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Gerald N. De La Fuente
Iowa State University

Ursula K. Frei
Iowa State University, ufrei@iastate.edu

Benjamin Trampe
Iowa State University, btrampe@iastate.edu

Daniel Nettleton
Iowa State University, dnett@iastate.edu

Wei Zhang
Iowa State University

See next page for additional authors

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Abstract

The maize in vivo maternal doubled haploid (DH) system is an important tool used by maize breeders and geneticists around the world. The ability to rapidly produce DH lines of maize for breeding allows breeders to quickly respond to new selection criteria based on the ever changing biotic and abiotic stresses that maize is subjected to across its growing area. There are two important steps in the generation of DH lines using the in vivo maternal DH system: 1) the production and identification of haploid progeny, and 2) the doubling of genomes to create fertile, diploid inbred lines that can be used for topcross and per se evaluation. For this study, the focus is the first step, the production and identification of haploid progeny. A diallel mating between six inbred lines of maize, three highly inducible lines (CR1HT, PA91HT1, WF9) and three lines with low inducibility (NK778, A427, A637) was produced to study the genetic makeup of inducibility in temperate maize germplasm. A maximum estimated rate of inducibility was found in A427/A637 at 14.6%. Significant general combining ability (GCA) specific combining ability (SCA), reciprocal (REC), environmental (ENV), as well as GCA by ENV and SCA by ENV interactions were found. Misclassification rates ranged from 0-45.2% in the 30 hybrids considered. This study supports the use of germplasm with improved inducibility for breeding to improve rates of inducibility in germplasm which has low induction rates.

Disciplines

Agricultural Science | Agronomy and Crop Sciences | Plant Breeding and Genetics | Statistical Models

Comments

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Authors

Gerald N. De La Fuente, Ursula K. Frei, Benjamin Trampe, Daniel Nettleton, Wei Zhang, and Thomas Lubberstedt

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1 # A Diallel Analysis of a Maize Donor Population Response to *In Vivo*

2 # Maternal Haploid Induction I: Inducibility

3 Gerald N. De La Fuente[1], Ursula K. Frei[1], Benjamin Trampe[1], Daniel Nettleton[2], Wei Zhang[2],

4 and Thomas Lubberstedt[1,*]

5 [1]Department of Agronomy, Iowa State University, 100 Osborn Drive, Ames, IA 50011

6 [2]Department of Statistics, Iowa State University, Snedecor Hall, Ames, IA 50011

7 [*]Corresponding author: thomasl@iastate.edu

8

9

10 **Abstract**

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12 breeders and geneticists around the world. The ability to rapidly produce DH lines of maize for
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24 SCA by ENV interactions were found. Misclassification rates ranged from 0-45.2% in the 30
25 hybrids considered. This study supports the use of germplasm with improved inducibility for
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27

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28 **Introduction**

29 Maize (*Zea mays* L.) is a diverse crop used primarily for food and feed. However, it also
30 provides various raw materials for industrial applications. Maize transitioned from open
31 pollinated varieties to highly productive single cross hybrids used today. The ability to rapidly,
32 efficiently, and economically run breeding cycles is essential to both a successful breeding
33 program, and to the overall security of a robust food, feed, and fuel supply. In response to this
34 need breeders implemented tools such as winter nurseries and embryo rescue techniques to
35 increase the number of generations per year. Doubled haploids (DHs) reduced the number of
36 generations needed to develop parental inbred lines (two generations), compared to traditional
37 line development (pedigree method) in five or more selfing generations.

38 Chase (1947) found haploid plants to occur naturally in maize at a low frequency. Their
39 utility for maize breeding and genetics was not immediately understood (Chase, 1947). However,
40 it was not until further progress was made to develop maternal haploid inducers and chromosome
41 doubling techniques, that maize DHs became commonplace in breeding programs (Coe, 1959;
42 Geiger, 2009, Rober et al., 2005). Today, a large percentage of commercial and public maize
43 breeding is conducted through the use of the DH system (Liu et al., 2016). The ability to generate
44 a new fully homozygous and homogeneous inbred line in 12 months or less provides a time
45 advantage that allows breeders to quickly respond to new market demands and shifts in selection
46 targets.

47 The maize DH system used today is known as the *in vivo* maternal haploid system.
48 Though this method is most popular due to its ease of use and limited genotype dependency, two
49 other methods exist: *in vivo* paternal haploid induction, and *in vitro* anther culture for haploid
50 production (Geiger, 2009). Herein, the focus will be on the *in vivo* maternal haploid induction

51 and the genetics controlling specific steps in the process. This system involves two key
52 biological steps: 1) production of haploid progeny, and 2) doubling of genomes.

53 As with all inbred line development programs, the breeding cycle begins with the cross of
54 two (or more) parents of interest. Note that it is possible to produce DH lines from any type of
55 line, population, cross, or backcross. Since speed is critical, breeders tend to use F_1 breeding
56 crosses as the donor parent. The donor parent is the female in this system, and will provide, in
57 theory, all of the genetic information which is passed on to the haploid progeny. These donor F_1
58 plants are pollinated by a maternal haploid inducer. The inducers are termed 'maternal' since the
59 resulting haploid progeny from the induction cross are produced on the female in the cross. The
60 use of the *in vivo* maternal induction system has implications: 1) the genotype dependency of this
61 system is much lower than *in vitro* haploid systems in maize (Geiger, 2009), 2) this system is
62 more economical as it allows for use of isolation nurseries to generate induction crosses, and 3)
63 the cytoplasm of the resulting lines will be from the maternal donor parent.

64 The phenomenon of haploid induction in maize, though it is extensively used, is poorly
65 understood. Two competing hypotheses exist. One hypothesis is that some percentage of the
66 pollen from the inducer is able to 'induce' the egg cell to begin parthenocarpic development
67 leading to a functional haploid embryo without fusion of the sperm and egg cell (Chalyk et al.,
68 2003). The second, and more supported hypothesis, involves the union of the sperm of the
69 inducer and the egg of the donor, which stimulates development of the embryo (Wedzony et al.,
70 2002; Zhang et al., 2008). The genome of the inducer is subsequently eliminated from the
71 embryo, which is becomes haploid, containing only the genome of the donor parent which is a
72 result of one meiotic recombination during egg cell development. Normal triploid endosperm is
73 formed in induction crosses. Without production of a normal healthy endosperm, the survival of

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74 the seed would not be possible without special care. The production of haploid kernels is a
75 complex phenomenon involving genetic control by both the maternal inducer and the donor
76 (Prigge et al., 2011; Rober et al., 2005). The genetic control of induction, the ability to induce
77 haploids – a trait carried by the inducer, has been extensively investigated through both breeding
78 and genetic analyses (Prigge et al., 2012; Prigge et al., 2011; Rober et al., 2005). QTL studies
79 have been conducted and two major QTL, explaining over 60% of the phenotypic variation have
80 been identified which both trace back to 'Stock 6' (Prigge et al., 2012). The pollen specific gene,
81 *MATRILINEAL* has been identified as the major gene effecting the ability of maternal inducer
82 lines to induce haploid progeny (Kelliher et al., 2017), however, without providing evidence for
83 any of the two hypotheses. Since the discovery and development of 'Stock 6' (Coe, 1959), many
84 new maternal inducers have been developed with increasing rates of haploid induction (Rober et
85 al., 2005; Prigge et al., 2011). A maternal haploid inducer with a high induction rate will provide,
86 on average, a high number of haploid kernels. However, this is a two sided phenomenon.
87 Inducing a large number of diverse germplasm, any given maternal haploid inducer will likely
88 produce a range of rates of inducibility (ability to be induced to create haploid kernels – a trait of
89 the donor) pointing to a quantitative control on the donor side due to several factors (Rober et al.,
90 2005; Prigge et al., 2011; Kebede et al., 2011). Although the genetics of haploid induction have
91 been well studied, to our knowledge few previous studies exist that consider the effect of the
92 donor population, and these were mostly conducted in tropical germplasm (Rober et al., 2005;
93 Prigge et al., 2011; Kebede et al., 2011; Wu et al., 2014).

94 The efficient and economic production of DH lines relies on the ability to produce
95 sufficient numbers of haploid kernels. In all germplasm pools (even in elite adapted material)
96 there exists some germplasm with low inducibility. This limits the potential pool of breeding

97 materials for those programs which conduct breeding primarily or exclusively with DHs. For this
98 reason, and to prime and facilitate future mapping experiments we screened a set of diverse
99 maize inbreds for inducibility. Though most inbreds produced an inducibility rate near the
100 average (data not shown), three lines were identified, which produced average induction rates in
101 excess of 25% when induced with RWS/RWK-76 (Rober et al., 2005). These three lines
102 (CR1HT, Wf9, and Pa91Ht1), along with three lines with low inducibility (A427, A637, and
103 NK778) were mated in a full diallel to study the genetic components of inducibility. A diallel
104 mating scheme was chosen because F_1 donors are typically the generation used for haploid
105 induction. It is also of interest to answer the question, whether or not poor lines can be
106 'supplemented' by lines, which are superior for inducibility, if specific combinations produce
107 superior inducibility rates, and finally, if there is an effect of the direction of the cross.
108 The objectives of this study were to 1) investigate the practical use of inducibility in the
109 maternal DH system, 2) evaluate the inheritance of inducibility, and 3) evaluate, whether a
110 highly inducible line can improve rates of inducibility in a line with lower inducibility.
111
112 **Materials and methods**
113 *Germplasm*
114 A preliminary experiment was conducted during summer of 2012, in which 160 inbred
115 lines were planted in two replications and pollinated with the RWS x RWK-76 inducer. Seed was
116 bulk harvested from the inbred lines and the haploid seed was then sorted from the hybrid seed
117 so that an inducibility rate could be calculated. Data from the preliminary experiment was also
118 used to model inducibility and the associated variances. As seen in Figure 1, the trend is as
119 expected. As the number of kernels sorted goes up, the probability of detection for small mean

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120 differences reaches 1 faster. The ability to detect 5% differences was sought, and with a sample
121 size of 1000 kernels, detection of a 5% difference would occur with a probability of 0.999.
122 Sorting of 1000 kernels was also a manageable number and chosen for this study. Based on the
123 results from the aforementioned work, six inbred lines were selected for use in a complete
124 diallel: three of which are highly inducible (IND) and three of which are poorly inducible based
125 on initial trials. The lines and details of their heterotic grouping, flowering time, pedigree, and
126 origin are presented in Table 1. Seed from all six lines was acquired from the USDA North
127 Central Regional Plant Introduction Station.
128
129 *Production of diallel*
130 The six inbreds were mated in a full diallel without the inclusion of parents producing 30
131 unique F_1 combinations between the six inbreds. The diallel crosses were first made in winter
132 2013 at Tuniche Seed Services in Graneros, Chile. Due to nicking issues (mostly with PA91HT1
133 and A637) the diallel crosses were repeated in summer of 2014 in Ames, IA, at the Iowa State
134 University Agricultural Engineering and Agronomy Farm (AF). All ears from each specific cross
135 were bulk harvested.
136
137 *Trials and trait scoring*
138 The diallel was grown in two separate environments over the course of two years. The
139 first environment was in summer of 2014 (AF14) at AF, and the second in summer of 2015
140 (AF15) at AF. Both trials were grown on loam soils in rainfed conditions, under standard
141 agronomic practices for central Iowa maize production. Weed control was conducted via a
142 preplant application/incorporation of Atrazine with a UAN fertilizer carrier solution, and a layby

143 cultivation as well as hand weed control as needed. The experiment was grown in a four
144 replication randomized complete block design with 5.4 m plots in AF14 and 3.8 m plots in AF15
145 both on 0.76 m row spacing. All plots were manually detasseled and shoots were covered prior to
146 silk emergence. All plots were pollinated with bulk pollen from either RWS/RWK-76 or an F_2
147 generated from this F_1 whose induction rate is not statistically different (data not shown). All
148 plants in the plot were attempted to be pollinated. Plots were then bulk harvested, dried, and
149 shelled. A random sample of 1000 (or as many as possible up to 1000) kernels were selected as
150 the sample size based on simulation studies conducted with data from the preliminary
151 experiment.

152 Kernels were sorted into hybrids and putative haploids based on the *R1-nj* seed based marker
153 system (Nanda and Chase, 1966). Kernels showing coloration in the aleurone (successful fusion
154 of inducer sperm with central cells) and in the embryo (successful fusion of inducer sperm with
155 egg cell) were scored as hybrid. Those kernels which showed coloration in the aleurone, but not
156 in the embryo (successful induction of haploid embryo) were scored as putative haploids.
157 Inducibility rate (IR) was calculated as number of putative haploids divided by number of total
158 kernels. However, the *R1-nj* system is not 100% accurate (Kebede et al., 2011; Prigge et al.,
159 2011). Haploid seed was grown to calculate a misclassification rate (following Kebede et al.,
160 2011) to adjust for misclassified haploid kernels. This was done in conjunction with another
161 experiment (conducted in summer of 2015), where the haploid seed was planted in the field for
162 observation. For each entry, a single row 5.5 m plot with 0.7 m spacing was planted across two
163 locations with four replications in each. For each plot a total of 28 seeds were planted. After
164 approximately three weeks of growth, the hybrid plants differ from haploids due to increased
165 vigor. Hybrid plants were counted as misclassified haploids and then divided by the total number

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166 of planted seed (non-germinated seed were assumed to be haploids). The corrected induction rate
167 (IRc) was calculated as:
168 (1) IRc = number of haploids * (1-misclassification rate)/total number of planted seed.
169
170 *Statistical Analyses*
171 The combining ability analysis was conducted using DIALLEL-SAS05 (Zhang et al.,
172 2005) considering all F_1s and reciprocals, also known as method 3 (Hallauer, 1988). In our
173 experiments, we did not randomly sample germplasm, but characterized defined lines. Thus, a
174 fixed effect model was considered. Estimates for general combining ability (GCA), specific
175 combining ability (SCA), reciprocal (REC), GCA x environment, SCA x environment and REC
176 x environment computed using DIALLEL-SAS05.
177 SAS PROC MIXED (version 9.4, SAS Institute, 2013) was implemented using angular
178 transformed IRc. The angular transformation was used to normalize the distribution of the data.
179 The model considered here was $Y_{ijk} = Env_i + Rep(Loc)_{i(j)} + Entry_k + Env*Entry_{ik} + e_{ijk}$, where
180 Y_{ijkl} is the angular transformed IRc, Env_i is the random effect of the ith environment, $Rep(Loc)_{i(j)}$
181 is random effect of the jth replication nested in the ith environment, $Entry_k$ is the fixed effect of
182 the kth entry (F_1s from diallel), $Env*Entry_{ik}$ is the random interaction between the ith
183 environment and the kth entry, and e_{ijk} is the residual error. Due to the nature of the experimental
184 design, heritability in the narrow sense or h^2 could be estimated as follows (Hallauer, 1988).

$$(2)\quad \hat{h}^2 = \frac{4\sigma^2_{GCA}}{\left(\frac{\sigma^2}{r}\right) + 4\sigma^2_{SCA} + 4\sigma^2_{GCA}}$$

185
186

187 **Results**

188 For this experiment, across both environments, a total of 233,665 (120,000 in AF14, and
189 113,665 in AF15) kernels were sorted. A total of 27,174 putative haploids were identified with
190 visual sorting giving an IR of 11.6% with uncorrected IR values ranging from 2.6%-32.5%. For
191 AF14, the IR was 13.1% and for AF15 IR was 10.2%. PA91HT1/WF9 and NK778/WF9 had the
192 highest misclassification rates at 45.2% and 32.1%, respectively (Table 2). Misclassification
193 rates ranged from 0%-45.2% with averages for each parental line of: 5.8% (A427), 4.1% (A637),
194 4.5% (CR1HT), 11.8% (PA91HT1), 16.2% (WF9), and 9.2% (NK778). After accounting for
195 misclassification, the average IRc was 10.5% with values ranging from 2.4%-30.5%.

196 As seen in table 3, the combining ability analysis showed significant sources of variation
197 for GCA (p =<0.001), SCA (p =0.0019), REC (p =0.0028), ENV (p =<0.001), GCA by ENV
198 interaction (p =0.0012), and SCA by ENV interaction (p =0.016). In general, the estimated
199 effects in the combining ability analysis are low (Table 4). For GCA, A637, CR1HT, PA91HT1,
200 WF9 and NK778 had significant GCA estimates at 0.05%, 0.063%, -0.01%, 0.003%, and -
201 0.14%, respectively. For SCA, two specific crosses had significant estimates: A637/NK778 (-
202 0.05%) and CR1HT/NK778 (0.06%). For reciprocal estimates, only A427/PA91HT1 had a
203 significant reciprocal effect of 0.06%.

204 The highest estimated IRc was found for A427/A637 (15%) and the lowest estimated IRc
205 is NK778/PA91HT1 (7%). PA91HT1/WF9, WF9/PA91HT1, PA91HT1/NK778 and
206 NK778/WF9 have high rates of misclassification (Table 2). Using equation 2, as mentioned in
207 the materials and methods, heritability in the narrow sense was estimated to be h^2=0.67. Which
208 can be interpreted as a substantial portion of the genetic variation seen for inducibility is of the
209 additive form which would support observations made for GCA.

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210 **Discussion**

211 The effective and efficient use of the maize *in vivo* maternal DH system relies on the
212 ability to efficiently produce and sort, and effectively identify haploid progeny. There are
213 multiple avenues to increase the efficiency of this system: 1) development and use of improved
214 inducer lines (Prigge et al., 2011; Coe, 1959; Geiger, 2009; Rober et al., 2005), 2) improved
215 methods of haploid selection through new traits and/or automation (Boote et al. 2016; De La
216 Fuente et al., 2017; Jones et al., 2012; Melchinger et al. 2015; Smelser et al., 2015), and 3) the
217 method considered in this and other studies (Kebede et al., 2011; Prigge et al, 2011), namely
218 improvement of rates of induction in donor materials.

219

220 *Misclassification rates*

221 Misclassification rates of haploid seed can become a significant problem if the rates are
222 too high. For example, if a breeder needs to large quantity of haploid seed to produce the
223 required number of DH lines, and the misclassification rate is high, many of the plants in the
224 field will be hybrids and will need to be removed. This is a waste of time, materials, and field
225 space all of which add costs to the breeding program. The objective of DHs is speed and
226 efficiency, and wasting time and money is counterproductive. As a general guide,
227 misclassification rates are best kept below 10%, though this can be heavily influenced by factors
228 such as the person classifying the seeds and the depth of the coloration on the kernel. In this
229 study, misclassification rates ranged from 0%-45.2%. For the specific lines included in the study,
230 A427, A637, and CR1HT had misclassification rates that were within acceptable limits 4.5-5.8%
231 while WF9 had an unacceptable rate of misclassified haploids (16%) averaged across all crosses.
232 Misclassification rates were, on average, lower than those reported in other studies (Kebede et

233 al., 2011; Prigge et al., 2011; Rober et al., 2005). Both Prigge et al. (2011) and Rober et al.
234 (2005) report that the inclusion of unadapted landraces and populations increase the
235 misclassification rates. This could be an explanation of why misclassification rates in this study
236 are lower. Alleles that modify the *R* locus may still be segregating in unadapted materials, while
237 in elite yellow dent corn, those alleles have been removed over time with selection being for no
238 aleurone coloration and yellow or white endosperm. Based on the results presented here and the
239 genetic contributions of PA91HT1, WF9, and NK778 and their parents to the dent germplasm it
240 may be desirable for breeders to obtain a small sample (i.e., 100-250 seed) and grow or cut the
241 seed to come up with an estimate for the misclassification rate so that it can be accounted for
242 when estimating the number of haploid seed that need to be planted. By growing the seed, the
243 phenotypic difference between haploid and hybrid will be seen. Cutting the seed allows for a
244 clearer view of the embryo coloration which may be difficult to see through the pericarp layer.
245
246 *Induction Rates*
247 Induction rates in this study average 10% across both environments and all 30 hybrids,
248 which is consistent with averages seen at the ISU-DH Facility (Frei, personal communication).
249 When compared to other studies, which evaluated tropical germplasm, IRs in this study are
250 slightly higher than those reported: 6.74% (Kebede et al., 2011), and 7.63% (Prigge et al., 2011).
251 The higher induction rates seen in this study are likely due to the more adapted and elite nature
252 of the germplasm in consideration. It is possible that elite germplasm from temperate adapted
253 environments provide better overall IR. However, the higher overall rates may also be due to the
254 fact that of the six lines selected, half of them were selected to have high inducibility.
255

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256 *Environmental effects on induction rates*

257 Induction rates presented for AF14 (13.1%) were higher than those for AF15 (10.2%). A
258 significant effect of environment in the combining ability analysis also supports this difference.
259 An effect of environment was also reported by Kebede et al. (2011) and Prigge et al. (2012).
260 However, in this study a significant effect of GCA by ENV was found unlike that reported by
261 Kebede et al. (2011). The significant GCA by ENV interaction could have come from the
262 abnormally cool season and high amounts of rainfall that was experienced at AF15 during
263 pollination, which lead to fungal ear diseases at the end of the season. This may have reduced the
264 IR, and has been previously reported in maize (Geiger, 2009).
265

266 *Genetic Components of IRc*

267 GCA and SCA estimates in this study were, in general, very low and not significantly
268 different from zero. This is not surprising when the range in average corrected IR for each of the
269 inbred lines in the diallel was only 10%-12%. Ranges reported for a similar study in tropical
270 maize (Kebede et al., 2011) were 2.9-9.7% across environments. Their inclusion of more
271 contrasting germplasm provided higher estimates for GCA. The highest estimate for GCA in this
272 study was 0.06% for CR1HT while the highest reported in Kebede et al., (2011) was 1.06%.
273 However, similar low percentage effects were reported for other lines in their study. In contrast
274 to results from Kebede et al. (2011), this study reports significant SCA and REC effects.
275 Significant SCA effects were reported for A637/NK778 (-0.05%) and CR1HT/NK778 (-0.06%).
276 It may be that SCA effects were not found in Kebede et al. (2011) due to the fact that their
277 germplasm was all sourced from breeding lines and breeding material from CIMMYT
278 (International Center for Maize and Wheat Improvement), while germplasm for this study was

279 sourced from both private and public breeding programs from different times and locations.
280 More diversity in the six parents in this study may have led to the SCA seen. It is also possible,
281 that the different inducer hybrid RWS x UH400 used in Kebede et al. (2011) may have also
282 caused this difference due to interaction between the donor germplasm and the specific inducer
283 used. In this study, a significant effect of REC was estimated and WF9/PA91HT1 had a
284 significant REC effect (-0.06%). No other study reported, or has considered reciprocal effects for
285 IR. REC effects would be an important consideration as it could allow breeders to increase their
286 IR by planning the direction of their crosses.
287
288 *Breeding for increased IR*
289 This study is in agreement with others (Kebede et al., 2011; Prigge et al., 2011; Rober et
290 al., 2005) that there is significant variation in IR and that the environment has an effect on the
291 average induction rate, and that selection could be possible for improvement of IR. This study in
292 addition concludes, that there are significant interactions between germplasm and environment,
293 and that specific combinations and the direction of the cross have an effect on IR. Breeding for
294 increased IR in already established elite breeding pools of dent and flint maize using the DH
295 system would likely be a waste of effort. Increased IR is being unintentionally selected for
296 through the use of the DH process as germplasm which do not produce enough haploids will be
297 removed and those that produce sufficient amounts are retained in the breeding program.
298 However, as breeding programs evolve and react to new biotic and abiotic stress as well as end
299 user requests, it is important to be able to go outside of the adapted breeding pool and work with
300 unadapted germplasm. As is reported by others (Kebede et al., 2011; Prigge et al., 2011; Rober et
301 al., 2005) when unadapted germplasm is used, the rates of misclassification go up and the rates

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302 of induction go down. It may then be possible for breeders to utilize more adapted lines with
303 high induction rates and low misclassification rates to cross with unadapted material to raise the
304 rates of induction and reduce the rates of misclassification through selection. Significant GCA
305 effects in all lines except A427 points to the importance of additive genetic effects. Only two of
306 the hybrid combinations produced a significant SCA estimate and both were negative and
307 occurred in combination with NK778 which also had the lowest GCA for inducibility. If we
308 consider the Germplasm Enhancement of Maize (GEM) project, which is an established and
309 successful tropical introgression breeding program, the strategy is to cross unadapted tropical
310 materials with two adapted commercial inbred lines. Since estimates for SCA were both
311 negative, there would be no need to pair the adapted inbreds with specific tropical germplasm.
312 Also, since reciprocal effects were only present in two crosses there is no need to consider the
313 direction of the cross, other than to maximize seed yield on the female in the induction cross.
314 Therefore, the most important aspect to consider is to use at least one adapted parent which has a
315 high positive GCA estimate (in this study CR1HT as a non-stiff stalk, and A637 as a stiff stalk).
316 Since GCA and ENV are the most important factors, continued selection for higher rates of
317 inducibility and selecting high seed yield environments will provide an optimal pairing of
318 environment and genotypes to use as adapted parents, when introgressing unadapted germplasm
319 using the doubled haploid breeding strategy.

320

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Tables

Table 1. Pedigree, origin, and general information for six parents included in diallel.

Line Name	Heterotic Group	Origin	Pedigree	GDD† to Silk	IR (preliminary exp.)
A427	non-stiff	Minnesota	CC36 x A405	1318	0.15
	stalk				
A637	stiff stalk	Minnesota	CO106 x A321	1522	0.06
CR1HT	non-stiff	Nebraska	W117Ht x Mo17Ht	1178	0.27
	stalk				
PA91HT1	stiff stalk	Hawaii	Oh40B, L317, WF9	1640	0.29
WF9	stiff stalk	Indiana	Indiana strain of	1522	0.33
			Stiff Stalk Synthetic		
NK778	stiff stalk	Minnesota	W117 x B37Ht	1400	0.15

†GDD=growing degree days

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Table 2. Corrected average induction rate (IRc), misclassification rate, and confidence intervals for the 30 hybrids of the diallel in order of pedigree.

Hybrid	IRc	Misclassification Rate	Lower C.I.	Upper C.I.
	%	%	%	%
A427/A637	14.6	6	14.59	14.61
A427/CR1HT	10.3	4.8	10.29	10.31
A427/PA91HT1	9.6	6	9.58	9.62
A427/WF9	11.7	6	11.69	11.71
A427/NK778	7.3	4.8	7.28	7.32
A637/A427	10.7	4.8	10.69	10.71
A637/CR1HT	11.7	1.2	11.70	11.70
A637/PA91HT1	11.6	3.6	11.59	11.61
A637/WF9	13.9	3.6	13.89	13.91
A637/NK778	8.9	1.2	8.89	8.91
CR1HT/A427	11.1	2.4	11.09	11.11
CR1HT/A637	12.6	4.8	12.59	12.61
CR1HT/PA91HT1	11.9	3.6	11.89	11.91
CR1HT/WF9	11.9	6	11.88	11.92
CR1HT/NK778	10.2	6	10.18	10.22
PA91HT1/A427	8.7	11.9	8.68	8.72
PA91HT1/A637	12.4	3.6	12.26	12.54
PA91HT1/CR1HT	11.2	4.8	11.06	11.34
PA91HT1/WF9	7.5	45.2	7.36	7.64
PA91HT1/NK778	8	4.8	7.98	8.02
WF9/A427	9.8	11.9	9.78	9.82
WF9/A637	11	8.3	10.96	11.04
WF9/CR1HT	12.8	7.1	12.77	12.83
WF9/PA91HT1	10.3	21.4	10.24	10.36
WF9/NK778	10.2	20.2	10.15	10.25
NK778/A427	7.9	0	7.88	7.92
NK778/A637	7.5	4.8	7.48	7.52
NK778/CR1HT	11.9	4.8	11.79	12.01
NK778/PA91HT1	7.1	13.1	6.99	7.21
NK778/WF9	7.3	32.1	7.19	7.41
Overall Mean	10.4	8.6	-	-

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Table 3. Analysis of variance table for diallel analysis. Analysis was conducted using SAS program Diallel SAS 5.0.

Source of Variation	DF	SS	MS	F-value	P-value
Env	1	0.13186	0.13186	83.61	<0.001***
Reps(Env‡)	6	0.02054	0.003424	2.17	0.0479*
Hybrid	29	0.272023	0.00938	5.95	<0.001***
GCA§	5	0.17054	0.0341	21.6278	<0.001***
SCA¶	9	0.043443	0.004827	3.0607	0.00198**
REC#	15	0.05803	0.003869	2.4533	0.0028**
Hybrid x Env	29	0.09077	0.0031	1.98	0.0038**
GCAxEnv	5	0.033306	0.006661	4.2238	0.0012**
SCAxEnv	9	0.033108	0.00367	2.3326	0.01664**
RECxEnv	15	0.024355	0.001624	1.0296	0.42717^{ns}
M††xEnv	5	0.007553	0.00151	0.9579	0.4452^{ns}
N‡‡xEnv	10	0.016802	0.00168	1.0654	0.39151^{ns}
Error	174	0.2744	0.001577		

*significant at the 0.05 probability level
**significant at the 0.01 probability level
***significant at the 0.001 probability level
†ns, nonsignificant at the 0.05 probability level
§ GCA = general combining ability
¶ SCA = specific combining ability
# REC = Reciprocal
†† M = maternal
‡‡ N = non-maternal

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Table 4. Combining ability analysis. Corrected IR reported in percent. Diagonal (bolded) values are GCA, above the diagonal are SCA, and below the diagonal are reciprocal effects.

Inbred	A427	A637	CR1HT	PA91HT1	WF9	NK778
A427	**-0.0003^{ns}**	-0.0333^{ns}	-0.0210^{ns}	0.0014^{ns}	0.0032^{ns}	-0.0033^{ns}
A637	0.0835*	0.**0500***	-0.0339^{ns}	0.0256^{ns}	0.0035^{ns}	-0.0478*
CR1HT	-0.0044^{ns}	-0.0050^{ns}	**0.0630***	0.0046^{ns}	0.0005^{ns}	-0.0569*
PA91HT1	0.0058^{ns}	-0.0067^{ns}	0.0029^{ns}	**-0.0129***	-0.0339^{ns}	0.0000^{ns}
WF9	0.0258^{ns}	0.0702^{ns}	-0.0041^{ns}	-0.0645*	**0.0027***	0.0019^{ns}
NK778	-0.0025^{ns}	0.0161^{ns}	-0.0324^{ns}	0.0092^{ns}	0.0700^{ns}	**-0.1357***

*Significant at the 0.05 probability level.
†ns, nonsignificant at the 0.05 probability level

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Figure Legends

Figure 1. Probability of detection of simulated mean differences ranging from 1% to 16% with number of kernels sorted ranging from 100 to 4000. Minimizing the number of kernels sorted helps reduce the amount of labor needed, and also the amount of error by limiting the number of people that sort the kernels. A balance between this and maximizing the probability of detection for a given mean difference is the objective.

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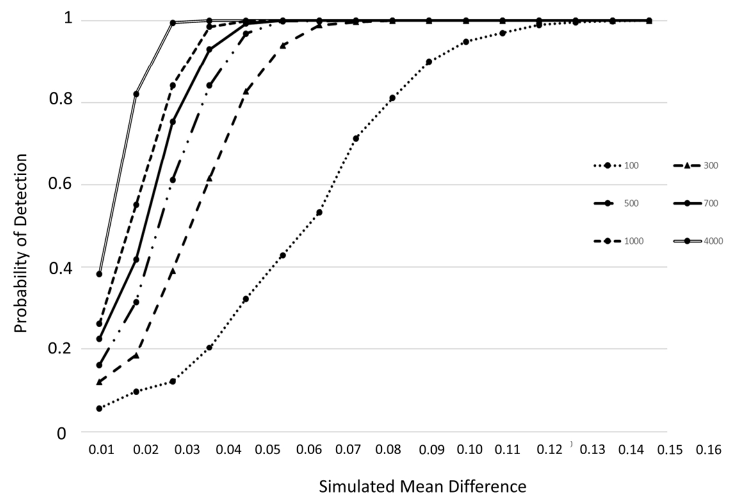

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190x107mm (300 x 300 DPI)