ISSN: 0975-833X

Availableonlineathttp://www.journalcra.com

InternationalJournalofCurrentResearch
Vol. 9, Issue, 11, pp.61045-61053, November, 2017

INTERNATIONAL JOURNAL OFCURRENTRESEARCH

RESEARCH ARTICLE

EVALUATION OF UH400 INDUCER FOR ADAPTED AND INDUCING HAPLOID ABILITY IN VIETNAMESE CONDITION

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ARTICLEINFOABSTRACT

ArticleHistory:
Received 22nd August, 2017
Received in revised form
14th September, 2017
Accepted 29th October, 2017
Published online 30th November, 2017

Key words:
Inducer,
Haploid,
Double Haploid,
Maize Inbred line.

Adapted evaluation of the UH400 line in the Northern of Vietnam with ten seasons from 2012 to 2015, it could be maintained and multiplication in spring and Autumn-winter season and sowing time for most suitable from early to middle of February annual. UH400 line can inducing the inbred lines with ratio from 4 to 10 percentage depends on the genotype, seeds of haploid type identified by the morphological marker *R1-nj*, this was confirm by molecular marker SSR. Doubling haploid lines, which were induced by colchicine with concentration was 0.06%, were obtained about 50 percentages of the survived DH lines. These DH haploid lines were evaluated on the field trial together inbred lines in Autumn-winter season in 2015. They showed appropriated growth and development in Northern of Vietnam and agronomical characteristics were indifferent compared within bred lines, they have yield vary from 14.21 to 44.12 quintal/ha. These DHs could be used for hybrid maize breeding programs in Vietnam.

Citation: Vu Van Liet, Nguyen Viet Long, Nguyen Van Ha, Pham Quang Tuan, Hoang Thi Thuy, Vu Thi Bich Hanh, Tran Thi Thanh Ha, Nguyen Thi Nguyet Anh and Le Thi Kim Hue. 2017. "Evaluation of uh400 inducer for adapted and inducing haploid ability in vietnamese condition", *International Journal of Current Research*, 9, (11), 61045-61053.

INTRODUCTION

Modern-day maize production is based on the heterosis phenomenon, which accounts for the vigour of hybrid progeny relative to the homozygous parents (Shull, 1909). Heterosis is larger when crossing genetically divergent lines than when crossing those that are genetically close or related. Shull, G. H., 1908 reported the pure- line method in corn breeding. The author showed that self-fertilization in the maize from 6 to 8 generations will be developed inbred lines with homogenous, that inbred lines of maize showed general deterioration in yield and vigor, but that hybrids between two inbred immediately and completely recovered (Shull, 1908); in many cases their yield exceeded that of the varieties from which the inbred lines were derived. Furthermore, they had a highly desirable uniformity. In a subsequent paper in 1909, he outlined the procedures that later became standard in corn-breeding programs (Shull, 1909). The conventional processes of inbred line development, it takes at least 6 - 8 generations with ~99% homozygosity (Forster and Thomas, 2005; Geiger and Gordillo, 2009; Chang and Coe, 2009).

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The DH technology shortens the breeding cycles significantly by rapid development of completely homozygous lines (within 2–3 generations). The haploid inducers are specialized genetic stocks, when crossed to a diploid (normal) maize plant, resulted in progeny kernels in an ear with segregation for diploid (2n) kernels and certain fraction of haploid (n) kernels due to anomalous fertilization. Kernels with a haploid embryo have are triploid (3n) endosperm, and therefore, these kernels are capable of displaying germination similar to those kernels with a diploid embryo (Coe and Sarkar, 1964). The DH technology in maize breeding, which based on in vivo haploid induction, is recognized worldwide as an important mean for enhancing breeding efficiency. Major advantages of DH lines in hybrid breeding include (i) maximum genetic variance from the very first generation, (ii) perfect compliance with DUS criteria, (iii) short time to market, (iv) simplified logistics, (vi) reduced expenses for selfing and maintenance breeding (Andrés Gordillo and Hartwig H. Geiger, 2010).Chase (1947, 1951) reported a spontaneous haploid induction rate in maize of 0.1% and suggested that haploids could be used for line development in hybrid breeding. However, initially the low spontaneous haploid induction rate did not meet the needs of breeders. A much higher induction rate (up to 2.3%), Coe (1959) found an inbred line called Stock 6 with an induction

rate of 1 to 2%. This line became the ancestor of all subsequently developed inducer lines. A number of haploid inducer lines with high HIR and for commercial use have been derived over the years, with Stock 6 as the founder; these include: (1) KMS (Korichnevy Marker Saratovsky) and ZMS, both derived from Stock 6 (Tyrnov and Zavalishina1984, cited in Chebotar and Chalyk, 1996); (2) WS14, developed from a cross between lines W23ig and Stock 6 (Lashermes and Beckert, 1988); (3) KEMS (Krasnador Embryo Marker Synthetic), derived from a cross (Shatskaya *et al.,* 1994); (4); MHI (Moldovian Haploid Inducer), derived from a cross KMS × ZMS (Eder and Chalyk, 2002); (5) RWS (Russian inducer KEMS+ WS14), descendant of the cross KEMS×WS14 (Röber *et al.,* 2005); (6) UH400, developed at University of Hohenheim from KEMS (Chang and Coe, 2009); (7) PK6 (Barret *et al.,* 2008); (8) HZI1, derived from Stock 6 (Zhang *et al.,* 2008); (9) CAUHOI, developed at China Agricultural University from a cross between Stock 6 and Beijing High Oil Population (Li *et al.,* 2009), and (10) PHI (Procera Haploid Inducer), derived from a cross between MHI and Stock6 (Rotarenco *et al.,* 2010). The temperate inducers UH400, RWS, and RWS×UH400 were successfully employed for haploid induction and DH line development in CIMMYT's tropical and subtropical source germplasm from 2007 to 2011. Although these temperate inducers are poorly adapted to tropical lowland conditions (Prigge *et al.,* 2011). Base on the cooperation between Vietnam National University of Agriculture (VNUA) and Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim (UH), 70593 Stuttgart. VNUA received UH400 from UH in order to development of DH lines for hybrid maize breeding program. The objectives of this study were evaluated adaptability and inducing haploid in maize in Vietnamese condition.

MATERIALS AND METHODS

In vivo induction of haploid materials

The materials used in this study consisted of UH400 inducer line (University of Hohenheim, Germany) and 29 inbred lines, which were developed at VNUA from domestic and exotic germplasm on S_6 to S_8 generation, of which there are 19 lines (symbolized D1 to D19) were induced to double haploids (DHs) and 10 inbred lineswere not induced (symbolized IL1 to IL10) as check varieties as the following:

Methods

Evaluation of UH400 adaptation in Northern of Vietnam condition

The experimental plots (10 m^2/plot) consisted of two rows, each ten meters long and two meters width, a row-spacing of 0.50 m and plant-spacing of 0.25 m. Seeds were sown excessively and thinned to 80 plants $plot^{-1}$ (55,000 plants ha^{-1}). Experiment was implemented from 2012 to 2015 to evaluate adaptation, the UH400 was sown 10 times at Gialam, Hanoi, where belong to Red River Delta, the Northern of Vietnam as follows:

- In 2012, SD1 (sowing date 1): Aug 8, 2012; SD2: Sep 5, 2012; SD3: Nov 28, 2012;
- In 2013, SD4: Feb 7, 2013; SD5: Feb 17, 2013; SD6: Mar 12, 2013;
- In 2014, SD7: Sep 15, 2014; SD8: Oct 15, 2014; SD9: Nov 29, 2014
- In 2015, SD 10: Feb 13, 2015

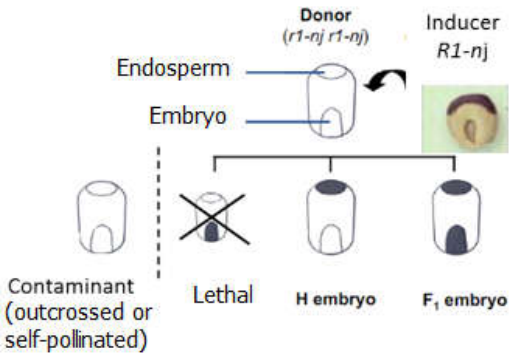
No.	Inbred lines	Generation	Germplasm original
19 inbred lines used to induce maternal haploid			
1	D1	S8	Vietnam
2	D2	S5	US
3	D3	S6	Vietnam
4	D4	S6	US
5	D5	S8	China
6	D6	S8	Vietnam
7	D7	S6	China
8	D8	S6	China
9	D9	S6	US
10	D10	S6	China
11	D11	S5	US
12	D12	S5	US
13	D13	S6	Vietnam
14	D14	S6	China
15	D15	S6	China
16	D16	S6	China
17	D17	S6	China
18	D18	S6	China
19	D19	S6	US
10 inbred lines (check varieties)			
20	IL1	33.12131111	Vietnam
21	IL2	122.52121111	US
22	IL3	123.64211111	US
23	IL4	126.11271111	China
24	IL5	D22.12131111	Vietnam
25	IL6	D29.11312111	Vietnam
26	IL7	D6.12121111	Vietnam
27	IL8	D5.5.5.3,3	US
28	IL9	D162.2.2.1	China
29	IL10	D1.1.1.3	China
30	UH400	Inducer	Germany

Each of the SD times was planted on a bed with 10m length, two rows per bed, spacing row to row was 50 cm and plat to plant was 25 cm. Data were recorded for growth duration, sowing to tasseling, sowing to silking, agronomical characteristics, field tolerance to biotic and abiotic stresses, yield and yield components per plant.

Design and implementation ofmaternal haploid induction

Planting the alternation between UH400 with maternal inbred lines on the plots (10 m length), planted two rows per plot, spacing row to row was 50 cm and plant to plant was 25 cm. The same field design for the induction nursery with isolation using open pollinations and aninduction nursery using manual pollinations. Base on the growth duration from sowing to flowering time source population (days to silking) and UH400 (days to anthesis) is synchronous. At tasseling and silking period, each plant was isolated by glassine paper bag, removal of the tassels from source populations immediately after they appear to minimize pollen contamination. The selected 10 plants of UH400 per plot for selfing to maintain and seed multiplication, crossing between UH400 and maternal inbred lines by collected pollens of the UH400 plants mixed to pollinate for each plant on the rows of the maternal inbred lines and pollinated twice. Harvesting is conducted when all the ears in that population reach physiological maturity. Assessment of the haploid inducer lines for haploid inducing ratio (HIR) requires suitable this study used *R1-nj* (R1-Navajo) anthocyanin marker system for assessment of HIR in UH400, kernel on the ears classified three types of kernels are (1) normal diploid or hybrid kernels with purple coloration on the endosperm (aleurone) and the embryo (scutellum); (2) Haploid kernels with purple endosperm but no coloration on the

embryo; and (3) Kernels without purple coloration on the embryo and endosperm, which could be due to pollen contamination as describe from Andrés Gordillo và Hartwig H. Geiger, 2010 and Vijay Chaikam and BM Prasanna, 2011.



Inducing chromosome doubling of maternal haploids by colchicines

Haploid plants contain only one copy of each chromosome in their cells. Haploids kernels derived by maternal in vivo induction contain chromosomes only from the maternal inbred lines. Doubling chromosomes of haploid kernels to creates DH were used colchicines ($C_{22}H_{25}NO_6$) as decribled by W. Schipprack (2012). Briefly, kernels were geminated on the paper, cutting the coleoptiles being about 2 cm,treatment of seedlings with colchicine in 0.06% for 12 hours then washing by distilled water, planting of treated seedlings in greenhouse, transplanting of D_0 plants at three leave stage in the field and evaluted DH lines in autermn-winter season together with inbred lines no inducing.

Checking chromosome doubling of maternal haploids by DNA markers

We used 5 primers indicated for the maize crop, according to Battistelli et al. (2013) and Veiga et al. (2012) in a study of QTLs linked to resistance to cercospora disease and maize grain production. Of the 5 primers are *BNLG 1175, BMC 1714, BNLG 1520*, *BNLG 1233*, and *BNLG 1258* were considered to be polymorphic between the parents, whose bands had good size and definition on the gels. These primers were used in the progeny to analyze and determine the paternity of haploids.

Primers	Sequences	Repeat	Bin
bnlg1175	Left End: ACTTGCACGGTCTCGCTTAT Right End: GCACTCCATCGCTATCTTCC	Repeat: AG(38)	2.04
bnlg1520	Left End: TCCTCTTGCTCTCCATGTCC Right End: ACAGCTGCGTAGCTTCTTCC	Repeat: AG(22)	2.09
bnlg1233	Left End: GAACACCAGAGGAGAGTGGG Right End: TTCACTTGTCCACCACTGGA	Repeat: AG(24)	2.08
bnlg1258	Left End: GGTGAGATCGTCAGGGAAAA Right End: GAGAAGGAACCTGATGCTGC	Repeat: AG(24)	2.08
bmc1714	Left End: CATCATGGAGGCATATGTCG Right End: ACACATTTAGACCCACCCCA	AG(25)	9.04

Chromosomal duplication assessment by microsatellites, DNA from the inducer UH400 line was extracted from the 19 DH lines (crosses between UH400 x 19 inbred lines), 10 inbred lines and UH400 line. Sampling was conducted to collect DNA from plants that produced pollen and had green leaves and were classified as haploid, haploid/diploid, diploid, or diploid/tetraploid. The extraction procedure was performed according to Pereira et al. (2007). Briefly, 3 g leaves were collected from each plant and ground using a mortar and pestle in liquid nitrogen, 10 mL extraction buffer, and 20 µL β-mercaptoethanol. The extraction buffer consisted of 2% CTAB, 100 mM Tris, pH 8.0, 1.4 M NaCl, and 1% polyvinylpyrrolidone. The ground material was placed in centrifuge tubes and incubated in a 65°C in water bath for 30 min. The tubes were gently shaken during the incubation. After centrifugation, the supernatant containing the nucleic acids was collected. The nucleic acids were extracted using 10 mL chloroform: isoamyl alcohol (24:1) and precipitated by adding 30 mL of 95% ethanol and 7.5 M ammonium acetate (6:1). This mixture was placed in a -20°C freezer for approximately 12h. Following precipitation, the nucleic acids were transferred to microcentrifuge tubes, centrifuged, and dried. Subsequently, the nucleic acids were rehydrated in TE buffer (1 mM Tris and 0.1 mM EDTA). Shortly after the second extraction with chloroform: isoamyl alcohol (24:1), the supernatant was precipitated by adding at least three volumes of 3 M sodium acetate: 95% ethanol (1:20).

The precipitated nucleic acids were kept in TE buffer. A third extraction was performed with chloroform-isoamyl-alcohol using an equal volume of TE. The supernatant was collected and the DNA was precipitated (for approximately 12 h in the freezer) with sodium acetate alcohol until filling the tube (1.5 µL). After centrifugation for 10 min at 10,000 rpm, the nucleic acids were rehydrated using TE buffer. Aliquots of DNA from each specimen were quantified on a 1% agarose gel alongside a standard DNA molecular weight marker from λ phage DNA with the concentrations of 100, 200, and 300 ng/µL. Upon visual comparison of the intensities of the DNA bands of the samples and standards, the DNA of each sample was quantified and the concentration subsequently adjusted to 10 ng/µL for use in the reactions.Each PCR volume totaled 11.06 µL. This reaction was prepared by mixing the following reagents at the respective concentrations (Pereira *et al.,* 2007): 2.25 µL genomic DNA; 1 µL solution containing deoxyribonucleotide acids (dATP, dGTP, dCTP, and dTTP); 0.6 µL Taq DNA polymerase; 0.8 µL primer pair; 1.0 µL reaction buffer (50 mM Tris, pH 8.3, 2.0 mM MgCl2, 20 mM KCl, 10 µg BSA, 0.25% Ficoll 400, and 10 mM tartrazine); and 4.45 µL distilled water to the final volume. Amplifications were performed in 0.2-mL tubes on a Model Mastercycler Eppendorf thermocycler. The cycling program consisted of 5 min at 95°C for DNA denaturation, 8 cycles for 20s at 94°C for denaturation, 20s for primer annealing at 55° or 60°C (depending on the primer tested), and 1 min at 72°C for DNA extension. We performed 24 cycles during which only the annealing temperature (from 52° to 65°C) differed from the initial cycles. In addition, there was a final extension step performed for 4 min at 72°C. The PCR products were subjected to vertical electrophoresis for 1 h and 40 min at 130V on a 2% agarose gel. After completion, the gel was stained using ethidium bromide (0.5 µg/ ml) for 10 min, followed by rinsing in running water. The gel was then immersed in a developing solution until bands appeared, evaluated under fluorescent light, and photographed using a digital camera.

Evaluation of DH lines and inbred lines

In Autumn-Winter season 2015 was implemented to evaluate of 19 DH lines and 10 inbred lines no inducing in experiment in RCBD with two replications, plot area are 14m^2 at Crop Research and Development Institute, VNUA. Data were recorded for growth duration, agronomical characteristics, field

tolerance to biotic and abiotic stresses, yield and yield components.

Statistical analysis

The grain yield data were subjected to combined analysis of variance (ANOVA) cross sowing dates. Since, genotype and location interaction (GEI) and used AMMI (Additive main effects and multiplicative interactions) analysis by software IRRISTAT ver.5.0.

RESULTS AND DISCUSSION

Adaptation of the UH400 inducer in Northern condition of Vietnam

UH400 inducer from Germany was sown in 10 times (symbolized SD1 to SD10) that focus in the spring season and autumn-winter season from 2012 to 2015. The line was growth in Vietnamese condition (Table 1). Sowing times have effected to growth and development duration of UH400 inducer, sowing to germinated from 5 to 10 days, SD9 and SD10 showed slower germination, corresponding to from sowing to tasseling ranged 41 to 58 days, silking ranged 43 to 59 days and sowing to physiological mature ranged from 80 to 108 days, sowing times showed too short duration also have lower yield. Anthesis to silking interval (ASI) is an importance trait related to adaptation of the crops as maize, shorted ASI will be more adaptation and more tolerance to stressese. Over 10 sowing times, the UH400 has short interval ranged from 1 to 4 days, thus it is appropriate for growing in northern of Vietnam.Morphologies of UH400 inducer at Gialam, Hanoi showed that plant height at short level ranged from 102.5 – 132.5 cm and variation between sowing times at significant difference; ear height ratio per PH about 30 – 35% and not significant difference. Color of the stem, leaves is almost purple and kernel color has black spot on the crown. Color characteristics were not changed compared original from University of Hohenheim indicated this trait quite stability in ecological condition and across of the 10 sowing times.

Figure 1. Morphological and color of the UH400 inducer at Gialam, Hanoi

Adaptation of the UH400 inducer on the fertility traits showed poorly seed setting in Vietnamese condition thus small tasseling, number of branch ranged from 13.2 – 14.5 branches, low pollen quantity in 2 to 4 score level, silk ability at medium level such as selection the sowing date is an importance factor.

This study indicated that favorable sowing date have higher seed setting, sowing date are SD4, SD5 and SD10 in February of 2013 and 2015 was highest seed setting from 80 to 90% per ears. Result also appropriated with other studies as H.H. Geiger, G.A. Gordillo (2009), DH lines have highest yield was 323 kg ha^{-1}.The damage ability of the UH400 inducer on the field showed appear stem borer (*Ostrinia nubilalis* Hübner); Turcicum leaf blight (*Helminthosporium turcicum*); maydis leaf blight(*Helminthosporium maydis)*, special in the sowing times with high temperature. Stem borer damaged ranged from 5.5 to 25.5% in the sowing date have high temperature, three sowing dates were have slighter susceptible are SD4, SD5 and SD10 ranged from 5.5 to 9.7%. Turcicum leaf blight disease ranged from 2 to 5 score, leaf blight disease score vary from 3 to 5, three sowing times SD4, SD5 and SD10 showed susceptible more slightly (Table 4).

Figure 2. Kernel of the UH400 inducer at Gialam, Hanoi

Yield and yield components of the UH400 inducer in Vietnamese condition showed that the ear length ranged from 9.5 to 12.1 cm, ear diameter from 2.0 to 2.5 cm, number of kernel per ear ranged from 10.0 to 15.5 kernel and 1000 - kernel weight range from 115.5 to 195.5g. Plant yield ranged from 5.14 to 11.95 g/plant, the highest yield was SD4 and SD5 following is SD10.Genotype and environment interaction analysis according to Eberhart-Russel and AMMI model (additive main effects and multiplicative interaction) and as desirable from Guilherme Moraes Ferraudo *et al.,* (2014) with characteristics are growth duration (GD), plant height (PH), Anthesis-silking interval (ASI), number of leaves (NL), 1000-kernel weight, individual yield and yield components (IY). Analysis value will used to determine the best sowing time to maintain and multiplication of the UH400 inducer in Northern condition of Vietnam (Table 6). Result of analysis by AMMI model indicated that UH400 can grow and develop in autumn-winter and spring season in Northern of Vietnam. But the best sowing time was from early to middle February in both 2013 and 2015. Because UH400 inducer is the temperate inducer, so that in autumn-winter and spring season in the Northern with lower temperature than other season.

Evaluation of in vivo maternal haploid induction

Haploid inducing ratio (HIR) of the UH400 inducer when crossed with 19 maternal inbred lines used an *R1-nj* (*R1-Navajo*) anthocyanin marker system for assessment of HIR in inducer lines that incorporate R1-*nj*.

Table 1. Growth stages of the UH400 inducer through 10 sowing dates at Gialam, Hanoi

Sowing date	Days of germinating	Days to tasseling	Days to silking	Days of ear leaf senescence	ASI (days)
SD1 (Aug 11,2012)	5	41	43	80	4
SD2 (Sep 5, 2012)	6	46	49	86	3
SD3 (Nov 28, 2012)	8	54	57	91	3
SD4 (Feb 7, 2013)	5	52	55	86	3
SD5 (Feb 17, 2013)	5	50	53	82	3
SD6 (Mar 12, 2013)	5	50	54	80	4
SD7 (Sep15, 2014)	7	45	49	95	4
SD8 (Oct 15, 2014)	7	50	52	102	2
SD9 (Nov 29, 2014)	10	58	59	108	1
SD10(Feb13, 2015)	5	53	54	103	1

Table 2. Some agronomical and morphological characteristics of UH400 inducer through 10 sowing times at Gialam, Hanoi

Sowing date	PH(cm)	EH(cm)	No. Leaves	SD (cm)	SC	LC	KC
SD1 (Aug 11,2012)	100.5	28.5	14.2	1.62	Purple	Purple	W,B
SD2 (Sep 5, 2012)	117.2	30.5	15.0	1.60	Purple	Purple	W,B
SD3 (Nov 28, 2012)	102.7	22.4	11.7	1.58	Purple	Purple	W,B
SD4 (Feb 7, 2013)	102.2	25.1	11.7	1.65	Purple	Purple	W,B
SD5 (Feb 17, 2013)	109.3	28.8	11.7	1.86	Purple	Purple	W,B
SD6 (Mar 12, 2013)	132.5	45.2	15.3	1.80	Purple	Purple	W,B
SD7 (Sep15, 2014)	102.5	35.5	15.2	1.65	Purple	Purple	W,B
SD8 (Oct 15, 2014)	120.0	38.2	15.5	1.86	Purple	Purple	W,B
SD9 (Nov 29, 2014)	117.3	41.3	15.6	1.80	Purple	Purple	W,B
SD10(Feb13, 2015)	110.5	40.0	15.0	1.88	Purple	Purple	W,B

Notice: PH: plant height; EH: ear height; No. leaves: number of leaves; SD: stem diameter; SC: stem color; LC: leaves color; KC: kernel color; W: white, B: black spot on cap

Table 3. Some characteristics of tassel, anthesis and silking ability of UH400 inducer through 10 sowing times at Gialam, Hanoi

Sowing date	Tassel color	Tassel height (cm)	Pollen quantity (score)	Silking ability
SD1 (Aug 11,2012)	Purple	17.2	2	Medium
SD2 (Sep 5, 2012)	Purple	25.5	2	Medium
SD3 (Nov 28, 2012)	Purple	25.5	4	Medium
SD4 (Feb 7, 2013)	Purple	23.3	3	Fast
SD5 (Feb 17, 2013)	Purple	23.0	3	Fast
SD6 (Mar 12, 2013)	Purple	28.1	4	Fast
SD7 (Sep15, 2014)	Purple	22.1	3	Medium
SD8 (Oct 15, 2014)	Purple	25.2	2	Medium
SD9 (Nov 29, 2014)	Purple	25.5	4	Medium
SD10(Feb13, 2015)	Purple	24.7	3	Fast

Notice: 1 score: little, 5 score: abundantly

Table 4. Field pest susceptibility of UH400 inducer across 10 sowing times at Gialam, Hanoi

Sowing date	Stem borer (%)	Ear borer (%)	Turcicum leaf blight (score)	Maydis leaf blight(score)	Stalk rot(%)
SD1 (Aug 11,2012)	23.5	2.8	4	4	5.0
SD2 (Sep 5, 2012)	25.5	5.4	r	5	3.5
SD3 (Nov 28, 2012)	18.2	5.0	5	5	2.0
SD4 (Feb 7, 2013)	9.7	3.0	2	3	2.0
SD5 (Feb 17, 2013)	8.5	3.0	2	3	2.0
SD6 (Mar 12, 2013)	10.3	2.0	5	4	0.0
SD7 (Sep15, 2014)	25.4	10.2	4	5	0.0
SD8 (Oct 15, 2014)	17.2	0.0	4	5	0.0
SD9 (Nov 29, 2014)	10.3	0.0	4	4	5.3
SD10(Feb13, 2015)	5.5	2.0	3	3	0.0

Notice: Score 1: slighter, score 5: strong susceptibility

Table 5. Plant yield and yield components through10 sowing times at Gialam, Hanoi

Sowing date	ear/plant	Earlength (cm)	Ear diameter (cm)	No. row /ear	No. kernel/row	1000-kernel weight (g)	Yield (g/ plant)
SD1 (Aug 11,2012)	1.0	10.0	2.3	10.0	2.5	155.5	5.14
SD2 (Sep 5, 2012)	1.0	12.1	2.4	10.2	2.9	115.5	6.31
SD3 (Nov 28, 2012)	1.0	10.1	2.0	10.4	2.6	157.1	8.94
SD4 (Feb 7, 2013)	1.0	10.8	2.5	11.3	3.2	158.4	11.95
SD5 (Feb 17, 2013)	1.0	10.2	2.5	10.7	3.3	159.9	11.62
SD6 (Mar 12, 2013)	1.0	11.2	2.5	10.3	3.2	113.7	6.73
SD7 (Sep15, 2014)	1.0	10.3	2.3	15.5	3.5	175.3	7.24
SD8 (Oct 15, 2014)	1.0	9.5	2.4	15.5	3.0	173.6	5.69
SD9 (Nov 29, 2014)	1.0	10.6	2.5	10.5	5.2	167.5	6.84
SD10(Feb13, 2015)	1.0	11.5	2.5	12.5	5.0	195.5	9.24

Table 6. Genotype and environment interaction analysis over 10 sowing times of the UH400 inducer by AMMI model

Sowing date	Yield (g/plant)	T_{est}	AMMI
SD4 (Feb 7, 2013)	11.950	13.700	0.521
SD5 (Feb 17, 2013)	11.620	13.370	0.521
SD10(Feb13, 2015)	9.240	6.990	0.460
SD3 (Nov 28, 2012)	8.940	12.690	0.260
SD7 (Sep15, 2014)	7.240	8.990	0.391
SD9 (Nov 29, 2014)	6.840	8.590	0.391
SD6 (Mar 12, 2013)	6.730	8.480	0.391
SD2 (Sep 5, 2012)	6.310	8.060	0.391
SD8 (Oct 15, 2014)	5.690	7.440	0.444
SD1 (Aug 11,2012)	5.140	6.890	0.260

Table 7. Haploid inducing ratio of UH400 inducer with 19 inbred lines at Gialam,Hanoi

No.	Crosses		Total kernels /ear	Kernels type 2	Diploid kernels	Haploid kernels	HIR (%)
	Female	Male					
1	D1	UH400	49	11	33	5	10.09
2	D2	UH400	24	7	16	1	4.16
3	D3	UH400	78	17	56	5	6.41
4	D4	UH400	72	4	62	6	8.33
5	D5	UH400	65	4	57	4	6.15
6	D6	UH400	47	2	42	3	6.38
7	D7	UH400	55	3	50	2	3.64
8	D8	UH400	57	4	50	3	5.26
9	D9	UH400	43	3	36	4	9.30
10	D10	UH400	36	2	31	3	8.33
11	D11	UH400	48	2	41	5	10.04
12	D12	UH400	32	5	25	2	6.25
13	D13	UH400	24	2	20	2	8.33
14	D14	UH400	37	2	33	2	5.41
15	D15	UH400	38	3	32	3	7.89
16	D16	UH400	54	3	47	4	7.41
17	D17	UH400	58	3	51	4	6.89
18	D18	UH400	41	2	37	2	4.87
19	D19	UH400	64	2	57	5	7. 81

Table 8. Germination and survived ratio after chromosome doubling by colchicine treatment

No.	Crosses	Symbol	No. HK	No. KG	GR (%)	SP	SPR (%)
1	D1xUH400	DH1	210	150	71,4	72	48
2	D2xUH400	DH2	60	41	68,3	32	78
3	D3xUH400	DH3	160	110	68,8	75	68
4	D4xUH400	DH4	80	50	71,4	23	48
5	D5xUH400	DH5	150	90	71,4	60	48
6	D6xUH400	DH6	160	120	71,4	88	48
7	D7xUH400	DH7	120	90	71,4	68	48
8	D8xUH400	DH8	90	67	74,4	60	90
9	D9xUH400	DH9	70	47	67,1	38	81
10	D10xUH400	DH10	180	105	58,3	50	48
11	D11xUH400	DH11	200	100	50,0	31	31
12	D12xUH400	DH12	167	120	71,9	43	36
13	D13xUH400	DH13	158	93	58,9	36	39
14	D14xUH400	DH14	205	152	74,1	44	29
15	D15xUH400	DH15	230	139	60,4	42	30
16	D16xUH400	DH16	177	152	85,9	55	36
17	D17xUH400	DH17	159	147	92,5	65	44
18	D18xUH400	DH18	215	172	80,0	54	31
19	D19xUH400	DH19	220	135	61,4	37	27

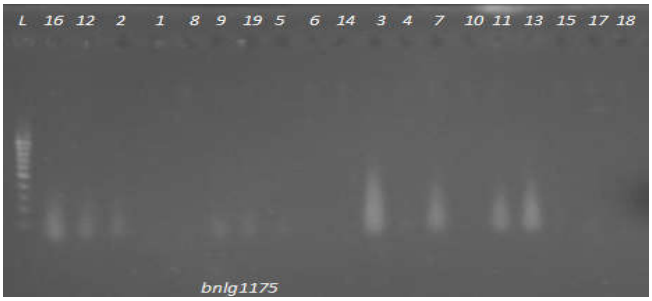
Note: No. HK: number of haploid kernel; No. GK: number of germinated kernel; GR: germinated ratio; SP: number of survived plant; SPR: survived plant ratio;

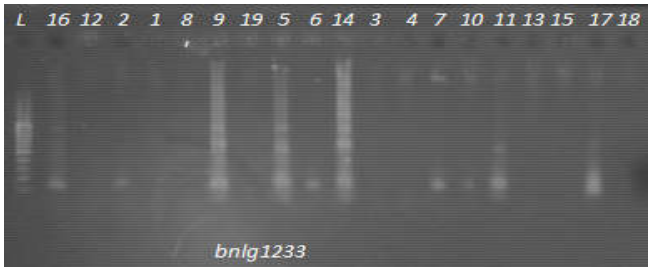
The results were obtained from the induction cross, that were (1) purple coloration on the both endosperm and embryo (so that this kernel may be normal diploid or hybrid kernels); (2) kernel type are without purple coloration on the embryo and endosperm (could be due to pollen contamination) and (3) kernel type are haploid with purple endosperm but no coloration on the embryo. HIR was gained from 3.64 to 10.09%, two genotypes have highest HIR were D1 and D11 lines, D1 have derived from a local maize population and D11 derived from US germplasm. This result was similar to to W. Skippack (2014), who reported UH400 inducer have HIR is 8%, of course HIR depend on the genotypes were used maternal donor are open pollination population, synthetic, hybrid, landrace, dent or flint maize variety (V.Mirdita,W. Schipprack, and A.E. Melchinger, 2014). Chromosome doubling of maternal haploids by colchicine treatment on the seeds was germinated to create DH lines, this study showed low germination ratio from 50 to 92.5%. Before colchicine treatment, shoot tissues were cut at about 1 cm from the tip. Solution with 0.06% colchicine, and 0.5% DMSO was used for chromosomal doubling. Seedlings were cut shoot deep into colchicine solution for 12 h, then washing by distilled water and sown on the tray. Recording the survived seedlings until harvesting time. Our result was alike other reported as Ngoc-

Chi Dang *et al.,* (2011) with survived about 50%. Vijay Chaikam and George Mahuku (2012) reported survived ranged from 40 to 80% depend on genotypes, respectively.

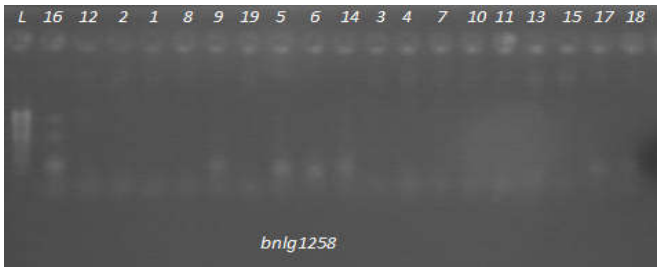
Chromosomal duplication assessment by microsatellites

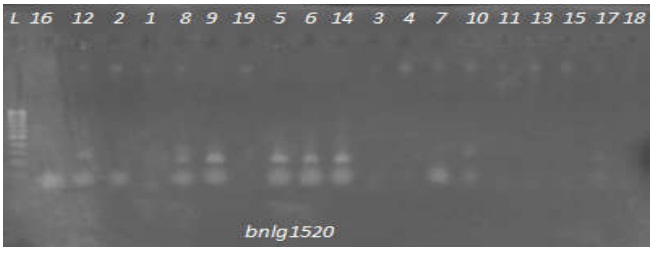
Chromosomal duplication assessment by microsatellites SSR with 5 primers aimed confirms DH identified based on *R1-nj* marker. Method implemented according to Veiga et al. (2012) was considered to be polymorphic between the parents, whose bands had good size and definition on the gel. Five primers were used to analyze and determine the paternity of haploids are *bnlg 1175,bmc1714,bnlg1520, bnlg1233 and bnlg 1258.* Primer *bnlg 1175* identified 7 double haploid lines, with lanes are 16, 12, 2, 3, 7, 11 and 13 (DH16, DH12, DH2, DH3, DH7, DH11 and DH13) with size 100bp



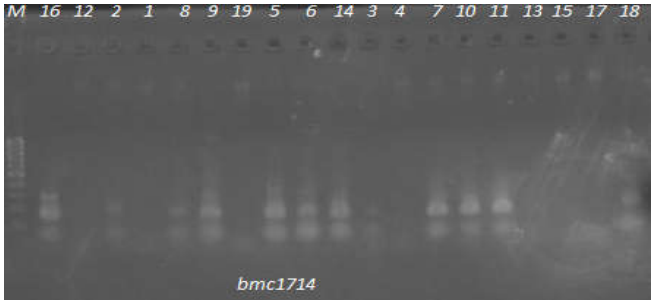


Primer *bnlg 1233* identified 6 double haploid lines, with lanes are 16, 2, 6, 7, 10 v and 17 (DH16, DH2, DH6, DH7, DH10 and DH17) with size 100bp





Primer *bnlg 1258* identified 12 double haploid lines, with lanes are 1, 12, 2, 3, 7, 8,19,4,7, 10, 11 and 13 (DH1, DH12, DH2, DH3, DH4, DH7, DH8, DH10, DH11, DH13 and DH19) with size 100bp, there are two lanes 17 and 18 have size 200bpPrimer *bnlg 1258* identified 12 homozygous lines per 1 alien, lines DH12, DH2, DH1, DH8, DH19, DH4, DH7, DH10, DH11, và DH13 have a ban with 100bp, 2 lines DH17 and DH18 have a band with size 200bp.Primer *bnlg 1250* identified 3 double haploid lines, with lanes are 16, 2 and 7(DH16, DH2 and DH7) with size 100bp.



Primer *bnlg1714* identified 1 double haploid lines, with lanes are 19 (DH9) with size 100bp.The SSR markers were used to confirm that duplicated plants, as confirmed by *R1-nj*, were actually haploid and also indicated that in vivo UH400 inducer was inducing there types of the kernel are normal diploid or hybrid kernels, contamination and haploid kernel. The identified haploid kernel base on *N1-rj* marker is confidence to develop double haploid lines in maize

Evaluation of DH lines on the field condition in autunm-winter season 2015

Seedlings that were treated by colchicine were then transplanting into pots and placed in the greenhouse, the well-established seedlings transplanted in the field after two weeks. Nineteen of putative DH lines (D_0 seedlings) to the field together 10 inbred lines (no inducing) in experiment with RCBD design, two replications, plot area was 14 m^2, grown two rows per a bed with spacing 60 cm and plant to plant 25 cm. Data collection was included growth and development, some agronomical characteristics, field tolerance, yield and yield components. Growth duration of the DH lines were ranged from 93 to 106 days and IL from 92 to 103 days and was not significant difference, days from sowing to Anthesis ranged 52 to 60 days and silking 52 to 63 days. The anthesis-silking interval is an importance relating to yield and tolerance of the maize DH and inbred lines, twenty nine lines in this study were ranged from 1 to 4 days are ASI appropriately and also have not difference between DH and inbred lines.Some agronomical characteristics as plant height, ear height, number of leaves, and ratio ear plant per plant height showed not significant difference between DH and IL.

The coefficient of variation for plant height was 3.38 with DH lines and 11.8 with inbred lines, respectively. Coefficient of variation for plant height, ear height indicated that DH lines more uniformity than inbred lines (table 10) The DH and inbred lines were damaged by some insects and diseases on the field condition in autumn-winter season 2015 consisted of black cutworm (*Agrotis ipsilon*Hufnagel), armyworm (*Pseudaletia unipuncta* (Haworth), stalk borer ((*Papaipema nebris* Guenée), tassel aphid (*Rhopalosiphum maidis*(Fitch), tropical rust (*Physopella zeae*), Turcicum leaf blight (*Setosphaeria turcica*), Maydis leaf blight (*Cochliobolus heterostrophus*). Yield and yield components of DH and inbred lines in autumn-winter season 2015 showed the kernel row number per ear ranged from 10.9 to 14.7 with from 18.1 to 29.2 kernels per row, 1000-kernel weight ranged from 101.4 to 329.4 g.

Table 9. Growth duration of the maize double haploid and inbred lines in autunm-winter season 2015

No.	Line	Growth duration (day)	Sowing to Anthesis (day)	Sowing to silking (day)	ASI (day)
1	DH1	96	54	55	1
2	DH2	97	54	58	4
3	DH3	97	55	57	2
4	DH4	96	53	56	3
5	DH5	98	57	58	1
6	DH6	106	60	61	1
7	DH7	97	54	57	3
8	DH8	97	48	52	4
9	DH9	95	54	55	1
10	DH10	96	50	54	4
11	DH11	98	55	57	2
12	DH12	93	55	56	1
13	DH13	93	50	53	3
14	DH14	92	51	54	3
15	DH15	97	54	55	1
16	DH16	94	57	59	2
17	DH17	97	54	57	3
18	DH18	93	52	54	2
19	DH19	94	52	55	3
20	IL1	92	52	54	2
21	IL2	89	50	52	2
22	IL3	92	54	56	2
23	IL4	96	47	51	4
24	IL5	97	50	52	2
25	IL6	97	58	59	1
26	IL7	98	51	53	2
27	IL8	101	62	63	1
28	IL9	103	60	62	2
29	IL10	90	50	52	2

Note: DH= double haploid, IL = inbred line, ASI = Anthesis-silking interval

Table 10. Some agronomical characteristics of DH and inbred lines in autumn-winter season 2015 at Gialam, Hanoi

No.	Line	PH (cm)	No. leaves	EH (cm)	EH/PH
1	DH1	186.6	17.0	68.1	0.36
2	DH2	179.5	17.0	60.6	0.34
3	DH3	189.5	18.0	65.0	0.34
4	DH4	138.2	15.2	46.5	0.34
5	DH5	169.7	16.4	53.7	0.32
6	DH6	203.4	17.4	61.5	0.30
7	DH7	178.3	16.3	56.5	0.32
8	DH8	172.9	16.3	55.6	0.32
9	DH9	177.3	15.6	54.9	0.31
10	DH10	173.8	16.4	60.2	0.35
11	DH11	168.0	15.7	52.1	0.31
12	DH12	192.5	16.6	65.4	0.34
13	DH13	177.1	15.8	54.1	0.31
14	DH14	173.5	16.4	54.5	0.31
15	DH15	192.7	17.3	62.1	0.32
16	DH16	184.0	17.7	56.9	0.31
17	DH17	176.5	16.5	54.2	0.31
18	DH18	177.9	16.8	59.2	0.33
19	DH19	206.9	17.4	71.9	0.35
$LSD_{.05}$		*9.4*	*1.2*	*8.7*	
CV%		*3.4*	*2.8*	*5.3*	
20	IL1	159.8	16.0	49.8	0.31
21	IL2	161.5	16.6	61.9	0.38
22	IL3	147.5	15.7	39.9	0.27
23	IL4	186.6	17.7	67.9	0.36
24	IL5	204.9	19.1	76.0	0.37
25	IL6	190.4	17.8	62.4	0.33
26	IL7	166.4	17.9	64.4	0.39
27	IL8	173.9	17.0	56.6	0.33
28	IL9	197.1	18.3	68.7	0.35
29	IL10	183.6	18.6	56.9	0.31
$LSD_{0.5}$		*25.0*	*1.3*	*14.1*	
CV%		*11.8*	*3.7*	*11.5*	

Recorded yield of the DH and inbred lines ranged from 14.21 to 44.12 quintal/ha.

Table 11. Yield and yield component of the DH and inbred lines in autumn-winter season 2015

No.	Line	No. Row/year	No. kernel/ row	1000 –kernel weight (g)	Yield (quintal/ha)
1	DH1	12.5	23.4	271.6	37.86
2	DH2	11.7	16.7	260.2	22.87
3	DH3	12.7	29.2	278.8	40.48
4	DH4	11.6	20.7	203.2	18.43
5	DH5	10.9	18.3	309.6	28.69
6	DH6	14.4	22.2	265.6	40.51
7	DH7	11.4	20.1	329.4	39.68
8	DH8	12.6	21.0	311.9	27.54
9	DH9	12.2	21.8	273.1	37.45
10	DH10	12.7	18.6	253.4	28.90
11	DH11	11.2	21.1	295.9	27.42
12	DH12	12.6	21.3	262.1	21.83
13	DH13	11.2	19.7	277.3	20.79
14	DH14	11.2	20.6	203.7	26.73
15	DH15	12.3	18.1	295.7	35.54
16	DH16	13.0	21.5	242.3	30.66
17	DH17	11.9	20.7	311.1	38.01
18	DH18	13.2	18.7	281.6	25.51
19	DH19	13.5	28.4	313.8	44.12
20	IL1	11.0	20.9	158.4	16.34
21	IL2	11.7	17.2	259.2	21.75
22	IL3	14.7	22.5	101.4	18.39
23	IL4	14.0	22.0	213.1	34.77
24	IL5	11.9	18.2	262.1	26.19
25	IL6	12.4	17.0	202.1	14.21
26	IL7	10.3	17.1	258.3	24.08
27	IL8	11.6	20.1	192.4	21.23
28	IL9	13.9	22.3	265.9	40.65
29	IL10	13.0	20.5	215.7	28.26
LSD		*0.98*	*4.2*	*31.7*	*5.47*
CV%		*1.60*	*3.9*	*9.9*	*8.7*

This result according to G. Maluku (2012) harvested ears from the D_0 nursery varying variationin the amount of seed produced from one or two seeds to more than 50 kernels and low yield level.

Conclution

The UH400 inducer can maintaining and multiplication in autumn-winter and spring season in Northern region of Vietnam, but the best maintained time in early to middle February.The inducing ratio of the UH400 inducer from 4 to 10% with tropical inbred lines, haploid kernels can identified by *N1-rj* marker and doubling by colchicine in 0.06% concentration was gained survival ratio were 50% in order to evaluation of the DH lines on the field. Results showed that have not difference between DH lines and inbred lines on the growth and development, agronomical characteristics, yield and yield components. Yield of the DH and inbred lines ranged from 14.21 to 44.12 quintal/ha. These lines could be used for combining ability evaluation to hybrid maize variety development.

REFFERNCES

Adam Edward Vanous, 2011. Optimization of doubled haploid production in maize (*Zea mays* L.), Graduate Theses and Dissertations, Iowa State University, Paper 11974.

Andrés Gordillo and Hartwig H. Geiger, 2010. Optimum Hybrid Maize Breeding -Strategies Using Doubled Haploids, University of Hohenheim Institute of Plant Breeding, Seed Science, and Population Genetics,70593 Stuttgart, Germany 46th Illinois Corn Breeders School, Champaign, 1st,of March 2010

Chang MT, Coe EH 2009. Doubled haploids. In: AL Kriz, BA Larkins (eds) Biotechnology in Agriculture and Forestry. Vol. 63. Molecular Genetic Approaches to Maize

improvement. Springer Verlag, Berlin, Heidelberg, pp. 127–142.

Chase, S.S. 1969. Monoploids and monoploid-derivatives in maize (*Zea mays* L.). TheBotanical Reviews 35: 117–167.

Chase, S.S. 1947. Techniques for isolating monoploid maize plants. J. Bot. 34: 582.

Chase, S.S. 1951. Production of homozygous diploids of maize from monoploids. Agron.J. 44:263–267.

Chase, S.S. 1952. Monoploids in maize. Iowa State College Press, Ames, Iowa, pp. 389–399.

Coe, E.H. 1959. A line of maize with high haploid frequency. Am. Naturalist 93: 381–382.

Forster, B.P.,Thomas, W.T.B. 2005. Doubled haploids in genetics and plant breeding. PlantBreed Rev. 25: 57–88.

Geiger, H.H. 2009. Doubled haploids. In: JL Bennetzen, S Hake (eds.) Maize handbook - volume II: genetics and genomics. *Springer Science and Business Media, New York,* pp.641–657.

Guilherme Moraes Ferraudo, Dilermando Perecin, 2014, Mixed Model, AMMI and Eberhart-Russel Comparison via Simulation on Genotype × Environment Interaction Study in Sugarcane, Applied Mathematics, 2014, 5, 2107-2119

Mirdita, V., Schipprack, W. and Melchinger, A.E. 2014. Identification of in vivo-induced haploid seeds in maize based on oil content, 50th Annual Illinois Corn Breeders School

Geiger, H.H., Gordillo, G.A. 2009. Doubled haploid in hybrid maize breeding, Maydica 54 (2009): 485-499

Ngoc-Chi Dang, Magali Munsch, Ingrid Aulinger, Wen Renlai and Peter Stamp, 2011, Inducer line generated double haploid seeds for combined waxy and opaque 2 grain quality in subtropical maize (*Zea mays*. L.) , Euphytica , 10681-011-0423-0, Spinger Science + Business media

Prasanna, B.M. Vijay Chaikam and George Mahuku (eds).2012. Doubled Haploid Technology in Maize Breeding: Theory and Practice. Mexico, D.F.: CIMMYT.

Rober F.K., G.A. Gordillo, H.H. Geiger, 2005. In vivo haploid induction in maize-Performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica 50: 275-283.

Schipprack, W. 2012.Inbred Line Development in Maize Using the *In-Vivo* Haploid pproach,Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart

Shull, G. H. 1909. A pure line method of corn breeding. Am.Breeders Assoc. Vol. V 5pages: 51–59. Published May,1959

Shull, G.H. 1908. The composition of a field of maize. J. *Hered.* 4, 296–301

Vijay Chaikam and George Mahuku, ChromosomeDoubling of MaternalHaploids, 2012. Doubled Haploid Technology in Maize Breeding: Theory and Practice. Mexico, D.F.: CIMMYT

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