Indian J. Genet., 80(2) (2020)
DOI:

Genetic variability of popping quality traits and microsatellite-based characterization of popcorn inbreds for utilization in breeding programme

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(Received: December 2019; Revised: March 2020; Accepted: April 2020)

Abstract

Popcorn is a popular snack item worldwide. The genetic base of popcorn germplasm is quite narrow, which resulted in limited number of popcorn hybrids. Here, a set of sub-tropically adapted 39 popcorn inbreds of exotic- and indigenous-origin, were characterized using morphological traits and molecular markers for their effective utilization in hybrid breeding. Significant variation for grain popping percentage (GPP: 86.0-98.0%), percent popping expansion (PPE: 1493-3213%) and volume after popping (VAP: 210-315ml) was observed. The mean PPE (2069%) and GPP (93.6%) among popcorn inbreds were higher than the popcorn check variety, VL popcorn (PPE: 1739%, GPP: 92%). Positive correlations were observed among GPP, PPE and VAP. Molecular characterization using 72 SSRs produced 237 alleles with a mean of 3.3 alleles per locus. Polymorphism information content ranged from 0.05 to 0.68, while Jaccard's dissimilarity coefficients varied from 0.27-0.82. Principal coordinate analysis also depicted diverse genetic nature of inbreds. Cluster analysis grouped the inbreds into three major clusters each having 16, 18 and 5 inbreds. Contrasting inbreds were identified for development of mapping populations to identify loci governing desirable popping quality. The study also identified potential heterotic combinations. The information generated here assumes great significance in breeding for popcorn hybrids.

Key words Diversity, SSRs, popcorn, popping, genetic distance

Introduction

Popcorn (*Zea mays* var. *everta*) has emerged as one of the most popular snacks worldwide (Ren et al. 2018; Senhorinho et al. 2019). It is an important source of income to the farming community. Popcorn grains are much harder than the traditional dent- and flint- corn, as it contains higher proportion of hard endosperm compared to soft portion (Jain-Poster et al. 2015). Upon heating at 180-190^{o}C, water molecules inside the kernel get vaporized and cause the pressure to explode through pericarp and form large flakes (Hoseney et al. 1983). Higher number of popped grains and expanded popping volume are the major factors that determine the acceptability by the consumers (Zunjare et al. 2015).

Despite high commercial value and popularity, number of diverse popcorn hybrids is limited compared to dent- and flint- corn types. The germplasm base of popcorn is quite narrow compared to dent- and flint-types (Hallauer et al. 2010). In India as well, very few number of popcorn cultivars have been developed (Yadav et al. 2015). The recent initiatives under the maize breeding programme in India have led to the development of new sub-tropically adapted diverse popcorn inbreds from exotic- and indigenous- sources. Characterization of these newly available inbreds and understanding their genetic relationships therefore hold immense significance for their utilization in the breeding programme (Devi et al. 2017, Vittorazzi et al. 2018). Molecular markers have often been utilized for accurate measurement of genetic relationship due to their abundance, whole genome coverage and environment neutral behavior. Several research groups across countries especially from Brazil, Argentina and Chile have analyzed genetic diversity of popcorn lines

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Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

using RAPD (Resh et al. 2015), ISSR (Kantety et al. 1995) and SSR/microsatellite (Carvalho et al. 2013) markers. Among various marker systems, SSRs are the preferred choice due to their co-dominance behavior, high reproducibility and cost effective simple assay (Chin et al. 1996). So far, studies on popcorn germplasm from India are very limited, therefore comprehensive characterization of the newly developed popcorn inbreds holds immense significance in the breeding programme. The present investigation was therefore aimed to (i) assess the genetic relationships among sub-tropically adapted popcorn inbreds using microsatellite markers, and to identify (ii) diverse and contrasting parents for the development of mapping populations to identify loci governing popping quality traits, and (iii) potential heterotic combinations to develop promising popcorn hybrids.

Materials and methods

Plant materials

A set of 39 popcorn inbreds consisted of exotic- and indigenous- origin and three checks [VL Popcorn (composite: popcorn), HKI163 (inbred: semi dent) and UMI1230 (inbred: flint)] were analyzed for popping quality traits (Table S1). The genotypes were planted in a randomized complete block design (RCBD) with two replications per entry and one row (3 m) per replication (2.25 m^2 plot area) during *Kharif* 2017 at IARI, experimental farm, New Delhi. Standard agronomic practices were used for raising and maintenance of the plants. Three random plants from each row were selfed for popping quality analyses.

Popping quality

The popping quality attributes of popcorn genotypes were analyzed using the methods described by Babu et al. (2006) and Zunjare et al. (2015). The ears were gradually dried until the kernels reached the optimum moisture (10-12%) for popping. A sample of 100-selfed kernels from each inbred were individually popped in a microwave with a temperature of 180^oC for 3 mins (Model: LG Grill Intellowave, MH6558F, India). The original volume of raw kernels and volume after popping (VAP) the kernels was measured using a 250 ml measuring cylinder. The percent popping expansion (PPE) was calculated as [{(volume after popping-volume before popping)/volume before popping} × 100], while the grain popping percentage (GPP) was calculated as the number of popped kernels out of 100 kernels. VL Popcorn, a released composite with desirable popping quality, and UMI1230 and HKI163, parental inbreds of released hybrids with normal grain type were used as check for quality analyses.

DNA isolation and PCR assay

Genomic DNA from 39 popcorn inbreds was isolated from leaves of three-week old seedlings using modified Cetyl trimethyl ammonium bromide (CTAB) procedure (Saghai-Maroof et al. 1984). A total 72 SSRs spanning all 10 chromosomes of maize were used for molecular characterization. SSR genotyping was carried using the standard PCR cycle conditions (Devi et al. 2017). Amplified products were resolved on 4% agarose gel (Lonza, Rockland, ME USA) and were visualised using gel documentation system (AlphaImager, USA).

Statistical analyses

The phenotypic data sets were analyzed for Analysis of Variance (ANOVA) using WINDOSTAT 8.5. Pearson's simple correlations between GPP, PPE and VAP were computed using MS Office-Excel (2007). Gene diversity, major allele frequency, unique and rare alleles, and polymorphism information content (PIC) values were computed using Power Marker 3.25 (Liu and Muse 2005). Genetic dissimilarity was calculated using Jaccard's coefficient. Cluster analysis following unweighted neighbour joining method and principal coordinate analysis (PCoA) was undertaken using DARwin6.0 (Perrier et al. 2003).

Results

Genetic variation for popping quality

ANOVA revealed significant genetic variation for GPP, PPE and VAP among 42 maize genotypes (Table S2). Among popcorn inbreds excluding checks, GPP ranged from 86.0 to 98.0% (mean: 93.6%), while PPE and VAP varied from 1493 to 3213% (mean: 2069%) and 210 to 315ml (mean; 266ml), respectively. However, popping quality attributes of semi-dent type inbred, HKI163 (GPP: 17.5%, PPE: 81%, VAP: 53ml) and flint type inbred, UMI1230 (GPP: 16.5%, PPE: 124%, VAP: 75ml) were very poor. Majority of the popcorn inbreds however were better than VL Popcorn (GPP: 92.0%) and PPE: 1739%). Considering all the three traits ($\geq$90% GPP, $\geq$1900% PPE and $\geq$280ml VAP), PC105, PC112, PC120, PC133, PC130, PC119, PC121, PC113, PC109, PC122, PC116, PC102, PC132, PC125 and PC104 were identified as the most promising inbreds with desirable popping quality. PPE and GPP were positively correlated (r=0.77**), while GPP and VAP showed stronger positive association (r=0.85**). PPE and VAP were also positively correlated

(r=0.81**). Popcorn inbreds also possessed similar anthesis (Mean: 46.4 days, range: 43.0-56.5 days) and female flowering time (Mean: 48.5 days, range: 46.0-58.5 days) (Table 1).

Table 1. Flowering behavior and popping quality attributes of popcorn inbreds

S.No.	Genotype	MF (days)	FF (days)	GPP (%)	PPE (%)	VAP (ml)
1.	PC101	46.5	48.5	96.5	1686	230
2.	PC102	47.0	48.0	96.0	2142	280
3.	PC103	46.0	47.5	93.5	1995	220
4.	PC104	46.5	48.5	95.0	2467	280
5.	PC105	44.5	46.0	97.0	2007	315
6.	PC106	45.5	47.0	96.0	1867	285
7.	PC107	46.0	47.5	91.5	1692	215
8.	PC108	44.5	47.0	87.0	1650	210
9.	PC109	47.0	49.0	94.0	1936	285
10.	PC110	48.0	49.5	92.5	1744	275
11.	PC111	51.5	54.5	91.0	1850	260
12.	PC112	48.5	50.0	94.5	2150	315
13.	PC113	46.0	47.5	93.5	2668	290
14.	PC114	44.5	47.0	92.0	2527	275
15.	PC115	44.5	46.5	95.0	2433	275
16.	PC116	45.0	46.5	96.5	2385	285
17.	PC117	44.5	46.5	98.0	2383	270
18.	PC118	45.5	48.0	93.5	2120	255
19.	PC119	45.5	46.0	91.0	3213	295
20.	PC120	46.0	48.0	95.0	3000	310
21.	PC121	49.5	49.5	93.5	2468	295
22.	PC122	45.5	46.5	93.5	2381	285
23.	PC123	45.5	48.5	92.5	1954	265
24.	PC124	46.5	49.0	86.5	1532	220
25.	PC125	44.5	47.5	95.0	1900	280
26.	PC126	45.0	48.5	95.0	1858	235
27.	PC127	46.5	49.0	93.5	1729	265
28.	PC128	46.5	47.5	91.0	1817	230
29.	PC129	45.0	48.0	93.5	1570	250
30.	PC130	44.0	46.5	95.0	1904	300
31.	PC131	47.0	49.0	98.0	1995	220
32.	PC132	46.0	48.5	93.5	2467	280
33.	PC133	49.5	51.0	91.5	1980	310
34.	PC134	47.0	49.5	91.5	2099	275
35.	PC135	44.0	46.0	86.0	1493	215
36.	PC136	44.0	47.0	95.0	2023	240
37.	PC137	47.0	48.5	95.5	1829	270
38.	PC138	43.0	46.0	95.0	1924	260
39.	PC139	46.5	49.0	97.0	1869	285
40.	HKI163	53.0	55.0	17.5	81	53
41.	UMI1230	56.5	58.5	16.5	124	75
42.	VL Popcorn	44.5	47.5	92.0	1739	300
	Mean	46.4	48.5	89.9	1968	258
	CD (5%)	1.82	2.38	3.17	21.90	446.52

MF: days to 50% anthesis, FF: days to 50% silking, GPP: Grain popping percentage, PPE: Percent popping expansion, VAP: Volume after popping, CD: Critical Difference

Assessment of molecular diversity

Molecular profiling using 72 SSRs generated a total of 237 alleles among 39 popcorn inbreds (Fig. 1). The

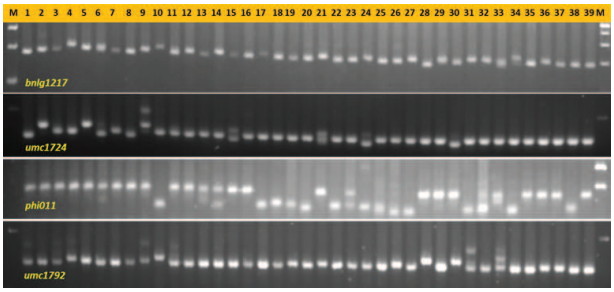

Fig. 1. Marker profile of selected SSRs among 39 popcorn inbreds. M: 100 bp ladder, 1: PC101, 2: PC102, 3: PC103, 4: PC104, 5: PC105, 6: PC106, 7: PC107, 8: PC108, 9: PC109, 10: PC110, 11: PC111, 12: PC112, 13: PC113, 14: PC114, 15: PC115, 16: PC116, 17: PC117, 18: PC118, 19: PC119, 20: PC120, 21: PC121, 22: PC122, 23: PC123, 24: PC124, 25: PC125, 26: PC126, 27: PC127, 28: PC128, 29: PC129, 30: PC130, 31: PC131, 32: PC132, 33: PC133, 34: PC134, 35: PC135, 36: PC136, 37: PC137, 38: PC138 and 39: PC139

number of alleles varied from 2 to 7 with an average of 3.3 alleles per locus (Table 2). The SSR locus *viz*., *umc1225*, *bnlg1396* and *umc1256* were the most polymorphic with $\geq$6 alleles/locus. The PIC value ranged from 0.05 (*umc2046*) to 0.68 (*phi308707 and umc2323*) with a mean of 0.41. Among the SSRs used for genetic diversity analysis, 26 loci had di-repeat motif, 30 loci had tri-repeat, eight loci had tetra-repeat motif and one locus possessed hepta-repeat motif. One marker *viz*., *phi115* was found to have both di and tetra-repeat motif (Table 2). The mean PIC (0.47) of di-repeat SSRs was higher than tri-repeat (0.38), tetra-repeat (0.34) and hepta-repeat (0.29). A total of eight unique alleles were identified, including three alleles by *umc1225*, *umc1792* and *umc1724* in PC109, five alleles by *bnlg149*, *bnlg1396*, *phi011*, *phi038* and *umc1833* in PC134, PC128, PC133, PC119 and PC103, respectively. A total of 28 rare alleles were also identified in the study. The major allele frequency ranged from 0.35 (*umc1833*) to 0.97 (*umc2046*) with an average of 0.64. Gene diversity varied from 0.05 (*umc2046*) to 0.72 (*umc1833, phi308707, umc1256* and *umc2323*) with a mean of 0.47. The mean heterozygosity was quite low (0.03) with the highest value of 0.47 (Table 2).

Table 2. Primer details and summary statistics of genotyping assay undertaken among popcorn inbreds

S.No.	Marker	Bin	SSR repeat	Annealing temp. (°C)	Major allele frequency	No. of alleles	Gene diversity	Hetero-zygosity	Polymorphism information content
1	*bnlg149*	1.00	-	55	0.51	4	0.56	0.03	0.47
2	*bnlg1014*	1.01	(AG)14	55	0.72	3	0.42	0.00	0.35
3	*umc1754*	1.06	(CGAT)5	55	0.47	3	0.61	0.16	0.53
4	*umc1833*	1.07	(TG)8	56	0.35	5	0.72	0.31	0.67
5	*phi038*	1.08	AT	57	0.45	5	0.69	0.16	0.64
6	*phi011*	1.09	AGC	55	0.62	4	0.55	0.13	0.49
7	*phi308707*	1.10	AGC	55	0.37	4	0.72	0.38	0.68
8	*mmc0221*	1.11	(CA)10	55	0.50	3	0.57	0.15	0.47
9	*umc1605*	1.12	(GGC)4	58	0.87	2	0.22	0.00	0.20
10	*umc1622*	2.00	(AAG)5	58	0.90	3	0.19	0.00	0.18
11	*bnlg1064*	2.03	(AG)16	57	0.51	5	0.63	0.32	0.58
12	*nc132*	2.05	AG	57	0.95	2	0.10	0.10	0.09
13	*bnlg1396*	2.06	(AG)5	55	0.58	6	0.58	0.03	0.51
14	*umc1256*	2.09	(CAT)5	55	0.38	6	0.72	0.00	0.67
15	*umc1780*	3.01	(ACC)4	55	0.76	2	0.36	0.00	0.30
16	*umc1892*	3.01	(GA)8	55	0.67	2	0.44	0.00	0.35
17	*phi193225*	3.02	AAC	56	0.41	3	0.64	0.38	0.56
18	*nc030*	3.04	CT	55	0.72	2	0.40	0.00	0.32
19	*umc1311*	3.06	(TCTT)4	55	0.45	3	0.61	0.28	0.53
20	*umc1273*	3.08	(AAG)4	55	0.51	4	0.56	0.13	0.46
21	*umc1010*	3.09	(GA)10	55	0.45	5	0.71	0.13	0.66
22	*phi074*	4.04	CAA	55	0.67	2	0.44	0.00	0.35
23	*bnlg1217*	4.05	(AG)33	55	0.54	3	0.56	0.00	0.47
24	*umc1329*	4.06	(GCC)7	55	0.51	3	0.58	0.20	0.49
25	*bnlg1784*	4.07	(AG)13	55	0.63	2	0.46	0.00	0.36
26	*phi092*	4.08	GCAA	55	0.92	3	0.14	0.05	0.14
27	*umc2046*	4.09	(CCG)4	56	0.97	2	0.05	0.00	0.05
28	*umc1738*	4.10	(CGCT)5	56	0.69	4	0.46	0.15	0.40
29	*umc1716*	4.11	(GAG)4	55	0.79	4	0.35	0.23	0.33
30	*nc130*	5.00	AGC	55	0.74	2	0.38	0.00	0.31
31	*phi024*	5.01	CCT	58	0.76	4	0.40	0.34	0.37
32	*umc1990*	5.04	-	57	0.67	2	0.44	0.00	0.35
33	*umc2026*	5.05	(AT)7	57	0.58	2	0.49	0.00	0.37
34	*bnlg1306*	5.07	(AG)21	55	0.77	3	0.38	0.15	0.34
35	*umc1225*	5.08	(AG)6	55	0.72	7	0.47	0.16	0.45
36	*umc1792*	5.08	(CGG)5	55	0.67	5	0.51	0.10	0.47
37	*bnlg1600*	6.00	(AG)21	55	0.46	3	0.64	0.03	0.56
38	*umc1257*	6.02	(CAC)4	55	0.95	2	0.10	0.00	0.09
39	*umc1083*	6.02	(GA)16	55	0.44	5	0.65	0.15	0.58

40	*nc010*	6.04	GTAC	55	0.71	4	0.45	0.47	0.40
41	*umc1352*	6.05	(GCC)6	55	0.95	2	0.10	0.00	0.09
42	*umc1912*	6.06	(GCG)6	57	0.85	3	0.27	0.13	0.25
43	*umc2323*	6.07	(GCA)4	55	0.42	5	0.72	0.06	0.68
44	*phi089*	6.08	ATGC	55	0.94	2	0.12	0.13	0.11
45	*mmc0171*	7.00	(GA)29	55	0.46	5	0.69	0.17	0.64
46	*umc1241*	7.00	(GTCTTTG)4	57	0.77	2	0.36	0.00	0.29
47	*umc1015*	7.03	(GA)45	57	0.41	5	0.69	0.31	0.64
48	*umc1888*	7.03	(ATA)6	55	0.64	3	0.48	0.05	0.39
49	*umc1125*	7.04	(CTCG)5	55	0.60	3	0.50	0.13	0.40
50	*umc1359*	7.04	(TC)12	55	0.51	3	0.59	0.26	0.51
51	*phi420701*	8.00	CCG	55	0.63	3	0.51	0.13	0.43
52	*umc1139*	8.01	(GAC)4	55	0.54	3	0.59	0.28	0.51
53	*umc1872*	8.02	(GCA)6	55	0.77	3	0.38	0.33	0.34
54	*phi115*	8.03	AT/ATAC***	55	0.63	2	0.47	0.33	0.36
55	*umc1172*	8.04	(CCA)4	55	0.85	2	0.26	0.00	0.23
56	*bnlg162*	8.05	-	55	0.51	5	0.60	0.05	0.53
57	*umc1724*	8.06	(CGA)4	55	0.74	5	0.42	0.08	0.39
58	*umc1384*	8.07	(CA)8	58	0.67	3	0.49	0.13	0.43
59	*bnlg1056*	8.08	(AG)16	55	0.54	4	0.57	0.05	0.49
60	*umc1957*	9.00	-	56	0.42	3	0.64	0.38	0.56
61	*nc134*	9.03	-	57	0.91	2	0.16	0.03	0.15
62	*umc1492*	9.04	(GCT)4	55	0.50	4	0.64	0.23	0.58
63	*phi040*	9.05	TTA	55	0.79	2	0.33	0.00	0.27
64	*umc1733*	9.06	(CATC)4	55	0.85	2	0.26	0.00	0.23
65	*dupssr29*	9.07	(GA)24	55	0.83	3	0.29	0.00	0.27
66	*phi117*	10.00	ACC	55	0.82	2	0.29	0.00	0.25
67	*umc2053*	10.01	(CGA)4	55	0.60	3	0.53	0.18	0.46
68	*umc1318*	10.01	(GTC)5	55	0.47	3	0.58	0.26	0.48
69	*umc1337*	10.02	(TA)8	55	0.53	2	0.50	0.00	0.37
70	*umc1938*	10.03	-	57	0.67	2	0.44	0.00	0.35
71	*bnlg1518*	10.04	(AG)15	56	0.44	5	0.64	0.05	0.57
72	*bnlg1839*	10.07	AG(24)	56	0.56	3	0.58	0.03	0.51
		Mean			0.64	3.29	0.47	0.12	0.41
		Range			0.35-0.97	2-7	0.05-0.72	0.00-0.47	0.05-0.68

Genetic relationships

Cluster analysis of 39 popcorn lines was conducted based on genetic distance (Fig. 2). The genetic dissimilarity coefficient varied from 0.27 (PC135 and PC138) to 0.82 (PC105 and PC124) with a mean value of 0.62. Cluster diagram grouped the inbreds into three major clusters (Fig. 2). Cluster-B having four sub-clusters (B1 to B4) was the largest group with 18 inbreds, while 16 genotypes belonged to Cluster-A (sub-cluster: A1 to A3). Cluster-C emerged as the smallest group with only five inbreds. PCoA analyses revealed that inbreds were distributed across four quadrangles (Fig. 3). Twelve inbreds were in top left quadrangle, while eight were in top right quadrangle. Nineteen inbreds could be observed in the bottom half, with ten and nine inbreds in the left and right quadrangle, respectively.

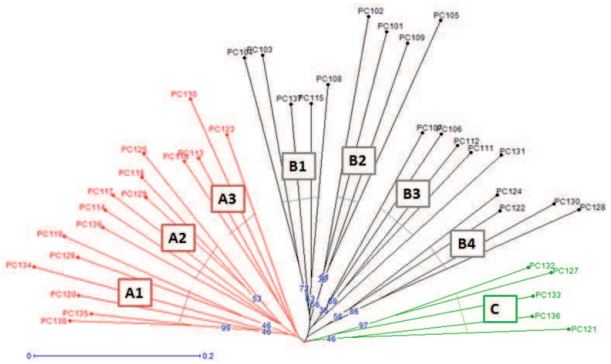

Fig. 2. Cluster analysis depicting genetic relationship among popcorn inbreds. Bootstrap value of e"30 is presented

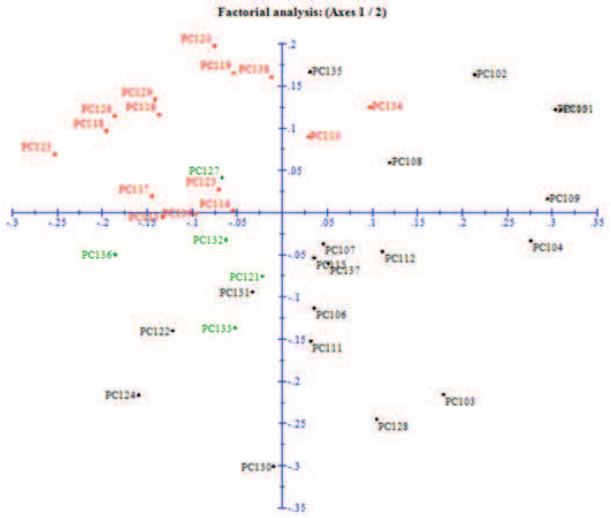

Fig. 3. Principal Coordinate Analysis (PCoA) among popcorn inbreds

Discussion

Genetic variability of popping quality traits

The present set of popcorn inbreds showed wide variation for GPP, PPE and VAP, thereby suggesting the ample scope for genetic improvement for these traits. Among the traits, GPP is the most important factor that influences PPE and VAP. Further, significant effect of genotypes on the percentage of unpopped kernels has been observed. Song et al. (1994) reported that kernel size and genotypes significantly affect the expansion volume and the number of unpopped kernels. Flake size, 1000 kernel weight, kernel size, percentage of unpopped kernels and protein content possess a positive direct effect on expansion volume (Soylu et al. 2007). Besides GPP, popping expansion volume determines the value of a popcorn genotype, as commercial buyers procure popcorn kernel on weight basis but sells the popped popcorn by volume. Greater expansion not only improves the quality, it also contributes to higher yields of the popped product thereby greater return (Kumar et al. 2013). Ceylan et al. (2001) reported that genotypes and kernel size directly affect the popping volume, while Song et al. (1994) reported that moisture content, popping temperature, kernel size and shape, genotype, kernel density, drying condition and kernel damage are the determining factors of popping volume. The variation in popping quality is due to various genetic loci distributed throughout the genome. Babu et al. (2006), Jain-Poster and Thomas (2015) and Lu et al. (2003) identified several quantitative trait loci (QTLs) for various popping related traits including expansion volume.

Molecular characterization

Large number of alleles detected across SSRs in the present study depicts the diverse nature of the popcorn inbreds (Li et al. 2004). Several researchers especially from Brazil, Argentina, Chile, China and USA have characterized popcorn germplasm using SSRs, and observed diverse nature of the loci. Li et al. (2004) while working with 56 popcorn lines detected 306 alleles with an average of 1-3 alleles per locus. Santacruz-Varela et al. (2004) detected 191 alleles in 56 diverse popcorn populations. In contrast, relatively low number of alleles was observed by Eloi et al. (2012) and Saavedra et al. (2013) among popcorn genotypes, respectively. Markers with high PIC observed in the present study are also indicative of the diverse nature of the inbreds. More than half of the SSRs possessed PIC higher than the mean. Pena et al. (2016) while analyzing the diversity of popcorn germplasm reported higher PIC in many of the SSR loci.

Gene diversity is defined as the probability that two randomly chosen alleles from the population are different (Liu and Muse 2005). The present study also found wide range of gene diversity among the inbreds. Bracco et al. (2009) reported gene diversity ranging from 0.04-0.55, while Eloi et al. (2012) found the highest gene diversity of 0.76 in diverse popcorn populations. Low value of major allele frequency of any marker is indicative of highly diverse nature of the locus (Devi et al. 2017). In the present study as well, diverse nature of the locus was quite apparent. Choudhary et al. (2015) also reported low average major allele frequency in non-popping type maize inbreds.

The study also identified unique- and rare-alleles, suggesting the selection of diverse nature of inbreds (Mehta et al. 2017). This is possibly due to inclusion of a number of popcorn inbreds developed from source populations procured from Mexico, Argentina and Chile, apart from local germplasm source. Further, selection of higher number of SSRs with smaller repeats, which mostly tend to be more polymorphic than longer-repeat motifs could also be responsible (Vigouroux et al. 2005). Further, unique alleles are useful in DNA fingerprinting as they can be unambiguously identified from other inbreds (Muthusamy et al. 2015).

In the present study, very low degree mean heterozygosity was detected among the inbreds, thereby confirming the higher degree of homozygosity obtained during the inbred development. The higher degree of heterozygosity detected in some of the loci could be due to residual heterozygosity (Choudhary et al. 2015). Inbreds developed through conventional breeding tends to express heterozygosity in few loci despite several generations of inbreeding (Pandey et al. 2015). While, inbreds developed through doubled haploid technique achieves complete homozygosity in a single generation. Further, mutation at specific SSR locus or amplification of similar sequences from different genomic regions due to duplication could also be the reason of heterozygosity (Devi et al. 2017). Eloi et al. (2012) while working with popcorn populations reported heterozygosity as high as 0.82 in specific SSR locus. The open pollination in populations allows majority of the loci to perpetuate in heterozygous conditions.

Utilization in popcorn breeding

Information generated here can be effectively utilized in the popcorn improvement programme. Inbreds with contrasting popping attributes can be used to identify the loci for further improvement by developing segregating mapping populations. Vittorazzi et al. (2018) has reported sufficient genetic variability at phenotypic and genotypic levels among the 38 popcorn genotypes. Babu et al. (2006) mapped QTLs for popping quality traits using popcorn × flint corn, while Lu et al. (2003) and Jain-Poster and Thomas (2015) identified QTLs through mapping population developed from popcorn × dent corn. Mapping QTLs using contrasting popcorn inbreds identified in the study would likely to identify the minor QTLs that might have been undetected using crosses involving flint and dent corn. Recently, Senhorinho et al. (2019) has identified four SNPs that were significantly correlated with popping expansion, and therefore can be useful for marker-assisted selection to breed genotypes with higher popping. In the present study, PC120 and PC119 were the most promising inbreds for popping quality, while PC135, PC124 and PC108 were the most unfavourable genotypes. Considering the clustering pattern, following mapping populations *viz*., PC119 × PC108, PC119 × PC124, PC120 × PC108 and PC120 × PC124, can be developed to localize the position of QTLs governing the popping quality traits in maize.

Knowledge of the heterotic patterns among the inbreds is crucial for the maximum exploitation of heterosis (Hallauer et al. 2010). Melani and Carena (2005) indicated that the success of a popcorn breeding programme depends on parental selection and the accurate identification of heterotic groups. Molecular markers have often been used to accurately measure genetic similarity and predict hybrid performance (Vigouroux et al. 2005). Considering their genetic relationship from clustering pattern and desirable kernel popping quality, following possible heterotic cross combinations can be attempted. PC119, PC120, PC125, PC116, PC113 of cluster-A, can be crossed with inbreds of cluster-B (PC104, PC102, PC105, PC112, PC122 and PC130) and cluster-C (PC132, PC133 and PC121). Similarly, desirable popcorn inbreds of cluster-B and -C can be crossed together. Earlier, various research groups identified potential heterotic combinations for various quality traits *viz*., waxy (Devi et al. 2017), sweet corn (Mehta et al. 2017), provitamin A (Choudhary et al. 2015) and iron and zinc (Chakraborti et al. 2011) in maize. Further inbreds with wide genetic distance tend to harbour diverse alleles, thus F_2 segregants with accumulation of the favourable alleles from diverse parents of the above mentioned crosses can also be selected carefully for further improvement of popping quality. Pandey et al. (2015) while analyzing the genetic diversity of QPM inbreds using SSRs, identified diverse inbreds for accumulation of favourable alleles for endosperm- and amino acid- modifier loci. Further, a synthetic population/pool comprising of inbreds of the same cluster can also be created for the development of improved popcorn lines for their utilization in the breeding programme. This is the first report of comprehensive analysis of popcorn inbreds adapted to Indian condition using both popping quality attributes and molecular marker analyses.

Conclusion

Wide variation for popping attributes established scope of further genetic improvement. Genetically diverse promising inbreds with desirable popping attributes provide opportunity for attempting selected cross combinations for heterotic hybrids, besides serving as component lines for constitution of pools to develop new and improved popcorn lines. Crosses identified for generating mapping populations could help in identification of loci governing the popping attributes. The information generated here holds significant promise in the popcorn breeding programme.

Authors' contribution

Conceptualization of research (FH, JCS); Designing of the experiments (FH, VM); Contribution of experimental materials (VM, FH, JCS); Execution of field/lab experiments and data collection (DP, SKJ, RC, AB, HSC, VB); Analysis of data and interpretation (RUS, VM); Preparation of manuscript (FH, VM, RUZ).

Declaration

The authors declare no conflict of interest.

Acknowledgements

We thank ICAR-Indian Agricultural Research Institute, New Delhi for the financial support. We thank Dr. B.M. Prasanna, CIMMYT, Mexico for providing the source germplasm of selected popcorn inbreds developed at IARI. We also thank Director, ICAR-IIMR, Ludhiana for providing the popcorn lines from Winter Nursery Centre. The help of Mr. Manish Kapasia in managing the field and pollination programme is acknowledged.

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Supplementary Table S1. Pedigree information of popcorn inbreds

S. No.	Genotype	Source population	Institution
1.	PC101	TL10B-6925-7x8-Mexico	
2.	PC102	TL998-6903-43-Mexico	
3.	PC103	VL Pop Corn	
4.	PC104	VL Pop Corn	ICAR-IARI, New Delhi
5.	PC105	CM137/NAI147-TLB	
6.	PC106	Pop Bulk	
7.	PC107	VL Pop Corn	
8.	PC108	ANG-POP	WNC, ICAR-IIMR, Hyderabad
9.	PC109	CHILE-1994-Mexico	ICAR-IARI, New Delhi
10.	PC110	VL Baby Corn	ICAR-IARI, New Delhi
11.	PC111	BPC98	
12.	PC112	BPC141	
13.	PC113	BPC85	
14.	PC114	BPC720	
15.	PC115	BPC17-1	
16.	PC116	BPC28-2	
17.	PC117	BPC85	
18.	PC118	BPC66	
19.	PC119	BPC56	
20.	PC120	BPC-2C	
21.	PC121	BPC73	
22.	PC122	BPC17	
23.	PC123	BPC280-2	
24.	PC124	BPC26	
25.	PC125	BPC-C-10	WNC, ICAR-IIMR, Hyderabad
26.	PC126	BPC23-1-1	
27.	PC127	BPC15	
28.	PC128	BPC114	
29.	PC129	BPC80	
30.	PC130	BPC127	
31.	PC131	PC111	
32.	PC132	BPC-ARGENTINA	
33.	PC133	BPC106	
34.	PC134	BPC126	
35.	PC135	BPC123	
36.	PC136	BPC104	
37.	PC137	BPC114	
38.	PC138	BPC127	
39.	PC139	BPC25-1	
40.	HKI163	CML161	CCSHAU, Uchani
41.	UMI1230	C7254-105-3	TNAU, Coimbatore
42.	VL Pop corn	Composite of six inbreds	ICAR-VPKAS, Almora

Supplementary Table S2. ANOVA for PPE, GPP and VAP among inbreds

Sources of variation	df	PPE		GPP		VAP	
		MSS	Prob.	MSS	Prob.	MSS	Prob.
Genotype	41	559.65	0.000	637112.86	0.000	5603.61	0.000
Replication	1	3.05	0.272	9408.58	0.663	0.12	0.976
Error	41	2.46	-	48883.95	-	117.62	-
Total	83	565.16	-	695405.39	-	5721.35	-

df = degrees of freedom, MSS = Mean sum of squares, Prob. = Probability, PPE = Percent popping expansion, GPP = Grain popping percentage, VAP = Volume after popping