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Development of in vivo haploid inducers for tropical maize breeding programs

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Abstract Lack of adapted haploid inducers currently impedes adoption of the doubled haploid technology in tropical maize breeding programs. Our objective was to generate inducers with improved adaptation to tropical conditions. We developed segregating generations from crosses between temperate inducers having haploid induction rates (HIR) of 8–10 % and tropical CIMMYT maize lines (CML; HIR = 0 %) and evaluated these for HIR and agronomic performance under tropical lowland field conditions. The applied pedigree breeding scheme comprising mass selection on individual F_2 plants for highly heritable and visually scorable traits, followed by family-based selection for HIR and other agronomic characteristics in advanced selfing and backcross (BC) generations seems highly suitable for breeding improved haploid inducers with adaptation to different agroecologies. The most advanced tropical inducer candidates (TIC) combine HIR of up to 10 % with improved pollen production, disease resistance, and plant vigor compared to temperate inducers under tropical conditions. Agronomic characteristics were significantly improved in the BC to CML compared to BC to inducers, while mean HIR of both populations were similar, indicating that backcrossing to the adapted parent was suitable to improve adaptation of new inducers without sacrificing high HIR. When screening random open-pollinated maize accessions, HIR of up to 3 % were observed, suggesting that novel genetic variation may be present in maize accessions that could be exploited to improve HIR in maize. In conclusion, tropical inducer development proceeds well, but evaluation of TIC in multi-environment trials needs to be completed before large-scale dissemination can commence.

Keywords Haploid inducer · Doubled haploid · Maize · Haploid induction rate

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Introduction

Reliable production of haploids is one of the key elements of successful implementation of the doubled haploid (DH) technology in maize breeding programs. For in vivo induction of maternal haploids, pollen of maize inducer genotypes is used to pollinate source germplasm from which DH lines are to be developed. The haploid induction rate (HIR) of a maize inducer denotes the proportion of seeds with haploid embryo detected in the total number of seeds harvested on source germplasm pollinated with inducer pollen.

Modern maize inducers have HIR of about 8–10 % on average (e.g., Röber et al. 2005; Zhang et al. 2008; Geiger 2009; Rotarenco et al. 2010). These inducers carry the dominantly acting marker gene *R1-nj* that causes purple coloration of the scutellum and the aleurone of seeds (Nanda and Chase 1966; Neuffer et al. 1997). This scutellum and aleurone coloration can be used as embryo and endosperm marker, respectively, to identify putative haploid seeds (Prigge and Melchinger 2012). All of the above inducers were developed from temperate germplasm and principally evaluated for HIR and agronomic performance under temperate climatic conditions.

To examine the applicability of temperate haploid inducers for tropical DH line development programs, Prigge et al. (2011) evaluated three inducers (i.e., RWS, UH400, and the hybrid RWS × UH400) adapted to Central European climate for HIR and agronomic performance in the tropics. While HIR obtained under tropical lowland conditions were similar to those obtained under temperate conditions, the temperate inducers showed symptoms of poor adaptation. These included the lack of synchrony between anthesis of inducers and silking of tropical source germplasm and susceptibility to tropical leaf diseases, particularly leaf blight caused by *Excelohilum turcicum*. Poor pollen production and plant vigor requiring hand pollinations for induction crosses as well as limited seed set after self-pollination for maintenance, which had already been experienced under temperate conditions, even worsened in the tropics. Hence, the authors concluded that HIR were sufficiently high to allow deployment of these temperate inducers for initiation of DH breeding programs in the tropics, but better adapted inducers would greatly simplify inducer handling and, consequently, enhance efficiency of large-scale induction of haploidy in tropical maize breeding programs (Prigge et al. 2011).

The objective of our study was to initiate a breeding program at the International Maize and Wheat Improvement Center (CIMMYT) aiming at developing haploid inducers with tropical adaptation. More specifically we (1) outline a breeding scheme for haploid inducer development and present potential tropical inducer candidates (TIC) derived from crosses between temperate inducers and tropical CIMMYT maize lines (CML), (2) examine the suitability of backcrosses (BC) to the adapted parent versus BC to the inducer parent with respect to HIR levels and agronomic performance, and (3) study the occurrence of haploid induction ability in tropical open-pollinated maize accessions.

Materials and methods

Breeding populations were developed from two sets of inducers and CML to study the suitability of different generations and effects of germplasm for tropical inducer development. In Exp. 1, crosses were made in winter 2006/2007 between three CMLs from CIMMYT-Mexico (CML494, CML451, and CL02450) as female parents and inducer hybrids RWS × UH400 and RWS × RWK as pollinators. The selection scheme is exemplified for a cross between CML451 and inducer hybrid RWS × RWK (Fig. 1). Briefly, mass selection for adaptational traits was performed on individual F_2 plants, followed by ear-to-row cultivation of selected progeny and assessment of HIR along with adaptational traits. In addition, the first and second BC generations to the corresponding inducers were produced for each CML by inducer combination. Selection for traits outlined in Fig. 1 resulted in 4 $F_{4:5}$, 4 BC_1F_4, and 2 BC_2F_3 TIC for evaluation. These materials were planted in a randomized complete block design with two

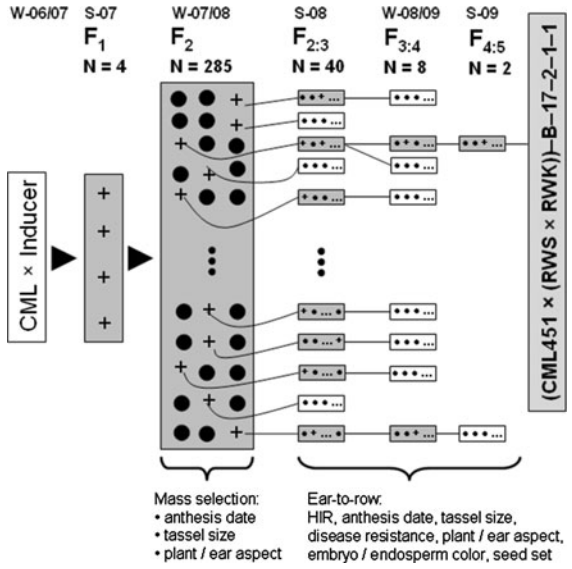

Fig. 1 Example breeding scheme for development of haploid inducers with tropical adaptation in Exp. 1. *CML* CIMMYT maize line, *N* number of progenies evaluated in generations F_1 to $F_{4:5}$ (i.e., F_4-derived F_5 lines) in summer (S) and winter (W) seasons 2006–2009 in Mexico

replicates in two tropical lowland environments, namely Agua Fría, Puebla (20°N lat, 104 m elevation) and Cotaxtla, Veracruz (18°N lat, 24 m elevation), in Mexico in summer 2009 (average minimum and maximum temperature was 21.7 and 32.4 °C, respectively, from May to October). Inducers RWS, UH400, and RWS × UH400 were included as reference inducers. Days to anthesis, tassel size [scale 1–5; for this and all other visually scored traits 1 = excellent and 5 = poor], and plant aspect during flowering [1–5] were assessed in both environments, while purple embryo coloration of selfed ears [1–5] and HIR of TIC were only assessed in Agua Fría. HIR were determined as the proportion of haploids in the total testcross progeny of each TIC employing the *liguleless* tester as female and bulked pollen from a minimum of five plants per TIC as male according to Prigge et al. (2011). Haploids in the testcross progeny were characterized by missing ligule and auricle as well as upright leaves enveloping the shoot (Fig. 2). Pearson–Clopper confidence intervals (CI) for HIR were estimated at $\alpha = 0.10$ using standard formulae (Hartung et al. 2005, p. 204). For agronomic traits, standard analysis of variance across two locations was conducted with Plabstat (Utz 2004) using adjusted entry means and error mean squares.

In Exp. 2, crosses were made in summer 2007 between five CML from CIMMYT-Zimbabwe (CML312SR, CML384, CML395, CML442, CML444) as female parents and the temperate inducer line UH400 and inducer hybrid RWS × UH400 as pollinators. Four to six random F_1 plants from each cross combination were (a) selfed to produce the F_2 generation, (b) backcrossed to the corresponding inducer to produce the first BC generation to the inducer (BC_1F_1-IND), and (c) backcrossed to the corresponding CML to produce the first BC generation to the CML (BC_1F_1-CML). Individual F_2 and BC_1F_1 plants were grown at Agua Fría in summer 2008 and mass selection was practiced by only selfing those plants for advancement that had no apparent leaf disease symptoms, anthesis date >68 days, excellent plant vigor [score 1, on a scale 1–5], and excellent tassel size [score 1, on a scale 1–5]. After additional scoring of the seeds produced on selfed ears with regard to purple embryo and endosperm coloration [1 = excellent purple color to 5 = poor purple color], a total of 168 ($F_{2:3}$), 68 (BC_1F_2-IND), and 82 (BC_1F_2-CML) progeny were selected. These were grown ear-to-row in winter 2008/2009 and mild selection for the above traits was applied to develop 109 $F_{3:4}$, 30 BC_1F_3-IND, and 51 BC_1F_3-CML families. One ear of each family from these three populations was grown ear-to-row in single-row plots allocated at random to the field in summer 2009 at Agua Fría and evaluated for days to anthesis and plant height. HIR was assessed as in Exp. 1. Wilcoxon's signed rank test was used to test for significant differences between HIR of $F_{3:4}$, BC_1F_3-IND, and BC_1F_3-CML populations, whereas a *t* test was used to test different means of days to anthesis and plant height of these populations. Spearman's rank correlations were determined between HIR and six agronomic traits.

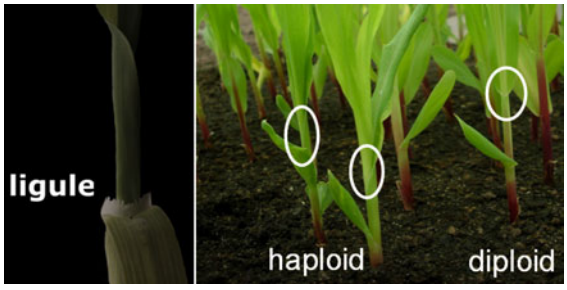

Fig. 2 Haploid identification system used in Exp. 1–3: after pollination of the *liguleless* tester with inducer pollen, diploids in the testcross progeny show a ligule (*left*) and auricle, while haploids are characterized by missing ligule and auricle and upright-positioned leaves enveloping the shoot (*right*)

In Exp. 3, we screened 10 randomly chosen open-pollinated populations from Paraguay and Guatemala (Table 4) held as accessions in CIMMYTs germplasm collection for HIR. These accessions were grown for regeneration by the germplasm collection team in Tlaltizapan, Morelos (19°N lat, 930 m elevation) in Mexico during summer season 2008. They were selected for our study simply based on the fact that their anthesis date matched the silking date of *liguleless* tester plants that had been planted about 2 weeks later than the accessions. Days to anthesis and plant height of the accessions were recorded in their regeneration plots. HIR was determined for each accession by pollinating up to 10 *liguleless* tester plants with bulked pollen from five plants per accession, and the testcross progeny was evaluated for haploidy as in Exp. 1 and 2. CI for HIR were estimated as in Exp. 1.

Results and discussion

Breeding scheme and choice of germplasm

Evaluation in two tropical lowland environments during the summer season 2009 in Exp. 1 revealed

superior agronomic performance of the majority of potential TIC compared with the mean performance of the three temperate inducers RWS, UH400, and RWS × UH400 (Table 1). While anthesis date was only slightly delayed compared to the temperate inducers, TIC showed notably improved tassel size and plant vigor. Larger tassels were generally associated with abundant pollen production and plant vigor was associated with improved seed set after self-pollination (data not shown). Lack of these two characteristics constituted the major constraints of temperate inducers under tropical conditions (Prigge et al. 2011). At the same time, half of the TIC showed HIR > 5 % and most showed excellent purple embryo coloration (Table 1), two important prerequisites for deployment of TIC for induction of haploidy in tropical breeding programs. Our results demonstrate that it is possible to combine high HIR from temperate inducers with improved adaptation to tropical conditions from CML through phenotypic selection. Weak and mostly non-significant correlations between HIR and six important traits for haploid inducer breeding observed in Exp. 2 (Table 2) corroborate this finding.

All CMLs used in our study are common tester lines from CIMMYTs maize breeding programs and they were selected as parental components for tropical inducer development because of their excellent agronomic characteristics under tropical and subtropical conditions. During winter season 2008/2009, CML were tested for HIR using the *liguleless* tester as outlined above and found to have no haploid induction ability (data not shown). Comparison of suitability of individual CML by inducer combinations in Exp. 1 is not reasonable because of the small numbers of TIC per combination. Yet, there was no general trend for superiority of a particular inducer, CML, or generation ($F_{4:5}$, BC_1F_4, or BC_2F_3) with regard to HIR and agronomic characteristics in Exp. 1 (Table 1).

Our strategy of backcrossing to the inducer in Exp. 1 was driven by the fact that it is fairly simple to adapt maize to different agroecologies, given the wide range of climatic conditions under which maize can be grown, whereas haploid induction ability is a rare trait specific to inducer genotypes. However, albeit showing improved tassel and plant characteristics, TIC evaluated in Exp. 1 did not show a notable shift towards delayed anthesis (Table 1). This implies that TIC are flowering much earlier than common tropical source germplasm such that staggered planting of TIC would still be necessary to achieve synchronization with silking of germplasm to be pollinated. As a consequence, we additionally generated BC to the CML in Exp. 2 involving African CML and temperate inducers. As expected, comparisons of $F_{3:4}$ with the BC to the inducers (BC_1F_3-IND) and the CML (BC_1F_3-CML) revealed a significantly later anthesis date of BC_1F_3-CML compared to $F_{3:4}$ and BC_1F_3-IND, whereas HIR of the three populations did not differ significantly (Fig. 3). Further, significantly taller progeny were observed in BC_1F_3-CML compared to BC_1F_3-IND. This suggests that development of inducers with improved adaptation to the tropics can be achieved without sacrificing a high level of HIR by backcrossing to the adapted parent. HIR is likely controlled by one or few major quantitative trait loci (QTL; Deimling et al. 1997; Barret et al. 2008; Prigge et al. 2012). Consequently, it seems possible to identify genotypes in the BC_1F_3-CML population that are fixed for the inducer allele(s) at few QTL controlling HIR but resemble the genomic constitution of the adapted CML parent at the majority of the QTL controlling adaptational traits such as anthesis date, plant height, etc. Further, mean HIR of the BC_1F_3-IND population may be lower than expected because of segregation distortion against the inducer allele at loci affecting HIR (Barret et al. 2008; Prigge et al. 2012). In fact, this may also happen to BC_1F_3-CML progenies homozygous for the HIR-enhancing allele at the relevant loci in subsequent generations. Further evaluations of HIR across several locations and seasons as well as assessment of other important traits such as pollen and seed production capacities, purple embryo and endosperm coloration, and disease resistance will be necessary to better appraise the suitability of BC to the adapted parent for tropical inducer development.

Phenotyping for HIR

In all experiments, HIR was assessed in one environment based on a limited number of testcross seeds (N). Significant effects of environments on HIR of inducers were reported (Röber et al. 2005; Prigge et al. 2011), indicating the need for multi-environment evaluations of potential TIC. Further, $N = 200$ testcross seeds must be seen as a lower limit for HIR determination, as the use of larger N is expected to provide more reliable estimates of HIR. However, during early generations

Table 1 Haploid induction rates (HIR) and associated 90 % confidence intervals (CI), intensity of purple embryo coloration (EMC) on selfed ears, days to anthesis (DTA), tassel size (TAS), and plant aspect at flowering time (PAF) of haploid inducers from the University of Hohenheim (UHOH; mean of three inducers) and 10 tropical inducer candidates (TIC) evaluated during summer season 2009 at two tropical lowland locations in Mexico in Exp. 1

No.	Pedigree	HIR[a]	CI	*N*	EMC[b]	DTA	TAS[b]	PAF[b]
Mean of UHOH inducers		–	–	–	–	43.9	2.6	4.0
Performance of TIC			%	No.	1–5	Days	1–5	1–5
1	((CML494 × (RWS × UH400)) × (RWS × UH400))-36-1-1	10.2	7.1–14.1	226	1	43.3	1.3	2.0
2	((CML494 × (RWS × UH400)) × (RWS × UH400))-36-1-2	10.0	6.7–14.2	200	1	45.3	1.5	2.3
3	(CML451 × (RWS × RWK))-B-17-2-1	8.7	6.3–11.7	332	2	46.0	1.0	1.0
4	(((CML494 × (RWS × UH400)) × (RWS × UH400)) × (RWS × UH400))-1-2	6.8	4.8–9.2	385	1	43.0	1.0	1.8
5	((CL02450 × (RWS × RWK)) × (RWS × RWK))-6-1-1	5.2	3.2–7.8	291	1	45.5	1.3	1.8
6	((CML494 × (RWS × RWK)) × (RWS × RWK))-1-2-2	5.0	3.3–7.2	381	2	44.8	1.3	3.5
7	(CML451 × (RWS × RWK))-B-10-4-1	3.8	2.1–6.0	320	1	54.8	1.0	2.4
8	(CML494 × (RWS × RWK))-B-27-1-2	1.7	0.8–3.2	402	2	49.9	1.3	2.8
9	(CML451 × (RWS × UH400))-B-16-2-1	1.5	0.6–2.9	412	3	47.3	1.3	4.4
10	(((CL02450 × (RWS × UH400)) × (RWS × UH400)) × (RWS × UH400))-1-2	1.3	0.3–3.4	226	2	45.8	1.3	3.3

– Not assessed

[a] Determined from *N* testcross seeds produced by pollinating the *liguleless* tester with inducer pollen in one environment

[b] Scored on a scale from 1 to 5 with 1 = very good and 5 = very poor

Table 2 Spearman's rank correlation coefficients between haploid induction rates (HIR) and six agronomic traits assessed in $F_{3:4}$ and backcross generations to the inducer (BC_1F_3-IND) and the adapted parent (BC_1F_3-CML) from crosses of temperate inducers and tropical CIMMYT maize lines (CML) in Exp. 2

Population	*N*	Days to anthesis	Plant height (cm)	Tassel size[a]	Ear aspect[a]	Embryo color[a]	Endosperm color[a]
$F_{2:4}$	109	−0.11	-0.12	0.00	0.08	-0.02	0.20*
BC_1F_3-IND	30	0.11	0.03	–	0.27	0.12	0.48**
BC_1F_3-CML	51	−0.04	0.01	–	0.28*	0.01	0.05

N denotes the number of progeny per population

*, ** Significant at $P < 0.05$ and 0.01, respectively

[a] Scored on a scale from 1 to 5 with 1 = very good and 5 = very poor

large numbers of families must be screened for HIR, such that large numbers of testcross seeds per family are barely feasible. Since it takes 2–4 weeks (depending on growth conditions) for testcross plants to grow to the four-leaf stage so that monitoring for the *liguleless* phenotype can commence, off-season determination of HIR between two growing seasons per year would consume immense resources (labor, greenhouse space) if based on large *N*. In general, it seems reasonable to employ smaller *N* (e.g., 200–400 testcross seeds) in early generations and confirm HIR of potential new inducers in advanced generations with larger *N* (e.g., 1000 testcross seeds), preferably in multiple environments.

Of general concern during haploid inducer development is the accuracy of HIR estimates. In Exp. 1 and 3, 90 % CI of variable magnitude were observed for HIR estimates, but there was a trend for increasing CI when *N* decreased and/or when HIR increased (Tables 1, 4). For example, in Exp. 1 TIC nos. 5 and 6 had similar HIR of about 5 %, yet the CI of TIC no. 6 was smaller because its HIR was based on a larger *N* (Table 1). In contrast, identical $N = 226$ led to a CI of 7 % in TIC no. 1, whereas the CI of TIC no. 10 was only 3 % because of its 10-fold smaller HIR compared to TIC no. 1. This dependence of the CI on *N* and on the magnitude of HIR complicates reliable phenotyping and impedes selection of superior inducer genotypes in breeding programs. It can be explained by the statistical properties of the trait in vivo haploid induction ability though, as elaborated in detail by Prigge et al. (2012). Briefly, if we consider the total testcross progeny of a given inducer genotype *i* as N_i independent Bernoulli trials, with each seed representing either a success (i.e., haploid) or a failure, then the HIR observed for *i* (denoted by $\hat{p}_i$) can serve as an estimator of the true probability of success p_i. It follows that the expectation of $\hat{p}_i$ is $E(\hat{p}_i) = p_i$ and its variance is $\mathrm{var}(\hat{p}_i) = \frac{1}{N_i} p_i(1 - p_i)$. Consequently, smaller sample sizes N_i and larger true probability of haploidy induction p_i lead to higher error variance, i.e., less reliable phenotypic values. While N_i can be increased by the experimenter to improve accuracy of HIR estimates, p_i is a genetic property of the genotype under consideration and the handling of this aspect during haploid inducer breeding warrants further research.

In our experiments, we predominantly used the *liguleless* tester as female parent for testcross production. This tester generally provides more reliable estimates of HIR than identification of haploids based on purple embryo coloration (Röber et al. 2005; Prigge et al. 2011), because its recessive *liguleless* phenotype is only revealed in haploids and is clearly distinguishable by visual scoring. However, the *liguleless* tester was also adapted to central European climate and, thus, exhibited the same adaptational problems as the temperate inducers. While reduced pollen production was not an issue because the tester was employed as female parent during testcross seed production, strong leaf blight susceptibility and poor seed set resulted in low *N* per pollinator and were a major constraint for accurate determination of HIR. Further, poor standability required that individual *liguleless* plants be tied between ropes fixed at the edges of each planting row. Hence, a better adapted tester carrying a recessive morphological trait (such as *liguleless* or *glossy*) with improved agronomic performance under tropical conditions is urgently needed for tropical inducer development and maintenance programs.

As the *liguleless* characteristic is associated with erect leaf position (Neuffer et al. 1997), we screened

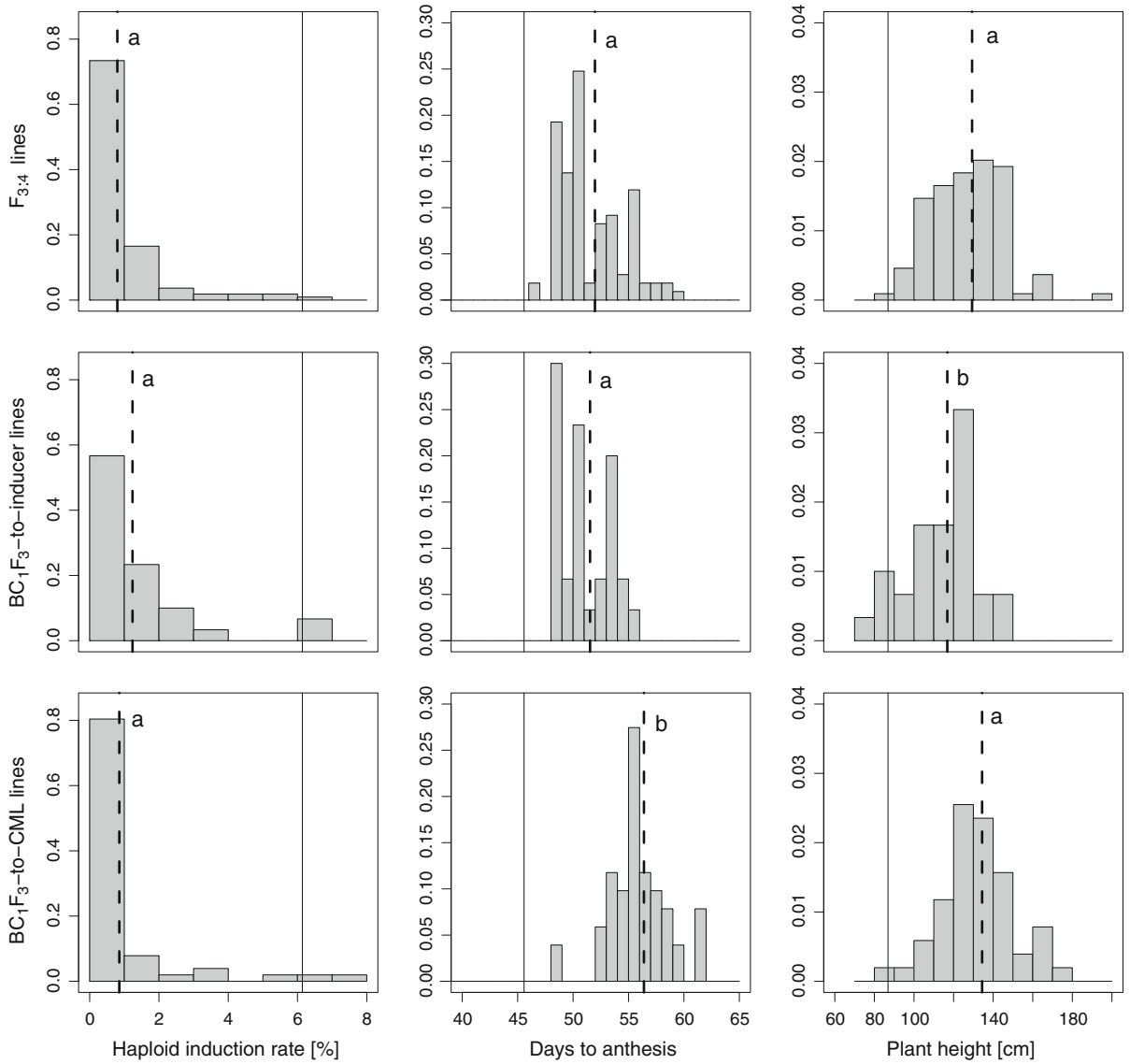

Fig. 3 Frequency distributions of haploid induction rates (HIR), days to anthesis, and plant height of 109 $F_{3:4}$ progeny (*top*), 30 BC_1F_3 backcross to the inducer progeny (*center*), and 51 BC_1F_3 backcross to the CIMMYT maize line (CML) progeny (*bottom*) evaluated in summer 2009 at Agua Fría, Mexico in Exp. 2. The *dashed line* represents the population mean, the *solid line* represents the mean of three inducers (RWS, UH400, and RWS × UH400) included as checks in the experiment. *Common letters* for a trait mean in all populations indicate no significant differences between populations according to Wilcoxon's signed rank test (for HIR) or *t* test (for days to anthesis and plant height)

16 tropical CMLs matching this leaf phenotype for absence of ligules in the four-leaf stage in winter season 2009/2010. CML508, a recycled line from CIMMYT-Zimbabwe with mid-altitude/subtropical adaptation, showed no ligule at this developmental stage. Further research should clarify whether this CML can be employed as tester for HIR monitoring in tropical inducer breeding programs. In addition, temperate *liguleless* inbreds could be crossed with tropical CML to develop suitable testers with tropical adaptation via phenotypic selection in the segregating progeny.

While HIR determination based on the *liguleless* phenotype generally provides more reliable estimates of HIR because of unambiguous identification of haploids, HIR determined based on the *R1-nj* seed

coloration provides practically relevant estimates, as the latter haploid identification system is used in DH breeding programs (Röber et al. 2005; Prigge and Melchinger 2012). Further, *R1-nj*-based HIR determination using common hybrids as females also allows evaluation of the intensity of purple embryo and endosperm coloration, which inducers transmit to the testcross progeny when used as pollinators. Obviously, visual identification of haploids is easier for those inducers leading to more intense embryo coloration in the testcross progeny. For this reason, we determined HIR based on the *R1-nj* seed coloration marker in addition to the *liguleless* phenotype in various generations of Exp. 1.

Independent of the haploid identification system, HIR remained at 9–10 % for TIC no. 1 in advanced generations of Exp. 1, whereas HIR of TIC no. 3 decreased compared with previous generations (Table 3). Such variations in HIR depending on the generation have also been observed in temperate inducer breeding programs (W. Schipprack 2011, personal communication) and they render selection of superior genotypes extremely difficult. Inaccurate HIR caused by fairly small *N* monitored during early generations may be responsible for this, as outlined above. Further, since different generations are commonly evaluated for HIR in different seasons, genotype by environment interactions may cause the discrepancy (Prigge et al. 2011). Finally, small-effect modifier genes (Allard 1999) influencing major HIR-controlling QTL may segregate in different generations, thus leading to variable phenotypes.

Prospects for further improvement of haploid induction ability in maize

In general, increased HIR of e.g. 20 % would allow breeders to substantially reduce the space allocated to

Table 3 Performance history of two tropical inducer candidates (TIC) (nos. 1 and 3, see Table 1) developed from temperate inducer by tropical CIMMYT maize line (CML) crosses evaluated for haploid induction rate (HIR) and agronomic characteristics at Agua Fría station, Mexico in summer (S) and winter (W) seasons from 2007 to 2010 in Exp. 1

Generation	HIR (%) (N[a])		Days to anthesis	Tassel size[d]	Endosperm color[d]	Embryo color[d]	Plant aspect[d]	Growing season
	lg[b]	*R1-nj*[c]						
TIC no. 1: ((CML494 × (RWS × UH400)) × (RWS × UH400))-36-1-1-1-B								
BC_1F_1	–	–	62	1	–	–	–	W07/08
BC_1F_2	–	–	53	1	–	–	–	S08
BC_1F_3	–	4.3 (211)	74	1	2	1	1	W08/09
BC_1F_4	10.8 (226)	–	47	1	3	1	2	S09
BC_1F_5	10.7 (626)	–	84	2	3	1	3	W09/10
BC_1F_6	10.5 (8441)	9.1 (12618)	50	–	1	–	–	S10
TIC no. 3: (CML451 × (RWS × RWK))-B-17-2-1-1-B								
F_2	–	–	69	1	–	–	–	W07/08
$F_{2:3}$	4.0 (616)	–	58	1	–	–	–	S08
$F_{3:4}$	–	3.5 (317)	75	1	3	2	3	W08/09
$F_{4:5}$	8.7 (332)	–	50	1	2	1	1	S09
$F_{5:6}$	3.6 (1362)	–	85	2	1	2	2	W09/10
$F_{6:7}$	5.5 (4068)	5.1 (27609)	57	–	1	–	–	S10

– Not assessed

[a] Number of testcross seeds used for HIR determination (in brackets after HIR value)

[b] Based on the *liguleless* phenotype of testcross seeds using the *liguleless* tester as female

[c] Based on the *R1-nj*-encoded purple embryo coloration in testcross seeds using single cross hybrids as females (mean HIR across four hybrids): haploids show nonpigmented embryo and purple endosperm, while diploids show purple embryo and endosperm (Prigge and Melchinger 2012)

[d] Scored on a scale from 1 to 5 with 1 = very good and 5 = very poor

Table 4 Haploid induction rates (HIR) determined with the *liguleless* tester and their associated 90 % confidence intervals (CI), days to anthesis, and plant height of genebank accessions evaluated in Tlaltizapan (Mexico) during summer season 2008

Accession name	Accession ID[a]	Race	HIR (%)	CI (%)	N^b	Days to anthesis	Plant height (cm)
Paraguay							
PARA138	4135	Cristal Semidentado	0.9	0.4–1.6	807	79	285
PAZM2020	19019	Tupi Pytâ	1.1	0.4–2.3	448	63	300
PAZM6067	21524	Pichinga Redondo	1.0	0.2–3.0	207	70	270
PAZM10096	21634	Pichinga Redondo	0.5	0.2–1.1	974	70	190
PAZM13011	19065	Avati Mitâ	3.1	1.4–5.8	224	72	265
Guatemala							
GUAT1008	27516	Tuxpeño	1.1	0.2–3.3	187	61	270
GUAT1010	27518	Tuxpeño	0.4	0.0–1.7	272	59	250
GUAT1100	27604	Tuxpeño	2.0	0.5–5.0	153	59	225
GUAT1030	27538	Tuxpeño	0.1	0.0–0.3	1457	58	275
GUAT1038	27546	Tuxpeño	0.7	0.0–3.2	145	55	225

[a] Identification from CIMMYTs germplasm collection

[b] Number of testcross seeds evaluated for haploidy

production of induction crosses in DH breeding programs. However, no transgressive segregants exhibiting HIR > 10 % were observed in Exp. 1 and 2 (Table 1; Fig. 3), indicating that significantly increased HIR may not be accomplished with currently available materials. Further, strong segregation distortion against the inducer allele at a major QTL on chromosome 1 controlling HIR suggests that HIR is associated with transmission failure of the inducer gamete (Barret et al. 2008; Prigge et al. 2012). Hence, novel sources of HIR are unlikely to be identified in elite germplasm because natural and artificial selection would disfavor haploid induction ability. In contrast, open-pollinated populations may be more promising because HIR-enhancing alleles may "hide" from natural selection in heterozygous genotypes. To examine this hypothesis, we screened 10 randomly chosen accessions from Paraguay and Guatemala for HIR using the *liguleless* tester in Exp. 3. Preliminary results from one season revealed that nine of them had higher HIR than the rate of spontaneously occurring haploids estimated at 0.1 % (Chase 1952). Accession PAZM13011 from Paraguay even showed HIR = 3.1 % (Table 4). Certainly, these results should be confirmed in further evaluations, yet they suggest that novel sources of HIR may be found in maize accessions held in global germplasm collections. In combination with existing inducer stocks, these could help develop inducers with increased HIR.

In Exp. 1, TIC nos. 1, 2, and 3 excelled in HIR and agronomic characteristics during the summer season 2009 (Table 1). The three temperate inducers, which were included as checks in this experiment, did not produce enough pollen to assess HIR, possibly because of heat stress, whereas TIC did not exhibit this problem. Further, performance of TIC no. 1 was confirmed in advanced generations (Table 3) and, thus, this inducer genotype may represent a suitable alternative for induction of haploidy in tropical maize breeding programs. However, evaluations under different environmental conditions should be conducted to verify HIR levels and agronomic performance before large-scale dissemination is commenced. In addition, chromosomal regions harboring major QTL for HIR should be fine-mapped and further mapping experiments should be conducted to identify putative small-effect and/or modifier genes. Marker-based preselection for the major QTL on chromosome 1 (Prigge et al. 2012) among progeny segregating for HIR, followed by phenotypic or genomic selection for HIR among pre-selected families may be suitable to capture small-effect QTL to develop well-adapted inducers with high HIR for various agroecologies.

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