Current Applied Science and Technology Vol. 20 No. 3 (September-December 2020)

Genetic Diversity of Commercial Field Corn Hybrids in Thailand as Verified by SSR Markers and Their Inbreeding Depression

Terdsak Suwanatape[1], Sansern Jampatong[2] and Choosak Jompuk[1]*

[1]Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand
[2]National Corn and Sorghum Research Center, Faculty of Agriculture, Kasetsart University, Nakhon Ratchasima, Thailand

Received: 22 January 2020, Revised: 26 April 2020, Accepted: 5 June 2020

Abstract

Commercial field corn hybrids are often used for germplasm or for extracting new inbred lines in hybrid breeding programs. However, the commercial single-cross hybrids should be identified before using their genetic materials to increase the effectiveness of new inbred lines and their hybrids. The objective of this study was to evaluate the genetic diversity of commercial single-cross hybrids as verified by Simple Sequence Repeat (SSR) markers and to assess their inbreeding depression using Troyer's genetic diversity. Fifteen commercial field corn single-cross hybrids were selected from private seed companies and public sector agencies and evaluated for genetic diversity using 40 SSR markers. There was a high correlation between Jaccard's index and simple matching index. The UPGMA dendrogram clustered the hybrids into 8 clusters, which showed hybrids within each of four clusters coming from the same genetic sources, except for a group of CP201 and NS3 hybrids that came from different sources. Besides, Troyer's genetic diversity was applied to analyze the genetic diversity of six single-cross hybrids selected from the previous results. The SSR markers and the Troyer's genetic diversity application gave the same direction of corn genetic diversity with a moderate correlation ($r=0.66$, $P<0.01$). Troyer's genetic diversity ranged from 0.47 to 0.91, with an average of 0.72. The commercial field corn single-cross hybrids used in Thailand are still quite diverse, especially those from different seed companies. Therefore, breeders have a good chance to extract inbreeding lines from the commercial single-cross hybrids, and to make a new hybrid with high yield after the grouping of single-cross hybrids through the use of SSR markers or Troyer's genetic diversity.

Keywords: SSR marker, genetic diversity, inbreeding depression, field corn hybrid
DOI 10.14456/cast.2020.28

---

*Corresponding author: Tel.: (086) 9818658 Fax: (034) 351887
E-mail: agrcsj@ku.ac.th

1. Introduction

Corn has been an important economic crop for the feed mill business in Thailand, and Thailand was ranked the 20th largest maize producer in the world [1]. Germplasm is an essential key factor in plant breeding programs for yield improvement and the development of new hybrid varieties. For field corn, commercial single-cross hybrids are often used as the source of germplasm from which to extract new inbred lines in hybrid breeding programs in Thailand. For example, Ki21 (Pacific 9-S_8-45), Ki22 ({Pacific 11 × Suwan 1(S)C_6}-S_8-30), and Ki48 (Pioneer 3013-S_8-57-2) were extracted from single-cross hybrids from private companies [2]. On the other hand, sources of germplasm from public sector agencies, such as the National Corn and Sorghum Research Center, Kasetsart University (Ki48 and Ki60) [2] and the Nakorn Sawan Field Crops Research Center, Department of Agriculture (Takfa1 and Takfa3) [3], were successfully used to develop inbred lines that were sold to private seed companies. From 1980 to 2004, in the USA, new corn inbred lines were extracted from different types of crosses including two-parental lines (77%), BC1 (9%), BC2 (2%), three-parental lines (5%), four or more parental lines (2%), synthetic (1%), synthetic × inbred (2%), and commercial hybrid (3%) [4]. Heterotic patterns, or groups of heterosis, play an essential role in the selection of germplasm sources for the development of new sources of inbred lines. Therefore, methods to identify and group germplasm are of importance in breeding programs. Molecular genetic markers are potent tools to separate and assign single-cross hybrids or inbred lines into heterotic patterns [5, 6]. SSR markers offer more cost-effectiveness over other marker types, as well as reliability for the grouping of corn inbred lines [7-9]. Also, in conventional breeding, the grain yield of hybrids, which are a function of inbreeding depression [expressed as Troyer's genetic distance (TGD)], can be used to determine heterotic groups [10, 11]. Various publications have studied the relationship between molecular marker-based genetic distance (MGD) and hybrid grain yield in tropical and temperate corn cultivars, resulting in different levels of correlation [5, 7, 12]. The objective of this study was to evaluate the genetic diversity of commercial single-cross hybrids as verified by SSR markers and to assess their inbreeding depression expressed as Troyer's genetic distance (TGD) for further improvement of corn germplasm.

2. Materials and Methods

2.1 Plant materials

Fifteen commercial field corn single-cross hybrids were selected from private seed companies and public sector agencies in Thailand (Table 1). SSR markers were used to analyze the genetic distance between these 15 varieties directly.

Table 1. Description of fifteen commercial field corn single-cross hybrids from 5 private seed companies and 2 public sector agencies in Thailand

Private seed companies and public sectors	Varieties
1. Charoen Pokphand Produce Co., Ltd.	CP201, CP888
2. Monsanto (Thailand) Co., Ltd.	DK9901
3. Nakorn Sawan Field Crop Research Center	NS3[1/]
4. Syngenta Co., Ltd.	NK6248, NK7328, NK6253
5. Pacific Seeds (Thai), Ltd.	PAC339, PAC559, PAC777
6. Pioneer Hi-Bred Thailand Co., Ltd.	PIO4311, PIO4546, PIO4181
7. National Corn and Sorghum Research Center	KSX5402[2/], KSX5603[3/]

[1/] Takfa × Takfa 3, [2/] Ki 48 × Ki 60, and [3/] Kei1303 × Ki60

Besides, six single-cross hybrids were selected from the previous analysis to study genetic diversity, as described by Troyer genetic diversity (TGD) [10], comprising PAC777, PAC559, PIO4546, PIO4181, NK6248, and NK7328. These six varieties came from the same and different clusters, as shown in Figure 1. PIO4546 and PIO4181 were in cluster 3, while PAC777 and PAC559 were in cluster 5. On the other hand, NK6248 and NK7328 were in clusters 6 and 4, respectively. These varieties were used in Griffing's method IV resulting in fifteen double-cross hybrids and were self-pollinated to obtain six S_1 lines.

2.2 Field experiments

Field trials were conducted in the dry season of 2016 with three locations at Sukhothai, Saraburi, and Phitsanulok provinces and in the rainy season of 2016 with three locations at Saraburi, Phetchabun, and Phitsanulok provinces. Randomized complete block design (RCBD) with two replications was applied in each location. Each plot consisted of four-rows, 5-meters long, with spacing of $0.75 \times 0.25\ m^2$, and with 21 plants per row. Before sowing, approximately 312.5 kg/ha of basal fertilizer 27-12-6 was applied, and approximately 312.5 kg/ha fertilizer 46-0-0 was applied one month after planting. Herbicide was used according to local practices.

2.3 DNA extraction and genotyping with SSR markers

The leaves of ten-day-old F_1 plants were collected for DNA extraction as described by Sinkangam *et al*. [13]. Two SSR primers, umc1225 (5.08) and bnlg238 (6.00), were used to screen the purity of the sample, which confirmed the purity of DNA, after which it was bulked for the next process. Then, 40 SSR primers were applied to screen the DNA samples that passed the purity test. They were then scored 0 or 1 representing absence or presence of a DNA band, respectively. The SSRs were listed in Table 2. Primer sequences used were sourced from MaizeGDB (www.maizegdb.org; verified 15 September 2019).

2.4 Data analyses

2.4.1 Jaccard's index genetic distance and Cluster dendrogram

The Jaccard's index was calculated based on this formula; $d_{ij} = 1-[a/(a+b+c)]$, where d is the distance between i and j, a attributes both i and j, b attributes only i, and c attributes only j [14]. Moreover, the simple matching's index genetic distance was calculated based on this formula; $d_{ij} = 1-[(a+b)/(a+b+c)]$ [15]. The unweighted pair group method with arithmetic mean (UPGMA) dendrogram of Jaccard's index was obtained from a cluster analysis of fifteen commercial single-cross hybrids using R statistics [16]. For field experiments, average grain yields were measured at 15% moisture content.

2.4.2 Inbreeding depression

The inbreeding depression was analyzed using Troyer's genetic diversity (TGD) as described by Troyer *et al*. [10]. Six single-cross hybrids were selected to study by this method according to the formula; $TGD = 1- [(H-C)/ (H-S)]$, where H is the average yield of the two hybrids, C is the double-cross hybrids, and S is the average yield of the two self-hybrids.

2.4.3 Correlation between Jaccard's index and Troyer's genetic diversity

The Pearson correlation (r) was analyzed between two indices of genetic distances: Jaccard's and Troyer's.

3. Results and Discussion

3.1 SSR primers

3.1.1 Polymorphic information content (PIC)

Forty SSRs were used to analyze 15 single-cross hybrids spanning over ten chromosomes. The number of alleles per SSR locus ranged from 2 to 11, with an average of 5.5 (Table 2). The polymorphic information content (PIC) was significantly different (P<0.05), ranging from 0.33 to 0.88. The average PIC was about 0.67, which was more significant than the value reported by Senior *et al*. [17] with an average of 0.59, indicating greater diversity of corn varieties in Thailand.

Table 2. Numbers of alleles, repeat type and polymorphic information content (PIC) values of 40 SSR loci in 15 single-cross hybrids

Number	SSR locus	Bin location no.^{a}	No. of alleles	Repeat type^a	PIC value
1	bnlg1014	1.01	7	AG(14)	0.80
2	umc2232	1.05	2	(CAC)4	0.47
3	bnlg1025	1.07	7	AG(23)	0.78
4	umc1118	1.11	3	(GAGCA)4	0.51
5	bnlg1327	2.02	6	(GA)6	0.75
6	umc2247	2.04	5	CT(25)	0.59
7	bnlg1138	2.06	7	AG(14)	0.81
8	bnlg1940	2.08	11	AG(18)	0.88
9	umc1970	3.01	6	-	0.71
10	umc1501	3.05	4	(AAG)5	0.67
11	bnlg197	3.06	10	-	0.87
12	umc2152	3.09	3	(TG)8	0.51
13	umc2278	4.01	6	(TCTC)4	0.73
14	umc1652	4.04	4	(CCG)5	0.68
15	umc1940	4.09	3	-	0.35
16	bnlg1890	4.11	11	AG(26)	0.86
17	bnlg1879	5.03	8	AG(14)	0.82
18	umc2296	5.03	3	(AGT)4	0.60
19	umc1221	5.04	9	(CT)7	0.88
20	umc1225	5.08	6	(AG)6	0.74

Table 2. (cont.)

21	bnlg238	6.00	7	-	0.84
22	umc1979	6.04	4	-	0.66
23	umc1352a	6.05	2	(GCC)6	0.44
24	umc1490	6.07	2	(AC)6	0.49
25	umc1426	7.00	3	(AGAGG)4	0.33
26	umc1393	7.02	5	(GTC)4	0.68
27	umc1710	7.04	5	(CTG)5	0.73
28	umc2333	7.05	5	(CCGT)4	0.65
29	umc1735	8.03	8	(AG)40	0.85
30	umc1807	8.03	2	(AGC)5	0.49
31	bnlg666	8.05	9	-	0.80
32	umc1005	8.08	6	(GT)15	0.77
33	bnlg2122	9.01	8	AG(17)	0.74
34	umc1170	9.02	5	(TC)12	0.71
35	umc2338	9.05	2	(GCC)4	0.44
36	umc2346	9.06	4	(TA)7	0.61
37	umc1380	10.00	4	(CTG)5	0.59
38	umc2180	10.03	4	(GGCC)4	0.50
39	bnlg1028	10.06	3	AG(12)	0.62
40	bnlg1450	10.07	10	AG(34)	0.86
Total			219		
Mean			5.50		0.67

[a] Loci and repeat class were referred from MaizeGDB

3.1.2 Indices genetic distances and cluster analysis

Genetic distances based upon variation in SSR profiles between 15 single-cross hybrids were shown according to Jaccard’s index and the simple matching index (Table 3). Jaccard’s index genetic distance ranged from 0.18 to 0.80, with an average of 0.62. On the other hand, the simple matching index genetic distance ranged between 0.06-0.39 with an average of 0.28. However, these indices had a high correlation ($r = 0.98$, $P<0.01$). Simple matching and Jaccard’s indices are rather simplistic and similar in their calculation and have similar metric properties [18]. Nevertheless, the numerator of these indices is different (a vs. a+d) such that the behavior of this similarity index may be data specific [19]. Using SSR-based data to obtain Jaccard’s index, the UPGMA dendrogram showed that the fifteen single-cross hybrids could be classified into groups of hybrids on the index of 0.5, separating into eight clusters (Figure 1); CP201 and NS3 in cluster 1, KSX5603 and KSX5402 in cluster 2, PIO4181, PIO4546 and PIO4311 in cluster 3, NK7328 and NK6253 in cluster 4, PAC559, PAC777 and PAC339 in cluster 5, and NK6248, DK9901 and CP888 in clusters 6, 7 and 8, respectively. Four clusters consisted of hybrids coming from the same genetic sources, including

Table 3. Pairwise genetic distance values of 15 single-cross hybrids; Jaccard’s index (below diagonal) and simple matching’s index (above diagonal)

Hybrids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. NK6248	-	0.28	0.26	0.31	0.29	0.32	0.3	0.28	0.28	0.27	0.28	0.28	0.27	0.3	0.28
2. NK7328	0.63	-	0.28	0.29	0.25	0.21	0.27	0.26	0.26	0.28	0.26	0.2	0.28	0.22	0.27
3. PAC777	0.59	0.61	-	0.21	0.29	0.34	0.27	0.25	0.33	0.33	0.18	0.24	0.32	0.28	0.36
4. PAC559	0.68	0.66	0.51	-	0.29	0.36	0.35	0.27	0.27	0.28	0.19	0.26	0.31	0.31	0.39
5. PIO4546	0.65	0.59	0.63	0.66	-	0.15	0.29	0.21	0.32	0.30	0.29	0.22	0.06	0.23	0.22
6. PIO4181	0.69	0.53	0.70	0.76	0.40	-	0.33	0.25	0.35	0.34	0.29	0.23	0.16	0.27	0.25
7. CP201	0.65	0.60	0.59	0.72	0.63	0.69	-	0.21	0.26	0.31	0.34	0.27	0.29	0.32	0.28
8. NS3	0.64	0.60	0.57	0.62	0.53	0.60	0.50	-	0.26	0.27	0.29	0.23	0.22	0.25	0.27
9. DK9901	0.63	0.61	0.69	0.62	0.69	0.75	0.58	0.60	-	0.23	0.29	0.29	0.33	0.33	0.32
10. CP888	0.60	0.63	0.69	0.63	0.67	0.72	0.66	0.61	0.55	-	0.31	0.32	0.31	0.31	0.33
11. PAC339	0.63	0.59	0.45	0.48	0.65	0.66	0.70	0.66	0.65	0.67	-	0.27	0.32	0.33	0.36
12. NK6253	0.63	0.49	0.56	0.61	0.53	0.57	0.60	0.57	0.66	0.68	0.61	-	0.23	0.28	0.28
13. PIO4311	0.62	0.63	0.67	0.68	0.18	0.43	0.64	0.53	0.71	0.67	0.68	0.56	-	0.26	0.23
14. KSX5603	0.67	0.54	0.62	0.68	0.56	0.62	0.67	0.59	0.71	0.67	0.70	0.63	0.60	-	0.16
15. KSX5402	0.65	0.63	0.74	0.80	0.55	0.61	0.63	0.64	0.71	0.72	0.76	0.65	0.56	0.43	-

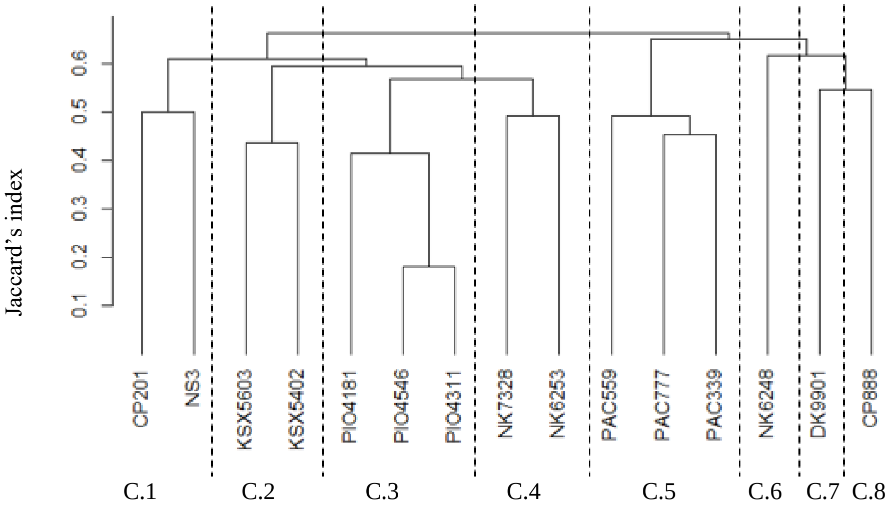

Figure 1. UPGMA dendrogram of 15 single-cross hybrids based on differences in 40 SSR loci of Jaccard's index classified a group of hybrids on the index at 0.5 into eight clusters

hybrids from the National Corn and Sorghum Research Center in cluster 2, Pioneer Hi-Bred in cluster 3, Syngenta in cluster 4, and Pacific Seeds in cluster 5. There was only the group of CP201 and NS3 in cluster 1 that came from different sources, while the remaining three groups of one hybrid each came from each company. These results indicated that hybrids from the same companies or public sector agencies were in the same cluster for at least 4 out of 5 clusters, showing relatedness of the parental lines of hybrids used by each company and public sector agency. For example, hybrids from the public sector, referred to as KSX5603 and KSX5402, used one common parent known as Ki 60 [2]. And Jaccard's index was about 0.43. The highest correlation of genetic distance was between PIO4546 and PIO4311, with an index value of about 0.18, and these hybrids came from the same company. On the other hand, hybrids from different companies were more diverse than those from the same sources, except for CP201 and NS3 in cluster 1. These were more closely related because one parental line of both NS3 (Takfa 3) and CP201 was extracted from the same genetic source, Cargill 5124001 variety (personal communication), indicating the relatedness of these hybrids. Results showed the effectiveness of SSR primers to identify the diversity or relatedness of corn germplasm in Thailand. Based on cluster analysis, six single cross hybrids were selected to study the genetic diversity (GD) as described by Troyer *et al.* [10] including NK6248 (cluster 6), PAC559 and PAC777 (cluster 5), NK7328 (cluster 4) and PIO4546 and PIO4181 (cluster 3). Also, the UPGMA dendrogram from the simple matching index of genetic distance was identical to the dendrogram from Jaccard's index of genetic distance, except for the fact that the value of the index differed.

3.2 Troyer genetic diversity (TGD)

The grain yield of single-cross hybrids (F_1) ranged from 10.26 to 11.67 t/ha and gave an average yield of 11.04 t/ha. At the same time, the grain yield of their selfing (S_1) ranged from 6.48 to 8.36 t/ha and had an average yield of 7.26 t/ha (Table 4). The average grain yield of selfing lines was less than their hybrids by about 34%, indicating inbreeding effect.

Fifteen double-cross hybrids derived from a diallel cross of 6 single-cross hybrids gave grain yields ranging from 8.69 to 10.46 t/ha with an average of 9.54 t/ha (Table 5). The crosses of

PIO4546 × PAC559 and PIO4181 × PAC559 showed the same highest yield of 10.46 t/ha, while the cross of PAC559 × NK7328 gave the lowest yield of 8.69 t/ha.

Table 4. Grain yield (t/ha) of six single-cross hybrids and their selfing (S_1) across 6 environments

	NK6248	**NK7328**	**PAC777**	**PAC559**	**PIO4546**	**PIO4181**	**Mean**
Selfing (S_1)	7.04	6.48	7.22	6.73	8.36	7.73	7.26
Single-cross hybrids (F_1)	11.12	10.79	11.09	11.33	11.67	10.26	11.04

Table 5. The average grain yield (t/ha) of the double-cross hybrids (C) across 6 environments

Hybrids	**NK6248**	**NK7328**	**PAC777**	**PAC559**	**PIO4546**	**PIO4181**
NK6248	-					
NK7328	9.16	-				
PAC777	9.87	9.08	-			
PAC559	9.36	8.69	9.04	-		
PIO4546	9.66	9.41	9.89	10.46	-	
PIO4181	10.18	9.10	10.05	10.46	9.41	-
Mean						**9.54**

To analyze the genetic diversity (GD) as described by Troyer *et al*. [10], the average grain yield of selfing (S_1) and single-cross hybrids (F_1) from Table 4 were taken to measure the average performance of the two hybrids (H), and the two self-hybrids as shown in Table 6.

Table 6. The average grain yield performance (t/ha) of the two hybrids (H) (below diagonal), and the two self-hybrids (S) (above diagonal) across 6 environments

Hybrids	**NK6248**	**NK7328**	**PAC777**	**PAC559**	**PIO4546**	**PIO4181**	**Mean**1/
NK6248	-	6.76	7.13	6.89	7.70	7.38	7.17
NK7328	10.96	-	6.85	6.60	7.42	7.10	6.95
PAC777	11.10	10.94	-	6.98	7.79	7.47	7.24
PAC559	11.22	11.06	11.21	-	7.54	7.23	7.05
PIO4546	11.39	11.23	11.38	11.50	-	8.04	7.70
PIO4181	10.69	10.53	10.68	10.79	10.97	-	7.44
Mean2/	11.07	10.94	11.06	11.16	11.29	10.73	-

1/ self-hybrids, and 2/ hybrids

Troyer genetic diversity (TGD) for each pair of single-cross hybrids was presented in Table 7. The TGD ranged from 0.47 to 0.91 and had an average of 0.72. The TGD of hybrids from the same company ranged from 0.47 to 0.57, including NK6248 and NK7328 (TGD=0.57), PAC559 and PAC777 (TGD= 0.49), and PIO4546 and PIO4181 (TGD=0.47). The results indicated that for each pair of hybrids, both hybrids had one common parent, and the other two inbred lines were genetically unrelated [10]. On the other hand, the TGD of hybrids from different companies ranged from 0.47-0.91, indicating that some of the hybrids from various sources were derived from very diverse parents, e.g., PIO4181 × PAC777 (TGD=0.80), NK 6248 × PIO4181 (TGD=0.85), and PIO4181 × PAC559 (TGD=0.91). Theoretically, TGD = 0.75 means that both hybrids have two genetically unrelated parents, and the other two inbred lines are 50% related. Two unrelated hybrids derived from 4 genetically distinct inbred lines have TGD equal to 1 [10]. The TGD of some hybrids from different sources exhibited as hybrids from the same company, e.g., NK6248 × PAC559

(TGD=0.57), NK6248 × PIO4546 (TGD=0.53), NK7328 × PAC777 (TGD=0.55), NK7328 × PAC559 (TGD=0.52), NK7328 × PIO4181 (TGD=0.58), and PIO4546 × PAC777 (TGD=0.58), but the relatedness was not of a high degree. This result may be different from Jompuk *et al*. [11], indicating that some pairs of hybrids from various sources showed a relatively high degree of relatedness of parents (TGD=0.37). However, genetic distance indices have a value ranging from 0-1, which depends upon the percentage of relatedness of the four parents involved in both hybrids, which is a function of inbreeding depression. Furthermore, heterosis is a cumulative effect of dominant genes and the absence of epistasis [10, 11].

Table 7. Troyer's genetic diversity (TGD) of 6 commercial single-cross hybrids in Thailand

Hybrids	NK6248	NK7328	PAC777	PAC559	PIO4546	PIO4181
NK6248	-					
NK7328	0.57	-				
PAC777	0.69	0.55	-			
PAC559	0.57	0.47	0.49	-		
PIO4546	0.53	0.52	0.58	0.74	-	
PIO4181	0.85	0.58	0.80	0.91	0.47	-
Mean	0.64	0.54	0.62	0.64	0.57	**0.72**

3.3 Correlation between Jaccard's index and Troyer's genetic diversity (TGD)

The correlation coefficients between SSR analysis-based Jaccard's index and grain yield-based Troyer's genetic diversity among six single-cross hybrids were significant across six locations ($r=0.66$, $P<0.01$). This result indicated that both Jaccard's index and Troyer's genetic diversity were directionally identical as indications of corn diversity in Thailand. The yield heterosis and DNA-marker based differences had a positive correlation, which agreed with the results of previous studies using tropical corn [20, 21] and temperate corn [5, 22]. Therefore, both SSR-based analysis and Troyer's genetic diversity of grain yield can be used to separate the heterotic groups of single-cross hybrids before using them as germplasm sources, offering breeders the choice of either molecular markers or conventional breeding methods, depending on their facilities. Most of the inbred lines derived from well-adapted germplasm, either from a narrow genetic base synthetic variety or from well-adapted single crosses which in turn ended up with similar hybrids in the market [23]. So, separating heterotic groups of germplasm sources by the methods in this study will help breeders avoid using too narrow a genetic base in their breeding programs.

4. Conclusions

SSR markers and Troyer's genetic diversity (TGD) indicated the same direction of genetic diversity in commercial field corn hybrids in Thailand. There was a moderate correlation between molecular markers and inbreeding depression, as verified by Troyer's genetic diversity. TGD ranged from 0.47 to 0.91, with an average of 0.72. The UPGMA dendrogram clustered the hybrids into 8 clusters, which showed hybrids within each of four clusters coming from the same genetic sources, except for a group of CP201 and NS3, which came from different sources. The commercial field corn hybrids sold in Thailand are still more diverse, especially those from different companies. Therefore, corn breeders have a good chance to extract inbreeding lines from the commercial single-cross hybrids available on the market, and to make a new single-cross hybrid with high yield, after the grouping of single-cross hybrids by SSR markers or by Troyer's genetic diversity method.

5. Acknowledgements

The authors are grateful to the Charoen Pokphand Produce Co., Ltd., Sathorn, Bangkok, Thailand, for supporting this research.

References

[1] Napasintuwong, O., 2015. Structure of maize seed industry in Thailand. *Proceedings of the 37th National Corn and Sorghum Research Conference*. The Greenery Resort Khao Yai, Nakhon Ratchasima, Thailand, August 5-7, 2015, 30-38.

[2] Jampatong, S., 2015. Kasetsart inbred lines released from 1982-2012. Handout of the demonstration field. *Proceedings of the 37th National Corn and Sorghum Research Conference*. The Greenery Resort Khao Yai, Nakhon Ratchasima, Thailand, August 5-7, 2015, 1-10.

[3] Koshawatana, C., Grudloyma, P. and Indan, W., 2010. Inbred planting technique for Nakhon Sawan 3 hybrid maize seed production. *Kasetsart Journal (Natural Science)*, 44, 776-782.

[4] Mikel, M.A. and Dudley, J.W., 2006. Evaluation of North American dent corn from public to proprietary germplasm. *Crop Science*, 46, 1193-1205.

[5] Melchinger, A.E., 1993. Use of RFLP markers for analyses of genetic relationships among breeding materials and prediction of hybrid performance. In: D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen and R.F. Wilson, eds. *International Crop Science I*. Madison: Crop Science Society of America, pp. 621-628.

[6] Smith, J.S.C., 1988. Diversity of United States hybrid maize germplasm: Isozymic and chromatographic evidence. *Crop Science*, 28, 63-69.

[7] Xu, S.-X., Liu, J. and Liu, G.-S., 2004. The use of SSRs for predicting the hybrid yield and yield heterosis in 15 key inbred lines of Chinese maize. *Hereditas*, 141, 207-215.

[8] Xia, X.C., Reif, J.C., Hoisington, D.A., Melchinger, A.E., Frisch, M. and Warburton, M.L., 2004. Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: Lowland tropical maize. *Crop Science*, 44, 2230-2237.

[9] Xia, X.C., Reif, J.C., Melchinger, A.E., Frisch, M., Hoisington, D.A., Beck, D. and Warburton, M.L., 2005. Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers. II: Subtropical, tropical midaltitude, and highland maize inbred lines and their relationships with elite U.S. and European maize. *Crop Science*, 45, 2573-2582.

[10] Troyer, A.F., Openshaw, S.J. and Knittle, K.H., 1988. Measurement and genetic diversity among popular commercial corn hybrids. *Crop Science*, 28, 481-485.

[11] Jompuk, C., Samphantharak, K. and Chowchong, S., 2000. Genetic diversity of corn hybrids from different sources in Thailand as verified by their heterotic pattern and inbreeding depression. *Kasetsart Journal (Natural Science)*, 34, 205-209.

[12] Barbosa, A.M.M., Geraldi, I.O., Benchimol, L.L., Garcia, A.A.F., Souza Jr., C.L. and Souza, A.P., 2003. Relationship of intra- and inter-population maize single crosses hybrid performance and genetic distances computed from AFLP and SSR markers. *Euphytica*, 130, 87-99.

[13] Sinkangam, B., Stamp, P., Srinives, P., Jompuk, P., Mongkol, W., Porniyom, A., Dang, N.C. and Jompuk, C., 2011. Integration of quality protein in waxy maize by means of simple sequence repeat markers. *Crop Science*, 51, 2499-2504.

[14] Jaccard, P., 1901. Distribution de la flore alpine dans le Bassin des Drouces et dans quelques regions voisines. *Bulletin de la Société Vaudoise des Sciences Naturelles*, 37(140), 41-272.

[15] Everitt, B.S., Landau, S., Leese, M. and Stahl, D. 2011. *Cluster Analysis*. 5th ed. Chichester: John Wiley & Son Inc.

[16] R Core Team., 2019. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*. [online] Available at: https://www.r-project.org/ index.html

[17] Senior, M.L., Murphy, J.P., Goodman, M.M. and Stuber, C.W., 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science*, 38, 1088-1098.

[18] Jackson, D.A., Somers, K.M. and Harvey, H.H., 1989. Similarity coefficients: Measurements of co-occurrence and association or simply measures of occurrence? *American Naturalist*, 133, 436-453.

[19] Hubalek, Z., 1982. Coefficients of association and similarity based on binary (presence-absence) data: An evaluation. *Biological Reviews,* 57, 669-689.

[20] Betran, F.J., Ribaut, J.M. Beck, D. and Gonzalez de Leon, D., 2003. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. *Crop Science*, 43, 491-496.

[21] Phumichai, C., Doungchan, W., Puddhanon, P., Jampatong, S., Grudloyma, P., Kirdsri, C., Chunwongse, J. and Pulum, T., 2008. SSR-based and grain yield-based diversity of hybrid maize in Thailand. *Field Crops Research*, 108, 157-162.

[22] Smith, O.S., Smith, J.S.C., Bowen, S.L., Tenborg, R.A. and Wall, S.J., 1990. Similarities among a group of elite maize inbreds as measured by pedigree, F1 grain yield, grain yield, heterosis, and RFLPs. *Theoretical and Applied Genetics,* 80, 833-840.

[23] Hallauer, A.R., Russell, W.A. and Lamkey, K.R., 1998. Corn breeding. In: G.F. Spraque and J.W. Dudley, J.W., eds. *Corn and Corn Improvement.* Agron. Monogr. 18. ASA, CSSA and SSSA, Madison, WI, pp. 463-564.