ANALYSIS OF GENETIC DIVERSITY IN MAIZE BREEDING MATERIALS FOR DEVELOPMENT OF DROUGHT TOLERANT HYBRIDS IN SOUTHERN VIETNAM

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Drought causes a significant reduction in corn yield all over the world as well as Vietnam. The incidence, the frequency and the tension of drought are very difficult to forecast. It is the fact that corn hybrids have been exposed to drought more frequent any where any times. Thus, there has always been an interest in breeding for drought tolerant corn in any breeding programme. Impacting the genetic improvement of drought tolerance in corn hybrids requires an effort to establish long term commitments and objectives. Corn plants can achieve drought tolerance through different approaches involving escape, avoidance and tolerance. Therefore, many different traits have been attributed as selection targets to improve levels of drought tolerance and yield under water deficit conditions. A corn breeding research programme in ASI has focused on developing drought tolerant hybrids by using PCR to diversify materials for a single cross development. There are hundreds of drought tolerant inbred lines preserved in germplasm storage of the programme but not fully exploited efficiently. This study has identified and applied the diversity of 62 drought tolerant inbreds in developing new tolerant hybrids for Southern Vietnam. The accession of 62 inbreds was observed for diversity by phenotyping and DNA marker in the year 2010. The study revealed abundant genetic variability among 62 inbred lines indicating good potential in utilization. Based on the genetic variation, 4 genetic-diversified groups have been set up. Eighty four crosses among genetic groups have been developed and tested in 2011. Based on the test, five best crosses were retained for further field examination.

The collection of 62 inbred lines were developed focusing on extracting from drought tolerant hybrids and tropical populations in a few years before this study. Phenotypic evaluation was undertaken in dry season 2009 under two water regimes: water well (WW) and water stress (WS, stop irrigation before and after flowering 10 days). Genetic variability was determined with 8 polymorphic loci (Table 2). The polymorphic information content (PIC) value of each SSR locus was calculated as:

$$PIC = 1 - \sum P_i^2$$

Pi is the frequency of i allel. Diversity groups were follwed UPGMA (Nei et al, 1979).

Frequency of allel: $FP = 1 - \sum [n_i \times (n_i - 1)]/[N \times (N-1)]$

Genetic distances among inbred lines : $GD = 1 - S$ with $S = 2Nij/(Ni+Nj)$

Nij Number of band between lines i and j.

Ni, Nj Total number of band of lines i and j.

Clustering analysis was performed by NTSYS-pc (Rohlf, 2002).

Phenotypic evaluation. The 62 inbred lines have been evaluated under two water regimes: fully irrigated (water well-WW) and stop irrigation before and after 10 day at flowering (water stress- WS). Range of most traits showed highly significant at P<0.01 indicate very high genetic variation among the inbreds (Table 1). This means that there are good potential of utilization in these inbred lines to develop new crosses.

Table 1. Major parameters of 62 inbred lies based on phenotypic evaluation at two water regimes: water well (WW) and water stress (WS)

Traits	WW			WS		
	Average	Range	CV (%)	Average	Range	CV (%)
P-F (days)	54.62	47-59*	5.67	62.78	55-68*	8.75
ASI (days)	2.37	1.2-5.2*	12.32	7.20	4.5-9.6**	14.95
PH (cm)	169.35	158-189**	5.40	163.85	147-182**	5.7
EH (cm)	81.36	73-96**	6.20	77.54	70-92**	6.9
Row/Ear	14.72	12-18*	5.80	14.23	12-17*	7.2
Kernel/Row	25.38	20-32**	12.95	22.41	19-31**	13.76
KW/Ear (g)	65.35	43-82**	15.76	47.15	31-71**	16.95
EL (cm)	14.63	10-16**	11.69	13.21	9-16*	13.63
W1000 kernel(g)	258	210-264**	7.69	215	189-245**	5.65
Yield (tonne/ha)	3.14	2.1-3.8**	7.80	2.31	1.6-2.8**	8.45

*Notes: *: Significant at P<0.05 **: Significant at P<0.01. P-F: Planting - flowring, ASI: anther-silking interval, SW/ear: Kernel Weight/ear, PH:Plant height: EH:Ear height. EL: ear length, W1000 kernel: weight of 1000 kernels.*

Evaluation of genetic diversity based on SSR

No of allele. Total 21 alleles have been revealed in 62 inbred lines (Table 2) with clear polymorphism and average 2,63 alleles per SSR locus.

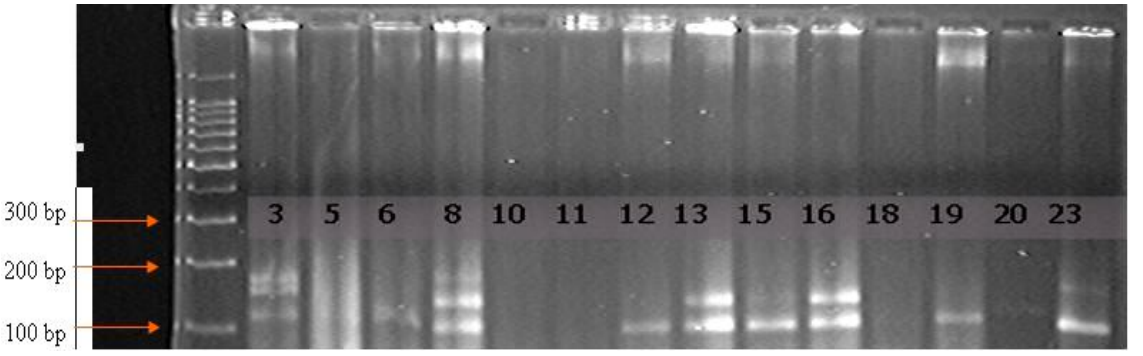

Figure1. PCR product at locus ***bnlg1064*** on chromosome 2

Table 2. List of the polymorphic markers used in the research

No	Marker	Chromosome	No of allele	PIC value
1	umc1354	1	5	0.76
2	phi19600	2	3	0.47
3	bnlg1064	2	3	0.60
4	phi99852	6	2	0.44
5	phiI328175	7	2	0.49
6	phi233376	8	2	0.50
7	umc1279	9	2	0.49
8	umc1154	10	2	0.49
	Average		**2.63**	**0.53**

PIC value

The PIC values reflect the genetic diversity level in a population of 62 inbred lines High PIC indicates high level of genetic diversity and *vice versa*. The set of 62 inbred line revealed the average PIC value 0,53, ranging from 0,44 to 0,76. PIC value at marker *umc1354* highest: 0,76. Marker *phi*99852 revealed lowest PIC value: 0,44 (Table 2).

Differences among inbred lines

Process of PCR produced stable results with 8 pairs of primers. The comparison of phenotypes with genotypes may be identified if the homozygous collections came from the same origin. Analysis showed significant differences in phenotype and genotype by SSR screening of the lines. Based on the analysis, 62 inbred lines could be classified into 4 genetic distant groups as followed (Table 3 and Figure 2).

Table 3. The four genetic distant groups of 62 inbred lines

Group	Name of inbred lines	No of line
I	VL3 TD5-2, HH07-2, L22-2, A1-2, HH07-3, VL41	7
II	VL12, VL46, HH07-5, 30D-2, MR07-1-2, MR07-2, R8, VL29, L22-17-6, L22-24, CML465, D11, MR06-9, D12.	14
III	NK67, HH06-8, V3A, VT6-1, VE8, V67-2, VC4, FNK67-3,VL45,HH07-4, NK67-1, VK1, MR06-8, T04-2, L22-10, T04-3, L22-8, L22-4, L22-8-1, L22-11, A1-3, RM97, NW292, VL20, L22-12, CLR-CY0363, VE1	27
IV	Nh T04-1, V10, H06-2, VL36, H06-4, NK67-2, DF2, D1, H06-5, H06-6, H06-7, M97, A1-1, CML 161	14

In a breeding programme, managing the development, evaluation and utilization are critical process. Normally, a large number of inbred lines need to be assessed and implemented efficiently. If all the possible crosses are going to be made, in this case we have (62-61)/2 = 1891 single crosses. This is almost impossible due to a large number of crosses, especially

when field evaluation for their potentials is a time and resource consumption. Among these crosses, a large number must come from lines that closed in genetic relation with poor heterosis.

Genetic distance grouping can assist breeders to utilize lage number of inbred lines most efficiently. Intelligent exploitation of corn inbred lines in breeding based on the knowledge of genetic relationship and understanding of the partitioning of genetic diversity among them. In this study, single crosses must be implemented between inbred lines from different groups, avoiding lines from the same group as indicated in Table 3.

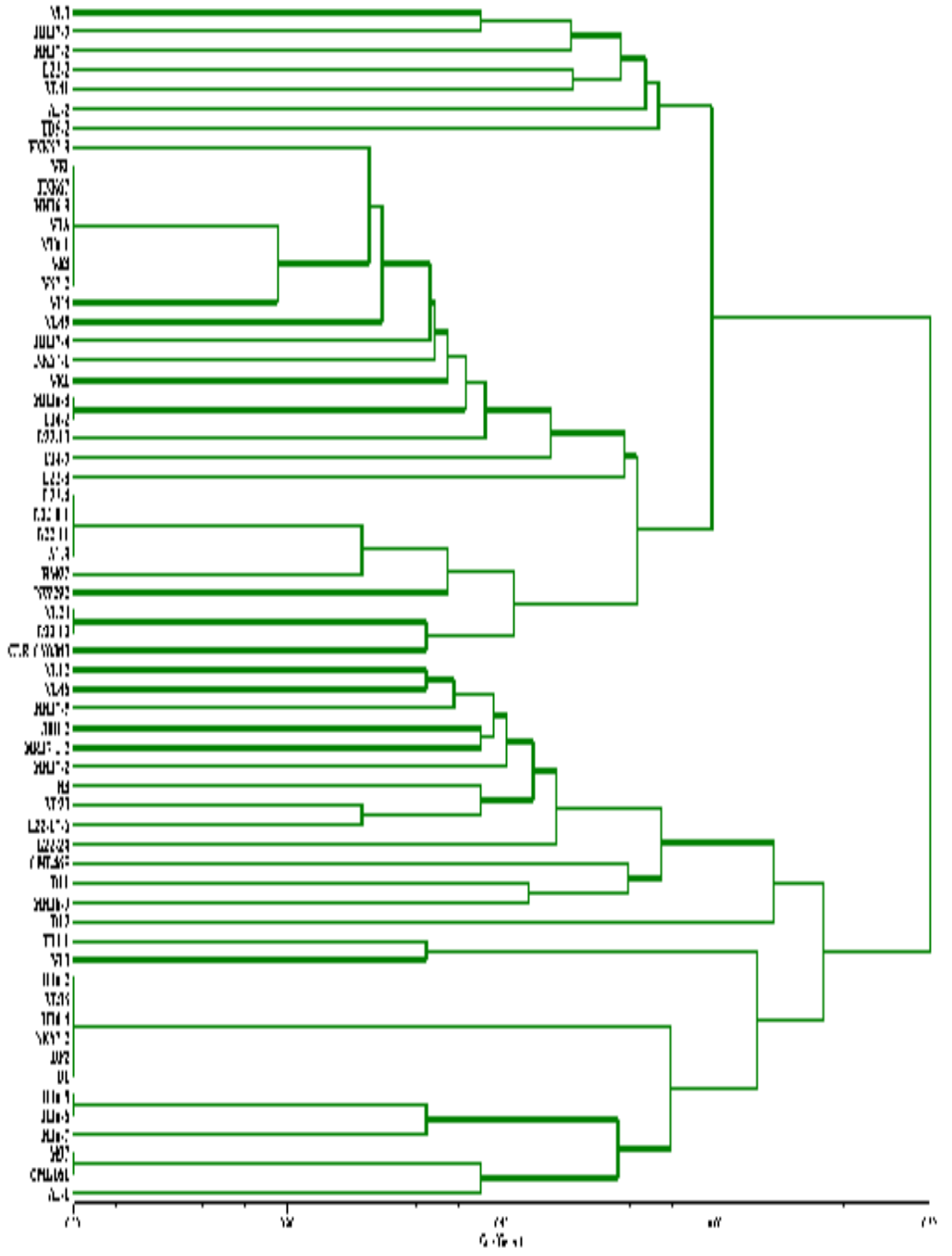

Figure 2. Dendrogram of 62 corn inbred lines based on SSR data

The set of 62 inbred lines extracted from drought tolerant hybrids showed significant differences in the morphological and genetic aspects. These results indicated an abundant genetic diversity among the set of inbred lines, implicating good potentials for development of new hybrids between them. Based on the grouping, crosses to be developed should be among inbred lines of different genetic groups as indicated in Table 3.