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Molecular characterization of cotton (*Gossypium hirsutum* L.) using simple sequence repeat (SSR) markers

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Abstract
Cotton is a major commercial crop with vast and diversified uses of its products. The improvement of any crop mainly depends upon the nature and magnitude of genetic variability present in the base population. The objective of this study was to assess molecular characterization among the 40 *G. hirsutum* genotypes employing SSR markers. A total of 50 SSR markers were used for analysis among which 19 showed polymorphism with a total of 47 SSR alleles which were produced across 19 loci with estimated fragment length of 100 to 700 bp. The number of alleles varied from 2 to 4 with average number of alleles per primer pair was 2.47. The PIC values ranged from 0.36 (BNL 3445) to 0.98 (CCRI 0230) with an average of 0.67 indicating more informativeness of the primers used. The general cluster analysis was performed using the unweighted pair-group method on arithmetic averages (UPGMA) method based on similarity coefficients and the 40 genotypes were grouped into 7 clusters among which three were solitary clusters. Jaccard's similarity matrix was constructed using binary data. Similarity coefficients among 40 genotypes ranged from 0.03 (between RAH 1033 and L 788) to 0.80 (TCH 1741 and ARBH 1402) indicating vast genetic base. The genetically diverse genotypes having least similarity coefficient value, RAH 1033 and L 788, can be used in future hybridization programme to exploit heterosis.

Keywords: Cotton, molecular diversity, PIC value, jaccard's similarity coefficient

Introduction

Cotton, a major cash crop of the world, also known as king of fibre crops, is the foremost natural fiber of mankind and is cultivated in more than 75 countries. There are around fifty species in cotton among which New world cotton Gossypium hirsutum is mainly cultivated for its fibre properties and yield. Cotton is a source of seed oil and protein meal along with its natural fibre.

In cotton, presence of variability among the genotypes is pre-requisite for the improvement of yield and yield traits. The association of yield and yield traits is very well studied and exploited in the plant breeding programmes in cotton [1, 2]. Diversity analysis using the yield and yield traits is studied and utilized in the crop breeding programmes including cotton [3, 4, 5]. Yield being a complex trait, improvement is continuous process and heterosis in the form of hybrids is exploited in cotton for the improvement of yield. The development of hybrids for yield has gained lot of importance in the present scenario of genetically modified crops and this is dominated by the private companies. The improved lines are characterized using DUS characters for their identity and protection[6]. But the recent advances in biotechnology has led to the application of molecular markers for the characterization and diversity analysis in various crops including cotton. Different molecular markers like RAPD, RFLP, AFLP, SSR, ISSR etc. re being used for this purpose but SSR markers are preferred as they are robust, reliable, quick, efficient and reproducible with greater discriminative ability. Therefore, the present study was planned to characterize 40 diverse cotton accessions using SSR markers to reveal the information of genetic divergence.

Materials and Methods
Plant material: The present study was conducted with 40 cotton genotypes collected from different parts of the country at Regional Agricultural Research Station, Lam, Guntur.

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Genomic DNA extraction: 2-3 young fully expanded leaves were collected and grinded in liquid nitrogen using pestle and mortar. About 0.5 g of the grinded tissue was transferred in 2.0 ml sterilized eppendrof tube. DNA isolation and purification was carried out using modified cetyl-tetramethyl ammonium bromide (CTAB) method as suggested by (Sharma *et al*., 2003) [7] with slight modifications. Quality and quantity of DNA was checked by 0.8% gel electrophoresis and calculating the absorbance ratio at 260/280 by using Nanodrop (Thermo Scientific, 2000).

Microsatellite markers and PCR amplification: In the present study, 50 SSR primer pairs were used. PCR amplifications were carried out in a 20 µl reaction mix comprising of 2.0 µl Template DNA (50 ηg/µl), 2.0 µl of 10X buffer with 15 mM MgCl₂ 0.5 µl of 2.5 mM dNTPs, 0.5 µl of Forward Primer (10 pM/µl) 0.5 µl of Reverse Primer (10 pM/µl) and µl of Taq polymerase 0.2 (3 U/µl). PCR amplification conditions include initial denaturation at 94 ºC for 5 min followed by 30 cycles of denaturation at 94 ºC for 30 sec, Annealing at 55-60 ºC for 1min, Extension at 65 ºC 1min 10 sec, Final extension at 94 ºC for 25 min and hold at 4ºC. PCR was performed using Agilent technologies thermocycler (Agilent Sure cycler 8800). The amplified products were separated using 3% Agarose (HiMedia) gel containing ethidium bromide (10mg/ml). The PCR products were run in 1× TAE at 150 V for 120-150 minutes (until the tracking dye migrated to the end of the gel) in a horizontal gel electrophoresis system (AA Hoefer Electrophoresis unit). A 100 bp DNA ladder was run along with side of the amplified products to determine their approximate size. The band patterns were recorded in a gel documentation system (Syngene GBox F3).

SSR data analysis: SSR alleles were scored manually and designated as '1' for presence of band and '0' for absence of band. Polymorphism Information Content (PIC) value is a measure of the informativeness of the marker. Molecular markers with high PIC values are described as highly polymorphic and detect higher level of genetic variation in an organism. PIC was calculated, according to the method of Anderson *et al*. (1993) [8]. It is reported that PIC value of 0.70 and above is highly informative while PIC value from 0.44 - 0.70 is moderately informative [9]. Genetic similarities of the genotypes were estimated from the matrix of the binary data using Jaccard's similarity coefficient. Cluster analysis of elite lines was performed utilizing SPSS software ver.20.

Results and Discussion
Polymorphism revealed by SSR: A total of 50 primers were used to screen the 40 diverse genotypes. Out of 50 SSR primers, 19 were found informative revealing polymorphism among the genotypes. A total of 47 SSR alleles were produced across 19 loci, with estimated fragment length of 100 (BNL 1604) to 700 bp (Gh.EXPA 1). The number of alleles detected varied form 2 (CCRI 0230, BNL 1045, BNL 1604, BNL 1721, BNL 2440, BNL 2646, BNL 3594, BNL 3650, BNL 3071 and BNL 2449) to 4 (BNL 1162). The average number of alleles per primer pair was 2.47. The DNA profile of 40 genotypes with different SSR primers is presented in plate 1-3. It is higher than that reported by Liu *et al*. (2000) [10] and Kavithamani and Amalabalu (2017) [11] while it is lower than that of Lacape *et al*. (2007) [12] and Zhang *et al*. (2011) [13]. The number of alleles per primer pair depends on markers used, platform used for resolution of

amplified products and plant material genotyped. The relatively higher values obtained in the present investigation may be due to genotypes used from different genetic backgrounds.

Polymorphism Information Content: The PIC values in the present study with 19 amplified primers in 40 genotypes varied from 0.36 (BNL 3445) to 0.98 (CCRI 0230) with an average of 0.67. The markers, BNL 1604 and BNL 3445, were moderately informative with PIC values 0.48 and 0.36 while the remaining SSR markers displayed higher PIC values of >0.70 and were highly informative (Table 1). It was observed that the primers mentioned above were efficient in genetic diversity analysis in cotton. The maximum PIC values of 0.80 (Zhang *et al*., 2011 and Kavithamani and Amalabalu, 2017) [11, 13] and 0.88 (Jamwal, 2011) [14] were reported by different scientists in cotton in their studies.

Cluster analysis: SSR amplification results were used to generate a Jaccard's similarity matrix. Genetic similarity coefficients among 40 genotypes ranged from 0.03 (between RAH 1033 and L 788) to 0.80 (TCH 1741 and ARBH 1402) (Table 2) indicating that the cultivars used in the present study has a wide genetic base. Thus, it is suggested that the genotypes having least similarity percentage between them can be used as potential parents in hybridization programme to get heterotic F₁ hybrids.
The general cluster analysis for 40 genotypes was analyzed using the unweighted pair-group method on arithmetic averages (UPGMA) method based on similarity coefficients and dendrogram was constructed using SPSS software ver. 20. [15] (Fig 1). The cotton genotypes were grouped into 7 major clusters. The genotypes present in each cluster are mentioned in the Table 3. Cluster I had nine genotypes which were sub-clustered into IA and IB. The sub-cluster IA had 8 genotypes *viz.,* CNH 1118, RS 2767, L 1060, SCS 1061, SCS 1214, L 799, RAH 1033 and H 1442. The genotypes, RAH 1033 and H 1442, recorded 64% similarity but grouped differently. The sub-cluster IB had only one genotype *i.e.* BS 26. Cluster II had only one genotype *i.e.* LH 2220.
Cluster III was the second largest cluster with 11 genotypes. Cluster IIIA had 4 genotypes viz., SURAJ, TSH 0499, RS 2765 and L 1008. The genotypes, SURAJ and TSH 0499, recorded 63% similarity and grouped in different clans. Cluster IIIB has 7 genotypes viz., GJHV 510, RAH 1066, TSH 0533-1, H 1471, HS 292, CCH 14-1 and F 2501. The genotypes, HS 292 and H 1471, recorded 77% similarity.
Cluster IV was the largest cluster with 16 genotypes and further subdivided into 2 subclusters. Cluster IVA had 8 genotypes viz., TCH 1741, ARBH 1402, BS 23, F 2493, SCS 1207, GISV 267, SAKTHI SULTAN and L 389. The genotypes, ARBH 1402 and TCH 1741, recorded 80% similarity and grouped in same clan. The genotypes, CCH 14-2, LH 2256, CNH 5, HS 294, ARBH 1401, CPD 1402, GJHV 497 and PBH 10, were grouped into cluster IIB. The genotypes, CCH 14-2 and CNH 5, of cluster IVB recorded 73% similarity and grouped differently. The clusters, V, VI and VII were solitary clusters with single genotype each *i.e.*, LRK 516, CSH 2838 and L 788, respectively. Similar trend of results were reported by Rehman *et al*. (2009) [16], Sapkal *et al*. (2011) [17] and Ambreen *et al*. (2013) [18].
The genotypes from the diversified clusters can be used as parents for hybrid or varietal development programme. The molecular study identified the genetically diversified genotypes like RAH 1033 and L 788 (least similarity

coefficient value among these genotypes 0.03) to exploit them in future hybridization programme for better heterosis for yield and yield components.

Table 1: Number of alleles, base pair range produced by the primer *in vivo* and PIC of polymorphic markers studied in cotton genotypes

S. No.	SSR Marker	No. of alleles	bp range	PIC
1	Gh.EXPA 1	3	600-700	0.91
2	Gh.EXPA 3-2	3	500-600	0.87
3	CCRI 0230	2	100-200	0.98
4	BNL 1045	2	200-250	0.9
5	BNL 1162	4	200-250	0.85
6	BNL 1404	3	200-250	0.92
7	BNL 1434	3	250-300	0.88
8	BNL 1604	2	100-150	0.48
9	BNL 1721	2	150-200	0.91
10	BNL 2440	2	200-250	0.83
11	BNL 2544	3	200-250	0.88
12	BNL 2646	2	150-200	0.89
13	BNL 3594	2	150-200	0.86
14	BNL 3650	2	300-350	0.92
15	BNL 3994	3	150-200	0.89
16	BNL 3071	2	150-200	0.87
17	BNL 3445	3	150-200	0.36
18	BNL 2690	2	200-250	0.97
19	BNL 2449	2	150-200	0.88
Total		47		
Average per primer pair		2.47		

Table 3: Clustering pattern of 40 cotton genotypes based on molecular data analysis

| Cluster No.	Number of genotypes	Sub cluster no	Name of genotypes
I	9	IA	CNH 1118, RS 2767, L 1060, SCS 1061, SCS 1214, L 799, RAH 1033, H 1442
		IB	BS 26
II	1	-	LH 2220
III	11	IIIA	SURAJ, TSH 0499, RS 2765, L 1008
		IIIB	GJHV 510, RAH 1066, TSH 0533-1, H 1471, HS 292, CCH 14-1, F 2501
IV	16	IVA	TCH 1741, ARBH 1402, BS 23, F 2493, SCS 1207, GISV 267, SAKTHI SULTAN, L 389
		IVB	ARBH 1401, CPD 1402, CNH 5, CCH 14-2, GJHV 497, HS 294, LH 2256, PBH 10
V	1	-	LRK 516
VI	1	-	CSH 2838
VII	1	-	L 788
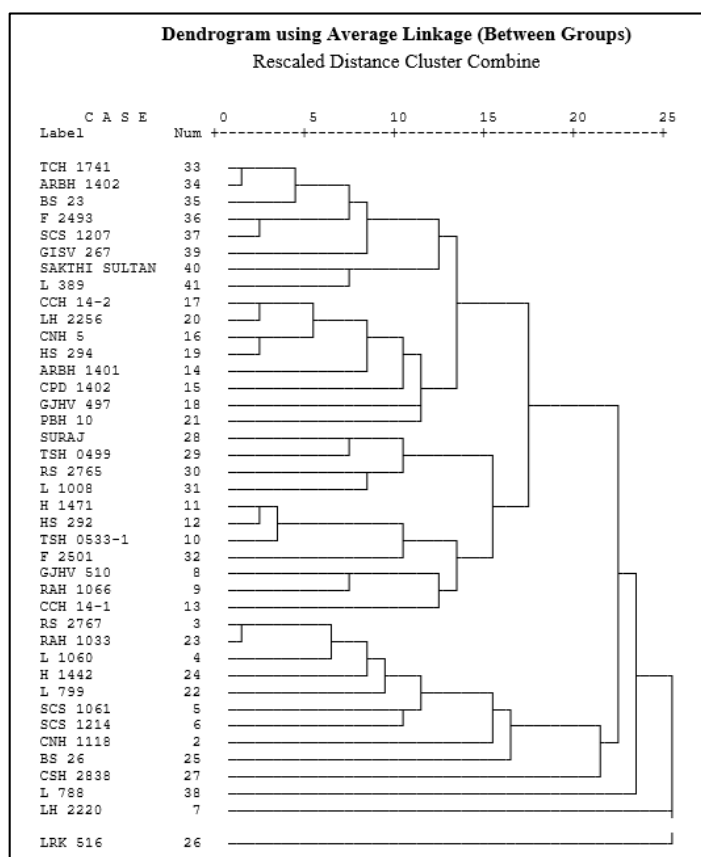
Fig 1: Clustering of cotton (*G. hirsutum* L.) genotypes using SPSS software based on SSR markers
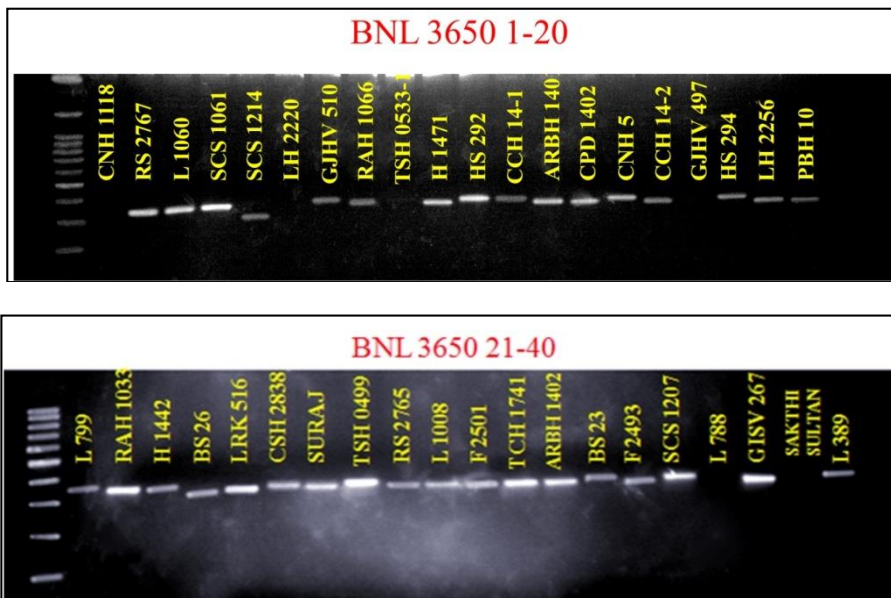
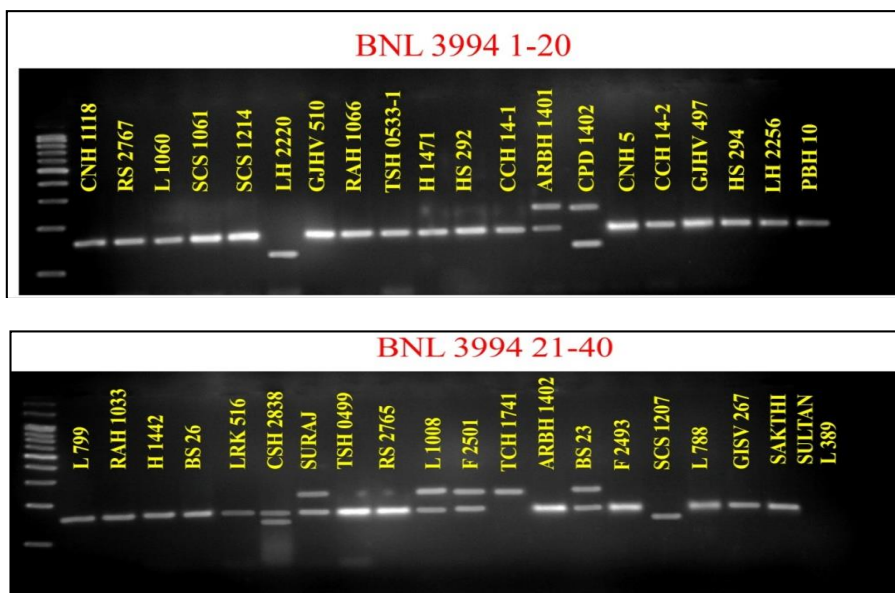**Plate 1:** DNA profile of 40 genotypes of cotton with SSR marker BNL 3650

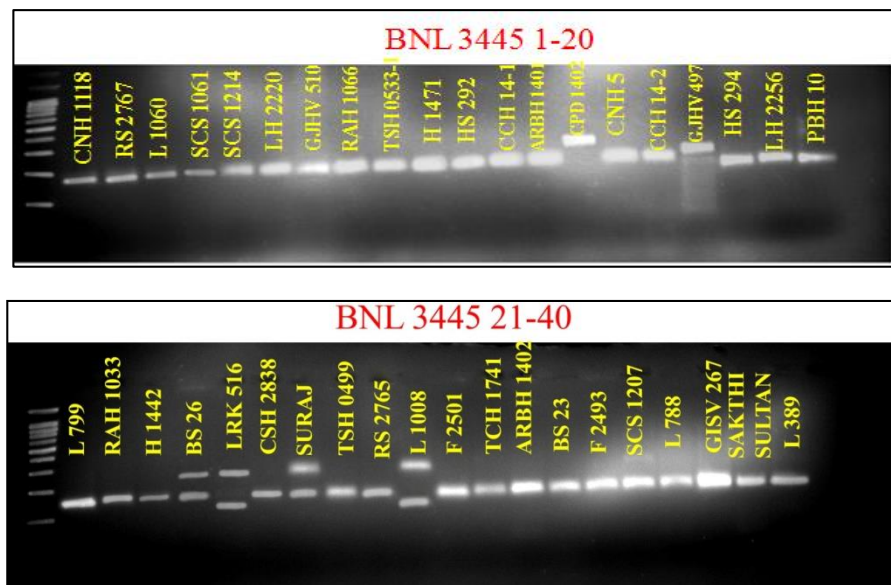**Plate 2:** DNA profile of 40 genotypes of cotton with SSR marker BNL 3994

Plate 3: DNA profile of 40 genotypes of cotton with SSR marker BNL 3445

Table 2: Summary of Jaccard's similarity coefficient values between 40 genotypes of cotton (*Gossypium hirsutum* L.)

Jaccard Measure

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	1.00	0.50	0.48	0.37	0.32	0.20	0.19	0.21	0.15	0.11	0.14	0.10	0.11	0.12	0.06	0.06	0.07	0.06	0.10	0.07	0.43	0.44	0.40	0.33	0.05	0.18	0.29	0.25	0.13	0.17	0.11	0.11	0.08	0.11	0.12	0.08	0.15	0.06	0.11	
2		1.00	0.70	0.58	0.56	0.26	0.39	0.26	0.31	0.29	0.29	0.16	0.22	0.25	0.15	0.15	0.22	0.17	0.10	0.18	0.61	0.63	0.37	0.16	0.29	0.40	0.50	0.34	0.38	0.23	0.20	0.23	0.17	0.17	0.15	0.06	0.21	0.11	0.18	
3			1.00	0.46	0.60	0.24	0.29	0.24	0.37	0.31	0.31	0.25	0.23	0.27	0.20	0.13	0.20	0.18	0.14	0.20	0.53	0.59	0.55	0.45	0.12	0.26	0.51	0.43	0.28	0.29	0.31	0.25	0.27	0.18	0.21	0.27	0.13	0.25	0.15	0.19
4				1.00	0.54	0.19	0.33	0.24	0.37	0.35	0.31	0.17	0.27	0.23	0.13	0.16	0.16	0.14	0.11	0.12	0.48	0.59	0.44	0.40	0.17	0.22	0.34	0.43	0.28	0.33	0.31	0.25	0.24	0.15	0.17	0.13	0.06	0.22	0.15	0.19
5					1.00	0.29	0.50	0.40	0.60	0.51	0.51	0.29	0.37	0.39	0.27	0.21	0.29	0.22	0.16	0.25	0.51	0.56	0.39	0.34	0.14	0.27	0.50	0.42	0.25	0.30	0.42	0.36	0.31	0.25	0.28	0.31	0.12	0.29	0.23	0.32
6						1.00	0.29	0.21	0.14	0.13	0.13	0.08	0.13	0.11	0.07	0.07	0.09	0.08	0.08	0.09	0.22	0.25	0.20	0.10	0.07	0.17	0.21	0.13	0.07	0.06	0.06	0.06	0.10	0.13	0.16	0.11	0.10	0.08	0.09	
7							1.00	0.64	0.63	0.53	0.53	0.42	0.38	0.31	0.35	0.35	0.28	0.25	0.25	0.24	0.29	0.38	0.35	0.16	0.12	0.32	0.46	0.43	0.32	0.37	0.31	0.32	0.27	0.25	0.31	0.31	0.10	0.25	0.18	0.23
8								1.00	0.51	0.43	0.48	0.54	0.37	0.30	0.25	0.25	0.17	0.16	0.20	0.17	0.27	0.33	0.34	0.14	0.04	0.20	0.46	0.38	0.26	0.32	0.26	0.23	0.22	0.23	0.30	0.30	0.11	0.20	0.16	0.21
9									1.00	0.70	0.76	0.48	0.51	0.44	0.40	0.35	0.38	0.34	0.30	0.28	0.25	0.34	0.31	0.20	0.17	0.32	0.62	0.53	0.36	0.41	0.53	0.45	0.39	0.32	0.31	0.35	0.17	0.33	0.26	0.32
10										1.00	0.77	0.50	0.63	0.57	0.57	0.51	0.46	0.51	0.36	0.35	0.27	0.33	0.12	0.07	0.25	0.58	0.50	0.38	0.47	0.54	0.62	0.60	0.46	0.61	0.46	0.46	0.09	0.31	0.21	0.34
11											1.00	0.50	0.63	0.51	0.46	0.51	0.52	0.46	0.41	0.46	0.31	0.32	0.33	0.19	0.16	0.29	0.48	0.45	0.30	0.47	0.60	0.56	0.50	0.46	0.45	0.46	0.16	0.43	0.33	0.50
12												1.00	0.53	0.46	0.52	0.46	0.28	0.34	0.40	0.39	0.20	0.18	0.26	0.10	0.04	0.16	0.38	0.27	0.32	0.29	0.35	0.36	0.35	0.36	0.44	0.46	0.20	0.33	0.30	0.37
13													1.00	0.60	0.60	0.66	0.39	0.55	0.61	0.56	0.30	0.24	0.25	0.11	0.11	0.20	0.42	0.40	0.33	0.41	0.44	0.50	0.53	0.50	0.58	0.50	0.20	0.46	0.32	0.37
14														1.00	0.66	0.53	0.47	0.54	0.42	0.41	0.35	0.28	0.25	0.17	0.08	0.14	0.45	0.33	0.34	0.43	0.46	0.48	0.51	0.43	0.64	0.48	0.46	0.48	0.14	0.35
15															1.00	0.73	0.54	0.76	0.60	0.47	0.18	0.17	0.17	0.10	0.04	0.06	0.36	0.22	0.25	0.35	0.41	0.48	0.57	0.53	0.51	0.60	0.14	0.40	0.38	0.40
16																1.00	0.61	0.76	0.76	0.54	0.18	0.17	0.17	0.10	0.08	0.14	0.28	0.25	0.25	0.39	0.41	0.48	0.57	0.59	0.69	0.53	0.19	0.50	0.44	0.45
17																	1.00	0.63	0.47	0.47	0.18	0.25	0.17	0.12	0.11	0.19	0.25	0.26	0.22	0.29	0.46	0.42	0.46	0.48	0.46	0.41	0.13	0.38	0.42	0.52
18																		1.00	0.61	0.47	0.16	0.18	0.15	0.11	0.04	0.12	0.35	0.24	0.28	0.46	0.48	0.57	0.53	0.51	0.54	0.16	0.44	0.37	0.39	
19																			1.00	0.63	0.16	0.11	0.15	0.15	0.10	0.12	0.27	0.17	0.20	0.30	0.32	0.33	0.41	0.48	0.57	0.48	0.27	0.44	0.43	0.39
20																				1.00	0.28	0.16	0.13	0.12	0.11	0.13	0.18	0.15	0.17	0.25	0.31	0.32	0.31	0.37	0.40	0.36	0.13	0.38	0.36	0.52
21																					1.00	0.62	0.52	0.34	0.15	0.26	0.30	0.27	0.15	0.22	0.16	0.17	0.16	0.13	0.20	0.18	0.03	0.21	0.13	0.22
22																						1.00	0.64	0.41	0.18	0.28	0.39	0.50	0.33	0.34	0.21	0.15	0.18	0.17	**0.03**	0.19	0.12	0.20		
23																							1.00	0.32	0.19	0.24	0.40	0.41	0.39	0.39	0.22	0.26	0.29	0.26	0.22	0.25	0.14	0.27	0.12	0.20
24																								1.00	0.28	0.09	0.22	0.19	0.14	0.25	0.27	0.12	0.15	0.12	0.19	0.17	0.14	0.20	0.20	0.16
25																									1.00	0.23	0.07	0.11	0.09	0.14	0.11	0.12	0.07	0.12	0.11	0.08	0.14	0.17	0.16	0.17
26																										1.00	0.28	0.34	0.25	0.23	0.20	0.21	0.16	0.13	0.12	0.06	0.15	0.13	0.08	0.13
27																											1.00	0.63	0.46	0.54	0.44	0.41	0.40	0.33	0.32	0.32	0.24	0.30	0.17	0.14
28																												1.00	0.59	0.56	0.45	0.38	0.37	0.30	0.29	0.22	0.12	0.31	0.11	0.14
29																													1.00	0.60	0.38	0.44	0.43	0.44	0.34	0.30	0.25	0.36	0.16	0.17
30																														1.00	0.51	0.58	0.48	0.42	0.35	0.23	0.41	0.27	0.28	
31																															1.00	0.67	0.65	0.51	0.50	0.46	0.20	0.48	0.42	0.44
32																																1.00	**0.80**	0.70	0.56	0.59	0.25	0.55	0.39	0.46
33																																	1.00	0.74	0.60	0.63	0.25	0.53	0.37	0.39
34																																		1.00	0.74	0.65	0.36	0.66	0.50	0.46
35																																			1.00	0.76	0.34	0.70	0.60	0.50
36																																				1.00	0.24	0.55	0.50	0.52
37																																					1.00	0.43	0.42	0.18
38																																						1.00	0.58	0.54
39																																							1.00	0.63
40																																								1.00

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