International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

ISSR Marker Analysis of *Acacia Auriculiformis* in First Generation Orchard Populations

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Abstract: ***Acacia auriculiformisA.Cunn.exBenth. is an important fast growing tree species in plantation forestry programs and also used for pulp and paper production . In India, phyllodinous Acacias viz, Acacia auriculiformis and Acacia mangium have been used as the source of paper pulp and timber. Utility value and growth performance of these Acacia species led to the initiation of genetic improvement programs in the form of seedling seed orchards, progeny trials and hybrid production. With the technical collaboration of Australian Tree Seed Center, CSIRO, Australia, many short term and long term projects have been undertaken in the past fifteen years at the Institute of Forest Genetics & Tree Breeding (IFGTB), Coimbatore. The genetic structure of forest trees and the pattern of genetic variation that are maintained in the successive generations are essentially a function of the mating systems. The present work targeted the assessment of genetic diversity using Inter Simple Sequence Repeats (ISSR) in Acacia auriculiformis seedling seed orchards of first generation established by IFGTB in Panampalli, Kerala. Ten ISSR markers were profiled for fifty three individual phenotypes with two replications in the seedling seed orchard (SSO) population. The results from the ISSR data revealed that the total number of bands was 215 in which polymorphic bands were 184 and mean percentage of polymorphism was 85.5. The study showed that in the first generation orchard population have exhibited higher gene diversity (Nei's genetic diversity of 0.45)in Acaciaauriculiformis.***

Keywords: *Acacia auriculiformis*, ISSR markers, Polymorphism, genetic diversity, seed orchard

1. Introduction

In recent years research on domestication by genetic studies focusing on species of commercial interest from temperate and tropical countries (Moran, 1992; Butcher *et al*., 1999; Moran *et al.*, 2000). The patterns of variation using molecular markers (allozymes and DNA markers) can help more efficient sampling strategies for domestication programs and for identifying key populations for conservation.

Genetic diversity among seeds is an important aspect of plantation forestry especially when using seed crops (e.g. seed orchard crops). Seed orchards have become an important global seed source.(Zobel and Tolbert, 2003). The genetic quality of seed orchard crops and performance of resulting trees depend on many factors, including clone fertility, genetic diversity, mating design and combining ability (Hosius*et al*, 2006; Ertekin, 2010). Therefore the determining the genetic composition of seed orchard crops is important. When establishing new plantations and performing genetic research studies, genetic parameters such as genetic relatedness, inbreeding and genetic diversity should be measured and monitored in seed orchard crops. Genetic variation have also been reported for *A. auriculiformis* (Northern Black Wattle) (Wickneswari&Norwati, 1993), *A. mearnsii* (Black Wattle) (Searle *et al.*, 2000), *A. acuminata*(Jam) (Maslin *et al.*, 1999; Byrne *et al.*, 2001). A genetic linkage map has been constructed for *A. mangium* to locate genes associated with quantitative traits which can be used for marker assisted selection (Butcher *et al*., 2000a, 2000b; Butcher & Moran, 2000).

Acacia auriculiformis, an exotic, fast growing timber species, native of Australia which was introduced to India during World bank sponsored projects from CSIRO, Australia to Institute of Forest Genetics and Tree Breeding during 1996. The Institute has established first generation seedling seed orchards in Kerala, and Tamil Nadu. The present study envisaged the estimation of genetic diversity in the first generation orchard population, Panampalli, kerala through DNA markers. The level of genetic diversity in seed orchards is important to plantation forests because high levels will increase resistance to pest, diseases or climate change. Hence the present study was taken up to estimate the diversity in the orchard population.

2. Materials and Methods

Leaves samples were collected from fifty three individual trees in the seedling seed orchard population, Panampalli, Kerala.

DNA Isolation

The genomic DNA was isolated using DNeasyminikit(Quiagen) followed by the procedure prescribed it . The quality and quantity were checked by using 0.8% Agarose gel Electrophoresis and were stored at -20^{0}C until further use. Invitroamplificatiuon of DNA using polymerase reaction (PCR) was performed in0.2ml PCR tubes using 10-15ng of genomic DNA of each sample in a final volume of 10ul reaction mixture. A total of ten primers were used for analysis.(Table-1). The PCR reaction mixture contained 1ul of template DNA, 4.5 ul of ddH2O, 1ul of 10xTaq Buffer A, 1ul of 100mM dNTP mix, 1ul of 5ul primer, 1 ul of 10mM $Mgcl_2$, 0.5ul of Taq DNA polymerase (3U/ul). Amplification of DNA was done by the thermal cycler (Eppendorf). Each reaction was performed using initial denaturation of Template DNA at 94°C for 5 min followed by 40 cycles of PCR amplification following: 30 sec of denaturation at 94°C, 45 sec of primer annealing at 52°C and 2 min of primer extension at 72°C. Final incubation was at 72°C for 10 min so as to complete primer extension. The amplified products were electrophoretically resolved on a 1.8 %

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agarose gel in 1X Tris Borate- EDTA (TBE) and visualized under UV light after staining with 0.1%ethidium bromide.

Table 1: Total number of bands and level of polymorphism in ISSR profiling of *Acacia auriculiformis*

S.NO	*Primer*	*Total Bands*	*Polymorphic Bands*	*Polymorphism (%)*
1	ISSR 1	21	18	81.8
2	ISSR 2	23	23	100
3	ISSR 3	16	15	93.75
4	ISSR 4	22	18	81.8
5	ISSR 5	19	14	73.68
6	UBC 812	25	22	88
7	UBC 842	22	22	100
8	UBC 868	21	21	100
9	BDB(ACA)5	19	19	100
10	TA(CAG)4	28	19	68
	TOTAL	215	184	85.5

3. Data Interpretation and Diversity Analysis

All the gels were scored manually for monomorphic and polymorphic bands. The diversity estimates and dendrogram was derived based on the unweighted neighbor-joining method and the analysis was performed usingPOPGEN32 software.

4. Results and Discussion

Ten different ISSR primers were used to evaluate the level of genetic diversity amongst the different samples of the orchard population. All the ISSR primers were polymorphic. The amplified product was scored on the basis of presence and absence of bands (Fig 1). The scoring of bands was done independently and only the distinct well separated bands were used to generate the input 1, 0 matrix that was used for all further computations. A total of 215 bands were amplified in fifty three Acacia samples using 10 ISSR primers. Out of these 215 bands, 184 bands were polymorphic (Table 1). Maximum numbers of bands were generated from the primer TA(CAG)4giving 28 bands out of which 19 were polymorphic, followed by primer UBC 812 generating 25 bands with 22 polymorphic bands. Highest level of polymorphism was obtained with the primer UBC 842, UBC 868, BDB(ACA)5, ISSR 2 (100%) followed by primer ISSR-3 (93.75%). Lowest polymorphism (40%) was observed in TA(CAG)4 primer.ChiveuChemulanga*et al.*2008 assessed four Kenyan populations of *A. senegal* using 10 RAPD and 5 ISSR primers. A total of 55 polymorphic bands with an average of 3.6polymorphic loci per RAPD& ISSR primer were detected. The mean Nei's gene diversity index (*H*) for the populations was 0.283 and mean observed number of alleles per locus (*A*) was 1.982. In the present study exhibited the mean Nei's gene diversity index (H) was 0.381 and mean observed number of alleles per locus (A) was 1.984. The Shannon's Information Index (I) was 0.562 and the Percentage of polymorphic loci was 98.490. (Table-2).

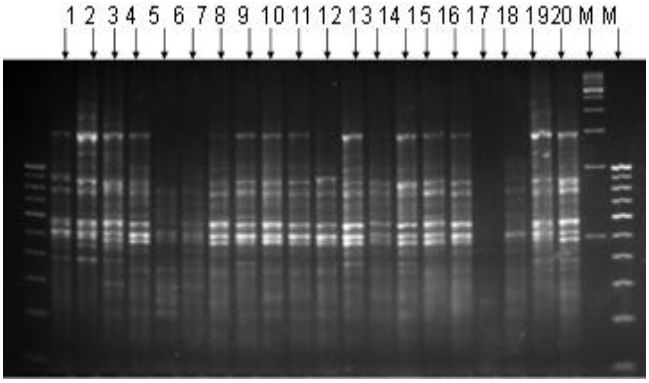

Figure 1: ISSR profiling in *Acacia auriculiformis* using BDB(ACA)5 primer

Table 2: Nei's Genetic diversity estimates in orchard populations of *Acacia auriculiformis*

Tree No	Observed no.of alleles (na)	Effective no. of. Alleles (ne)	Nei's Gene diversity (h)	Shannons's Information Index (I)	% of polymorphic loci
1	2	1.62	0.3727	0.5552	100
2	2	1.67	0.395	0.5815	100
3	2	1.7	0.402	0.588	100
4	2	1.83	0.4509	0.6428	100
5	2	1.75	0.4242	0.6142	100
6	2	1.77	0.4311	0.6218	100
7	2	1.68	0.3973	0.584	100
8	2	1.78	0.4343	0.625	100
9	2	1.57	0.3472	0.5248	100
10	2	1.74	0.4218	0.6117	100
11	2	1.81	0.444	0.6347	100
12	2	1.74	0.4137	0.5994	100
13	2	1.81	0.4456	0.6372	100
14	2	1.77	0.4298	0.62	100
15	2	1.83	0.4435	0.6327	100
16	1.9	1.7	0.3839	0.5527	90
17	1.7	1.55	0.2988	0.4297	70
18	1.9	1.72	0.3908	0.5602	90
19	2	1.74	0.4144	0.6009	100
20	2	1.83	0.4522	0.6438	100
21	2	1.75	0.4237	0.6128	100
22	2	1.78	0.4261	0.615	100
23	2	1.8	0.4366	0.6261	100
24	2	1.84	0.4519	0.6434	100
25	2	1.8	0.4417	0.6325	100
26	1.9	1.71	0.3944	0.5659	90
27	2	1.6	0.3733	0.5595	100
28	2	1.68	0.3929	0.5763	100
29	2	1.74	0.4202	0.6092	100
30	2	1.7	0.4063	0.594	100
31	2	1.59	0.3649	0.5483	100
32	2	1.65	0.3752	0.5509	100
33	2	1.64	0.3867	0.5729	100
34	2	1.61	0.3755	0.561	100
35	2	1.59	0.3598	0.5406	100
36	2	1.65	0.3873	0.5733	100
37	2	1.67	0.3963	0.5836	100
38	2	1.62	0.3676	0.5483	100
39	2	1.68	0.3982	0.5846	100
40	2	1.72	0.4152	0.6039	100
41	2	1.7	0.4006	0.5857	100
42	1.8	1.34	0.2365	0.3453	80
43	2	1.58	0.3532	0.5333	100
44	2	1.46	0.3075	0.4816	100
45	2	1.55	0.3386	0.5156	100

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46	2	1.37	0.2646	0.4299	100
47	2	1.39	0.2605	0.416	100
48	2	1.51	0.3263	0.5032	100
49	2	1.44	0.2762	0.4327	100
50	2	1.6	0.3649	0.547	100
51	2	1.54	0.3477	0.5301	100
52	2	1.42	0.2832	0.4503	100
53	2	1.44	0.2888	0.4557	100
mean	1.984906	1.656038	0.38180	0.562053	98.49057

5. Cluster Analysis

The dendrogram revealed the presence of six distinct clusters (Fig 2). Fifty three genotypes were grouped based on their genetic relationships. Among this, thirty six genotypes were further grouped (1,9), (27, 33), (35, 38) (31,36),(28,30), (40,41), (43,45), (42, 46),(44,63), (50,51),(47,52),(48,49),(2,3), (6,8), (10,22), (11,23), (7,14), (18,21) based on their genetic closeness. The rest of the fifteen genotypes viz, 32,29,34,4,5,19,13,,20,12,24,25,16,15,26,17 were standing out reflects as genetically divergent genotypes. The study demonstrates the utility of using dominant DNA marker such as Inter Simple Sequence Repeats (ISSR) markers to characterize interspecific relationships and to evaluate germplasm diversity in *Acacia auriculiformis*population in the seedling seed orchard. Thus this material could be exploited in the future for breeding programs to develop new Acacia varieties/ hybrids.

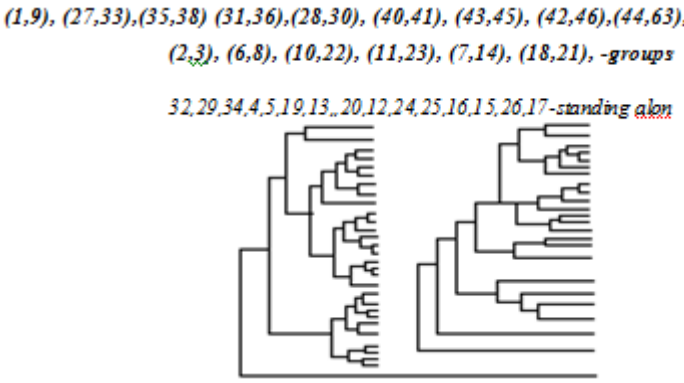

Figure 2: Dendrogram of orchard populations in *Acacia auriculiformis*

6. Acknowledgement

The authors are thankful to Director General, Indian Council of Forestry Research and Education, Dehradun for the financial support and the Director, Institute of Forest Genetics and Tree Breeding, Coimbatore for providing facilities to carry out this study.

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