RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (3) 598-608, 2023
http://doi.org/10.54910/sabrao2023.55.3.1
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

GENETIC PURITY AND DIVERSITY ASSESSMENT OF PARENTAL CORN INBRED LINES USING SSR MARKERS FOR PHILIPPINE HYBRID BREEDING

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SUMMARY

Accurate data and relevant insights on parental corn inbred lines' and hybrids' genetic purity and diversity are essential for hybrid development and seed production. Here, the genetic purity and diversity of 19 parental yellow corn inbred lines were assessed using SSR markers. A total of 91 SSR markers were utilized, of which 61 were highly polymorphic and had high polymorphism information content value (PIC = 0.379). Genetic purity and diversity parameters were calculated from the generated SSR marker data. Observed pairwise genetic distances ranging from 0.257 to 0.808 implied high genetic dissimilarities among the accessions. Cluster analysis separated the parental lines into three distinct subclusters, which can potentially be a basis for generating heterotic groupings among the parental lines. Eight (8) out of the 19 parental lines showed considerable residual heterozygosity of ≤10%. Inbred line CML 431 displayed complete homozygosity across all 61 SSR markers. Inbred lines that have residual heterozygosity of >15% need purification through further breeding and selection. Out of the resultant F_1 hybrids analyzed, only four (4) showed genetic impurity of ≤10%. It may be attributed to the intrinsic genetic impurity of parental line CML 452 (21.67%). In contrast, hybrids generated from two genetically pure parents (e.g., CML 431 and CML 575) showed low to no off-types. Overall, genetic purity and diversity determination of promising parental lines can be valuable for future yellow corn breeding programs in the Philippines.

Keywords: corn, SSR markers, genetic purity, genetic diversity, hybrid development

Key findings: The genotyping strategy employed in the study proved cost-efficient and effective in terms of identifying whether the corn genotypes are genetically pure or contaminated. Corn breeders can utilize the generated clusters as a basis for designing cross-combinations. Finally, the achieved set of SSR markers determined can help further assess successful hybridization among the corn genotypes used in this study.

Citation: Fernandez ECJ, Nuñez JPP, Gardoce RR, Manohar ANC, Bajaro RM, Lantican DV (2023). Genetic purity and diversity assessment of parental corn inbred lines using ssr markers for Philippine hybrid breeding. *SABRAO J. Breed. Genet.* 55(3): 598-608. http://doi.org/10.54910/sabrao2023.55.3.1.

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: February 26, 2023; Accepted: April 25, 2023.
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INTRODUCTION

Corn (*Zea mays* L.) is the second most valuable crop in the Philippines, based on overall value and total area planted (De los Santos *et al.,* 2007). January-March 2022 quarter alone produced around 2.44 million MT of corn, with 80.4% attributed to yellow corn production (Philippine Statistics Authority, 2022a). By the end of 2022, the total harvest area contracted was 627,880 ha, while corn production dipped to 1.98 million MT (Philippine Statistics Authority, 2022b). Adequate production of yellow corn is vulnerable to biotic and abiotic stresses, causing low- and poor-quality grain yields. Thus, there is a continuous demand to improve harvest and develop disease-resistant and stress-tolerant varieties by utilizing heterosis in yellow corn hybrid breeding.

In corn hybrid development, genetic purity maintenance of parental inbred lines and genetic purity testing of resultant F_1 hybrids are crucial quality control genotyping parameters. These assessments are critical due to the stringent intellectual property requirements governing plant breeding and variety registrations across many countries (Semagn *et al.,* 2012). Further, maintaining relatively high genetic purities is vital for the robust agronomic performance of genotypes (Josia *et al*., 2021). Conversely, a parent-offspring test confirms the parentage of a specific hybrid if purely derived from two intended parental inbred lines without any pollen contamination (Gowda *et al.,* 2017). In addition, knowledge of the genetic variation of parental inbred lines would aid in the success of a genetic breeding program. Identifying the most suitable parents (i.e., parents with the best combining abilities) would yield the most genetic gain breeders want.

Using molecular markers can accurately determine genetic purity and parentage confirmation, as well as, analyze genetic diversity. Unlike morphological approaches, such as, the grow-out test (GOT) and biochemical markers, molecular markers, in particular, simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs) are highly polymorphic, not environmentally influenced, reproducible, expressed at all developmental stages, often linked to traits of interest, have known positions in the genome, and can be automated (Gowda *et al.,* 2017). Further, molecular markers are more efficient, saving time and resources (Ertiro *et al.,* 2015; Mushtaq *et al*., 2016).

Parental corn inbred lines have acceptable genetic purity if the proportion of heterozygous loci does not exceed 5%. Inbred lines with residual heterozygosity above 5% are either impure due to genetic contamination or not fixed unless their deliberate maintenance at an early generation during development (Gowda *et al.,* 2017; Josia *et al*., 2021). In addition, by its highly cross-pollinated nature, corn may maintain up to 10% residual heterozygosity in inbred lines (Nepolean *et al.,* 2013). Overall, inbred lines with >95% genetic purity suit commercial corn seed production.

In general, assessing the genetic purity of inbred lines, parent-offspring tests, and genetic diversity analyses are imperative in maintaining the quality of materials and determining the success of hybrid breeding programs in corn.

MATERIALS AND METHODS

Experimental materials

The 19 S5/S6 parental yellow corn inbred lines from CIMMYT and the Institute of Plant Breeding-University of the Philippines Los Baños (IPB-UPLB), Philippines (Table 1) entered genotyping using 91 SSR markers. These markers' selection basis was their bin

Table 1. Passport data and information regarding the 19 yellow corn inbred lines used in the study.

Genotype	Source Germplasm	As parent	Origin	Remarks[1]
CML 431	AMATL	Male	CIMMYT, El Batan, Mexico	DMR; common rust resistance; SCA with 432
CML 432	KTX3752	Male	CIMMYT, El Batan, Mexico	DMR; common rust resistance; SCA with 431
CML 473	P31	Male	CIMMYT, El Batan, Mexico	DMR; ear rot and common rust resistance
CML 425	P31	Male	CIMMYT, El Batan, Mexico	DMR; common rust resistance
Entry 3a	CIMMYT189	Male	CIMMYT, El Batan, Mexico	Extracted from drought-tolerant IPB breeding populations
Entry 3b	CIMMYT189	Male	CIMMYT, El Batan, Mexico	Extracted from drought-tolerant IPB breeding populations
Entry 4b	CIMMYT313	Male	CIMMYT, El Batan, Mexico	Extracted from drought-tolerant IPB breeding populations
Entry 8b	IPBYIL-1	Male	Institute of Plant Breeding, UPLB, Philippines	ACB, rust, stalk rot and earworm resistance.
Entry 8c	IPBYIL-2	Male	Institute of Plant Breeding, UPLB, Philippines	ACB, rust, stalk rot and earworm resistance.
Entry 10	IPBYIL-3	Male	Institute of Plant Breeding, UPLB, Philippines	ACB, rust, stalk rot and earworm resistance.
Entry 1b	CIMMYT155	Male	CIMMYT, El Batan, Mexico	Extracted from drought-tolerant IPB breeding populations
CML 563	WL Composite	Male	CIMMYT, El Batan, Mexico	TLB resistance; drought and waterlogging tolerance;
CML 454	P27	Male	CIMMYT, El Batan, Mexico	Heterotic group B; ear rot and common rust resistance; SCA with CML 287
Pi 17	SMC E9 (Philippine inbred)	Female	Institute of Plant Breeding, UPLB, Philippines	High combining ability with Pi 23
Pi 23	P 3228 (Philippine Inbred)	Female	Institute of Plant Breeding, UPLB, Philippines	High combining ability with Pi 17
CML 287	P24	Female	CIMMYT, El Batan, Mexico	Heterotic group A tester; ear rot and common rust resistance
CML 452	P28	Female	CIMMYT, El Batan, Mexico	Heterotic group B; ear rot and common rust resistance; SCA with CML 287
CML 188	G33Q	Female	CIMMYT, El Batan, Mexico	QPM
CML 575	RCY	Female	CIMMYT, El Batan, Mexico	Heterotic group B; TLB resistance; tar spot and ear rot resistance; MLN and Low N tolerant

[1]Based on passport data derived from CIMMYT Global Maize Program (2015); DMR = downy mildew resistance; QPM = quality protein maize; TLB = turcicum leaf blight; SCA = specific combining ability; MLN = maize lethal necrosis; ACB = Asian corn borer.

locations for each chromosome, extracted from the Maize Genetics and Genomics Database (maizeGDB.org; Woodhouse *et al.* 2021). Polymorphic markers were identified across these parental inbred lines. Further, the genotypes were classified into male (13) and female (6), then crossed to generate promising F_1 hybrids. All supplementary files are accessible through this link: 10.6084/m9.figshare.22696624.

Leaf sampling, DNA extraction, and genotyping

The parental genotypes' planting ensued at E1 experimental station, Barangay Tranca, Bay, Laguna, Philippines, during the dry season (January-April 2022), with the resulting F_1 progenies planted inside a glasshouse at IPB-UPLB. Leaf samples collected from the second

youngest leaf flash of corn plants underwent DNA extraction following the CTAB DNA isolation protocol for corn (CIMMYT, 2005), with modifications. A total of 300 mg of leaf sample was pulverized in liquid nitrogen using a mortar and pestle. Then, 700 µL of CTAB extraction buffer was added to homogenize the tissue. Sample incubation at 65 °C for 60 min in a water bath transpired. Afterward, adding 700 µL of chloroform: isoamyl (24:1) continued, followed by gentle mixing for 1 min at room temperature. Samples were centrifuged at 7,378 × g for 3 min, with each resulting aqueous phase transferred to a new 1.5 mL tube. Adding an equal volume of ice-cold absolute isopropanol (2-propanol) preceded gentle mixing to precipitate the nucleic acid components, followed by centrifugation again at 7,378 × g for 3 mins to form the pellet at the bottom of the tube while discarding each supernatant. The DNA pellet was washed with a solution consisting of 500 µL 76% EtOH, 0.2 M sodium acetate for 20 mins, and again using a solution of 500 µL of 76% EtOH, 10 mM ammonium acetate for 5 mins, then air-dried. The DNA was re-suspended by adding a 50–100 µL Tris-EDTA (TE) buffer with RNase. Isolated DNA for long-term storage was at -20 °C.

Electrophoresis determined the quality and yield of the genomic DNA in 1% UltraPure™ agarose (Invitrogen Corp., Carlsbad, California, USA) in 1× Tris-borate EDTA (TBE) running buffer at 90 V for 40 min, 0.5 µg mL^{-1} ethidium bromide staining, and UV illumination at 300 nm using the Enduro GDS Touch Imaging System (Labnet International, Inc, Edison, New Jersey, USA). DNA concentrations of the samples' estimates were through densitometric comparison with known concentrations of lambda (λ) DNA molecular weight standards (Roche, Basel, Switzerland), then a normalized concentration of 10 ng/µL was prepared for each DNA sample.

Pooling five individual DNA samples per parental genotype constituted a bulk DNA sample for each entry to screen the polymorphic marker core set. On the other hand, 30 singular DNA prepared for each cross underwent genotyping (i.e., hybridity, purity testing). A total of 91 SSR markers used to genotype the parentals resulted in one to three SSR markers identified as specifically polymorphic to the two parents of each hybrid utilized for parent-offspring tests.

PCR amplification reactions carried through with 10 µL total reaction volume consisting of 30 ng genomic DNA, 1× PCR buffer (10 mM Tris pH 9.1 at 20 °C, 50 mM KCl, 0.01% Triton™ X-100) (Vivantis Technologies, Malaysia), 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Promega Corporation, Madison, Wisconsin, USA), 0.2 µM forward and reverse primer (Invitrogen Corp., Carlsbad, California, USA, and Research Genetics Co), and *Taq* DNA polymerase (Vivantis Technologies, Malaysia). These proceeded in the Bio-Rad 96-well T100 PCR thermal cycler (Bio-Rad Laboratories Inc., Hercules, California, USA) with the following temperature profile: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation (94 °C, 30 s), annealing (53 °C – 60 °C depending on the primer pair, 45 s), extension (72 °C, 1 min), and final extension at 72 °C for 5 min. Electrophoresis resolved the PCR amplicons using 8% non-denaturing polyacrylamide gel in 1× Tris-borate EDTA buffer at 100 V for 45–60 min in C.B.S. Scientific Triple Wide Mini-Vertical System™ (C.B.S. Scientific Company San Diego, California, USA) and visualized using 0.5 µg mL^{-1} ethidium bromide staining and UV illumination using the Enduro GDS Touch Imaging System (Labnet International, Inc, Edison, New Jersey, USA). Polyacrylamide gel electrophoretograms were scored manually for the presence or absence of bands representing SSR alleles.

Genetic diversity analyses and parent-offspring test

Screening for polymorphic SSR markers across the parental genotypes went on. Using the polymorphic markers, the computation of the genetic purity of each parental inbred line used the formula below. Parental lines with residual heterozygosity of 5% – 10% and lower are acceptable inbreds.

$$\%\ Heterozygosity = \left(\frac{Number\ of\ heterozygous\ SSR\ loci}{Total\ number\ of\ SSR\ loci}\right)100$$

Since the study used pooled DNA, SSR markers were treated as dominant markers for the subsequent analyses (Mushtaq *et al*., 2016; Guevarra *et al.*, 2022). Allele bands were scored for presence (1) or absence (0), while missing data scoring was -1. Genetic diversity parameters, such as the total number of bands, expected (H_E) heterozygosity, and Shannon's information index (I) calculations were performed using GenAlEx software version 6.51b2 (Peakall and Smouse, 2006) while the polymorphic information content (PIC) was calculated using the formula below for binary data (Roldan-Ruiz *et al.,* 2000).

$$PIC = 2f(1-f)$$

Where f is the frequency of present bands.

The genetic relationships of the parental inbred lines calculations employed an unweighted neighbor-joining algorithm in DARwin 6.0 software (Perrier and Jacquemoud-Collet, 2006). Pairwise dissimilarity matrices obtained used Jaccard's similarity coefficient, generating a dendrogram. For node construction, a bootstrap analysis followed based on 10,000 bootstrap values in DARwin 6.0. The resulting dendrogram refinement engaged FigTree v1.4.4 (Rambaut, 2010). Cluster distinctiveness validation used the calculated cophenetic correlation coefficient (r). Successful true hybrids in two inbred lines, CML 431 and CML 452, with their crosses were identified using one to three polymorphic discriminant markers for both parents.

RESULTS

The results revealed that out of 91 SSR screened markers, 61 were polymorphic across the 19 parental inbred lines. The minimum number of identified polymorphic markers for each chromosome was three for chromosome four, whereas, the maximum was nine for chromosomes two and five. The summary of values for gene diversity, Shannon's information index, and PIC of the 61 polymorphic SSR markers assessed on the 19 parental inbred lines are in Table 2. A total of 160 individual alleles/bands were detected using the 61 polymorphic SSR markers. Gene diversity ranged from 0.052 to 0.500, with an average of 0.335, while PIC values ranged from 0.100 to 0.500, with an average of 0.379. The highest PIC value occurred in loci 56 (dupssr 05), with the lowest PIC values in locus 19 (bngl 166), 26 (umc 1516), 46 (phi 53), 48 (bngl 197), 85 (phi 126), 88 (bngl 238), 116 (bngl 240), and 132 (bngl 666). Shannon's diversity index ranged from 0.123 to 0.693, with an average value of 0.505.

Based on the 61 polymorphic SSR markers, the heterozygosity or the genetic purity of the 19 inbred lines had a mean value of 14.84% (Table 3). Out of the 19, eight parental lines may be true inbreds based on their heterozygosity values (≤10%). Only CML 431 showed 100% genetic purity. In contrast, the remaining parental accessions had residual heterozygosity values ranging from 11.67% to 31.15%. CML 452, used as a female parent, showed a high level of heterogeneity (21.67%) based on the 61 polymorphic markers.

Cluster analysis based on Jaccard's similarity coefficient revealed three distinct groupings (Figure 1). Formed clusters were unique based on a high cophenetic correlation coefficient of r = 0.9508. Cluster I had two parental inbred lines, namely, CML 575 and CML 287. Cluster II comprised eight inbreds, further subdivided into two distinct subclusters, with CML 432 having a distinct subcluster. Lastly, cluster III consisted of two subclusters covering nine parental lines. Some closely related genotypes clustered, such as, entries 3a and 3b, from CIMMYT189; then CML 473 and CML 425, derived from P31, combined. Interestingly, all IPBYIL accessions (i.e., Entries 8b, 8c, and 10) did not cluster.

The parent-offspring test conducted for lines CML 431 and CML 452 and their corresponding hybrids revealed varying genetic impurity levels, ranging from 56% to zero or complete heterozygosity for all hybrids (Table 4). Of the 15 test crosses, four have reputable genetic impurities (≤10%), with test cross CML 575 × CML 431 being completely pure based on the three SSR markers used, specifically for this cross (Supplementary Table 1). Most of the test-crosses with CML 452 used as

Table 2. Summary of genetic diversity parameters of the 19 parental inbred lines using 61 SSR markers.

Parameters	H_E	I	PIC
MEAN	0.335	0.505	0.379
SE	0.011	0.014	0.009

H_E = gene diversity; PIC = polymorphic information content; I = Shannon's information index; SE = standard error.

Table 3. Residual heterozygosity of the 19 parental inbred lines based on 61 polymorphic SSR markers.

Accession	Heterozygous loci (#)	Homozygous loci (#)	Heterozygosity (%)
CML 431	0	56	0.00
CML 432	2	54	3.57
CML 473	3	54	5.26
CML 425	4	57	6.56
Entry 3a	13	48	21.31
Entry 3b	8	51	13.56
Entry 4b	16	45	26.23
Entry 8b	5	54	8.77
Entry 8c	3	55	5.17
Entry 10	8	51	13.56
Entry 1b	14	45	23.73
CML 563	18	41	30.51
CML 454	17	41	29.31
Pi 17	6	55	9.84
Pi 23	5	54	8.47
CML 287	7	53	11.67
CML 452	13	47	21.67
CML 188	19	42	31.15
CML 575	7	53	11.67

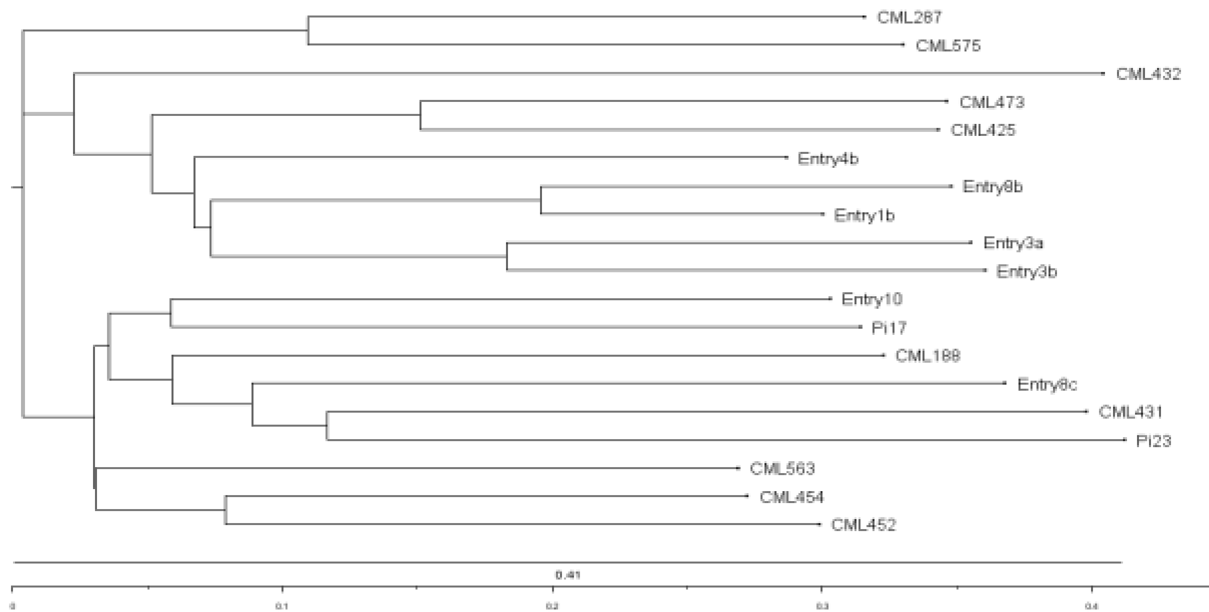

Figure 1. Unweighted neighbor-joining dendrogram depicting the genetic similarities among inbred lines based on 61 polymorphic SSR markers.

Table 4. Genetic purity (%) of hybrids derived from parental inbred lines CML 431 and CML 452.

Female × Male Cross	Mean number of off-types	Mean % off-types
Pi 17 x CML 431	4.00	13
CML 287 x CML 431	1.33	4
CML 452 x CML 431	12.67	44
CML 452 x CML 432	1.33	4.0
CML 452 x CML 473	11	37
CML 452 x CML 425	15.5	55
CML 452 x Entry 3a	15	56
CML 452 x Entry 3b	5	13
CML 452 x Entry 4b	11	39
CML 452 x Entry 8c	3	10
CML 452 x Entry 10	11	39
CML 452 x Entry 1b	16	53
CML 452 x CML 563	13	43
CML 188 x CML 431	9.5	36
CML 575 x CML 431	0	0

the female parent showed high levels of heterozygosity, except for cross-combinations CML 452 × CML 432 and CML 452 × Entry 8c. Supplementary Table 1 shows the list of recommended markers used to test the hybridity of each hybrid. Selected markers could discriminate the alleles found in the two parents of each crossbreed.

DISCUSSION

Sixty-seven percent (61 out of 91) of the SSR markers obtained from the corn database (Woodhouse *et al.,* 2021), previously mapped in the corn genome, produced polymorphic banding patterns. At least three polymorphic SSR markers emerged for each chromosome in corn. The highest numbers appeared in chromosomes two and five, each with nine, whereas, only three polymorphic markers showed for chromosome four. The SSR markers, found polymorphic for two specific parental inbred lines used in this study, would be helpful to validate the genetic purity of resultant F_1 hybrids from their cross. The study tested several SSR markers to confirm the purity of hybrids derived from parental inbred lines CML 431 and CML 452 with other parentals.

PIC estimates the informativeness of a marker about the number of alleles expressed and relative frequencies (Smith *et al.,* 1997; Abakemal *et al*., 2015). For dominant markers, for which we have treated our SSR markers as such due to binary scoring, the PIC value indicates the probability of finding that marker as present or absent in two randomly selected individuals in a population (Serrote *et al.,* 2020). Monomorphic markers show zero value, whereas those present in 50% of individuals have a 0.50 value. The study reports PIC values ranging from 0.100 to 0.500, with a high average of 0.379. PIC is low when values range from 0 to 0.10, medium (0.10 to 0.25), high (0.30 to 0.40), and very high (0.40 to 0.50; Serrote *et al*., 2020). Lopes *et al*. (2015) reported a similar PIC value of 0.41 in their genetic diversity study of 22 sweet corn cultivars, using 45 polymorphic SSR markers. More recently, Elec *et al.* (2022) also conveyed a moderately informative PIC value of 0.37 in their study of 30 waterlogging-tolerant corn inbred lines, using 32 SSR markers. Meanwhile, low PIC values found in several loci (e.g., PIC = 0.100 at Locus 19) due to skewed allele frequency is consistent with the findings in Brazilian popcorn analyzed using 250 microsatellite markers and has not excluded such markers in data analysis (Da Silva *et al*., 2015). Skewed allele frequency in the lines may also be due to its inherited nature, with fixing of alleles in certain loci relative to others.

Knowledge of the genetic relatedness of parental inbred lines would give insight into the performance of their potential single-cross

hybrids (Garcia *et al*., 2004). Cluster analysis implementing genetic distance estimates would provide measures of genetic relatedness of various accessions or individuals in the population. The highest genetic distance of 0.808 showed between corn inbred lines CML 432 and Pi 23. The lowest genetic distance of 0.257 occurred between entries 1b and 8b. A high genetic gap (0.794) resulted between CML 431 and CML 432, accessions with good specific combining abilities (CIMMYT Global Maize Program, 2015). Inversely, entries 8b, 8c, and 10 have moderate genetic distances despite being extracted from the same commercial F_1 hybrid. It suggests that the lines have differentiated during early-advanced generations after F_1 extraction. On average, the inbred lines had a genetic distance value of 0.624 among each other. In a similar study, Adeyemo *et al.* (2011) reported an average genetic distance estimate of 0.45 among 38 tropical yellow corn inbred lines, using 75 polymorphic SSR markers. Here, the genetic distance estimates revealed the accessions' diverse genetic backgrounds, which could further benefit corn breeding programs.

In cross-pollinated crops, such as corn, maintaining the genetic purity of inbred lines is crucial for successful hybrid breeding programs and seed production (Josia *et al.,* 2021). Changes in the constitution of the genotype (i.e., genetic contamination) would affect the quality of developed hybrids and seeds produced. In this study, only CML 431 showed complete homozygosity, while CML 432, CML 473, and Entry 8c showed respectable residual heterozygosity values (≤5%). CML 425, Entry 8b, Pi17, and Pi23 also showed acceptable residual heterozygosity values (≤10%) for corn. Since corn is cross-pollinated, inbreds may tend to segregate for a few loci or characters despite continuous selfing cycles over many generations (Nepolean *et al.,* 2013; Josia *et al.,* 2021). Pollen contamination could have occurred during seed regeneration. Other possible causes of residual heterozygosity may include pollen and/or seed contamination, SSR loci mutations, and amplification of similar but distinct SSR regions (Nepolean *et al.,* 2013; Dias-Maioli *et al.,* 2021), maintenance, and bulking (Warburton *et al.,* 2010). CML 287 and CML 575 may also be true inbreds since their residual heterozygosity values are close to 10%. Other accessions, in particular, CML 188 (31.15%), CML 454 (29.31%), and CML 563 (30.51%), showed very high heterozygosity values. These lines with more than 15% residual heterozygosity are likely to have gained contamination with pollen from unrelated genetic materials and need discarding (Gowda *et al.,* 2017). However, the higher level of heterogeneity observed in some of these inbred lines may be attributable to either that the inbred line is in the early generation of inbreeding or there was pollen contamination and seed admixture during maintenance breeding. Further, it is also possible that higher levels of residual heterozygosity were intentionally maintained at early generations due to strong inbreeding depressions (Josia *et al*., 2021). For instance, inbred lines tested by the Ethiopian Institute of Agricultural Research (EIAR) showed higher levels of heterozygosity due to the use of early-generation ($<S_4$) inbred lines (Ertiro *et al*., 2017). Also, we observed that the Philippine inbred line Pi23 (with a low heterozygosity level of 8.47%) exhibited very low vigor and plant stand (unpublished data) attributed to continuous cycles of selfing and purification, making it challenging to use as a parental for corn hybrid development. Minimizing the strong effect of inbreeding depression requires maintaining a specific degree of heterozygosity in certain parentals for a successful corn hybrid program. Hence, the success of a corn hybrid program necessitates implementing effective corn genetic stock management by utilizing molecular marker technologies to determine the heterozygosity state of the parentals.

The basis for validating parentage is to confirm whether the generated hybrid is a true resultant F_1 progeny from the parental inbred lines with little to no pollen contamination (Gowda *et al*., 2017). It would allow us to determine if the pollination activities performed during hybrid development were diligent. Pollen contamination is particularly likely in corn due to self-pollination or cross-pollination from neighboring plants. In hybrid seed production, breeders opt to ensure generating

of genuine hybrid seeds to exploit heterosis, hence, requiring parentage confirmation.

Here, only four resultant hybrids (CML 287 × CML 431, CML 452 × CML 432, CML 452 × Entry 8c, and CML 575 × CML 431) have respectable genetic impurities (≤10%) out of the crosses derived from CML 431 and CML 452 as source parents. The results may refer to the genetic impurities of our inbred lines rather than possible pollen contaminations during pollination activities. Hybrids derived from parent CML 452 have varying levels of heterozygosity. It may be due to the high residual heterozygosity of this parent (21.67%) leading to genetically impure F_1 progenies apart from hybrids CML 452 × CML 432 and CML 452 × Entry 8c. The study detected a low number of off-types in these two resultant hybrids. Possibly, CML 452 is already fixed with the SSR loci used in testing the hybrids, thus further supporting our claim that the purity of the hybrids was not due to pollen contamination but from the inherent heterozygosity of the parents used. In a similar study, Daniel *et al*. (2012) also reported that hybrids with higher levels of genetic contamination resulted from segregating inbred lines. More recently, the same results came from Josia *et al*. (2021), where 90 out of 158 experimental hybrids failed the parent-offspring test due to a higher contamination percentage. In contrast, CML 431 crossed with inbred parents, also with low residual heterozygosity values, i.e., Pi17, CML 287, and CML 575, generated genetically pure hybrids. Overall, these results confirm that the purity of generated hybrids relies on the purity levels of parental inbred lines used. CML 452 purification is ongoing via selfing and thorough morphological and molecular selection.

CONCLUSIONS

This study presented the analysis of the genetic diversities of inbred lines, which could help exploit heterosis in corn hybrid breeding for economically important traits. The dendrogram generated can help choose genetically distinct parentals to initiate agronomically superior F_1 yellow hybrids. The study also revealed that the set of SSR markers used was reliable in assessing whether the inbred lines used have acceptable genetic purities and, thus, are ready for hybrid production. Ideally, we expect that the inbred lines used to be genetically pure with not more than 5%–10% residual heterozygosity. However, out of the 19 inbred lines, only eight accessions can be concluded to be true inbreds. Consequently, the high residual heterozygosity of parent CML 452 led to genetically impure resultant F_1 hybrids. The inbred lines that failed the genetic purity test conducted using SSR markers should thus proceed with further purification through the ear-to-row method and proper pollination techniques before hybrid production.

ACKNOWLEDGMENTS

This study was part of the Institute of Plant Breeding (IPB) core-funded project entitled "Generation and maintenance of mapping populations and genetic stocks in corn and sorghum" and UPLB Basic Research Project entitled "Differential gene expression analysis of corn in response to downy mildew disease caused by *Peronosclerospora philippinensis.*" The authors acknowledge the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico, for the provision of the CML lines used in the study. Our thanks to Mr. Marvin M. Obando, Mr. Victor V. Moliñawe, Mr. Melvin J. Malison, Mr. Rodelio R. Pia, and the Cereals section of IPB, for their contributions to the generation of hybrids and Ms. Wilermie D. Hernandez, for the laboratory procedures. Further, more thanks to Ms. Ayn Kristina M. Beltran, for her technical knowledge in corn breeding.

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