Comparative Meiotic Chromosome Studies in Nine Accessions of *Tecomella undulata* (Sm.) Seem., Threatened Tree of Indian Desert

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Abstract

Meiotic studies were carried out in nine different accessions of *T. undulata* collected from three districts of Rajasthan, India. Data collected on chromosome associations, chiasma frequency and their distribution pattern concluded that the somatic chromosome number of *T. undulata* is 2n = 2x = 36 which is at variance with published literature. The high frequency of 18:18 chromosome distribution at anaphase I and an overwhelming percentage of stainable pollens observed are indicative of overall genomic stability as supported by complete absence of accessory chromosomes (B) and supernumerary nucleoli. Numerical alteration of chromosomes might have played an important role in origin and adaptation of *T. undulata* to the adverse climate conditions of Indian desert.

Key words: genetic variation, meiotic studies, threatened tree, *Tecomella undulata*, Bignoniaceae.

Introduction

Tecomella undulata (Sm.) Seem. belonging to family Bignoniaceae and is commonly known as desert teak (ver. Rohiro). In India family Bignoniaceae is represented by 21 genera and about 25 species including the non-indigenous ornamental plants. Of these genera *Tecomella* Seem. is a monotypic genus and one of the most important deciduous, ornamental shrub/tree of the arid zone regions in India (SHANKARANARAYAN and NANDA, 1963). The natural stands of *T. undulata* are restricted to the western parts and a few to southeastern parts of Pakistan. The species has been identified as an important for environmental conservation in arid zones as a stabilizer of shifting sand dunes, providing shelter for wild life. It is also a very useful species for afforestation of the drier tracts due to its drought and fire resistant properties (SHANKARANARAYAN and NANDA, 1963). It is a common agroforestry tree species in the Thar desert of Rajasthan for its higher survival rates even in extreme drought conditions (ANONYMUS, 2003). *T. undulata* is highly valuable in terms of economy for its quality wood (BHANDARI, 1990; SINGH, 1992) and medicinal properties (JOSHI and SINGH, 1974, 1977; JOSHI et al., 1977; AHMAD et al., 1994; ANONYMOUS, 2003; OUDHIA, 2005).

Indiscriminate felling for timber and fuel by the local populace, coupled with poor regeneration has severely depleted the natural populations of this valuable tree, with an associated loss of valuable germplasm. The species has been designated as a "threatened" plant species in Rajasthan province of India (PANDEY et al., 1983; SHETTY and SINGH, 1987; TRIPATHI and JAIMINI, 2002). Moreover United Nations Environment Programmes (UNEP), World Conservation Monitoring Centre (WCMC) Nairobi, Kenya, has included *T. undulata* into "Category 1- Indeterminate" of their list of threatened plants to emphasize the status of *T. undulata* and the urgent need for conservation (ANONYMOUS, 2003; ANONYMOUS, 2005). Despite the greater importance of *T. undulata* as economical, ethnobotanical and medicinally important tree, attempts for its conservation, sustainable utilization and/or genetic improvement, are by and large lacking. A quick perusal of the published literature reveals that JINDAL et al. (1992) have studied the growth pattern in half-sibling progeny of *T. undulata*, established at an experimental site in India and recorded significant differences among progenies in overall growth over a period of six years. They also found the need for additional selection procedures for higher genetic gains of tree height in place of single tree selection from a family. VIR et al. (1994) have observed differential resistance to stem borer (*Stegmatophora* sp.) and also recommended the need for management of leaf skeletonizer (*Ptialus tecomella*) through applied control measures. JINDAL and PANCHOLY (1994) have observed that viability of seeds with low moisture content can be maintained for longer periods, if stored in air tight containers.

Cytogenetic and molecular mechanisms controlling the organization and adaptation of the genome in this tree species remains quite ambiguous to date. The true somatic chromosome number for *T. undulata* has been debated. SINGH (1992) reported 2n = 2x = 32 as the somatic chromosome number of *T. undulata* through the analyzing root tips of invitro regenerants, while an entirely unrelated number n = 11 been reported by SHANKARANARAYAN and NANDA (1963). The chromosome number reports of the other genera of this family includes *Tecoma grandiflora* Del.2n = 38 (VENKATASUBBAN, 1944), *Tecoma radicans* (Linn.) Seem. 2n = 32 (KONDO, 1972) and 2n = 40 (SAX, 1933; RAGHAVAN and VENKATASUBBAN, 1940; VENKATASUBBAN, 1944). Recently genetic diversity has been reported in the natural populations of *T. undulata* using AFLP analysis (ANONYMOUS, 2003; ANONYMOUS, 2005). In view of conflicting information on the chromosome number in this species, a study was conducted on the cytologenetic parameters in populations of *T. undulata* collected from the different areas of Rajasthan to determine the true chromosome number and investigate cytogenetic variability within the species.

Material and Methods

Populations of *T. undulata* in three districts, Jodhpur, Barmer and Nagaur, of Rajasthan state were sampled (*Fig. 1*). Three representative trees of each location with distinct phenological variations like plant height (shrub/small tree/large tree), flower color (red/orange/yellow), pod length (large/medium/small) have been marked and labeled properly. The voucher specimens are deposited with Botanical Survey of India, Jodhpur (BSJO), and accession numbers were obtained (*Table 1*). Flower buds of about 4–6 mm size were collected for meiotic studies from selected mature trees and fixed in freshly prepared Carnoy's fluid for a minimum of 24 hours at room temperature and latter stored in 70% alcohol at 10 °C.

Anthers were squashed in 1% acetocarmine solution for meiotic studies. For each phenotype within an accession, 25 pollen

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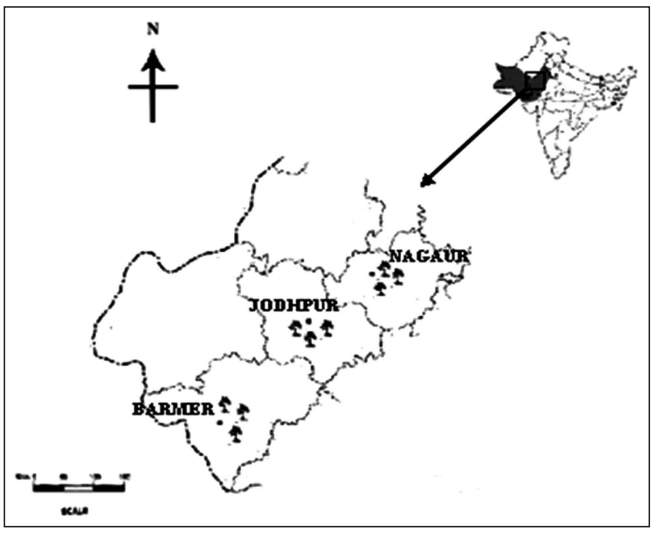

Figure 1. – Map depicting the locations in Rajasthan where *Tecomella undulata* accessions were sampled (Inlet shows the natural geographical distribution).

mother cells (PMCs) were analyzed at diplotene/diakinesis/metaphase I to estimate the range of chromosome associations and recombinational frequencies through chiasma analysis. At anaphase I/II 15-20 cells were analyzed for distributional pattern of chromosomes. The details regarding various accessions analyzed in the present investigations, their place of collection, total number of PMCs analyzed, chromosome number and their associations at diplotene/diakinesis/metaphase I are summarized in *Table 1*. The data on total and mean number of chiasmata, and its range along with number of chiasmata terminalized, terminalization coefficient and the percentage pollen stainability is summarized in *Table 2*. The distribution pattern of chromosomes at anaphase I has been detailed in *Table 3*. Most of these observations are illustrated in Figs. 2–19. To estimate the percentage of viable male gametes, pollen grains were stained in 1:1 glycerine: acetocarmine mixture, and stained pollen were considered viable. Agfa Copex Pan photonegative film (ASA-20) was used to make photomicrographs from temporary preparations.

Results

The chromosome number of *T. undulata* was found to be 2n = 36 in all accessions and phenotypes within accessions. When the meiotic data collected from different accessions belonging to three locations were compared, it was observed that one accession each from Nagaur and Barmer locations showed aberrant PMCs with 17 bivalents (*Table 1*), while no such deviation were observed in any of the accessions from Jodhpur location. In contrast, when the chromosome associations were analyzed it was observed that all three locations had at least one accession exhibiting quadrivalent associations (*Table 1*), although very rarely. There were no marked variations between plants collected from the three different locations with respect to total number of bivalents. Marked differences (*Table 1*), however, were found in ring and rod types in BSJO-25144 collected from Nagaur location. The number of univalents observed in PMCs of plants collected from Nagaur was highest when compared to those of Barmer location. The corresponding value was moderate for plants of Jodhpur location (*Table 1*).

The mean number of chiasmata per PMC was comparatively low in different accessions from Nagaur as compared to the other two sites (*Table 2*). One accession each from Nagaur and Jodhpur location (BSJO-25144, BSJO-25150) (*Table 2*) were characteristic in having very few chiasmata terminalized, which was also reflected in their terminalization coefficient values. On contrary, all the other accessions studied had more number of chiasmata terminalized with greater terminalization coefficient value. From studies on percentage pollen stainability (*Table 2*), it is clear that no variability exhibited in the plants collected from three different locations.

Furthermore, it is quite interesting to note that one accession from each of the three locations (BSJO-25146, BSJO-25149, BSJO-25152) showed lagging univalents and/or at anaphase I, while other accessions had shown equal distribution of chromosomes at anaphase I, in majority of the PMCs (*Table 3*).

From the data summarized in *Table 1*, it is clear that all accessions were characteristic in exhibiting 18 bivalents at diplotene/diakinesis/metaphase I in all the PMCs analyzed, with the exception of BSJO-25146, BSJO-25147. In a rare

Table 1. – Mean number and range of associations at diplotene/diakinesis/metaphase I in *Tecomella undulata* accessions.

Accession Number	Place of collection (District)	No. of cells analyzed	Cells With n=18		Cells With n=17		Chromosome associations													
							Quadrivalents			Bivalents									Univalents		
										Total			Ring			Rod					
			No.	%	No.	%	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range
BSJO-25144	Ren (Nagaur)	25	25	100	-	-	-	-	-	440	17.60 ±0.80	15-18	288	11.52 ±2.80	5-17	152	6.08 ±2.62	1-12	20	0.80 ±1.60	0-6
BSJO-25145	Kuchera (Nagaur)	25	25	100	-	-	1	0.04 ± 0.20	0-1	431	17.24 ± 0.81	16-18	413	16.52 ±1.20	14-18	18	0.72 ±1.18	0-4	34	1.36 ±1.57	0-4
BSJO-25146	Kheduli (Nagaur)	15	14	93.33	1	6.67	1	0.07 ± 0.25	0-1	254	16.93 ±0.93	15-18	253	16.87 ±0.88	15-18	1	0.07 ±0.25	0-1	26	1.73 ±1.91	0-6
BSJO-25147	Hatitala (Barmer)	30	29	96.67	1	3.33	2	0.07 ±0.25	0-1	533	17.77 ±0.56	16-18	529	17.63 ±0.71	15-18	4	0.13 ±0.34	0-1	4	0.13 ±0.50	0-2
BSJO-25148	Sansiyon ka tala (Barmer)	25	25	100	-	-	-	-	-	443	17.72 ±0.72	15-18	434	17.36 ±1.49	11-18	9	0.36 ±1.20	0-6	14	0.56 ±1.44	0-6
BSJO-25149	Dhandhalawas (Barmer)	25	25	100	-	-	-	-	-	442	17.68 ±0.84	15-18	438	17.52 ±0.85	15-18	4	0.16 ±0.37	0-1	16	0.64 ±1.67	0-6
BSJO-25150	University campus (Jodhpur)	25	25	100	-	-	-	-	-	445	17.80 ±0.40	17-18	441	17.64 ±0.62	16-18	4	0.16 ±0.54	0-2	10	0.40 ±0.80	0-2
BSJO-25151	Guda Vishnoiyan (Jodhpur)	24	24	100	-	-	2	0.08 ±0.40	0-1	420	17.50 ±1.12	14-18	417	17.38 ±1.35	14-18	3	0.13 ±0.33	0-1	16	0.67 ±1.70	0-6
BSJO-25152	Indroka (Jodhpur)	25	25	100	-	-	-	-	-	433	17.32 ±0.73	16-18	406	16.24 ±1.82	10-18	27	1.08 ±1.70	0-7	34	1.36 ±1.47	0-4

Table 2. – Mean number, range of chiasmata, terminalization coefficient and pollen stainability in *Tecomella undulata* accessions.

Accession Number	No. of cells analyzed	Chiasmata				Terminalization coefficient	Percentage pollen stain ability
		Mean	Range	Terminalized	Unterminalized		
BSJO-25144	25	29.12 ±3.18	22-35	22.8 ±4.39	6.32 ±3.03	0.78	93.67
BSJO-25145	25	33.92 ±1.70	30-36	31.84 ±2.26	2.08 ±1.55	0.94	91.67
BSJO-25146	15	34.07 ±1.80	30-36	31.67 ±4.69	2.40 ±3.05	0.93	93.86
BSJO-25147	30	35.67 ±0.65	34-36	34.17 ±1.57	1.50 ±1.45	0.96	89.19
BSJO-25148	25	35.08 ±2.02	28-36	33.44 ±3.49	1.64 ±1.69	0.95	90.00
BSJO-25149	25	35.20 ±1.65	30-36	32.52 ±3.52	2.68 ±2.46	0.92	92.13
BSJO-25150	25	35.44 ±0.90	34-36	28.60 ±4.90	6.84 ±4.71	0.81	93.22
BSJO-25151	24	35.21 ±2.00	29-36	33.83 ±2.36	1.38 ±1.93	0.96	91.30
BSJO-25152	25	33.56 ±2.19	27-36	31.44 ±3.07	2.12 ±2.39	0.94	92.21

Table 3. – Anaphase I distribution in *Tecomella undulata* accessions.

Accession number	2n	Number of cells analyzed	Chromosome distribution (Number of cells)
BSJO-25144	36	15	18:18 (15)
BSJO-25145	36	15	18:18 (15)
BSJO-25146	36	14	18:18 (13) 18: 1B: 16 (1)
BSJO-25147	36	16	18:18 (16)
BSJO-25148	36	15	18:18 (15)
BSJO-25149	36	16	18:18 (15) 18: 1 U: 17 (1)
BSJO-25150	36	15	18:18 (15)
BSJO-25151	36	15	18:18 (15)
BSJO-25152	36	14	18:18 (12) 15:6U*: 15 (2)

U = Univalents, B = Bivalents, * = Chromatid bridge.

occurrence one PMC with 17 bivalents was encountered in two accessions viz. BSJO-25146 and BSJO-25147. Occasional quadrivalents were encountered in four accessions BSJO-25145, BSJO-25146, BSJO-25147 and BSJO-25151 in one, one, two and two PMCs respectively. However, their number never exceeded one per PMC. The mean value of total number of bivalents per PMC was highest (17.8) in BSJO-25150 and lowest (16.93) in BSJO-25146, while the remaining accessions had in between values.

Among bivalents, ring types were encountered more frequently than rod types in all the accessions analyzed presently. The mean value for ring type ranged between 11.52 (BSJO-25144) and 17.64 (BSJO-25150) while the corresponding values for rod type were 0.07 (BSJO-25146) and 6.08 in BSJO-25144 as expected. Univalents were characteristically present in all the accessions, with a lowest mean value of 0.13 per PMC in BSJO-25147, and the highest being 1.73 in BSJO-25146.

From the data presented in *Table 2* it is apparent that mean number of chiasmata per cell ranged from 29.12 (BSJO-25144) to 35.67 (BSJO-25147), while the other accessions had the values ranging between these two extremes. A minimum of 22 chiasmata per PMC was observed in BSJO-25144, while a maximum number of 36 chiasmata were found in all the accessions. BSJO-25144 was characteristic in having minimum number of terminalized chiasmata per PMC (22.8) while a maximum 34.17 was recorded in BSJO-25147. Similarly BSJO-25151 had minimum number of un-terminalized chiasmata whereas BSJO-25150 had recorded highest value of 6.84. The terminalization coefficient mostly ranged between 0.92 to 0.96 in all the accessions. However BSJO- 25144 with 0.78 and BSJO-25150 with 0.81 were the exceptions (*Table 2*).

Majority of the PMCs analyzed at anaphase I /II had shown equal distribution of chromosomes (18:18) in all the accessions investigated. Few PMCs in BSJO-25146 (1), BSJO-25149 (1) and BSJO-25152 (2) had shown lagging bivalent (0-1) and/or lagging univalents (1-6) respectively. The percentage pollen stainability hovered around 90 percent in all these accessions.

Discussion

The diploid chromosome number of *T. undulata* (2n = 36) found in the present study differed from previous reports. Singh (1992) studied mitosis using root tips of invitro regenerated plants, which can have altered chromosome numbers due to the regeneration process (Orton, 1980; Constantin, 1981). The meiotic study of Shankaranarayan and Nanda (1963) found 2n = 32, but was conducted on a single tree from a single location. In contrast, the present investigation analyzed approximately 200 PMCs from trees inhabiting different locations and has consistently showed the gametic number of n = 18 with two insignificant exceptions.

The lack of variation in chromosome number and occurrence of only bivalents or bivalent-univalent associations, with the rare occurrence of multivalent associations support *T. undulata* as being a diploid species. The chromosome number reports of the other genera of this family includes *Tecoma grandiflora* 2n = 38 (Venkatasubban, 1944), *Tecoma radicans* 2n = 32 (Kondo, 1972) and 2n = 40 (Sax, 1933; Raghavan and Venkatasubban, 1940; Venkatasubban, 1944), which indicate that more study is needed on other Bignoniaceae species before a final conclusion is made on basic number.

From the meiotic data dealing with chromosome associations in nine different accessions, it is evident that three accessions

have showed variation with respect to mean number of univalents per cell. Occurrence of univalents in a relatively higher proportion is indicative that there is certain degree of structural heterogeneity in the gametic makeup of the bivalents. This observation further draws support by high mean value for rod bivalents in BSJO-25144 and BSJO-25152 accessions. Such phenomenon is reported in a number of tropical tree genera like *Anogessius pendula* Edgew., *A. sericea* var. *sericea* Bran-

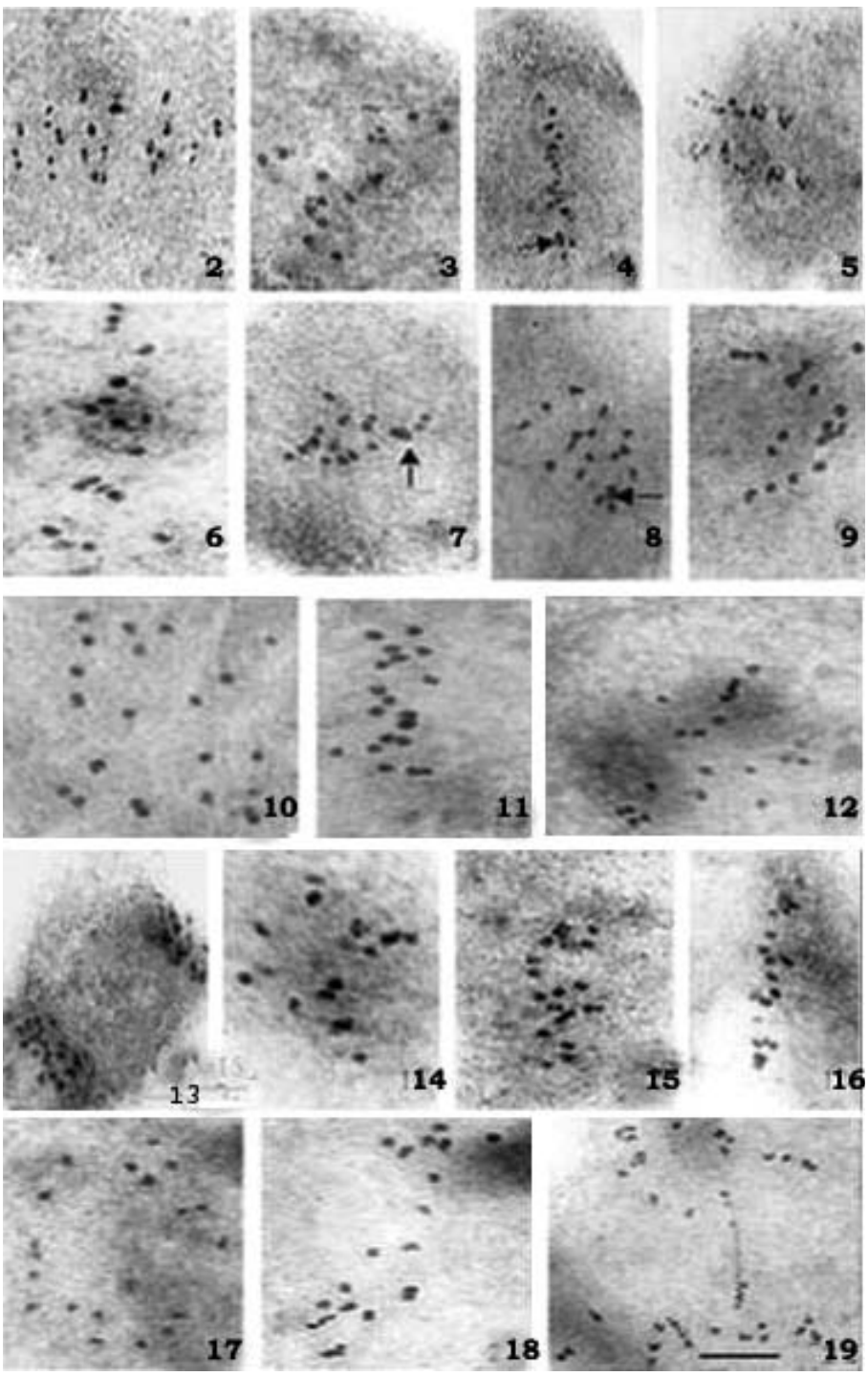

Figure 2–19. – Male meiosis in *Tecomella undulata*; *T. undulata* BSJO- 25144, 2. Metaphase I 18II. (3-5) *T. undulata* BSJO- 25145, 3. Diakinesis 18II. 4. Metaphase I 1IV + 16II. 5. Anaphase I. (6-7) *T. undulata* BSJO- 25146, 6. Metaphase I 18II. 7. Metaphase I 1IV + 16II. (8-9) *T. undulata* BSJO- 25147. 8. Diakinesis 1IV + 16II. 9. Diakinesis 18II. (10-11) *T. undulata* BSJO- 25148. 10. Diakinesis 16II + 4I. 11. Metaphase I 18II. (12-13) *T. undulata* BSJO- 25149, 12. Metaphase I 18II. 13. Anaphase I, *T. undulata* BSJO- 25150, 14. Metaphase I 18II. (15-16) *T. undulata* BSJO- 25151, 15. Metaphase I 18II. 16. Metaphase I 17II + 2I. (17-19) *T. undulata* BSJO- 25152, 17. Diakinesis 17II + 2I. 18. Metaphase I 16II + 4I. 19. Anaphase I with chromatid bridge. Scale bar = 10 µm. (Arrow head showing quadrivalents).

dis., *A. latifolia* (Roxb. Ex DC) Wall. Ex Guill. and Perr. (KUMAR and RAO, 2002), *Salvadora persica* L., *S. oleoides* Decne. (KUMAR et al., 2002), *Capparis decidua* (Forsk) Edgew (KUMAR and RAO, 2003) and *Balanites ageyptiaca* (L.) Del., (unpublished). Occasional encounter of multivalents in the form of quadrivalents, in three accessions, did not influence the mean number of chromosome association significantly. The mean chiasma frequency has been observed to be very high in as many as five out of nine accessions studied presently. Extreme small size of the bivalents has been the major obstacle in locating and counting of chiasmata. However, most of the chiasmata are traced to distal position leading to early terminalization of bivalents, even at the beginning of diakinesis itself. Distally located chiasmata may have prevented some chromosome segments in the bivalents from undergoing genetic recombinations and enhancing linkage among the constituent genes (CHUA and ROEDER, 1998; MOORE, 1998; SCHNABLE et al. 1998; SYBENGA; 1996, 1999, ZICKLER and KLECKNER, 1999: HUSBAND, 2004). Such observations were earlier reported in large number of plants (SYBENGA, 1972, SINGH, 1993) including tropical hardwood tree species (RAO and KUMAR, 2004).

The equal distribution of chromosomes/chromatids at anaphase I is normal in the meiotic process in plants and is indicative of overall genomic stability and its manifestation. Few PMCs which showed lagging univalents/bivalents did not apparently influence the viability of the gametes.

The complete absence of accessory chromosomes (B) and supernumerary nucleoli in PMC s followed by high percentage of pollen stainability indicates an overall genomic stability in *T. undulata* and the apparent phenotypic variation cannot be linked to chromosome level genetic variation. Recently a report (ANONYMOUS, 2003; ANONYMOUS, 2005) concerning the genetic diversity analysis in 42 genotypes using AFLPs markers has been published which found the significant genetic divergence among the genotype studied. They have attributed the broad genetic base of *T. undulata* to out crossing nature and argued about the utility of their study in identifying the diverse genetic stocks and determining the conservation priorities of *T. undulata* genotypes. However, such information further needs to be corroborated by information generated by more defined molecular markers.

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Genotype by Environment Interaction in *Pinus sylvestris* L. in Southern Sweden

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Abstract

To estimate the amount of genotype by environment interaction (G x E) data was obtained within the Swedish breeding program of *Pinus sylvestris* L. The calculations were based on estimates of G x E expressed by the genetic correlations across trials. In total, 66 progeny trials were included coming from 17 different test series. The number of parents tested per progeny trial was in average 52. Some parents were tested in several series and in total 812 parents were represented in the study. The results of our study showed that the amount of G x E for growth traits in *Pinus sylvestris* in southern Sweden was low. The median genetic correlation across trials for height, height increment and diameter were in the range 0.75–0.80 and the pattern of interaction was largely unpredictable from site differences in site index, latitude, longitude and altitude.

Key words: Scots pine, genetic correlation, stability, progeny testing.

Introduction

Clone and progeny testing in field tests is one of the most important and costly components of a tree breeding program. The objective is to compare the selection candidates under conditions similar to those under which mass-propagated material will be grown. Several considerations have to be made to design an efficient testing, but a key element is to find the optimum number of trials to be used. In Sweden, the testing of *Pinus sylvestris* L. was designed to identify selection candidates that perform well across sites within seed zones assuming that the genotype by environment interaction (G x E) is low within seed zones. The division of zones was based on the general adaptation pattern of *Pinus sylvestris*, which in Sweden is characterised by a clinal variation along a latitudinal temperature gradient (LANGLET, 1936; EICHE, 1966). The population differentiation is lower in southern Sweden (JOHNSSON, 1970) as opposed to the stronger effects reported from studies in northern Sweden (ERIKSSON et al., 1980; PERSSON, 1994) where the climate is harsher. As a consequence, larger seed zones have been used in southern than in northern Sweden. Within seed zones the optimum number of trials is highly dependent on the amount of G x E and this optimum number of trials is further explored in an adjacent study (HANNRUP et al., 2007). The large body of *Pinus sylvestris* progeny tests now being available enables an accurate quantification of the amount of G x E within seed zones of this species in southern Sweden.

An array of statistical methods has been used to quantify the amount of G x E such as traditional analysis of variance, regression methods and multivariate techniques (for review of methods see SKROPPA, 1984). In *Pinus* species, such studies have commonly indicated statistical significant interactions, but the interactions have not been sufficiently strong to warrant any further subdivision of breeding populations or seed zones (MATHESON and RAYMOND, 1984; MCKEAND et al., 1990; PSWARAYI et al., 1997). Alternatively, genetic correlations across sites have been used to quantify the amount of G x E. FALCONER (1952) introduced the concept of genetic correlations across environments by regarding a character measured in two different environments as two separate traits. As long as the correlation across sites is

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