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ORIGINAL ARTICLE

Microsatellite marker-based characterization of waxy maize inbreds for their utilization in hybrid breeding

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Abstract Waxy corn possesses 95–100% amylopectin, compared to 70–75% in normal maize, owing to mutation in *Wx* gene encoding a granule-bound starch synthase I. Amylopectin is used as an ingredient in textile, adhesive and paper industries. Further, waxy green cob is popular as breakfast item in South Asia and an important constituent of diet in north-eastern states of India as well. We developed a series of waxy inbreds from diverse exotic sources and through introgression breeding. To characterize and unravel the genetic relationships, 24 diverse waxy inbreds were analysed using 77 SSRs distributed throughout the genome. The study generated a total of 203 polymorphic alleles, with a mean of 2.69 alleles per locus. A total of nine unique and 20 rare alleles were detected. The polymorphism information content ranged from 0.08 to 0.68 with an average value of 0.40. Molecular profiling suggested sufficient attainment of homozygosity among the inbreds. Jaccard's dissimilarity coefficient between pairs of genotypes varied from 0.26 to 0.83 which revealed the diverse nature of the inbred lines. Cluster analyses grouped 24 genotypes into three major clusters. Principle coordinate analysis based on SSR also depicted the diverse origin of the genotypes as per the pedigree more reliably than agro-morphological traits. These inbreds were also promising for various cob and grain characteristics including grain yield. The study identified a set of potential cross-combinations that can be planned to develop highly heterotic waxy hybrid combinations. This is the first report of development and characterization of waxy inbreds in India.

Keywords Waxy · Maize · SSR · Diversity · Genetic distance

Introduction

Waxy corn also known as 'sticky maize' or 'glutinous maize' is a popular choice in South Asia (Xiaoyang et al. 2017). It contains 95–100% amylopectin, a branched-chain starch, in contrast to 70–75% in normal maize (Zhou et al. 2016). Waxy maize was first discovered in China, and Yunnan–Guangxi region is considered to be the centre of its origin (Zheng et al. 2013). The *waxy* (*wx*) locus is located on chromosome 9, and wild type allele (*Wx*) encodes a granule-bound starch synthase (GBSS-I), which catalyses amylose synthesis from ADP-glucose in the endosperm (Klosgen et al. 1986; Mason-Gamer et al. 1998). Waxy maize is thought to be originated from the cultivated flint maize through mutation (Fan et al. 2008; Zheng et al. 2013). Various types of mutations, viz. insertion of transposon, retroposon and fragments of few nucleotides and deletion of nucleotides, result in mutant allele (*wx*). These mutations cause the synthesis of altered transcript with premature stop codon or change in amino acids in key domain or splicing or translational errors that in turn stops the activity of *Wx* allele or inhibits the activity of GBSS-I, thereby resulting in lower amylose and higher amylopectin in grain (Bao et al. 2012; Zhang et al. 2013).

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Waxy maize is an important component of diet in many countries, viz. Thailand, Vietnam, Laos, Myanmar, China, Taiwan, Philippines and Korea. It is consumed as 'green corn', especially during breakfast, and also popular as vegetable. Due to high amylopectin, it possesses the property of high viscosity and is easily digested in human gut (Lu and Lu 2012). These excellent characters make waxy maize widely used in frozen food processing and livestock feeding industries. Further, amylopectin is a popular ingredient in textile, adhesive and paper industries (Bao et al. 2012). Waxy corn, therefore, holds an immense promise as an economically potential crop worldwide because of starch composition and economic value (Tian et al. 2009).

The germplasm base of waxy corn is narrow compared to normal maize, as few countries have active waxy corn breeding programme. Only few reports from China (Yu et al. 2012; Zheng et al. 2013; Hao et al. 2015), Vietnam (Liet and Tinh 2009; Hung et al. 2012) and Korea (Park et al. 2008; Sa et al. 2015) are available on molecular characterization of waxy inbreds. In India, so far waxy trait has not been utilized in the breeding programme, despite the fact that people in north-eastern states of the country prefers waxy maize as a food over traditional maize. Further, the green cobs can be popularized as a breakfast item in the urban areas of India and would serve as a source of livelihood to farming community by exporting the processed products to many of the South Asian countries. Specialty corn breeding programme at ICAR–Indian Agricultural Research Institute (IARI), New Delhi, has developed a set of waxy inbreds from diverse source populations and through introgression breeding. Characterization of these inbreds and understanding their genetic relationships assume significance for their effective utilization in waxy hybrid breeding programme.

Molecular markers have been a preferred choice over morphological data primarily due to their abundance, whole genome coverage and environment neutral behaviour. Assessment of genetic relationships using molecular markers has often been utilized for accurate measurement of genetic similarity and to predict hybrid performance (Smith et al. 1997; Ajmone-Massan et al. 1998; Senior et al. 1998). The present investigation was, therefore, carried out to characterize the newly developed waxy corn inbreds to (1) assess the genetic relationships among inbreds using microsatellite markers and (2) identify potential heterotic combinations for their utilization in hybrid breeding.

Materials and methods

Plant material

A set of 24 waxy corn inbreds (MGUWX-101–124) developed at IARI, New Delhi, were employed for the present study, and pedigree of the inbreds is presented in Table S1. Of the 24 inbreds, 9 were generated from exotic populations segregating for *wx* allele, and 15 were developed through targeted introgression of *wx* allele into elite inbreds. Among the inbreds, 15 were of white endosperm type, while 9 inbreds possessed yellow endosperm. These inbreds were maintained through repeated selfing to avoid any possible contamination from foreign pollen.

Agronomic performance of inbreds

Each of the 24 waxy corn inbreds was grown in two rows during *kharif* season 2015 at IARI experimental station, New Delhi, under standard package of practices for cultivation. The individual plants were selfed and harvested with care to avoid any contamination of inbreds. These inbreds were evaluated for days for 50% anthesis, days for 50% silking, ear length, ear width, number of kernel rows per ear, number of kernels per row, 100-kernel weight and grain yield.

Genomic DNA isolation and PCR amplification

Genomic DNA was extracted from seeds of the selected waxy genotypes using modified SDS extraction protocol (Dellaporta et al. 1983). DNA concentration was measured on 0.8% agarose gel. The final concentration of extracted DNA was made to 10 ng/µl and used as stock solutions for polymerase chain reactions (PCR). Seventy-seven microsatellite or simple sequence repeat (SSR) markers distributed throughout the genome were used for the present study. The primer sequence and the bin locations of the selected SSRs were obtained from the Maize Genomic Database (www.maizegdb.org). The oligonucleotide primers were synthesized from M/S Macrogen in purified and lyophilized form. The dilutions were made for a final concentration of 10 µM with Milli-Q water. PCRs were carried out with touch-down protocol on 96-well thermal cycler (Veriti 96-well thermal cycler, Applied Biosystems). All PCRs were performed in a final volume of 20 µl with the following reagent concentrations: 50 ng of template DNA, 1× One PCR^{TM} mix (Ready-to-mix PCR mix, GeneDirex), 0.5 µM of each of the forward and reverse

primers. The amplification conditions were as follows: initial denaturation at 95 °C for 5 min, four cycles consisting of denaturation at 95 °C for 45 s, primer annealing ranged between 57 and 64 °C for 45 s with a decrease of 0.5 °C/cycle, primer extension at 72 °C for 45 s, followed by 35 amplification cycles consisting of denaturation at 95 °C for 45 s, primer annealing ranged between 55 and 62 °C for 45 s, primer extension at 72 °C for 45 s and final extension at 72 °C for 8 min. The amplified products were resolved at 120 voltage using 4.0% Seakem LE agarose gel (Lonza, Rockland, ME, USA). AlphaImager® (M/s Alpha Innotech, San Leandro, CA) gel documentation system is used for visualization of resolved PCR products.

Statistical analyses

The allele size was evaluated by comparing with 100-bp DNA ladder. Parameters like total number of alleles, major allele frequency, gene diversity, observed heterozygosity per locus and polymorphism information content (PIC) were determined using PowerMarker V3.0 (Liu and Muse 2005). The allele appearing in only one genotype was considered as unique allele, while allele with a frequency of <0.05 was considered as rare allele. A dendrogram was constructed using neighbour-joining method (3000 bootstrapping) implemented in DARwin 6.0 software to provide a general visualization of genetic relationship among inbreds (Perrier et al. 2003). Genetic dissimilarity between two genotypes was calculated using Jaccard's coefficient. The principal coordinate analysis (PCoA) was estimated to depict the diverse origin of the genotypes using both molecular and morphological data (Perrier et al. 2003).

Results and discussion

Molecular characterization

A total of 203 alleles were generated across the 24 waxy inbreds with a mean of 2.69 alleles and a range of 2–4 alleles per locus (Table 1). Several researchers, viz. Yu et al. (2012) [60 alleles, 2.73 average alleles/locus and 2–4 alleles/locus], Zheng et al. (2013) [104 alleles, 5.20 average alleles/locus and 2–8 alleles/locus], Hung et al. (2012) [117 alleles, 3.26 average alleles/locus and 1–6 alleles/locus] and Park et al. (2008) [127 alleles, 4.20 average alleles/locus and 2–9 alleles/locus], observed similar trend while working on genetic diversity of waxy maize genotypes. These waxy accessions and inbreds belonged to China, Vietnam and Korea and were analysed using 20–30 SSRs. In the present study, waxy inbreds developed for first time in India were characterized using 77 SSRs distributed throughout the genome (Table 1). In contrast, Sa et al. (2015) reported higher number of alleles [1268 alleles, 6.34 average alleles/locus and 2–14 alleles/locus] among 40 waxy inbreds of South Korea using 200 SSRs. Further, Hao et al. (2015) analysed 110 waxy maize accessions using 2751 single nucleotide polymorphisms (SNPs) and detected two alleles/locus. In the present study, allele size ranged from 60 bp (*phi028*) to 300 bp (*bnlg1635* and *phi113*) across markers, which revealed the presence of high genetic diversity of the loci analysed. The average major allele frequency was 0.61, with a range of 0.35 (*umc1015*)–0.96 (*umc2046*) (Table 1). Sa et al. (2015) reported major allele frequency of 0.46 with a range of 0.20–0.95 among waxy inbreds. Lesser frequency of the major allele is indicative of diverse nature of the locus, and nearly one-fourth of the SSR loci analysed in the present study showed major allele frequency of ≤ 0.5, indicating the wide diversity in the panel of inbreds. The gene diversity ranged from 0.08 (*umc2046*) to 0.73 (*umc1015*) with a mean of 0.48. Gene diversity of 0.51 (range 0.13–0.80) and 0.66 (range 0.10–0.89) was reported by Sa et al. (2015) and Park et al. (2008), respectively. Hao et al. (2015) reported gene diversity of 0.40 among waxy germplasm of China.

The PIC among waxy inbreds developed in India ranged from 0.08 (*umc2046*) to 0.68 (*umc1015*) with an average value of 0.40 (Table 1). A total of 17 SSR loci were found to have a PIC value ≥ 0.50, suggesting the higher ability of these loci to discriminate between the inbred lines. Closely related lines will exhibit lower PIC value, whereas genetically diverse lines will show higher PIC values (Muthusamy et al. 2015; Zunjare et al. 2015). The PIC of 0.31 and 0.46 was also observed in waxy germplasm by Hao et al. (2015) and Hung et al. (2012), respectively. However, Sa et al. (2015) [PIC = 0.62] and Zheng et al. (2013) [PIC = 0.70] reported high PIC in their studies on waxy maize.

The present study also detected nine unique and 20 rare alleles, thus providing a prospect for unambiguous separation of the respective inbreds from others. Similar observation of unique and rare alleles was reported among maize inbreds for several kernel quality traits, viz. provitamin A (Sivaranjani et al. 2014; Choudhary et al. 2015; Muthusamy et al. 2015), lysine and tryptophan (Pandey et al. 2015b), iron and zinc (Chakraborti et al. 2011; Pandey et al. 2015a) and sweetness (Mehta et al. 2017), in India. The heterozygosity observed among the SSRs varied from 0.00 to 0.29, with a mean of 0.05 (Table 1), indicating that the inbreds used in the study have attained high degree of homozygosity upon inbreeding. Hung et al. (2012) reported the presence of higher heterozygosity among majority of the 22 waxy inbreds, suggesting the need for further inbreeding to stabilize the inbreds. However, some loci, viz. *umc1076* (0.29), *umc1332* (0.21), *umc1823* (0.21), *phi113* (0.21), *bnlg1740* (0.18), *bnlg1635* (0.17), *umc1067* (0.17), *nc010* (0.17) and *umc1125* (0.17),

Table 1 Primer details and summary statistics of genotyping assay of 24 inbred lines used in the study

S. no.	Markers	Bin	Repeats	Major allele frequency	Number of alleles	Gene diversity	Heterozygosity	PIC
1	*bnlg1803*	1.02	$(AG)_{15}$	0.50	3	0.60	0.05	0.52
2	*umc2217*	1.04	$(TG)_6$	0.52	2	0.50	0.04	0.37
3	*umc1076*	1.05	Long CA	0.77	2	0.35	0.29	0.29
4	*bnlg1884*	1.05/1.06	$(AG)_{13}$	0.56	2	0.49	0.04	0.37
5	*bnlg1057*	1.06	$(AG)_{17}$	0.63	2	0.47	0.00	0.36
6	*bnlg1919*	1.06	$(CT)_8$	0.78	2	0.34	0.00	0.28
7	*bnlg1556*	1.07	$(AG)_{18}$	0.43	4	0.63	0.04	0.55
8	*dup ssr12*	1.08	$(AC)_{15}$	0.42	4	0.65	0.04	0.58
9	*umc2586*	1.08	$(CA)_4$	0.57	2	0.49	0.00	0.37
10	*umc2245*	2.01	$(CAA)_7$	0.89	2	0.19	0.04	0.17
11	*umc1823*	2.02	$(TG)_{36}$	0.73	3	0.41	0.21	0.36
12	*umc1542*	2.02	$(AG)_{10}$	0.67	3	0.48	0.04	0.42
13	*bnlg1064*	2.03	$(AG)_{16}$	0.38	4	0.68	0.13	0.62
14	*umc1026*	2.04	$(CT)_9$	0.58	2	0.49	0.00	0.37
15	*bnlg1635*	2.05	$(GAAGG)_4$	0.40	4	0.67	0.17	0.61
16	*umc2220*	2.07	$(ACT)_4$	0.79	2	0.33	0.00	0.28
17	*bnlg198*	2.08	–	0.37	3	0.66	0.07	0.59
18	*umc2103*	3.00	(GCG)5	0.54	3	0.56	0.00	0.47
19	*umc1892*	3.01	$(GA)_8$	0.69	3	0.48	0.04	0.43
20	*bnlg1144*	3.02	–	0.42	4	0.70	0.00	0.65
21	*phi374118*	3.02	ACC	0.63	2	0.47	0.00	0.36
22	*umc1030*	3.04	$(CT)_{21}$	0.58	2	0.49	0.00	0.37
23	*bnlg1957*	3.05	$(AG)_{10}$	0.52	3	0.55	0.13	0.45
24	*bnlg1456*	3.05	$(AG)_{15}$	0.54	3	0.59	0.08	0.52
25	*umc1311*	3.06	$(TCTT)_4$	0.67	3	0.48	0.13	0.41
26	*umc1273*	3.08	$(AAG)_4$	0.54	2	0.50	0.00	0.37
27	*bnlg1126*	4.03	$(AG)_{20}$	0.80	2	0.32	0.00	0.27
28	*nc004*	4.03	AG	0.90	3	0.19	0.04	0.18
29	*phi074*	4.04	CAA	0.67	2	0.44	0.00	0.35
30	*umc1067*	4.04	$(GCC)_4$	0.54	4	0.56	0.17	0.48
31	*umc1382*	4.05	$(AAC)_7$	0.92	2	0.15	0.00	0.14
32	*bnlg1217*	4.05	$(AG)_{33}$	0.50	3	0.62	0.13	0.55
33	*umc1869*	4.06	$(GGT)_6$	0.81	4	0.32	0.08	0.29
34	*umc1194*	4.07	GGCC	0.52	3	0.52	0.08	0.40
35	*umc2046*	4.09	$(CCG)_4$	0.96	2	0.08	0.00	0.08
36	*nc130*	5.00	AGC	0.71	2	0.41	0.00	0.33
37	*phi113*	5.03	GTCT	0.46	4	0.65	0.21	0.58
38	*umc1784*	5.03	$(CCG)_4$	0.63	2	0.47	0.00	0.36
39	*umc1332*	5.04	$(CTA)_5$	0.67	3	0.49	0.21	0.43
40	*umc2298*	5.04	$(GCG)_4$	0.65	2	0.45	0.00	0.35
41	*umc2373*	5.04	$(GCT)_4$	0.52	3	0.57	0.04	0.47
42	*bnlg2323*	5.04	$(AG)_{25}$	0.54	3	0.53	0.00	0.43
43	*umc1990*	5.04	–	0.71	2	0.41	0.00	0.33
44	*bnlg278*	5.05	–	0.42	3	0.64	0.08	0.57
45	*bnlg1019*	5.06	$(CT)_{17}$	0.46	3	0.59	0.08	0.50
46	*bnlg1306*	5.06/5.07	$(AG)_{21}$	0.56	3	0.58	0.04	0.51
47	*bnlg1600*	6.00	$(AG)_{21}$	0.75	2	0.38	0.00	0.3
48	*bnlg0426*	6.01	–	0.70	4	0.46	0.04	0.41

Table 1 continued

S. no.	Markers	Bin	Repeats	Major allele frequency	Number of alleles	Gene diversity	Heterozygosity	PIC
49	*umc1006*	6.02	$(GA)_{19}$	0.79	3	0.34	0.08	0.31
50	*nc10*	6.04	GTAC	0.58	3	0.50	0.17	0.40
51	*bnlg2317*	6.04	$(GGC)_6$	0.76	3	0.37	0.04	0.32
52	*nc013*	6.05	–	0.71	2	0.41	0.00	0.33
53	*umc1413*	6.05	$(TC)_{16}$	0.50	4	0.59	0.13	0.50
54	*bnlg1732*	6.05	$(AG)_{15}$	0.68	2	0.43	0.00	0.34
55	*umc2319*	6.05	$(GAGGAG)_5$	0.63	2	0.47	0.00	0.36
56	*umc1912*	6.06	$(GCG)_6$	0.54	2	0.50	0.00	0.37
57	*bnlg1740*	6.07	$(AG)_{21}$	0.52	3	0.59	0.18	0.51
58	*umc2059*	6.08	$(CAG)_8$	0.54	2	0.50	0.08	0.37
59	*bnlg2325*	7.01	$(TGG)_7$	0.53	2	0.50	0.11	0.37
60	*umc1016*	7.02	$(CT)_{25}$	0.44	3	0.64	0.13	0.56
61	*bnlg339*	7.03	–	0.54	3	0.60	0.00	0.53
62	*umc1837*	7.03	$(TA)_8$	0.55	3	0.56	0.00	0.48
63	*umc1015*	7.03	$(GA)_{45}$	0.35	4	0.73	0.04	0.68
64	*umc1125*	7.04	$(CTCG)_5$	0.50	2	0.50	0.17	0.38
65	*umc1944*	7.04	–	0.75	2	0.38	0.00	0.30
66	*phi072*	7.05	AAAC	0.83	3	0.29	0.08	0.26
67	*phi116*	7.06	ACTG/ACG	0.50	2	0.50	0.00	0.38
68	*bnlg162*	8.05	–	0.48	3	0.63	0.04	0.56
69	*bnlg1056*	8.08	$(AG)_{16}$	0.58	3	0.56	0.08	0.49
70	*phi028*	9.01	GAA	0.56	2	0.49	0.04	0.37
71	*phi022*	9.03	GTGC	0.57	3	0.51	0.09	0.40
72	*phi061*	9.03	TTCT-GTAT	0.54	3	0.53	0.00	0.43
73	*umc2358*	9.06	$(CGC)_5$	0.54	2	0.50	0.00	0.37
74	*umc1938*	10.03	–	0.75	2	0.38	0.00	0.30
75	*bnlg1506*	10.05	$(AACA)_4$	0.60	3	0.53	0.04	0.45
76	*bnlg1839*	10.07	$(AG)_{24}$	0.83	2	0.28	0.00	0.24
77	*umc1196*	10.07	CACACG	0.71	2	0.41	0.00	0.33
Mean				0.61	2.69	0.48	0.05	0.40

detected high heterozygosity in the present set of waxy inbreds (Table 1). This may be because of some loci regardless of repeated cycles of inbreeding over many generations which tend to segregate due to residual heterozygosity (Kaur et al. 2011). Moreover, mutation at specific allele or amplification of similar sequences from different genomic regions due to duplication (Semagn et al. 2006; Zunjare et al. 2015) may also be other possible reasons. Conventionally bred inbreds often exhibited some degree of heterozygosity as compared to doubled-haploid-based inbreds (Sivaranjani et al. 2014; Pandey et al. 2015a).

Genetic relationships among inbreds

Cluster analysis of 24 waxy corn inbreds was conducted based on genetic dissimilarities from SSR data using neighbour-joining method. The genetic dissimilarity between the genotype pairs was found to range from 0.24 to 0.81 with a mean of 0.65, indicating the genetically diverse nature of the inbreds used in the study. Yu et al. (2012) and Hung et al. (2012) found average genetic distance of 0.55 and 0.62, while working with 80 and 22 waxy inbreds, respectively. Cluster diagram grouped the 24 genotypes into three distinct clusters, viz. A, B and C (Fig. 1). Cluster A had eight genotypes and was further subdivided into two subgroups (A1 and A2). Cluster A1 comprised of four inbreds, viz. MGUWX-121, MGUWX-120, MGUWX-122 and MGUWX-119. Cluster A2 also had four inbreds (MGUWX-104, MGUWX-103, MGUWX-102 and MGUWX-101). Cluster B possessed five genotypes, viz. MGUWX-110, MGUWX-109, MGUWX-124, MGUWX-108 and MGUWX-107. There were eleven genotypes in cluster C, of which cluster C1

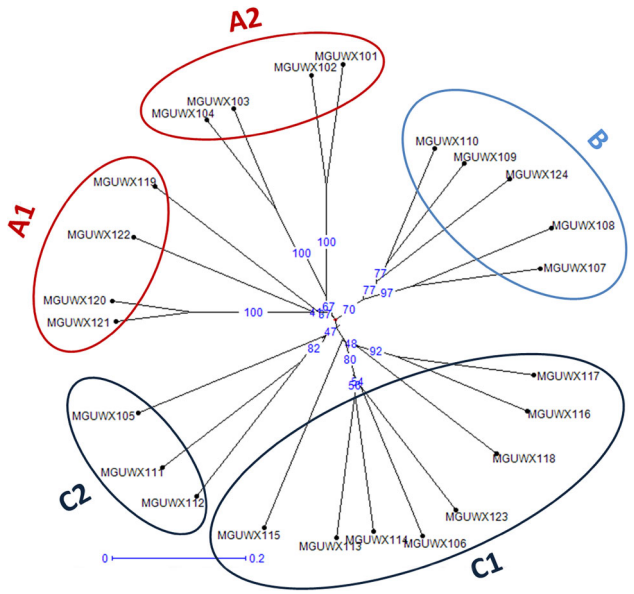

Fig. 1 Cluster analysis revealed by 77 SSRs depicting genetic relationships among waxy 24 inbreds. Bootstrap value of ≥30 is presented. A, B and C indicate the major clusters circled with varying colours, while 1 and 2 indicate subclusters within each major cluster

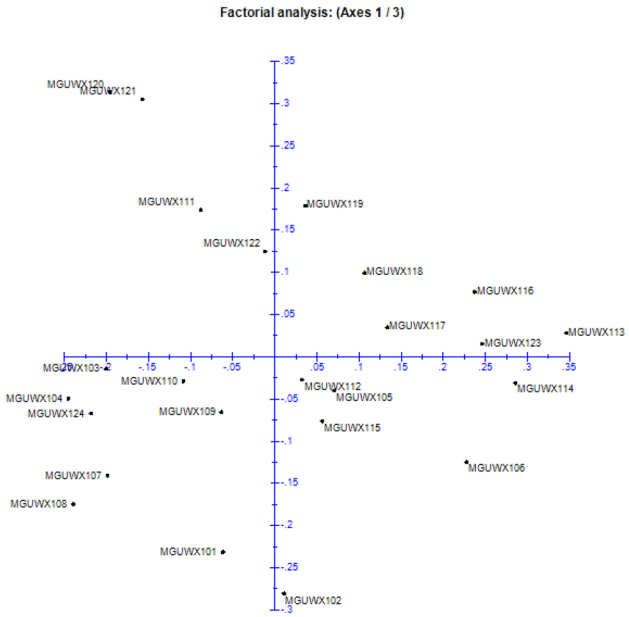

Fig. 2 Principal coordinate analysis (PCoA) among 24 waxy inbreds characterized through 77 SSRs. Total per cent variation explained by axes −1, −2, −3 and −4 is 15.03, 12.33, 10.24 and 9.52, respectively

was composed of eight inbreds (MGUWX-117, MGUWX-116, MGUWX-118, MGUWX-123, MGUWX-106, MGUWX-114, MGUWX-113 and MGUWX-115), while C2 consisted of three inbreds, viz. MGUWX-112, MGUWX-111 and MGUWX-105. In general, two to three major groups have been also observed in various studies on waxy genotypes (Park et al. 2008; Zheng et al. 2013; Hao et al. 2015; Sa et al. 2015). However, a higher number of clusters, i.e. six and nine, were observed among the waxy inbreds of Vietnam and China, respectively, (Hung et al. 2012; Yu et al. 2012).

The clustering of the inbred lines based on the markers' information was highly consistent with their pedigree information. The inbreds developed from the same source population were generally under the same cluster. For example, MGUWX-101 and MGUWX-102 derived from VQL1 and MGUWX-103 and MGUWX-104 derived from VQL2 through backcross breeding were together in A2 cluster. Similarly inbreds derived from similar pedigree, viz. (i) MGUWX-109 and MGUWX-110, (ii) MGUWX-107 and MGUWX-108, (iii) MGUWX-111 and MGUWX-112, (iv) MGUWX-113 and MGUWX-114, (v) MGUWX-106 and MGUWX-123 and (vi) MGUWX-120, MGUWX-121 and MGUWX-122, clustered together in their respective groups.

The genotypic PCoA showed that the inbreds were distributed in all the four quadrangles, signifying their genetic variability (Fig. 2). PCoA generated using morphological data also showed the presence of inbreds across four quadrangles suggesting the presence of diversity for agronomic traits among the waxy inbreds (Fig. 3). In both genotypic and phenotypic PCoA, MGUWX-108 and MGUWX-110 were together in the left bottom quadrangle, while MGUWX-114 and MGUWX-115 could be clustered in the right bottom quadrangle. Further, based on the pedigree data, (i) MGUWX-101 and MGUWX-102,(ii) MGUWX-103 and MGUWX-104, (iii) MGUWX-107 and MGUWX-108, (iv) MGUWX-113 and MGUWX-114, and (v) MGUWX-120, MGUWX-121 and MGUWX-122 were found to be closely placed in genotypic PCoA. In case of phenotypic PCoA as well, the inbreds with similar pedigree grouped together, except MGUWX-113 and MGUWX-114. However, the distribution of inbreds in different quadrangles was quite different in genotypic and phenotypic PCoA. This is could be due to environmental factors that influence phenotypic expression of the traits. DNA markers, such as SSRs, are particularly suited for genetic characterization and diversity studies, as compared to morphological markers primarily due to their environment neutral nature, numerous and wide distribution throughout the genome (Smith and Smith 1992; Collard et al. 2005; Prasanna et al. 2010).

Identification of potential cross-combinations

In hybrid breeding, per se performance of the parental inbreds assumes great significance for effective and economic hybrid seed production. A highly heterotic hybrid may fail if the parental inbreds bear poor characteristics such as non-synchrony in flowering, poor ear and grain

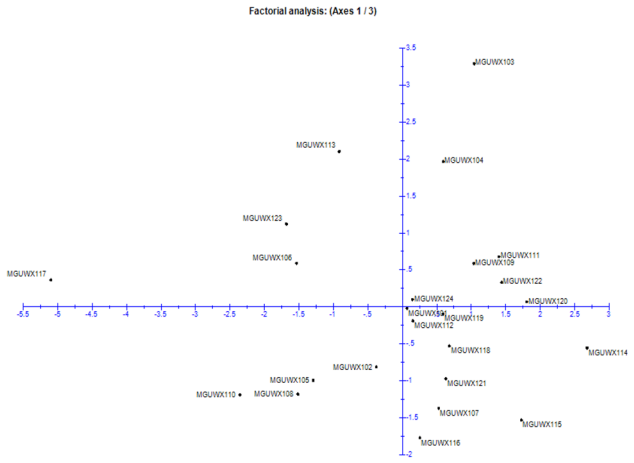

Fig. 3 Principal coordinate analysis (PCoA) among 24 waxy inbreds characterized through agronomic traits. Total per cent variation explained by axes −1, −2,−3 and −4 is 31.40, 24.18, 19.13 and 10.92, respectively

characteristics and low grain yield potential. Waxy inbreds analysed in the present study flowered during 47–51 days after sowing and can be used as parent in the hybrid breeding programme, where synchrony of flowering among the two parental inbreds is of utmost significance (Table 2). Cob and grain characteristics depicted the agronomic superiority of the inbreds. The grain yield potential of the waxy inbreds ranged from 1.78 to 2.67 tonnes/ha, thereby suggesting the promising nature of the inbreds. Genetic diversity studies on waxy maize genotypes by Hao et al. (2015), Liet and Thinh (2009), Park et al. (2008), Yu et al. (2012) and Zheng et al. (2013) were based solely on molecular markers. In contrast, Sa et al. (2015) evaluated waxy inbreds for ten morphological traits coupled with SSR-based diversity analyses. The present study, therefore, suggests that 24 waxy inbreds can be effectively utilized in the waxy corn hybrid programme, as they possess desirable per se performance.

Identification of combination of inbreds having high heterosis for grain yield is a costly and time-consuming activity in maize breeding programme. Information related to the genetic relationship among maize inbreds pertaining to within and between clusters is useful in planning crosses for hybrid development (Melchinger et al. 1990; Lanza

Table 2 Morphological characterization of waxy inbreds used in the study

S. no.	Inbreds	MF (days)	FF (days)	EL (cm)	EW (cm)	NKR (number)	NKPR (number)	KW (g)	YLD (t/ha)
1.	MGUWX-101	49	52	9.1	3.0	15.3	19.3	17.6	2.06
2.	MGUWX-102	50	51	9.1	2.4	15.3	20.7	17.9	2.20
3.	MGUWX-103	47	48	8.0	3.9	18.3	16.0	17.0	2.10
4.	MGUWX-104	47	49	8.3	3.6	16.0	18.0	17.2	2.20
5.	MGUWX-105	50	52	12.3	2.9	14.7	16.7	18.4	2.38
6.	MGUWX-106	48	50	11.8	2.9	16.3	18.7	24.9	2.31
7.	MGUWX-107	47	52	10.2	2.7	12.7	14.0	16.8	2.35
8.	MGUWX-108	50	52	12.3	3.0	12.3	17.0	21.6	2.42
9.	MGUWX-109	51	52	9.0	3.0	16.3	11.3	23.0	1.78
10.	MGUWX-110	50	52	13.3	2.9	15.0	21.0	17.1	2.49
11.	MGUWX-111	48	51	8.0	3.5	12.0	14.0	20.3	2.03
12.	MGUWX-112	49	51	9.8	3.3	11.3	14.3	21.6	2.28
13.	MGUWX-113	51	53	8.4	4.0	16.7	13.0	30.6	2.17
14.	MGUWX-114	47	50	8.2	2.7	10.3	12.0	18.5	1.88
15.	MGUWX-115	47	50	8.5	2.3	11.0	13.3	16.5	2.28
16.	MGUWX-116	48	51	9.5	2.4	10.3	15.7	21.3	2.38
17.	MGUWX-117	49	51	14.9	3.1	16.7	26.0	30.8	2.67
18.	MGUWX-118	48	51	8.4	2.7	13.7	15.3	19.0	2.28
19.	MGUWX-119	48	50	9.2	2.8	12.7	15.3	22.1	2.17
20.	MGUWX-120	50	52	7.6	2.8	15.3	13.0	19.0	1.78
21.	MGUWX-121	51	53	9.0	2.9	13.7	14.3	17.4	2.06
22.	MGUWX-122	48	50	9.5	3.2	12.7	14.0	17.2	1.99
23.	MGUWX-123	48	50	10.9	3.8	12.0	20.7	25.6	2.42
24.	MGUWX-124	50	52	8.8	3.0	16.7	16.0	17.4	2.17
Mean		48.8	51.0	9.8	3.0	14.1	16.2	20.4	2.20

MF days to 50% anthesis, *FF* days to 50% silking, *EL* ear length, *EW* ear width, *NKR* number of kernel rows, *NKPR* number of kernels per row, *KW* 100 kernel weight, *YLD* grain yield

et al. 1997; Ajmone-Marsan et al. 1998; Drinic et al. 2002; Aguiar et al. 2008; Park et al. 2008). Since the probability of creating hybrids having high heterosis by crossing among different groups is higher than those crossed within the same group (Hung et al. 2012), a set of potential cross-combinations were identified in the present study. Among the inbreds, nine were of yellow endosperm type, while 15 were having the white endosperm. To develop yellow endosperm-based waxy hybrids, crosses among the inbreds of clusters A2 (MGUWX-101 and MGUWX-103), B (MGUWX-107, MGUWX-109 and MGUWX-110), C1 (MGUWX-113) and C2 (MGUWX-105, MGUWX-111 and MGUWX-112) can be undertaken to achieve higher heterosis for grain yield. For white endosperm-based waxy hybrids, inbreds of clusters A2 (MGUWX-102 and MGUWX-104), B (MGUWX-108 and MGUWX-124), A1 (MGUWX-119, MGUWX-120, MGUWX-121 and MGUWX-122) and C1 (MGUWX-106, MGUWX-114, MGUWX-115, MGUWX-116, MGUWX-117, MGUWX-118 and MGUWX-123) can be crossed to generate potential heterotic hybrid combination. Further, mosaic cobs having mixture of yellow and white kernels have also become popular among the consumers. To develop such type of novel waxy hybrids, yellow inbreds of cluster C2 can be crossed with white inbreds of clusters A1, A2, B and C1. Similarly, MGUWX-101 and MGUWX-103 of cluster A2 (yellow inbreds) can be crossed with white inbreds of A1, B and C1. Further, crosses between yellow endosperm-based inbreds of cluster B (MGUWX-107, MGUWX-109 and MGUWX-110) and white endosperm inbreds of clusters A1 and C1 can also be attempted. Yellow waxy inbred, MGUWX-113 of cluster C1 can be crossed to white inbreds of A1. Molecular marker-based genetic diversity among waxy germplasm of Vietnam (Hung et al. 2012) and Korea (Park et al. 2008) has been used to predict potential waxy hybrid combinations. Further, inbreds of same cluster may be crossed among themselves to develop improved inbreds by accumulating favourable alleles from similar heterotic groups (Melchinger et al. 1990; Lanza et al. 1997; Ajmone-Marsan et al. 1998). Considering the availability of very few reports of molecular characterization of waxy inbreds, the information generated here holds significance in the waxy maize breeding programme in India.

Conclusion

This is the first report of development and molecular characterization of waxy inbreds from India. The present study reported wide genetic diversity among the 24 waxy corn inbreds developed in India. The unique alleles as observed in some of the inbreds can be effective in fingerprinting analysis and registration of the inbreds. The waxy inbreds were also promising for grain yield and other agronomic traits, thereby suggesting their direct utilization in breeding programme. This study also highlighted the coherence of genetic relationship with pedigree data. The identified potential cross-combinations can be effectively exploited in waxy corn hybrid breeding programme to develop high yielding waxy corn hybrids.

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Author contribution ELD conducted the experiment; FH involved in development of waxy inbred; RC and AB contributed to genotyping; RUZ, RC, AB and SKJ participated in data analysis; RUZ and SD involved in phenotyping for morphological characteristics; MV, RUZ and RG participated in field evaluation and maintenance of lines; FH and MV involved in design of experiment and drafting the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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