

## CHLOROPLAST rps8 GENE OF COTTON REVEALS THE CONSERVED NATURE THROUGH OUT PLANT TAXA

TAYYABA SHAHEEN, MEHBOOB-UR-RAHMAN\* AND YUSUF ZAFAR

*Plant Genomics and Molecular Breeding (PGMB) Lab, National Institute for Biotechnology and Genetic Engineering (NIBGE), P.O. Box 577, Jhang Road, Faisalabad, Pakistan*

### Abstract

Chloroplast genome sequences have been utilized extensively for studying evolutionary dynamics, indicating a very low rate of base substitutions among different plant taxa. An initiative has been taken to reveal phylogenetic relationship using rps8 gene derived from chloroplast genome of *Gossypium arboreum* L. (A genome species) and also to validate the authenticity of A genome as a mother parent in the present day cotton species *Gossypium hirsutum* L. The rps8 gene sequence derived from *G. arboreum* was aligned with the DNA sequences of the corresponding rps8 genes of 34 diverse angiosperms. Differences in rps8 gene of *G. arboreum* with the corresponding gene sequences were in the range of 0% for *G. hirsutum* to 17.41% for *Epiphagus virginiana*. The average nucleotide differences among all the rps8 genes were 12.5%. Cluster analysis grouped all the members into three major clusters and one small cluster. Most of the plant species belonging to the same family grouped in one cluster. It has been demonstrated that the A genome is the mother parent in the evolution of *G. hirsutum*, and the gene is relatively conserved across the different plant taxa.

**Keywords:** Phylogenetic; rps8; dissimilarity; *G. arboreum*

### Introduction

Over the last two decades chloroplast genetic information has been widely used as genetic markers for plant and algal phylogenetic studies (Clegg, 1993). Conserved nucleotide substitution rate of chloroplast DNA, facilitates comparison of variations among different plant taxa. Chloroplast genome is uniparentally inherited, and is effectively nonrecombining thus helps simplify theories of phylogenetic inference (Provan *et al.*, 2001).

Small genomes, especially of organelles and bacteria, are well suited to global comparisons of gene order. Like eukaryotic genomes, they are subjected to structural changes such as inversion, transposition or translocation, as well as gene loss and (more rarely) gene gain. Nucleotide diversity provides basic information about evolution and it is also a source of most of morphological changes. Chloroplast DNAs in most land plants share highly conserved gene content and similar gene orders (Palmer, 1985). The presently available chloroplast genome sequences of about 35 photosynthetic land plants and seven charophycean green algae exhibit a high degree of conservation in overall structure and gene arrangement (Turmel *et al.*, 2006). Ribosomal protein S8 (rps8) is one of the proteins from the small ribosomal subunit and its function has been widely studied in *Escherichia coli* (Zengel & Lindahl, 1994). The rps8 is thought to be an essential ribosomal protein in bacteria, because its mutants cannot assemble the large and small ribosomal subunits (Wower *et al.*, 1992). It regulates the translation of its mRNA,

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\* Corresponding Author: Mehboob-ur-Rahman; [mehboob\\_pbd@yahoo.com](mailto:mehboob_pbd@yahoo.com)

(Cerretti *et al.*, 1988; Gregory *et al.*, 1988). Crystal structures are available for *Bacillus stearothermophilus* S8 and for *Thermus thermophilus* S8, and the structure of *Methanococcus jannaschii* S8 has been explained which is bound to a fragment of 16S rRNA (Tishchenko *et al.*, 2001). In addition, the structure of S8 is known in the intact 30S ribosomal subunit from *T. thermophilus* (Wimberly *et al.*, 2000).

Understanding chloroplast genome and the mechanism of gene regulation will become increasingly important in future including the development of environmentally safe transgenic crops (Scott & Wilkinson, 1999; Zhang *et al.*, 1999). Globally, cotton is an economically important crop (Rahman *et al.*, 2002). Limited studies have been reported to understand the evolution of cotton (Rong *et al.*, 2004). In the present study we reported the DNA sequence of *rps8* gene in *G. arboreum* and its phylogenetic relationship to illustrate its evolution and degree of conservation of this gene among taxa.

### Materials and Methods

**Isolation of total DNA:** Total genomic DNA was isolated from a local cotton variety of *G. arboreum* var. Ravi by a method proposed by Iqbal *et al.*, 1997.

**Primer designning:** Primers were designed on the basis of the reported sequence of *rps8* gene in *G. hirsutum*. Forward 5'-TCGTATACCGGACAGA-3' and reverse 5'-ACCATGGGTAAGGACACT-3' primers were designed to amplify region of 424 bases from the *G. arboreum*. Polymerase chain reaction (PCR) was performed in a total volume of 20 $\mu$ l, using 2.5 $\mu$ l (15ng/ $\mu$ l) of cotton DNA, 10 X PCR buffer without MgCl<sub>2</sub> (10mM Tris-HCl, 50mM KCl, PH 8.3), 3mM MgCl<sub>2</sub>, 0.1mM each of dATP, dGTP, dCTP and dTTP and 0.5 units of taq DNA polymerase, 0.15 mM of each primer. Taq DNA polymerase together with 10 X PCR buffer, MgCl<sub>2</sub> and dNTPs were from MBI Fermentas. Polymerase chain reaction consisted of 35 cycles of 94 °C for 1 min, 94 °C for 30 sec, 50 °C for 30 sec, 72 °C extension for 1 min and final extension at 72 °C for 10 min. PCR amplification produced single product of size 424 bases. PCR products were resolved on 2% agarose gel.

**Cloning and sequencing of PCR product:** PCR product was cloned into T/A cloning vector ptz57R/T and sequencing was done on ABI automated DNA sequencer. Sequence was edited manually to get exon region of 405 bases of *rps8* gene (Accession No, A.M. 422014).

**Phylogenetic study:** DNA sequence of the exon region of the corresponding *rps8* gene of 34 angiosperms belonging to diverse families were obtained from the Gene Bank. All sequences were aligned using clustalX and phylogenetic analyses were conducted by bootstrap analysis excluding gaps with PAUP version 4 beta 8.

### Results and Discussion

*Gossypium arboreum* L. containing A genome is thought to evolve from its ancestor 6-11 MYA and then reunited with D genome to produce tetraploid cotton species nearly 1.1-1.9 MYA (Wendel & Cronn, 2003). In the present study, *rps8* gene derived from *G. arboreum* chloroplast was found 100% similar with the corresponding gene in *G.*

*hirsutum* and comparatively high homology in sequences of rps8 (average similarity= 87.50%) was found among the 34 plant taxa. Maximum dissimilarity (17.41%) was detected in the corresponding gene of *Epiphagus virginiana*. Genome rearrangements influence gene order and configuration of gene clusters in the chloroplast genomes. (Cui *et al.*, 2006)

**Table.1 Species and family name of 35 Angiosperms.**

Sr. #	Name	Family
1	<i>Gossypium arboreum</i>	Malvaceae
2	<i>Gossypium hirsutum</i>	Malvaceae
3	<i>Eucalyptus globulus</i>	Myrtaceae
4	<i>Vitis vinifera</i>	Vitaceae
5	<i>Cucumis sativus</i>	Cucurbitaceae
6	<i>Panax ginseng</i>	Araliaceae
7	<i>Nicotiana tabacum</i>	Solanaceae
8	<i>Solanum tuberosum</i>	Solanaceae
9	<i>Atropa belladonna</i>	Solanaceae
10	<i>Lycopersicon esculentum</i>	Solanaceae
11	<i>Lactuca sativa</i>	Asteraceae
12	<i>Helianthus annuus</i>	Asteraceae
13	<i>Solanum bulbocastanum</i>	Solanaceae
14	<i>Lotus japonicus</i>	Fabaceae
15	<i>Arabidopsis thaliana</i>	Brassicaceae
16	<i>Brassica rapa</i>	Brassicaceae
17	<i>Calycanthus fertilis</i>	Calycanthaceae
18	<i>Ranunculus macranthus</i>	Ranunculaceae
19	<i>Nuphar advena</i>	Nymphaeaceae
20	<i>Yucca schidigera</i>	Agavaceae
21	<i>Medicago truncatula</i>	Fabaceae
22	<i>Nymphaea alba</i>	Nymphaeaceae
23	<i>Acorus americanus</i>	Acoraceae
24	<i>Vigna angularis</i>	Fabaceae
25	<i>Spinacia oleracea</i>	Brassicaceae
26	<i>Oenothera elata</i>	Onagraceae
27	<i>Epifagus virginiana</i>	Orobanchaceae
28	<i>Oenothera ammophila</i>	Onagraceae
29	<i>Amborella trichopoda</i>	Amborellaceae
30	<i>Phalaenopsis aphrodite</i>	Orchidaceae
31	<i>Glycine max</i>	Fabaceae
32	<i>Nicotiana sylvestris</i>	Solanaceae
33	<i>Nicotiana tomentosum</i>	Solanaceae
34	<i>Acorus calamus</i>	Acoraceae
35	<i>Populus alba</i>	Salicaceae

Table.2 Genetic distances of selected 34 angiosperm (dicots) rps8 gene sequences with *G. arboreum* rps8 gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	-																			
2	0.0000	-																		
3	0.0815	0.0815	-																	
4	0.0855	0.0855	0.0780	-																
5	0.0988	0.0988	0.1111	0.1031	-															
6	0.1111	0.1111	0.0914	0.0858	0.1210	-														
7	0.1185	0.1185	0.1012	0.0881	0.1333	0.0815	-													
8	0.1185	0.1185	0.1012	0.0881	0.1333	0.0815	0.0000	-												
9	0.1185	0.1185	0.1012	0.0881	0.1333	0.0815	0.0049	0.0049	-											
10	0.1182	0.1182	0.1030	0.0882	0.1332	0.0831	0.0101	0.0101	0.0101	-										
11	0.1210	0.1210	0.0988	0.0804	0.1358	0.0790	0.0173	0.0173	0.0173	0.0125	-									
12	0.1182	0.1182	0.1030	0.0882	0.1332	0.0831	0.0101	0.0101	0.0101	0.0050	0.0125	-								
13	0.1101	0.1101	0.1123	0.0996	0.1328	0.0818	0.0947	0.0947	0.0947	0.0895	0.0869	0.0895	-							
14	0.1198	0.1198	0.1097	0.1070	0.1300	0.0636	0.0969	0.0969	0.0969	0.0918	0.0891	0.0917	0.0331	-						
15	0.1207	0.1207	0.1055	0.0907	0.1358	0.0832	0.0127	0.0127	0.0127	0.0076	0.0151	0.0076	0.0920	0.0942	-					
16	0.1309	0.1309	0.1333	0.1206	0.1136	0.1556	0.1630	0.1630	0.1630	0.1580	0.1580	0.1580	0.1425	0.1523	0.1606	-				

1= *G. arboreum*; 2= *G. hirsutum*; 3= *E. globulus*; 4= *V. vinifera*; 5= *C. sativus* 6= *P. ginseng*; 7= *N. tobacum*; 8= *N. sylvestris*; 9= *N. tomentosiformis*; 10= *S. tuberosum*; 11= *A. belladonna*; 12= *L. esculentum*; 13= *L. sativa*; 14= *H. annuus*; 15= *S. bulbocastanum*; 16= *L. japonicus*; 17= *A. thaliana*; 18= *B. rapa*; 19= *C. fertilis*; 20= *R. macranthus*; 21= *N. advena*; 22= *G. max*; 23= *Y. schidigera*; 24= *N. alba*; 25= *A. americanus*; 26= *A. calamus*; 27= *V. angularis*; 28= *S. oleracea*; 29= *O. elata*; 30= *E. virginiana*; 31= *P. alba*; 32= *O. ommophila*; 33= *A. trichopoda*; 34= *P. aphrodite*; 35= *M. truncatula*

Table 1 continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
17	0.1383	0.1383	0.1333	0.1386	0.1383	0.1506	0.1506	0.1506	0.1506	0.1506	0.1436	0.1457	0.1436	0.1454	0.1530	0.1461	0.17037-			
18	0.1407	0.1407	0.1383	0.1385	0.1383	0.1556	0.1556	0.1556	0.1556	0.1556	0.1485	0.1506	0.1485	0.1504	0.1579	0.1461	0.1679	0.02716-		
19	0.1332	0.1332	0.1199	0.0966	0.1451	0.1402	0.1253	0.1253	0.1252	0.1276	0.1228	0.1276	0.1552	0.1702	0.1303	0.17821	0.17816	0.16831-		
20	0.1363	0.1363	0.1306	0.1155	0.1383	0.1362	0.1286	0.1286	0.1286	0.1309	0.1312	0.1309	0.1482	0.1529	0.1311	0.16631	0.17862	0.17385	0.11634-	
21	0.1362	0.1362	0.1179	0.1207	0.1556	0.1410	0.1410	0.1410	0.1410	0.1385	0.1385	0.1385	0.1610	0.1579	0.1412	0.17617	0.15825	0.14845	0.0988	0.13131
22	0.1506	0.1506	0.1457	0.1357	0.1482	0.1506	0.1556	0.1556	0.1506	0.1507	0.1531	0.1507	0.1578	0.1677	0.1532	0.08889	0.17778	0.17778	0.17071	0.16889
23	0.1378	0.1378	0.1321	0.1064	0.1423	0.1452	0.1483	0.1483	0.1508	0.1508	0.1481	0.1508	0.1635	0.1707	0.1535	0.1712	0.17892	0.1741	0.09201	0.1308
24	0.1509	0.1509	0.1327	0.1303	0.1553	0.1354	0.1505	0.1505	0.1504	0.1481	0.1480	0.1481	0.1708	0.1573	0.1508	0.17824	0.17068	0.15585	0.10831	0.14418
25	0.1573	0.1573	0.1615	0.1369	0.1668	0.1546	0.1520	0.1520	0.1545	0.1549	0.1596	0.1548	0.1915	0.1829	0.1524	0.195	0.2072	0.18713	0.11484	0.14756
26	0.1592	0.1592	0.1634	0.1388	0.1661	0.1513	0.1538	0.1538	0.1563	0.1567	0.1614	0.1567	0.1931	0.1793	0.1543	0.19669	0.20387	0.18393	0.11691	0.1469
27	0.1728	0.1728	0.1704	0.1706	0.1630	0.1803	0.1753	0.1753	0.1704	0.1680	0.1728	0.1680	0.1802	0.1902	0.1706	0.10864	0.18519	0.18272	0.20403	0.18685
28	0.0989	0.0989	0.1079	0.0840	0.1176	0.1301	0.1141	0.1141	0.1240	0.1226	0.1227	0.1274	0.1410	0.1446	0.1336	0.12692	0.13113	0.12654	0.14161	0.10935
29	0.1069	0.1069	0.0790	0.0933	0.1168	0.1092	0.1195	0.1195	0.1194	0.1165	0.1119	0.1165	0.1131	0.1183	0.1167	0.13981	0.14809	0.15542	0.15177	0.13405
30	0.1741	0.1741	0.1747	0.1769	0.1737	0.1402	0.1430	0.1430	0.1482	0.1480	0.1507	0.1479	0.1582	0.1473	0.1506	0.19347	0.18481	0.18133	0.20205	0.19712
31	0.1161	0.1161	0.1086	0.0930	0.1111	0.1284	0.1309	0.1309	0.1309	0.1331	0.1309	0.1331	0.1430	0.1479	0.1256	0.14074	0.16543	0.15802	0.13982	0.14337
32	0.1145	0.1145	0.0855	0.0859	0.1293	0.1254	0.1215	0.1215	0.1222	0.1170	0.1170	0.1218	0.1298	0.1362	0.1221	0.14031	0.15263	0.14734	0.15759	0.14592
33	0.1425	0.1425	0.1308	0.1099	0.1447	0.1306	0.1164	0.1164	0.1162	0.1238	0.1192	0.1240	0.1466	0.1445	0.1270	0.17551	0.18287	0.17228	0.08889	0.12324
34	0.1550	0.1550	0.1623	0.1440	0.1608	0.1393	0.1553	0.1553	0.1577	0.1606	0.1518	0.1605	0.1806	0.1811	0.1611	0.18033	0.18638	0.17287	0.12505	0.14704
35	0.1658	0.1658	0.1720	0.1450	0.1402	0.1768	0.1648	0.1648	0.1573	0.1561	0.1679	0.1512	0.1829	0.1841	0.1643	0.08183	0.17639	0.17518	0.20296	0.20465

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Table I continued

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
21	-														
22	0.1688	-													
23	0.12573	0.18696	-												
24	0.02539	0.17335	0.13381	-											
25	0.1247	0.20007	0.12149	0.1276	-										
26	0.12671	0.20175	0.11832	0.12964	0.00253	-									
27	0.1842	0.0642	0.20755	0.18888	0.22545	0.22719	-								
28	0.13895	0.15523	0.13619	0.1634	0.15927	0.15902	0.17172	-							
29	0.1545	0.15971	0.14968	0.16436	0.18454	0.18627	0.18092	0.13356	-						
30	0.19444	0.20138	0.20892	0.20186	0.19837	0.1948	0.20374	0.20021	0.19654	-					
31	0.16578	0.18025	0.15521	0.16009	0.18459	0.18622	0.19506	0.13745	0.11957	0.19077					
32	0.14452	0.1648	0.17281	0.16235	0.18753	0.18737	0.18902	0.11714	0.01842	0.22268	0.1311	-			
33	0.09161	0.16163	0.11608	0.10812	0.12055	0.12282	0.18433	0.16685	0.16629	0.19514	0.1514	0.17893	-		
34	0.15252	0.18024	0.09991	0.15133	0.14885	0.14537	0.18937	0.17936	0.17624	0.22077	0.1652	0.20324	0.1372	-	
35	0.17977	0.10277	0.1829	0.19497	0.2226	0.22213	0.11395	0.1483	0.19426	0.22041	0.1948	0.19413	0.1764	0.1819	-

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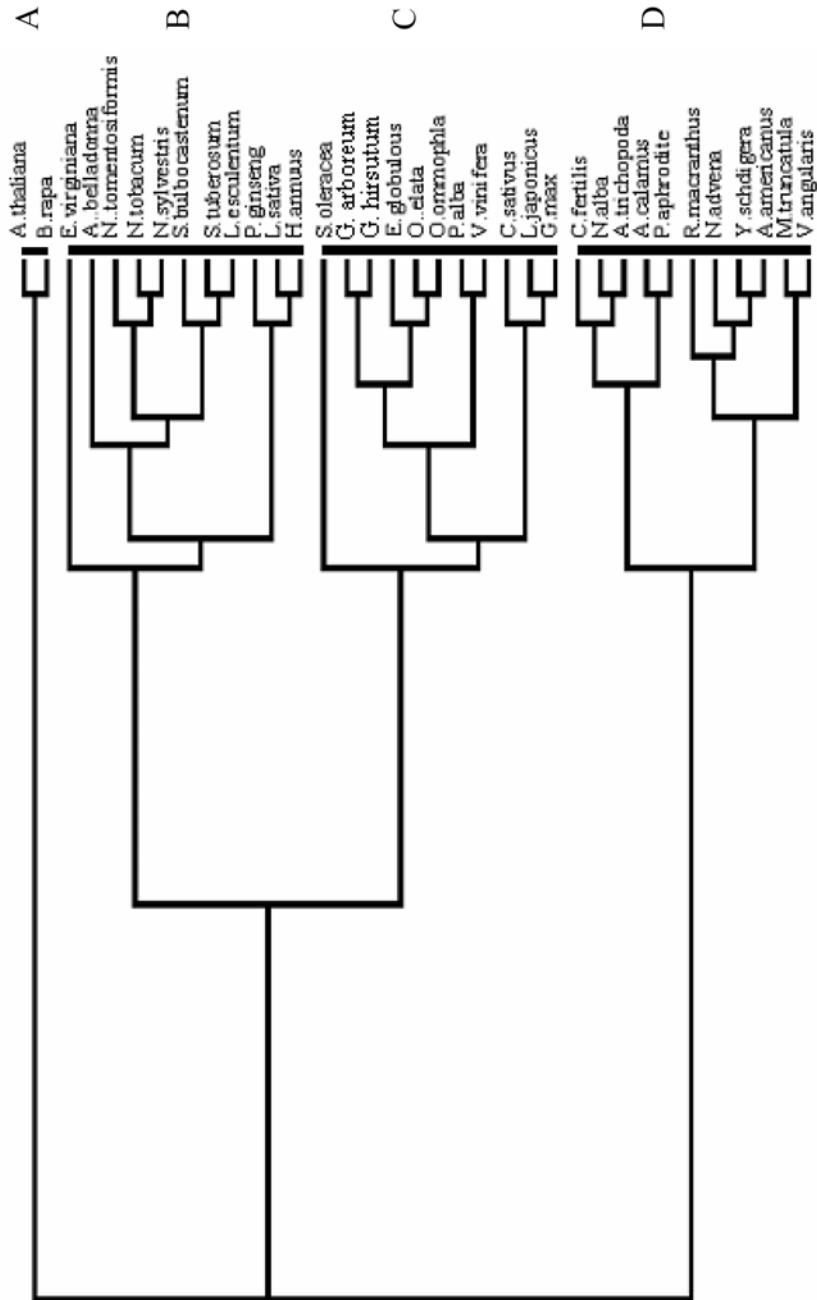


Figure 1. Phylogenetic tree of rps 8 gene

It may be demonstrated that this gene has not been affected during the course of evolution in the genus *Gossypium*. Secondly, the evolution of *G. hirsutum* took place 1.1-1.9 MYA that is not a very long time in the geological time scale (Herland *et al.*, 1990). It may also be concluded that 100% similarity of the *rps8* gene of *G. arboreum* with *G. hirsutum* is also one of the indicators that the A genome contributed as female parent in the present day cotton (Wendel & Cronn, 2003).

Cluster analysis grouped the 35 species in three major clusters and one small cluster (Fig 1). Small cluster 'A' includes *Arabidopsis thaliana* (brassicaceae) and *Brassica rapa* (Brassicaceae). Cluster 'B' consisted of two subclusters; subcluster 'b<sub>1</sub>' comprised of *Atropa belladonna* (Solanaceae), *Nicotiana tobaccum* (Solanaceae), *Nicotiana sylvestris* (Solanaceae), *Nicotiana tomentosum* (Solanaceae), *Solanum tuberosum* (Solanaceae), *Lycopersicon esculentum* (Solanaceae), and *Solanum bulbocastenum* (Solanaceae) and subcluster b<sub>2</sub> comprised of *Panax ginseng* (Araliaceae), *Lactuca sativa* (Asteraceae) and *Helianthus annuus* (Asteraceae) (Fig 1). Cluster 'C' consisted of two subclusters, subcluster 'c<sub>1</sub>' includes *G. arboreum* (Malvaceae), *G. hirsutum*, (Malvaceae), *Eucalyptus globulosa* (Myrtaceae), *Oenothera elata* (Onagraceae), *Oenothera ommophila* (Onagraceae), *Vitis vinifera* (Vitaceae) and *populus alba*, 'c<sub>2</sub>' subcluster consisted of *Cucumis sativus* (Cucurbitaceae), *Lotus japonicus* (Fabaceae) and *Glycine max* (Fabaceae). Cluster 'D' included two subclusters. Subcluster 'd<sub>1</sub>' contained *Calycanthus fertilis* (Calycanthaceae), *Nymphaea alba* (Nymphaeaceae), *Amborella trichopoda* (Amborellaceae), *Acorus calamus* (Acoraceae) and *Phalaenopsis aphrodite*, however, subcluster d<sub>2</sub> comprised of *Ranunculus macranthus* (Ranunculaceae), *Nuphar advena* (Nymphaeaceae), *Yucca schidigera* (Agavaceae) *Acorus americanus* (Acoraceae), *Medicago truncatula* (Fabaceae) and *Vigna angularis* (Fabaceae).

The relationship portrayed in the cluster is compatible with evolutionary information and the evolutionary trees constructed on the basis of sequences of 18S rDNA, *rbcL*, and *atpB* genes (Soltis *et al.*, 2000). All the clusters and subclusters indicated a very conceivable pattern of evolution. Members of the same family and related families were grouped in same clusters and subclusters. Moreover, the *rps8* gene showed a very low degree of differences among diverse dicot families belonging to a wide range of geographical distribution including dicots like trees, shrubs, climbing shrubs and herbs, aquatic angiosperms and basal angiosperms (Burleigh & Mathews, 2004) illustrating that this gene possesses a conserved DNA sequence, which was least effected during the course of evolution of angiosperms under the influence of external factors including climate and habitat. Such commonalities have been reported in 17 chloroplast genes including 14 slow evolving genes (10 of photosystem II, three ribosomal protein genes and one NADH dehydrogenase gene) (Graham & Olmstead, 2000). Amborellaceae is considered as a sister to Calycanthaceae, *Nymphaea alba* (Nymphaeaceae), *Nuphar advena* (Nymphaeaceae), water lilies (*Nymphaea alba* and *Nuphar advena*) and Amborella are considered as sister to all other angiosperms and are considered as basal angiosperms (Nickerson & Drouin, 2004). Although they are primitive angiosperms but showed a high degree of similarity with *G. arboreum*, a member of an advance family. Chloroplast DNAs of most of the land plants shares a highly conserved gene content and with notable exceptions, a largely co-linear gene order. Conserved gene order may reflect a slow intrinsic rate of neutral chromosomal rearrangements, or selective constraint. (Cui *et al.*, 2006).



Conserved structure of ribosome throughout the prokaryotic and eukaryotic lineages is the main reason of the conserved nature of this gene, reflecting the early origin of their essential function (Kay & Jacobs-Lorena, 1987; Katsukoshi *et al.*, 1998). Also, the protein encoded by the corresponding gene is known to be important for ribosomal structure and function such as S4, S5, S10, S12, S13, L1, L2, L5 and L11 which are highly conserved throughout bacterial kingdom as expected (Katsukoshi *et al.*, 1998). Secondly, the angiosperms have typically been viewed as being monophyletic (Doyle & Donoghue, 1993). Thus being a ribosomal gene and least prone to external factors, the rps8 gene has not been as affected as any other non-conserved gene could be. Thus this study suggests that this gene has a conserved DNA sequence and also confirms the maternal status of A genome species in the present day cotton species.

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### References

- Burleigh, G. G. and S. Mathews. 2004. Phylogenetic signal in nucleotide data from seed plants: implications for resolving the seed plant tree of life. *Am. J. Bot.*, 91: 1599-1613.
- Cerretti, D.P., L.C. Mattheakis, K.R. Kearney, L. Vu and M. Nomura 1988. Translational regulation of the *spc* operon in *Escherichia coli*: Identification and structural analysis of the target site for S8 repressor protein. *J. Mol. Biol.*, 204: 309-329.
- Clegg, M.T. 1993. Chloroplast gene sequences and the study of plant evolution. *Proc. Natl Acad. Sci.*, USA. 90: 363-367.
- Cui, L., J. Leebens-Mack, L.S. Wang, J. Tang, L. Rymarquis, D.B. Stern, and C.W. dePamphilis. 2006. Adaptive evolution of chloroplast genome structure inferred using a parametric bootstrap approach. *BMC Evol Biol.* 6:13.
- Doyle, J.A. and M.J. Donoghue. 1993. Phylogenies and angiosperm diversification. *Paleobiology*. 19: 141-167.
- Graham, S.W. and R.G. Olmstead. 2000. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *Am. J. Bot.* 87(11): 1712-1730.
- Gregory, R.R., P.B.F. Cahill, D.L. Thurlow, and R.A Zimmermann. 1988. Interaction of *Escherichia coli* ribosomal protein S8 with its binding sites in ribosomal RNA and messenger RNA. *J. Mol. Biol.*, 204: 295-307.
- Harland, W.B., R.L. Armstrong, A.V. Cox, L.E. Craig, A.G. Smith, and D.G. Smith. 1990. A geologic time scale, Cambridge University Press: Cambridge, pp.1-263.
- Iqbal, M.J., N. Aziz, N.A. Saeed, Y. Zafar, and K.A. Malik. 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. *Theor. Appl. Genet.*, 94: 139-144.
- Katsukoshi, F., B. Tomoya, and I. Katsumi. 1998. Genomic analysis of genes encoding ribosomal proteins in eight eubacterial species and *saccharomyces cerevisiae*. *Genome Informatics*, 9: 3-12.
- Kay, M.A. and M. Jacobs-Lorena. 1987. Developmental genetics of ribosome synthesis in *Drosophila*. *Trends Genet.*, 3:347-351.
- Nickerson, J. and G. Drouin. 2004. The sequence of the largest subunit of RNA polymerase II is a useful marker for inferring seed plant phylogeny. *Mol. Phylogen. Evol.*, 31: 403-415.
- Palmer, J.D. 1985. Evolution of chloroplast and mitochondrial DNA in plants and algae. In Molecular Evolutionary Genetics. R.J. MacIntyre (ed) *New York, Plenum Press*; 131-240.

- Provan, J., W. Powell, P.M. Hollingsworth. 2001. Chloroplast microsatellites: new tools for studies in plant ecology and evolution. *Trends Ecol. Evol.*, 16:142-147.
- Rahman, M., D. Hussain, and Y. Zafar. 2002. Estimation of genetic divergence among elite cotton (*Gossypium hirsutum* L.) cultivars/genotypes by DNA fingerprinting technology. *Crop Sci.*, 42: 2137-2144.
- Rong, J., C. Abbey, J.E. Bowers, C.L. Brubaker, C. Chang, P.W. Chee, T.A. Delmonte, X. Ding, J.J. Garza, B.S. Marler, C. Park, G.J. Pierce, K.M. Rainey, V.K. Rastogi, S.R. Schulze, N.L. Trolinder, J.F. Wendel, T.A. Wilkins, T.D. Williams-Coplin, R.A. Wing, R.J. Wright, X. Zhao, L. Zhu, and A.H. Paterson. 2004. A 3347-Locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). *Genetics*, 166: 389-417.
- Scott, S.E. and M. Wilkinson. 1999. Low probability of chloroplast movement from oilseed rape (*Brassica napus*) into wild *Brassica rapa*. *Nature Biotechnol.*, 17:390-392.
- Soltis, D.E., P.S. Soltis, M.W. Chase, M.E. Mort, T.D. Albach, M. Zanis, V. Savolainen, W.H. Hahn, S.B. Hoot, M.F. Fay, M. Axtell, S.M. Swensen, L.M. Prince, W.J. Kress, K.C. Nixon and J.S. Farris. 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. *Bot. J. Linn. Soc.*, 133(4): 381-461.
- Tishchenko, S.V., A. Nikulin, N.P. Fomenkova, N. Nevskaya, O. Nikonov, P. Dumas, H. Moine, B. Ehresmann, C. Ehresmann, W. Piendl, et al. 2001. Detailed analysis of RNA-protein interactions within ribosomal protein S8-rRNA complex from the archaeon *Methanococcus jannaschii*. *J. Mol. Biol.*, 311: 311-324.
- Turmel, M., C. Otis and C. Lemieux. 2006. The chloroplast genome sequence of *Chara vulgaris* sheds new light into the closest green algal relatives of land plants. *Mol. Biol. Evol.*, 23:1324-1338.
- Wendel, J.F. and R.C. Cronn. 2003. Polyploidy and the evolutionary polyhistory of cotton. *Adv. Agron.*, 78: 139-186.
- Wower, I., M.P. Kowaleski, L.E. Sears and R.A. Zimmermann. 1992. Mutagenesis of ribosomal protein S8 from *Escherichia coli*: Defects in regulation of the spc operon. *J. Bacteriol.*, 174: 1213-1221.
- Wimberly, B.T., D.E. Brodersen, J.W.M. Clemons, R.J. Morgan-Warren, A.P. Carter, C. Vonrhein, T. Hartsch, and V. Ramakrishnan. 2000. Structure of the 30S ribosomal subunit. *Nature*, 407: 327-339.
- Zengel, J.M. and L. Lindahl. 1994. Diverse mechanisms for regulating ribosomal protein synthesis in *Escherichia coli*. *Prog. Nucleic Acid. Res. Mol. Biol.*, 47: 331-370.
- Zhang, Z., B.R. Green and T. Cavalier-Smith. 1999. Single gene circles in dinoflagellate chloroplast genomes. *Nature*, 400:155-159.

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