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Review Article

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Breeding Waxy Maize Hybrid for Fresh Quality: Integration between Domestic and Exotic Germplasm

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Abstract Waxy maize **(***Zea mays* L. var. *certain*) hybrid program has been established since 2003 at Vietnam National University of Agriculture (VNUA), began upon collection activity of waxy maize nationwide germplasm. During 2003 to 2012, total 160 accessions of local waxy maize were collected based on the four ecosystem regions includedNorth-West mountain (Lai Chau, Dien Bien and Son La province); North-east mountain (Cao Bang, Bac Kan, Ha Giang, Tuyen Quang, Lang Son and Lao Cai provinces); mountain region of North coastal Central (Quang Tri province) and Highland central (Dac Lak, Gia Lai and Lam Dong provinces).Along with the collected local waxy maize germplasm, the 36 exotic waxy maize germplasm was also imported, exchanged and commercial waxy maize hybrids in Vietnam market. Evaluation of waxy maize germplasm diversity and identified usefultraits like drought tolerance, diseases -resistance, eating quality, especially thinner pericarp thickness, anthocyanin content for utilization and maize database establishment in VNUA. Currently, the mostgrown commercial maize varieties are hybrids. However, some waxy corn varieties derived in themountainous regions of Vietnam, where most ethnic minority people are counties are open-pollinated varieties (OPVs). Therefore, the improved OPVs are pivotal properties in these regions. Two local waxy maize varieties wereselected by thefull-sibmethod toenhancepopulationsare Khau li and Xa li luot. The results indicated that the selection method has significantly help to improve the numerous traits of interest of this population such as plant height, yield components and fresh ear yield Khau li which are shown to be higher 0.61 ton/ha and Xa li luot is 0.67 ton/ha via three consecutive cycles. Development of the inbred line for the hybrid breedin gprogram in CRDI was initiate dby use of 43 domestic and 36 exotic germplasm for inbred waxy maize line development. During 2009 to 2016, it has generated and evaluated 1683 of inbred lines on the genetic diversity, eating quality, anthocyanin content, drought tolerance, GCA, SCA base on phenotyping traits and applying molecular breeding**.** Hybrid waxy maize breeding wassuccessfully generated withfour waxy maize hybrids are VNU16, VNUA 69, MH8 and HUA 601 which revealed high fresh ear yield good eating quality, while a purple waxy maize hybrid is NT141 with high-quality and high anthocyanin content. Five waxy maize hybrids were taken into Vietnam Nation Inspection and Testing Network from 2013; they were shown higher yield and quality to compare with the controlled variety. The integration between the use of domestic and exotic germplasm could be successfully improved the diversity of waxy maize germplasm and hybrid breeding for traits of interest such as high fresh with husk yield and good eating quality.

Keywords Waxy maize, domestic, exotic, germplasm,population improvement, hybrid, fresh quality.

Introduction

Pre-breeding is one of the most promising alternative ways to link genetic resources and breeding programs. Pre-breeding refers to all activities designed to identify desirable characteristics and/or genes from unadapted (exotic or semi-exotic) materials, including those that, although adapted have been subjected to any kind of selection for improvement. As pre-breeding is being carried out, the resulting materials are expected to have merit to be included in ordinary breeding programs. Although there are some different concepts of exotics, Hallauer and Miranda [1]considered that exotics for pre-breeding purposes included any germplasm that does not have immediate usefulness without selection for adaptation for a specific area. In this sense, exotic germplasms are represented by races, populations, inbred lines, etc. Consequently, the results of crosses between adapted and exotic materials, where different proportions of introgression are obtained and evaluated, have been denominated as semi-exotic materials. According to report of Hallauer [2], the utilization of semi-exotic populations has been the most common procedure to evaluate exotic germplasms. To obtain promising results with exotics, their reproduction is necessary for a few generations in order to allow genetic recombination accompanied by mild selection. Before useful recombinants can be selected a minimum of five generations of random mating with mild selection pressure is indicated [3].

Maize can be classified into three types, including normal, waxy, and sweet, depending on the starch composition of the endosperm in the seed. Normal corn and waxy corn differ regarding texture or starch content (amylose and amylopectin) [4]. Amylose-free (*waxy*), *i.e.*, amylopectin maize has been a vegetable and staple food in East and South-East Asia for centuries, resulting in hundreds of landraces (LR). Eating preferences could have resulted in the additional selection for different starch properties of *waxy* maize, of interest in the food and feed industry [5]. A great advantage of waxy maize is its specific structure of starch, due to its unique and high amylopectin content (95-98%), which creates unlimited possibilities of industrial use [6].

Waxy maize (*Zea mays* L. var. *certaina* Kulesh), with many excellent characters regarding starch composition and economic value, has grown in China for a long history and its production has increased dramatically in recent decades. However, the evolution and origin of waxy maize remain obscure. Some reports disclosed that the genetic diversity of Chinese waxy maize including typical landraces and inbred lines by SSR analysis and the results showed a wide genetic diversity in the Chinese waxy maize germplasm. We analyzed the origin and evolution of waxy maize by sequencing 108 samples and downloading 52 sequences from GeneBank for the waxy locus in some accessions from genus *Zea*. A sharp reduction of nucleotide diversity and significant neutrality tests (Tajima's D and Fu and Li's F*) were observed at the waxy locus in Chinese waxy maize but not in nonluminous maize. Phylogenetic analysis indicated that Chinese waxy maize originated from the cultivated flint maize and most of the modern waxy maize inbred lines showed a distinct independent origin and evolution process compared with the germplasm from Southwest China. The results indicated that an agronomic trait could be quickly improved to meet production demand by selection [7].

Kernel pericarp thickness and ear architectural traits are important selection criteria in fresh waxy corn breeding programs as they are associated with a consumer sensory and visual preferences. Due to the necessity to breed new fresh waxy corn hybrids for U.S. market, genetic research on preference traits, specifically pericarp thickness associated with tenderness perception, and ear traits relevant to yield and consumer preference were conducted on a population derived from South Korean germplasm. Since there is limited genetic information on the pericarp and ear traits related to consumer preference of fresh waxy corn germplasm, estimating genetic relationships among the traits, identifying and validating QTL for the traits,and evaluating the testcross performance of the traits would be useful in selection programs designed to improving these traits in fresh waxy corn breeding programs [8].

The local waxy maize varieties in Northern of Vietnam have been cultivated for a long time and commonly utilizedfor fresh eating, especially in the northern, central and central highland region of Vietnam where many ethnic minority people live and they used waxy maize as the principle food. Therefore, local waxy maize varieties with high quality and diversity and suitable to local consumers, but low yield because most of them are open pollination varieties. Our attempts during 10 years have focused on integrating domestic with exotic waxy

maize germplasm *via* hybridprogram in order to generate the varieties with high yield and quality, simultaneously adapted to Vietnam condition.

Breeding waxy maize hybrids: Waxy maize germplasm

The current CRDI (Crop Research and Development Institute) belonging to Vietnam National University of Agriculture, Hanoi, where is maintaining local waxy maize germplasm which can be traced to samples of landraces collected and regenerated over 2003-2017. The active collection was launched to mainly focus on the local waxy maize varieties for breeding in order to improve waxy maize in Vietnam. Initiative in 2003, 24 local maize varieties collected from the Northwest mountain region were evaluated. The results have indicated that that there is being existed a large amount of phenotypic diversity involving in some specific characteristics such as growth duration, plant height, and color grain, eating quality and yield components. The diversity depended on the sub-ecological conditions and ethnic minority groups, respectively. The accessions have been classified into three sub-species: *Zea mays indurata*, *Zea mays semlindenrata* Kulesh Sturt. And *Zea mays* L. var. *certaina* Kulesh and nine varieties. This is a valuable genetic resource to develop open-pollinated and hybrid varieties with high yield and good quality, tolerant to adverse upland conditions [9].

The finding on the local waxy maize diversity was made which has encouraged the breeders in CRDI to establish the waxy maize breeding program. Waxy maize resource collection has been implemented during from 2006 to 2012by CRDI Program. Maize collections were gathered from the main four ecosystems of Vietnam included: North-West mountains (Lai Chau, Dien Bien and Son La provinces); North-East mountains (Cao Bang, Bac Kan, Ha Giang, Tuyen Quang, Lang Son and Lao Cai province);mountain region of North Coastal Central (Quang Tri province) and Highland Central (Dac Lak, Gia Lai and Lam Dong provinces).These two regions were assembled the largest collections included North - west mountainous regionswith three provinces: Lai Chau, Dien Bien and Son La, with total accessions were63 samples, Northeastmountainous regions collected in Cao Bang, Bac Kan, Ha Giang, Tuyen Quang, Lang Son, Lao Cai provinces, 87 samples and others are 8 sampleswere collected as shown in the Table 1.

Table 1: Number of collections from 13 provinces registered into genbank of CRDI in field genbank and seed genbank from 2003 to 2012

Province	Number accessions	Kernel color			
		White	White + purple	Orange	Purple
Son La	21	13	5	1	2
Dien Bien	35	24	7	1	3
Lai Chau	7	6	1	-	-
Cao Bang	10	8	2	-	-
Bac Kan	13	10	3	-	-
Lao Cai	22	12	9	1	-
Ha Giang	33	22	6	3	2
Tuyen Quang	9	9	-	-	-
Lang Son	2	2	-	-	-
Quang Tri	1	1	-	-	-
Dak Lak	5	5	-	-	-
Gia Lai	1	1	-	-	-
Lam Dong	1	-	1	-	-
Total	160	113	34	6	7

Total 160 accessions were made by adding code from GN1 to GN60, respectively. They wereevaluatedin field experiments for their phenotypic specific traits of interest included: growth duration, stages from sowing to tasselling and silking, plant height, ear height, number of leaves, characters of tassel, ear length, ear diameter, stem color, kernel color, tassel color, cob color, drought, insect and disease tolerance in the field condition.The yield and yield components were also recorded. Data were updated on the database in CRDI and utilization for further waxy maize breeding program.

Genetic diversity evaluation of accessions in genbankwas consecutively implemented. For instance, the genetic diversity among 54 maize accessions consisting of 34 waxy and 20 normal maize accessions, collected from 6 provinces in North Mountain region of Vietnam which were assessed by RAPD markers. The RAPD markers produced a total of 134 fragments is amplified in waxy maize accessions, the polymorphic fragments are 83 bands/134 total of fragments (61.8%). The RAPD markers produced 65 DNA fragments in normal maize accessions and polymorphic fragments are 53 bands/65 total of fragments (81.5%). The dendrogram was constructed by using the UPGMA method. Genetic similarity coefficient ranged from 0.59 to 0.81 with waxy corn accessions and ranged from 0.61 to 0.83 with normal maize accessions. The molecular data grouped the waxy maize accessions into 11 main clusters and normal maize into seven groups. This information on the genetic diversity between local maize accessions has been useful for selection of maize accession for developing the inbred parent lines in hybrid maize breeding. The classification of germplasm into genetic groups also is a base to design methodologies and make policies for conservation and utilization of local maize genetic resource in Vietnam. RAPD markers with ten primers are OPA-12, OPA-18, S208, OPW- 08, OPM-12, OPA-15, OPAW- 07, OPE-18, OPP- 05 and OPP-14 could be used to identify the genetic diversity between local maize accessions and grouping into heritability groups. This information may be useful for collection, conservation as well as various breeding programs in the Highlands of Vietnam [10].

The importance of assessment utilization of plant genetic resources for the improvement of modern cultivars is well recognized. The waxy maize breeding program in Vietnam has been recognized as the challenges of incorporating diversity into their elite breeding pools because of local waxy maize germplasm are lacking undesirable agronomic traits and combining ability associated with exotic maize germplasm, the time required to extract valuable material, and the rapid nature of breeding cycles in commercial programs. Exotic germplasm possesses high levels of genetic diversity for valuable traits. Moreover, the yield gap between elite and exotic germplasm widens, which increases the effort needed to use exotic germplasm and to identify beneficial alleles and for their introgression [11]. On the cooperative exchange of waxy maize germplasm, gathers from hybrid waxy maize varieties that commercially in Vietnam market, we were used as exotic waxy maize germplasm to enhance domestic germplasm (Table 2).

Table 2: Exotic waxy maize germplasm were evaluated and utilization in CRDI from 2003 to 2012

No.	Name	Coding	Origin	Type of Germplasam
1	White waxy	M1	Xiengkhuang, Laos	OPV
2	Purple waxy maize	M2	Phongsali,Laos	OPV
3	Phon Kham I	M3	NAFRI, Laos	OPV
4	Pho sa may I	M4	NAFRI, Laos	OPV
8	Na phoc 1 (purple)	M8	NAFRI, Laos	OPV
9	Duoc (yellow)	M9	Huaphanh, Laos	OPV
10	D_4 inbred line	M10	NAFRI, Laos	Inbred line
11	White waxy	M11	NAFRI, Laos	OPV
13	White waxy	M13	Taiwan, China	OPV
14	Ki14	M14	Korea	Inbred line
15	Hi31	M15	Korea	Inbred line
16	HQ3	M16	Korea	OPV
17	HQ4 hybrid	M17	Korea	OPV
18	BH20 line	M18	Korea	Inbred line
19	BH30 line	M19	Korea	Inbred line
20	WT1 line	M20	Korea	Inbred line
21	PH1	M21	Philippine	Inbred line
24	V-3	M24	Taiwan, China	Inbred line
25	Tim ĐL-6	M25	Taiwan, China	Inbred line
26	White waxy	M26	China	OPV
27	CL2(608)	M27	China	Inbred line
28	CL3	M28	China	Inbred line
29	CL5	M29	China	Inbred line
30	CL6	M30	China	Inbred line
31	CL11	M31	China	Inbred line
32	CL16	M32	China	Inbred line

33	Ban Kao-S2	M33	Thailand	Inbred line
34	Ubon-S2	M34	Thailand	Inbred line
35	Argon	M35	Thailand	OPV
36	AG208	M36	Loctroi company	Hybrid

Exotic germplasm was used to cross with local waxy maize line or OPV receives F_1 progeny, self-pollinated for inbred line development, from $S_1 – S_4$ generation carry out evaluated agronomical characters and GCA to select the elite waxy maize lines and most S_8 generation take into specific combining ability to develop hybrid waxy maize variety

Breeding waxy maize hybrids: population improvement

The improved populations can be used as germplasm source for high yield and yield components in corn breeding programs [12]. The landrace presently cultivated in the mountainous region of Vietnam are mostly open-pollinated and long times grew so that they were uniformly decelerated and differ in yield, ear size, kernel color and eating quality. Our study was to investigate responses of two local waxy maize populations are Khau li and Xa li luot by full-sib selection. Khau li was population collected from Pac ta commune, Tan Uyen district, Cao Bang province, Xa li luot was population collected from Hau Thao commune, Sa Pa district, Lao Cai province (Table 3).

Table 3: Passport data of the two waxy maize germplasmwas used for full-sib selection

Local name	Code Access	Ethnic minority	Collected in local	Year collected	Cultivation practice
Khau li	GN151	Thai	Pac Ta, Tan Uyen, Lai Chau	2008	Upland and rainfed cultivation
Xa li luot	GN166	Dao	Hau Thao, Sa Pa, Lao Cai	2009	Upland and rainfed cultivation

Two populations were selected because of their good eating quality, tendency, Xa li luotis anaroma, short growth duration (Xa li luot is 90days and Khau li is 101days) appropriateness for fresh eating ear harvesting, drought and some insect and diseases tolerance and its popularity consumers. Population Development and selection procedures: In Spring season 2009, full-sib section experiment for improvement was initiated in two local varieties at CRDI, Vietnam National University of Agriculture and successful Autumn-winter 2013 as following Figure 1:

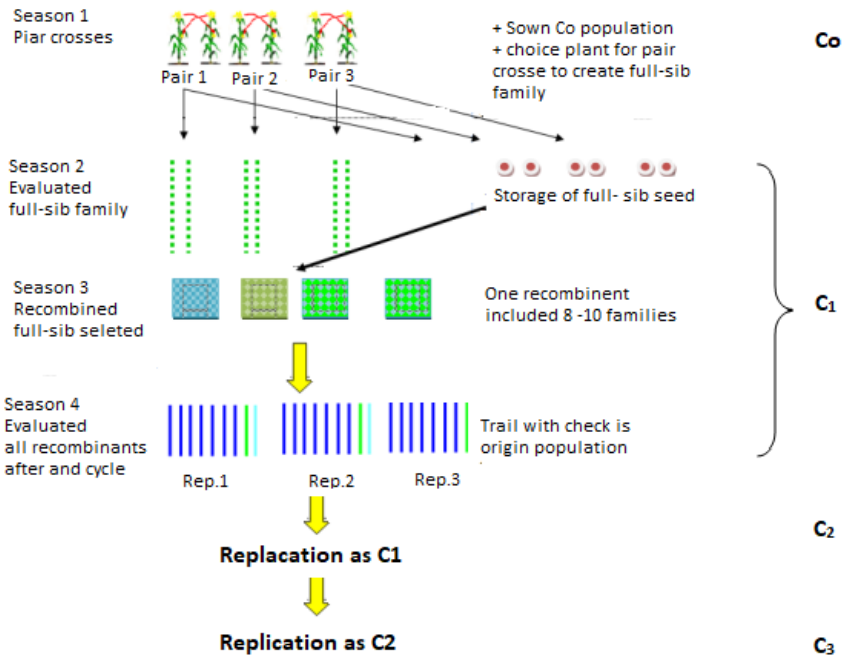

Figure 1: Schematic diagram for population development of two waxy maize population are Khau li and Xa li luot through full-sib selection for three cycles in Gialam, Hanoi, Vietnam

Khau li population through three cycles selections and identified 5 recombinants which shown to be promising and were taken into into evaluation in replication experiments in Autumn-winter 2013 for their yield and yield components as the results shown in Table 4.

Table 4: Yield and yield components of Khau li recombinants (TTH) in Autumn-winter 2013 at Gia Lam, Hanoi

Recombinants	EL (cm)	ED (cm)	No. Row/ear	Number kernel/row	Yield (t /ha)
TTH1-1-1	16.10	3.90	14.00	20.5	3.02
TTH1-1-2	16.10	3.95	14.00	20.0	3.03
TTH1-1-3	16.60	4.15	14.60	23.5	3.26
TTH1-1-4	16.60	4.10	14.20	21.3	3.08
TTH1-1-5	16.60	4.13	14.60	20.4	3.16
S_0 population(check)	16.05	4.00	13.80	19.5	2.65
CV%	*14.5*	*14.0*	*5.0*	*17.8*	*13.7*
LSD0,05	*0.36*	*0.69*	*0.90*	*5.42*	*4.29*

EL: ear length; ED: ear diameter; No. Row/ear: number of kernel row/ear

Similarly that, Xa li luot population through three cycle's selections also identified 5 recombinants. They were further evaluated in replication experiments in Autumn-winter 2013 for the yield and yield components as shown in Table 5.

Table 5: Yield and yield components of Xa li luotre combinants (TTH) in Autumn-winter 2013 at Gialam, Hanoi

Recombinants	EL (cm)	ED (cm)	No. Row/ear	Number kernel/row	Yield (t /ha)
TTH9-8-1	16.10	3.90	13.80	23.2	3.42
TTH9-8-2	15.10	3.70	13.60	20.3	3.23
TTH9-8-3	15.60	4.00	13.60	22.1	3.14
TTH9-8-4	14.80	4.10	13.80	20.9	3.28
TTH9-8-5	14.60	3.60	13.80	20.4	2.96
S_0 population (control)	14.20	3.50	13.20	19.0	2.75
CV%	*14.5*	*14.0*	*5.0*	*17.8*	*13.7*
$LSD_{0,05}$	*2.36*	*0.69*	*0.90*	*5.42*	*4.29*

TTH1-1-3 was selected and is an improvement Khau li population, the TTH9-8-1 was selected and wasan improvement Xa li luot population, both improvement populations were demonstrated in Lao Cai province (Figure 2)

Figure 2: Demonstration of the improvement populations are Khau li and Xa liluot after three selection cycles in Lao Cai province 2013

Breeding waxy maize hybrids: fresh and nutrient quality

Waxy maize hybrid breeding program in CRDI included two ways, first waxy maize hybrids with high quality and thinner pericarp thickness and second purple waxy maize hybrid were conducted. Genetic diversity has frequently been estimated by maize breeders to select for the besthybrid combinations.

Developed inbred lines

Developed waxy maize inbred line

Shull [13] published a paper on the A pure- line method in corn breeding. The author showed that self-fertilization in the maize from 6 to 8 generations which shouldbe developed inbred lines with homogenous. This becomes the standard method to develop an inbred line in hybrid maize breeding. At present inbred line development was appliedto the DH technology by *in vivo* maternal haploid induction using haploid inducers [14]. In our hybrid waxy maize breeding program was used two methods were self-pollination. Materials were used developing inbred line include OPV and F_1progeniesof crosses between local with exotic germplasm or extraction of inbred lines from commercial waxy maize hybrid. Number accessions of local waxy maize germplasm were chosen for inbreeding are 43 accessions, inbred lines at S_3 take into GCA analysis and discard lines have not had GCA at significant level, the remain lines continuously develop for inbreeding to S_6 or more was get 579 elite lines, and take into evaluation of specific combining ability to selected promising hybrids are 81 lines, base on SCA value selected best expression crosses for compared experiment with three replication at CRDI, through evaluated result from 2015 to 2017 were selected 8 promising hybrids submitted National Inspection and Testing Network (Table 6).

Table 6: Germplasms used development of waxy maize inbred line and number elite line participated in crosses and number promising hybrid submitted to Nation Inspection and testing network

TT	Coding	Local name	Type of germplasm	S_6 and more generation	Number crosses joined	Promising hybrid
1	GN2	Khau lion lun	OPV	56	15	2
2	GN5	Pooc cu lau	OPV	25	10	1
3	GN6	Sli lo	OPV	12	0	
4	GN33	Nep Lao SL	OPV	12	4	0
5	GN34	Nep Thai	OPV	6	0	
6	GN35	Khau Lion LC	OPV	6	0	
7	GN36	Khau Lilec	OPV	4	0	
8	GN40	Dray Rang	OPV	8	6	1
9	GN47	Hai thang ruoi	OPV	34	12	1
10	GN48	Ngo nep Khanh Hoa	OPV	40	15	1
11	GN53	Ngo nep Bac Ha	OPV	8	0	
12	GN55	Tam me pen	OPV	6	0	
13	GN58	Nep trang Khanh Thien	OPV	25	8	1
14	GN121	Mai plot	OPV	13	0	
15	GN125	Tay Nu II	OPV	15	0	
16	GN127	Ngo nep Viet Lam II	OPV	21	0	
17	GN128	Hu Nu	OPV	4	0	
18	GN129	Bap Nu	OPV	10	0	
19	GN130	Giống nep tim	OPV	5	0	
20	GN131	Bap Nu Xam Bum	OPV	21		
21	GN132	Pooc culau	OPV	10		
22	GN135	Hu Nu Khao	OPV	12	0	
23	GN136	Khau Hudi ban	OPV	5	0	
24	GN137	Khau Hu Khao	OPV	10	0	
25	GN139	Hu Nu	OPV	15	0	
26	GN141	Ngo nep tim Dien Bien	OPV	5	1	
27	GN142	Ngo nep thuanhoa	OPV	5	0	
28	GN151	Khau li	OPV	10	5	0
29	GN154	Nep trang	OPV	15	0	

30	GN155	Nep trang Nua Khao I	OPV	15	0	
31	GN157	Bap Khỉ Ma I (purple)	OPV	10	0	
32	GN 164	Bap Nua Khao 2	OPV	15	0	
33	GN166	Xa li luot	OPV	10	5	1
34	GN169	Nap Trang (Bap Nua)	OPV	5	0	
35	GN173	Nep Trang (Tay Nua)	OPV	10	0	
36	GN176	Bap Nua Lai (purple)	OPV	5	0	
37	GN177	Ngo Nep Tra Linh	OPV	10	0	
38	GN180	Ngo Nep (Nhan Muc)	OPV	10	0	
39	GN181	Ngo nep thang	OPV	21	0	
40	GN182	Ngo nep Na Hang	OPV	10	0	
41	GN196	Nep Trang	OPV	10	0	
42	GN198	Tay Nua	OPV	20	0	
43	GN240	Pooc cu long do	OPV	10	0	
		Total		**579**	**81**	**8**

Exotic germplasm collected from some countries include Laos, China, Korea, Philippine, Taiwan, China include OPV, inbred lines and commercial hybrids from Syngenta and Loc troi Company with total 53 samples (Table 7). Exotic germplasms used for inbred lines by self-pollination, extract line from a hybrid, crossed with local inbred lines then inbreeding from F_1 progenies reach to S_6 or more generation with total 1.104 inbred lines was created from 43 accessions.

Table 7: Exotic germplasm used development of waxy maize inbred line and elite number line participated in crosses and number promising hybrids was submitted to Nation Inspection and testing network

Coding	**Origin**	**Type**	**S_6 or more generation**	**No. Crosses joined**	**Promising hybrid**
M1	Laos	OPV	43	35	0
M2	Laos	OPV	76	28	0
M3	NAFRI,Laos	OPV	41	33	1
M4	NAFRI,Laos	OPV	89	29	0
M8	NAFRI,Laos	OPV	85	13	0
M9	Laos	OPV	52	22	0
M10	NAFRI,Laos	OPV	1	6	0
M11	NAFRI,Laos	OPV	23	20	0
M13	China	OPV	71	18	0
M14	Korea	Hybrid	1	6	1
M15	Korea	Hybrid	1	6	0
M16	Korea	OPV	68	21	4
M17	Korea	OPV	84	18	3
M18	Korea	Hybrid	1	6	1
M19	Korea	Hybrid	1	6	0
M20	Korea	Hybrid	1	12	1
HQ5	Korea	Hybrid	6	3	1
M21	Philippine	Inbred line	1	6	0
M24	Taiwan,Chiana	Hybrid	1	3	0
M25	Taiwan,Chiana	Hybrid	1	3	0
M26	China	OPV	75	12	1
M27	China	Hybrid	1	5	0
M28	China	Hybrid	1	5	1
M29	China	Hybrid	1	2	0
M30	China	Hybrid	1	4	0
M31	China	Hybrid	1	2	0
M32	China	Hybrid	1	4	0
M33	Thailand	Hybrid	1	5	0
M34	Thailand	Hybrid	1	5	0
M35	Thailand	OPV	18	9	0
TL2	Thailand	Hybrid	12	2	1
AG2	Loc troi Company	Hybrid	13		0
AG3	Loc troi Company	Hybrid	16	18	2

AG5	Loc troi Company	Hybrid	22	15	0
AG8	Loc troi Company	Hybrid	11	3	0
AG9	Loc troi Company	Hybrid	8	5	0
AG10	Loc troi Company	Hybrid	12	2	0
AG11	Loc troi Company	Hybrid	11	17	1
AG12	Loc troi Company	Hybrid	9	3	0
AG13	Loc troi Company	Hybrid	6	3	0
AG14	Loc troi Company	Hybrid	12	0	0
AG15	Loc troi Company	Hybrid	15	5	0
AG16	Loc troi Company	Hybrid	24	3	0
AG17	Loc troi Company	Hybrid	15	23	1
AG19	Loc troi Company	Hybrid	14	5	0
AG21	Loc troi Company	Hybrid	24	5	0
AG22	Loc troi Company	Hybrid	14	2	0
AG23	Loc troi Company	Hybrid	18	4	0
AG26	Loc troi Company	Hybrid	20	5	0
AG208	Loc troi Company	Hybrid	31	25	2
QT 523	China	Hybrid	23	15	0
QT 608	China	Hybrid	21	10	2
Wax44	Syngenta Company	Hybrid	5	15	1
	Total		1104	532	24

Evaluation of waxy inbred lines

During from 2009 to 2016, we have evaluated 1683 of waxy maize inbred lines on the diversity, quality, drought tolerance, GCA, SCA base on their phenotype traits and assisted molecular markers. The recent studies were attained some significant results as following:

- Assessing genetic diversity and heterotic grouping of 24 waxy maize inbred lines in S_8 to S_{10} generations wereperformed by using morphological characters and molecular markers (SSRs). Based on genetic diversity analysis of morphological traits, 24 inbred lines were grouped into six heterotic groups at similarity value of 0.25. Genetic diversity analysis of inbred lines by 19 SSR markers analysis which was detected 19 loci with a total of 75 alleles and average of 4 alleles per locus. Polymorphic Information Content (PIC) varied from 0.36 to 0.81, respectively. At genetic similarity value of 0.83, 24 inbred lines were divided into five groups. Of 24 lines tested, 5 lines, viz. D_5, D_9, D_8, D_{23}, andD_{15} were identified to be drought tolerant. These lines belonged to the different heterotic groups and therefore they can be used for hybrid waxy maize breeding for the drought-prone environment such as a rain-fed farming condition for Northern mountainous provinces [15].
- Selection of waxy maize inbred lines for thinner pericarp is a priority for enhancing tenderness in fresh waxy corn breeding. We have assessed 48 of local maize varieties and inbred lines to identify genetic materials that have thinner pericarp for the waxy maize breeding with improved quality. In 2012, 48 varieties and inbred lines were evaluated and selected lines with kernel pericarp thickness are thinner,pericarp of cultivars and inbred lines (measured by micrometer) ranged between 51.6 to 118.9 µm. Six potential lines were selectedwith desirable pericarp thinness are D_{27}, D_{34}, D_{36}, D_{14}, D_{22} and D_{35} (D_{27} with 51.6 µm). Using SSR markers are detected QTL controlling thinner pericarp traits. Based on the phenotypic evaluation and genetic markers, six inbred lines are D_{14}, D_{22}, D_{27}, D_{47}, D_{36} and D_{44}, possessing desirable agronomic characteristics; thinner pericarp was selected for high-quality waxy maize breeding program in Vietnam [16].
- The experiment was carried out to evaluate and select superior purple waxy corn lines derived from self-pollination (S_3 to S_6 generation) with high grain yield, marketable fresh cob yield, anthocyanin content, good eating quality and desirable agronomical characteristics. These lines were selectedfrom exotic and domestic germplasm. Phenotypic data collected included growth and developmental characteristic, yield and yield components, marketable fresh cob yield, total anthocyanin content, eating quality, pericarp thickness and sugar content, tenderness as well as theirtaste. Eighteen purple waxy corn lines out of 45 lines were selected based on selection index computed from ideotype plant

analysis with 12 traits. These lines had high anthocyanin content (22.4 to 260.10 µg/L), acceptable grain yield (2.0 to 3.5 t/ha) and marketable fresh cob yield (3.8 to 6.4 t/ha), good eating quality and suitable agronomical characteristics. These lines were recommended for further purple waxy corn inbred line and hybrid development [17].

The combining ability

The concepts of general combining ability (GCA) and specific combining ability (SCA) defined by Sprague and Tatum [18] have been used extensively in the breeding of several economic crop species. For maize yield, they found that GCA was relatively more important than SCA for unselected inbred lines, whereas SCA was more important than GCA for previously selected lines. Our program was implemented GCA during development inbred lines by method line x tester and SCA conducted in S_6 to S_8 generation to identifiedpromising hybrids. The recent studies showed as below:

- The combining ability of eight waxy corn (*Zea mays* var. *Ceratina*) inbred lines, namely D_2, D_3, D_4,D_5, D_6, D_8, D_{10} and D_{17}, developed from three waxy corn populations of three Ethnic Minority People Groups (Thai, Mong and Van Kieu) was evaluated using a Griffing's diallel mating design method 4. The parent lines were evaluated in 2009 winter season 2009 and total of 28 F_1 crosses involving in these eight inbred lines which were produced and evaluated in 2010 spring season. The field trials were arranged in a simple lattice design with 2 replications in Gia Lam, Ha Noi. Simultaneously, analysis of genetic distance among the inbred lines was performed based on 11 phenotypic traits from three heterotic groups' classification and evaluation. Three lines with good GCA, namely D_2, D_4 and D5 could be utilized in developing the single cross hybrid breeding program. The lines D_2 and D_4 showed SCA value at significant level with grain yield of 3.06 t/ha, it showed higher than the controlled variety MX4 but was not displayed significantly difference in comparison with VN2. Nevertheless, the growth duration of this combination is shorter than the controlled varieties regarding to thedate to fresh ear harvest by about 10 to 14 days. This is conforming to the fresh waxy corn production in winter season in the Red River Delta region. It was also found that the lines belonging to different heterotic group exhibit higher SCA, indicating that genetic distance between lines correlated with SCA [19].
- Another studywas also conducted to evaluate the combining ability of thinner pericarp waxy maize inbred on the eating and nutrient quality traits such as tenderness, sweetness and amylose content through diallel cross using method 4 of Griffing. Six waxy maize inbred lines and their crosses were evaluated in a complete block design with 2 replications with plot size of $14m_2$ and HN88 as the hybrid variety check. The results showed that six parental lines and the crosses disclosed short growth duration. The grain yield of parental lines ranged from 2.448 to 2.961 t/ha and fresh ear yield of the crosses was higher than the check variety. Four waxy maize inbred lines are D_1, D_2, D_5, and D_6 had high values for general combining ability on the fresh ear yield, thinner pericarp trait, protein, amylose content and other traits. Our findings suggest that development of hybrid waxy maize variety for high quality should focus on parental inbred lines with combining ability for the quality traits [20].

Breeding waxy maize hybrids

Waxy maize hybrid program established from 2003, began upon collection of waxy maize germplasm, evaluation, selection, inbreeding and developing of the hybrid breeding. Hybrid waxy maize breeding wassuccessful with4waxy maize hybrids have high fresh eating quality are VNUA16, VNUA 69, MH8 (ADI688), HUA 601 and a purple waxy maize hybrid is NT141 with high quality and anthocyanin content.

Waxy maize hybrid VNUA69

VNUA69 waxy maize hybrid is a single cross between D6 line (female) with D_4 line (male). D_6 line gathered in breeding continuously 8 generation from the F_1 progeny of crosses GN47 accession x 608 line, among them, a line import from GXAAS (Guangxi Academy of Agricultural Science), China; GN47 is an open-pollination local waxy maize variety collected in Yen Bai province. D_4 line (male) received from in breeding continuously 8 generation from the F_1 progeny of crosses GN2 x AG208, among them, GN2 (open-pollination local waxy maize variety collected in Dien Bien province), AG208 is hybrid, it was imported by Angiang Plant Protection Company (nowaday is Loc Tro company) from China (Figure 3).

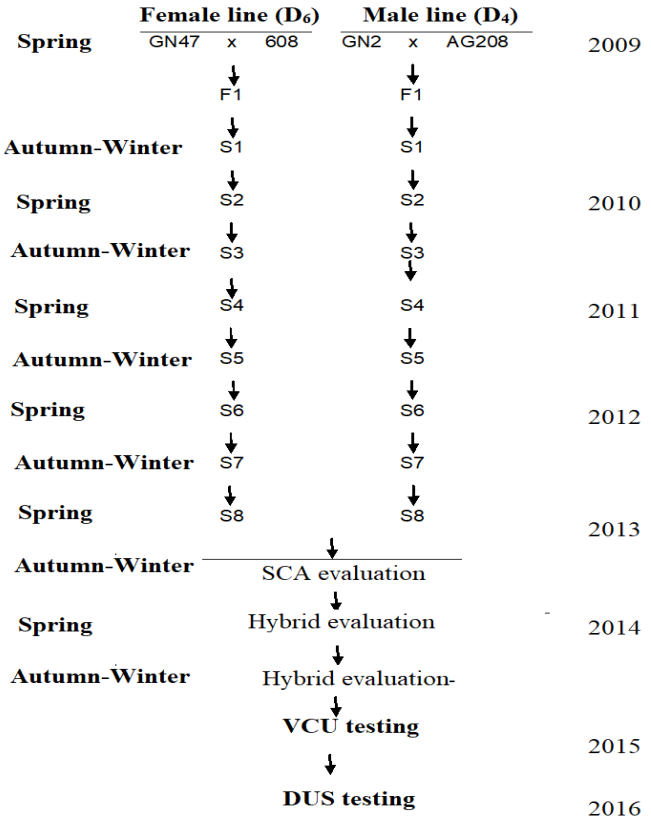

Figure 3: Scheme of the hybrid waxy maize VNUA69 breeding

Table 8: Fresh ear yield and yield component of VNUA69 hybrid though three seasons at Gialam,Hanoi

Place	**VNUA69**			**HN88 (Check)**		
	Winter 2014	**Spring 2015**	**Winter 2015**	**Winter 2014**	**Spring 2015**	**Winter 2015**
Ear length (cm)	18.0	18.5	18.2	18.5	18.2	17.9
Ear diameter (cm)	5.3	5.4	5.2	4.8	4.8	4.4
No. Kernel row	18-20	16-18	16-18	12-14	12-14	14-16
No. kernel/row	29.1	37.9	28.6	33.5	34.7	33.0
Kernel 1000 weight (g)	295.8	299.3	303.0	241.3	234.8	247.2
Fresh ear yield (t/ha)	13.03	13.38	12.82	11.12	11.65	11.07

Waxy maize hybrid VNUA69 was evaluated in National Inspection and Testing Networkwhich belongs to National Technical Regulation on Testing for Value of Cultivation and Use of Maize varieties of the Ministry of Agricultural and Rural development No. QCVN01-56:2011/BNNPTNT and National Technical Regulation on Testing for Distinctness, Uniformity, and Stability of Maize Varieties of the Ministry of Agricultural and Rural Development No. QCNV01-66:2011/BNNPTNT (Table 8, Table 9).

Table 9: Fresh ear yield of waxy maize hybrid VNUA69 through three seasons in Nation Inspection and testing Network

Season	Local	Fresh ear yield harvested after silking 18 – 20 d (t/ha)					Average
		Ha Noi	Hai Duong	Thai Binh	Thanh Hoa	Nghe An	
	Hybrid						
Autumn 2015	VNUA69	13.017	12.160	10.719	10.853	11.830	11.716
	HN88 (Check)	10.068	11.833	11.174	9.187	10.373	10.527
CV%		*6.0*	*5.2*	*6.8*	*6.2*	*6.8*	
LSD(0,05)		*8.7*	*9.1*	*10.2*	*10.7*	*10.1*	

Spring 2016	VNUA69	13.024	11.211	12.903	12.773	12.714	12.525
	HN88 (Đ/c)	11.594	11.652	13.166	10.780	11.286	11.696
CV%		*4.0*	*8.2*	*6.1*	*4.0*	*4.5*	
LSD(0.05)		*7.09*	*14.69*	*11.52*	*6.70*	*7.59*	
Autumn 2016	VNUA69	12.855	11.400	11.429	11.538	10.103	11.465
	HN88 (Đ/c)	11.639	11.005	10.982	10.282	11.157	11.004
CV%		*3.9*	*3.6*	*6.0*	*6.5*	*6.7*	
LSD(0.05)		*7.26*	*6.37*	*10.53*	*10.58*	*11.52*	

Source: Nation testing for crop variety and material Centre 2015-2016

Table 10: Fresh eating quality of the VNUA69 waxy maize hybrid (scale) through three seasons in Nation Inspection and testing Network

No.	Traits	VNUA69			HN88 (Check)		
		Winter 2015	Spring 2016	Winter 2016	Winter 2015	Spring 2016	Winter 2016
1	Sweetness	4	3.8	3	3	3.4	3
2	Aroma	3	2.9	2	2	2.1	2
3	Tast	2	2.4	2	2	2.2	2
4	Tenderness	3	2.2	2	2	1.9	2
5	Cooking ear color	Milky white	Milky white	Milky white	Milky white	Milky white	Milky white

Source: Nation testing for crop variety and material Centre 2015-2016

In Testing for Distinctness, Uniformity and Stability of VNUA69 waxy maize variety. Uniformity was evaluated on the 120 plants, there are 7 off-type plants that an unexceeded number of maximum plant of the permission regulation (Table 11)

Table 11: Distinctness, Uniformity and Stability of VNUA69 waxy maize variety

	Trait	Testing year	VNUA69	HN88 (check)	Least significant difference /LSD $_{0,05}$
9	Tassel: anthocyanin pigmentin flower glume root (1/3 flower in primary branch)	2016	8	6	2
11	Tassel: Anthocyanin pigment of Anther(1/3 flower in middle branch, fresh anther)	2016	6	2	2
13.(*)	Tessel: Angle between main and secondary branch (1/3 tassel lower)	2016	2	4	2
15. (*)	Tassel: Number of primary branch (branch)	2016	10.15	17.40	1.72
18	Stem: Anthocyanin pigment in brace roots	2016	8	6	2

Source: Nation testing for crop variety and material Centre 2015-2016

Stability: VNUA was showed through two seasons DUS testing confirm that VNUA 69 is stable compared with mega- variety (check) in DUS testing Table 11.

Waxy maize hybrid MH8 (ADI688)

Waxy maize hybrid MH8 is a single cross variety between D611 (female) x D158 (male). The female D611 line was derived in F_1 progenyof crosses D25 x HN88 inbreeding developed to S_7 generation.HN88 is waxy maize hybrid variety imported from China by Nation Seed Company and growing large area in Vietnam. D25 line was developed by self-pollinated an OPV local variety is GN66 (germplasm collected in Dien Bien province). Male line D158 was extracted from HQ5 hybrid imported from Korea by self-pollinated come to S_7 specific combining ability evaluation get MH8 and took into next studies and transferred the breeder's right to ADI company and rename ADI688;

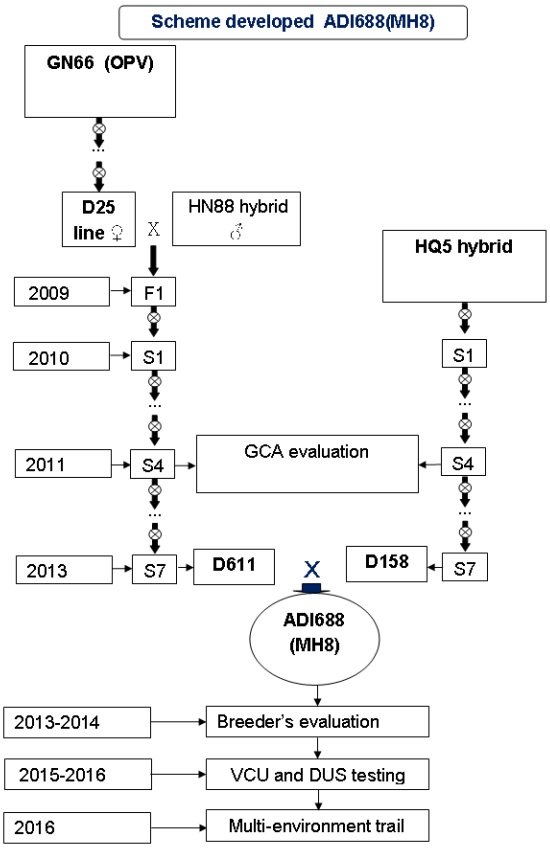

Figure 4: Scheme of the hybrid waxy maize MH8 breeding

The new variety MH8 was showed good performance and high fresh ear production in yield trial, regional trials and sensory evaluation trials. The new variety has high fresh ear yield with husk about 11,294 t/ha, good ear shape and good eating quality (Figure 4).

Waxy maize hybrid HUA601

The hybrid waxy corn crosses 601 developed by Maize Research Institute (MRI), Guangxi Academy of Agriculture Science (GXAAS), China was cooperation with Crop Research and Development Institute (CRDI), Vietnam National University of Agriculture (VNUA), Vietnam to evaluate and to select in Vietnamese condition. Hybrid waxy corn HUA601 was evaluated at CRDI in two seasons autumn-winter season of 2011 and spring season of 2012. HUA601 showed yield higher than that of the check variety MX10. In autumn-winter season of 2011, HUA601 has yield 9.2t/ha (check variety 6.50t/ha) and 9.25t/ha (check variety 7.05t/ha) in spring season of 2012. HUA601 was also well- tolerance to biotic and abiotic stresses in field condition, and has good eating quality. Result was evaluated from Vietnam National Plant Testing Network for VCU in five sites and three seasons that were winter of 2012, spring of 2013 and winter of 2013 have average growth duration from sowing to fresh harvesting were 90 – 91 days that belong to early mature group, plant height from 170.5 to 172.3 cm, fresh yield from 86.60 (winter season of 2012) to 12.7t/ha (winter season of 2013). Yield and yield components of HUA601 were higher than that of check variety (Wax44) special in testing sites Hanoi, Vinh

Phuc and Thai Binh. HUA601 has good eating quality and tolerance to drought and some insects such as stem and ear borer (*Ostrinia nubilalis* Hübner), disease maize rusts (*Puccinia sorghi*), maydis leaf blight(*Helminthosporium maydis*). HUA601 was DUS testing in spring of 2013 and spring of 2014 on the distinctness, uniformity and stability. Distinctness testing showed that HUA601 showed two traits, which was number 18 stalk (anthocyanin color in air root) and trait number 40 ear (color of the back kernel), were clearly different compared to check and seminar variety. HUA601 was stability and uniform with high yield in both of tested seasons. Production testing with 30.1 ha in five provinces that were Lao Cai, Thai Binh, Nghe An, Phu Tho and Ha Nam during the spring season in 2014. HUA601 had yield ranged from 9.99 tons/ha to 11.82tons/ha and autumn-winter season gained 9.77 tons/ha to 12.53 tons/ha. The objectives of this study was to determine the effect of fertilizer and plant density with yield and quality of the hybrid waxy corn HUA601 was identified fertilizer quantity and plant density per ha for highest of the fresh yield and good quality are 160kgN- 90kgP_2O_5 - 70kgK_20 and optimum planting was 57.000 plants/ha [21].

Waxy maize hybrid VNUA16

In the light of Industry recognization, it is the big challenges of incorporating diversity into their elite breeding pools because of lack of adaptation and undesirable agronomic traits associated with exotic maize germplasm, the time required to extract valuable materials, and the rapid nature of breeding cycles in commercial programs. Breeding cross combinations potential germplasm: GEM A/GEM B GEM A/Private A GEM A/Ex-PVP GEM A/Exotic //GEM B GEM A/Exotic//Ex-PVP Exotic/Private A//Elite Exotic///Private B [22]. Waxy maize breeding program at CRDI applied this concept to develop waxy maize single cross variety VNUA16.

The female line D8.1 derived from F_1 progeny of GN125 x AG3 through self-pollination to reach S_8 generation, of which GN125 is OPV waxy maize variety collected from Cao Bang province and AG3 is a hybrid from China. The male line D12 was derived F1 progeny of GN58 x AG208, inbreeding through 8 generations gained the D12 inbred line and take into SCA evaluation to identify promisingcrossed are VNUA16.

The new variety VNUA16was showed good performance and high fresh ear production in yield trial, regional trials and sensory evaluation trials. The new variety has short growth duration from sowing to fresh ear harvesting about 65 – 70 d in Winter season and 80 -85 d in the spring season, high fresh ear yield with husk about 11. 0 to 14.0 t/ha, good ear shape and good eating quality.

Purple Waxy maize hybrid NT141

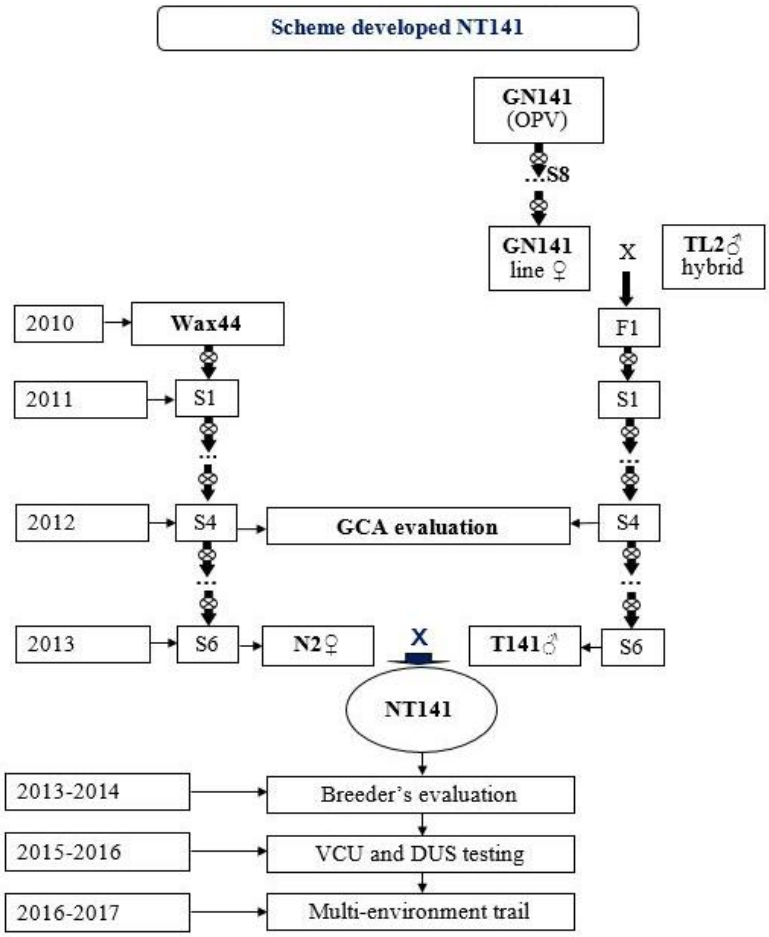

Figure 5: Scheme of the purple hybrid waxy maize NT141 breeding

NT141is a new purple waxy maize hybrid derived from crosses between N2 line x T141, among them N2 female line was selected from Wax44, a commercial waxy maize hybrid in Vietnam of Syngenta Company to reach S_6 generation. T141 male line is inbreeding initiative F_1 progeny of GN141 x TL2 crosses to reach S_6 generation. GN141 is local purple waxy maize collected in Dien Bien province and TL2 is purple waxy hybrid have origin Thailand, it a commercial hybrid in Vietnam (Figure 5).

NT141 was evaluated at CRDI and submitted in National Testing Network. The result was shown short growth duration from sowing to fresh ear harvesting about 74 to 78 d and to mature about 87 to 90 d. Plant height from 165cm to 175cm, height ear from 55cm to 65 cm, stem with purple–green color,vein and kernel have purple. Tolerance to lodging and some diseases in field condition, good eating quality tenderness, sweetness, taste, and special high anthocyanin content from 103.2 in winter season to 114.2 mg/100g; high fresh ear yield with husk about 9.00 to 11.5 t/ha (Figure 6).

Figure 6: Ear of the purple hybrid waxy maize NT141 breeding A: Fresh ear; B: Dried ear

Current opportunities and future prospect to generate waxy maize hybrid in Vietnam

The germplasm enhancement of waxy maize in Vietnam has valuable to increase diversity in breeding, our program has made great efforts to integrate between the domestic and exotic germplasm which collected from some provinces of the four ecosystem regions of Vietnam and some countries in Asia as Thailand, China, Philippines and Korea. The waxy maize program consisted germplasm, genetics, phenotyping, and selection, combined with waxy maize product targets, are the foundation of successful hybrid waxy maize breeding.

Kernel quality and early maturity are also indispensible in maize and waxy maize breeding programs. Currently, most maize varieties grown commercially are hybrids. However, some waxy corn varieties in mountainous regions of Vietnam, where most ethnic minority people are countries are open-pollinated varieties (OPVs). Therefore, the improved OPVs are important in these regions, and they also have had potential as germplasm sources for hybrid development. OPVs with high yield or good adaptation are excellent germplasm sources for waxy maize enhancement via the breeding programs.

Hybrid waxy maize breeding with superior yield, good quality adapted to Northern regions of Vietnam was combined adaptation with exotic germplasm. An initiative from the collection, evaluation, selection, the inbred line development and combining ability analysis have been implemented. The works have beeb enabled to contribute for successful waxy maize hybrid in the target traits as quality, short growth duration, and high anthocyanin content. To the best our knowledge, the result attained, lessons learned, and current opportunities for further improving the quality and yield of waxy maize for Vietnam are promising and needed to be further researched.

National and International collaborations and cooperation between public and private agencies in the country will significantly increase to exchange waxy maize germplasm and characterize germplasm in unique environments to provide information on performance, yield, quality, biotic and abiotic stress resistance and other traits. This mission will be remarkably contributed to develop sustainable agriculture production as well as economic stability and the nutrition and well-being of society in this country.

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