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Weighing in on a method to discriminate maize haploid from hybrid seed

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Abstract
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Keywords
doubled haploid, Sorting kernels, maize breeding

Disciplines
Agronomy and Crop Sciences

Comments
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Weighing in on a method to discriminate maize haploid from hybrid seed

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With 2 tables

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Abstract

The doubled haploid breeding method can produce maize inbred lines faster than traditional methods, but there are challenges associated with it. Sorting haploid from hybrid seed based on visual colour markers is time consuming and can be difficult due to colour inhibitors that obscure pigmentation needed to distinguish between haploid, hybrid and out-crossed seed. In this study, weight was evaluated as a method to sort haploid from hybrid seed. A first experiment utilized two families for analysis in a preliminary study. Eleven haploid and hybrid kernels from both families were weighed for a total of 44 experimental units. A second experiment was carried out using six families, using the same format as the previous, for 132 experimental units. Hybrid seed weighed significantly more than haploid seed in both experiments. However, the interaction between line and kernel type was significant in the second experiment. In conclusion, efficacy of sorting haploid from hybrid kernels based on weight depends on the genotypes involved.

Key words: doubled haploid — sorting kernels — maize breeding

Materials and Methods

In the winter of 2011, 5 haploid seeds and 5 hybrid seeds of B73 were sent to Strube Research GmbH & Co. KG, in Germany for evaluation on various seed characteristics. Using a simple ANOVA, weight was identified as being significant (data not shown) for kernel type (haploid or hybrid). From this point, the Agriculture Experimentation Station Consulting Group from the Department of Statistics at Iowa State University for statistical support ran simulations from the data set of the B73 trial and determined that 22 haploids and 22 hybrids were needed for evaluation to test weight as a method to differentiate kernel types with 90% confidence on the outcome.

In the summer of 2011, A632, G80, Mo17 and LH82 were pollinated using RWSxRWK-76 to produce haploid seed, and PHB47 and PHZ51 were pollinated in the summer of 2012 with the same haploid inducer. G80, LH82, PHB47 and PHZ51 are expired Plant Variety Protection (Ex-PVP) lines, and A632 and Mo17 are popular public inbred lines. A632, PHB47 and G80 all belong to the stiff stalk heterotic group and Mo17, LH82 and PHZ51 belong to the non-stiff stalk heterotic group. In the winter of 2011/2012, the haploid and hybrid progeny from the A632 and Mo17 crosses were selected and screened for seed size using 0.31 cm and 0.15 cm diameter screens (Experiment 1). Screening was used to create a pool of haploids and hybrids that would be of similar dimensions for the study and to take year affect out of the equation. Eleven putative haploid seeds and eleven hybrid seeds, as determined by...
R1-nj expression, were selected at random for each line from the screened pools of seed (44 experimental units). The selected haploid and hybrid seeds of these two lines were sent to Strube Research GmbH & Co. KG, Solingen, Germany, for preliminary evaluation of weight as a method for distinguishing haploid from hybrid seed. Strube Research GmbH & Co. KG, Germany, used a Mettler Toledo scale with a sensitivity of 0.1 mg to weigh each kernel. An ANOVA was run using the following statistical model, with LS means calculated, and a 90% confidence interval used:

\[ Y_{ijk} = \mu + L_i + T_j + LT_{ij} + \varepsilon_{ijk} \]

where:  
- \( \mu \) = the response observed for the \( \text{ijk} \)th experimental unit,  
- \( L_i \) = the line at the \( i \)th level,  
- \( T_j \) = kernel type at the \( j \)th level,  
- \( LT_{ij} \) = the interaction between the line and kernel type at the \( ij \)th level,  
- \( \varepsilon \) = the standard error at the \( ij \)th level,  
- \( i \) and \( T \) are fixed effects, and a normal distribution was used. Data were analysed using the below code for SAS 9.2.

```sas
proc glm;
class pedigree type;
model weight = pedigree|type/lines pdiff
lsmeans pedigree|type/lines pdiff;
run;
```

In the winter of 2012/13, 11 haploid and 11 hybrid seeds of all six lines (132 experimental units) were screened for seed size using 0.31 cm and 0.15 cm diameter screens and weighed using an Ohaus scale that measures with a resolution of 0.1 mg at the North Central Regional Plant Introduction Station in Ames, Iowa (Experiment 2). Haploid and hybrid A632 and Mo17 kernels from the cross with the inducer genotype were reselected, at random, and measured, to confirm the accuracy of the scale and results of the second experiment. This experiment was analysed using the same model mentioned above, but with a 95% confidence level due to use of more experimental units than the prior experiment.

### Results

In Experiment 1, the average kernel weight among lines was non-significant (\( \text{Pr} = 0.5150 \)), and kernel type was significant (\( \text{Pr} = 0.0007 \)), with hybrid seed weighing significantly more than haploid seed. The average kernel weight of haploid kernels was 273.2 mg and the average hybrid kernel weight was 316.4 mg. The interaction of the line and kernel type was 273.2 mg and the average hybrid kernel was significantly more than haploid seed. The average kernel weight of A632 PI 587140 was 303.6 mg on average (Table 1).

In Experiment 2, there were significant differences among haploid and hybrid kernels for weight. For the lines tested, there was no significant impact of heterotic pool identity on haploid kernel weight could be utilized to enrich selection for haploid kernels. It was not surprising that the type of line and interaction between the line and kernel type was significant in the second experiment due to the larger number of lines and experimental units evaluated. As the interaction between line and kernel type was significant in experiment two, the efficacy of successfully identifying haploids in multiple backgrounds may not be sufficient for some breeding programmes due to the need for a stringent threshold to avoid false positives. We evaluated whether heterotic grouping impacts haploid – hybrid seed weight differentiation. In Experiment 2, three stiff stalks (G80, PHB47 and A632) and three non-stiff stalk lines (LH82, PHZ51 and Mo17) were evaluated for haploid and hybrid weight, and only A632 and PHZ51 showed significant differences among haploid and hybrid kernels for weight. For the lines tested, there was no significant impact of heterotic pool identity on haploid – hybrid seed differences (data not shown).

These experiments verified that haploid kernels weighed significantly less than hybrids in both experiments, and that kernel weight could be utilized to enrich selection for haploid kernels. Haploids were induced and putative haploid kernels sorted based on weight alone and by colour marker alone, grow them out, evaluate and compare the rates of false positives (hybrids) found within the same putative haploid groups.

### Discussion

These experiments verified that haploid kernels weighed significantly less than hybrids in both experiments, and that kernel weight could be utilized to enrich selection for haploid kernels. It was not surprising that the type of line and interaction between the line and kernel type was significant in the second experiment due to the larger number of lines and experimental units evaluated. As the interaction between line and kernel type was significant in experiment two, the efficacy of successfully identifying haploids in multiple backgrounds may not be sufficient for some breeding programmes due to the need for a stringent threshold to avoid false positives. We evaluated whether heterotic grouping impacts haploid – hybrid seed weight differentiation. In Experiment 2, three stiff stalks (G80, PHB47 and A632) and three non-stiff stalk lines (LH82, PHZ51 and Mo17) were evaluated for haploid and hybrid weight, and only A632 and PHZ51 showed significant differences among haploid and hybrid kernels for weight. For the lines tested, there was no significant impact of heterotic pool identity on haploid – hybrid seed differences (data not shown).

<table>
<thead>
<tr>
<th>Letter</th>
<th>Line</th>
<th>Accession #</th>
<th>Kernel type</th>
<th>Average weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A632</td>
<td>PI 587140</td>
<td>Hybrid</td>
<td>329.1 mg</td>
</tr>
<tr>
<td>A</td>
<td>A632</td>
<td>PI 587140</td>
<td>Hybrid</td>
<td>348.8 mg</td>
</tr>
<tr>
<td>A</td>
<td>A632</td>
<td>PI 558532</td>
<td>Hybrid</td>
<td>303.6 mg</td>
</tr>
<tr>
<td>A</td>
<td>A632</td>
<td>PI 587140</td>
<td>Haploid</td>
<td>287.2 mg</td>
</tr>
</tbody>
</table>

Same letter are NOT significantly different.

Table 1: Experiment 1: Comparison of LS means for interaction of the line and kernel type

Table 2: Experiment 2: Comparison of LS means for interaction of the line and kernel type

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<table>
<thead>
<tr>
<th>Letter</th>
<th>Line</th>
<th>Accession #</th>
<th>Kernel type</th>
<th>Average weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>G80</td>
<td>PI 601037</td>
<td>Haploid</td>
<td>362.3 mg</td>
</tr>
<tr>
<td>A</td>
<td>PHZ51</td>
<td>PI 601322</td>
<td>Hybrid</td>
<td>357.0 mg</td>
</tr>
<tr>
<td>A</td>
<td>A632</td>
<td>PI 587140</td>
<td>Hybrid</td>
<td>348.8 mg</td>
</tr>
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<td>A</td>
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Same letter are NOT significantly different.
Using weight to discriminate haploid from hybrid seed would likely be less efficient for enrichment of haploids compared to colour markers or using a high oil inducer. Utilizing a high oil inducer would also be a more accurate, high-throughput method than using the weight of the kernels. One study found that haploid identification using a high oil inducer can be 90% accurate (Chen and Song 2003, Chang and Coe 2009). However, if a high oil inducer line is unavailable, then differentiating haploid and hybrid seed based on weight will be much faster than the use of colour markers alone. New methods could be developed evaluating three dimensional (3D) computed tomography (CT) scanning technology. 3D CT uses a sealed microfocal X-ray tube that utilizes a manipulation system, a flat panel x-ray detector and a system of computers for acquiring the data, and reconstruction and processing of the 3D image. This technology is currently used by Strube Research GmbH & Co. KG on sugar beet seeds to evaluate the morphological and anatomical features of the seed that correlate to the seedling emergence capabilities. The use of the 3D CT scanning technology could be used as a non-destructive, high throughput way to observe differences in the seed between haploids and hybrids.

Currently, visual sorting is the most common method for distinguishing haploid seed from hybrid seed. Data reported in this study suggest that moving to an automated system that uses weight evaluation to differentiate haploid individuals from hybrid is an option to consider. One could set a very stringent limit on weight that would result in loss of haploid individuals in the higher weight category. This would reduce field effort and resources needed for roguing hybrids, but more induction crosses would be necessary to obtain sufficient haploid seeds to satisfy a required number of doubled haploid individuals, postweight screening process. One could also set a very loose limit on weight, resulting in an increase of hybrid individuals found in the field after sorting. This would increase hybrid roguing efforts, but would also increase the number of haploids making it through the screening process and reduce the number of induction crosses needed. Using either method, there would be a dramatic decrease in the amount of time and labour required to separate haploid from hybrid seed compared to visual identification. Judgement will be required to determine the balance between excluding haploid individuals in the upper weight distribution versus including more hybrid individuals that will be mixed in with the heavier haploids.

**Acknowledgements**

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**References**


