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5. RESULTS OF BIOTECHNOLOGICAL APPLICATIONS IN HYBRID MAIZE BREEDING FOR HIGH YIELD, GOOD STRESS TOLERANCE

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ABSTRACT

After 10 years of research and application of biotechnology in breeding maize hybrids, the Maize Research Institute has gained several remarkable achievements. Most notably in developing the doubled haploid (DH) breeding technology, and applying molecular markers to analyse genetic diversity and resistant ability of maize inbred lines. A nursery of DH lines was established for the development of commercial maize hybrids with high yield, good stress tolerance, such as LVN145, LVN146, LVN092, and other commercially desirable traits. A system of molecular markers has been established to effectively analyze genetic diversity, cluster heterotic groups, improve maize materials resistance to *Rhizoctonia solani*, and evaluate drought tolerance ability. Some new varieties were created using biotechnological techniques that have been applied in cultivation and have partly contributed to improving yield, productivity and production efficiency of maize production.

Keywords: Biotechnology, anther culture, DH line, genetic diversity, maize breeding

I. INTRODUCTION

Biotechnology is of high importance in developing agriculture in general and maize in particular. The Maize Research Institute has carried out studies of the application of biotechnology in breeding maize hybrids as follows:

- Studying the application of doubled haploid line developing technology;
- Using molecular markers to analyse genetic diversity, clustering of heterotic group, and drought and diseases tolerance among materials;
- Developing new high yield maize hybrids through combining traditional methods and biotechnological methods.

After 10 years of research and application, the Maize Research Institute has gained remarkable achievements in maize variety breeding.

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II. RESEARCH RESULTS

2.1. Developing DH lines

Based on the support of the experts from Agricultural Genetics Institute, the researchers of Maize Research Institute has approached and researched DH technology since 1996 to develop DH maize lines. The first DH line was developed in 2003, and the technical procedure of anther culture also was accomplished in this year. In two year 2004 and 2005, the focus on identifying materials with a high ability to induce embryo-like structures. Based on the results of anther culture of over 1000 local and imported maize germplasm, two germplasm (Xiem Song Boi and imported population Q431) were determined to have a high ability to induce embryo. A milestone in DH line development was achieved in 2005, for the first time allowing the exploitation of breeding valuable materials which have improved the induced embryo ability in anther culture by crossing these materials with AC24 donor (germplasm having culture respond ability). These achievements started a new trend in maize research; transferring normal maize lines into high quality protein maize (QPM) lines or developing waxy maize lines. The efficiency of DH technology is summarized in table 1.

Table 1: Results of doubled haploid lines

No.	Crop season	Number of used germplasm	Rate (Percent)		
			Embryo regeneration	Plant regeneration	Fertile Plants
1	Spring 2006	75	2.41	24.05	13.84
2	Autumn 2006	52	3.41	20.15	11.46
3	Spring 2007	80	6.90	22.48	14.93
4	Autumn 2007	53	9.67	17.13	11.19
5	Spring 2008	55	7.15	19.38	11.62
6	Autumn 2008	63	5.23	17.11	12.87
7	Spring 2009	54	7.04	18.72	14.32
Average			5.97	19.86	12.89

- The results (table 1) show that: The rate of embryo regeneration improved from 2.41% (in 2006) to 7.04% (in 2009); the rate of fertile plants from 13.84% (in 2006) to 14.32% (in 2009); the rate of plant regeneration declined from 24.05% (in 2006) to 18.72% (in 2009) because new materials have lower regeneration ability.

- During 2006-2009 stage, the value of using these lines in maize hybrid breeding was higher and more efficient than ever before 2005. Before 2005, only the C156N line (from (LVN10 x Xiem Song Boi) material) involved the commercial crossed combination of LVN145. From 2006 to 2009, some DH lines (such as: C152N, V72, V64, V272 etc.) have been used as parents in some commercial hybrids that exhibit high yield and good stress tolerance. These results are partly based on: 1) using germplasm having culture respond ability (AC24) that was selected from Population Q431 to improve the culture respond ability of many germplasm and 2) improving the genetic base of commercial materials.

- Using germplasm that have improved the genetic background in anther culture, we can created the DH lines with high yield (over 0.30 t/ha) e.g. C156N - DH line. This is one advance of DH method. This shows that the DH method can be applied to create the high yield line through improving genetic materials.

- Almost of the DH lines had high general combining ability and high specific combining ability,

their combinations had high purity, stability and wide adaptability (LVN146, LVN092, LVN152 and so on).

- In 2010, a rich and varied DH lines nursery (including 130 normal maize lines and 22 waxy lines) was selected and used for hybrid breeding. Combining traditional breeding methods, some hybrids with commercial value were selected such as: LVN145, LVN146, LVN092, LVN154, LVN152 [2, 4, 6].

2.2. Genetic diversity analysis using molecular markers

SSR markers were used to analyze inbred lines nursery at Maize Research Institute (MRI). Based on the molecular analysis we can estimate the genetic diversity of materials breeding, to reduce the time and field works and orient crossing through clustering genetic groups, raising the probability of elite hybrid pairs. The results of the genetic diversity analysis of maize inbred lines are summarized in table 2.

Table 2: The results of genetic diversity analysis using SSR markers

Crop season/Set	No. of inbred lines	No. of heterotic groups	Coefficient of Genetic similarity (GS)	Genetic distance (GD)
Spring 2006	96	4	0.71	0.29
Autumn 2006	51	7	0.75	0.25
Spring 2007	23	8	0.78	0.22
Autumn 2007 - Set 1	30	10	0.37	0.63
Autumn 2007 - Set 2	34	4	0.32	0.68
Spring 2008	30	4	0.35	0.65
Autumn 2008	80	7	0.35	0.65
Spring 2009	73	6	0.31	0.69

Results of 5 years of applying molecular markers indicated that:

- Based on the genetic diversity analysis, the level of genetic distance (GD) of inbred lines nursery raised from 0.26 to 0.65 between 2006 and 2010. Thus the genetic diversity of inbred lines nursery was increased.

- Analysis of genetic diversity has helped breeders reduce crosses by 30-50% in the field. Experimental results also demonstrate that crosses between lines in the same group result in low heterosis. Alternatively, crosses between different genetic groups results in high heterosis.

- The correlation value between genetic distances with the specific combining ability of inbred lines ranged from 0.60 to 0.65. The genetic distance of inbred lines is not correlated with their general combining ability.

- The analysis of genetic polymorphisms useful for the renovation and recreation of inbred lines, the new line has many advantages over the original line.

- A pair of lines with high heterosis often occurs in genetic groups that have high genetic distance, but the occurrence of high heterosis in a pair of lines does not coincide with high genetic distance.

- Based on the analysis of genetic diversity one can design a top-cross diagram, diallen experiments and choose testers that have the average genetic distance of line equality with the average genetic distance of groups.

- To get an effective genetic diversity analysis one should note that the lines used for molecular

markers analysis must had favorable criteria such as being morphologically uniform, exhibit stress tolerance and have a high value in commercial hybrid breeding.

2.3. Using molecular markers to estimate the abiotic resistance

- Isolation of *Rhizoctonia* strains cause in cabbage, soybeans, rice and maize. Species assessment conducted through the gene encoded 16S and 18S ribosome. The results showed that the collected samples were belong of *Rhizoctonia solani* species, however in Vietnam conditions has made the difference between maize pathogenic clones compared with other ones. *Rhizoctonia solani* has caused on maize in northern Vietnam. Preliminary results were determined phi065 and phi123 markers correlated sheath blight resistance caused by isolated strains, identified the two combinations (F93 and F90) and three lines (C156N, C347 and C110) lines resisted sheath blight disease, [1].

- Through filed estimation and isolation of Rp1 gene have been identified C152N, V272, C7N (Dekalgold) lines which had rust resistance, is currently used in rust resistant maize breeding service to maize production in provinces of Highlands.

- Identifying phi088 marker associated with rab15 gene encoding for abscisic acid synthesis and phi084 marker linked genes encoding for protein 1-NaCl related to drought tolerance of lines such as VNL38 , DF1, DF2, C26, C36 , C156N.

- Isolated and sequenced dhn1 gene participating Late Embryogenesis Abundant (LEA) protein synthesis in maize drought tolerance [3].

2.4. Some maize breeding results

From 2001-2010 the Department of Biotechnology has combined traditional breeding and biotechnological methods, the results have created a diverse inbred line nursery that are required to breeding the high yield hybrids, some DH lines participated in commercial hybrids (table 3).

Table 3: Results of promising hybrid combinations

Crop season/Set	No. of inbred lines	No. of heterotic groups	Coefficient of Genetic similarity (GS)	Genetic distance (GD)
Spring 2006	96	4	0.71	0.29
Autumn 2006	51	7	0.75	0.25
Spring 2007	23	8	0.78	0.22
Autumn 2007 - Set 1	30	10	0.37	0.63
Autumn 2007 - Set 2	34	4	0.32	0.68
Spring 2008	30	4	0.35	0.65
Autumn 2008	80	7	0.35	0.65
Spring 2009	73	6	0.31	0.69
Autumn 2009	22	4	0.44	0.56
Spring 2010 - Set 1	50	4	0.38	0.62
Spring 2010 - Set 2	32	6	0.40	0.60
Spring 2010 - Set 3	90	9	0.35	0.65

By 2010, identifying six commercial hybrid maize varieties used in the different levels: LVN145 were recognized as new varieties in 2007; LVN146 were recognized as new varieties in 2011; LVN092 were recognized as trial production in 2011; LVN152 has been prospected variety; F449 and F600 combinations have been prospects;. The hybrid combinations have high yield and stable (Table 4), relatively wide adaptability, partly to meet the needs of production, the annual supply from 250-300 tons of each variety.

Table 4: Yield of some parent lines and their F1 combination in different ecological regions

No.	Combination	Line yield (kg/ha)		Combination yield (kg/ha)					
		Male	Female	Northern	Central	Southern East	Western Highland	Mekong Delta	Average
1	LVN145	14.5	25.5	72.0	81.0	-	-	-	76.5
2	LVN885	25.5	30.0	69.0	78.0	73.0	77.0	-	74.3
3	LVN146	30.7	38.0	74.8	104.1	86.4	107.6	108.4	96.3
4	LVN092	30.0	35.5	69.8	81.0	80.6	90.3	86.1	81.6
5	LVN152	35.0	38.0	76.4	87.3	90.4	88.0	93.0	87.0
6	F449	30.7	40.0	71.0	78.4	98.9	90.1	116.0	90.9
7	F600	38.0	40.0	73.4	81.0	96.1	92.3	110.6	90.7
8	LVN4 (LC1)*	-	-	68.8	-	-	-	-	68.8
9	LVN99(LC2)*	25.5	38/0	72.4	-	-	-	-	72.4
10	C919(LC3)*	-	-	75.0	78.2	68.4	78.5	96.9	79.4
11	NK67(LC4)*	-	-	78.0	-	86.2	90.2	110.3	91.2
12	CP888(LC5)*	-	-	69.4	-	77.5	86.6	-	77.8

Note: LC – Local check

III. THE ORIENTATIONS OF MAIZE BIOTECHNOLOGICAL RESEARCH PERIOD 2012-2015 AT MAIZE RESEARCH INSTITUTE

In the economic globalization, rapidly development of technology, the increasing demands for high production and consumption, which requires continuously improving productivity and quality of the product are solved with the supporting of policies, mechanisms and financial resources of the state, the issues of biotechnology development in plant breeding in general, including in particular on maize become more urgent. In the next time, we should focus research the following:

- To complete the technological procedure of create high yield inbred lines by the doubled haploid and traditional method.
- To develop a tolerant genes database at the molecular level service for maize stress tolerant breeding.
- To apply of molecular markers in genetic clustering, regenerate and create new excellent breeding materials.
- To complete the procedure of drought tolerant gene transfer technology into maize.

IV. CONCLUSION

- Completing technology procedure of the doubled haploid technology, creating a maize DH lines nursery with high yield and good stress resistance, wide adaptation, satisfied goals for hybrid maize breeding. A number of elite lines were used as parents in commercial maize combinations.
- Genetic diversity analysis has contributed an important part to improving the effectiveness of genetic clustering, hybrid maize breeding, estimating resistance of maize breeding materials.
- Releasing a number of new hybrid maize varieties which had high yield, good stress resistance, wide adaptation such as LVN145, LVN146 (recognized as new varieties); LVN154, LVN092 (recognized as trial) contributing to improve the productivity, product quality and the requirements of maize commodity today.

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6. RESULTS OF HYBRID MAIZE RESEARCHING AND PRODUCING IN VIETNAM

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ABSTRACT

Maize research in Vietnam started in 1952 with the establishment of the Institute of Crops Production Research, now known as the Vietnamese Academy of Agricultural Sciences. From the late 1950s to early 1960s maize research was led by Hanoi Agricultural University and the Institute of Agricultural Science for Southern Vietnam. Since 1971, when the Maize Research Institute (MRI) was founded, research on maize, especially hybrid maize, has developed in a systematic way parallel to high level global standards. After 40 years of research, especially from 1992 until now, the hybrid maize's revolution has been the main factor for maize development in Vietnam. Thirty eight nationally recognized hybrids including field corn, waxy corn, sugar corn, baby corn, high-quality protein corn, etc. have been kept up with targets: high yield, drought resistance, early maturity. Hybrid maize production and trading have brought the high efficiency for maize production in nationwide.

Key words: Hybrid, maize, research, production.

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