

Studies on applying SNP markers to breeding drought-tolerant maize hybrids

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Abstract:

The results of testing the International Maize and Wheat Improvement Center's (CIMMYT's) hybrid combinations, developed from hybrid 790 F_{2,3} lines and 10 parental lines, using two testers (CML451 and CLO2450) under optimal and managed drought conditions in Ninh Thuan, Vietnam, show that the average grain yield of the biparental (BP) groups of heterosis groups A and B is, respectively, 2.58-3.65 tons/ha and 2.56-2.76 tons/ha in drought conditions, and 4.24-5.02 tons/ha and 5.41-5.93 tons/ha in well-watered conditions, respectively. By genotyping eight BP populations with 39,846 single nucleotide polymorphism (SNP) markers, CIMMYT experts identified 15 important gene regions that regulate grain yield associated with 15 SNP markers on chromosomes 3, 4, 5, 6, 7, 8, 9, and 10 which is useful for applying molecular markers in breeding drought-tolerant maize. On that basis, the Maize Research Institute of Vietnam studied and genotyped three populations, including 450 F₂ families, with 96 SNPs using the Kompetitive Allele Specific PCR (KASP) genotyping method. The result was that 57 SNP markers related to drought tolerance were found useful to these populations. In addition, 27 F₂ families demonstrating drought tolerance and high grain yield were selected as primary materials for breeding maize hybrids tolerant to stresses and adaptive to climate change.

Keywords: drought, GWAS, KASP, maize, optimal conditions, SNP markers.

Classification number: 3.1

Introduction

Maize (*Zea mays* L.) is one of the three most important cereal crops after wheat and paddy rice. World maize production amounted to 1,075.6 million tons in the 2017/2018 crop year (USDA, 2019). However, climate change has become a considerable challenge for global maize production and led to a 3.8% reduction in yield from 1980 to 2008 [1].

Vietnam is one of the countries most affected by climate change, with a number of serious droughts occurring in the 2015-2017 period. With around 80% of the cultivated area under rainfed condition, drought is considered the biggest challenge for maize production in Vietnam [2]. Therefore, the research and selection of drought-tolerant maize varieties that have high grain yield and the ability to adapt to climate change are of great interest to maize breeders. However, drought tolerance is a low-heritability trait that is regulated by multiple genes; it requires substantial money and time to accomplish these daunting research and selection tasks. Fortunately, genomic selection (GS) by means of mapping quantitative trait loci (QTL) relating to drought tolerance using molecular markers is an efficient and time-saving tool in plant breeding. It results in the achievement of greater breeding value through selection at the early stages of the improvement cycle [3]. Currently, using single nucleotide polymorphisms (SNPs) is becoming more common in plant breeding through marker-assisted selection and is replacing simple sequence repeats (SSRs) for crops, such as maize, whose genomes have been completely sequenced [4].

Applying SNP markers using the Kompetitive Allele Specific PCR (KASP), a technique for genotyping, has been widely used in research because it is cheaper than BeadXpress and GoldenGate platforms, more effective and flexible in many applications, saves time, and produces fewer genotyping errors [4]. Currently, KASP is used by

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CIMMYT for the global maize improvement programme and in quality control QC analysis, QTL mapping, marker-assisted recurrent selection (MARS), genome-wide association studies (GWAS), allele mining [5].

Genetic selection based on SNP markers (KASP and Tagman) is more than 2-4 times effective than the traditional selection method that is based only on phenotype. In drought conditions, the genetic gains in grain yield per cycle using the GS method with KASP markers is 86 kg/ha, without changing the traits of maturity and plant height [6]. Furthermore, F. Bankole, et al. [7] indicate that, in each selection cycle using MARS with SNP markers, grain yield increased by 7% in drought conditions, and the frequency of favourable alleles increased from 0.510 at the original population (C_0) to 0.515 at the selection cycle C_2 of the MARS population.

In Vietnam, the application of SNP markers by means of the KASP method has been used to evaluate and select maize materials tolerant to stresses, especially drought. With the support and advice of CIMMYT's experts in cooperation programmes, the application of SNP markers in MRI's drought-tolerant maize breeding has been studied.

Materials and methods

CIMMYT's hybrid combinations

One thousand five hundred and eighty hybrid combinations developed by CIMMYT from 790 $F_{2,3}$ lines and two testers (tester 1: CML451; tester 2: CLO2450) and 20 hybrid combinations (of 10 parental lines with these testers) were evaluated for drought tolerance with five local checks of LVN10, LVN61, VN8960 (MRI, Vietnam), NK67 (Syngenta), and C919 (Monsanto) in the 2013/2014 dry season in Ninh Thuan.

Leaf samples

The 790 $F_{2,3}$ families developed by CIMMYT by crossing two drought tolerant maternal lines with eight elite ones (divided into two heterosis groups) were collected for genotyping with 1,250 SNP markers which were identified from 1,536 SNP markers, as per Yan, et al. [8].

MRI's three F_2 populations

These F_2 populations were developed from CML161 (one drought-tolerant female line) and the MRI's three male elite ones (TA6, P24 and G12). One hundred and fifty F_1 individuals of each population were selected based on the criteria of growth and development, drought tolerance, and pest resistance in order to self-pollinate and form F_2 seeds; the F_2 seeds of each population were planted in 150 rows (row length: 4.0 m; distance between hills: 0.7 m). The F_2

families of each population (150 F_2 families per population) were evaluated under managed drought conditions in Ninh Thuan. Leaf samples of three F_2 populations including 450 F_2 families (6-8 plants per family) grown at MRI were collected for genotyping with 96 SNP markers (*The physical positions of these SNP markers was determined on the maize genome chromosomes according to B73 RefGen_v2 at Maize GDB*) using the KASP method to select F_2 families capable of drought tolerance.

Methodologies of phenotyping

The experiments were conducted in field conditions designed using Latin squares (*Alpha lattices*). For trials testing CIMMYT's hybrid combinations: row length: 4.0 m; distance between rows: 0.75 m; distance between hills in the row: 0.25 m. The experiments were evaluated under managed drought and optimal conditions in Ninh Thuan according to CIMMYT's guidelines [9]. For testing MRI's F_2 populations: row length of 4.0 m at a spacing of 0.7 m by 0.2 m; evaluated under managed drought conditions in Ninh Thuan using CIMMYT's guidelines [10].

Methodologies of the GWAS

Genotypic and phenotypic data on grain yield were analysed using 55K models (56,110 SNP markers) and GBS v2.7 (954,179 SNP markers). Genotyping with 55K MaizeSNP50 from Illumina (www.illumina.com), while the SNP marker positions of GBS and 55K were sourced from Panzea_2.7GBS (http://plants.ensembl.org/Zea_mays/Info/Index). Based on standard requirements, a minimum allele frequency >0.05 for 55K and >0.02 for GBS; 39,846 SNP markers from 55K chips and 435,975 SNP markers from GBS were selected for genotyping.

Methodologies of genotyping using the KASP method

KASP is a technique for genotyping with SNP markers [4] and consists of three components: the KASP assay mix, the KASP master mix, and DNA samples. The procedures were conducted according to the instructions of LGC Genomics Ltd (details at <http://www.lgcgroup.com>).

Phenotypic analysis

Analysis of variance of the genotype and phenotype (σ_g^2 and σ_p^2) and heritability (h^2) were calculated using the formula suggested by Lush, et al. [11] with GenStat 12.0, METAR 2.1, and Fieldbook software (CIMMYT, 2010). The multivariate restricted maximum likelihood model and SAS ver. 9.2 software were used to calculate genetic variance and covariance.

Genotypic analysis

For GWAS with SNP markers, the multi-locus mixed

additive model was used [12]. The genotypic analysis was conducted with Variation Suite ver. 8.3.4 software.

Results

Testing of hybrid combinations developed from 790 F_{2:3} families by CIMMYT in drought and optimal conditions in Ninh Thuan province, Vietnam

Phenotyping results: through testing, it was shown that the grain yield of hybrid combinations developed from crossing 790 F_{2:3} families, parental lines of heterosis group A, and parental lines of heterosis group B with two testers in drought conditions decreased from 27.23 to 54.16%, from 16.2 to 100.0%, and from 5.88 to 83.3%, respectively, compared to well-watered conditions (Tables 1 and 2).

Table 1. Average grain yield of hybrid combinations (heterosis group A with two testers) in the 2013/2014 dry season under managed drought and optimal conditions in Ninh Thuan.

Heterosis group A	Conditions	Grain yields (tons/ha)									
		BP1 × testers	BP2 × testers	BP3 × testers	BP4 × testers						
F _{2:3} × CT±Std	Drought	3.65±1.87	2.86±1.77	3.18±1.68	2.58±1.53						
	Optimal	5.02±1.53	4.24±1.71	4.66±1.82	4.58±1.79						
	Reduction %	27.23	32.53	31.72	43.75						
Variation	Drought	0.30±6.89	0.00±6.32	0.00±6.54	0.00±6.22						
	Optimal	0.23±8.29	0.23±7.86	0.00±7.6	0.00±7.23						
σ _e ²	Drought	2.18	1.80	1.98	1.29						
	Optimal	1.30	1.80	1.19	1.47						
σ _g ²	Drought	0.45	0.41	0.46	0.78						
	Optimal	0.77	0.72	1.20	0.95						
h ²	Drought	0.29	0.31	0.32	0.55						
	Optimal	0.54	0.44	0.67	0.56						
P.e × testers±Std		P1 × testers	P2 × testers	P3 × testers	P4 × testers						
P.e × tester 1 (CML415)	Drought	5.56±0.86	0.00±0.00	2.83±1.91	0.97±0.42						
	Optimal	6.29±1.35	0.23±0.11	4.8±0.83	3.01±2.31						
	Reduction %	11.61	100.00	41.04	67.77						
P.e × tester 2 (CLO2450)	Drought	4.62±0.53	0.00±0.00	4.77±0.63	1.19±0.70						
	Optimal	5.84±0.99	0.53±0.04	5.97±1.37	3.86±0.69						
	Reduction %	20.89	100.00	20.10	69.17						
P.dr × testers±Std		P9 × Testers									
P.dr × tester 1 (CML415)	Drought	2.17±1.23									
	Optimal	3.79±1.26									
	Reduction %	42.74									
P.dr × tester 2 (CLO2450)	Drought	4.08±1.22									
	Optimal	4.87±0.72									
	Reduction %	16.22									
LSD _{0.05}	Drought	2.90	2.60	2.80	2.20						
	Optimal	2.20	2.60	2.10	2.40						
CV (%)	Drought	40.90	47.10	43.20	43.40						
	Optimal	22.80	31.60	23.60	26.40						
Local checks		LVN10	LVN8960	NK67	C919	LVN61					
Conditions		Drought	Optimal	Drought	Optimal	Drought	Optimal	Drought	Optimal		
Grain yields (tons/ha)		3.15	4.79	4.25	5.26	5.00	6.12	4.47	7.01	4.76	7.36
Reduction %		8.02	12.15	13.05	35.62	20.35					

Note: x: cross; ±Std: standard deviation; ±: variation between minimum and maximum values; BP: Bi-parent; P: parental lines; P.e: elite lines (P1 to P8); P.dr: drought tolerant lines (P9 and P10); reduction %: the rate of reduction in grain yield in drought conditions compared to optimal conditions (%); σ_e²: error variation; σ_g²: genotype variation; CV (%): coefficient of variation; LSD_{0.05}: least significant difference at a 95% confidence level.

Table 2. Average grain yield of hybrid combinations (heterosis group B with two testers) in the 2013/2014 dry season under managed drought and optimal conditions in Ninh Thuan.

Heterosis group B	Conditions	Grain yields (tons/ha)									
		BP5 × testers	BP6 × testers	BP7 × testers	BP8 × testers						
F _{2:3} × testers±Std	Drought	2.76±1.64	2.75±1.59	2.72±1.58	2.56±1.64						
	Optimal	5.89±1.54	5.65±1.43	5.93±1.59	5.41±1.25						
	Reduction %	53.14	51.40	54.16	52.72						
Variation	Drought	0.00±6.97	0.00±7.18	0.00±5.91	0.00±6.39						
	Optimal	0.20±9.57	0.00±8.57	0.53±8.79	0.00±8.67						
σ _e ²	Drought	2.17	0.34	1.80	1.79						
	Optimal	1.48	0.57	1.49	0.95						
σ _g ²	Drought	0.15	0.00	0.48	0.12						
	Optimal	0.75	0.00	0.67	0.51						
h ²	Drought	0.12	0.34	0.35	0.12						
	Optimal	0.50	0.57	0.48	0.52						
P.e × testers±Std		P5 × testers	P6 × testers	P7 × testers	P8 × testers						
P.e × testers 1 (CML415)	Drought	2.99±1.38	0.75±0.76	2.73±1.18	1.43±1.27						
	Optimal	4.84±0.43	4.49±0.74	6.27±0.74	5.01±0.52						
	Reduction %	38.22	83.30	56.46	71.46						
P.e × testers 2 (CLO2450)	Drought	3.42±1.04	2.68±0.93	0.83±0.71	3.17±1.18						
	Optimal	3.60±0.79	5.45±0.18	4.32±0.23	6.36±1.06						
	Reduction %	5.88	50.83	80.79	50.16						
P.dr × testers±Std		P10 × testers									
P.dr × testers 1 (CML415)	Drought	1.12±0.63									
	Optimal	5.14±1.17									
	Reduction %	78.21									
P.dr × tester 2 (CLO2450)	Drought	3.17±2.09									
	Optimal	6.35±0.70									
	Reduction %	50.08									
LSD _{0.05}	Drought	2.90	2.60	2.60	2.60						
	Optimal	2.40	2.00	2.40	1.90						
CV (%)	Drought	53.50	47.90	48.50	51.90						
	Optimal	20.70	18.30	20.80	18.00						
Local checks		LVN10	LVN8960	NK67	C919	LVN61					
Conditions		Drought	Optimal	Drought	Optimal	Drought	Optimal	Drought	Optimal		
Grain yields (tons/ha)		3.15	4.79	4.25	5.26	5.00	6.12	4.47	7.01	4.76	7.36
Reduction %		8.02	12.15	13.05	35.62	20.35					

Note: x: cross; ±Std: standard deviation; ±: variation between minimum and maximum values; BP: Bi-parent; P: parental lines; P.e: elite lines (P1 to P8); P.dr: drought tolerant lines (P9 and P10); reduction %: the rate of reduction in grain yield in drought conditions compared to optimal conditions (%); σ_e²: error variation; σ_g²: genotype variation; CV (%): coefficient of variation; LSD_{0.05}: least significant difference at a 95% confidence level.

In drought conditions, the average grain yield of hybrid combinations of BP groups of heterosis group A with testers reached 2.58-3.65 tons/ha, of which the combinations developed from BP1 had the highest yield (3.65 tons/ha) and the least reduction (27.23%) (Table 1). Hybrid combinations created from F_{2,3} families of heterosis group B with these testers showed no differences in grain yield, with the range of 2.56 to 2.76 tons/ha (Table 2).

In optimal conditions, the average grain yield of hybrid combinations among BP groups of heterosis groups A and B with testers reached 4.24-5.02 tons/ha and 5.41-5.93 tons/ha, respectively. The yield of hybrid combinations of the two drought-tolerant lines (P9 and P10) with these testers in drought conditions decreased by 42.74-78.21% for tester 1 and by 16.22-50.08% for tester 2 compared to those in optimal conditions. The results indicate that hybrid combinations derived from P9 and P10 with tester 2 demonstrate better drought tolerance. In drought conditions, the yield of hybrid combinations developed from elite lines with two testers also decreased, by 27.23-43.75% for group A and by 51.40-54.16% for group B, compared to those in the optimal condition. Thus, the progenies of group A showed a smaller reduction in grain yield than did those of group B did in dehydrated conditions. In other words, the hybrid combinations that originated in group A had better tolerance to drought than did those that originated in group B (Tables 1 and 2).

Compared to the grain yield of five local checks, the highest yield of hybrid combinations developed from F_{2,3} families with testers in drought and optimal conditions was, respectively, 6.89 tons/ha and 8.29 tons/ha (for group A), and 7.18 tons/ha and 9.57 tons/ha (for group B) - higher than these of local checks (3.15-5.00 tons/ha in drought conditions; and 4.79-7.36 tons/ha in optimal conditions, with a reduction in grain yield of 8.02-35.62%) (Tables 1 and 2).

This result is significant because it was found that among 790 F_{2,3} families developed from eight BP lines, some showed better drought-tolerance ability, were higher in grain yield than their parental lines, and, especially, reached a yield equivalent to the five local checks. These families can potentially be selected as materials and germplasms for a drought-tolerant maize breeding programme in order to adapt to climate change.

Genome-wide association analysis for grain yield of eight progenic populations F_{2,3}

The MRI is a member of the project “Abiotic stress tolerant maize for increasing income and food security among the poor in South and Southeast Asia”. Experts from CIMMYT conducted GWAS with 39,846 SNP markers

for 790 F_{2,3} families of eight populations. As the result, 15 genomic regions controlling the trait of grain yield in drought conditions were identified. These regions associating with the SNPs markers include S3_151334181, S4_224910359, S5_208101878, S6_67260174, S7_40327099, S8_144372859, S9_88734345, S9_82359236, S9_154651413, S9_151662859, S9_100305550, S9_96774495, S9_11501850, S10_137460286, and S10_147354987 (from Panzea_2.7 GBS) on chromosomes 3, 4, 5, 6, 7, 8, 9, and 10 (Table 3) and can be significant for drought-tolerant maize breeding programmes.

Table 3. The list of 15 genomic regions controlling grain yield for BP populations of the F_{2,3} generation through GWAS of each chromosome.

SNP markers	Chr	GWAS Marker DD-dd	Marker location	Loci										Minor allele	Allele frequency	Major allele
				P1	P2	P3	P4	P5	P6	P7	P8	BP/P9	BP/P10			
S3_151334181	3	0.59	151.334.181	C/C	C/C	G/G	C/G	C/G	C/G	C/C	C/C	C/C	C/C	G	0.11	C
S4_224910359	4	8.50	224.910.359	C/C	T/T	T/T	T/T	C/T	T/T	C/C	C/C	C/C	C/C	T	0.30	C
S5_208101878	5	7.32	208.101.878	T/T	T/T	T/T	T/G	T/T	T/T	T/G	T/T	T/T	G/G	G	0.26	T
S6_67260174	6	0.53	67.260.174	C/C	C/C	C/C	C/A	C/A	C/A	A/A	A/A	C/C	C/C	A	0.25	C
S7_40327099	7	7.99	40.327.099	G/G	G/G	G/G	G/A	G/G	G/G	A/A	G/A	G/G	G/G	A	0.08	G
S8_144372859	8	1.99	144.372.859	T/T	C/C	C/C	C/C	C/C	C/C	T/T	T/T	C/C	C/T	T	0.31	C
S9_88734345	9	8.70	88.734.345	A/A	A/A	A/A	A/A	A/A	A/G	A/A	A/A	A/A	G/G	G	0.20	A
S9_82359236	9	7.84	82.359.236	C/C	C/C	C/C	C/A	C/C	C/C	C/C	C/C	C/C	A/A	A	0.19	C
S9_154651413	9	4.30	154.651.413	A/A	C/C	C/C	A/C	A/A	A/A	A/A	C/C	A/A	C/C	C	0.43	A
S9_151662859	9	-2.64	151.662.859	T/T	T/T	A/A	T/T	T/A	T/T	T/T	T/T	T/T	T/T	A	0.06	T
S9_100305550	9	-5.50	100.305.550	G/G	T/T	T/T	T/T	T/T	G/G	G/G	T/G	T/T	T/T	G	0.18	T
S9_96774495	9	-5.54	96.774.495	G/G	A/A	A/A	A/A	A/A	G/G	G/G	A/A	A/A	A/A	G	0.18	A
S9_11501850	9	-5.85	11.501.850	C/C	C/C	C/C	C/C	C/C	C/C	G/G	G/G	C/C	C/C	G	0.10	C
S10_137460286	10	0.02	137.460.286	G/G	C/C	G/G	C/G	G/G	C/G	C/C	C/G	C/C	C/C	G	0.26	C
S10_147354987	10	-1.87	147.354.987	T/C	T/T	C/C	T/C	T/T	C/C	T/T	T/C	T/T	C/C	C	0.46	T

Note: P: parental lines; BP/P: populations developed from parent pairs; DD-dd: homozygous.

Identifying materials tolerant to drought with SNP markers by means of KASP method

Based on the results of phenotyping and genotyping with SNP markers of the cooperation programme with CIMMYT, the MRI conducted initial research on identifying materials tolerant to drought with SNP markers by means of the KASP method and evaluated their drought tolerance in Ninh Thuan in the 2018/2019 dry season.

Results of phenotyping three populations including 450 F₂ families: it has been shown that the yield of three populations in drought conditions, in which had been selected 27 F₂ families with grain yields equivalent to local check DK7328 and higher than the yield of NK67,

Table 4. Results of evaluating the grain yield of three populations (including 450 F₂ families) under managed drought conditions in Ninh Thuan.

Statistical Indices	Grain yields (tons/ha)					
	CML161 x TA6	CML161 x P24	CML161 x G12			
F ₂ ±Std	1.20±0.52	1.33±0.52	1.00±0.45			
Variation	0.01÷3.14	0.00÷3.25	0.66÷3.40			
σ _p ²	3.01	4.83	4.12			
σ _e ²	0.13	0.14	0.10			
σ _g ²	1.67	3.44	3.08			
h ²	0.56	0.71	0.75			
LSD _{0.05}	1.02	1.03	0.88			
CV (%)	42.91	39.64	45.41			
Parental lines	CML161	TA6	CML161	P24	CML161	G12
Grain yields (tons/ha)	0.580	0.247	0.948	0.368	0.812	0.513
Local checks	NK7328	NK67	NK7328	NK67	NK7328	NK67
Grain yields (tons/ha)	2.455	2.327	2.801	2.618	3.041	2.803

Note: x: cross; ±Std: standard deviation; σ_p²: phenotype variation; σ_e²: error variation; σ_g²: genotype variation; h²: heritability; CV (%): coefficient of variation; LSD_{0.05}: least significant difference at a 95% confidence level.

varies from 1.00 to 1.33 tons/ha (Tables 4 and 5). The heritability (h²), which was from 0.56 to 0.75, showed that the relationship between phenotype and genotype of these populations was positive. Genotypic variance (σ_g²) on grain yield in drought conditions ranged from 1.67 to 3.44, leading to the conclusion that variation in grain yield was mainly affected by male lines (TA6, P24, and G12).

Results of genotyping three populations including 450 F₂ families and parental lines: through genotyping 450 F₂ families and four parental lines with 96 SNP markers using the KASP technique combined with CIMMYT's researched data, the initial results showed that there were 57 meaningful SNP markers in these populations of 450 F₂ families and that these markers could be related to yield in drought conditions (Table 6). The trait of grain yield is controlled by many genes and the interaction among major and minor loci that affect this trait in drought conditions. Hence, potential SNP markers identified through the KAPS method can be useful for breeding drought-tolerant maize.

Table 5. Selected families of three populations under managed drought conditions in Ninh Thuan.

F ₂ families	Pedigree	GY (tons/ha)	F ₂ families	Pedigree	GY (tons/ha)
Population 1: CML161xTA6			Population 2: CML161xP24		
BP1_110	(CML161xTA6)-110	3.14	BP2_188	(CML161xP24)-188	3.25
BP1_107	(CML161xTA6)-107	2.92	BP2_181	(CML161xP24)-181	3.10
BP1_101	(CML161xTA6)-101	2.91	BP2_239	(CML161xP24)-239	3.08
BP1_127	(CML161xTA6)-127	2.80	BP2_177	(CML161xP24)-177	3.06
BP1_102	(CML161xTA6)-102	2.80	BP2_226	(CML161xP24)-226	3.00
BP1_7	(CML161xTA6)-7	2.55	BP2_275	(CML161xP24)-275	2.99
BP1_93	(CML161xTA6)-93	2.45	BP2_196	(CML161xP24)-196	2.96
BP1_33	(CML161xTA6)-33	2.34	BP2_207	(CML161xP24)-207	2.94
NK7328	Local check 1	2.46	BP2_203	(CML161xP24)-203	2.91
NK67	Local check 2	2.33	BP2_281	(CML161xP24)-281	2.85
LSD _{0.05}		1.02	BP2_295	(CML161xP24)-295	2.79
CV (%)		42.91	BP2_227	(CML161xP24)-227	2.79
Population 3: CML161xG12			BP2_166	(CML161xP24)-166	2.76
BP3_344	(CML161xG12)-344	3.40	BP2_271	(CML161xP24)-271	2.71
BP3_335	(CML161xG12)-335	3.32	K7328	Local check 1	2.80
BP3_301	(CML161xG12)-301	3.27	NK67	Local check 2	2.62
BP3_307	(CML161xG12)-307	3.20	LSD _{0.05}		1.03
BP3_331	(CML161xG12)-331	3.12	CV (%)		39.64
K7328	Local check 1	3.04			
NK67	Local check 2	2.80			
LSD _{0.05}		0.88			
CV (%)		45.41			

Note: x: cross; GY: grain yield; CV (%): coefficient of variation; LSD_{0.05}: least significant difference at a 95% confidence level.

Table 6. The list of 57 SNP markers for drought tolerance useful to MRI's populations using KASP method.

SNP markers	Chr	Maker location	Alleles	SNP markers	Chr	Maker location	Alleles
S10_10246089	10	10246089	A G	S3_220896526	3	220896526	C T
S10_122267546	10	122267546	G C	S3_220901626	3	220901626	A G
S10_136938361	10	136938361	A T	S4_10217574	4	10217574	A T
S10_139313697	10	139313697	G A	S4_123979624	4	123979624	A G
S10_139318575	10	139318575	C T	S4_229316522	4	229316522	A T
S10_139321312	10	139321312	G A	S5_11882524	5	11882524	T G
S10_139321315	10	139321315	T G	S5_167585338	5	167585338	A G
S10_145942320	10	145942320	A G	S5_179010958	5	179010958	A C
S10_19372348	10	19372348	G A	S5_179024804	5	179024804	T C
S10_19372355	10	19372355	C T	S5_212872911	5	212872911	C G
S1_182392227	1	182392227	A G	S5_213035501	5	213035501	A T
S1_182423684	1	182423684	C T	S5_39454290	5	39454290	A G
S1_182423923	1	182423923	T C	S5_42746321	5	42746321	A T
S1_183911148	1	183911148	A G	S5_42746324	5	42746324	T C
S1_188224557	1	188224557	C G	S6_105833772	6	105833772	G A
S1_21714481	1	21714481	T G	S6_105833891	6	105833891	T G
S1_288696288	1	288696288	A C	S6_106407839	6	106407839	A G
S2_118910398	2	118910398	A G	S6_106736814	6	106736814	T C
S2_15997107	2	15997107	T C	S7_13851342	7	13851342	G A
S2_213597045	2	213597045	C G	S7_157204971	7	157204971	A G
S2_51286162	2	51286162	G A	S7_19272803	7	19272803	G T
S3_1467367	3	1467367	G C	S8_160298809	8	160298809	A G
S3_1467368	3	1467368	G A	S8_75390111	8	75390111	T C
S3_156797754	3	156797754	A C	S8_94438283	8	94438283	T C
S3_169044562	3	169044562	A G	S9_116356519	8	116356519	C T
S3_187437395	3	187437395	A C	S9_152528782	9	152528782	T G
S3_187712524	3	187712524	A G	S9_20311986	9	20311986	A C
S3_187712599	3	187712599	C G	S9_22106256	9	22106256	T A
S3_220888091	3	220888091	T C				

The identification of significant SNP markers will support and improve breeding drought-tolerant maize. Based on the application of these SNPs at major gene regions associated with drought tolerance, materials tolerant to drought can be found. Through genotyping with 96 SNP markers and phenotyping under managed drought conditions, it was initially shown that the drought tolerance of 450 F₂ families is inherited from the female line (CML161), twenty-seven F₂ families with these SNP markers related to drought tolerance and the grain yield from 2.34 to 3.40 tons/ha, equivalent to the local checks of DK7328 (2.46 to 3.04 tons/ha) and NK67 (2.33 to 2.80 tons/ha) were found through testing in the field. These families could be primary materials for the MRI's drought-tolerant maize breeding in the future.

Discussion

Through cooperation programmes with CIMMYT, studies on applying SNP markers in drought-tolerant maize breeding were conducted with the participation of scientists from the MRI, which helped the institute gain access to modern research technologies.

A number of good materials with drought tolerance, that are adapted to climate change, and that moreover improve the research capacity of MRI scientists regarding the application of SNP markers in maize breeding and towards mastering maize breeding technology with SNP markers have initially been developed.

Based on CIMMYT's results pertaining to genotyping materials with SNP markers in the course of the Affordable, Accessible Asian Drought Tolerant Maize and Abiotic Stress-Tolerant Maize for Increasing Income and Food Security among the Poor in South and Southeast Asia projects, and with advice and support from CIMMYT experts, the MRI studied and genotyped 450 F₂ families with 96 SNP markers using the KASP method. Fifty-seven SNP markers related to drought tolerance were found useful in these populations. The research results also show that the allele call rate was 87%, which is equivalent to that in studies that currently apply SNP markers using the KASP method, which have found a rate of 50-97% [13, 14].

This research can be used as a guideline for the MRI

breeding maize tolerant to stresses by combining traditional and biotechnological methods in accordance with current conditions in Vietnam. At the same time, in order to develop drought-tolerant materials that are adapted to climate change in hybrid maize breeding programmes, it is necessary to continue research that applies SNP markers to the MRI's existing germplasm and to enhance cooperation with CIMMYT and other international institutes regarding the application of SNP markers in maize breeding.

Conclusions

Based on CIMMYT cooperation programmes involving phenotyping and genotyping with SNP markers by means of QTL mapping and GWAS, the MRI initially carried out genotyping three populations including 450 F₂ families with 96 SNP markers by KASP method, it was shown that 57 SNP markers related to drought tolerance were found useful to these populations and, through testing them in drought conditions, 27 F₂ families with drought tolerance and high yield were selected as primary materials for breeding stress-tolerant maize hybrids that are adapted to climate change.

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The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] D.B. Lobell, et al. (2011), "Climate trends and global crop production since 1980", *Science*, **333**(6042), pp.616-620.
- [2] Mai Xuan Trieu (2014), "Maize production in Vietnam: current status and future prospects", *12th Asian Maize Conference and Expert Consultation on Maize for Food, Feed, Nutrition and Environmental Security*, Bangkok, Thailand, CIMMYT, pp.332-338.
- [3] K. Koehler (2014), *Application of Genomic Selection in Commercial Corn Breeding and Crop Improvement*, Dow AgroSciences.
- [4] K. Semagn, R. Babu, S. Hearne, M. Olsen (2014), "Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement", *Molecular Breeding*, **33**(1), pp.1-14.
- [5] R.J. Elshire, J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, S.E. Mitchell (2011), "A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species", *PLOS ONE*, **6**(5), Doi: 10.1371/journal.pone.0019379.
- [6] Y. Beyene, et al. (2015), "Genetic gains in grain yield through genomic selection in eight bi-parental maize populations under drought stress", *Crop Science*, **55**, pp.154-163.
- [7] F. Bankole, et al. (2017), "Genetic gains in yield and yield related traits under drought stress and favorable environments in a maize population improved using marker assisted recurrent selection", *Frontiers in Plant Science*, **8**(808), Doi: 10.3389/fpls.2017.00808.
- [8] J. Yan, et al. (2010), "High-throughput SNP genotyping with the GoldenGate assay in maize", *Molecular Breeding*, **25**(3), pp.441-451.
- [9] P.H. Zaidi (2002), *Drought Tolerance in Maize: Theoretical Considerations & Practical Implications*, CIMMYT, Int.
- [10] M. Zaman Allah, et al. (2016), *Phenotyping for Abiotic Stress Tolerance in Maize: Drought Stress*, CIMMYT, Mexico.
- [11] J.L. Lush, A.E. Mollin (1942), "Litter size and weight as permanent characteristics of sows", *Technical Bulletin*, **836**, pp.1-40.
- [12] A. Korte, et al. (2012), "A mixed-model approach for genome-wide association studies of correlated traits in structured populations", *Nature Genetics*, **44**(9), pp.1066-1071.
- [13] D.L. Hyten, et al. (2010), "High-throughput SNP discovery through deep resequencing of a reduced representation library to anchor and orient scaffolds in the soybean whole genome sequence", *BMC Genomics*, **11**, Doi: 10.1186/1471-2164-11-38.
- [14] U. Majeed, E. Darwish, S.U. Rehman, X. Zhang (2019), "Kompetitive Allele Specific PCR (KASP): a singleplex genotyping platform and its application", *Journal of Agricultural Science*, **11**(1), pp.11-20.