Research Article

Ekin
Journal of Crop Breeding and Genetics

3(1):25-40, 2017

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Genetic Variation in Drought Linked Morpho-physiological Characters and Microsatellite DNA Loci in Rice (*Oryza sativa* L.)

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Citation:
Bhatia P., Jain R., Jain S., Singh M., Chowdhury V., 2017. Genetic Variation in Drought Linked Morpho-physiological Characters and Microsatellite DNA Loci in Rice (*Oryza sativa* L.). Ekin J. 3(1):25-40.

Received: 10.10.2016 ***Accepted: 14.12.2016*** ***Published Online: 29.01.2017*** ***Printed: 31.01.2017***

ABSTRACT

Evaluation for drought tolerance and SSR (microsatellite) markers based molecular polymorphism were investigated in F_6 plant population raised via single seed descent method from a cross between drought tolerant *japonica* rice variety Azucena and drought sensitive premium traditional Basmati rice variety Taraori Basmati HBC 19. A total of 50 F_6 plants were evaluated individually for drought tolerance on 1-9 scale on the basis of agronomic characteristics, root and shoot traits, relative water content and visual observations; the average score ranged between 1to 8.3. Fourteen plants each in the category of drought tolerant and drought sensitive were selected from F_6 population for SSR marker analysis using 30 SSR markers covering all the chromosomes. The 28 Azucena × HBC19 F_6 plants had an allele from either of the two parental lines (homozygous condition) or alleles from both the parental rice varieties (heterozygous condition). Frequency of HBC19 specific alleles was higher in comparison to Azucena in selected drought tolerant and drought sensitive Azucena x HBC19 F_6 plants, which may be indicative of segregation distortion. At ten SSR loci new/recombinant alleles were obtained which indicate the active recombination between genomes of two rice varieties. Cluster tree analysis and principal component analysis demonstrate high level of diversity between Azucena and HBC19 with the clustering of 28 Azucena × HBC19 F_6 plants with HBC19.

Keywords: Genetic diversity; drought stress; microsatellite; *Oryza sativa*; root traits; recombinant inbred lines

Introduction

Rice (*Oryza sativa* L.) is a staple food for almost half of the world's population and it is grown in tropical, subtropical and temperate regions of the world. More than 90% of the world's rice is grown and consumed in Asia, where rice is cultivated on 135 million ha with an annual production of 516 million tonnes. In India, area under low land rice is about 14.4 million hactares which accounts to 32.4 percent of the total rice crop area in the country. Yields of rainfed lowland rice are drastically reduced by drought due to unpredictable, insufficient and uneven rainfall during the growing period. Further, upland rice which accounts for 13% of the total area is always prone to drought during a part of the growing season. In developing countries like India, rainfall is the main source of water available to crops and irrigation facilities are often lacking, so the problem of water stress is more acute in these countries. Thus, emphasis has been given to alleviate this problem in recent years.

Under drought conditions, the performance of crops may be improved by number of morphological, physiological and phenological characters (Hemamalini *et al.,* 2000). Several scientists have suggested adaptive mechanisms of plants in response to water stress (Fukai and Cooper, 1995; Nguyen *et al.,* 1997). Root system is one

of the most significant components of drought tolerance. Nguyen *et al.,* (1997) reported that traits such as root thickness, depth of rooting, and deep root to shoot ratio have been found to be associated with this mechanism. Maximum root depth and dry weight of roots below 30 cm were good indicators of drought resistance in rice (Ahmadi, 1983). Desirable root characteristics could be useful in selecting rice genotypes for drought resistance breeding. However, phenotypic selection for most root traits is challenging and labor intensive. Molecular marker technology is a powerful tool to overcome these drawbacks. It has been successfully utilized for molecular dissection of complex agronomical traits, marker assisted breeding and in linkage mapping (for review see Flowers *et al.,* 2000). Molecular marker technology can significantly enhance the efficiency and accuracy of breeding process. A number of genes have been mapped which include genes/QTLs for several agronomically important traits such as yield, quality and resistance against abiotic stresses including salinity and water stress (Forster *et al.,* 2000; Zhang *et al.,* 1999). Among abiotic stresses maximum progress has been made towards the salinity tolerance and there have been only a few studies to map QTLs for drought tolerance (Babu *et al.,* 2003). Several types of marker such as RFLP, RAPD and AFLP, microsatellites (SSRs) have been used for drought tolerance in rice (Hemamalini *et al.,* 2000). However, PCR based markers such as AFLPs and microsatellites have revealed a great potential in the analysis of genetic diversity, gene tagging and genome mapping studies because they are very informative, technically simple, require less time, and need small amounts of DNA. Microsatellites are tandemly repeated short sequences of DNA with repeat unit of less than 6 bp in length. They exhibit high level of polymorphisms and have been successfully applied in the study of genetic diversity in wheat (Plaschke *et al.,* 1995), barley (Saghai-Maroof *et al.,* 1984) and rice (Xiao *et al.,* 1996). Rice grain yield under drought conditions may be improved with the help of marker-assisted breeding approaches due to the availability of genome wide molecular markers, inexpensive genotyping platforms and sequence information of rice genome.

In this paper, we report the genetic evaluation and microsatellite marker analysis of F_6 advance population derived from a cross between a drought tolerant *japonica* rice variety (Azucena) and Taraori Basmati and its application in linkage mapping for drought tolerance and Basmati rice breeding.

Material and Methods

Plant Materials

A population of 211 Azucena x HBC19 F_6 plants was raised through single seed descent method of which 50 were used for drought tolerance analysis. Azucena is a drought tolerant *japonica* rice variety and HBC19 (Taraori Basmati) is a commercially important traditional Basmati variety, which is quite sensitive to drought.

Evaluation for drought tolerance

The dehusked F_5 plant seeds along with parental genotypes were germinated in large size pots in the green house of the CCS Haryana Agricultural University, Hisar. Two sets of 50 Azucena x HBC19 F_6 lines were taken for recording observations. Each set contained four plants per line. Water stress was given to one set of plants by with-holding water at 60 days after sowing while the other set comprised plants under control conditions and these plants were regularly irrigated. Observations consisted of plant height (PH) in cm, tiller number (TN), grain yield (GY) in g/plant, thousand grain weight (TGW) in g, maximum root length (MRL) in cm, shoot and root fresh weight (SFW, RFW) in g, shoot and root dry weight (SDW, RDW) in g, root:-shoot ratio (RSR), harvest index (HI), relative water content (RWC), leaf drying(LD) and recovery of water stressed plants (RWSP). RWC of youngest expanded leaf was calculated as suggested by Weatherly (1950). Drought tolerant index (DTI) was then calculated for agronomic characteristics (PH, TN, GY, TGW), shoot and root trait (SFW, RFW, SDW, RDW, MRL, RSR), HI and RWC (Ribaut *et al.,* 1997) and on the basis of DTI all the F_6 plants were individually grouped under 1,3,5,7 and 9 score categories for drought tolerance. Further, grouping of these F_6 plants was done on the basis of visual symptoms of leaf drying and recovery on a 1-9 scale as per IRRI's standard evaluation system, where lower score stated for tolerant and higher scale for sensitive (Gregorio *et al.,* 1997). Average scores were calculated for each of the F_6 plants and data was used for the selection of drought tolerant and drought sensitive surviving plants.

DNA isolation and microsatellite DNA loci amplification

Genomic DNA was extracted from leaf samples using modified CTAB method (Saghai-Maroof *et al.,* 1984) from parents and fourteen F_6 plants each selected for the both extremes i.e. most drought sensitive and most drought tolerant plants. Thirty microsatellite primer pairs (Table 1, Research Genetics, Inc.) were used to amplify microsatellite DNA loci using genomic DNAs as templates. PCR reaction was conducted in a volume of 20 µl containing 50 ng template DNA, 1X Taq DNA polymerase buffer, 100 µM of each of four dNTPS, 0.4 µM each primer, 1.2 mM $MgCl_2$ and 1 unit Taq DNA polymerase (Perkin Elmer). The

PCR amplifications were performed on a PTC100 (MJ Research) thermal cycler under the following conditions- a hot start at 95°C for five minutes; followed by 35 amplification cycles of denaturing at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 2 minutes and final extension at 72°C for 7 minutes. Amplification products were resolved on 4% polyacrylamide gels using aluminium backed sequencing system model # 535 (Owl Scientific, Inc., USA) with silver staining.

Molecular weights of electromorphs were estimated using 10 bp DNA ladder from Gibco BRL, Md.

Data Analysis

The band patterns were scored for each microsatellite primer pair in each rice genotype. Presence and absence of each band in each rice genotype was coded as 1 and 0, respectively. The 0/1 matrix was used to calculate similarity genetic distance using simqual sub-program of NTYSYS-pc program (Rohlf, 1990). The resultant distance matrix was employed to construct dendrograms by the cluster tree analysis sub-program of NTYSYS-pc.

Results

Evaluation of Azucena x HBC19 F_6 population for drought tolerance

It has been suggested that traits, particularly RFW, RDW, RSFW, RSDW, MRL, RWC and visual symptoms (LD and RWSP) are more important for drought resistance in rice. In this study, some shoot traits such as PH, TN, TGW, GY, SFW and SDW were also recorded as summarized in Table 2. Significant variation in all the investigated traits indicated the presence of high genetic diversity among of Azucena x HBC19 F_6 genotypes. Mean drought tolerant index (DTI) which is the average of DTI values calculated on the basis of agronomic characteristics, shoot and root traits and RWC ranged from 42.1% (F_6 genotype no. 9) to 90.6% (Azucena). Regarding MRL, thirteen F_6 genotypes were observed to have higher DTI than Azucena. All the 52 genotypes (Azucena, HBC19 and 50 F_6 genotypes) were further scored for drought tolerance. Mean score values were calculated on the basis of scores given to DTI values and visual symptoms and it was found to be varied between 1 to 8.3. Out of 50 F_6 genotypes, two genotypes (genotype no. 14 and 46) were as tolerant as Azucena (mean score value -1.6). Genotype 48 was highly susceptible to drought conditions. Maximum numbers of plants (20 plants) were found to be moderately tolerant with mean score values of 4-5, followed by 12 plants in tolerant category with mean score values in the range of 3-4.

Microsatellite Marker Analysis

Microsatellite (SSR) DNA fingerprint database was generated for 28 selected plants (14 drought tolerant and 14 drought sensitive plants) from a population of Azucena × HBC19 F_6 lines using 30 SSR markers covering all the 12 chromosomes. The 28 Azucena × HBC19 F_6 plants had an allele from either of the two parental lines (homozygous condition) or alleles from both the parental rice varieties (heterozygous condition). Silver stained gels displaying allelic polymorphism among selected F_6 plants for SSR markers RM 332 and RM 247 have been shown in Fig 1a, b. Number of of F_6 plants with parental alleles in heterozygous condition varied from 1 (RM 170, RM 21, RM 232, RM 218, RM 332, RM 316, RM 24, and RM 247) to maximum of 6 (RM 169 and RM 180). 27 of 28 selected F_6 plants amplified HBC19 specific alleles at RM 207 locus, while 14 F_6 plants showed Azucena specific alleles with RM 18. In some cases, new (rare) alleles were also observed in combination with a parental allele or in the homozygous state. 10 (RM 304, RM 171, RM 241, RM 335, RM 180, RM 22, RM 332, RM 247, RM 204 and RM 310) of 30 SSR markers amplified rare (new) alleles, which were different to those present in two parental rice varieties. Number of F_6 plants with rare allele(s) varied from 1 (RM 304) to 10 (RM 22 and RM 332). At 5 SSR loci (RM 304, RM 241, RM 180, RM 247 and RM 204) rare alleles were present alone, while for rest of SSR loci rare allele was present alone as well as an allele from either of the parents.

The frequency distribution of Azucena and HBC19 specific alleles in 28 selected plants is shown in the Figure 2). Plant number 5 showed maximum number of Azucena specific alleles with Azucena alleles present at 9/30 loci in homozygous condition while the maximum number of Azucena alleles (sum of homozygous and heterozygous state) were observed at as many as 11 of 30 loci in plant number 5 and 6. While plant no. 15 and 25 had as many as 26 HBC19 specific alleles (sum of homozygous and heterozygous state), the plant no. 8 and 25 had maximum no. (25 alleles) HBC19 specific alleles in homozygous condition. All 28 F_6 plants had higher number. (>15 alleles) of HBC19 specific alleles.

SSR allelic database for 28 Azucena x HBC19 F_6 plants and the two parental rice varieties was used for generating similarity matrices data (Table 3) and UPGMA tree cluster/PCA analysis. The similarity coefficient ranged from 0.39 to 0.86 and dendrogram resolved 28 F_6 plants and their parents into two groups (Fig. 3). Group 1 was further divided into two subgroups. Subgroups- II had plant numbers 14 and 23.

Subgroup– I had HBC19 and rest of F_6 plants. Group 2 had a lone parent plant Azucena which merged with group 1 at similarity coefficient of 0.37. The groups identified by PCA were very similar to those linked by cluster analysis (Fig.4).

Discussion

Molecular marker technologies have revolutionized the genetic analysis of crop plants and its application has been suggested for the molecular dissection of complex physiological traits such as drought tolerance (Steele *et al.*, 2013; Sehgal et al., 2012). Using DNA markers, comprehensive molecular marker/ linkage maps have been developed in variety of crops. However, a mapping population such as recombinant inbred lines (RILs), double haploid lines (DHLs) and backcross/ F_2/ F_3 families is a prerequisite for the development of most of the maps. The main objective of the present study was to develop the mapping population, F_6 lines and RILs, to increase the efficiency of QTLs mapping for drought tolerance. F_6 lines were derived from the cross between Azucena (drought tolerant *japonica* rice variety with good root growth) and HBC19 (drought sensitive *indica* rice variety with poor root growth). Drought tolerant and drought sensitive plants were selected on the basis of agronomic characteristics (plant height, number of productive tillers per plant, 1000 grain weight and single plant yield), shoot and root related traits (root length, root weight, shoot weight and root: shoot weight ratio), relative water content, harvest index and visual symptoms like leaf drying and recovery from drought. Ingran *et al*., (1990) reported that among the selection indices used to screen rice, visual scoring of stressed plants was the best method of scoring for drought resistance. DeDatta *et al*., (1988) used visual scoring method to evaluate rice germplasm during the vegetative stage. Malabuyoc *et al*., (1985) reported that drought recovery ability is more important than drought tolerance. Various parameters used to assess the drought tolerance clearly showed tremendous variation for drought tolerance in Azucena x HBC19 F_6 population.

This was evident from the variation in the overall mean score of individual F_6 line (1-8.3) calculated on the basis of score given to each parameter. Yogameenakshi *et al.,* (2003) evaluated rice varieties for drought tolerance on the basis of yield and drought tolerant traits viz., days to 50 per cent flowering, plant height, number of productive tillers per plant, panicle length, 100 grain weight, proline content, relative water content, root length, dry root weight, root: shoot weight ratio, harvest index and single plant yield. Kanbar *et al.,* (2004) also evaluated transgresant backcrosses of rice for drought resistance on the basis of root morphological traits. Most of F_6 plants were moderately drought tolerant, followed by 12 plants in tolerant category. Two plants were as tolerant as parental drought tolerant rice variety Azucena. These studies indicate that it should be feasible to improve the drought tolerance by developing new elite combinations of genes/QTLs from different sources by marker-assisted selection in plant breeding programs.

SSR markers have been preferably employed for DNA fingerprinting and varietal identification (Olufowote *et al.,* 1997; Bligh *et al.,* 1999), linkage mapping and marker-assisted selection (Guvvala *et al.,* 2013; Joseph *et al.,* 2004;), assessment of genetic diversity and phylogenetic relationships (Jain *et al.,* 2004), detection of cases of adulteration (Bligh, 2000) in *Oryza* species. In this study, a total of 30 polymorphic SSR markers were tested on 28 selected F_6 plants comprising of 14 drought tolerant and 14 drought sensitive plants. The two parental rice varieties, Azucena and HBC19 had a similarity coefficient of 0.21, which indicates that two parents are considerably genetically divergent. Evaluation of population of Azucena x HBC19 F_6 plants derived through single seed descent method, showed considerable variation for drought tolerance. Selected 28 F_6 plants had alleles from either or both the parental rice varieties, Azucena and HBC19. Most of the selected F_6 plants (24 plants) had both the parental alleles at one or more (up to 5) of the 30 SSR loci. Frequency of HBC19 specific alleles was higher in comparison to Azucena in selected drought tolerant and drought sensitive Azucena x HBC19 F_6 plants, which may be indicative of segregation distortion. However, it is difficult to be conclusive since only limited number of markers/F_6 plants were analyzed for SSR diversity. Segregation distortion has been frequently reported in wide crosses of rice (Maekawa and Kita, 1985). A number of genetic markers have been found to show segregation distortion in wide crosses. Many instances of segregation distortion have been reported through studies of isozymes (Wu *et al*., 1988; Guiderdoni *et al*., 1989) and RFLP alleles (McCouch *et al*., 1988; Saito *et al*., 1991). The genetic basis of the segregation distortion may be the abortion of male or female gametes or selective fertilization of particular gametic genotypes. Lin *et al*., (1992) studied segregation distortion via male gametes in hybrids between *indica* and *japonica* or wide-compatibility varieties of rice (*Oryza sativa* L.). Notably several new/rare alleles also appeared in selected F_6 plants, which were entirely different from those present in parental rice genotypes. The origin of these rare alleles may be another interesting area to

work on. Occurrence of such new or rare (recombinant) alleles may have resulted from crossing over. Some of the microsatellite loci are hot spots because here mutations occur up to 100 times more frequently than the normal mutation rate, a hotspot is a center of high activity within a larger area of low activity, a hot spot can be a position on the DNA where mutations occur with an unusual high frequency or a position on the DNA where recombination occur with an unusual high frequency. Brar *et al*., (1996) also detected some non-parental bands for some of the RFLP markers during their studies on the molecular characterization of introgression of genes for brown plant hopper and bacterial blight resistance, which have been transferred from wild *Oryza* species to cultivated rice.

However, both morpho-physiological traits and SSR markers provided independent, yet different estimates of genetic variation among F_6 rice plants. However, both markers were proficient at distinguishing the genotypes. It was evident from the present study that the genetic relationships estimated from SSR-based markers enhanced the resolution of diversity and thus provided an improved representation of variability. Analysis of genetic diversity suggested differentiation that is more ecotypic. Appropriate parents with regard to drought – resistance components (e.g. root traits, RWC) may be selected using such estimates of diversity at morpho-physiological and DNA levels so as to develop a population for mapping QTLs of interest. Research can be pursued to look for marker association with important genes/traits/QTLs using appropriate population.

Acknowledgement

This research is a part of Ph.D thesis submitted to CCS Haryana Agricultural University, Hisar. All co-authors listed have made substantial, direct, and intellectual contribution to the work. The authors are grateful to Dr. Seema Bhutani, Dr. Poonam Rana and Dr. Vikram Singh for their contributions during research project.

Table 1. A brief description of microsatellite markers used for SSR analysis

Marker	Clone no.	Map position	Repeat type & length	Forward primer	Reverse primer	Size range (bp)
RM 1	GA12	8	$(GA)_{18}$	GGAAAGAATGATCTTTTCATCC	CTACCATCAAAACCAATGTTC	77-113
RM 17	GA56	12	$(GA)_{21}$	TGCCCTGTTATTTTCTTCTCTC	AACACAGCAGGTACGCGC	168-184
RM 18	GA97	7	$(GA)_4AA(GA)(AG)_{16}$	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGCTGTAC	151-163
RM 21	GA275	11	$(GA)_{21}$	ACAGTATTCGGTAGGCAGG	GCTCCATGAGGGTGGTAGAG	133-164
RM 22	GA580	3	$(GA)_{22}$	GGTTTGGGAGCCCATAATCT	CTGGGCTTCTTTCACTCGTC	187-197
RM 24	GA5	1	$(GA)_{29}$	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG	152-198
RM 31	GA257	5	$(GA)_{15}$	GATCACGATCCACTGGAGCT	AAGTCCATTACTCTCCTCCC	141-153
RM 38	GA334	8	$(GA)_{16}$	ACGAGCTCTCGATCAGCCTA	TCGGTCTCCATGTCCCAC	250-260
RM 42	GA376	8	$(GA)_{26}$	ATCCTACCGCTGACCATGAG	TTTGGTCTACGTGGCGTACA	159-165
RM48	GA479	2	$(GA)_{17}$	TGTCCCACTGCTTTCAAGC	CGAGAATGAGGGACAAATAAC	199-219
RM169	OSM69	5	$(GA)_{12}$	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGGCCGTTCATCCCTCC	164-194
RM170	D15716	6	$(CCT)_7$	TCGCGCTTCTTCCTCGTCGACG	CCCGCTTGCAGAGGAAGCAGCC	106-119
RM171	OSM71	10	$(GATG)_5$	AACGCGAGGACACGTACTTC	ACGAGATACGTACGCCTTTG	318-343
RM174	D48756	2	$(AGG)_7 (GA)_{10}$	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG	207-222
RM180	M2	7	$(ATT)_{10}$	CTACATCGGCTTAGGTGTAGCAACACG	ACTTGCTCTACTTGTGGTGAGGGACTG	107-204

Continuing table 1

Marker	Clone no.	Map position	Repeat type & length	Forward primer	Reverse primer	Size range (bp)
RM204	CT19	6	$(CT)_{25}$	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	146-174
RM207	CT41	2	$(GA)_{25}$	CCATTCGTGAGAAGATCTGA	CACCTCATCCTCGTAACGCC	123-142
RM218	CT125	3	$(TC)_{24}ACT(GT)_{11}$	TGGTCAAACCAAGGTCCTTC	GACATACATTCTACCCCCGG	125-153
RM232	CT339	3	$(GA)_{24}$	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG	143-166
RM235	CT368	12	$(GA)_{24}$	AGAAGCTAGGGCTAACGAAC	TCACCTGGTGGAAAATGAG	90-132
RM241	CT404	4	$(GA)_{31}$	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTC	104-149
RM247	CT462	12	$(GA)_{16}$	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	127-178
RM252	CT206	4	$(GA)_{19}$	TTCGCTGATCCCGAGAACG	ATGACTTGATCCCGAGAACG	184-267
RM257	CT522	9	$(GA)_{24}$	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	104-192
RM259	CT550	1	$(GA)_{17}$	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGCGCCCATGT	152-174
RM304	GT25	10	$(GT)_2(AT)_{10}(GT)_{33}$	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG	138-175
RM310	GT137	8	$(GT)_{19}$	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC	85-120
RM316	GT264	9	$(GT)_8(TG)_9(TTTG)_4(TG)_4$	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC	150-290
RM332	CTT38	11	$(CTT)5\text{-}12\text{-}(CTT)_{14}$	GCGAGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC	162-183
RM335	CTT50	4	$(CTT)_{25}$	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	104-155

Table 2. Overall mean score of F_6 rice plants for drought tolerance

	Drought Tolerant Index (DTI)																	
Genotypes	**PHw**	**TN**	**TGW**	**GY**	**SFW**	**SDW**	**RFW**	**RDW**	**RSFW**	**RSDW**	**MRL**	**HI**	**RWC**	**Mean DTI**	**Score on the basis of mean DTI**	**LD Score**	**RWSP Score**	**Mean Score**
Azucena	79.3	82.4	79.9	89.6	93	77	95	83.2	97.7	108.2	106	112	75.1	90.6	1	3	1	1.6
HBC19	82.6	41.2	67.1	50.2	36	56	50.4	62.3	154	106.5	71.2	87	62.1	71.3	5	9	9	7.6
1*	93.8	66.7	78	59.8	91	80	34.1	45.2	41.7	53.4	94.5	98.5	70.4	69.7	7	7	5	6.3
2	76.2	54.5	88.7	54.2	65	37	62.6	41.4	99	125.2	88.8	128	72.6	76.3	3	7	3	4.3
3	79.5	75	69.9	70.9	90	76	64.8	91.7	68.9	118.7	86.4	88.7	59.2	75.5	3	7	3	4.3
4*	73	66.7	89.1	49.8	74	70	93.9	81.9	132	117.8	97.2	67.6	53.8	70.7	5	7	5	5.6
5	88.2	63.6	93.4	68.1	92	61	76.4	54	81.3	72.7	111	111	78.2	80.8	1	5	5	3.6
6	75.9	61.5	77	72.6	84	76	43.8	86.3	65.9	110.5	123	95.5	48.3	71.9	5	5	3	4.3
7	83.2	66.7	77.3	61.1	70	47	91.2	85.5	114	182.6	70.9	118	60.5	71.7	5	5	5	5
8*	64.5	68.8	75.9	56.6	84	73	67.4	63.7	75.4	76.5	125	76.3	83.6	76.1	3	3	3	3
9	46.1	57.1	78	50.2	44	55	86.4	85	196	166.2	70.7	86	79.1	42.1	9	1	1	3.6
10	68.2	62.5	81.2	25.7	58	62	82.7	65.1	112	117.8	79.6	42.6	35.8	72.7	5	5	5	5
11*	76.1	50	75.7	39.8	77	51	46.9	64.6	69.6	126.9	61.3	77.3	71.8	68.3	7	5	5	5.6
12	77.9	57.1	82.6	73.5	86	70	91.6	74.3	94.2	131.5	50.6	98.8	61.1	73.9	5	3	3	3.6

Continuing table 2

Genotypes	Drought Tolerant Index (DTI)																	
	PHw	TN	TGW	GY	SFW	SDW	RFW	RDW	RSFW	RSDW	MRL	HI	RWC	Mean DTI	Score on the basis of mean DTI	LD Score	RWSP Score	Mean Score
13*	86.9	71.4	87.9	70.9	94	70	30.3	72	54	93.4	94.7	90	63.7	73.7	5	3	1	3
14*	70	66.7	77.4	50.9	64	44	88.3	79.2	98.4	236.3	75.8	103	62.9	85.9	1	3	1	1.6
15*	65.2	37.5	85.6	6.1	40	31	91.4	54	169	168.3	48	16.4	61.9	61.2	9	7	3	6.3
16	81.7	50	77.6	71.8	94	64	21.5	32.9	35	53.1	99.4	131	63.2	74.7	5	5	3	4.3
17	73.7	71.4	64.6	76.6	76	81	96.4	67.9	131	83.2	104	104	68.8	84.4	1	7	5	4.3
18	90.7	60	88.1	65	45	52	51.8	39.4	134	87.1	86.5	130	83.1	86.7	1	7	5	4.3
19	93.6	76.5	84.5	70.9	83	81	90.3	82.9	97.9	96.2	138	87	81.3	75.7	3	3	3	3
20	73	63.6	54	68.4	79	64	86	66.4	89	132.1	122	101	67.9	81.9	1	7	3	3.6
21*	86.7	72.7	70.7	79.3	84	80	97.3	80	108	111.4	117	111	90.1	82.7	1	3	3	2.3
22*	74	69.2	87.9	81.5	91	52	84	87.5	97.1	133.9	119	117	76.9	76.7	3	7	5	5
23	53.8	58.8	87.1	42.4	57	73	94.7	90.2	171	125.5	87.4	52.6	77.6	82.3	1	7	5	4.3
24	64.1	55.6	65.5	48.4	45	61	76.2	79.6	153	132.4	140	76.3	76.7	60.1	9	3	1	4.3
25	83.9	66.7	71.4	55.1	43	53	35.8	43.9	134	94.6	116	109	49.4	77.7	3	5	3	3.6
26*	73.1	71.4	80	58.7	42	43	96.8	44	114	95.4	94.4	129	58.3	76.9	3	7	5	5

Continuing table 2

Drought Tolerant Index (DTI)																		
Genotypes	**PHw**	**TN**	**TGW**	**GY**	**SFW**	**SDW**	**RFW**	**RDW**	**RSFW**	**RSDW**	**MRL**	**HI**	**RWC**	**Mean DTI**	**Score on the basis of mean DTI**	**LD Score**	**RWSP Score**	**Mean Score**
27	93.6	50	78.7	50.5	73	63	76.7	82.2	104	123.4	102	73	85.4	89.6	1	7	5	4.3
28*	68.7	63.6	75	36.8	41	37	43.3	53.9	129	152.8	61.6	111	53.3	78.7	3	7	5	5
29	88.4	44.4	76.4	47.7	88	73	67.7	72.8	96.7	99.6	113	72.1	63	77	3	5	3	3.6
30*	63.9	57.1	78.2	79.3	76	67	77.8	79	127	122.7	139	113	72.5	59.9	9	7	5	7
31*	56.1	60	83.4	57.1	45	63	92.5	81.6	210	155.7	102	96	47.9	79.7	3	7	5	5
32*	73.2	70	85.6	46.3	80	71	48.6	44.3	41.1	53.4	106	73.5	73.5	66.6	7	7	1	5
33*	89.1	44.4	68.4	76.6	87	56	45.5	22.9	63.9	46.1	68	167	74.7	85.1	1	5	3	3
34*	91.5	44.4	72.4	55.9	59	61	91.9	60.8	128	98.1	90.5	100	80.7	80.7	1	5	5	3.6
35*	79.2	33.3	77.6	43.4	59	59	20.2	53.7	42.5	105.1	95.4	74.3	66.9	62.3	9	3	3	5
36*	68.2	30.4	77.3	23	28	26	13.5	17.1	77.8	67.8	76	102	72.9	64.2	9	5	3	5.6
37	52.2	22.2	78.3	31.6	27	25	11.7	8.6	46.3	34.5	55.7	163	66.2	81.7	1	7	3	3.6
38	92.6	40	87.1	68.6	76	50	56.6	48.9	90.9	109.3	103	141	62.5	78.8	3	5	3	3.6
39*	81.7	54.5	68.1	54.7	81	78	98.7	86.1	111	91.1	75.2	68.3	71.4	77.7	3	7	5	5
40*	88.9	50	87.7	72.3	88	57	83	47.7	79.9	104.5	88.6	143	67.2	82.7	1	3	3	2.3

Continuing table 2

	Drought Tolerant Index (DTI)																	
Genotypes	**PHw**	**TN**	**TGW**	**GY**	**SFW**	**SDW**	**RFW**	**RDW**	**RSFW**	**RSDW**	**MRL**	**HI**	**RWC**	**Mean DTI**	**Score on the basis of mean DTI**	**LD Score**	**RWSP Score**	**Mean Score**
41*	83.9	42.9	81.8	61.2	38	30	19.5	33.7	63.5	139.8	44	197	75.7	70	7	1	1	3
42*	59.1	66.7	69.4	51.7	38	39	10	49	58.6	112	30.2	127	46.7	55.1	9	1	1	3.6
43*	52.2	60	81.5	66.5	35	25	22.6	54	85.5	219.3	60.6	244	64.4	83.7	1	3	3	2.3
44	61.7	66.7	74.3	51.9	42	49	25.6	53.4	67.1	110.8	77.2	98.6	72.4	65.3	7	3	3	4.3
45	61.7	42.9	73.2	48	61	55	84.4	84.3	115	173.8	94.2	81.1	75.1	57.7	9	3	3	5
46*	67.9	42.9	70.7	77.9	48	56	95.1	82.5	170	138.8	48.5	127	65	84.7	1	1	1	1
47*	66.4	57.1	64.6	61.3	68	44	82.4	100	128	246.8	60.5	124	69.9	90.2	1	3	1	1.6
48*	55.5	42.9	64.6	59.7	37	37	105	69.7	197	195.9	91	136	82.6	51.5	9	9	7	8.3
49*	82.5	76.9	80.4	87.3	79	71	115	80.1	114	95.4	151	116	59	85.7	1	5	5	3.6
50*	78.9	66.7	90.9	77.3	83	63	98.9	80.5	107	127.3	84.4	115	84.5	88.9	1	5	5	3.6

* Plants selected for SSR analysis

Plant height (PH) in cm, ttiller number (TN), grain yield (GY) in g/plant, thousand grain weight (TGW) in g, maximum root length (MRL) in cm, shoot & root fresh weight (SFW, RFW) in g, shoot & root dry weight (SDW, RDW) in g, root:shoot ratio (RSR), harvest index (HI), relative water content (RWC), leaf drying(LD) and recovery of water stressed plants (RWSP).

Figure 1a. A silver stained gel showing allelic polymorphism among selected F_6 plants of cross Azucena × HBC19 and parental lines at RM 332 locus. L represent the 10 base pair ladder, lanes 1-30 represents drought tolerant F_6 plants (1-14), drought sensitive F_6 plants (15-28), Azucena (29) and HBC19 (30).

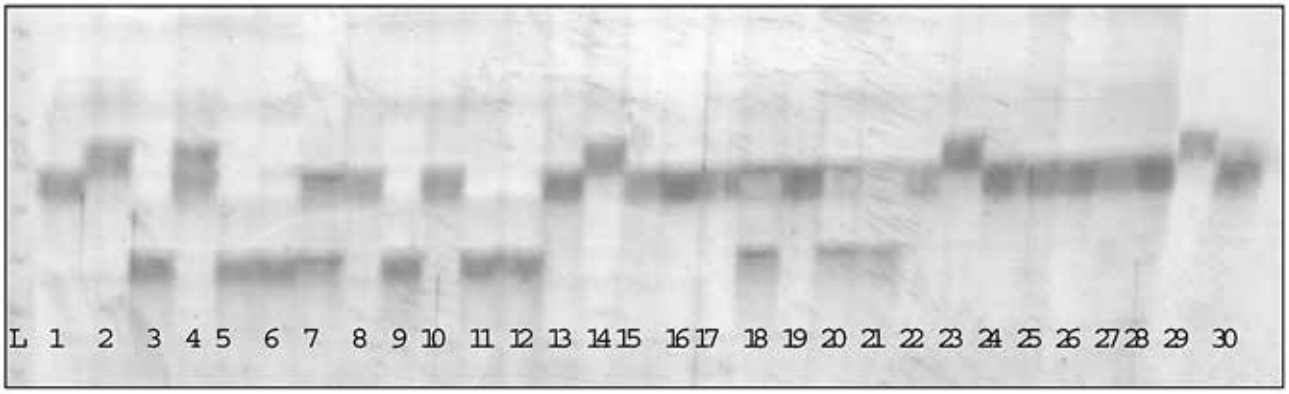

Figure 1b. A silver stained gel showing allelic polymorphism among selected F_6 plants of cross Azucena × HBC19 and parental lines at RM 247 locus. L represent the 10 base pair ladder, lanes 1-30 represents drought tolerant F_6 plants (1-14), drought sensitive F_6 plants (15-28), Azucena (29) and HBC19 (30).

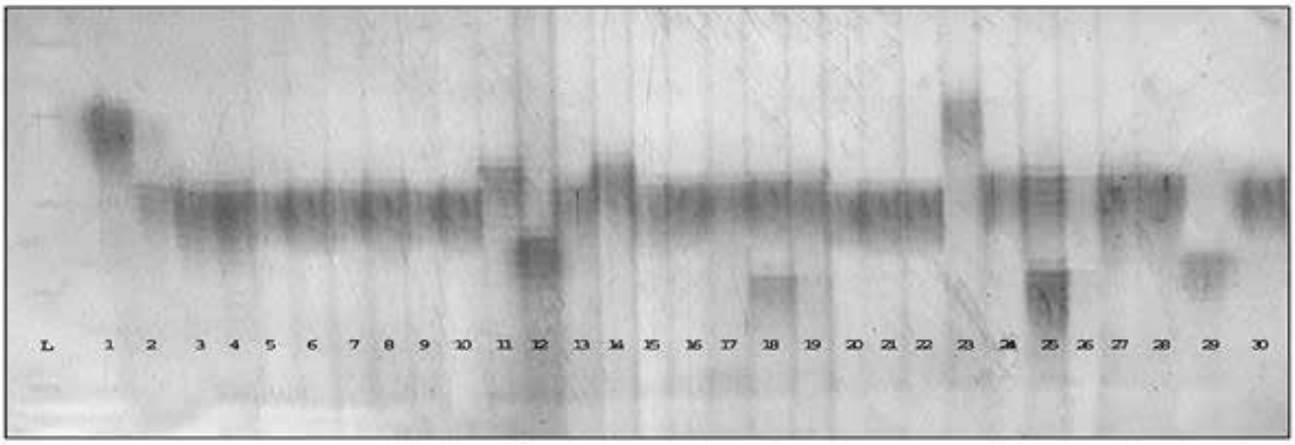

Figure 2. Distribution of Azucena and HBC19 specific alleles in 28 selected F_6 plants of Azucena x HBC19

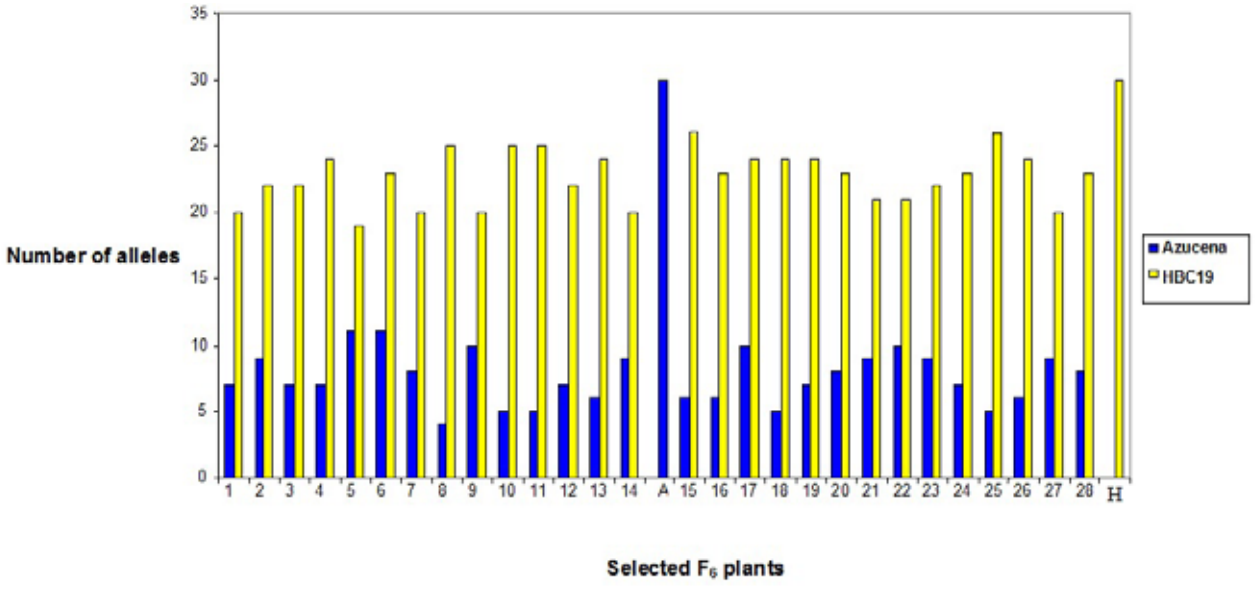

Figure 3. Dendrogram of selected 28 Azucena x HBC19 F_6 plants based on SSR diversity at 30 loci.

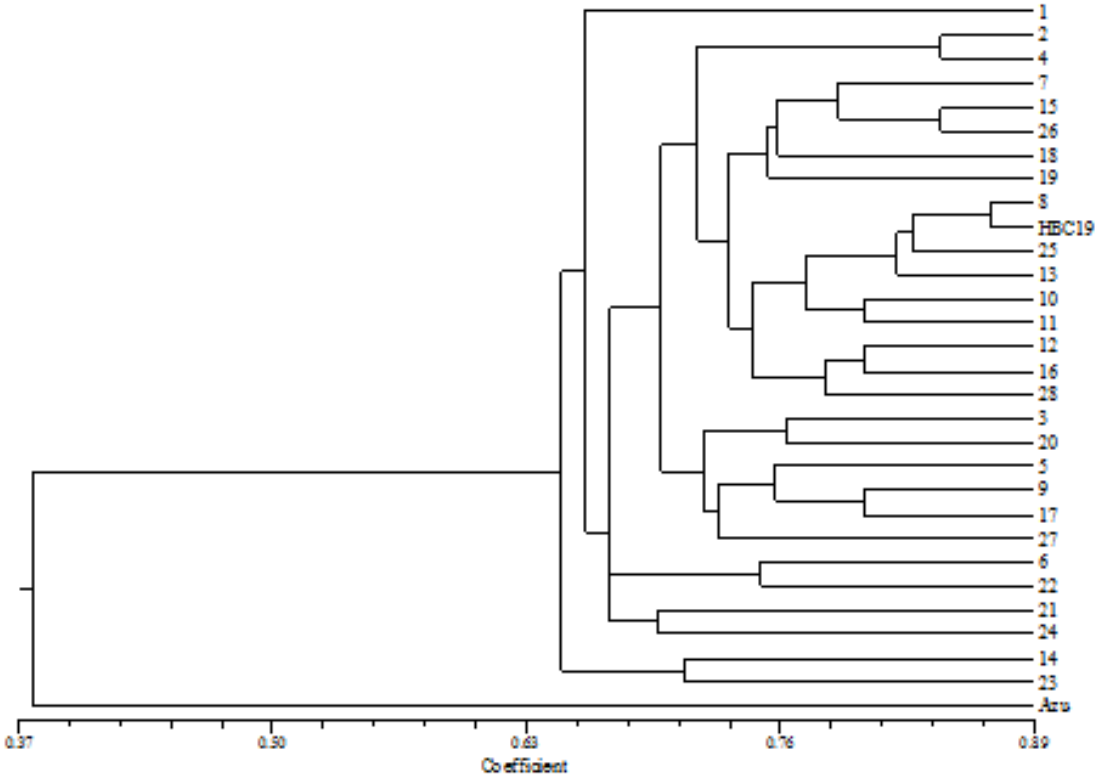

Figure 4. Three dimensional PCA scaling of selected Azucena x HBC19 F_6 plants using SSR diversity data at 30 loci.

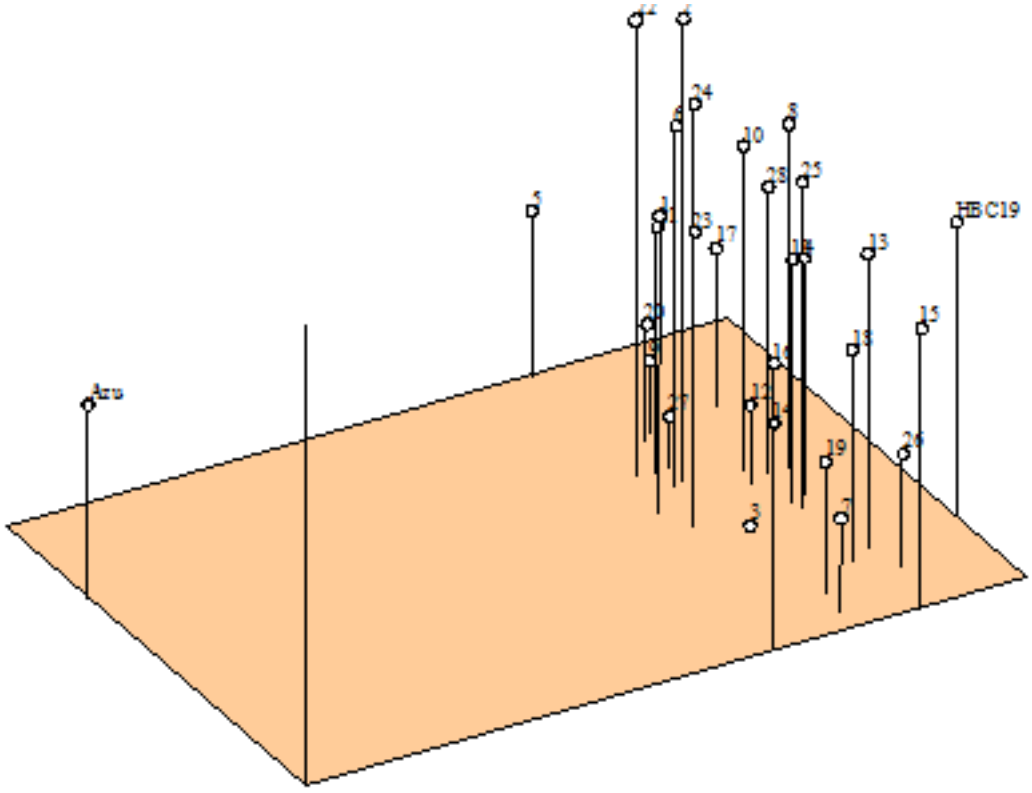

Table 3. Similarity matrix data of 28 Azucena x HBC19 F_6 plants and parental rice varieties using the allelic database at 30 SSR loci

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Azu	HBC19	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1.00																													
2	0.68	1.00																												
3	0.67	0.62	1.00																											
4	0.71	0.84	0.75	1.00																										
5	0.59	0.67	0.68	0.67	1.00																									
6	0.61	0.71	0.67	0.66	0.70	1.00																								
7	0.59	0.57	0.74	0.67	0.61	0.70	1.00																							
8	0.72	0.70	0.63	0.70	0.76	0.70	0.66	1.00																						
9	0.61	0.66	0.72	0.68	0.72	0.66	0.64	0.72	1.00																					
10	0.70	0.67	0.61	0.72	0.71	0.70	0.66	0.84	0.67	1.00																				
11	0.76	0.71	0.67	0.74	0.67	0.71	0.72	0.78	0.74	0.80	1.00																			
12	0.64	0.64	0.74	0.75	0.71	0.64	0.74	0.68	0.72	0.71	0.75	1.00																		
13	0.72	0.72	0.71	0.78	0.63	0.78	0.79	0.82	0.67	0.76	0.75	0.71	1.00																	
14	0.50	0.61	0.62	0.63	0.57	0.61	0.67	0.67	0.61	0.62	0.66	0.62	0.67	1.00																
15	0.64	0.75	0.74	0.80	0.58	0.72	0.76	0.74	0.62	0.74	0.72	0.68	0.82	0.75	1.00															
Azu	0.38	0.43	0.39	0.38	0.47	0.43	0.42	0.32	0.41	0.34	0.30	0.37	0.37	0.41	0.34	1.00														
HBC19	0.72	0.78	0.76	0.83	0.68	0.75	0.74	0.87	0.75	0.82	0.80	0.76	0.84	0.72	0.87	0.21	1.00													
16	0.68	0.61	0.72	0.74	0.72	0.55	0.75	0.83	0.74	0.75	0.71	0.80	0.75	0.61	0.67	0.36	0.80	1.00												
17	0.67	0.67	0.76	0.75	0.79	0.72	0.66	0.76	0.80	0.74	0.70	0.79	0.74	0.57	0.68	0.39	0.82	0.80	1.00											
18	0.66	0.63	0.70	0.68	0.64	0.63	0.78	0.78	0.71	0.72	0.79	0.75	0.67	0.68	0.75	0.30	0.83	0.76	0.70	1.00										
19	0.59	0.62	0.71	0.72	0.61	0.59	0.71	0.68	0.67	0.63	0.64	0.66	0.71	0.67	0.76	0.37	0.82	0.72	0.71	0.75	1.00									
20	0.67	0.67	0.76	0.75	0.74	0.67	0.66	0.68	0.72	0.71	0.72	0.68	0.71	0.62	0.68	0.42	0.74	0.70	0.71	0.64	0.68	1.00								
21	0.58	0.68	0.64	0.68	0.70	0.66	0.67	0.70	0.71	0.70	0.68	0.67	0.59	0.63	0.67	0.41	0.75	0.68	0.72	0.71	0.67	0.72	1.00							
22	0.59	0.72	0.61	0.67	0.66	0.75	0.63	0.71	0.59	0.71	0.62	0.63	0.68	0.54	0.71	0.45	0.74	0.67	0.68	0.70	0.66	0.71	0.70	1.00						
23	0.55	0.71	0.59	0.68	0.64	0.66	0.64	0.67	0.71	0.62	0.61	0.78	0.70	0.71	0.62	0.41	0.75	0.71	0.72	0.68	0.67	0.62	0.63	0.70	1.00					
24	0.62	0.70	0.58	0.64	0.63	0.62	0.66	0.74	0.62	0.66	0.64	0.63	0.68	0.59	0.63	0.39	0.76	0.72	0.74	0.67	0.68	0.55	0.70	0.71	0.67	1.00				
25	0.71	0.74	0.70	0.79	0.64	0.68	0.64	0.80	0.74	0.72	0.71	0.67	0.80	0.68	0.72	0.36	0.86	0.76	0.78	0.76	0.67	0.64	0.71	0.62	0.71	0.72	1.00			
26	0.67	0.70	0.79	0.80	0.66	0.67	0.82	0.74	0.70	0.74	0.72	0.79	0.82	0.64	0.84	0.34	0.87	0.78	0.79	0.75	0.79	0.66	0.67	0.61	0.67	0.68	0.72	1.00		
27	0.63	0.63	0.67	0.76	0.70	0.66	0.64	0.72	0.76	0.72	0.68	0.70	0.70	0.61	0.70	0.41	0.75	0.74	0.72	0.63	0.70	0.75	0.63	0.57	0.61	0.54	0.66	0.72	1.00	
28	0.70	0.72	0.68	0.80	0.68	0.70	0.66	0.76	0.67	0.76	0.72	0.79	0.71	0.59	0.76	0.34	0.82	0.78	0.76	0.75	0.66	0.66	0.59	0.74	0.70	0.68	0.75	0.74	0.75	1.00

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