CULTIVAR RELEASE

Deep purple – an open-pollinated variety to induce haploids in tropical maize

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Abstract: *Deep purple is an open-pollinated variety, well adapted to tropical conditions, developed to induce maternal haploids in maize. The founders were W23, Stock6, and an experimental tropical maize population. The inducer population inherits the dominant the R1-nj marker, and the expected real haploid induction rate is 3%.*

Keywords: *Doubled haploids, recurrent selection, cultivar description, seed production*

INTRODUCTION

The doubled haploids (DH) technique has been applied in maize breeding to speed up maize inbred development. However, smaller breeding operations have not been able to fully take advantage of this rapid method of producing inbred lines. One of the main reasons for this lag is the lack of adapted or efficient haploid inducers, frequently observed in tropical countries where efforts at breeding inducers are more recent (Prigge et al. 2011). Clearly defining target traits, breeding approaches, and the most appropriate type of inducer cultivar for each breeding program is a fundamental step in a maize breeding program aiming at implementing the *in vivo* DH technique (Uliana Trentin et al. 2020). Furthermore, this technique has been used in temperate maize breeding programs for quite some time. Nevertheless, it is not commonly applied in tropical breeding programs because tropical inducers are scarce, and those available are still under patent protection. Hence, it is crucial to develop more tropical haploid inducers available to small and public breeding programs (Fritsche-Neto et al. 2023).

In this context, we developed a tropical maize haploid inducer population, and after two recurrent selection cycles with external testers and simultaneous selection, it is ready to be released to the scientific community. It is free of patent protection and can support small and limited budget breeding programs in critical regions, such as Africa, South America, and Southeast Asia.

PEDIGREE AND BREEDING METHODS

To obtain seeds from induction crosses marked by *R1-Navajo* (*R1-nj*), we used a haploid inducer population derived from a cross of two inducer lines

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(W23 and Stock6) with an experimental maize population adapted to tropical conditions. The inducer population inherits the dominant the R1-nj marker (Couto et al. 2019).

The breeding method used was an intrapopulation recurrent selection with external testers. The goal was to develop an inducer population rather than an inbred line, as an inducer population has a higher haploid induction rate and higher expressiveness for the *R1-nj* marker. At the beginning of each breeding cycle, we sampled 50 individual plants from the inducer population and crossed them with two different single-cross testers. These testers were from different germplasm (one flint and one dent germplasm), due to the known effect of the germplasm source on *R1-Navajo* expressiveness, which makes it hard to identify the haploid kernels and results in high false positives. Two replicates (ears) from each cross were evaluated, for a total of four ears per individual plant from the breeding population. Pollen collected from each inducer plant was used for selfing (S_1 progenies for recombination step) and fertilizing four single-cross plants (two from each tester - SC Flint and SC Dent) on the same day (Figure 1). The testers were commercial hybrids and may be used worldwide.

The four hybridized ears were harvested at the physiological maturity stage. Their seeds were counted (total number of seeds – TNS) and visually scored for ploidy level based on *R1-navajo* expression. Putative haploid seeds (PHS) had white embryos and purple endosperm, whereas seeds that had purple embryos and purple endosperm were classified as diploids. Seeds without any purple color were classified as inhibited (INH). Thus, the putative haploid induction rate (HIRp) was calculated as ($HIR_p = {}^{PHS}/_{TNS}$). In addition, $R1\text{-}nj\ EXP = 1 - ({}^{INH}/_{TNS})$ expressiveness (EXP) was used to account for the ability to observe a putative haploid seed $EXP = 1 - ({}^{INH}/_{TNS})$. Hence, the higher the expressiveness value, the lower the rate of inhibition in the seed.

The putative and inhibited seed fractions were sown in the field, following a completely randomized design with four replicates, to evaluate the “real” haploid induction rate (HIRr). Haploid plants were generally downgraded, smaller than diploid, and had short erect leaves in the field (Chaikam et al. 2017). Conversely, the diploid plants were vigorous and developed normally. After evaluating the real breeding program, we have generally used an “empirical” index to rank all the genotypes: $SI = 0.50HIR_r + 0.25HIR_p + 0.25EXP$. This index was constructed from the standardized values of the observations. Finally, the five best parents were selected to compose the recombination step. For that purpose, 40 S_1 plants of each superior progeny were randomly crossed under greenhouse conditions.

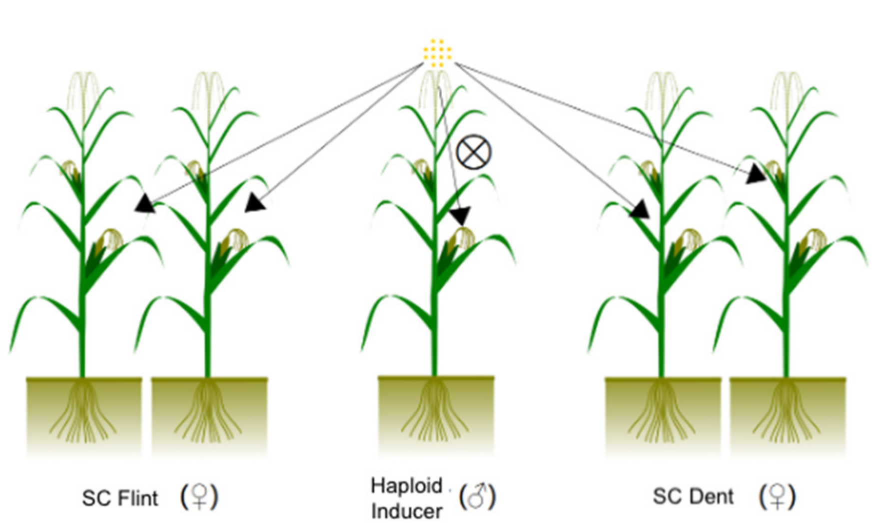

Figure 1. Crossing scheme and obtaining the progenies used in intrapopulation recurrent selection with external testers (single-crosses – SC). Each inducing plant was crossed with four other plants, two plants (replicates) of each external tester, and self-fertilized.

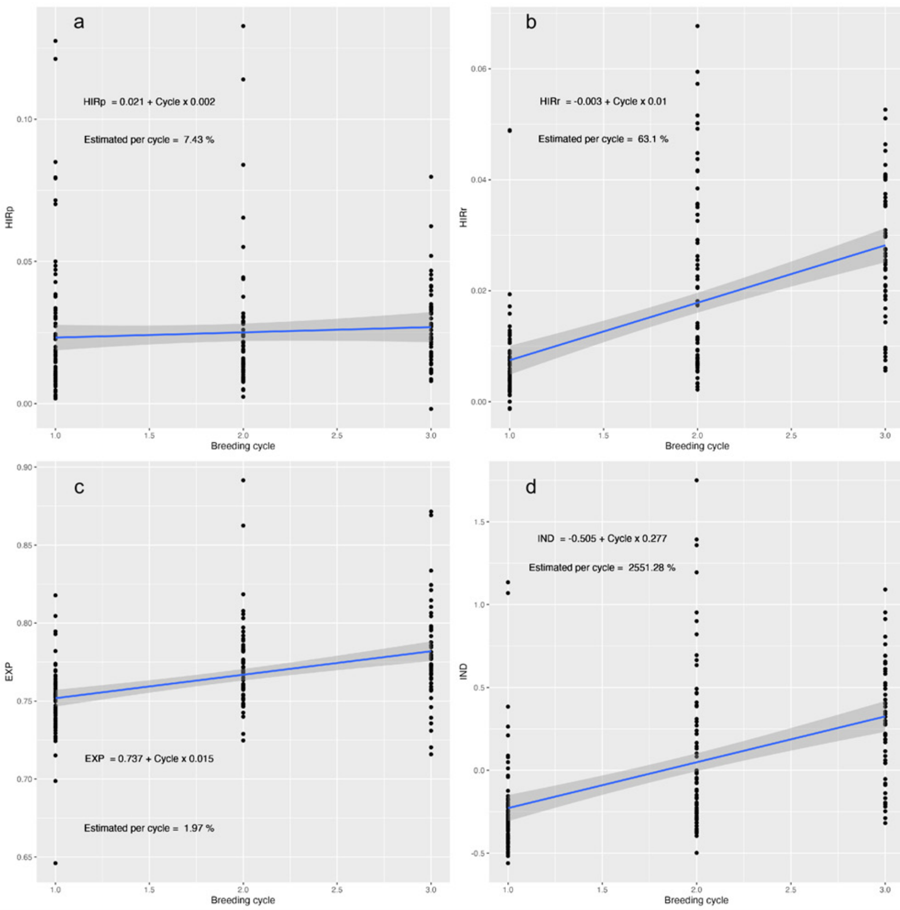

Figure 2. Genetic gains achieved and overall performance over two recurrent selection cycles for (a) putative haploid induction rate (HIRp), (b) real haploid induction rate (HIRr), (c) *R1-nj* expressiveness (EXP), and (d) the selection index used (IND).

PERFORMANCE

Genetic performance was estimated for each trait and the selection index was determined across breeding cycles, regardless of the tester effect. This is because the aim is to select inducer plants that perform well no matter the germplasm used to obtain haploids (Figure 2). In practical terms, we improved the population performance for HIRr from 0.8% to 2.8%. With two recurrent selection cycles, we achieved induction rates higher than those for CAUCHOI, a temperate climate inducer maize developed at China Agricultural University (Li et al. 2009). It is still a low value for a commercial inducer, especially upon comparing our population to temperate inducers, such as CAU5, CAU079, and UH400 with HIRs of 10%, 9%, and 8%, respectively (Prigge and Melchinger 2012, Xu et al. 2013). However, it is encouraging that it is possible to develop a maize haploid population inducer for tropical conditions and adapt it to those conditions, and better performances may be achieved in the next breeding cycles. Furthermore, the substantial increase of HIRr compared with HIRp exhibited a decrease in the false discovery rate, which is desirable and makes visual classification more accurate.

MAINTENANCE AND SEED DISTRIBUTION

The Deep Purple seeds may be requested from the corresponding authors at no cost. However, availability depends on the seed multiplication rate and demand. Additionally, logistical costs will be the responsibility of those requesting the seeds.

REFERENCES

Chaikam V, Lopez LA, Martinez L, Burgueño J and Boddupalli PM (2017) Identification of in vivo induced maternal haploids in maize using seedling traits. **Euphytica 213**: 8.

Couto EGO, Cury MN, Souza MB, Granato ICG, Vidotti MS, Garbuglio DD, Crossa J, Burgueño J and Fritsche-Neto R (2019) Effect of F1 and F2 generations on genetic variability and working steps of doubled haploid production in maize. **PLOS ONE 14**: e0224631.

Fritsche-Neto R, Sabadin F, DoVale JC, Borges KLR, Souza PH, Crossa J and Garbuglio DD (2023) Realized genetic gains via recurrent selection in a tropical maize haploid inducer population and optimizing simultaneous selection for the next cycles. **Crop Science 63**: 1-14.

Li L, XU X, Jin W and Chen S (2009) Morphological and molecular evidences for DNA introgression in haploid induction via a high oil inducer CAUHOI in maize. **Planta 230**: 367-376.

Prigge V, Sánchez C, Dhillon BS, Schipprack W, Araus JL, Bänziger M and Melchinger AE (2011) Doubled haploids in tropical maize: I. Effects of inducers and source germplasm on in vivo haploid induction rates. **Crop Science 51**: 1498-1506.

Prigge V and Melchinger AE (2012) Production of haploids and doubled haploids in maize. **Methods in Molecular Biology 877**: 161-172.

Uliana Trentin H, Frei UK and Lübberstedt T (2020) Breeding maize maternal haploid inducers. **Plants 9**: 5.

Xu X, Li L, Dong X, Jin W, Melchinger AE and Chen S (2013) Gametophytic and zygotic selection leads to segregation distortion through *in vivo* induction of a maternal haploid in maize. **Journal of Experimental Botany 64**: 1083-1096.