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Chromosome doubling by colchicine injection and haploidy induction in tropical genotypes of common and supersweet corn

Duplicação cromossômica por injeção de colchicina e indução de haploidia em genótipos tropicais de milho comum e superdoce

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Highlights

The tropicalized inducer PI4003 showed a maximum haploidy induction rate of 5%.
Colchicine solution injection has achieved an overall success rate of up to 34%.
Environments and populations interfere with the induction rate and DHs production.

Abstract

The double-haploid technology in maize was developed in temperate environments, using germplasm and inducers adapted for these environments, with the aim of accelerating the obtaining of homozygous inbred lines. Therefore, to advance this technology in tropical environments, research involving germplasm and inducers adapted for this environment is necessary. The objectives were to determine the haploidy induction rate in tropical common and supersweet corn populations, employing a tropicalized gymnogenetic inducer population, and to identify the effectiveness of chromosome doubling by colchicine injection. The haploidy inducer PI4003 was used as a male parent and crossed with 25 tropical maize populations (23 common and two supersweet). The putative haploid seeds, classified by the *R1-nj* gene marker, were sown in trays with peat during the 2020/21 and 2021/22 harvest seasons, and 100 µL of a solution of 0.125% colchicine and 0.5% dimethyl sulfoxide was injected into each haploid seedling

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at the V2 stage. The next day, the treated seedlings were transplanted to an irrigated field. The PI4003 inducer presented a haploidy induction rate ranging from 0.9% to 5%. There were significant differences to tropical genotypes and harvests for survival rates, reproduction rates, and overall success rates. The overall success rate ranged from 2.9% to 34%. The chromosome doubling method by colchicine injection proved to be effective, with lower colchicine consumption per haploid seedling treated and a reduction in the generation of toxic waste for disposal.
Key words: *Zea mays* var. *saccharate.* Haploidy induction rate. Double haploids. Gimnogenetic inducer.

Resumo

A tecnologia de duplo-haploide em milho foi desenvolvida em ambientes de clima temperado, empregando germoplasma e indutores adaptados para estes ambientes, com o objetivo de acelerar a obtenção de linhagens homozigotas. Neste sentido, para o avanço desta tecnologia em ambientes tropicais, são necessárias pesquisas envolvendo germoplasma e indutores adaptados para este ambiente. Os objetivos foram determinar a taxa de indução de haploidia em populações de milho comum e superdoce tropicais, empregando uma população indutora gimnogenética tropicalizada, e identificar a eficácia da duplicação cromossômica via injeção de colchicina. O indutor de haploidia PI4003 foi utilizado como parental masculino e cruzado com 25 populações de milho tropical (23 comuns e duas superdoces). As sementes haploides putativas, classificadas pela marcação do gene *R1-nj,* foram semeadas em bandejas com turfa nas safras 2020/21 e 2021/22, sendo injetado 100 µL de solução de 0,125% de colchicina e 0,5% de dimetilsulfóxido em cada plântula haploide no estádio V2. No dia seguinte, as plântulas tratadas foram transplantadas para campo irrigado. O indutor PI4003 apresentou taxa de indução a haploidia variando de 0,9% a 5%. Houve diferenças significativas entre genótipos tropicais e safras para as taxas de sobrevivência, taxas de reprodução e taxas de sucesso geral. A taxa de sucesso geral variou de 2,9% a 34%. O método de duplicação cromossômica via injeção de colchicina mostrou-se eficaz, com menor consumo de colchicina por indivíduo haploide tratado e redução na geração de resíduos tóxicos para descarte.
Palavras-chave: *Zea mays* var. *saccharate.* Taxa de indução de haploidia. Duplo-haploide. Indutor giminogenético.

Introduction

Hybrids derived from common or supersweet maize lines offer the advantage of greater productivity and uniformity compared to open-pollinated populations. These lines can be developed through six to eight generations of selfing or through double haploid (DH) technology, which involves two to three generations to achieve 100% homozygosity (Chaikam et al., 2019b).

The success of obtaining DHs depends on the refinement of steps involving haploidy induction, haploid identification, chromosomal doubling, and the multiplication of DH seeds (Chidzanga et al., 2017). While these steps have been extensively studied in common maize populations of temperate origin (Chase, 1969; Coe, 1959; Dang et al., 2012; Eder & Chalyk, 2002; Melchinger et al., 2016a; Trentin et al., 2022), research on maize of tropical origin is relatively limited (Baleroni

et al., 2021; Battistelli et al., 2013; Couto et al., 2015; Ribeiro et al., 2020), especially in the case of tropical supersweet maize (Khulbe et al., 2020; Sekiya et al., 2020; Trentin et al., 2022).

The first haploid inducers utilized were the gymnogenetic inducer Stock 6 and the androgenic inducer Wisconsin 23, both originating from temperate regions, exhibiting induction rates ranging from 1.0% to 3.2% (Coe, 1959; Kermicle, 1969). These rates were deemed low and posed limitations in achieving DHs. Subsequently, derived from these initial inducers, several temperate inducers were developed with induction rates as follows: 3% to 5% for WS14 (Lashermes & Beckert, 1988); 5.5% to 6.7% for MHI (Chalyk, 1999); 8% to 10% for RWS (Röber et al., 2005); 10% to 16% for PHI (Rotarenco et al., 2010); 9% to 16% for UH600 (Melchinger et al., 2016a); and 10.6% to 20.8% for the CHOI4 hybrid (Liu et al., 2022). These studies, among others, reveal the prevalence of employing gymnogenetic inducers for *in vivo* DH production. These inducers serve as pollen sources for donor populations, while androgenic inducers receive pollen from donor populations. The advantage lies in their higher haploidy induction rates, resulting in lines with the same cytoplasm as the donor population. Additionally, this method allows for induction through isolated field, eliminating the necessity for controlled manual pollination.

Temperate inducers, when grown in tropical environments, exhibit diminished vigor, low adaptability, heightened susceptibility to tropical diseases, extremely early flowering, and decreased production of pollen and seeds, thereby constraining the success of large-scale DH production (Prigge et al., 2012). Thus, the International Maize and Wheat Improvement Center (CIMMYT), in collaboration with the University of Hohenheim, introduced the first generation of tropical inducers known as Tropically Adapted Inducer Lines (TAILs). In tropical environments, this first generation of TAILs demonstrated superior agronomic performance compared to temperate inducers, achieving a haploidy rate ranging from 6% to 9% (Chaikam et al., 2016). Subsequently, CIMMYT advanced to the second generation of TAILs (CIM2GTAILs), characterized by enhanced vigor and adaptation to tropical conditions, increased pollen production, and an induction rate ranging between 9% and 14%, thereby reducing the costs associated with manual pollination (Chaikam et al., 2018). However, access to these tropicalized inducing lines is restricted and facilitated through use concession agreements (Chaikam et al., 2019b). Despite CIMMYT's endeavors, there persists a need for further research to develop inducers better adapted to tropical environments, possessing desirable agronomic characteristics, high induction rates, and being openly accessible.

The stage of chromosomal doubling in haploids, individuals with only one copy of each chromosome in their cells, is essential for restoring fertility and producing DH seeds. The spontaneous doubling rate in maize is typically less than 5% (Chaikam et al., 2019a; De La Fuente et al., 2020; Molenaar et al., 2019). Therefore, it is necessary to use methods that promote chromosomal duplication, through products such as colchicine (Chaikam et al., 2020), antimitotic

herbicides (Melchinger et al., 2016b), or nitrous oxide gas (Molenaar et al., 2018), which provide different doubling success rates. Among these agents, colchicine is the most commonly used to obtain DHs in maize (Chaikam et al., 2020), despite its drawbacks of high toxicity, elevated cost, potential carcinogenic, environmental hazards, and requiring special care in handling, application, storage, and disposal (Maqbool et al., 2020).

The primary protocols for chromosome doubling in maize haploids involve antimitotic solutions in newly germinated seedling dipping (Deimling et al., 1997), root dipping up to the crown region (Chaikam et al., 2020), seed soaking (Ren et al., 2018), or direct injection of a solution into the seedling basal meristem (Eder & Chalyk, 2002). To assess the comparative efficiency of these diverse protocols, it is important to employ uniform parameters that extend beyond the analysis of somatic tissue ploidy. Consideration should be given to the frequency of plants exhibiting the formation of fertile inflorescences and the production of DH seeds (Chaikam et al., 2020). In this context, Melchinger et al. (2016b) proposed the utilization of the following success rates: a) survival rate (SR): the proportion of treated haploids that survive until pollination time; b) reproduction rate (RR): the proportion of D1 ears with seed set obtained from D0 plants present at pollination; c) overall success rate (OSR): the proportion of D1 ears with seed set obtained from the treated haploids. In addition to these rates, the authors recommend considering the number of activities undertaken and the total costs associated with producing DH lines, encompassing the various steps of each method.

Research efforts aimed at improving the efficiency of obtaining DH lines in temperate environments, along with their adapted inducers, have made significant progress. However, there is a notable gap in published studies guiding and enhancing the success of obtaining DHs for common and supersweet maize in tropical environments. Much of the relevant information is restricted due to confidentiality maintained by private breeding companies. Therefore, the objectives of this study were to determine the effectiveness of chromosome doubling through colchicine injection in haploids derived from tropical common and supersweet maize populations. Additionally, the study explored the potential for haploidy induction in the tropicalized population PI4003 within an isolated field.

Material and Methods

This study encompassed 23 populations of yellow-endosperm common maize, two populations of supersweet maize, and the gymnogenetic inducer PI4003, derived from the crossbreeding of the old Stock 6 with an adapted tropical population. Within the common maize populations, 20 were classified as early cycle (PP01 to PP20), while three were designated as super-early (PS01 to PS03). All these populations belong to the company Tropical Melhoramento e Genética (TMG), Brazil, and demonstrate adaptation to diverse Brazilian environmental conditions. The two tropical populations of supersweet maize (SD3005 and SD3006) were developed by the Breeding Laboratory of the Department of Biology at the State University of Londrina (LGM-UEL), Paraná, Brazil, involving the introduction of the

shrunken (sh2) gene into tropical maize populations characterized by a green first leaf sheath.

The tropical inducer PI4003 was employed for pollinating 25 donor populations, which were segregated into two isolated fields within the TMG experimental field in Cambé, Paraná, Brazil. These fields were established during the 2019/20 and 2020/21 harvest seasons. The donor populations were planted in 120 meters double rows flanked by two rows of the inducing genotype sown five and ten days subsequent to these populations. Prior to male flowering, donor populations were detasseled.

The obtained F_1 seeds underwent classification based on the presence of the *R1-navajo (R1-nj)* gene. In this categorization, seeds displaying both purple endosperm and embryo were deemed diploid, while seeds featuring only purple endosperm were identified as putative haploid. Seeds lacking expression of *R1-nj* were discarded, as they were considered to originate from genetic contamination or marker gene inhibition.

The putative haploid seeds from each population were sown in 64-cell polypropylene trays filled with Sphagnum Peat and placed for germination in a greenhouse on August 27, 2020, and August 27, 2021. Simultaneously, trays containing diploid F_1 individuals from each population were sown to facilitate a comparison between diploid and haploid individuals and to aid in the elimination of false haploids present in the trays.

Nine days after each sowing, the seedlings from each population were counted at the V2 stage to estimate germination percentages. False haploids (mistakenly identified as haploids due to a flaw in the seed classification process) were eliminated. These false haploids were recognized for displaying high vigor and a morphological pattern typical of diploids. In the case of supersweet populations, diploid seedlings were identified by the purple color of the first leaf sheath, a typical characteristic of the inducer that is absent in the supersweet populations used, as observed by Sekiya et al. (2020).

In the LGM-UEL, procedures for chromosomal doubling of haploid seedlings at the V2 stage were conducted 11 days after sowing. This was achieved through the injection of 100 µL of a 0.125% colchicine and 0.5% dimethyl sulfoxide (DMSO) solution per seedling, using insulin syringe (1 ml). The solution was prepared at Mutagenesis Laboratory of UEL, using all safety procedures for working with colchicine. The injection was administered in the central portion of the stem close to the basal meristematic tissue (Eder & Chalyk, 2002).

The day after treatment, the seedlings were transplanted to a drip-irrigated field at the TMG experimental station in Cambé on September 9, 2020, and September 10, 2021, without shade cover, in conventionally prepared soil, using plowing and harrowing, with broadcast fertilization (Table 1).

Table 1
Number of putative haploid seeds (PHS), number of putative haploid seeds per ear (PHSe), haploid induction rate (HIR), PHS germination rate (GR), number of seedlings treated with colchicine and transplanted in the field (STF), and number of seedlings treated with colchicine and maintained after field roguing (STM), in different populations of common and supersweet maize. 2019/20, 2020/21, and 2021/22 harvests

Population	PHS	PHSe	HIR	GR	STF	STM
	2019/20 harvest			2020/21 harvest		
SD3005	1024	6.4	5.0%	77.5%	276 (27.0%)	268 (26.2%)
SD3006	655	5.0	1.1%	88.9%	155 (23.7%)	129 (19.7%)
PP01	6867	4.7	---	90.9%	2607 (38.0%)	1787 (26.0%)
PP02	8301	9.8	---	97.6%	2335 (28.1%)	1605 (19.3%)
PS01	11260	9.3	---	97.6%	3792 (33.7%)	3563 (31.6%)
PP03	6056	4.4	---	94.6%	1931 (31.9%)	1526 (25.2%)
PS02	15067	8.5	---	96.1%	5341 (35.4%)	4601 (30.5%)
PP04	11776	7.9	---	92.2%	3814 (32.4%)	3014 (25.6%)
PS03	12068	8.1	---	90.5%	5144 (42.6%)	3852 (31.9%)
X^2 within the harvest	---	---	*	*	*	*
	2020/21 harvest			2021/22 harvest		
SD3005	959	1.8	0.9%	91.8%	335 (34.9%)	333 (34.7%)
SD3006	1752	4.1	2.2%	97.5%	293 (16.7%)	292 (16.7%)
PP05	1497	7.9	---	90.1%	926 (61.9%)	755 (50.4%)
PP06	800	8.6	---	98.4%	214 (26.8%)	172 (21.5%)
PP07	694	6.3	---	98.1%	231 (33.3%)	140 (20.2%)
PP08	409	8.1	---	96.3%	198 (48.4%)	151 (36.9%)
PP09	509	4.4	---	94.3%	217 (42.6%)	190 (37.3%)
PP10	4362	4.3	---	90.2%	1931 (44.3%)	610 (14.0%)
PP11	1939	8.1	---	87.8%	921 (47.5%)	421 (21.7%)
PP12	1503	9.8	---	98.1%	389 (25.9%)	225 (15.0%)
PP13	2616	9.4	---	93.7%	1235 (47.2%)	1111 (42.5%)
PP14	830	9.3	---	96.5%	254 (30.6%)	180 (21.7%)
PP15	3447	8.1	---	97.9%	1717 (49.8%)	686 (19.9%)
PP16	986	4.4	---	99.5%	277 (28.1%)	219 (22.2%)
PP17	586	7.9	---	98.1%	282 (48.1%)	273 (46.6%)
PP18	1582	9.9	---	98.5%	547 (34.6%)	182 (11.5%)
PP19	1805	6.2	---	95.6%	781 (43.3%)	450 (24.9%)
PP20	795	8.5	---	97.4%	175 (22.0%)	144 (18.1%)
X^2 within the harvest	---	---	*	*	*	*
Harvest mean (2020/21)		---	---	94.0%[b]	34.8%[b]	27.8%[a]
Harvest mean (2021/22)		---	---	94.7%[a]	40.3%[a]	24.1%[b]
Total/Overall mean	100145	---	---	94.2%	36318 (36,3%)	26879 (26,8%)

*: significant at a 0.05 probability level using the chi-square test (X^2) within the harvest, respectively. Harvest means values followed by common letters do not differ significantly by X^2.

During the experiment in the 2020/21 and 2021/22 harvests, accumulated rainfall of 317 mm and 580 mm was observed, with average temperatures of 24.3 and 23.2 °C. respectively. Maximum temperatures varied from 40.0 °C to 12.6 °C, and 38.6 °C to 11.9 °C for the two respective harvests.

Mechanical transplantation in double rows varied in length, depending on the number of haploid individuals treated from each population. The establishment of DH selfing fields and their management adhered to recommendations for maize cultivation, with the goal of minimizing biotic and abiotic stress.

Approximately 30 days after transplanting and prior to flowering, plants that exhibited typical diploid characteristics (false haploids) were removed. These plants were identified as taller and more vigorous, featuring a thick stem, broad and intensely green leaves, and pronounced anthocyanin expression in various plant parts.

The obtained ears lacking grains marked by the *R1-nj* gene were considered DHs, and the number of seeds per ear was recorded. Segregating ears from each donor population were counted and discarded as they originated from diploid plants not eliminated before flowering.

Throughout the experiments, counting data were collected separately for each donor population induced to haploidy, including the number of putative haploid seeds (PHS); number of putative haploid seeds per ear (PHSe); number of diploid F_1 seeds resulting from induction in supersweet maize; number of seedlings treated with colchicine and transplanted in the field (STF); number of seedlings treated with colchicine and maintained after field roguing (STM); number of D_0 plants surviving at pollination (PSP); number of D_0 plants pollinated (D_0P); number of D_0 plants that produced D_1 seeds (D_0S); and number of seeds per DH ear. All these data have been adjusted to exclude the number of plants that produced ears segregating for the *R1-nj* gene, as observed during the harvesting of the DHs.

From the counting data, the following rates were estimated: haploid induction rate (HIR): number of PHS/total number of F_1 seeds marked by the *R1-nj* gene; germination rate (GR): number of haploid seeds germinated/ number of haploid seeds sown; survival rate (SR): PSP/STM; selfing rate (SFR): D_0P/ PSP; selfing success rate (SSR): D_0S/D0P; reproduction rate (RR): D_0S/PSP; and overall success rate (OSR): D_0S/STM.

The data were analyzed using the Chi-Square test (x^2) to compare success rates, assessing populations within each harvest and mean differences between harvests. The formula utilized is as follows: $X^2=\frac{1}{\bar{p}(1-\bar{p})}\sum (p_i-\bar{p})^2 N_i$, where N_i = sum of individuals employed in treatment i; n_i = total number of individuals from successful treatment i; p_i = proportion of success for treatment i, given by the n_i/N_i ratio; $\bar{p}$ = average proportion of success considering all treatments together, given by the $\Sigma n_i/\Sigma N_i$ ratio.

Furthermore, the DH ears from each population were counted and categorized into the following DH seed class ranges per ear: less than 5 seeds, 5 to 25 seeds, 26 to 50 seeds, 51 to 100 seeds, and more than 100 seeds.

Results and Discussion

In the 2019/2020 and 2020/2021 harvests, 73074 and 27074 putative haploid seeds were obtained, respectively, identified by the expression of the *R1-nj* gene in the seeds. The haploid induction rate (HIR) for supersweet maize populations varied from 0.9% to 5.0%, exhibiting different patterns between crops (Table 1). For common maize populations, where the count of diploid F1 seeds was not conducted, approximately 4 to 10 haploid seeds were acquired per ear in both harvests. Considering an average of 250 seeds per ear, the estimated induction rates for common maize populations ranged from 1.6% to 4.0%, falling within the HIR range of supersweet populations. These findings highlight the comparatively lower average induction potential of the PI4003 population, emphasizing the need for improvement as the rates observed in PI4003 were akin to temperate inducers from the 1980s (Lashermes & Beckert, 1988) and notably lower than the rates from 8% to 20.8% achieved by improved inducers (Chaikam et al., 2018; Liu et al., 2022; Melchinger et al., 2016a; Prigge et al., 2011; Rotarenco et al., 2010; Trentin et al., 2022).

The putative haploid seeds from each population exhibited favorable germination rates, ranging from 77.5% to 97.6% in the 2020/21 harvest and from 87.8% to 99.5% in the 2021/22 harvest (Table 1). These rates align with the results presented by Prasanna et al. (2012) and Zararsiz et al. (2019), who reported ranges of 85% to 90% and 63.6% to 95.2%, respectively.

The percentage of seedlings treated and transplanted in the field (STF), relative to the putative haploid seeds sown from each population, exhibited significant variation within each harvest. Values ranged from 23.7% to 42.6% in the 2020/21 harvest and from 16.7% to 49.8% in the 2021/22 harvest. These findings indicate distinct percentages of occurrence of false haploids depending on the induced populations, warranting the elimination of more than 50% of the seedlings from each population in trays before colchicine treatment (Table 1). Previous studies Kebede et al. (2011), Yu and Birchler (2016), Sekiya et al. (2020), and Trentin et al. (2022), also reported challenges in accurately identifying haploid individuals solely through the expression of the *R1-nj* gene in seeds, with a notable presence of false haploid seeds leading to an over estimation of induction rates.

Even with the pre-elimination of diploid seedlings in trays, field roguing of diploid individuals was still necessary. Estimates of the percentages of haploid seeds treated and maintained after field roguing (STM), based on the number of putative haploid seeds sown, displayed significant differences within harvests. Values ranged from 19.3% to 31.9% in the 2020/21 harvest and from 11.5% to 50.4% in the 2021/22 harvest (Table 1). As observed by Sekiya et al. (2020), the selection of haploids based on the green color of the first leaf sheath in supersweet maize proved effective in helping to eliminate diploids, which have a purple first leaf sheath. Similarly, concerning common maize populations, pre-selection in trays based on typical diploid morphological traits (greater seedling vigor, broader leaves, and more intense color), as described by Chaikam et al. (2017), played a prominent role in reducing the need for field roguing. Small differences were observed between STF and STM estimates in

several populations, with notable instances in PS01, PS02, PP06, PP09, PP13, PP17, and PP20. The major significant difference in the 2021/22 harvest was related to a hailstorm four weeks after transplanting, causing damage to the older leaves of the seedlings. This stress likely contributed negatively to reducing the success rates in obtaining DH lines. As observed in this study, Eder and Chalyk (2002) and Melchinger et al. (2016a) achieved better efficiency in obtaining DH lines under more favorable environmental conditions, nearly twice as high as in years with unfavorable conditions.

The survival rate (SR) after treatment with the injection of colchicine solution differed between harvests and between populations within each harvest (Table 2), highlighting the importance of lower-stress environments in increasing the survival rate of populations subjected to treatment with the antimitotic agent (Chaikam et al., 2019b). The populations exhibited significantly different survival rates in each harvest, ranging from 74.3% to 95.7% in the 2020/21 harvest and from 42.9% to 91.4% in the 2021/22 harvest (Table 2). Approximately, 78% of populations in the 2020/21 harvest and 61% of populations in the 2021/22 harvest had survival rates greater than or equal to 80%, aligning with the best results reported in the literature, which vary based on donor population, environmental conditions, and chromosome doubling methods. Values range between 47.2% and 92.6% for seedling chromosome dipping in a colchicine solution (Chaikam et al., 2020; Dang et al., 2012; Eder & Chalyk, 2002; Melchinger et al., 2016b; Zararsiz et al., 2019); 40.3% to 95.2% for root dipping in a colchicine solution (Chaikam et al., 2020); and 88.6% in the field and 90.8% in the greenhouse for injection with a colchicine solution (Eder & Chalyk, 2002).

Selfing rate (SFR) varied between populations in the harvests, ranging from 17.1% to 46.1% in the 2020/21 harvest and from 8.3% to 45.5% in the 2021/22 harvest, both under field conditions without using a shade cloth cover. The average selfing rate in the 2020/21 harvest was comparable to that observed by Eder and Chalyk (2002) in a greenhouse, a more favorable environment than the field. Even in the 2021/22 harvest, which resulted in a lower overall mean, the average SFR rate was almost double that observed by Eder and Chalyk (2002) in the field.

Selfing success rate (SSR) in the 2020/21 harvest ranged from 52.8% to 80.0%, but no significant difference between populations was detected in the 2021/22 harvest, averaging 46.8%.

Table 2
Number of D_0 plants surviving at pollination (PSP), survival rate (SR), number of D_0 plants pollinated (D_0P), selfing rate (SFR), number of D_0 plants that produced D_1 seed (D_0S), selfing success rate (SSR), reproduction rate (RR), and overall success rate (OSR), in different populations of common and supersweet maize. 2020/21 and 2021/22 harvests

Population	PSP	SR	D_0P	SFR	D_0S	SSR	RR	OSR
2020/21 harvest								
SD3005	201	75.0%	75	37.3%	50	66.7%	24.9%	18.7%
SD3006	115	89.1%	53	46.1%	36	67.9%	31.3%	27.9%
PP01	1664	93.1%	560	33.7%	385	68.8%	23.1%	21.5%
PP02	1536	95.7%	545	35.5%	310	56.9%	20.2%	19.3%
PS01	2646	74.3%	875	33.1%	462	52.8%	17.5%	13.0%
PP03	1373	90.0%	439	32.0%	351	80.0%	25.6%	23.0%
PS02	4116	89.5%	1025	24.9%	722	70.4%	17.5%	15.7%
PP04	2803	93.0%	1285	45.8%	1024	79.7%	36.5%	34.0%
PS03	3408	88.5%	582	17.1%	383	65.8%	11.2%	9.9%
X^2 within the harvest	---	*	---	*	---	*	*	*
Harvest average		87.8%[a]		30.5%[a]		68.5%[a]	20.8%[a]	18.3%[a]
2021/22 harvest								
SD3005	143	42.9%	56	39.2%	34	60.7%	23.8%	10.2%
SD3006	235	80.5%	90	38.3%	38	42.2%	16.2%	13.0%
PP05	649	86.0%	172	26.5%	86	50.0%	13.3%	11.4%
PP06	144	83.7%	56	38.9%	28	50.0%	19.4%	16.3%
PP07	110	78.6%	50	45.5%	28	56.0%	25.5%	20.0%
PP08	138	91.4%	45	32.6%	21	46.7%	15.2%	13.9%
PP09	164	86.3%	55	33.5%	17	30.9%	10.4%	8.9%
PP10	363	59.5%	57	15.7%	28	49.1%	7.7%	4.6%
PP11	329	78.1%	44	13.4%	21	47.7%	6.4%	5.0%
PP12	187	83.1%	46	24.6%	23	50.0%	12.3%	10.2%
PP13	931	83.8%	77	8.3%	34	44.2%	3.7%	3.1%
PP14	156	86.7%	31	19.9%	15	48.4%	9.6%	8.3%
PP15	438	63.8%	99	22.6%	48	48.5%	11.0%	7.0%
PP16	182	83.1%	71	39.0%	27	38.0%	14.8%	12.3%
PP17	231	84.6%	50	21.6%	28	56.0%	12.1%	10.3%
PP18	104	57.1%	30	28.8%	15	50.0%	14.4%	8.2%
PP19	356	79.1%	37	10.4%	13	35.1%	3.7%	2.9%
PP20	128	88.9%	49	38.3%	18	36.7%	14.1%	12.5%
X^2 within the harvest	---	*	---	*	---	ns	*	*
Harvest average		76.3%[b]		22.4%[b]		46.8%[b]	10.5%[b]	8.0%[b]
Total/Overall average	22850	85.0%	6554	28.7%	4245	64.8%	18.6%	15.8%

ns, *: not significant and significant at a 0.05 probability level using the chi-square test (X^2), respectively; harvest mean values followed by common letters did not differ significantly by X^2.

Reproduction rate (RR) differed between the assessed populations within each harvest. Estimates ranged from 11.2% to 36.5% in the 2020/21 harvest and from 3.7% to 25.5% in the 2021/22 harvest (Table 2). Likewise, the overall success rate (OSR) displayed differences across populations, spanning from 9.9% to 34.0% in the 2020/21 harvest and from 2.9% to 20.0% in the 2021/22 harvest (Table 2). These success rates for obtaining DH lines varied based on donor populations and harvests, averaging nearly double in the 2020/21 harvest compared to the subsequent harvest (Table 2). In a study by Melchinger et al. (2016b), reproduction rates ranged between 15.9% and 29.2%, with overall success rates between 10.0% and 22.1%, employing seedling dipping in a 0.06% colchicine solution. Chaikam et al. (2020) utilized the same method with different concentrations and exposure times across diverse populations, reporting reproduction rates between 3.1% and 11.7%, and overall success rates ranging from 2.7% to 10.5%. The root dipping method, extending up to the crown region, yielded reproduction rates of 11.42% to 30.3% and an overall success rate of 10.3% to 11.9%. However, in an experiment optimizing colchicine concentrations for the root dipping method, Chaikam et al. (2020) observed reproduction rates between 24.9% and 61.5%, with overall success rates ranging from 23.5% to 58.5%. These results reported in the literature for reproduction rates and overall success rates also demonstrated variation influenced by donor population, environmental conditions, colchicine exposure method, colchicine concentration, and exposure time to the antimitotic agent.

The quantities of colchicine solution required for treating 1000 individuals may vary among different breeding programs. Based on previous experiences at the LGM-UEL, a minimum of 100 mL is needed for direct injection into the basal meristematic tissue (132 mg of colchicine with a minimum of 95% purity, at a concentration of 0.125% colchicine). For seedling dipping, 1750 mL is required (737 mg of colchicine with a minimum of 95% purity, at a concentration of 0.04% colchicine), and for root dipping, 3750 mL is necessary (2763 mg of colchicine with a minimum of 95% purity, at a concentration of 0.07% colchicine). Therefore, in comparison to the injection method, approximately 6 and 21 times more colchicine is needed for seedling and root dipping methods, respectively. In addition to lower colchicine consumption, the injection method offers advantages such as requiring fewer steps, being a swift process with trained staff, and not generating toxic waste for disposal. However, this method has the drawback that application success is influenced by individual skills and demands a greater number of trained personnel to handle colchicine, potentially increasing contamination risks.

The frequencies of classes of DH seed quantities per ear obtained per tropical donor population using the colchicine injection method varied depending on the donor population, although they were similar in the mean for both harvests (Figure 1). The results align with the means reported by Chaikam et al. (2020) for root dipping in colchicine solution (38.9% of ears with less than five seeds, 36% in the range of 5 to 25 seeds, and 28% with more than 25 seeds). However, they surpassed the means obtained by these authors for seedling dipping in colchicine solution (56.8% of ears with less than 5 seeds, 44.3% in the range of 5 to 25 seeds, and 12.5% with more than 25 seeds).

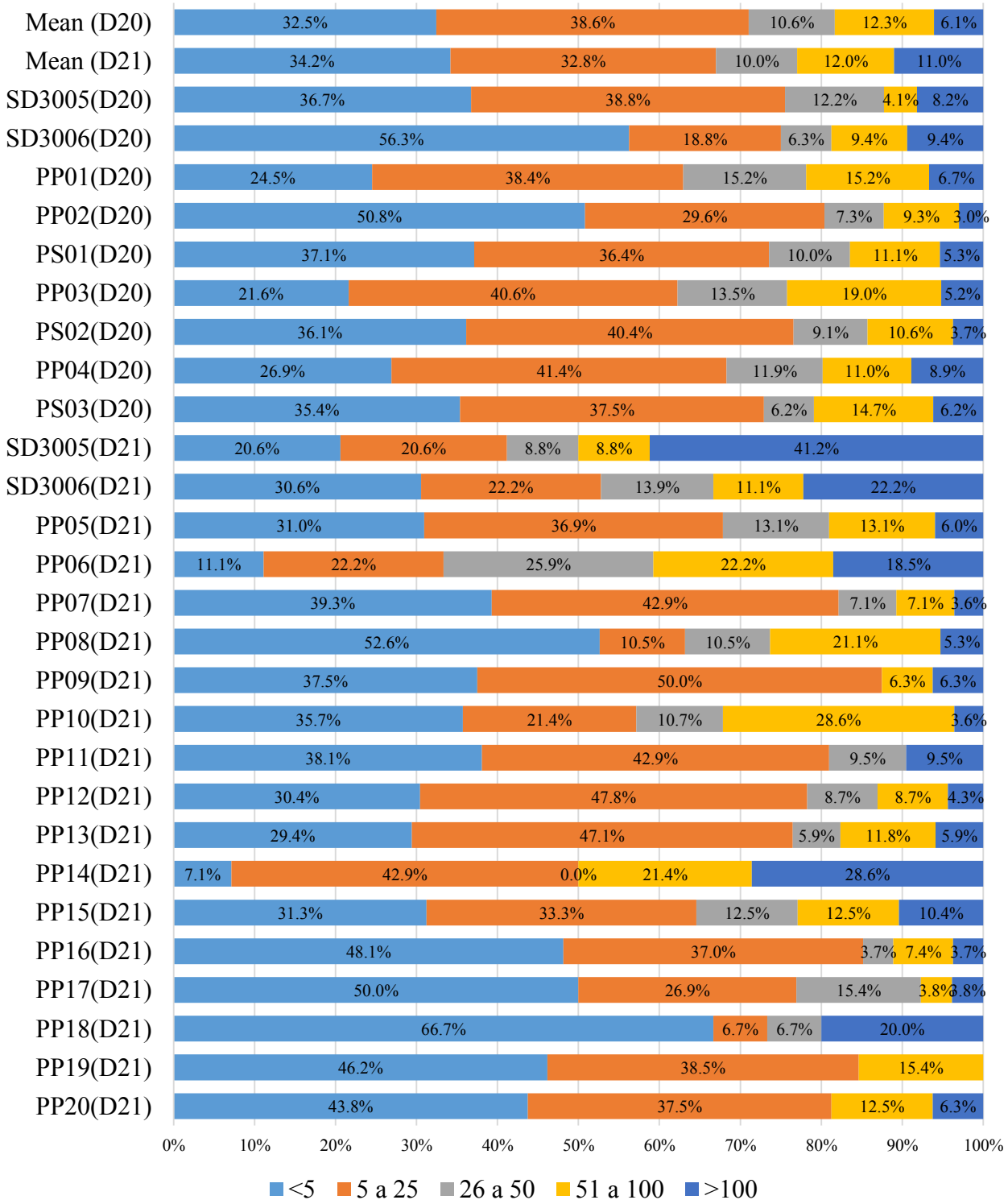

Figure 1. Percentages of double haploid ears obtained from different populations of supersweet and common maize in the 2020/21 (D20) and 2021/22 (D21) harvests, based on different ranges of seed numbers per ear, obtained by chromosomal doubling by colchicine solution injection into seedlings at the V2 stage.

Conclusions

The results obtained in this work reveal that the chromosome doubling method through the injection of colchicine solution into haploids from tropical maize populations, in a tropical environment, exhibits comparable efficacy to the best chromosome doubling methods presented in the literature. The PI4003 inducer demonstrates low rates of haploidy induction and high rates of false haploids in populations of common and tropical supersweet maize, indicating the need for improvement.

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