# Doubled Haploids in Tropical Maize:
## Influence of Inducer and Donor on in vivo Haploid Induction Rates

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## Question: Does HIR depend on donor & inducer?

Obtaining reliable haploid induction rates (HIR) is a key determinant for successful employment of inducer genotypes for maize doubled haploid (DH) line development using the in vivo haploid induction technique.

Depending on the donor germplasm from which DH lines are to be derived (e.g. landraces, improved open-pollinated varieties (OPV) or elite single or multiple crosses), induction nursery planning may have to be adjusted according to suitability of donors and induction ability of inducers.

The objective of this study was to investigate the influence of the genetic constitution of donors and inducers on HIR in tropical environments.

## Materials & Methods

### Experiment 1: Donor influence

- **Plant materials:** 150 tropical and subtropical donors (50 landraces, 50 improved OPV, 50 single crosses) as female parents and temperate inducer UH400 as pollen parent
- **Location in 2008A:** Agua Fria (Puebla, Mexico; 110m asl)
- **Design:** lattice with two replications
- Visual classification of seeds resulting from induction cross according to *R1-nj* red crown color marker (Nanda & Chase 1966; Fig. 1)
  - Haploid (H) seeds have colored endosperm (red crown) but non- colored embryo
  - Diploid seeds have colored endosperm and embryo or both non- colored
- HIR[%] calculated as (H seeds / Total seeds)*100 and arcsin sqrt transformed for ANOVA


**Figure 1: Phenoytpic classification of seeds resulting from induction cross.**

### Experiment 2: Inducer influence

- **Plant materials:** temperate liguleless (lglg) tester homozygous for recessive alleles conferring liguleless phenotype as female parent and temperate inducers UH400, RWS (Röber *et al*. 2005) and (RWSxUH400) as pollen parents
- **Locations:** 2008B in Tlaltizapan (Morelos, Mexico; 954m asl) and 2009A in Agua Fria
- **Design:** randomized complete block design with two replications
- Visual evaluation of testcross progeny at the four-leaf stage according to presence (diploid) or absence (haploid) of ligule and associated characteristic leaf sheath morphology (Fig. 2)
- HIR[%] calculated as (H plants / Total plants)*100 and arcsin sqrt transformed for ANOVA

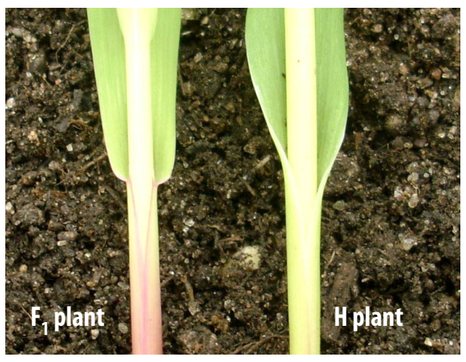


**Figure 2: Characteristic leaf sheath morphology at ligule presence in $F^1$ plants and ligule absence in haploid (H) plants of lglg x inducer progeny.**

## Results & Discussion

**Mean HIR** in **Experiment 1** was 5.5% for landraces (0-18.1%) and single crosses (0.7-16.1%) but only 3.7% for OPV (0-15.9%). For landraces and single crosses, half of the entries showed HIR between 3 and 7%, whereas half of the OPV entries had HIR between 2 and 4% (Fig. 1). In 20% of the OPV HIR of less than 3% were observed, while only 8 and 11% fell into this category for single crosses and landraces, respectively.

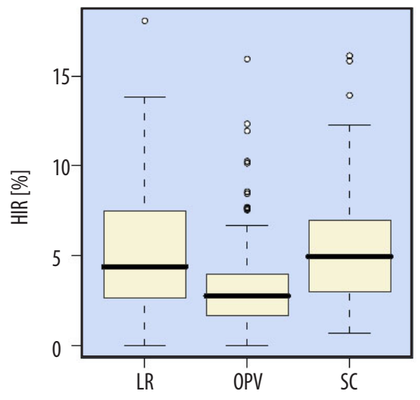


**Figure 3: Boxplot with median, quartiles, and extremes of in vivo haploid induction rates (HIR) obtained with inducer UH400 in 42 landraces (LR), 50 improved open-pollinated varieties (OPV), and 50 single crosses (SC) based on red crown marker.**

The **donor entries** had a significant effect on HIR which is in accordance with findings from temperate maize (Eder & Chalyk 2002). Although the groups (*i.e.* landrace, OPV, and single cross) also had a significant effect, their estimated variance component was only half as high as the variance component estimated for entries within groups (data not shown). This indicates that **no general superiority** of a certain donor type for in vivo haploid induction in tropical maize exists. It is indeed feasible to obtain sufficient H seeds from tropical maize landraces.

Moreover, HIR assessment seems to be largely dependent on the **seed classification system** employed. In induction environment Agua Fria 2008A, inducer UH400 attained a mean HIR of 8.4% using the temperate lglg tester while its HIR was only half as high using the red crown color marker in the 150 tropical donors (data not shown).

**Table 1: Means and ranges of in vivo haploid induction rates of three inducers assessed in two environments in Mexico with the liguleless tester (N = number of testcross plants evaluated). For ANOVA, HIR data were arcsin sqrt transformed.**

| *Environment* and Inducer | N | Haploid Induction Rate Mean [%]† | Range [%] |
|---|---|---|---|
| *Tlaltizapan 2008B* | *28064* | *11.01* | *8.3-13.1* |
| UH400 | 9037 | 8.38[a] | 8.3-8.4 |
| RWS | 10137 | 12.22[b] | 11.3-13.1 |
| RWSxUH400 | 8890 | 12.43[b] | 12.3-12.6 |
| $LSD_{.05}$ | | 2.84 | |
| *Agua Fria 2009A* | *9828* | *14.17* | *9.0-19.4* |
| UH400 | 2795 | 9.30[a] | 9.0-9.6 |
| RWS | 3218 | 19.05[b] | 18.7-19.4 |
| RWSxUH400 | 3815 | 14.42[c] | 13.8-15.1 |
| $LSD_{.05}$ | | 3.05 | |

† Identical letters in one environment indicate no significant differences based on least significant difference at α=0.05.

**Mean HIR** in **Experiment 2** was higher in Agua Fria A cycle than in Tlaltizapan B cycle (Tab. 1). In both environments, UH400 had the lowest HIR. RWS and the single cross RWSxUH400 performed similar in Tlaltizapan while in Agua Fria RWS's HIR was almost 5% higher than that of its single cross.

The **inducer genotype** had a significant effect on HIR which corresponds to results obtained with temperate maize (Röber *et al*. 2005). In general, HIR of the temperate inducers seem sufficiently high for large-scale application in tropical environments. However, **agronomic performance** of the temperate inducers is comparably poor in subtropical and tropical environments. This not only hinders inducer seed maintenance but also timely collection of sufficient inducer pollen for induction crosses.

Hence, developing a **tropically adapted inducer** with excellent pollen production as well as sufficiently high and stable HIR is a premise for DH line development in sub-tropical and tropical maize.

## Conclusions

- **Inducers and donors both have a significant influence on in vivo haploid induction rates**
- **Haploid induction is not the limiting factor in DH line development from tropical maize landraces**
- **HIR of the temperate inducers are sufficiently high for large-scale application in tropical environments but development of a tropically adapted inducer would greatly improve inducer seed maintenance and inducer pollen collection**
- **Temperature inducer RWSxUH400 is most suitable for tropical environments so far due to wide male flowering window and sufficient HIR**

## References

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