ORIGINAL ARTICLE

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Recurrent haploid selection in a population of sweet corn (*Zea mays* convar. saccharata)

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Abstract

Recurrent haploid selection is based on the phenotype of haploid plants. This selection method was reported to be very effective because of the high selection pressure. The aim of this study was to quantify the effect of five generations of recurrent haploid selection on a sweet corn population developed by intercrossing 40 hybrid cultivars. We compared the initial population, five cycles of recurrent haploid selection, and mass selection after five cycles in six environments. The results indicated that recurrent haploid selection had no positive effect on yield or quality. For the traits tillering, tip fill, total yield and total number of ears, significant changes from cycle to cycle were observed but in differing directions. The performance per se of single haploid plants has probably a low correlation to their combining ability in a population. Positive mass selection of the best ears resulted in no improvement of total yield, a reduction of marketable yield and about 2-day delay of flowering. In conclusion, recurrent haploid selection based on the phenotype of individual plants was not an efficient method to improve the sweet corn population investigated.

KEYWORDS
sweet corn, maize, breeding, recurrent haploid selection, mass selection, organic population improvement

1 | INTRODUCTION

Chalyk and Rotarenco (1999) proposed a method for recurrent population improvement called 'haploid recurrent selection'. The principal idea is to increase selection pressure by selecting the phenotype of haploid plants. Haploid plants are less vigorous than diploid plants and much more sensitive to biotic and abiotic stress. Haploid selection enables selection for recessive deleterious and lethal alleles and for traits expressed equally in the haploid phenotype and its diploid analogue (Chalyk et al., 2002).

Though maternal haploids in maize that are induced by pollination by an inducer genotype (Röber et al., 2005) are frequently male sterile, they do have some female flowers that are fertile (Chalyk, 1994; Geiger et al., 2006). These haploid plants can be selected and pollinated by a diploid plant, and the resulting diploid kernels can be used for population improvement. The selection procedure is explained in Figure 1.

Genetic load is mostly caused by deleterious, recessive alleles which generally need several cycles of inbreeding to be eliminated. Such genetic load reduces the vigour of lines and populations and plays an important role in causing inbreeding depression (Charlesworth & Charlesworth, 1987). Recurrent haploid selection is a quick and efficient way to select against genetic load. This is because alleles expressed in haploids are more exposed to natural selection
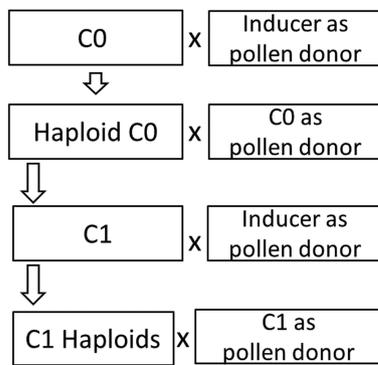
FIGURE 1 Recurrent haploid selection. One cycle needs two seasons.Plants deriving from the initial population C0 are induced in the winter nursery to produce haploid kernels. Superior plants are selected at the haploid stage and pollinated by C0. The best ears are selected as the result of the first cycle of selection (C1).

than in doubled haploid plants, due of the reduced vigour of haploids. Such deleterious alleles will not be masked by dominance. By applying recurrent haploid selection for more vigorous phenotypes, recessive deleterious alleles will be eliminated, and beneficial alleles will be favoured, even if these alleles are recessive.

Three and four cycles of recurrent haploid selection were applied to two synthetic maize populations in two different maturity groups (Chalyk & Rotarenco, 2001; Rotarenco et al., 2012). Selection caused positive increases in ear size (7%), ear diameter (5%), number of rows (6%) and weight per plant (16–21%) per cycle of selection (Chalyk & Rotarenco, 2001). To date, no other studies have evaluated the effect of this selection method.

This study investigated the effect of five cycles of recurrent haploid selection in organic management on a sweet corn population. The performance after each cycle of selection was compared to (i) the initial population and (ii) a population after five cycles of positive mass selection. The focus of selection was on plant vigour, ear quality traits and yield and fertility traits. Multiple trials were carried out under organic and conventional management.

2 | MATERIAL AND METHODS

2.1 | Plant material and selection

The initial population was developed from 40 yellow 'extra sweet' sweet corn hybrid cultivars carrying the *sh2* allele (Kramer et al., 2014). They were mixed and open pollinated in 2004 and subsequently selected with negative mass selection for six cycles from 2005 to 2011. The population selected in 2011 is called C0 in this experiment. Five cycles of recurrent haploid selection and of positive mass selection, respectively, were applied during the years 2012 to 2016. The resulting populations selected by recurrent haploid selection are called C1, C2, C3, C4 and C5; the population selected during the same period by five generations of positive mass selection is

called M5. All cycles were selected in fields near Rheinau, Switzerland (Table S1). Plants were open pollinated without pollen control.

For each cycle of mass selection, about 0.5 ha was sown, spaced 0.2 m × 0.75 m, equalling approximately 33,000 plants. The plants were selected in several steps: Natural selection eliminated seedlings with low vigour and plants that were not tolerant to the low temperatures that are common in the spring. Selection at flowering time entailed removing the tassels of extremely early female flowering plants prior to pollen maturity to restrict flowering to a limited period. Extremely late silking plants were marked and not selected. One-thousand mature ears of healthy and vigorous plants were selected. The ears were husked and 100 ears were selected for absence of *Fusarium*, ear size between 18 and 23 cm, ear diameter ∼5 cm, a cylindrical shape and row numbers >12. Only straight ears with good tip fill were selected. The seeds of these 100 ears are representing 0.3% of the initial population. Kernels were mixed for the next cycle of selection.

The recurrent haploid selection procedure we utilized is explained in Figure 1 and was described by Rotarenco et al. (2012). Haploid seeds were produced near Rancagua, Chile (34°04′06.7″S, 70°43′48.7″W) every year from winter 2012/2013 to 2016/2017 by pollinating 300 plants of the population with the Inducer. In the first year the RWS-inducer (Röber et al., 2005) was used. Haploid kernels were visually selected (Röber et al., 2005) and subsequently planted in pressed earth pots. By doing so, misclassified seedlings could be eliminated before transplanting. Spacing in the field was the same as for mass selection. An overview of selection criteria used in mass selection and recurrent haploid selection is given in Table 1. The haploid plants were selected in the field according to the following criteria: healthy vigorous plants with healthy ears. In the last cycle of selection, ears with row numbers >14 were selected. Generally, haploid plants are of low vigour with small and insufficiently pollinated ears, due to defective meiosis in haploids. Thus, it was impossible to select for the following traits: colour, angle of the ear to the stalk, full husk cover, ear shape, ears with good tip fill and without bird damage. Selecting plants having the ability to form anthers and even anthers shedding pollen was interpreted as an indicator for selecting plants with superior fitness. Haploid plants were open pollinated by diploid plants of the latest selection cycle. Pollination was improved on wind still days by collecting pollen of the population and applying the pollen mix on the haploids during the flowering period.

Table 2 indicates the selection intensity per cycle and method. In recurrent haploid selection, the proportion of selected plants varied from 3.4% to 19.4% and the number of selected plants varied from 25 to 180. These differences were a result of the varying number of kernels on ears of haploid plants. Thus, different numbers of plants were selected to get enough kernels for the next cycle. Most haploid ears had less than 20 seeds on the ear which means that different numbers of plants were selected to get enough kernels for the next cycle and that only ears with better fertility were selected. In C1, all selected haploids were completely male sterile. In C4 and C5, on average, about half of the haploids were shedding small amounts of pollen. Kernels of selected plants were bulked, and 300 kernels were used for

TABLE 1 Traits selected in mass selection and recurrent haploid selection

	Trait 2017–2018	Recurrent haploid selection	Positive mass selection
Plant development	**Total number of plants**	**Selection for survival (natural selection)**	**Selection for survival (natural selection)**
	Early vigour	**Selection of vigorous, healthy plants**	**Selection of vigorous, healthy plants**
	Tillering	No	No
	Flowering time	No	Yes
Ear quality	**Average diameter**	**Yes, ear diameter**	**Yes, ear diameter of ∼5 cm**
	Average length	**Yes, ear length**	**Yes, ear length of 18–20 cm**
	Colour	No	No
	Ear shape	No	Yes
	Tip fill	No	Yes
	Row number	Only in selection of C4 haploids selection of row numbers >14	Yes, row numbers ≥ 12
Yield	**Yield**	**Yes, by selecting the best ears**	**Yes, by selecting the best ears**
	Number of ears	**Yes, by selecting the best ears**	**Yes, by selecting the best ears**
Fertility	Fertility at the haploid state	Yes	No

Note: Traits selected by both methods are highlighted in bold.

TABLE 2 Selection intensity and numbers of selected plants in each cycle of recurrent haploid selection and positive mass selection

Cycle	C1	C2	C3	C4	C5
Number of haploid plants	1,883	740	865	468	500
Number of haploid plants selected	180	25	88	91	71
Percentage of selected haploids	9.5%	3.4%	10.1%	19.4%	14.2%
Number of selected ears by mass selection	100	100	100	100	100
Percentage of selected ears in mass selection	0.3% in each cycle				

the new haploid induction and 500 kernels for the recombination of the population in the winter nursery. The recombined population was used as pollinator in the next cycle (Figure 1).

2.2 | Performance tests 2017 and 2018

To produce seeds of homogenous quality for the comparison of selection methods, seeds were produced in the winter nursery 2016/2017. In the winter nursery 100 randomly selected seeds, per population, were sown to reproduce the cycles C0-C5 and M5 for the comparison.

During female flowering, pollen was collected every day from the entire population, mixed and applied on every female flower of the population, to assure maximum crosspollination. Seeds from the resulting ears were mixed in equal quantities. Seeds from the resulting ears were mixed in equal quantities. Trials were established in two environments 2017 and four environments 2018 in a randomized complete block design with eight replications. Details of the trials are given in Table S1.

2.2.1 | Plant vigour, ear quality and yield traits

Seedlings were thinned out after plants had three leaves. Two visual scorings per plot were carried out before flowering. The first scoring was done when the plants reached BBCH 18 (Lancashire et al., 1991), and the second scoring was done at BBCH 32. The average vigour per plot was scored on a scale of 1–9 (1 = lowest vigour, 9 = highest vigour) based on biomass, plant height, leaf number and leaf width.

Tillering is an undesired trait that can complicate the harvest. Scoring per plot was carried out when the mean height of the plants was about 70 cm (BBCH 34) with 1 = no tillering and 9 = maximum number and size of tillers.

Flowering day was recorded when 50% of the plants of a plot showed silks. Flowering day 1 was the date when the first plot in the trial was flowering. A plot flowering one day later was recorded as flowering day 2, and so forth. Harvesting took place at flowering day + 32 days in Göttingen 2017 and flowering day + 31 days in Göttingen 2018 in both management systems and at +33 days in Rheinau and Kleinhohenheim.

To take into account different climatic conditions during ripening, temperature was registered to calculate the accumulated temperature between beginning of flowering and harvest. The accumulated temperature was used to correct yield traits with a linear least square fitting of a straight line with slope and intercept obtained by a linear regression between the accumulated temperature and the measured traits:

$$\text{Regression of the uncorrected data}: y_1 = \widehat{a} + b\widehat{\ }x,$$

$$\text{Corrected data}: \widehat{y}_2 = y_1 - b\widehat{\ }x,$$

in which y_1 is the yield trait measurement (total yield, marketable yield, total number of ears and number of marketable ears); $b\widehat{\ }$ is the estimate of the slope; x is the accumulated temperature; $\widehat{a}$ is the estimate of the intercept and $\widehat{y}_2$ is the corrected yield trait estimate.

The biggest ear on every plant was harvested. Immature ears (silk not browning) were discarded. The number of plants per plot was counted at harvest. Ears were husked, weighed and visually classified into marketable ears and non-marketable ears. Ears shorter than 12 cm or longer than 23 cm were counted as non-marketable. Ears with odd shapes, with pollination less than 50%, and with unripe kernels were discarded. Marketable yield is the sum of all requirements, of the sweet corn market chain, to sell the ears and contains several traits, usually selected for, in variety improvement. These criteria include equally a maximum ear length, which is limited by the packaging sizes of the processed ears. Only the marketable ears were used to score quality traits. Tip fill was scored 1 (*tips without seeds*) to 9 (*fully filled*). Ratings under 5 were sorted out as non-marketable ears. Colour was scored 1 = *least intense in colour* (whitish) and 9 = *most intense colour*. Ear form was scored 1 = *ears with a pointed shape* to 9 = *cylindrical ears*. Five marketable ears were chosen randomly to count the number of kernel rows at the centre of the ear.

2.2.2 | Statistical analysis

Data analysis was done with R (R Core Team, 2018), figures were generated with the packages ggplot2 and ggrepel and variance components were estimated with the VCA-Package (Schuetzenmeister & Dufey, 2019). The package agricolae (de Mendiburu, 2009) was used for Tukey tests. Outliers were identified by calculating Cook distances in every environment for each trait. Highly influential data points with Cook's distances larger than four times the mean of the Cook's Distance for the selected dataset were removed. Heritability was calculated based on the variance components of the VCA-Package on the six trials with the following formula. Negative variance components were set to 0:

$$h^2 = \frac{VC_{gen}}{\left(VC_{gen} + \frac{VC_{gen*loc}}{l} + \frac{VC_{error}}{l*r}\right)}$$

VC_{gen} is the variance component estimate for the genotypes (C1–C5, M5); $VC_{gen*loc}$ is the variance component estimate for the interaction of the genotypes and locations and VC_{error} is the variance component estimated for the error, l is the number of locations and r is the number of replications.

For the analyses of variance, the following models were used: Experiments in six locations:

$$Y_{ijk} = \mu + g_i + l_j + b_{kj} + gl_{ij} + e_{ijk}$$

Including effect of years and cultivation method:

$$Y_{ijkl} = \mu + g_i + y_j + c_k + b_{jkl} + gy_{ij} + gc_{ik} + yc_{jk} + gyc_{ijk} + e_{ijkl}$$

in which Y_{ijk} or Y_{ijkl} are the observations of a plot, μ is the general mean, with the effects g_i for genotype i, l_j for location j, y_j for year j, c_k for cultivation method k, b_{kj} for block k within location j, b_{jkl} for block l within year j and cultivation method k, and with the respective interactions, for example gl_{ij} for the interaction between genotype i and location j and the error terms e_{ijk} or e_{ijkl}. The results of the analyses of variance are presented as variance components, because they are required to estimate heritability.

3 | RESULTS

3.1 | Performance of five cycles of recurrent haploid selection

3.1.1 | Analysis of variance for plant development, ear quality and yield

The results of the analysis of variance are presented in Tables 3–5. The location had a significant effect on all traits but tip fill. A significant interaction of genotype and location was observed for all selected traits of plant vigour and ear quality with the exception of ear length (Tables 3 and 4). For yield traits, however, very little interaction was observed (Table 5). All selected traits but the first early scoring were significantly influenced by the effect of the genotype. No clear effect of selection was found for non-selected traits and genetic changes occurred only in some traits.

Heritability ≥ 0.68 was measured for most of the plant development traits (Table 3). Both early scorings had a low heritability.

The non-selected or partially selected ear quality traits (ear shape, tip fill, colour and row number) were not significantly affected by the genotype (Table 4). The average row number was only selected in one generation and showed very low heritability. Tip fill could be selected more efficiently because of a high heritability. All selected quality traits, ear length and ear diameter, had a heritability ≥ 0.68.

Heritability for yield traits (Table 5) was medium to high (0.64–0.84).

TABLE 3 Variance components, heritability and significance for plant development traits after five cycles of recurrent haploid selection

Plant development	DF	Not/partially selected		Selected		
		Tillering	Flowering time	First vigour scoring	Second vigour scoring	Total number of plants
G	6	0.03*	0.45***	0.00	0.01**	0.97***
L	5	0.47***	8.72***	0.38***	0.02***	101.96***
L:B	42	0.04***	0.34***	0.39***	0.20***	1.50***
GxL	30	0.00	0.18***	0.06***	0.08***	1.70***
Error	252[a]	0.40	2.08	0.94	0.79	8.44
Heritability		0.77	0.86	0.06	0.20	0.68

Abbreviations: G, genotype; L, location; L:B, block nested in location; GxL, interaction of location and genotype; DF, degrees of freedom.

[a]The degrees of freedom are partly different in each trait, due to missing values.

*Significant at $p = .05$.

**Significant at $p = .01$.

***Significant at $p = .001$, F test of the appropriate mean squares.

TABLE 4 Variance components, heritability and significance for ear quality traits after five cycles of recurrent haploid selection

Ear quality	DF	Not/partially selected				selected	
		Colour	Ear shape	Tip fill	Row number	Ear diameter	Ear length
G	6	0	0	0.055	0.002	0.002***	0.094***
L	5	0.62***	0.32***	0.374	0.088***	0.092***	0.391***
L:B	42	0.06***	0.02**	0.003	0.025***	0.000	0.000
GxL	30	0.06***	0.02	0.018	0.000	0.002***	0.000
Error	252[a]	0.45	0.70	0.342	0.903	0.035	1.586
Heritability		0[b]	0[b]	0.84	0.09	0.68	0.74

Abbreviations: G, genotype; L, location; L:B, block nested in location; GxL, interaction of location and genotype; DF, degrees of freedom.

[a]The degrees of freedom are partly different in each trait, due to missing values.

[b]The estimate for heritability was set to zero for colour and ear shape, because of a negative estimate for the genotype variance component.

*Significant at $p = .05$.

**Significant at $p = .01$.

***Significant at $p = .001$.

TABLE 5 Variance components, heritability and significance for yield traits in five cycles of recurrent haploid selection

Yield	DF	Selected					
		Total yield	Number of ears	Number of marketable ears	% marketable ears	Marketable yield	% marketable yield
G	6	0.03*	1.43***	0.033***	0.001***	0.03***	0.001***
L	5	2.58***	71.75***	0.365***	0.007***	0.37***	0.005***
L:B	42	0.20***	1.30**	0.122***	0.001	0.12***	0.002***
GxL	30	0.00	0.00	0.000	0.001	0.00	0.001*
Error	252[a]	0.67	11.00	0.499	0.017	0.50	0.013
Heritability		0.64	0.86	0.76	0.70	0.76	0.69

Abbreviations: G, genotype; L, location; L:B, block nested in location; GxL, interaction of location and genotype; DF, degrees of freedom.

[a]The degrees of freedom are partly different in each trait, due to missing values.

*Significant at $p = .05$.

**Significant at $p = .01$.

***Significant at $p = .001$.

3.1.2 | Performance of plant development, quality and yield

The means of selected traits are shown in Figures 2–10. Non-selected traits are presented in Table 6.

Plant development traits
The number of plants is a selected trait. Mass selection had significantly fewer plants than the other entries, indicating a decreasing performance through mass selection. Recurrent haploid selection did not have a clear linear effect on the number of plants (Figure 2).

Plant vitality was a selected trait and evaluated by two scorings of plant vigour (Figures 3 and 4). Scores varied only from 6.1 to 6.5, and no significant differences were observed.

In tillering, a non-selected trait, C0 and C1 differed significantly from C4 (Table 6). A slight tendency of increased tillering over the generations of recurrent haploid selection could be observed.
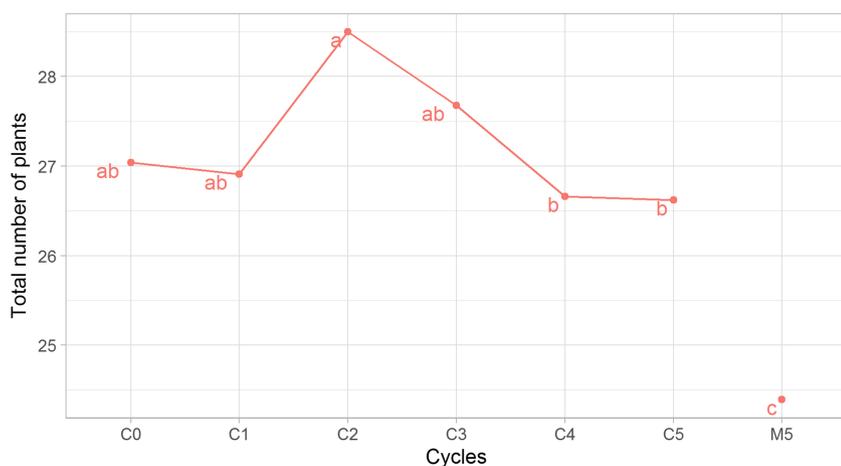
FIGURE 2 Total number of plants in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]
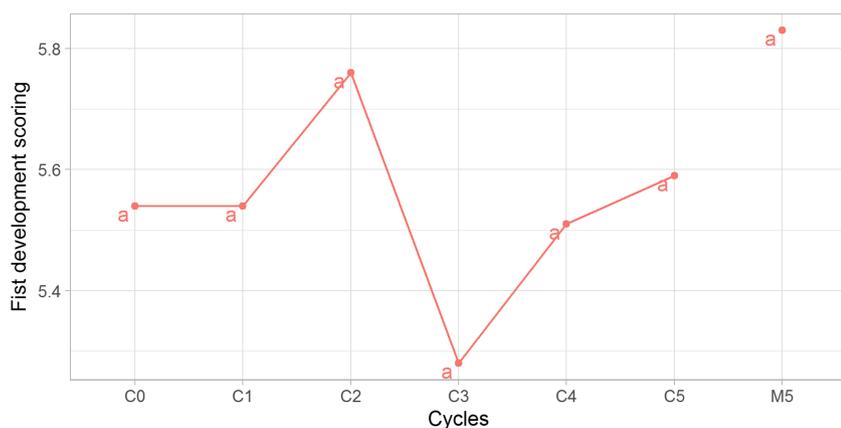

FIGURE 3 First (development) vigour scoring in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]
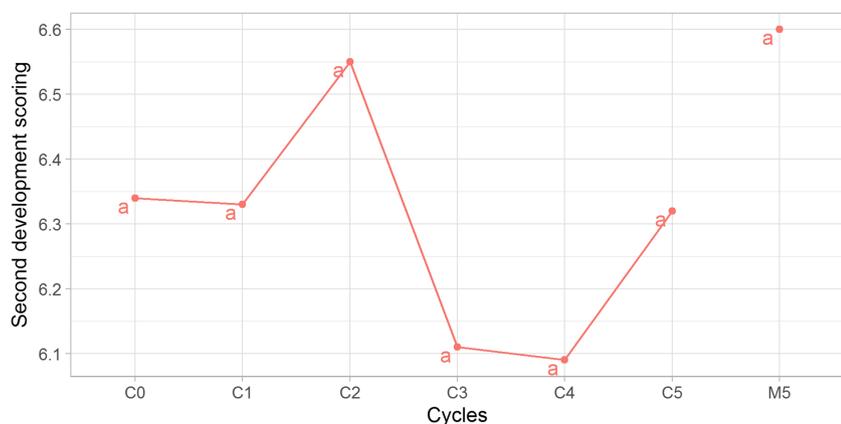

FIGURE 4 Second (development) vigour scoring in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 5 Average ear diameter in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary. com]
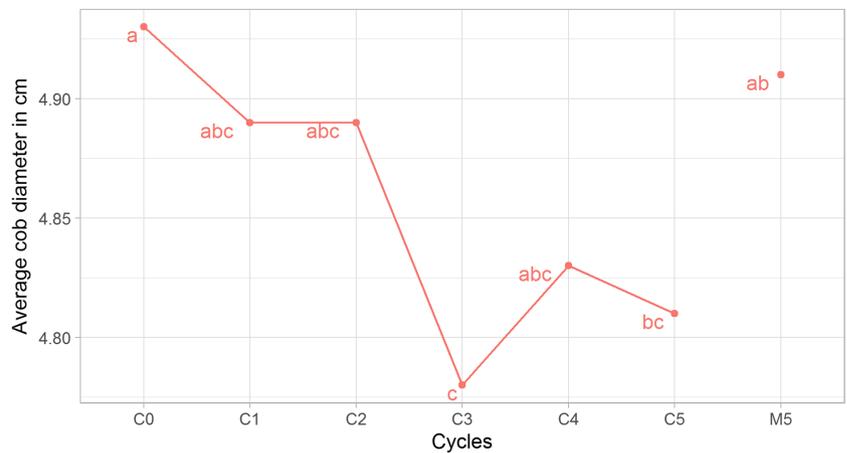
FIGURE 6 Average ear length in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary. com]
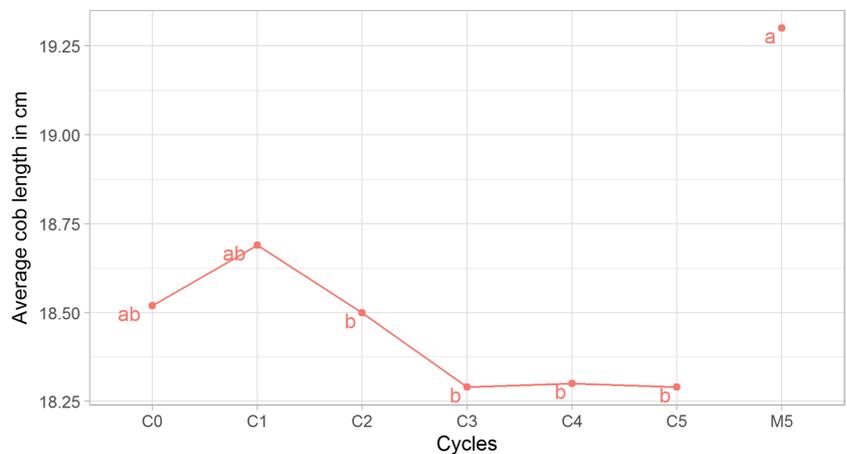
FIGURE 7 Total yield per plot in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary. com]
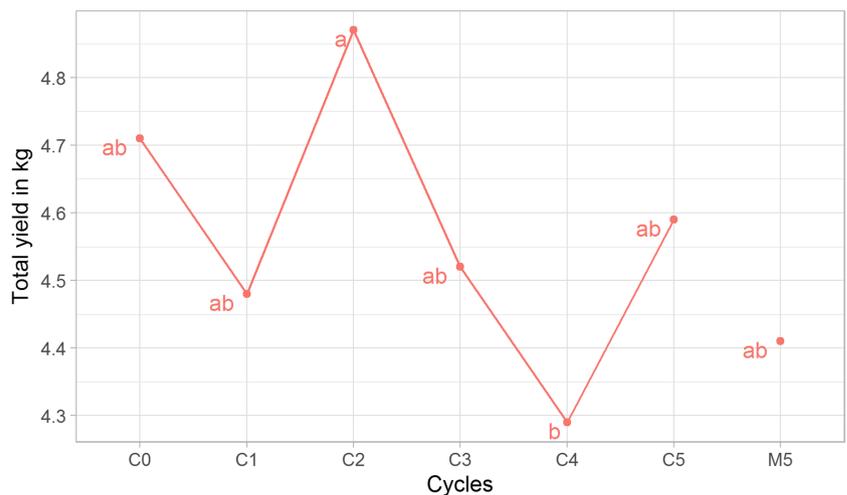
Flowering occurred 1.4 to 2.2 days later in M5 compared to the initial population and recurrent haploid selection (Table 6). Mass selection had delayed flowering time significantly.

Quality traits

Ear diameter significantly changed from C0 to C3 and C5, although absolute differences amounted to only 0.15 cm. Recurrent haploid selection reduced ear diameter slightly, while mass selection did not change it (Figure 5).

Ear length decreased in tendency from C0 to C5. The longest ears were obtained in M5, significantly longer than C2 to C5 (Figure 6).

Tip fill was selected in mass selection but could not be selected in recurrent haploid selection. Data analysis did not show a linear change of tip fill over the cycles of recurrent haploid selection (Table 6). A
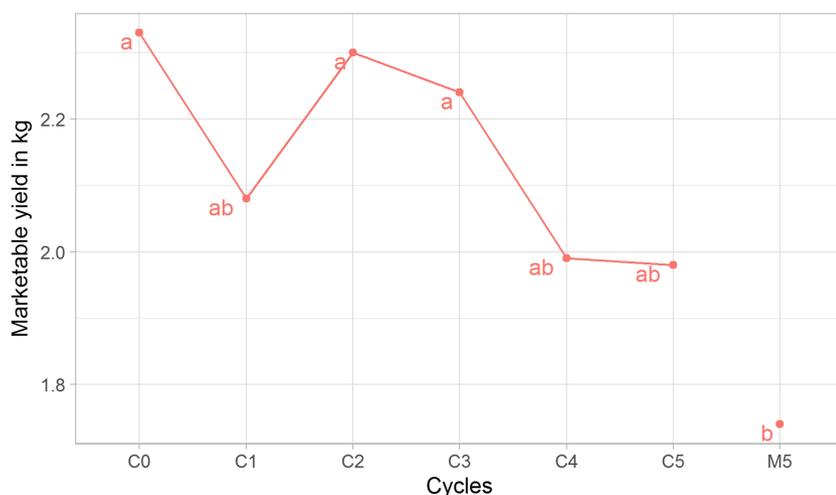
FIGURE 8 Marketable yield per plot in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]
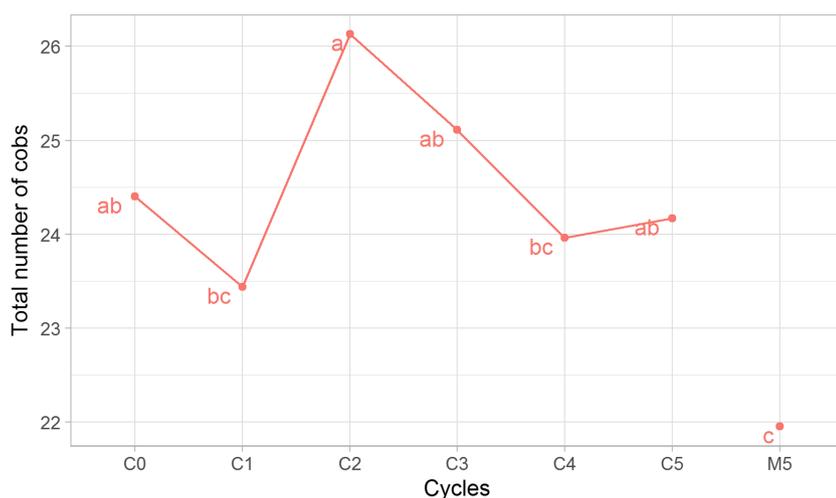
FIGURE 9 Total number of ears per plot in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]
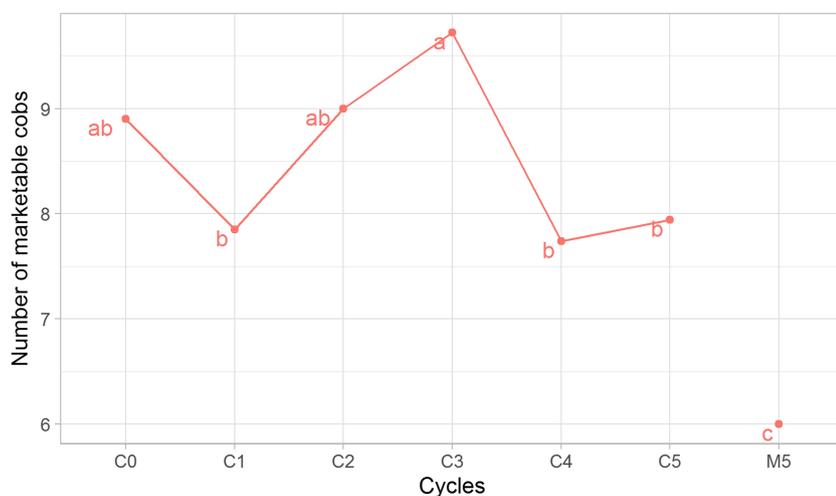

FIGURE 10 Number of marketable ears per plot in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018. Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]

significant increase from C1 to C2–C4 was observed but maximum differences did not exceed .7 score units.

No significant differences were observed in colour, ear shape and row number (Table 6).

Yield traits

Significant differences in total yield were only observed between C2 (highest yield) and C4 (lowest yield). No trend was observed for recurrent haploid selection (Figure 7). Marketable yield slightly decreased

during recurrent haploid selection, without being statistically significant (Figure 8). Significant differences were found mostly related to M5, which was lower than C0, C2 and C3 for marketable yield.

The results for the total number of ears (Figure 9) and marketable ears (Figure 10) were similar to the development of total yield. No directional linear effect of selection could be observed over five cycles of recurrent haploid selection and no improvement of the number of ears could be achieved. Mass selection decreased total number of ears and marketable ears significantly.

3.2 | Effect of the management system (organic vs. conventional)

The sweet corn population used in this study had been selected in organic management. The potential adaptation to organic management was investigated by comparing populations in paired organic–conventional trials at Göttingen for 2 years. These data are a subset of the data used in the preceding subchapter.

TABLE 6 Performance of the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in six environments 2017–2018 for non or partially selected traits

	Flowering day selected in mass selection)		Tillering (not selected)		Ear colour (not selected)		Ear shape (selected in mass selection)		Tip fill (selected in mass selection)		Row number (partially selected)	
C0	7.60	b	1.73	b	6.88	a	6.79	a	7.55	bc	16.30	a
C1	7.52	b	1.68	b	6.88	a	6.60	a	7.43	c	16.12	a
C2	7.35	b	1.85	ab	6.96	a	6.81	a	7.98	a	16.14	a
C3	7.65	b	2.03	ab	6.98	a	6.64	a	8.06	a	16.44	a
C4	8.11	b	2.18	a	7.06	a	6.79	a	8.08	a	16.22	a
C5	7.64	b	1.93	ab	6.83	a	6.57	a	7.73	abc	16.46	a
M5	9.47	a	2.05	ab	6.91	a	6.86	a	7.89	ab	16.36	a

Note: In columns, means followed by a common letter are not significantly different at $p = .05$ in Tukey's test.

FIGURE 11 Total yield per plot in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in two management systems (2017–2018). Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]
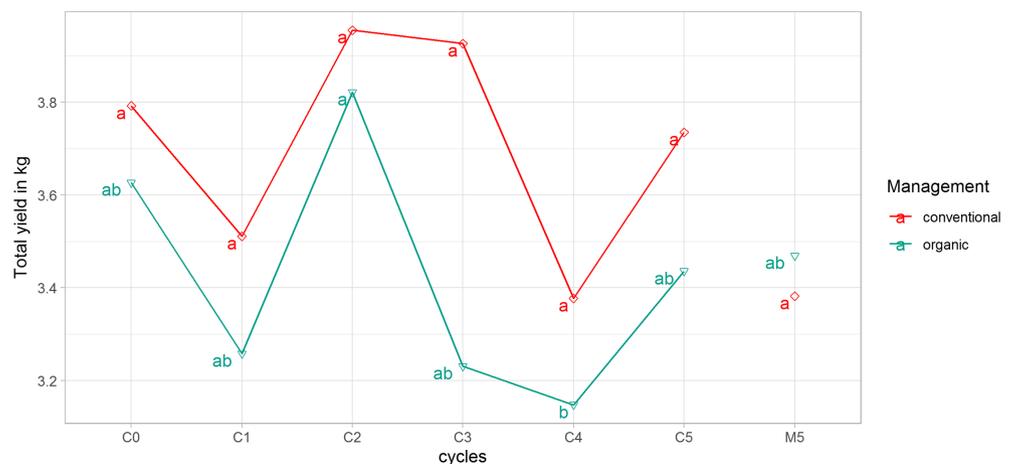

FIGURE 12 Number of marketable ears per plot in the initial population (C0), five cycles of recurrent haploid selection (C1–C5) and cycle five of positive mass selection (M5) in 2 management systems (2017–2018). Means followed by a common letter are not significantly different at $p = .05$ in Tukey's test. [Color figure can be viewed at wileyonlinelibrary.com]
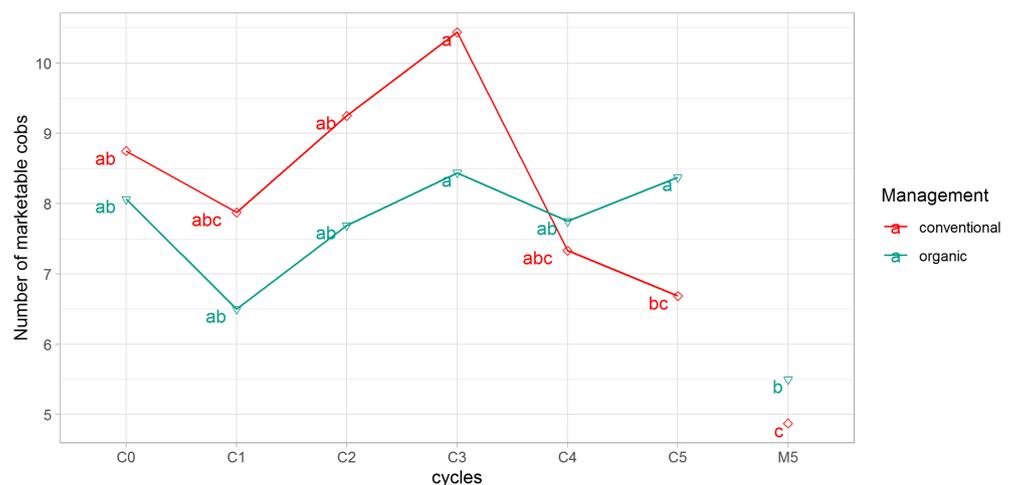
The analysis of total yield and the number of marketable ears (Figures 11 and 12) resulted in very similar observations for organic and conventional management. Exceptions were the statistically higher yield in C2 compared to C4 in organic management and the significantly reduced number of ears from C3 to C5 in conventional management. A decrease of marketable yield by mass selection occurred in both management system, although it was only significant in conventional management. Data for the remaining traits are given in the electronic supplement. No interactions of genotype and management system or year were observed for all traits except ear colour, which was a non-selected trait (Tables S2–S4). In conclusion, there was no indication of adaptation to organic management.

4 | DISCUSSION

4.1 | Heritability

Generally, the response to selection was low or even zero. This is not due to a lack of genetic variance in our material, because it was derived from 40 hybrids of different origins and because the heritability estimates in this study were compared to other studies and were found to be in the same range except the row number, where the genetic variability was low in our population (Table 7). This shows the

high precision of our experiments and a sufficiently high genetic variation in our material.

4.2 | The effect of recurrent haploid selection

In most traits, no clear changes during five cycles of selection could be detected. Contrary to expectations, there was no clear effect of recurrent haploid selection and no positive influence could be observed in several traits. The significant decrease of ear diameter, the slight decreases of ear length, marketable yield and the share of marketable yield were the only changes during five cycles of recurrent haploid selection. This occurred despite that yield and ear size had been actively selected. Which factors could have reduced a gain by selection?

Compared to the successful selection of Chalyk and Rotarenco (2001) and Rotarenco et al. (2012), the population used for this study was composed out of 40 hybrid cultivars. The two starting populations of the study by Rotarenco et al. were genetically much broader: The 'SP-Synthetic' contained four components and an English early landrace. The 'SA-Synthetic' was derived from four strains of which 3 were inbred lines. Many deleterious alleles may have been present. In our investigated sweet corn population, however, lethal alleles must have been eliminated already during the selection of the inbred

TABLE 7 Heritability of flowering time, quality traits and yield in various studies

Trait	Heritability in this study	Heritability in other studies	Reference
Tillering	0.77	0.50	Has & Has, 2009
Flowering time	0.86	0.58 (days to silking)	Asghar & Syed, 2010
		0.70	Has & Has, 2009
		0.53 (days to silking)	Nigussie & Saleh, 2005
		0.52 (days to silking)	Pereira & Amaral, 2001
Ear diameter	0.68	0.95	Gonçalves et al., 2018
		0.60	Asghar & Syed, 2010
		0.35	Nigussie & Saleh, 2005
		0.33	Has & Has, 2009
		0.95	Gonçalves et al., 2020
Ear shape	0	0.44	Has & Has, 2009
Ear length	0.74	0.72	Asghar & Syed, 2010
		0.75	Nigussie & Saleh, 2005
		0.58	Has & Has, 2009
		0.94	Gonçalves et al., 2020
Row number	0.09	0.98	Gonçalves et al., 2018
		0.84	Asghar & Syed, 2010
		0.12	Nigussie & Saleh, 2005
		0.65	Has & Has, 2009
Total yield	0.64	0.86	Gonçalves et al., 2018
		0.27	Nigussie & Saleh, 2005
		0.29	Has & Has, 2009
		0.96	Gonçalves et al., 2020

mother lines of the 40 parental hybrid cultivars. This could explain limited progress due to the lack of lethal alleles.

The starting populations improved by recurrent haploid selection of Rotarenco et al. (2004) were on a comparably low yield level with an ear length of 14.2 cm, after three cycles of selection length increased to 17.5 and 16.7 cm, respectively, for SP and SA. In the present study, the ear length of the initial population C0 had a good performance with an ear length of 18.5 cm. Similarly, 13.5 and 12.4 rows mentioned in Rotarenco et al. (2004) are row numbers long surpassed in modern cultivars. It is questionable whether haploid selection works on high performing populations.

Under recurrent haploid selection the individual haploid plants are selected. However, the crucial factor for the performance of populations is the combining ability of the selected genotypes. Already Chalyk et al. (2002) suggested a modification called 'haploid & diploid recurrent selection', in which selection of haploid plants is combined with selection of diploid plants based on their combining ability. Geiger et al. (2013) presented a selection method which is based on the evaluation of haploids, their corresponding DH lines and their testcrosses with testers. The results revealed a medium to high correlation for several traits. This selection method can be used to select against lethal alleles and recessive traits in the haploids and DH lines as well as for high additive and epistatic effects in the testcrosses. It could be more effective than recurrent haploid selection, but needs more steps for testing.

An even more severe limitation of the method is the untypical development of many haploid plants, for example, ears which are only partially filled because of reduced fertility. Important quality traits for sweet corn cannot be selected in haploids because of their unusual growth, reduced fertility and their higher susceptibility to environmental stress. Female fertility increased during five cycles of recurrent haploid selection in the experiment presented here (data not shown): Pollen production and seed set of haploid plants increased and haploid plants grew larger, probably doubling chromosomes spontaneously in parts of the haploid plant. Indirect selection may have occurred by selecting the haploids with the best female fertility. It is possible that selection for haploid female fertility could negatively influence plant development in diploids, affecting tip fill and the number of marketable ears. Tipfill is related to the ability of the plant to form grains in the tip of the ear and ears were only marketable, if they were well covered by grains. Both traits could be negatively influenced by selected female fertility in the haploid population, if this influences the female fertility in the diploid population. Fertility in haploid plants may be influenced by few loci (Molenaar et al., 2019) and may be linked to the ability of haploid plants to spontaneously double chromosomes to complete meiosis. The ability of the plants to double spontaneously might even be disadvantageous, if we assume that spontaneous doubling is abnormal and could have a negative effect for meiosis in diploid plants as well.

Recurrent haploid selection is based on single plants where the phenotypic value is very much influenced by the environment. In this study, selection was done in one location, Rheinau, during all five cycles which may have led to an adaptation to this location. Tests were performed in six environments including Rheinau. Thus, gain of selection could have been masked by adaptation to a single environment. To test this, the response to selection at Rheinau was compared to the mean of the other locations (data shown Figures S1 and S2); no general difference in selection response between Rheinau and the other locations could be observed.

Conditions during five cycles of selection in Rheinau were different for every year; examples for annual temperature and precipitation are given in Table S1. In a fluctuating environment, interactions with the genotype may occur. The best genotype in 1 year will not be the best in another year (Falconer & Mackey, 1995). Thus, in different cycles, different genotypes may have been selected. This can explain that for several traits, there was a significant difference between different cycles, but the response to selection was in different directions.

The response to selection can be limited by a small effective population size resulting in inbreeding depression and random changes due to genetic drift (Weyhrich et al., 1998). Selecting a large number of plants, on the other hand, leads to a low selection intensity. In this study, the number of selected plants was larger than 70 except in C2, when only 25 haploid plants were selected. Theoretically, this will lead to an inbreeding coefficient of 4% in this generation, probably counterbalanced by the high selection intensity of 3.4%. No significant decrease in performance from C2 to C3 could be observed. No clear signs of inbreeding as described in Allard (1999) like reduced plant height, smaller ears could be observed in the C4 and C5 or the M5.

4.3 | Mass selection

In this study, positive mass selection was applied in parallel to recurrent haploid selection as a point of reference. Mass selection has often been shown to be efficient in population improvement in maize. Summarizing the results of 19 studies with three or more cycles of mass selection for yield Hallauer and Miranda (1981) reported gains of selection between −1% and 19.1% with an average of 3.33% per cycle. Selection for ear length was applied successfully to sweet corn populations (Nigussie & Saleh, 2005). Tip fill was selected successfully and with high heritability in Shelton's study (Shelton & Tracy, 2015) and seemed to be an 'easy trait'. In those experiments, selection started with very broad populations and often the response to mass selection tended to plateau after some cycles.

In the present experiment, mass selection decreased performance in some cases did not significantly affect most of the traits and improved only few traits. Positive changes occurred in tip fill, vigour and length of the ears, but these changes were not significant. The population studied here had already undergone six cycles of mass selection before the experiment started with C0. It had already reached a high performance with, for example, 16.3 rows. No significant loss or gain of total yield was observed in mass selection nor in recurrent haploid selection, but there was a significant loss of marketable yield for mass selection. That loss is perhaps due to a higher amount of longer cobs in the mass selected population. This could be

observed also in the average cob length, where M5 is on average longer than C0. So mass selection was probably effective for increasing cob length, but this was decreasing marketable yield because of the limit of 23 cm for cob length in marketable cobs.

A limitation of mass selection is that the environmental conditions in the selection location may change from year to year. As already mentioned for the recurrent haploid selection, this may result in the selection of different alleles in different years and hence no general response to selection in the same direction.

One of the negative influences of selection was a significant delay of flowering by 2.5 days. This might not be very relevant for sweet corn growers, but it indicates the difficulty to maintain the flowering time of the population over five cycles. Removing male flowers of very late and very early flowering plants was apparently not efficient enough to restrict the flowering period. Apparently, ears from late flowering plants were indirectly selected because of higher yield. The reduction of marketable yield, however, was not caused by later flowering because yield data were corrected by the cumulated temperature during ear development. This lack of positive response to selection could be explained by an insufficient variability in the initial population having been selected and recombined already by mass selection for 6 generations before C0.

5 | CONCLUSIONS

In conclusion, the recurrent haploid selection was not efficient to improve yield and quality of the sweet corn population investigated. The most probable reasons are the already high performance of the initial population, a probably poor correlation between the performance of single haploid plants and their combining ability, and the fluctuating environmental conditions at the selection location from year to year. Other more frequently used methods like full-sib, S_1, or DH line selection are preferable.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

BH and HB conceived and planned the project. CA carried out experiments and data analysis. CA wrote the manuscript and all authors reviewed it.

DATA AVAILABILITY STATEMENT

The online version of this article contains supplementary material which is available to authorized users. Code availability is not applicable.

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REFERENCES

Allard, R. W. (1999). *Principles of plant breeding* (2nd ed.). Wiley.

Asghar, M., & Syed, M. (2010). Selection indices for yield and quality traits in sweet corn. *Pakistan Journal of Botany*, *42*, 775–789.

Chalyk, S. T. (1994). Properties of maternal haploid maize plants and potential application to maize breeding. *Euphytica*, *79*(1–2), 13–18. https://doi.org/10.1007/BF00023571

Chalyk, S. T., & Rotarenco, V. A. (1999). Using maternal haploid plants in recurrent selection in maize. *Maize Genetics Cooperation Newsletter*, *73*, 56–57.

Chalyk, S. T., & Rotarenco, V. A. (2001). The use of Matroclinous maize haploids for recurrent selection. *Russian Journal of Genetics*, *37*, 1382–1387. https://doi.org/10.1023/A:1013291711912

Chalyk, S. T., Rotarenco, V. A., & Bylich, V. G. (2002). New scheme of haploid recurrent selection in maize. *Maize Genetics Cooperation Newsletter*, *76*, 13–14.

Charlesworth, D., & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, *18*(1), 237–268. https://doi.org/10.1146/annurev.es.18.110187.001321

de Mendiburu, F. (2009). Una herramienta de analisis estadistico para la investigacion agricola. Dissertation, Universidad Nacional Agraria La Molina Lima-Peru.

Falconer, D. S., & Mackey, T. F. C. (1995). *Introduction to quantitative genetics* (4th ed.). Pearson Education Limited.

Geiger, H., Braun, M., Gordillo, G., Koch, S., Jesse, J., & Krützfeldt, J. (2006). Variation for female fertility among haploid maize lines. *Maize Genetics Cooperation Newsletter.*, *80*, 28–29.

Geiger, H., Gordillo, G., & Koch, S. (2013). Genetic correlations among haploids, doubled haploids, and testcrosses in maize. *Crop Science*, *53*(6), 2313–2320. https://doi.org/10.2135/cropsci2013.03.0163

Gonçalves, G. M. B., Ferreira, J. A., Durães, N. N. L., Crevelari, J. A., Viana, F. N., & Pereira, M. G. (2020). Yield potential of super sweet corn genotypes in progressive breeding stages. *Acta Scientiarum Agronomy*, *42*, e43789. https://doi.org/10.4025/actasciagron.v42i1.4378

Gonçalves, G. M. B., Pereira, M. G., Ferreira, J. A., Schwantes, I. A., Durães, N. N. L., Crevelari, J. A., & Amaral, A. T. (2018). Development and selection of super-sweet corn genotypes (sh2) through multivariate approaches. *Bragantia*, *77*(4), 536–545. https://doi.org/10.1590/1678-4499.2017340

Hallauer, A. R., & Miranda, J. B. (1981). *Quantitative genetics in maize breeding*. Iowa State University Press.

Has, V., & Has, I. (2009). Genetic inheritance of some important characters of sweet corn. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37 https://doi.org/10.15835/nbha3713129

Kramer, V., Shaw, J. R., Hannah, M. L., & Hannah, L. C. (2014). The sh2-R allele of the maize shrunken-2 locus was caused by a complex

chromosomal rearrangement. *Theoretical and Applied Genetics*, *128*(3), 445–452. https://doi.org/10.1007/s00122-014-2443-3

Lancashire, P. D., Bleiholder, H., Boom, T. V. D., Langelüddeke, P., Stauss, R., Weber, E., & Witzenberger, A. (1991). A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*, *119*(3), 561–601. https://doi.org/10.1111/j.1744-7348.1991.tb04895.x

Molenaar, W. S., Schipprack, W., Brauner, P. C., & Melchinger, A. E. (2019). Haploid male fertility and spontaneous chromosome doubling evaluated in a diallel and recurrent selection experiment in maize. *Theoretical and Applied Genetics*, *132*(8), 2273–2284. https://doi.org/10.1007/s00122-019-03353-w

Nigussie, M., & Saleh, G. (2005). Responses to recurrent selection methods in two sweet corn (*Zea mays L. saccharata*) populations. *Asian Journal of Plant Sciences*, *4*(6), 589–596. https://doi.org/10.3923/ajps.2005.589.596

Pereira, M. G., & Amaral, A. T. (2001). Estimation of genetic components in popcorn based on the nested design. *Crop Breeding and Applied Biotechnology*, *1*(1), 3–10. https://doi.org/10.13082/1984-7033.v01n01a01

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for statistical Computing, Vienna, Austria https://www.R-project.org/ accessed 19.02.2021.

Röber, F. K., Gordillo, G. A., & Geiger, H. H. (2005). In vivo haploid induction in maize - performance of new inducers and significance of doubled haploid lines in hybrid breeding. *Maydica*, *50*, 275–283.

Rotarenco, V., Chalyk, S., & Eder, J. (2004). Utilization of maize haploid plants in a recurrent selection procedure. *Journal of Genetics and Breeding*, *58*, 61–71.

Rotarenco, V., Dicu, G., Mihailov, M., & State, D. (2012). Selection and breeding experiments at the haploid level in maize (*Zea mays L.*). *Journal of Plant Breeding and Crop Science*, *4*(5), 72–79. https://doi.org/10.5897/jpbcs11.089

Schuetzenmeister, A., & Dufey, F. (2019). VCA: Variance Component Analysis. https://CRAN.R-project.org/package=VCA accessed 03.03.2020.

Shelton, A. C., & Tracy, W. F. (2015). Recurrent selection and participatory plant breeding for improvement of two organic open-pollinated sweet corn (*Zea mays L.*). *Populations. Sustainability*, *7*, 5139–5152. https://doi.org/10.3390/su7055139

Weyhrich, R. A., Lamkey, K. R., & Hallauer, A. R. (1998). Effective population size and response to S1-progeny selection in the BS11 MaizePopulation. *Crop Science*, *38* 5, 1149–1158. https://doi.org/10.2135/cropsci1998.0011183X003800050008x

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