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RESEARCH ARTICLE

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Assessment of genetic diversity at molecular and morphological levels of temperate maize landraces collected from diverse ecological niches in Kashmir

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Abstract

A study was conducted to carry out morpho-molecular characterization of 70 maize landraces collected from diverse ecological niches. The ANOVA revealed significant differences for all the traits studied except prolificacy and anthesis silking interval. Phenotypic performance-based clustering using Mahalanobis distance resolved these landraces into four major clusters. The PIC values for the 25 SSR markers ranged from 0.29 (*phi* 129) to 0.82 (*bnlg* 1335) with a mean value 0.51. Analysis of molecular variance revealed that the total genetic diversity is mainly due to within population diversity (93%). Average heterozygosity from all SSR loci was 0.15 and gene diversity ranged from 0.87 (*bnlg* 1335) to 0.32 (*phi* 021) with a mean value of 0.56 indicated a high level of polymorphism among landraces. The population structure analysis revealed the existence of two groups reflecting major divisions of the germplasm collected regardless of their geographical origin. Clustering method based on Jaccard similarity coefficient and an UPGMA further confirmed the two major clades with a significant level of similarity (0.28 to 0.76) among the landraces. Sub population1 was found to be genetically more diverse with an Fst value of 0.18 than sub population 2 with Fst value of 0.10. The results showed that the studied maize landraces are diverse making them ideal source population.

Keywords: Genetic diversity, heterotic pools, Mahalanobis D^2 statistic, maize, SSR.

Introduction

Maize (*Zea mays* L., 2n=20) is found to be the leading cereal globally, originated in Central America and Mexico but because of its spacious adaptability and higher productivity potential, it is grown over a broader range of environments around the world (Prasanna 2012). In India, it is cultivated over an area of 9 mha with a production of 28.86 mt and a productivity of 2.96 tha^{-1} (Anonymous 2020). The productivity of maize in India is very low as compared to global average of 5.6 tha^{-1} and can be detrimental to food security of our country. Maize is acknowledged worldwide as a major staple food crop and a model organism endowed with enormous genetic diversity. The flourishing plant breeding program mostly relies on continual sourcing, creation and deployment of novel useful genetic diversity with a focus to achieve persistent improvement in crop productivity and genetic gains (Smith et al. 2015). Labroda et al. (2005) studied the genetic variation at molecular level within temperate maize germplasm comprising of past introductions of exotic material in Brazil which provided more efficient and effective use in genetic improvement programs. Landraces and wild genetic resources of maize are known to be gifted with beneficial alleles useful for enhancing the quality of genetic base of existing breeding programs. In order to mine this huge amount of wealth, there is a need for detailed information about the genetic diversity and population structure. The population structure assessment provides greater understanding of the heterotic

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groups and the level of genetic variability present when defining core subsets identified for specific traits (Bedoya et al. 2017).

In Jammu and Kashmir, maize covers an area of 0.31 mha with a production of 0.48 mt (Anonymous 2019). Majority of area is rainfed and is grown under landraces with specific adaptability. Local knowledge has guided us to comprehend about varied farmer preferences based on grain type, grain colour, straw yield, etc. Bio-diversity existing in diverse heterogenous environments is being threatened by several factors including modern agriculture systems, urbanization, climate changes and a great human interference (MacLean-Rodriguez et al. 2019). It is noticed that erosion in landraces in this part of India is also taking place. Therefore, agrodiversity conservation is the key endeavour to support the sustainability of traditional and modern agriculture systems (MacLean-Rodriguez et al. 2021). However, the clear information on genetic diversity across maize landraces in the region is lacking. In the present study, 70 maize landraces were evaluated for various morphological traits and also screened using simple sequence repeat markers in order to estimate the magnitude of diversity.

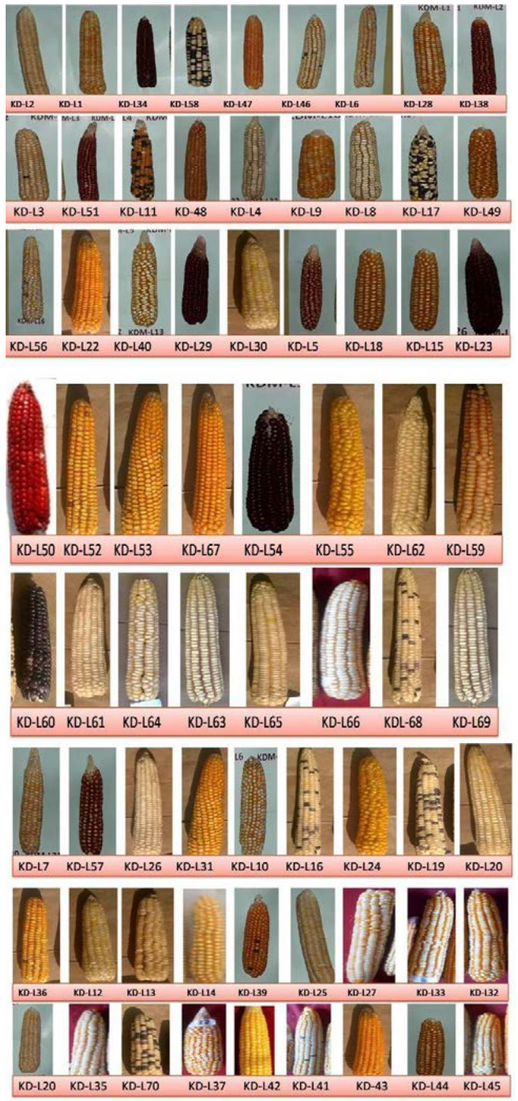

Fig. 1. Seventy landraces evaluated during experimentation

Materials and methods

A total of 70 maize landraces collected from different maize growing areas, namely, Kishtwar (10), Kulgam (9), Kupwara (10), Pulwama (11), Shopian (15), Srinagar (2), Tral (10) and one each from Poonch, Hail Kapran and Sidar Kapran of Kashmir valley were studied for population structure during 2018-19 (Fig. 1 and Fig. 2).

Field evaluation and phenotypic diversity analysis

In order to assess the phenotypic diversity, experimental material was evaluated in an augmented block design (Federer 1956) that consisted of 7 blocks, each containing 13 genotypes including 10 test entries and three check entries (KG-2, SM-C4 and SM-C7). The experimental plot comprised one row of two metre length with a planting geometry of 60 x 20 cm. Observations on various traits *viz*., plant height (cm), ear height (cm), ear diameter, number of kernel rows ear^{-1}, number of kernels row^{-1}, shelling percentage (%), 100-grain weight (g), prolificacy index, grain yield hectare^{-1}(kg) and protein content (%) were recorded on five randomly selected competitive plants. For maturity traits (days to 50 % tasseling, days to 50% silking, anthesis silking interval and days to maturity) data were recorded on plot basis.

Statistical analysis

Analysis of variance for all the phenotypic traits was obtained by SAS Proc GLM software (SAS Institute USA). Data recorded on various phenotypic characters were utilized for Mahalanobis D^{2}statistics (Mahalanobis 1936). Using D^2

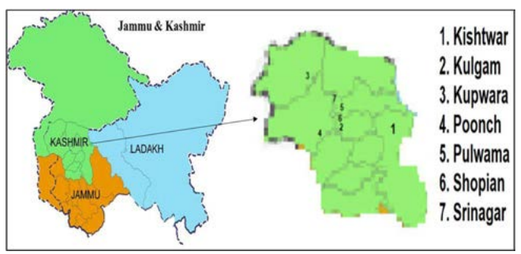

Fig. 2. Map of different districts, where landraces were collected: Mahalanobis euclidean distance for morphological, maturity, yield and quality traits in maize landraces (Not to be included)

values, different landraces were assembled into various clusters following Tocher's method as recommended by Rao (1952). Average inter and intra-cluster distances were obtained as per the method given by Singh and Chaudhary (1985). The computer programme "SPAR1" (Gupta 2005) was used for conducting D^2 analysis.

Molecular analysis

The plant genomic DNA isolation was carried out by CTAB (Cetyl- Tri Methyl Ammonium Bromide) protocol as described by Murray and Thompson (1980) with a little amendment. A total of 30 SSR markers dispersed across maize genome were selected for analyzing genetic diversity and population structure. The marker sequences were obtained from www.maizegdb.org (Supplementary Table S1). PCR amplification was performed in a thermocycler (Eppendorf, Hamburg, Germany) with initial template denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 45 s, primer annealing at 55-57°C for 45 s and extension at 72°C for 1 min. An additional extension period at 72°C for 7 minutes was given for the last cycle. The amplified PCR products were detected and resolved in 3.5% agarose (MolBio HIMEDIA) gel. The PCR products were visualized by staining with ethidium bromide (10mg/ml of double distilled water) and photographed under gel documentation unit (Bio-Rad Laboratories Inc., USA). This was followed by estimating the size of bands with the help of 50 bp DNA extension ladder (MolBioHimedia). Among the 30 SSR markers tested in this study, three markers showed meagre amplification and two markers exhibited no polymorphism. Therefore, excluding these five markers, genotypic data produced by 25 SSR markers was analyzed.

The Analysis of Molecular Variance (AMOVA) was accomplished by GenAlex software version 6.5 (Peakall and Smouse 2006) to determine whether the genetic variability was greater within the population or between the populations. The SSR genotypic data was analyzed using Power Marker V3.0 (Liu and Muse 2005) to obtain the information on major allele frequency (MAF), heterozygosity (H_O), gene diversity or expected heterozygosity (H_E), alleles per locus (N_a), number of effective alleles (N_E) and polymorphism information content (PIC). Further, the genetic structure analysis was performed as per the admixture model-based clustering method using the software package STRUCTURE 2.3.4 (Earl 2012). The STRUCTURE program was run three times for each *K* value, ranging from 1 to 10, with a burn-in of 50000 and Markov Chain Monte Carlo (MCMC) iterations of 100,000. The suitable *K* value was identified by uploading the data into the STRUCTURE HARVESTER software utilizing the Evanno method (Evanno et al. 2005) for easier detection of number of groups that best fit the data. An average likelihood value, LnP (D), was computed across all runs for each *K*. In order to nullify the overestimation of subgroup number by STRUCTURE, the ad-hoc criterion (D*K*) of Evanno et al. (2005) was utilized to find out the most suitable *K* value. A run of estimated numbers of the subgroups showing maximum likelihood was implemented to allocate the landraces that had membership probabilities of ≥0.80 to subgroups while as landraces with an estimated membership probabilities of <0.80 were allocated to the admixed group (Stich et al. 2005). Following STRUCTURE analysis, the differences among landraces were further confirmed by cluster analysis using computer software programme Numerical Taxonomy System (NTSYS) 2.2 (Rohlf 1993). The similarity matrix was generated using Jaccards coefficient and dendrogram was constructed using Tree plot options in Unweighted Pair Group Method using Arithmetic Averages (UPGMA) available in NTSYS.

Results

Phenotypic diversity analysis

The analysis of variance (Table 1) for 14 agro-morphological

Table 1. Analysis of variance for maturity, morphological, quality, yield and yield related parameters in 70 maize (*Zea mays* L.) landraces

Source of variation	DF	Days to 50% tasseling	Days to 50% silking	Anthesis silking interval	Plant height (cm)	Ear height (cm)	Days to maturity	Ear diameter (cm)
Treatments (eliminating blocks)	72	49.90*	50.62*	0.42	2728.01*	1120.10*	80.52*	0.12*
Blocks (ignoring treatments)	6	1.96*	2.52*	0.31	258.88	232.60	5.49	0.008
Entries (ignoring blocks)	69	18.32*	19.01*	0.38	1416.87*	709.65*	16.82*	0.10*
Checks	2	151.00*	188.19*	2.04*	33836.14*	8205.90*	586.71*	0.24*
Check v/s Entries	1	2026.33*	1956.30*	0.52	30980.07*	15269.74*	3463.59*	0.99*
Error	12	0.44	0.35	0.26	549.36	83.62	2.99	0.005
Treatments (eliminating blocks	72	6.37*	14.07*	0.024	33.16*	6911918.1*	27.96*	1.14*
Blocks (ignoring treatments)	6	0.38	10.53*	0.01	1.56	750868.5	0.71	0.15
Entries (ignoring blocks)	69	3.53*	9.95*	0.01	29.98*	627668.5	18.99*	0.54*
Checks	2	8.33*	49.47*	0.38*	94.79*	13162224.0*	33.27*	5.69*
Check v/s Entries	1	198.96*	227.45*	0.21*	129.25*	428024524.0*	636.32*	33.14*
Error	12	0.40	3.42	0.01	0.66	301593.8	1.36	0.06

*Significant at 5 % level of significance

traits revealed that mean squares for blocks were non-significant for all traits except days to 50% tasseling, days to 50% silking and kernels row^{-1}. The mean squares across 70 landraces were significant for all the traits except for prolificacy and anthesis-silking-interval. Cluster analysis for morphological, maturity, yield and quality attributes grouped 70 maize landraces into four major clusters with majority of landraces in cluster-II (19) followed by cluster-IV (18), cluster-I (13) and cluster III (8) whereas clusters-V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV and XVI were mono-genotypic (Table 2).

The average inter and intra-cluster distances are presented in Supplementary Table S2. Highest intra-cluster distance was obtained for cluster IV followed by cluster II, cluster III and cluster I. Highest inter-cluster distance was found between clusters I and III followed by clusters I and IV, clusters I and II.

Molecular diversity assessment

The AMOVA showed that 93 per cent of the overall genetic variability was exhibited by intra-population variance and the remaining 7 percent by inter-population variance (Table 3).

Table 2. Distribution of maize landraces into clusters based on D2 analysis for different traits

Cluster No.	No. of landraces	Name of the landraces
I		KD-L 30, KD-L 44, KD-L 10, KD-L 69, KD-L 61, KD-L 26, KD-L 67, KD-L 7, KD-L 43, KD-L 38, KD-L 28, KD-L 19 and KD-L 70
II		KD-L 12, KD-L 66, KD-L 11, KD-L 8, KD-L 63, KD-L 9, KD-L 42, KD-L 2, KD-L 62, KD-L 33, KD-L 57, KD-L 68, KD-L 4, KD-L 55, KD-L 60, KD-L 22, KD-L 26, KD-L 46, KD-L 64, and KD-L 54
III		KD-L 53, KD-L 56, KD-L 47, KD-L 48, KD-L 49, KD-L 32, KD-L 50 and KD-L 52
IV		KD-L 20, KD-L 21, KD-L 24, KD-L 23, KD-L 15, KD-L 39, KD-L 59, KD-L 58, KD-L 36, KD-L 27, KD-L 17 , KD-L 16, KD-L 25, KD-L 13, KD-L 14, KD-L 29, KD-L 18 and KD-L 34
V		KD-L 37
VI		KD-L 65
VII		KD-L 40
VIII		KD-L 1
IX		KD-L 41
X		KD-L 42
XI		KD-L 6
XII		KD-L 5
XIII		KD-L 45
XIV		KD-L 51
XV		KD-L 35
XVI		KD-L 31

KD = Karewa Dryland

Twenty five SSR makers detected a total of 108 alleles in 70 maize landraces. The number of alleles per locus ranged from 9 (*umc* 1918) to 2.00 (*bnlg* 198) with a mean of 4.72 alleles per locus but the effective number of alleles per locus was found to be lower for all SSR loci ranging from 7.24 for *bnlg* 1335 to 1.47 for phi 129 with an average value of 2.6 (Table 4). The level of heterozygosity ranged from 0.48 for SSR marker *bnlg* 1335 to 0.00 for SSR markers *phi* 129, *umc* 1293, umc 2210, *bnlg* 198, *umc* 1665 and *umc* 1545 with a mean of 0.16. The PIC value ranged from 0.82 (*bnlg* 1335) to 0.29 (*phi* 129) with an average of 0.49. The gene diversity ranged from 0.87 for *bnlg* 1335 to 0.32 for *phi* 021. Major allele frequency ranged from 0.81 for SSR marker *phi* 021 to 0.18 for SSR marker *bnlg* 1335 with a mean of 0.53.

Structure analysis and hierarchical clustering

The Bayesian analysis showed a structuring of landraces into two distinct groups based on sharp peak of D*K* which revealed that the most relevant partition was at k=2 (Fig. 3). Out of 70 maize landraces, sub-population I comprised 27 landraces (38.6%), whilst sub-population II consisted of 34 landraces (48.6 %) (Fig. 4). Fixation index (Fst) of 0.18 and 0.10 were recorded for sub-population I and sub-population II, respectively.

Following structural analysis, the 70 maize landraces were also analyzed using Jaccard's similarity coefficient

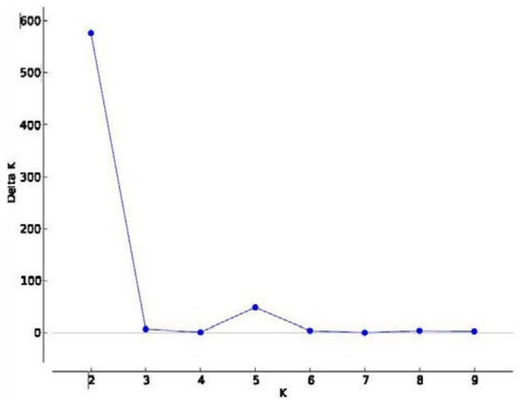

Fig. 3. Estimation of best number of populations (K) from an assumed range of 1-10 based on Evanno method

Table 3. Analysis of molecular variance (AMOVA) between the maize populations and within maize populations

Source of variation	d.f.	Sum of squares	Percentage of variation
Among Populations	2	67.692	7%
Among Individuals	67	798.630	68%
Within Individuals	70	126.500	24%
Total	139	992.821	100%

Table 4. Summary statistics of the genotyping assay for the 70 maize (*Zea mays* L.) landraces

S.No.	Marker	Major allele frequency	Allele No.	Gene diversity	Heterozygosity	Polymorphic information content	Number of effective alleles
1	bnlg 1335	0.18	8	0.86	0.48	0.84	7.24
2	umc 2210	0.54	4	0.49	0.00	0.37	1.98
3	umc 1568	0.46	4	0.64	0.18	0.57	2.85
4	umc1665	0.58	6	0.53	0.00	0.44	2.15
5	bnlg 1904	0.42	5	0.65	0.28	0.58	2.86
6	bnlg 198	0.52	2	0.49	0.00	0.37	1.96
7	bnlg 1176	0.46	8	0.8	0.28	0.78	5.00
8	bnlg 1265	0.55	5	0.63	0.3	0.58	2.80
9	phi021	0.81	7	0.32	0.05	0.34	1.53
10	phi064	0.50	3	0.49	0.20	0.46	1.99
11	phi129	0.78	3	0.34	0.00	0.3	1.47
12	bnlg 1720	0.61	5	0.52	0.06	0.44	2.11
13	umc 1640	0.58	4	0.55	0.14	0.51	2.19
14	phi024	0.52	4	0.6	0.24	0.52	2.70
15	bnlg 1605	0.68	4	0.49	0.18	0.47	1.87
16	bnlg105	0.77	4	0.36	0.14	0.31	1.62
17	umc 1887	0.63	4	0.47	0.14	0.37	1.98
18	phi033	0.56	4	0.58	0.11	0.51	2.42
19	phi065	0.48	5	0.52	0.02	0.41	2.17
20	umc 1859	0.50	4	0.63	0.21	0.63	2.40
21	umc 1545	0.49	4	0.62	0.00	0.57	2.71
22	umc 1293	0.65	4	0.45	0.00	0.38	1.88
23	bnlg 1138	0.60	4	0.55	0.25	0.5	2.31
24	umc 1918	0.37	9	0.78	0.40	0.75	4.54
25	Umc 1664	0.14	4	0.67	0.00	0.65	2.46
	Mean	0.53	4.72	0.56	0.15	0.51	2.60

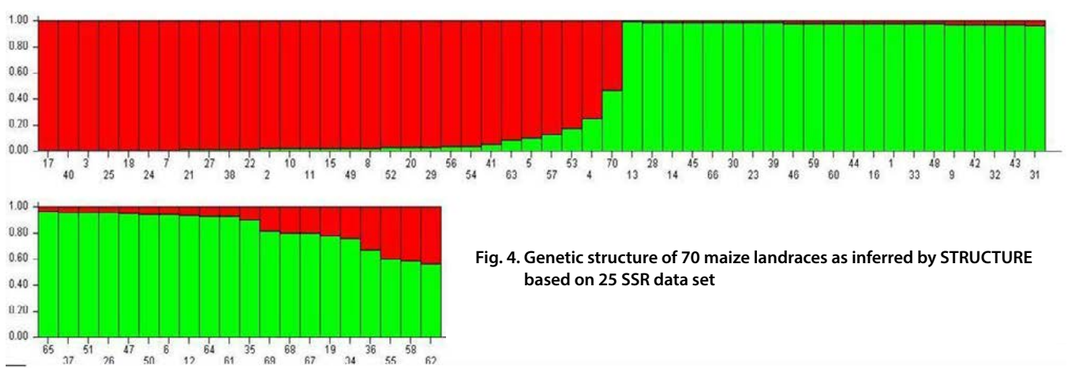

Fig. 4. Genetic structure of 70 maize landraces as inferred by STRUCTURE based on 25 SSR data set

followed by cluster analysis using UPGMA algorithm of NTSYS–PC package (Rohlf 1993). NTSYS (UPGMA) cluster analysis generated a dendrogram (not presented) that stratified 70 maize landraces in two clusters, Cluster-I and cluster-II with KD-L66 and KD-L36 as outliers. Cluster-I was further partitioned into two sub clusters namely cluster-IA & cluster-IB. Sub cluster-IA consisted of 31 landraces and sub cluster B consisted of 25 landraces. Cluster-II had only 12 landraces. The genetic similarity coefficient based on 25 SSR markers and 70 genotype panel ranged from 0.28 to 0.76 (Supplementary Table S3). Of the pair wise combinations generated KD-L49 and KD-L53 showed highest similarity

coefficient (0.76).

Discussion

To address the food security problem of the increasing population and to ease out the pressure on wheat and rice demand, it is imperative to boost the maize production. Maize, apart from being a staple diet, serves to be important fodder for cattle and a raw material for bio-fuel and industrial products. A wide genetic diversity is found within and among the maize populations. The knowledge of genetic diversity in maize is important for understanding the genetic structure, which in turn helps the breeders in choosing desirable parents for their breeding program (Al-Badeiry et al. 2014). Better understanding of existing genetic diversity facilitates the development of heterotic pools for breeding elite hybrids and composites (Ranatunga et al. 2009). The elucidation of genetic diversity among genotypes based on morphological markers alone is hampered by varying G x E interaction. The use of molecular markers provides estimates of genetic diversity with greater precision and throws light on kinship and ancestry. Therefore, in the present study, an attempt has been made to find out the extent of diversity at morphological and molecular level among 70 maize landraces which could help in devising a strategy for their use and inclusion in breeding programmes.

Phenotypic analysis

The analysis of variance revealed significant magnitude of variability is present in the landraces for all traits. Adequate genetic variability was also found in maize for various traits by Ali et al. (2012) except days to 50 % anthesis, days to 50 % silking, which were not in conformity with the results of present study as significantly high genetic variability was observed for days to 50 per cent tasseling and days to 50 per cent silking. Similarly, Aci et al. (2018) also found significant variation in Algerian maize landraces for most of the traits. It may be concluded that the sufficient variability found in the genetic material for flowering and yield traits is due to the fact that the landraces are adapted to an extensive range of environments. Distribution of landraces into different clusters revealed maximum grouping of these landraces to the tune of 27.14 % in cluster-II followed by 25.71 % in cluster-IV, 18.57 % in cluster-I and 11.42% in cluster-III. Similar studies were performed by Iqbal et al. (2015) where 153 maize genotypes were grouped into five clusters (Nikkhoy and Shiri 2017).

In the present investigation, clustering pattern revealed that landraces from a geographical origin were not assembled into the same cluster. This indicated that there is no correlation between geographic and genetic diversity. Such diversity among landraces collected from diverse geographic origins could be attributed to factors like free exchange of the germplasm or due to the unidirectional selection followed at many places, heterogeneity, genetic architecture of the populations, developmental traits, genetic drift, which might have played an important role in the diversity of landraces. Thus, geographical diversity was not found to be the only aspect in determining the genetic divergence. This lack of parallelism between geographic and genetic diversity was also obtained earlier. Inter-cluster distances with14 phenotypic traits revealed divergence among 70 landraces. Maximum inter-cluster distance was found between clusters I and III followed by clusters I, IV, I and II, respectively. The clusters with high genetic distance could be divergent heterotic groups, which may be confirmed through proper heterotic grouping methods. Thus, intergroup crosses may be used to obtain useful recombinants in the segregating generations. Further, intergroup crosses between the inbreds isolated from such groups are expected to produce highly heterotic hybrids and in turn these could be used to produce recycled recombinant inbreds.

Molecular analysis

On the basis of morphological data alone, categorization of landraces into different clades has limitations due to environmental factor and low reproducibility. Therefore, genotypic assay was carried out using 25 SSR markers. The AMOVA revealed that genetic diversity is greater within population than between them. This result was in agreement with observations made by Annicet et al. (2016) and Belalia et al. (2019). According to Nybom (2004) and Silva et al. (2015), allogamous species typically retain a greater magnitude of genetic variation within population and a low genetic differentiation between populations. Another aspect justifying the high variability within population is due to old age practice of exchange or mixture of seeds by farmers. An average of 4.72 alleles per locus detected high degree of polymorphism among 70 maize landraces. The effective number of alleles per locus is a robust parameter compared to absolute number of alleles that takes into account the distribution of allelic frequencies. The effective number of alleles was higher for markers *bnlg* 1335 (7.24) and *umc*1918 (4.76). Although, the average number of alleles per locus was highest in SSR loci *umc* 1918 (9) but only 50.4 % of total allele numbers are meaningful. However, in case of *bnlg* 1335 contribution of all alleles are equal as reflected from the highest effective number of alleles (90.5 %). The average number of alleles per locus was similar to that found by Al-Badeiry et al. (2014). A high mean number of allele per locus (10.9) has also been reported by Aci et al. (2018). They further reported that SSRs are highly informative with high PIC values revealing broad genetic diversity. It is therefore, advocated to use more number of SSRs markers to get robust conclusions. However, considerably lower number of alleles per locus was reported by Kumar et al. (2016), Pal et al. (2020), Mahato et al. (2021) and Iboyi et al. (2021) in maize, which may be due the type of material and number of samples analyzed in their study. Thus the study revealed a comparatively high allelic wealth in the 70 maize landraces confirming a broad genetic base of maize landraces from the valley. The reason for the discrepancy could be explained by several reasons: the size of the sample collection under study, the methodologies undertaken for molecular analysis, the expected diversity based on pedigrees and the SSR panel adopted (Adetimirin et al. 2008; Belalia et al. 2019). The genetic variability of maize landraces is affected by various factors throughout their evolutionary history. Out-crossing and fitness-relevant mutations created intra-population diversity, whereas natural or human selection and bottleneck effects lead to an upsurge in level of interpopulation diversity (Dreisigacker et al. 2005).

In the present investigation, 52 % of SSR loci had PIC > 0.50, which suggested that these markers were highly informative and explained the population grouping. These markers would help researchers to conduct further downstream studies related to genetic amelioration. The results were in congruence with the previous findings (Shukla et al. 2014: and Adu et al. 2019) but comparatively higher PIC value was determined as reported earlier by Iboyi et al. (2021) and Makore et al. (2021). The expected heterozygosity for SSR loci observed was similar to those reported previously by Pineda-Hidalgo et al. (2013), Synrem et al. (2017) and Belalia et al. (2019) but higher than those reported by Aci et al. (2013), Noldin et al. (2016), Pal et al. (2020) and Mahato et al. (2021) for Algerian maize landraces, Paraguayan race of maize accessions, 39 popcorn inbred lines and 12 sweet corn inbreds, respectively; while Yao et al. (2007) reported the higher mean values analyzing chinese maize landraces. The SSR markers *viz*., *phi 021 and phi 129* expressed allelic frequencies more than 0.75 which suggests non-neutrality of these alleles. The results were in accordance with the previous reports (Nepolean et al. 2013; Adu et al. 2019). The heterozygosity level observed in the landraces may be due to a number of possible factors including pollen or seed contamination and mutation at specific SSR loci (Bantte and Prasanna 2003). However, the level of observed heterozygosity was found to be less than expected heterozygosity for all SSR loci analyzed possibly because of inbreeding, since landraces within the Kashmir valley are confined to specific ecological niches with narrow geographical range which leads to considerable fixation of alleles. Also, the farmers cultivate landraces using their own saved seed, in many cases using small seed sample that might have caused random drift, thus having increased homozygosity.

The STRUCTURE analysis is a Bayesian model based study and helped to infer two sub-populations –I and II. Yield hectare^{-1} and anthesis silking interval were found to be unique traits for sub-populations –I whereas in sub-populations –II, ear height and protein content were important. These results were similar to those obtained by earlier by Sa et al. (2015), Annicet et al. (2016); Bedoya et al (2017) and Belalia et al. (2019). In contrast, Makore et al. (2021) revealed three distinct sub populations while analyzing 372 maize landraces using 116 SNPs. The structuring could be due to many reasons *viz*., direct natural or human selection, bottleneck effects in the recent past and shared ancestry (Dreisigacker et al. 2005). Fixation index (Fst) of 0.18 for population 1 and Fst value of 0.09 for population 2 was recorded and suggested moderate and low genetic differentiation for the two sub-populations, respectively. This result was in accordance with the results of Annicet et al. (2016) and Adu et al. (2019). This could be explained by the fact that there are moderate gene flows due to the closeness of adjacent fields or seed exchange between different regions. For a naturally cross pollinated crop like maize, these biological events are more apparent, as exchange of seed material between populations are favored by cross-pollination. Further, UPGMA based clustering approach also grouped the 70 maize landraces into two broad and genetically diverse clusters with KD-L36 and KD-L66 as outliers. Similar studies were carried out by Ranatunga et al. (2009) and Kanagarasu et al. (2013). As the landraces KD-L49 and KD-L53 belong to same site of collection, they shared a similarity of 76%. Since the cluster analysis based on both the approaches (UPGMA and STRUCTURE) grouped the landraces regardless of their geographical origin, it can be said that UPGMA clustering was in conformity with Bayesian clustering. The selection for beneficial alleles by farmers to meet their needs in terms of adaptation to local conditions and the exchange of seeds among farmers from distinct regions could be the cause for this non-relatedness of maize populations from the same region (Belalia et al. 2019). Furthermore**,** it was observed that there is lack of correspondence between morphological clustering pattern and molecular clustering approach. The molecular diversity did not correlate with distances based on morphological markers. The explanation lies in the fact that SSR markers used mostly originate from non-coding regions of the genome having mainly evolutionary utility while as agro-morphological traits are a manifestation of expressed part of the genome which is affected by artificial selection process. Another reason for the lack of correlation between the two approaches may be due to the criteria used for clustering. Thus, a combined approach using conventional and molecular studies can give a better insight into the variation pattern that can be harnessed for broadening the genetic base for various useful traits.

The microsatellite markers helped to group maize landraces of Kashmir into two sub-populations with moderate level of differentiation. The grouping was confirmed through UPGMA based clustering approach with inter-cluster distance of 0.70. The individuals can be selected

from either population to serve as parents for breeding of hybrids and composites for temperate highlands.

Authors' contribution

Conceptualization of research (SAD, ZAD); Designing of the experiments (NY, ZAD, AAL); Contribution of experimental materials (ZA, Dar); Execution of field/lab experiments and data collection (NY, ABS, ZAD, SG, PAS); Analysis of data and interpretation (NY, ABS, ZAD); Preparation of manuscript (NY, ABS).

Supplementary material

Three Supplementary tables supplied.

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Supplementary Table S1. List of selected microsatellite along with their sequences

S. NO.	Marker	Forward Primer Sequence	Reverse Primer Sequence	Bin Location
1	bnlg198	GTTTGGTCTTGCTGAAAAATAAAA	GCTGGAGGCCTACATTATTATCTC	2.08
2	bnlg1335	GAAGGTTGCTCTTCCACTGG	TGGTTTGTGCAAGTGTCACC	2.08
3	umc2210	GATGCTACCATTTCAGTGAGCGAT	AGCGGGTCGATCTTTCTCTTAGTT	8.05
4	umc1665	CAATCAGGAGCCAGGGAGATG	CTTAAACTTGTCGAGACGGTCCTG	8.05
5	umc1664	AATTGTTTACTGCGCTGAAACTCC	CCTCTTTGCCTGTACCGTGTATTC	1.06
6	umc1293	GTATCCGTTTCTCATGCAACACAC	GATCTCGATCTGCTTCATCATCTG	10.00
7	umc1859	ATATACATGTGAGCTGGTTGCCCT	GCATGCTATTACCAATCTCCAGGT	6.06
8	umc1568	AAGTCCAGCCAAGTTCATCAAAGA	ACTGTAACTAAACTGGGTGTGCCC	1.02
9	bnlg1720	CAACCCGGATGTCTCAAGTT	TTCGATGCGTATGTACTCAGC	1.09
10	phi064	CCGAATTGAAATAGCTGCGAGAACCT	ACAATGAACGGTGGTTATCAACACGC	1.11
11	bnlg1138	TGCTCTAGCCGACCTCAATT	ATGCCTGAACCGTGATTAGG	2.06
12	bnlg1904	AGGAGCATGCACTTGGTTCT	ACTCAACTGATGGCCGATCT	3.04
13	bnlg1605	CACCTCTGAACCCCTGTGTT	TCCTGCCCCCTTTGTTTTC	3.07
14	phi021	TTCCATTCTCGTGTTCTTGGAGTGGTCCA	CTTGATCACCTTTCCTGCTGTCGCCA	4.03
15	bnlg1265	GGTTGTCCGTAAAGGCAAGA	TGTGAAGGCCAGACAGTCAG	4.05
16	phi024	ACTGTTCCACCAAACCAAGCCGAGA	AGTAGGGGTTGGGGATCTCCTCC	5.01
17	bnlg105	GACCGCCCGGGACTGTAAGT	AGGAAAGAAGGTGACGCGCTTTTC	5.02
18	umc1887	CTTGCCATTTTAATTTGGACGTTT	CGAAGTTGCCCAAATAGCTACAGT	6.03
19	umc1545	GAAAACTGCATCAACAACAAGCTG	ATTGGTTGGTTCTTGCTTCCATTA	7.00
20	bnlg1176	ACTCCTCAAAACCTAGGTGACA	CACCGATGATGGTGAGTACG	8.05
21	phi033	ATCGAAATGCAGGCGATGGTTCTC	ATCGAGATGTTCTACGCCCTGAAGT	9.01
22	phi065	AGGGACAAATACGTGGAGACACAG	CGATCTGCACAAAGTGGAGTAGTC	9.03
23	phi129	TCCAGGATGGGTGTCTCATAAAACTC	GTCGCCATACAAGCAGAAGTCCA	6.05
24	umc1640	ACTACACGGTGTGAGATGTGATCG	GTCGTCGCAAGAACAACAAGG	10.07
25	umc1918	CACAAGAACATTATGACGACCGAG	AAGCAGGAGACATCGTTTAAGTCG	6.04

Supplementary Table S2. Average Intra cluster (Diagonal) and intercluster distances for maturity, morphological, quality, yield and yield related parameters in 70 maize (*Zea mays* L.) landraces

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	141.52	265.8	1067.25	313.56	672.92	297.58	444.84	244.9	297.82	465.55	436.03	248.43	210.06	1103.81	603.79	396.92
II		176.04	1184.39	333.86	490.88	329.58	593.24	294.09	348.03	335.7	326.6	350.71	348.84	942.56	482.97	500.65
III			169.08	1235.62	1898.71	1293.7	618.49	945.64	1281.78	500.19	1732.86	844.41	1008.2	309.84	762.15	1709.97
IV				219.69	529.15	685.78	369.95	470.21	390.74	483.7	680.92	660.83	513.33	1115.47	373.44	774.21
V					0	988.3	1064.03	398.39	264.92	717.62	549.9	871.49	868.48	1380.04	806.76	1071.88
VI						0	828.79	405.5	584.08	623.55	402.64	173.4	312.38	1291.03	1034.21	357.19
VII							0	705.59	742.38	448.81	1228.41	752.03	626.19	781.73	308.96	1128.75
VIII								0	118.6	337.43	280.14	132.66	230.25	786.95	673.69	473.12
IX									0	441.92	455.27	385.03	443.55	1138.44	623.01	590.6
X										0	730.54	422.86	577.61	322.47	190.49	956.53
XI											0	329.38	285.56	1311.78	1054.68	287.2
XII												0	139.78	828.5	891.12	348.66
XII													0	948.73	804.05	180.23
XIV														0	600.5	1634.98
XV															0	1162.58
XVI																0

Supplementary Table S3. Similarity matrix generated from NTSYS for 70 maize landraces (*Zea mays* L.) based on 25 SSR markers

	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
1	1.0																																		
2	0.3	1.0																																	
3	0.2	0.5	1.0																																
4	0.3	0.3	0.5	1.0																															
5	0.4	0.5	0.4	0.2	1.0																														
6	0.6	0.4	0.3	0.3	0.5	1.0																													
7	0.2	0.4	0.5	0.6	0.3	0.2	1.0																												
8	0.3	0.4	0.4	0.3	0.3	0.3	0.4	1.0																											
9	0.4	0.3	0.3	0.3	0.4	0.5	0.4	0.3	1.0																										
10	0.2	0.4	0.3	0.1	0.5	0.3	0.2	0.3	0.3	1.0																									
11	0.3	0.4	0.5	0.5	0.3	0.3	0.5	0.3	0.4	0.3	1.0																								
12	0.3	0.4	0.4	0.3	0.5	0.4	0.4	0.2	0.5	0.3	0.3	1.0																							
13	0.4	0.4	0.3	0.2	0.3	0.3	0.2	0.3	0.5	0.2	0.2	0.4	1.0																						
14	0.3	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.4	0.4	1.0																					
15	0.3	0.4	0.3	0.2	0.4	0.5	0.2	0.3	0.4	0.6	0.3	0.2	0.3	0.3	1.0																				
16	0.4	0.3	0.3	0.3	0.4	0.6	0.2	0.3	0.4	0.3	0.3	0.5	0.2	0.3	0.3	1.0																			
17	0.1	0.4	0.4	0.4	0.3	0.3	0.4	0.5	0.3	0.3	0.6	0.3	0.2	0.2	0.3	0.3	1.0																		
18	0.2	0.2	0.3	0.4	0.2	0.2	0.4	0.3	0.1	0.1	0.4	0.2	0.1	0.2	0.2	0.2	0.2	0.5	1.0																
19	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.2	0.3	0.3	0.3	0.4	0.2	0.3	0.2	0.6	0.3	0.2	1.0																
20	0.2	0.4	0.3	0.4	0.3	0.4	0.3	0.2	0.4	0.4	0.4	0.3	0.2	0.2	0.5	0.3	0.4	0.4	0.3	1.0															
21	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.2	0.2	0.2	0.3	0.2	0.5	0.3	0.2	0.2	1.0														
22	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0.3	0.4	0.2	0.1	0.2	0.3	0.2	0.4	0.5	0.1	0.4	0.2	1.0													
23	0.4	0.4	0.2	0.2	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.2	0.4	0.2	0.2	0.4	0.2	0.2	0.2	1.0												
24	0.2	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.2	0.4	0.2	0.2	0.2	0.4	0.1	0.4	0.4	0.2	0.4	0.3	0.4	0.2	1.0											
25	0.3	0.5	0.4	0.2	0.4	0.4	0.3	0.4	0.3	0.4	0.4	0.3	0.2	0.3	0.4	0.4	0.3	0.4	0.4	0.4	0.2	0.5	0.4	0.5	1.0										
26	0.4	0.5	0.4	0.3	0.5	0.3	0.2	0.3	0.4	0.3	0.3	0.4	0.5	0.4	0.3	0.3	0.3	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.4	1.0									
27	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.5	0.3	0.3	0.5	0.3	0.2	0.2	0.3	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.6	0.4	1.0								
28	0.5	0.3	0.2	0.3	0.3	0.4	0.2	0.3	0.5	0.2	0.3	0.3	0.6	0.3	0.3	0.4	0.1	0.0	0.4	0.2	0.2	0.2	0.4	0.1	0.2	0.5	0.3	1.0							
29	0.3	0.6	0.4	0.4	0.5	0.3	0.3	0.3	0.2	0.2	0.4	0.4	0.3	0.3	0.3	0.3	0.5	0.4	0.4	0.4	0.2	0.3	0.3	0.3	0.4	0.5	0.4	0.2	1.0						
30	0.4	0.2	0.2	0.3	0.2	0.4	0.3	0.2	0.4	0.2	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.2	1.0				
31	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.4	0.4	0.5	0.2	0.3	0.2	0.3	0.3	0.3	0.1	0.2	0.2	0.2	0.4	0.4	0.4	0.4	0.3	0.3	1.0				
32	0.4	0.4	0.3	0.3	0.3	0.4	0.2	0.2	0.4	0.2	0.3	0.5	0.5	0.4	0.3	0.4	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.4	0.2	0.4	0.4	0.3	0.4	1.0			
33	0.5	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.2	0.4	0.3	0.2	0.4	0.3	0.2	0.4	0.3	0.2	0.4	0.2	0.4	0.5	0.4	0.5	0.5	0.4	0.4	0.4	1.0	
34	0.3	0.4	0.3	0.4	0.4	0.5	0.4	0.3	0.6	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.5	0.3	0.4	0.4	0.3	0.4	1.0
35	0.5	0.4	0.4	0.3	0.5	0.5	0.3	0.3	0.5	0.3	0.4	0.5	0.3	0.2	0.3	0.6	0.3	0.2	0.5	0.3	0.3	0.2	0.4	0.2	0.4	0.4	0.5	0.5	0.5	0.2	0.3	0.3	0.5	0.4	1.0
36	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.4	0.3	0.2	0.3	0.2	0.2	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.3

37	0.3	0.4	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.4	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.1	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.2	0.3
38	0.4	0.5	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.2	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.6	0.5	0.5	0.3	0.5	0.3	0.3	0.4	0.4	0.4	0.5
39	0.4	0.5	0.4	0.3	0.3	0.3	0.3	0.2	0.4	0.2	0.4	0.3	0.5	0.4	0.3	0.3	0.2	0.2	0.3	0.4	0.2	0.3	0.4	0.3	0.3	0.5	0.3	0.6	0.4	0.3	0.5	0.3	0.4	0.4	0.4
40	0.3	0.4	0.5	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.5	0.3	0.3	0.3	0.5	0.2	0.4	0.4	0.3	0.4	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.2	0.4	0.2	0.4	0.3	0.3	0.3	0.3
41	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.4	0.2	0.3	0.2	0.4	0.3	0.3	0.5	0.3	0.2	0.3	0.3	0.2	0.4	0.4	0.3	0.3	0.3	0.1	0.4	0.3	0.2	0.3	0.4
42	0.5	0.3	0.2	0.3	0.4	0.4	0.2	0.2	0.4	0.3	0.2	0.4	0.2	0.3	0.3	0.5	0.2	0.1	0.4	0.3	0.1	0.2	0.4	0.2	0.3	0.3	0.3	0.4	0.2	0.3	0.3	0.3	0.5	0.3	0.5
43	0.3	0.3	0.2	0.4	0.3	0.4	0.3	0.3	0.4	0.2	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.5	0.3	0.5	0.3	0.3	0.4	0.3	0.4	0.4	0.4
44	0.4	0.3	0.2	0.3	0.4	0.4	0.3	0.2	0.4	0.2	0.2	0.4	0.4	0.3	0.3	0.4	0.2	0.1	0.3	0.2	0.3	0.1	0.3	0.1	0.2	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.5
45	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0.2	0.5	0.2	0.3	0.5	0.6	0.4	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.3	0.3	0.2	0.4	0.5	0.4	0.3	0.4	0.3
46	0.5	0.3	0.2	0.2	0.4	0.5	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.2	0.3	0.4	0.2	0.2	0.3	0.2	0.1	0.3	0.4	0.3	0.5	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.5	0.4	0.5
47	0.3	0.3	0.3	0.4	0.2	0.2	0.3	0.3	0.3	0.2	0.4	0.2	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.4	0.3	0.2	0.3	0.2	0.3	0.5	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.3
48	0.3	0.2	0.2	0.5	0.2	0.4	0.2	0.2	0.3	0.3	0.4	0.2	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.4	0.2
49	0.4	0.5	0.3	0.3	0.5	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.2	0.4	0.3	0.3	0.2	0.4	0.2	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.6
50	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.5	0.2	0.3	0.4	0.4	0.4	0.2	0.4	0.3	0.4	0.3	0.4	0.3
51	0.3	0.3	0.2	0.4	0.2	0.3	0.3	0.3	0.4	0.2	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.4	0.3	0.2	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.5	0.3
52	0.3	0.7	0.4	0.4	0.4	0.3	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.5	0.3	0.2	0.3	0.4	0.2	0.4	0.4	0.3	0.4	0.5	0.2	0.3	0.3	0.4	0.3	0.5
53	0.3	0.5	0.3	0.3	0.5	0.3	0.4	0.3	0.3	0.3	0.4	0.5	0.3	0.3	0.2	0.4	0.3	0.3	0.6	0.3	0.2	0.3	0.4	0.2	0.5	0.5	0.3	0.3	0.5	0.2	0.3	0.3	0.4	0.3	0.5
54	0.3	0.4	0.4	0.3	0.5	0.5	0.3	0.5	0.3	0.5	0.4	0.3	0.3	0.3	0.5	0.3	0.5	0.2	0.3	0.2	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.4
55	0.4	0.5	0.4	0.3	0.6	0.5	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.5	0.4	0.3	0.2	0.4	0.2	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.2	0.4	0.3	0.3	0.5	0.4
56	0.4	0.6	0.3	0.3	0.5	0.3	0.3	0.3	0.2	0.4	0.3	0.4	0.3	0.2	0.4	0.3	0.2	0.2	0.4	0.4	0.2	0.3	0.3	0.2	0.6	0.5	0.4	0.2	0.6	0.2	0.3	0.2	0.5	0.3	0.4
57	0.3	0.5	0.4	0.3	0.5	0.4	0.3	0.3	0.4	0.6	0.3	0.3	0.4	0.3	0.6	0.2	0.3	0.1	0.3	0.3	0.3	0.2	0.2	0.4	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3
58	0.4	0.4	0.3	0.2	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.2	0.4	0.3	0.3	0.3	0.4
59	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.5	0.3	0.4	0.3	0.6	0.3	0.3	0.3	0.2	0.2	0.2	0.4	0.2	0.3	0.5	0.3	0.4	0.5	0.4	0.5	0.3	0.4	0.4	0.3	0.4	0.5	0.4
60	0.3	0.3	0.3	0.4	0.3	0.4	0.2	0.2	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.4	0.4	0.4	0.3
61	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.2	0.4	0.3	0.2	0.3	0.2	0.2	0.2	0.5	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.3	0.2
62	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.2	0.4	0.4	0.4	0.4	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.2
63	0.4	0.4	0.5	0.3	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.5	0.4	0.3	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.5	0.4	0.4	0.4	0.3
64	0.5	0.3	0.4	0.4	0.2	0.4	0.3	0.3	0.4	0.2	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.5	0.4	0.4	0.3	0.3
65	0.3	0.5	0.3	0.3	0.4	0.4	0.3	0.2	0.3	0.5	0.3	0.4	0.6	0.4	0.4	0.2	0.2	0.1	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.4	0.5	0.3
66	0.3	0.4	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.4	0.3	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.4	0.0	0.1	0.3	0.2	0.5	0.2	0.2	0.3	0.2	0.3	0.3	0.3
67	0.4	0.3	0.4	0.3	0.3	0.5	0.2	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.2	0.4	0.2	0.4	0.4	0.4	0.5	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.3
68	0.4	0.3	0.2	0.2	0.3	0.4	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.4	0.3	0.2	0.3	0.2	0.3	0.2	0.4	0.4	0.4	0.4	0.2	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.3
69	0.5	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.2	0.4	0.3	0.2	0.3	0.2	0.3	0.3	0.5	0.2	0.3	0.4	0.3	0.4	0.2	0.4	0.3	0.5	0.4	0.3	0.4
70	0.3	0.4	0.3	0.2	0.3	0.5	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.2	0.3	0.3	0.3	0.3	0.5	0.4	0.4	0.3	0.4	0.3	0.3	0.4	0.3	0.2	0.4	0.4	0.4

36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	
36	1.0																																		
37	0.2	1.0																																	
38	0.3	0.3	1.0																																
39	0.3	0.4	0.4	1.0																															
40	0.4	0.2	0.5	0.3	1.0																														
41	0.4	0.2	0.4	0.3	0.3	1.0																													
42	0.3	0.3	0.3	0.2	0.2	0.3	1.0																												
43	0.2	0.4	0.2	0.4	0.2	0.2	0.4	1.0																											
44	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	1.0																										
45	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.0																									
46	0.2	0.4	0.4	0.3	0.3	0.3	0.5	0.3	0.3	0.3	1.0																								
47	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.2	0.4	1.0																							
48	0.2	0.2	0.3	0.4	0.2	0.3	0.2	0.4	0.2	0.3	0.2	0.4	1.0																						
49	0.4	0.3	0.5	0.3	0.4	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.2	1.0																					
50	0.4	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.4	1.0																				
51	0.3	0.4	0.4	0.3	0.2	0.5	0.3	0.5	0.3	0.3	0.4	0.5	0.5	0.4	0.4	1.0																			
52	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.3	0.3	0.2	0.3	0.4	0.2	0.7	0.2	0.3	1.0																		
53	0.4	0.3	0.5	0.3	0.4	0.6	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.8	0.3	0.3	0.6	1.0																	
54	0.3	0.2	0.3	0.2	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.5	0.4	1.0																
55	0.3	0.2	0.4	0.3	0.5	0.4	0.3	0.3	0.4	0.3	0.4	0.2	0.3	0.5	0.4	0.3	0.4	0.3	0.5	1.0															
56	0.3	0.4	0.5	0.3	0.4	0.3	0.4	0.3	0.3	0.2	0.5	0.3	0.2	0.4	0.2	0.3	0.6	0.5	0.3	0.4	1.0														
57	0.3	0.4	0.3	0.4	0.5	0.2	0.3	0.4	0.3	0.3	0.3	0.3	0.2	0.5	0.2	0.2	0.5	0.4	0.5	0.5	0.5	1.0													
58	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.5	0.2	0.3	0.5	0.5	0.6	0.4	0.4	0.6	1.0												
59	0.3	0.3	0.4	0.5	0.3	0.3	0.3	0.5	0.3	0.5	0.5	0.3	0.4	0.3	0.5	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0.4	1.0											
60	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.4	0.2	0.3	0.3	0.4	0.6	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.4	1.0										
61	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.4	0.4	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.3	0.2	0.4	0.4	0.3	0.4	1.0									
62	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.5	0.3	0.4	0.3	0.3	0.2	0.4	0.2	0.3	0.3	0.3	0.4	0.4	0.3	0.6	0.5	0.4	0.3	0.4	1.0								
63	0.3	0.3	0.4	0.4	0.5	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.5	0.4	0.3	0.4	0.5	0.4	0.5	0.3	0.5	0.4	0.4	0.3	0.3	0.5	1.0							
64	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.5	0.2	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.5	0.5	0.4	0.4	0.4	0.3	1.0						
65	0.3	0.4	0.3	0.4	0.3	0.2	0.3	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.3	0.5	0.3	0.3	0.5	0.4	0.4	0.6	0.4	0.4	0.4	0.5	0.6	0.5	0.4	1.0					
66	0.2	0.4	0.2	0.5	0.1	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.4	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.2	0.2	0.3	0.2	0.3	0.3	1.0				
67	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.4	0.3	0.5	0.3	0.5	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.5	0.5	0.4	0.3	0.4	0.5	0.4	0.2	1.0			
68	0.2	0.3	0.4	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.4	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.5	0.3	0.3	0.4	0.3	0.3	0.4	0.2	0.3	0.4	0.3	0.2	0.5	1.0		
69	0.3	0.2	0.4	0.4	0.3	0.4	0.3	0.2	0.4	0.4	0.4	0.2	0.3	0.4	0.6	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.4	0.2	0.2	0.2	0.4	0.3	0.3	0.3	0.4	0.3	1.0	
70	0.3	0.4	0.4	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.2	0.5	0.4	0.4	1.0