14.98%) for T₄₀.

4.2.2 Wood per cent (%)

From the perusal of data in Table 3 it is evident that wood per cent ranged from to (72.55%) T₁₅ to (85.02%) T₄₀. Clone T₃₉ (84.33%) and T₄₇ (82.01%) were statistically lower to the maximum T₄₀ in terms of wood per cent.

4.2.3 Moisture content (%)

Data in Table 3 for moisture content ranged from (33.48%) T₃₇ to (52.03%) T₄. T₄ performed statistically superior than T₇ (50.53%), T₁₂ (50.25%), T₉ (50.54%), T₁₈ (49.88%), T₈ (48.16%) and T₁₆ (47.29%).

4.2.4 Specific gravity (gcm⁻³)

Specific gravity varied significantly with the highest value of observed in T₁₃ (0.52gcm⁻³) and lowest value of (0.33gcm⁻³) in T₇. T₁₃ was statistically superior to T₄₂ (0.51gcm⁻³), T₁₀ (0.50gcm⁻³), T₉ (0.49gcm⁻³), T₂₃ (0.48gcm⁻³), T₄₁ (0.48gcm⁻³), T₃₀ (0.47gcm⁻³) and T₁ (0.46gcm⁻³).

Table 3: Mean values of wood characters

CLONE	Bark (%)	Wood (%)	Moisture (%)	Specific gravity (gcm⁻³)	Fibre length (mm)
T₁	21.89	78.12	43.53	0.46	0.61
T₂	25.15	74.86	46.68	0.44	0.59
T₃	23.34	76.67	46.50	0.41	0.63
T₄	26.47	73.53	52.03	0.42	0.68
T₅	24.90	75.10	45.22	0.39	0.66
T₆	26.56	73.45	47.16	0.37	0.68
T₇	26.87	73.13	50.53	0.33	0.73
T₈	23.51	76.49	48.16	0.36	0.70
T₉	24.03	75.97	50.04	0.49	0.65
T₁₀	23.99	76.01	46.49	0.50	0.72
T₁₁	26.33	73.67	45.58	0.37	0.66
T₁₂	26.71	73.29	50.25	0.40	0.67
T₁₃	24.33	75.67	43.40	0.52	0.66
T₁₄	25.33	74.67	35.09	0.43	0.62
T₁₅	27.45	72.55	40.39	0.37	0.66
T₁₆	23.19	76.81	47.29	0.43	0.72
T₁₇	22.95	77.05	43.92	0.41	0.65
T₁₈	24.71	75.30	49.88	0.45	0.65
T₁₉	21.64	78.36	46.25	0.46	0.72
T₂₀	22.08	77.92	39.99	0.43	0.64
T₂₁	20.83	79.17	44.27	0.40	0.65
T₂₂	26.02	73.99	42.02	0.44	0.70
T₂₃	24.18	75.82	46.21	0.48	0.68
T₂₄	23.77	76.23	43.79	0.38	0.69
T₂₅	24.78	75.22	42.33	0.42	0.76
T₂₆	24.30	75.71	39.77	0.41	0.77
T₂₇	25.04	74.96	43.09	0.36	0.72
T₂₈	25.53	74.47	43.97	0.41	0.65
T₂₉	23.97	76.03	45.15	0.45	0.67
T₃₀	23.91	76.10	43.50	0.47	0.63
T₃₁	23.29	76.71	46.28	0.35	0.72
T₃₂	24.98	75.02	44.93	0.41	0.69
T₃₃	26.62	73.38	35.81	0.36	0.66
T₃₄	25.72	74.29	35.99	0.39	0.63
T₃₅	23.07	76.93	35.48	0.37	0.61
T₃₆	24.43	75.57	37.32	0.41	0.67
T₃₇	26.78	73.23	33.48	0.38	0.68
T₃₈	25.65	74.35	36.31	0.35	0.66
T₃₉	15.67	84.33	47.29	0.47	0.71
T₄₀	14.98	85.02	40.24	0.43	0.76
T₄₁	17.99	82.01	46.45	0.48	0.73
T₄₂	24.23	75.78	40.29	0.51	0.65
CD_{0.05}	3.36	3.36	7.32	0.09	0.08
CV (%)	10.05	3.17	12.01	14.86	7.91

4.2.5 Fibre length (mm)

Table 3 revealed that fibre length ranged from 0.61mm to 0.77mm for clone T₃₅ and T₂₆ respectively. Clone T₂₆ (0.77mm) was found significantly higher than the remaining 41 clones. It was followed by T₂₅ (0.76mm), T₄₀ (0.76mm), T₇ (0.73mm), T₂₂ (0.70mm) and T₂₄ (0.69mm). The minimum value (0.61mm) was observed in T₂₆.

Wood is notably non-homogeneous both within and among trees of a species as well as among species and geographic sources. Genetic manipulation of wood in a breeding program can result in a higher proportion of desired wood. Wood properties have significant effect on the quality and yield of pulp and paper products and on strength and utility of solid wood products. Wood specific gravity and fibre length are ideal characteristics to manipulate genetically because of the large tree to tree variation, the strong heritability, its low genotype $\times$ environment interaction and has effects on yield and quality.

Wood samples of various clones showed a wide variation in bark per cent (14.98-27.45%), wood per cent (72.55-85.02%), moisture content (33.48-52.03%), specific gravity (0.33-0.52gcm⁻³) and fibre length (0.61-0.77mm) (Table 3).

Bark per cent ranged from 14.98% -27.45% in the present investigation. The present studies support the findings of Sennerby-Forsse (1995), Huse (2004) and Gupta (2005). Significant differences existed among the clones for fibre length. It ranged between (0.61-0.77mm). The results are in conformity with the findings of Gupta *et al.* (2014) and Raja (2014).

Bilal *et al.* (2014) while studying the variation of specific gravity in *Salix alba* of Kashmir Himalaya found the range of 0.42-0.48. Maximum value of 0.53 gcm⁻³ for clone 006/05 (*Salix alba*) was found by Gupta *et al.* (2014) while studying basic density in willow.

4.3 ESTIMATION OF VARIABILITY AND GENETIC PARAMETERS

Variability for growth, biomass and wood parameters was estimated in terms of mean, range, coefficient of variation, genotypic and phenotypic coefficient of variability. Genetic parameters were worked out with regards to estimate heritability (broad sense), genetic advance and genetic gain.

4.3.1 Growth and biomass parameters (Table 4)

Variability estimates and genetics parameter were calculated for growth and biomass traits viz., height, basal diameter, volume index, number of branches, number of nodes, internodal length, leaf area, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and total dry biomass.

Among all the parameters volume showed widest range of values (358.77-1071.92) followed by fresh shoot weight (129.84-380.59). The narrowest range was recorded for internodal length (2.22-3.27).

Regarding coefficient of variation, it was found maximum in number of branches (26.13%) and minimum was found in height (4.94%). Genotypic coefficient of variation (GCV) was found maximum (29.38%) for dry shoot weight followed by fresh shoot weight (28.33%), while minimum (4.23%) was exhibited by number of nodes. Phenotypic coefficient of variation (PCV) was estimated maximum (33.61%) for number of branches and minimum (7.17%) was found for height. The phenotypic coefficient of variation values were found slightly higher for all the parameters than genotypic coefficient of variation.

Heritability value was estimated maximum (0.85) for both dry shoot weight and total dry biomass followed by leaf area (0.80), fresh shoot weight (0.77), fresh root weight (0.75), volume index (0.69), basal diameter (0.63) and dry root weight (0.61) whereas; minimum heritability value (0.16) was recorded for number of nodes.

Genetic advance was recorded maximum (274.5) for volume and minimum (0.22) was recorded for internodal length. Genetic gain was estimated maximum (55.65%) for dry shoot weight it was closely followed by fresh shoot weight (51.30%). Minimum genetic gain (3.53%) was recorded for number of nodes.

4.3.2 Wood parameters (Table 5)

The widest range of value was recorded for moisture content (33.48-52.03) and narrowest range (0.61-0.77) was recorded in case of fibre length. Coefficient of variation was found maximum (14.86%) for specific gravity. Minimum value (3.17%) was recorded for wood per cent.

Table 4. Variability and genetic parameters of growth and biomass parameters

Character	Mean	Range	CV (%)	Coefficient of variation (%)		Heritability	Genetic advance	Genetic gain (%)
				Genotypic	Phenotypic			
Height (cm)	285.90	260.23-317.3	4.94	5.20	7.17	0.53	22.17	7.76
Basal diameter (mm)	14.52	11.47-18.6	9.13	12.05	15.12	0.63	2.87	19.77
Volume index (cm ³)	615.91	358.77-1071.92	17.61	26.10	31.49	0.69	274.5	44.58
Number of branches	14.33	8.5-22.5	26.13	21.13	33.61	0.40	3.92	27.36
Number of nodes	107.56	94.75-120	9.53	4.23	10.43	0.16	3.80	3.53
Inter nodal length (cm)	2.69	2.22-3.27	10.86	7.22	13.04	0.31	0.22	8.23
Leaf area (cm ²)	19.84	13.03-26.85	10.78	21.52	24.07	0.80	7.86	39.63
Fresh shoot weight (g)	251.58	129.84-380.59	15.36	28.33	32.33	0.77	129.05	51.30
Dry shoot weight (g)	132.58	69.02-208.05	12.57	29.38	31.96	0.85	73.78	55.65
Fresh root weight (g)	74.55	43.42-122.46	13.96	23.92	27.70	0.75	31.73	42.56
Dry root weight (g)	39.49	22.46-62.12	18.27	22.70	29.14	0.61	14.38	36.42
Total dry biomass (g)	172.07	97.7-267.91	10.86	25.53	27.75	0.85	83.29	48.40

Table 5. Variability and genetic parameters of wood characters

Character	Mean	Range	CV (%)	Coefficient of variation (%)		Heritability	Genetic advance	Genetic gain (%)
				Genotypic	Phenotypic			
Bark (%)	23.98	14.98-27.45	10.05	9.98	14.16	0.50	3.47	14.48
Wood (%)	76.02	72.55-85.02	3.17	3.15	4.47	0.50	3.47	4.57
Moisture (%)	43.63	33.48-52.03	12.01	8.62	14.90	0.35	4.69	10.75
Specific gravity (g cm ⁻³)	0.42	0.33-0.52	14.86	8.77	17.35	0.26	0.04	9.14
Fibre length (mm)	0.68	0.61-0.77	7.91	4.47	9.09	0.24	0.03	4.53



Plate 1. Growth performance of different willow clones



PN227 (*S. matsudana*) $\times$ SI-63-007(*S. alba*)



[PN227 (*S. matsudana*) $\times$ *S. tetrasperma*]

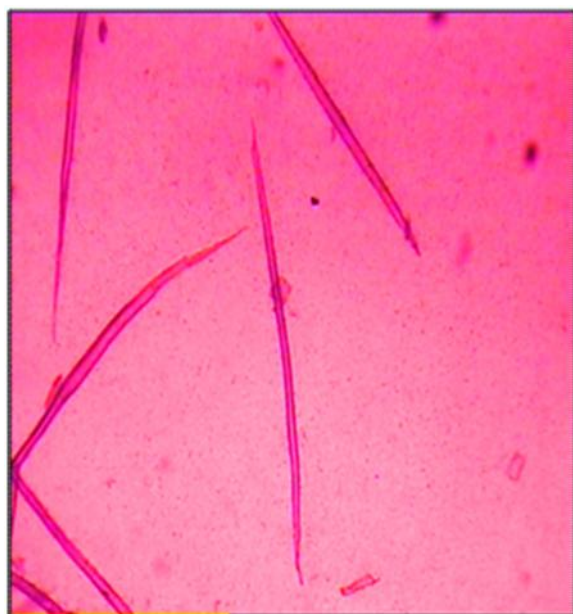


J799 (*S. matsudana* $\times$ *S. alba*)



KASHMIRI (*S. alba*)

Plate 2. Rooting behaviour of different willow clones



[J799 (*S. matsudana* × *S. alba*) Open
pollinated]



[PN227 (*S. matsudana*) × *S.*
tetrasperma]



J795 (*S. matsudana* × *S. alba*)



PN227 (*S. matsudana*) × 131/25 (*S.*
babylonica × *S. alba*)

Plate 3. Variation in fibre length of willow clones

Genotypic coefficient of variation was estimated maximum (9.98%) for bark per cent followed by specific gravity (8.77%) and moisture content (8.62%). Minimum genotypic coefficient of variation was recorded for wood per cent (3.15%). Phenotypic coefficient of variation was estimated maximum for specific gravity (17.35%) and minimum (4.47%) was estimated again for wood per cent. Heritability value was found maximum (0.50) for both bark per cent and wood per cent. Genetic advance was exhibited maximum by moisture content and genetic gain was estimated maximum for bark per cent (14.48). Minimum value for heritability value (0.24), genetic advance (0.03) and genetic gain (4.53%) was recorded for fibre length.

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 because of selection to be a mechanism of change. There must exist certain amount of observed genetic differences in a population amongst the individuals to be selected. A tree breeder doesn't create genetic variations but uses the already existing variation as a tool for improvement through selection. 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 among the desirable parameters and their correlation is important for selection in breeding programme (Johnson *et al.*, 1955).

All the growth and biomass characters showed a wide range of values (Table 1 &2), indicating the extent of variation existing in the clones. Phenotypic coefficient of variability was higher than the genotypic coefficient of variation for all characters (Table 4). High heritability was recorded for basal diameter, volume index, leaf area, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and total dry biomass. Widest range (0.22-274.50) was observed for genetic advance followed by genetic gain (3.53-55.65). This showed that the scope of further improvement through clonal selection. Similar results were reported by Tu and Pan (1991), Sharma *et al.* (2011) and Raja (2014) in *Salix* clones.

High heritability indicates the effectiveness of selection based on good phenotypic performance but does not necessarily mean a high genetic gain for a particular trait. High heritability estimates in broad sense will be reliable if accompanied by genetic gain (Burton and De Vane, 1953). Johnson *et al.* (1955) reported that heritability estimate along with

expected gain is more useful and realistic than heritability alone predicting the resultant effect for selecting best genotype.

In the present study high estimate of heritability along with high genetic gain was observed for volume index, leaf area and all biomass characters. This indicated that the higher proportion of additive gene effects for expression of these traits. Hence individual clone selection would be effective for improvement of these characters.

High heritability value (0.50) for bark and wood Per cent in comparison with fibre length (0.24) was obtained in the present study. Heritability value for fibre length was also recorded lower than the wood density in the present study. Sharma *et al.* (2014) while evaluating variability in wood properties of promising willow clones found the heritability value of (0.59) for wood and bark per cent. Monteoliva *et al.* (2005) while studying variability of wood density and fibre length in *Salix* clones found that the heritability values showed genetic control stronger for the density ($h^2 = 0.65$) than the fibre length ($h^2 = 0.32$).

4.4 ESTIMATION OF SIMPLE CORRELATION

4.4.1 Simple correlation among growth and biomass traits

The data in Table 6 revealed that the height was positive and significantly correlated with all growth and biomass traits except number of branches, internodal length and leaf area, where the correlation coefficient was positive but non-significant.

Basal diameter was found positive and significantly correlated with height, volume index, number of branches and all biomass traits. Correlation was recorded positive and non-significant for leaf area, internodal length and number of nodes.

Volume index was found positive and significantly correlated with all characters except internodal length, whereas it was found positive and non-significant.

Number of branches was positive and significantly correlated basal diameter and volume index. It was found positive and non-significant for number of nodes, internodal length, leaf area, fresh shoot weight and dry shoot weight. For fresh root weight, dry root weight and total biomass it was found negative and non-significantly correlated.

Table 6: Simple correlation coefficients among morphological and biomass traits.

Characters	Height	Basal diameter	Volume index	Number of branches	Number of nodes	Internodal Length	Leaf area	Fresh shoot weight	Dry shoot weight	Fresh root weight	Dry root weight	Total Dry biomass
Height	1.00	0.402**	0.406**	0.122	0.340*	0.246	0.217	0.568**	0.510**	0.473**	0.455**	0.597**
Basal diameter		1.00	0.974**	0.383*	0.215	0.282	0.223	0.333*	0.314*	0.307*	0.312*	0.414**
Volume index			1.00	0.364*	0.368*	0.235	0.305*	0.602**	0.548**	0.525**	0.431**	0.613**
Number of branches				1.00	0.267	0.172	0.181	0.156	0.257	-0.069	-0.285	0.275
Number of nodes					1.00	0.098	0.322*	0.341*	0.376*	0.402**	0.323*	0.356*
Internodal length						1.00	0.116	0.043	0.109	0.184	0.212	0.049
Leaf area							1.00	0.044	-0.042	0.067	0.075	-0.021
Fresh shoot weight								1.00	0.946**	0.683**	0.469**	0.940**
Dry shoot weight									1.00	0.655**	0.438**	0.981**
Fresh root weight										1.00	0.847**	0.763**
Dry root weight											1.00	0.604**
Total dry biomass												1.00

* Correlation is significant at the 5% level of significance.

** Correlation is significant at the 1% level of significance.

It was evident from Table 6 that number of nodes was found positive and significantly correlated with height, volume index and all biomass characters. It was positive and non-significant for basal diameter and internodal length. Internodal length was found positive and non-significantly correlated with all growth and biomass characters.

Leaf area showed positive and significant correlation with volume index and number of nodes. With rest of characters, it showed positive and non-significant correlation except dry shoot weight and total biomass that was found negative and non-significantly correlated.

Fresh shoot weight was recorded positive and significantly correlated with height, basal diameter, volume index, dry shoot weight, fresh root weight, dry root weight and total biomass. It was recorded positive and non-significantly correlated with number of branches, internodal length and leaf area. Dry shoot weight was positive and significantly correlated with height, basal diameter, volume index and rest of the biomass characters. It was positive and non-significant for number of branches and leaf area and negative and non-significant for internodal length.

Fresh root weight and dry root weight, as anticipated was found positive and significantly correlated with height, basal diameter, volume index, number of nodes, fresh shoot weight, dry shoot weight and total biomass. Both were found positive and non-significantly correlated with internodal length and leaf area, whereas negative and non-significant for number of branches.

Total biomass was found positive and significantly correlated with height, basal diameter, volume index, number of nodes and rest of the biomass traits. It was found positive but non-significantly correlated with internodal length and number of branches. Leaf area was found negative and non-significantly correlated with it.

4.4.2 Simple correlation coefficient of growth and wood traits

It was evident from the Table 7 that bark Per cent was recorded negative and significantly correlated with height, fresh root weight and dry root weight. It was positive and non-significant for basal diameter, volume index, number of branches, number of nodes, leaf area and internodal length. Fresh shoot weight, dry shoot weight and total biomass was found negative and non-significantly correlated with it.

Table 7: Simple correlation coefficients of morphological and biomass traits with wood traits

	Height	Basal diameter	Volume index	Number of branches	Number of nodes	Internodal length	Leaf area	Fresh shoot weight	Dry shoot weight	Fresh root weight	Dry root weight	Total biomass
Bark per cent	-0.395**	0.141	0.128	0.200	0.284	0.046	0.192	-0.195	-0.211	-0.456**	-0.423**	-0.278
Wood per cent	0.395**	-0.141	-0.128	-0.200	-0.284	-0.046	-0.192	0.195	0.211	0.456**	0.423**	0.278
Moisture content	0.245	0.428**	0.042	0.320*	-0.166	0.360*	-0.450*	0.040	0.093	-0.098	-0.305*	0.016
Specific gravity	0.214	0.002	-0.011	0.045	0.006	0.374*	-0.268	0.159	0.147	0.123	0.021	0.134
Fibre length	0.090	0.132	0.174	0.041	-0.113	0.219	-0.020	0.300	0.302	0.285	0.350*	0.343*

*** Correlation is significant at the 5% level of significance.**

**** Correlation is significant at the 1% level of significance.**

Wood per cent was recorded positive and significantly correlated with height, fresh root weight and dry root weight. It was found to be negative and non-significantly correlated with basal diameter, volume index, number of branches, number of nodes, leaf area and internodal length. It was recorded positive and non-significant for fresh shoot weight, dry shoot weight and total biomass.

Moisture content was found positive and significantly correlated with basal diameter, number of branches and internodal length. Leaf area and dry root weight was found negative but significantly correlated.

Specific gravity showed positive and significant correlation with internodal length. Volume index and leaf area showed negative and non-significant correlation, whereas rest of the characters showed positive and non-significant correlation. Fibre length was recorded negative and non-significant correlation with number of nodes and leaf area, whereas positive and non-significant for rest of the characters except dry root weight and total biomass. These were found positive and significantly correlated with fibre length.

Correlation is an important biometric tool which measures the degree and magnitude of association between various traits. It provides a good basis for indirect selection especially for those traits which require destructive sampling. The amount of genetic gain obtained from selection *i.e.* correlated response gives a better understanding of degree and magnitude of genetic association.

A close examination of Table 6, revealed that most of the correlation coefficients were found positive and significantly correlated at 5 and 1 per cent level of significance with the exception of positive and non-significant correlation showed by internodal length, number of branches and leaf area with most of the parameters. Biomass parameters showed positive and significant correlation with each other and all other growth parameters. Huse (2004) while, working on arborescent willow clones found that all the biomass parameters were positive and significantly correlated among themselves and with other parameters. Similar results were found by Gupta (2005) in *Salix alba*, Joshi (2009) and Raja (2014) on willow clones.

Bark per cent showed negative but significant and wood per cent showed positive and significant correlation for the characters; height, fresh root weight and dry root weight.

Specific gravity showed non-significant correlation with all growth and biomass parameters except internodal length which was positive and significant (Table 7). These were in conformity with the findings of Tunctaner (2002), Gupta (2005) and Joshi (2009).

4.5 RAPD (Random Amplified Polymorphic DNA) Studies

Molecular characterization of twenty five genotypes of willow clones were investigated using RAPD primers. From an initial screening of eleven arbitrary RAPD primers (Primers previously reported to be polymorphic in willow), only nine primer (Table 8) produced RAPD profiles with intense banding pattern, which showed some polymorphism between 25 accessions used in the study. The banding pattern of each primer generated a unique set of amplification products. RAPD analysis revealed high levels of genetic diversity within the references set of willow clones (Appendix-II).

Table 8. Nucleotide sequences of 9 decamer random primers which showed polymorphism

S.No.	Name of primers	Base sequences
1	OPJ – 5	CTCCATGGGG
2	OPA – 2	TGCCGAGCTG
3	OPA – 4	AATCGGGCTG
4	OPO – 4	AAGTCCGCTC
5	OPO – 17	GGCTTATGCC
6	OPO – 18	CTCGTATCCT
7	DECA – 5	CCAAGGGGGC
8	DECA – 7	CCGCCCCGAT
9	DECA – 12	CTTGCCCACG

The number of bands recognized by software, Alpha imager for each primer ranged from 3(OPJ-5, OPA-2 and DECA-5) to 6(OPA-4) (Table 9). All the primers used in analysis yielded a total of 39 scorable bands with an average of 4.33 bands per primer. Out of the total 39 scorable bands, all showed polymorphism.

4.5.1 RAPD pattern with 9 decamer primers

RAPD analysis with primer OPJ-5 yielded 3 bands; all bands were found to be polymorphic. This primer produced bands ranging from 150.22bp to 220.34bp. No monomorphic band was identified.

The total number of bands amplified with OPA-2 primer was found to be 3, all bands exhibited polymorphism. This primer produced bands ranging from 187.72bp-957.68bp.

With OPA-4 primer, total numbers of amplified products were found to be 6 and all bands exhibited polymorphism. The size of amplified bands ranged between 278.63bp to 814.73bp.

The RAPD banding pattern of OPO-04 produced a total of 5 bands. All were found to be polymorphic. The amplified bands ranged between 590.15bp to 2188.65bp.

Table 9. Total numbers of amplified and polymorphic fragments generated by PCR using RAPD primers

Primers	Total number of scorable bands	Total number of polymorphic bands	Total number of monomorphic bands	Band size (bp)
OPJ – 5	3	3	0	150.22-220.34
OPA – 2	3	3	0	187.72-957.68
OPA – 4	6	6	0	278.63-814.73
OPO – 4	5	5	0	590.15-2188.65
OPO – 17	5	5	0	183.98-2027.10
OPO - 18	5	5	0	218.62-504.73
DECA – 5	3	3	0	156.91-1542.14
DECA - 7	4	4	0	203.05-582.71
DECA - 12	5	5	0	182.33-1756.25
Total	39	39	0	150.22-2188.65

The total number of bands amplified with OPO-17 primer were found to be 5. All bands exhibited polymorphism. The size of amplified bands ranged between 183.98bp - 2027.10bp.

RAPD analysis with primer OPO-18 yielded 5 bands and all bands exhibited polymorphism. The size amplified bands ranged between 218.62bp - 504.73bp.

The total number of bands amplified with DECA – 5 primer were found to be 3 and all bands exhibited polymorphism. The size of amplified bands ranged between 156.91bp - 1542.14bp.

Table 10. Summary of RAPD amplified products obtained from 25 clones of willow

S.No.	Parameters	
1	Total number of primers examined	11
2	Number of polymorphic primers	9
3	Total number of bands amplified from polymorphic primers	39
4	Size range of amplified products	150.22bp - 2188.65bp
5	Average number of bands per polymorphic primer	4.33
6	Total number of polymorphic bands identified	39
7	Average number of polymorphic bands per polymorphic primer	4.33
8	Per cent of total polymorphic bands	100%

RAPD analysis with primer DECA – 7 yielded 4 scorable bands; all bands were found to be polymorphic. The produced size ranged from 203.05bp - 582.71bp.

The total number of bands amplified with DECA – 12 primer were found to be 5 and all bands exhibited polymorphism. The size of amplified bands ranged between 182.33bp - 1756.25bp.

4.5.2 RAPD data analysis

The RAPD fragments obtained after the amplification of genomic DNA from 25 accessions. The scoring of bands for presence as 1 (band present) and 0 (band absent) for each genotype was done by using Alpha Imager software. The data matrix so obtained was analyzed with NTSYS- 2.2 software to obtain the Jaccard's similarity correlation coefficient. The matrix so obtained has been presented in Table 11. The similarity coefficient values ranged from 0.19 to 0.61. This indicated a fair range of variability in the similarity coefficient values suggesting a wide genetic base of twenty five genotypes included in the experiment. The highest value (0.61) was found between genotypes T₅ and T₁₁.

The lowest value of 0.19 was exhibited between genotypes (T₁₂ and T₁₉) and (T₁₉ and T₂₇). The mean coefficient value of any genotype or accession may give an idea about its overall relatedness with all other genotypes or accessions in the study.

4.5.3 Dendrogram based on RAPD banding pattern

Dendrogram was created using the similarity coefficient and Unweighted Pair Group Mean Average (UPGMA) method in order to visualize genetic differentiation among

different willow clones and to see their clustering pattern (Fig. 1). A critical perusal of dendrogram reveals that the distribution of various clones into cluster was somewhat random. Genotype T₁₉ came out as an outlier and was found to be most divergent with 33 per cent similarity with the rest of clusters.

The dendrogram exhibited two clear clusters. Cluster A comprised further 5 subclusters. Subcluster A₁ comprises 8 genotypes viz., T₄, T₇, T₃, T₁₁, T₅, T₉, T₈ and T₁₂. Clone T₁₂ was found to be 57 per cent divergent with the rest of clones of subcluster A₁. Genotypes T₅ and T₁₁ recorded with maximum 61 per cent similarity. Overall subcluster A₁ was found to be 42 per cent similarity. Subcluster A₂ comprise genotype T₁₄ and T₁₅ and exhibited 59 per cent similarity. Subcluster A₁ and subcluster A₂ found to 41 per cent similar.

Subcluster A₃ comprises genotypes T₂ and T₁. Overall cluster recorded 41 per cent similarity. Subcluster A₄ comprises four genotypes viz., T₃₁, T₂₉, T₃₀ and T₃₂. T₃₁ and T₂₉ exhibited 55 per cent similarity and overall subcluster exhibited 52 per cent similarity.

Genotype T₂₅, T₂₇ and T₂₆ forms subcluster A₅. Genotype T₂₅ and T₂₇ exhibited 55 per cent similarity and overall subcluster recorded 47 per cent similarity.

Cluster B comprises two subclusters B₁ and B₂. Subcluster B₁ comprises T₃₄ and T₃₉ with 41 per cent similarity. Subcluster B₂ comprises genotype T₄₁, T₄₀ and T₄₂. Genotype T₄₂ found to be 60 per cent divergent with the rest of the genotypes of subcluster B₂. Overall subcluster B₂ was recorded 40 per cent similarity.

Molecular markers assess variations in the nucleotide sequence of DNA of different individuals. This variation is purely genetic and is not influenced by the environment and stage of development of the plant. Molecular markers are numerous and therefore a large genome can be easily assayed for existence of any variation, such genetic markers are easy to score. Use of molecular markers therefore provides an assessment of genetic diversity in a plant species and enables identification of elite genotypes.

Molecular techniques have been found to be more useful for determination of both interspecific and intraspecific genetic variation in plants. Random amplified polymorphic DNA (RAPD) markers, in particular, have been successfully employed for determination of intraspecies genetic diversity in several plants. Random amplified polymorphic DNA (RAPD) analysis has proved useful for estimating genetic diversity particularly to assist in

the conservation of rare species and plant genetic resources (Anderson and Fairbanks, 1990). RAPD analysis in particular has proven to be a rapid and efficient means of genomic mapping (Williams *et al.*, 1990) and have been successfully used for differentiating species of genus based on their similarities and geographical proximities (Thomas *et al.*, 2001).

RAPDs have been successfully used to assess the genetic diversity within and between populations of *Gliricidia sepium* and *G. maculate* (Chalmers *et al.*, 1992), *Populus* species (Sanchez *et al.*, 1998) and in willow (Barker *et al.*, 1999).

In order to examine the potential of RAPD markers for the ability to assess the variability of 25 clones of willow, the efforts were made in this direction.

A summary of amplified fragments from the study has been given in the Table 10. All the 9 primers were found to be polymorphic and amplified the genomic DNA of willow clones successfully. The number of RAPD markers generated per primer varied from 3 to 6. This is consistent with the studies carried out by Lin *et al.* (1994) on willow clones, Barker *et al.* (1999) on willow, Gupta (2005) on *Salix alba* clones and Joshi (2009) on *Salix* clones.

All the nine primers were found to produce distinct banding pattern for all the samples. Thirty nine amplified products were detected, all of which 39 (100%) found to be polymorphic. Gupta (2005) reported 100 per cent polymorphism in *Salix alba* clones. Choudhary *et al.* (2013) while studying cross-ability relationship among tree willows (*Salix* spp.) and molecular genetic variation among the progenies by using RAPD primers found 100 per cent and 70 per cent similarity of hybrid (*S. tetrasperma* (LG) $\times$ *S. mastudana* (TWE) with male and female parents respectively.

Jaccard's similarity correlation coefficient value ranged from 0.19 to 0.61. The lowest similarity value (0.19) was observed between genotypes T₁₉ [PN227 (*S. matsudana*) $\times$ NZ1179 (*S. matsudana* $\times$ *S. alba*)] and T₂₇ [PN227 (*S. matsudana*) $\times$ *S. tetrasperma*)] and T₁₂ [PN227 (*S. matsudana*) $\times$ AUSTREE (*S. alba* $\times$ *S. matsudana*)] and T₁₉ [PN227 (*S. matsudana*) $\times$ NZ1179 (*S. matsudana* $\times$ *S. alba*)], whereas, the highest value (0.61) was found between T₅ [PN227 (*S. matsudana*) $\times$ AUSTREE (*S. alba* $\times$ *S. matsudana*)] and T₁₁ [PN227 (*S. matsudana*) $\times$ AUSTREE (*S. alba* $\times$ *S. matsudana*)].

Table 11. Similarity coefficient value of RAPD data using Jaccard's similarity correlation coefficient

Clone	T ₄	T ₃₁	T ₇	T ₃₄	T ₃₀	T ₁₂	T ₄₁	T ₄₀	T ₁₄	T ₂	T ₁₃	T ₁₅	T ₂₅	T ₁	T ₁₉	T ₃₂	T ₈	T ₃₉	T ₉	T ₄₂	T ₂₉	T ₁₁	T ₂₆	T ₅	T ₂₇
T ₄	1.00																								
T ₃₁	0.50	1.00																							
T ₇	0.59	0.39	1.00																						
T ₃₄	0.27	0.32	0.32	1.00																					
T ₃₀	0.52	0.56	0.35	0.50	1.00																				
T ₁₂	0.45	0.26	0.43	0.27	0.30	1.00																			
T ₄₁	0.42	0.36	0.45	0.38	0.44	0.35	1.00																		
T ₄₀	0.39	0.32	0.32	0.43	0.44	0.40	0.47	1.00																	
T ₁₄	0.38	0.31	0.31	0.28	0.43	0.39	0.37	0.37	1.00																
T ₂	0.45	0.34	0.38	0.23	0.30	0.43	0.35	0.37	0.30	1.00															
T ₁₃	0.53	0.46	0.46	0.22	0.41	0.32	0.48	0.34	0.59	0.32	1.00														
T ₁₅	0.47	0.35	0.44	0.23	0.35	0.44	0.46	0.37	0.58	0.44	0.48	1.00													
T ₂₅	0.46	0.43	0.39	0.32	0.43	0.39	0.55	0.45	0.40	0.39	0.38	0.45	1.00												
T ₁	0.48	0.38	0.47	0.34	0.45	0.36	0.30	0.30	0.43	0.41	0.27	0.38	0.38	1.00											
T ₁₉	0.35	0.32	0.41	0.28	0.32	0.19	0.29	0.29	0.32	0.31	0.39	0.23	0.32	0.39	1.00										
T ₃₂	0.47	0.45	0.45	0.48	0.58	0.28	0.37	0.42	0.28	0.27	0.34	0.28	0.45	0.39	0.38	1.00									
T ₈	0.42	0.50	0.50	0.28	0.27	0.44	0.32	0.41	0.35	0.34	0.42	0.41	0.45	0.38	0.32	0.28	1.00								
T ₃₉	0.38	0.35	0.27	0.42	0.48	0.30	0.36	0.41	0.36	0.21	0.38	0.27	0.27	0.33	0.37	0.41	0.27	1.00							
T ₉	0.53	0.37	0.42	0.30	0.33	0.46	0.48	0.39	0.42	0.37	0.50	0.43	0.47	0.40	0.34	0.30	0.48	0.33	1.00						
T ₄₂	0.37	0.30	0.43	0.35	0.34	0.29	0.35	0.45	0.22	0.29	0.32	0.26	0.34	0.24	0.31	0.44	0.30	0.30	0.32	1.00					
T ₂₉	0.56	0.55	0.45	0.33	0.53	0.40	0.29	0.42	0.32	0.40	0.43	0.32	0.50	0.43	0.33	0.52	0.46	0.32	0.34	0.23	1.00				
T ₁₁	0.54	0.48	0.58	0.26	0.54	0.48	0.41	0.37	0.45	0.35	0.51	0.42	0.44	0.42	0.42	0.50	0.45	0.41	0.51	0.43	0.45	1.00			
T ₂₆	0.48	0.47	0.33	0.39	0.41	0.32	0.39	0.43	0.43	0.32	0.50	0.43	0.51	0.31	0.26	0.34	0.42	0.33	0.40	0.41	0.48	0.31	1.00		
T ₅	0.49	0.56	0.47	0.25	0.38	0.42	0.44	0.40	0.40	0.51	0.55	0.35	0.47	0.37	0.55	0.36	0.53	0.39	0.50	0.42	0.40	0.61	0.41	1.00	
T ₂₇	0.42	0.45	0.40	0.37	0.35	0.30	0.37	0.24	0.38	0.34	0.29	0.41	0.56	0.38	0.19	0.32	0.36	0.23	0.29	0.34	0.37	0.32	0.54	0.31	1.00

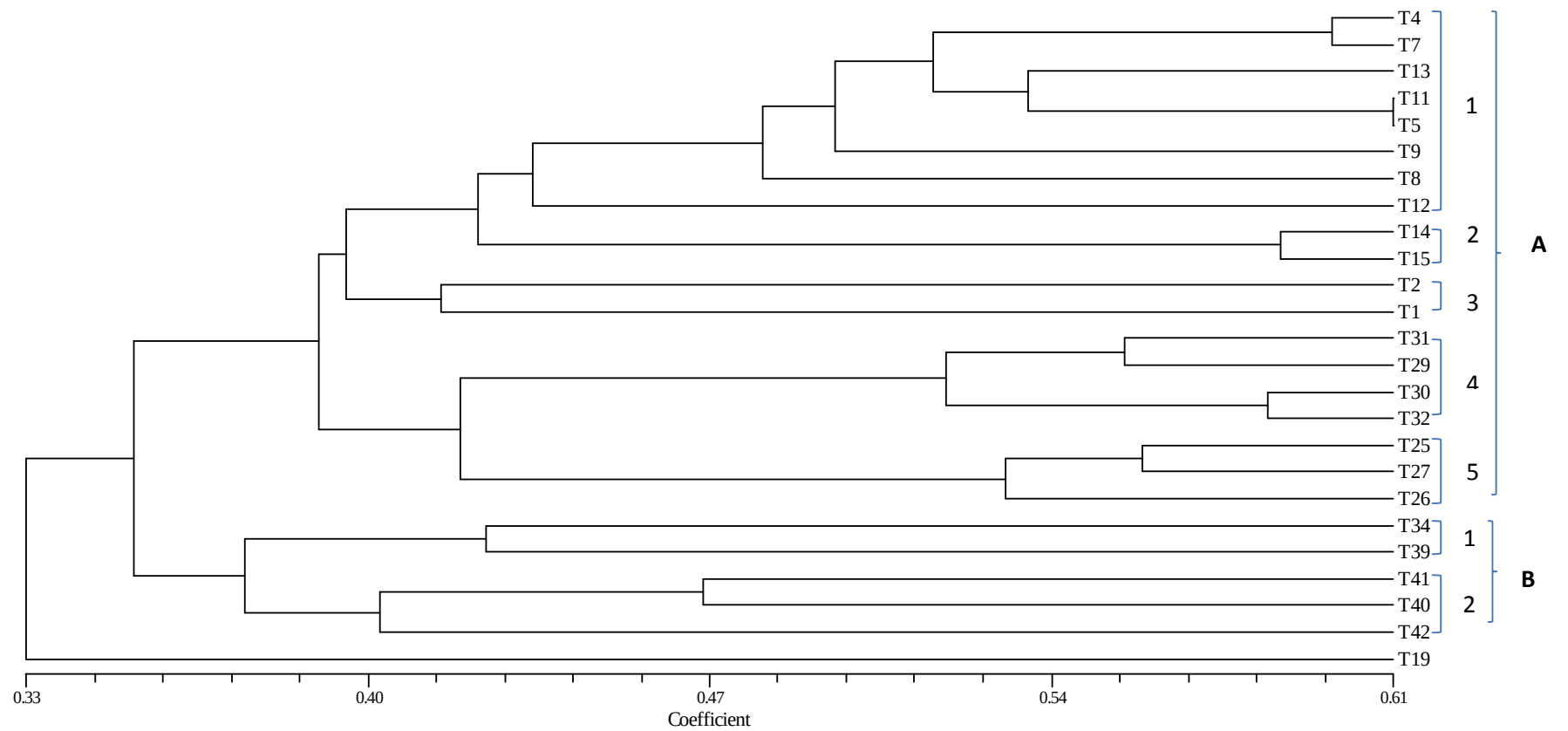


Fig. 1: Dendrogram based on UPGMA analysis of 25 clones of willow using RAPD markers

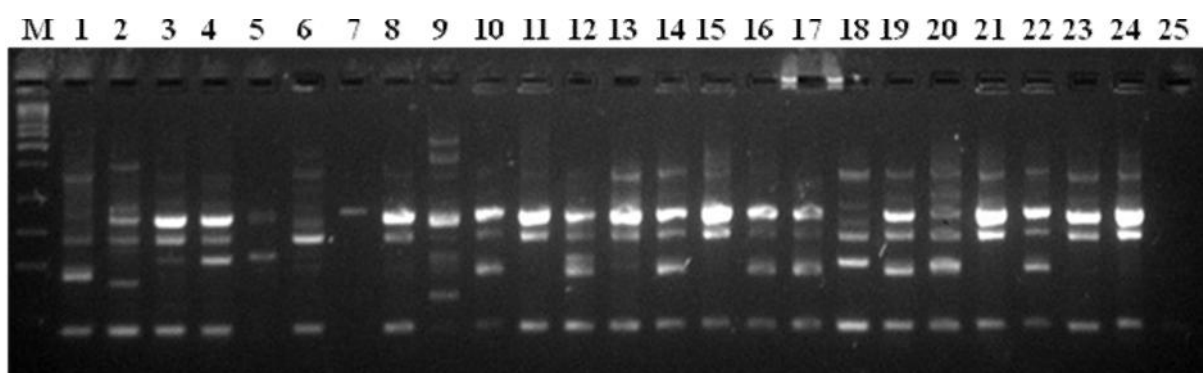


Plate 4(a) RAPD finger prints of willow clones using primer OPA-4

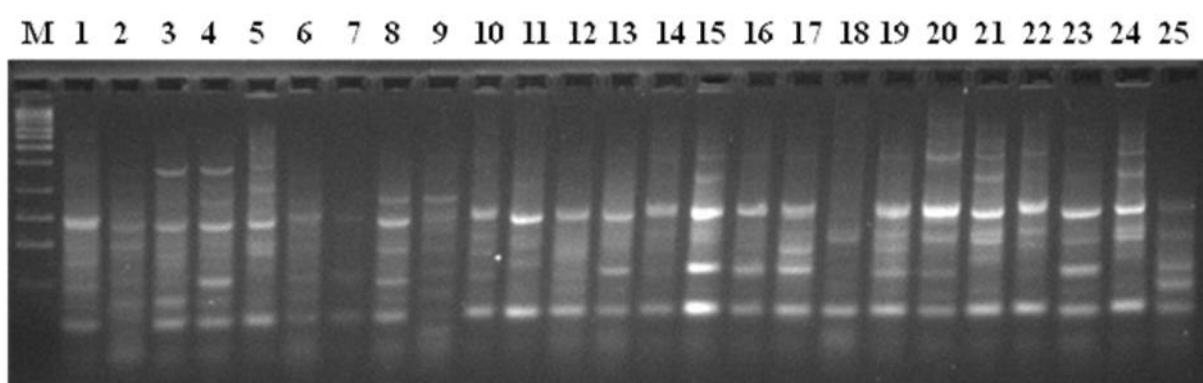


Plate 4(b) RAPD finger prints of willow clones using primer OPO-17

On the basis of UPGMA dendrogram, two clear clusters were obtained. Cluster A comprises 19 genotypes with five subclusters. The maximum similarity (61 per cent) was demonstrated by genotypes T₅ and T₁₁. Cluster B comprises two subcluster having five genotypes. Dendrogram also showed a major bifurcation, which sidelined T₁₉ [PN227 (*S. matsudana*) × NZ 1179 (*S. matsudana* × *S. alba*)] from rest of the genotypes. The genetic similarity values obtained with RAPD markers were analogous with the results of UPGMA dendrogram. Similar type of grouping of genotypes are reported by Chengjiang *et al.* (2004) for Sea buckthorn, Joshi (2009), Singh *et al.* (2013) and Raja (2014) for willow clones.

Chapter-5

SUMMARY AND CONCLUSION

The present investigation entitled “Nursery evaluation of willow (*Salix* spp.) clones” was carried out in the Department of Tree Improvement and Genetic Resources, Dr. Y. S. Parmar University of Horticulture and Forestry, Solan (HP) during 2015-16. Forty two genotypes (Appendix I) were selected out of the existed clonal material in the department to study for the extent and pattern of variation with respect to growth, biomass and wood characters and out of these 42 genotypes 25 genotypes were selected for RAPD technique to study the genetic variation among different genotypes.

The cuttings were planted in the departmental nursery during February-March 2015 and the data on raised nursery plants were collected in the month of November 2015 and studied for growth, biomass and wood characters. Analysis of variance revealed highly significant differences for all the characters. Maximum value for height (317.3cm) was observed for T₁[PN227 (*S. matsudana*) × 131/25 (*S. babylonica* × *S. alba*)], basal diameter (18.60mm) for T₂₇[PN227 (*S. matsudana*) × *S. tetrasperma*], volume index (1071.92cm³) for T₇[PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)], number of branches (22.5) for T₄₂ [Kashmiri (*S. alba*)], number of nodes (120) for T₆ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)], internodal length (3.27cm) for T₃₉ [J799 (*S. matsudana* × *S. alba*)], leaf area (26.85cm²) for T₃₃ [J799 (*S. matsudana* × *S. alba*) Open pollinated], fresh shoot weight (380.59g) for T₂₅ [PN227 (*S. matsudana*) × *S. tetrasperma*], dry shoot weight (208.05g) for T₂₅ [PN227 (*S. matsudana*) × *S. tetrasperma*], fresh root weight (122.46g) for T₄₁ [NZ1002 (*S. matsudana* × *S. alba*)], dry root weight (62.12g) for T₃₉ [J799 (*S. matsudana* × *S. alba*)] and total dry biomass (267.91g) for T₂₅ [PN227 (*S. matsudana*) × *S. tetrasperma*].

With respect to wood characteristics, the maximum value for bark per cent (27.45%) was observed for T₁₅ [PN227 (*S. matsudana*) × J194 (*S. matsudana* × *S. arbutifolia* × *S. matsudana*)], wood Per cent (85.02%) for T₄₀ [J795 (*S. matsudana* × *S. alba*)], moisture content (33.48%) for T₃₇ [J799 (*S. matsudana* × *S. alba*) Open pollinated], specific gravity (0.52gcm⁻³) for T₁₃ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)], and fibre length (0.77mm) for T₂₆ [PN227 (*S. matsudana*) × *S. tetrasperma*].

The variability and genetic estimates were computed for each trait. GCV ranged between (4.23-29.38%), PCV (7.17-33.61%) and heritability ranged between (0.16-0.85) for all growth and biomass parameters. Volume index, leaf area and all biomass characters showed high heritability with high genetic gain. For wood parameters GCV ranged between (8.77-9.98%), PCV (4.47-17.35%) and heritability (0.24-0.50). Bark Per cent showed high heritability (0.50) and moderate genetic gain (14.48%) among wood characters.

Correlation studies demonstrated the positive and significant correlation among most of the traits. Highly significant and positive correlation was observed for fresh shoot weight and total biomass (0.981) and basal diameter and volume index (0.974) among growth and biomass characters.

Correlation between growth and biomass characters with wood characters found to be highly positive and significant between height and wood Per cent (0.395), fresh root weight and wood Per cent (0.456), dry root weight and wood Per cent (0.423), basal diameter with moisture content (0.428), internodal length and moisture content (0.360), specific gravity and internodal length (0.374), fibre length and dry root weight (0.350) and fibre length and total dry biomass (0.343).

RAPD pattern was studied using 11 primers, out of which 9 were amplified. Total 39 bands were amplified and all were found to be polymorphic. The size range of polymorphic bands was 150.22bp – 2188.65bp. Jaccard's similarity correlation was obtained which ranged between 0.19 – 0.61. Maximum similarity correlation coefficient of 0.61 was observed for genotypes T₅[PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)] and T₁₁ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)]. The lowest value of 0.19 was recorded for genotypes T₁₂[PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)] with T₁₉ [PN227 (*S. matsudana*) × NZ1179 (*S. matsudana* × *S. alba*)] and T₁₉ [PN227 (*S. matsudana*) × NZ1179 (*S. matsudana* × *S. alba*)] with T₂₇ [PN227 (*S. matsudana*) × *S. tetrasperma*]]. Genotype T₁₉ [PN227 (*S. matsudana*) × NZ1179 (*S. matsudana* × *S. alba*)] appeared to be the most diverse with similarity value 0.33 from the rest of the genotypes on the basis of dendrogram.

CONCLUSIONS

- Analysis of variance revealed highly significant differences among the different clones for growth and biomass characters thus exhibiting more variability. Significant differences were also observed for wood characters of different clones.

- Clone T₁[(PN227 (*S. matsudana*) × 131/25 (*S. babylonica* × *S. alba*)] followed by T₂₉ [PN227 (*S. matsudana*) × SI-63-007 (*S. alba*)], T₇ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)] and T₃₉ [J799 (*S. matsudana* × *S. alba*)] were found to be significantly higher in performance in height parameter.
- Based on dry shoot weight, as an indicator of productivity T₂₅ [PN227 (*S. matsudana*) × *S. tetrasperma*], T₂₀ [PN227 (*S. matsudana*) × NZ1179 (*S. matsudana* × *S. alba*)], T₂₃ [PN227 (*S. matsudana*) × NZ1179 (*S. matsudana* × *S. alba*)] and T₄ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)] were found significantly outperforming from rest of the clones.
- Specific gravity was found highest in clone T₁₃ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)].
- High heritability corresponding with the high genetic gain was observed for volume index, leaf area and all biomass characters.
- Biomass characteristics were found to be positive and significantly correlated with most of the characters except internodal length, number of branches and leaf area. Hence, the selection of the clones can be effectively practised on key growth characteristics.
- Amplification of genomic DNA for 25 genotypes from 9 different random decamer primers generated a total of 39 bands and all were polymorphic in nature.
- The number of bands amplified per primer ranged from 3 to 6 with mean of 4.33 bands per primer. The size of amplification product ranged from 150.22bp – 2188.65bp.
- Highest numbers of RAPD marker (6) was obtained from primer OPA – 4.
- Similarity coefficient value between 25 genotypes of willow clones ranged from 0.19 to 0.61. This indicates the presence of a fair amount of variability in the genotypes. The highest value 0.61 is the similarity coefficient between T₅ and T₁₁.
- At similarity index value (0.33) T₁₉ [PN227 (*S. matsudana*) × NZ1179 (*S. matsudana* × *S. alba*)] was separated from the rest of genotypes giving information about its most diverse nature.
- The genetic diversity observed in the genotypes can be used for hybridization and improvement programmes.

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**DR. Y.S. PARMAR UNIVERSITY OF HORTICULTURE & FORESTRY
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DEPARTMENT OF TREE IMPROVEMENT AND GENETIC RESOURCES

Title of Thesis : “Nursery evaluation of willow (*Salix* spp.) clones”
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Admission Number : F-2014-23-M
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ABSTRACT

The present investigation entitled “Nursery evaluation of willow (*Salix* spp.) clones” was carried out in the Department of Tree Improvement and Genetic Resources, Dr. Y S Parmar University of Horticulture and Forestry Nauni, Solan (HP) during 2015-2016. Forty two clones of willow were procured from the existed clonal material in the department. Analysis of variance revealed highly significant differences among the clones. Clone T₁ [PN227 (*S. matsudana*) × 131/25(*S. babylonica* × *S. alba*)] was found to have maximum value for height, T₇ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)] was found to have maximum value for volume index and clone T₂₅ [PN227 (*S. matsudana*) × *S. tetrasperma*] was found to have maximum values for biomass traits. Maximum value for specific gravity was exhibited by clone T₁₃ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)]. Fibre length was observed maximum in T₂₆ [PN227 (*S. matsudana*) × *S. tetrasperma*]. Variability estimates depicted that volume index, leaf area and all biomass characters showed high heritability value with corresponding high genetic gain among the growth traits. Correlation coefficients were found positive and significant among the most of the characters; wood per cent was recorded to have negative correlation with others traits. RAPD pattern was studied using 11 primers out of which 9 showed amplification with 100 per cent polymorphism. Thirty nine scorable were identified in the range size of 150.22 - 2188.65bp. The Dendrogram generated using RAPD markers showed clone T₁₉ [PN227 (*S. matsudana*) × NZ1179 (*S. matsudana* × *S. alba*)] as most divergent with 33 per cent similarity. Maximum similarity of 61 per cent was observed between clone T₅ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)] and T₁₁ [PN227 (*S. matsudana*) × AUSTREE (*S. alba* × *S. matsudana*)].

Signature of the Student

Name: Leena Kumari

Date :

Signature of the Major Advisor

Name: Dr. I K Thakur

Date :

Countersigned

Head of the Department

APPENDIX – I

Original identity with scientific name

Clones	Number of clones	Original identity
T ₁ , T ₂	2	PN227 (<i>S.matsudana</i>)×131/25(<i>S.babylonica</i> × <i>S.alba</i>)
T ₃ , T ₄ , T ₅ , T ₆ , T ₇ , T ₈ , T ₉ , T ₁₀ , T ₁₁ , T ₁₂ , T ₁₃	11	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
T ₁₄ , T ₁₅	2	PN227(<i>S.matsudana</i>)×J194(<i>S.matsudana</i> × <i>S.arbutifolia</i> × <i>S.matsudana</i>)
T ₁₆ , T ₁₇ , T ₁₈ , T ₁₉ , T ₂₀ , T ₂₁ , T ₂₂ , T ₂₃ , T ₂₄	9	PN227 (<i>S.matsudana</i>)×NZ1179(<i>S.matsudana</i> × <i>S.alba</i>)
T ₂₅ , T ₂₆ , T ₂₇ , T ₂₈	4	PN227 (<i>S.matsudana</i>)× <i>S.tetrasperma</i>
T ₂₉ , T ₃₀ , T ₃₁ , T ₃₂	4	PN227 (<i>S.matsudana</i>)×SI-63-007(<i>S.alba</i>)
T ₃₃ , T ₃₄ , T ₃₅ , T ₃₆ , T ₃₇ , T ₃₈	6	J799 (<i>S.matsudana</i> × <i>S.alba</i>)Open pollinated
T ₃₉	1	J799 (<i>S.matsudana</i> × <i>S.alba</i>)
T ₄₀	1	J795(<i>S.matsudana</i> × <i>S.alba</i>)
T ₄₁	1	NZ1002(<i>S.matsudana</i> × <i>S.alba</i>)
T ₄₂	1	KASHMIRI (<i>S.alba</i>)
Total no. of clones	42	

APPENDIX – II

Original identity with scientific name of the clones used in molecular study

S.No.	Clones	Original identity
1.	T ₄	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
2.	T ₃₁	PN227 (<i>S.matsudana</i>)×SI-63-007(<i>S.alba</i>)
3.	T ₇	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
4.	T ₃₄	J799 (<i>S.matsudana</i> × <i>S.alba</i>)Open pollinated
5.	T ₃₀	PN227 (<i>S.matsudana</i>)×SI-63-007(<i>S.alba</i>)
6.	T ₁₂	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
7.	T ₄₁	NZ1002(<i>S.matsudana</i> × <i>S.alba</i>)
8.	T ₄₀	J795(<i>S.matsudana</i> × <i>S.alba</i>)
9.	T ₁₄	PN227(<i>S.matsudana</i>)×J194(<i>S.matsudana</i> × <i>S.arbutifolia</i> × <i>S.matsudana</i>)
10.	T ₂	PN227 (<i>S.matsudana</i>)×131/25(<i>S.babylonica</i> × <i>S.alba</i>)
11.	T ₁₃	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
12.	T ₁₅	PN227(<i>S.matsudana</i>)×J194(<i>S.matsudana</i> × <i>S.arbutifolia</i> × <i>S.matsudana</i>)
13.	T ₂₅	PN227 (<i>S.matsudana</i>)× <i>S.tetrasperma</i>
14.	T ₁	PN227 (<i>S.matsudana</i>)×131/25(<i>S.babylonica</i> × <i>S.alba</i>)
15.	T ₁₉	PN227 (<i>S.matsudana</i>)×NZ1179(<i>S.matsudana</i> × <i>S.alba</i>)
16.	T ₃₂	PN227 (<i>S.matsudana</i>)×SI-63-007(<i>S.alba</i>)
17.	T ₈	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
18.	T ₃₉	J799 (<i>S.matsudana</i> × <i>S.alba</i>)
19.	T ₉	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
20.	T ₄₂	KASHMIRI (<i>S.alba</i>)
21.	T ₂₉	PN227 (<i>S.matsudana</i>)×SI-63-007(<i>S.alba</i>)
22.	T ₁₁	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
23.	T ₂₆	PN227 (<i>S.matsudana</i>)× <i>S.tetrasperma</i>
24.	T ₅	PN227 (<i>S.matsudana</i>)× AUSTREE (<i>S.alba</i> × <i>S.matsudana</i>)
25.	T ₂₇	PN227 (<i>S.matsudana</i>)× <i>S.tetrasperma</i>

APPENDIX – III

ANOVA of mean values for growth and biomass characteristics of 42 genotypes of willow (*Salix* spp.)

Character	Mean sum of square (Source of variation)		
	Replication	Treatment	Error
df	3	41	123
Height (cm)	602.4417*	1082.195*	199.5686
Collar diameter (mm)	10.60798*	13.99389*	1.759158
Volume index (cm ³)	47449.25*	115157.3*	11765.07
Number of branches	42.06944*	50.67668*	14.02473
Number of nodes	501.4841*	187.8269*	105.0736
Inter nodal length (cm)	0.438479*	0.721411*	0.13942
Leaf area (cm ²)	13.89562*	77.47978*	4.574477
Fresh shoot weight (g)	4076.415*	21815.5*	1495.658
Dry shoot weight (g)	1139.998*	6348.073*	277.7105
Fresh root weight(g)	834.6109*	1380.426*	108.3847
Dry root weight (g)	515.3646*	373.2899*	52.03978
Total dry biomass (g)	2783.166*	8070.279*	349.0182

ANOVA of mean values for wood characteristics of 42 genotypes of willow (*Salix* spp.)

Character	Mean sum of square (Source of variation)		
	Replication	Treatment	Error
df	3	41	123
Bark (%)	20.71938*	28.69211*	5.803027
Wood (%)	20.71938*	28.69211*	5.803027
Moisture (%)	74.49179*	86.66402*	27.47579
Specific gravity	0.018236*	0.009249*	0.003896
Fibre length (mm)	0.013253*	0.006588*	0.002893

APPENDIX – IV

Variance of growth and biomass characters

Character	$\sigma^2 P$	$\sigma^2 G$	$\sigma^2 E$
Height (cm)	420.2253	220.6567	199.5686
Collar diameter (mm)	4.81784	3.058682	1.75918
Volume index (cm ³)	37613.14	25848.07	11765.07
Number of branches	23.18772	9.162989	14.02473
Number of nodes	125.7619	20.68835	105.0736
Inter nodal length (cm)	0.284918	0.145498	0.13942
Leaf area (cm ²)	22.8008	18.22633	4.574477
Fresh shoot weight (g)	6575.619	5079.962	1495.658
Dry shoot weight (g)	1795.301	1517.591	277.7105
Fresh root weight(g)	426.3949	318.0103	108.3847
Dry root weight (g)	132.3523	80.31254	52.03978
Total dry biomass (g)	2279.333	1930.315	349.0182

Variance of wood characters

Character	$\sigma^2 P$	$\sigma^2 G$	$\sigma^2 E$
Bark (%)	11.5253	5.72227	5.803027
Wood (%)	11.5253	5.72227	5.803027
Moisture (%)	42.07285	14.79706	27.47579
Specific gravity (gcm ⁻³)	0.005234	0.001338	0.003896
Fibre length (mm)	0.003817	0.000924	0.002893

BIO-DATA

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Scholarship/ Stipend/ Fellowship, any : Stipend
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 during the study period

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