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Constitution of Composite Cross Maize (*Zea mays* L.) Populations Selected for the Semi-Arid Environment of South Madagascar

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Abstract: In many African countries, such as Madagascar, a large part of the population is currently estimated to be undernourished, and self-subsistence agriculture represents the primary source of food available for the family. Smallholder farmers cultivate crops with limited agricultural input and use old landraces or obsolete hybrid varieties, with a total country-wide production that is far from being able to sustain the national food demand. In this study, we have developed two maize composite cross populations (CCPs) of different kernel colors, through a selection process among 30 half-sib lines, chosen both for their agronomic performance and their environmental adaptability to a Malagasy farm context. The best half-sib lines, identified through field tests, were clustered as a parental group for open-pollinated crosses. The new CCP created, after two years of seed multiplication in an open field, showed promising yields compared with the hybrids, parental varieties, and local landraces, with average values of 2.7 t/ha for the white CCP and 3.5 t/ha for the yellow one. The seeds produced were then distributed among 15 local farmers to begin a participatory breeding program. Our approach represents an innovative step to improve and stabilize maize yields, employing populations adapted to the cultivation environment and able to cope with different stresses, thereby helping farmers' life conditions.

Keywords: maize; inbred lines; composite cross population; half-sib lines; Madagascar

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1. Introduction

Maize (*Zea mays*) represents the major staple food source in several tropical areas, providing not only carbohydrates, but also several nutritional compounds (e.g., inulin, carotenoids, phenolic compounds, tocotrienols, and tocopherols) [1,2]. Nearly 300 million tons of maize are produced on over 90 million hectares across sub-Saharan Africa, Latin America, and South Asia each year. The average annual growth rate of maize cultivation areas from 1993 to 2013 was 2.7% in Africa, 3.1% in Asia, and 4.6% in South America [3]. In sub-Saharan Africa, the rise in maize cultivated areas was followed by only a 2.4% increase in grain production caused by the high year-to-year variability in grain yields due to a low input agronomic system and climate change [4]. In many of these regions, such as Madagascar, smallholder farmers primarily perform subsistence low input agriculture with a management system largely relying on few agronomic practices and the use of low productive landraces or outdated hybrids, thereby limiting their ability to produce food and nutritional security [5]. Madagascar is an African country with one of the highest poverty rates, with two thirds of the population estimated to be undernourished [6]. Subsistence agriculture is the first source of food availability [7] for Malagasy families since large-scale agriculture is almost absent [8,9]. In the last few years, maize cultivation as a staple food for the local diet has increased, reaching 400,000 tons a year and becoming the second crop cultivated in the island [3,10]; however, this production is far from able to sustain the food demands [11].

Climate variability may account for over 50% of the total variation in maize yields in these areas [12,13]. Possible future climate changes, including irregular rainfall, high temperature during the growing season, rainy storms and drought might further accentuate this variability, thus increasing the vulnerability of farmers [14,15]. Agronomic practices, such as tillage, fertilization, irrigation, and weed removal, commonly applied in many countries, may allow farmers to partly overcome these detrimental effects on their final yields, but these practices are not easily applicable in the socioeconomic contexts of many of these areas due to their high costs [16]. The development of new maize varieties or populations resilient to climate change and adapted to tropical environments and low input agriculture could be a partial solution to cope with these problems [17–19]. Specific breeding programs to select new and improved populations, starting from local landraces as a source of parental lines may be carried out to decrease the environmental effects affecting yields [20–22]. Local maize landraces, indeed, usually exhibit reduced productive performance compared to elite germplasm (i.e., hybrid and modern varieties), but their genetic variability may allow them to cope with environmental stress and to be more resilient to climate change [23–25]. Thereby, it became a potential source of favorable alleles to improve grain yield, pest resistance, and other favorable agronomic traits of new varieties [26,27]. Several methods, including diallel crosses, the evaluation of the phenotypic performance for specific traits, and estimation of the pedigree relationship, have been developed to select the parental lines to be employed for cross combinations or to be clustered into heterotic groups in order to create new varieties or populations [28–32]. Another useful and common tool to perform a preliminary selection for promising germplasm lines is to make inbred lined crosses and evaluate the F1 generation's agronomical performance in order to identify the general combining abilities of each inbred line and its potential as a parental line [30,33]. Although each of these methods have advantages and problems linked to the cost and time needed to actuate them (especially with the high numbers of lines to be screened), using information resulting from a combination of methods can actively contribute to identifying the best line combinations to produce hybrids or improved composite cross populations (CCPs) [34–37]. However, it is important to use methods that can estimate the different variance components (genetic, environmental, and their interactions) of each morphological or productive trait to facilitate the evaluation of individual genetic predictor values for the selections of the best lines. The choice of the best genotypes, in fact, must be based on genetic averages instead of phenotypic values, since genotypic components are the future averages of the selected individuals [38]. The mixed model methods are based on an estimation of the variance components by means of a restricted maximum likelihood model (REML) and the prediction of genotypic values by the best linear unbiased prediction (BLUP), thereby supporting an accurate selection process [39,40].

A plant breeding program relies on human selection acting on a heterogeneous population (i.e., CCPs) and represents a valuable method for developing populations adaptable to different agricultural contexts in subtropical areas [41–44]. Various agricultural conditions can drive the selection of more adaptable genotypes, which present increased fitness [45,46]. After several years of cultivation and multiplication in the same area under isolated conditions, these populations may reach equilibrium with stable yields [31].

The aim of this study was to screen 30 inbred maize lines as possible parental lines, testing their half-sib progeny for both their agronomic performance and general combining ability, to create two maize composite cross populations by crossing the best half-sib lines. Ultimately, two different CCP with different kernel colors were created. These populations have been multiplied for two cultivation cycles and compared for yield production with commercial hybrids and local varieties in open field conditions. Both populations were distributed to local farmers to begin a participatory breeding program for their cultivation and evolution according to Malagasy's environment and climate condition. This study began in the agronomical season of 2010/2011 and lasted until the end of the maize cultivation agronomical season (AS) of 2018/2019.

2. Materials and Methods

2.1. Study Area and Soil Management

Fields trials were carried out in two different areas, Andiolava (Lat. 22°29′40.97′′ S; Long. 45°38′45.73′′ E) and Satrokala (Lat. 22°19′49.24′′ S; Long. 45°43′4.23′′ E), located over the Plateaux de l'Horombe and the upland area (1100 m a.b.l.) in the southern part of Madagascar.

This area presents a sub-arid tropical and sub-tropical climate, characterized by two distinct seasons: a wet season from November to March and a dry season from April to October. The annual rainfall during the wet season ranges between 700 and 850 mm, while there is a lack of precipitation during the remaining part of the year [47]. The monthly average maximum and minimum air temperatures were recorded in winter (December, 30.4 °C) and summer (July, 12.9 °C). Solar radiation follows the rainfall and temperature patterns, with a maximum and minimum during winter and summer, respectively. Due to these climatic conditions (evaluated from the local weather station in Ranohira in the period of 1997–2018), the agricultural season for rainfed crops, such as maize, starts from October–November until May–June. The soil characteristics had marked differences between the sites (Table S1); Andiolava had a sandy-loamy texture, with a pH of 5.8, an organic matter content of 1.33%, and a cation exchangeable capacity (CSC) of 3.40 meq/100 g, while Satrokala had a sandy-clay texture, with acid soils (pH 4.8), a very low content of organic matter (0.89%), and a CSC of 3.34 meq/100 g. Given the different SOM content and texture, the soil colour of Andiolava was brown–dark while that of Satrokala was red–brown.

The fields used in all trials during the several years of experiments were managed in a biennial rotation system with green manure and were plowed each year with a disc harrow plow (depth 20 cm) before sowing.

All plant cultivation stages were carried out without any use of chemical fertilizer or irrigation to simulate the agricultural conditions that normally occur in Malagasy farms.

2.2. Inbred Line Constitutions and Their Agronomic Evaluations through Half-Sib Progeny

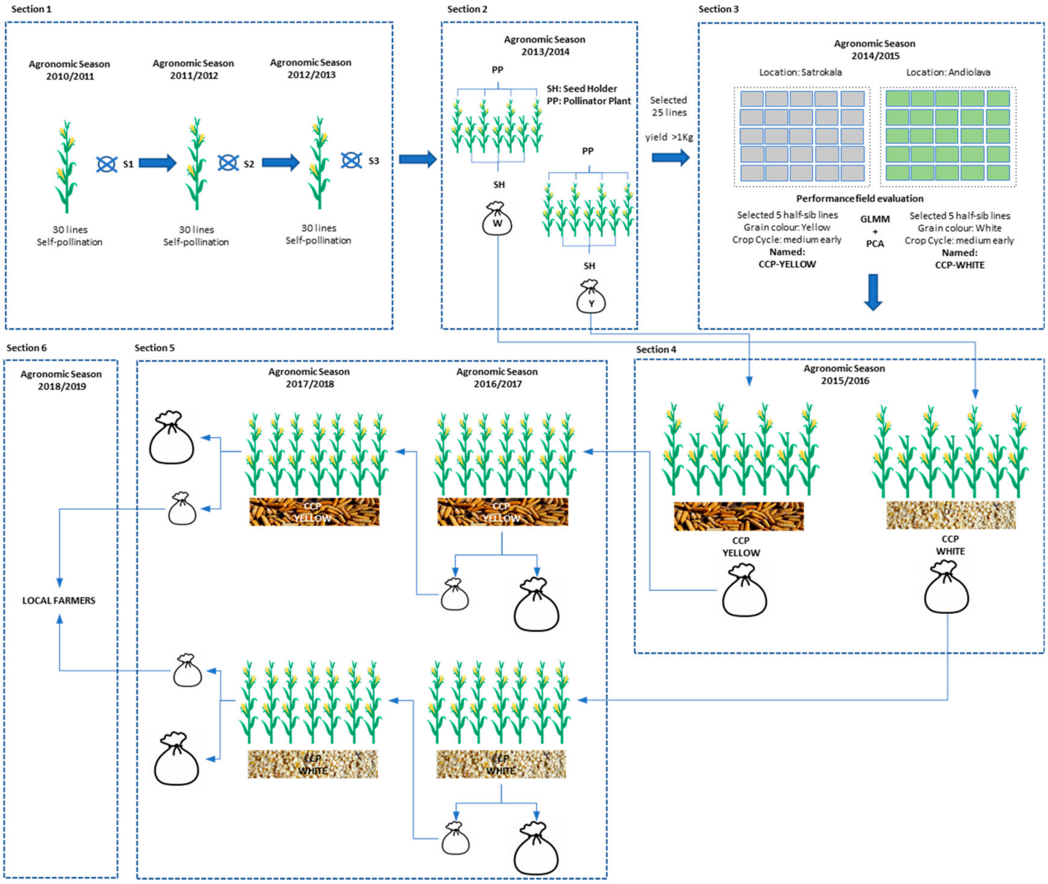
Thirty inbred lines (Table 1) were selected and reproduced from different maize accessions, starting from the AS of 2010–2011 (Scheme 1, Section 1). Of these entries, 10 lines came from an improved composite population selected in Somalia (Somtux) in the 1980s [48], 10 came from commercial hybrids, and 10 came from local Malagasy germplasm. The starting hybrid varieties and local landraces were chosen according to their commercial availability in the Malagasy market, while the Somtux population was considered for their good agronomic performance in tropical environments [49]. The selected inbred lines were characterized by different kernel colors (Table 1) and by a good uniformity in their different phenological developmental stages, assessed with the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) protocol following Meier [50].

Table 1. List of the inbred lines with their relative kernel colors and the seeds produced with the open cross-pollination of the emasculated seed holder plants.

Line Name	Origin	Kernel Colour	Half-Sib Yields (g)
SOM_P17	Somalian population	White	1338
SOM_P20	Somalian population	White	1343
SOM_P23	Somalian population	White	2456
SOM_P24	Somalian population	White	2091
SOM_P25	Somalian population	White	1793
SOM_P27	Somalian population	White	1950

Table 1. *Cont.*

Line Name	Origin	Kernel Colour	Half-Sib Yields (g)
SOM_P29	Somalian population	White	2238
SOM_P31	Somalian population	White	1896
SOM_P33	Somalian population	White	2013
SOM_P7	Somalian population	White	1527
FOTSY_P5	local landraces	White	258
FOTSY_P6	local landraces	White	1011
FOTSY_P7	local landraces	White	1338
FOTSY_P12	local landraces	White	2666
FOTSY_P9	local landraces	White	2303
MENA_P9	local landraces	Yellow	1859
MENA_P10	local landraces	Yellow	2704
MENA_P11	local landraces	Yellow	1187
MENA_P7	local landraces	Yellow	1317
MENA_P13	local landraces	Yellow	750
PAN6P_110_P4	commercial hybrid	Yellow	4081
PAN12_P6	commercial hybrid	Yellow	3122
MAS_55F_P5	commercial hybrid	Yellow	401
MAS_47D_P6	commercial hybrid	Yellow	2383
MAS_38D_P4	commercial hybrid	Yellow	1940
GASTI_P6	commercial hybrid	Yellow	3426
FRONTAL_P4	commercial hybrid	Yellow	2051
VENICI_P9	commercial hybrid	Yellow	1427
YOGGI_P12	commercial hybrid	Yellow	150
CLARITY_P9	commercial hybrid	Yellow	850



Scheme 1. Flow chart of the breeding process.

For each line (S3 generation), a half-sib progeny was created in Satrokala in the AS 2013–2014 (Scheme 1, Section 2). To avoid pollination between lines of different colours, breeding crosses were made in two different fields (more than 1000 m), subdividing the lines according to colour (15 lines in each field). An open cross-pollination method was adopted, where each inbred line was sown along a row, and each plant was emasculated and used as a maternal seed holder [51]. Each of these 30 seed-bearing lines was flanked by two rows using a mixed bulk of seeds from all the selected lines belonging to the same colour group, assuming the role of pollinator plants. Each row was 7 m long, with a distance of 0.70 m between rows and 0.20 m on the row (a planting density of 6 plants/m^2). At harvest time, in May, the grains produced by the seed holder plants (half-sib seeds) were collected, weighed, cleaned, and then stored in order to conserve them for the following years' tests and for future CCP constitution. Each progeny line maintained the name of its maternal inbred seed holder line.

In 2014–2015 AS (Scheme 1, Section 3), a selection of the half-sib progeny lines was made through two field tests with a completely randomized experimental design made by 25 plots each and located in Satrokala and Andiolava. Only the half-sib progenies belonging to the maternal plants that produced at least 1000 g of grains were chosen for these field trials. Each plot (5 m × 8 m) contained 6 rows with half-sib progeny plants from a single maternal line, with the same planting density previously adopted in crossing work. At harvest time, 10 plants were selected for each plot and used to record the following parameters: ear length (EL) and diameter (DIA), number of ranks per ear (NR), number of seeds per ear (NS), seed weight per ear (SW), and kernel colour. Total yields per plot were first calculated and then scaled up to obtain hectare production.

2.3. Population Constitution and Seed Multiplication

The 10 best half-sib progeny lines evaluated with the previous trials (Action B) were chosen for the constitution of two CCPs that will evolve in the Malagasy environment, differing by kernel color. The clustering accession for kernel colour was necessary for the constitution of populations with uniform traits. The cross-breeding operation was carried out in Satrokala during the AS 2015–2016 (Scheme 1, Section 4) using the remaining stored seeds of the half-sib progeny lines produced in 2013–2014 AS. Each selected line was sown in 2 rows 40 m long; at flowering time, each line was emasculated and alternated with 1 row of pollinator plants (mixed seed bulk from the selected lines) using the usual planting density of 6 plants/m^2. Two field plots of 1600 m^2 (one for each population) were used for the open pollination crosses, placed considerably far away than the 1 km as minimal distance needed to avoid cross-pollination [52]. At harvest time, the seeds were collected only from the seed holder plant rows of each plot (F1 generation), cleaned, and stored as base seed for open field sowing in the following years in Satrokala.

2.4. Seed Multiplication and Comparison between the New CCP Population with Hybrid and Local Variety Production in Open Fields

In the two years following the breeding crosses (AS 2016–2017 and 2017–2018), each population was reproduced in Satrokala in two isolated plots of 1 ha each (Scheme 1, Section 5). At the same time, part of the collected seeds was used to establish an open field trial to compare the yields of these populations with two hybrid varieties, Ranki and Gasti; two Malagasy landraces, Mena and Tombotsoa; and the Somtux. All these genotypes were cultivated in the same area under the same field conditions. Each maize entry was tested within a randomized block experimental design with three blocks of 7 plots each. Each plot of 0.5 ha was cultivated with a planting density of 6 plants/m^2, and, at harvest time, only the total yield per ha was evaluated.

In AS 2017–2018, the total seed production of the isolated fields for each new population was harvested, cleaned, and distributed to the local farmers to begin the participative breeding process starting from the following cultivation cycle in AS 2018–2019 (Scheme 1, Section 6).

2.5. Statistical Analysis

Data collected in the evaluation tests of the half-sib progeny were analyzed via analysis of variance using the software SPSS Statistics 25 (IBM company, Armonk, NY, USA) to compare their agronomic performance and to evaluate the general attitude of the inbred line used to combine them. According to a completely randomized block design replicated in two localities, a generalized linear mixed model (GLMM) was used to test the different factors (considering localities as random and genotypes as fixed), and a post hoc Tukey's test was done to compare the half-sib lines' performance. Furthermore, a comparison of the two years yields of the new maize populations, hybrid and local varieties, was performed with the same method to evaluate the variety factor as fixed, while the blocks and year were considered random.

In order to predict the genotypic values of the maize half-sib lines' traits, the data set was analysed with a restricted maximum likelihood model and with the best linear unbiased predictor (REML/BLUP) procedures, using the statistic software META-R [53]. Traits were explored separately for each location (individual REML) and pooled over the two areas (a combined REML across the environment). Yield predictors were not evaluated since we only had one observation for each locality without any replicate. For each trait, the following model was fitted to estimate the genetic parameters:

$$y = X_e + Z_g + W_i + \varepsilon$$

where y is the data vector; Xe is the vector of localities effects due to different environment (fixed); Zg is the genotypic effects (random); Wi is the vector of the effects of the genotype x environment (GxE) interactions (random), and ε is the residual errors (random). The total variation among lines that is attributable to genetic variation was categorized according to Hallauer and Miranda Filho [54] as low (<0.30), moderate (0.3–0.70), or high (0.70), with respect to maize breeding.

In addition, a multivariate Principal Component Analysis (PCA) on the vegetative and production predictors parameters (BLUPs) of the half-sib progeny was carried out using the R software v. 3.6 [55] with R/factoextra [56] in order to estimate the relative importance of each trait in capturing data variation.

3. Results

From the 30 inbred lines originally used as maternal lines for the half-sib progeny production (AS 2013–2014), 5 lines produced less than 1 kg of seed (10 plants production) (Table 1) and were removed from the field evaluation of the half-sib progeny of the following year. Three lines, PAN12_P6, PAN6P_110_P4, and GASTI_P6, showed the best general combined attitude and seed production, with more than 3 kg of grains from 10 plants (Table 1).

The GLMM results underlined a clearly significant effect of the cultivation environment over all the different parameters analysed. Yield (Figure 1) significantly varied between the two areas (F = 0.85; $p < 0.01$) but not among lines (F = 50.69; $p > 0.05$), likely because of the behaviour of some lines, which showed nearly the same yields in both environments. The ear length and diameter (Table 2) varied according to soil conditions and between the different half-sib lines, underlining how these parameters were significantly influenced by both cultivation condition (F = 47.8; $p < 0.001$ and F = 48.95; $p < 0.001$) and genetic factors (F = 2.01; $p < 0.05$ and F = 2.58; $p < 0.001$). The rank number per ear and the number of seeds per ear were influenced only from the cultivation conditions (F = 8.65; $p < 0.001$ and F = 80.27; $p < 0.001$ respectively), while the seed weight per ear varied according to environmental conditions (F = 2.3; $p < 0.001$) but also between half-sib lines (F = 185.4; $p < 0.001$).

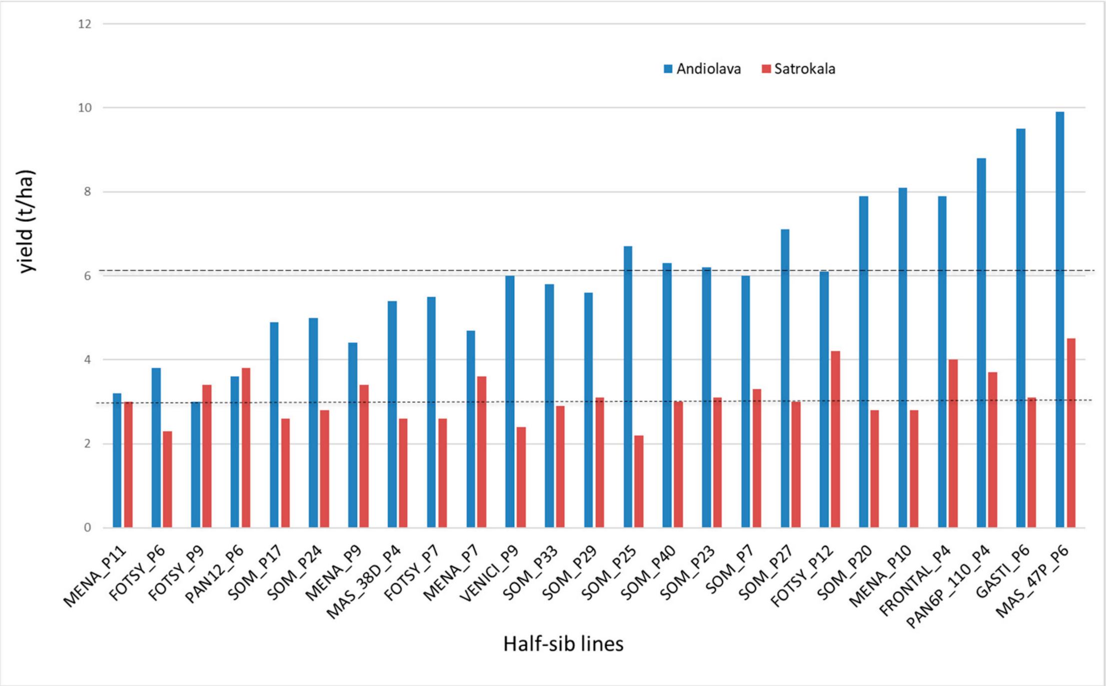


Figure 1. Half-sib progeny yields comparison in the two cultivation areas. Yields were calculated as plants plot yields and then referred to hectare. Dotted line represents the mean yield values obtained in Satrokala while the dashed lines the values of Andiolava.

Table 2. Mean values of vegetative and production parameters for the half-sib progeny comparison (AS 2014–2015) and their BLUP predictors combined across localities.

Variety	Ear Lenght		No. Rank/Ear		No. Seed/Ear		Seed Wheight (g)/Ear		Ear Diameter (cm)		Yields (t/ha)
	Mean	**BLUP**	**Mean**	**BLUP**	**Mean**	**BLUP**	**Mean**	**BLUP**	**Mean**	**BLUP**	**Mean**
VENICI_P9	14.35ab	10.10	14	10	422.80	328.69	118.10c	86.13	4.52ab	4.04	4.20
SOMTUX_P7	14.48ab	10.04	14	10	476.60	329.65	132.05ab	85.83	4.65a	3.98	4.65
SOMTUX_P40	14.59ab	10.22	14	12	524.10	331.15	131.55ab	87.29	4.47ab	4.04	4.65
SOMTUX_P33	13.32ab	9.91	16	12	470.90	331.33	110.35d	85.29	3.77b	3.93	4.35
SOMTUX_P29	14.32ab	10.09	16	10	489.50	332.34	122.30bc	86.37	4.44ab	4.01	4.35
SOMTUX_P27	14.10ab	10.32	16	12	529.60	330.46	139.45a	86.00	4.77a	3.95	3.90
SOMTUX_P25	13.04b	10.18	14	10	461.20	331.38	109.40d	86.72	4.47ab	4.06	3.00
SOMTUX_P24	13.80ab	9.93	14	10	441.20	329.89	110.00d	85.69	4.45ab	3.98	3.90
SOMTUX_P23	14.04ab	10.14	14	10	440.40	334.23	116.35c	87.59	4.39ab	4.04	4.65
SOMTUX_P20	14.69ab	9.97	14	12	456.10	331.90	116.40c	85.57	3.90b	3.83	5.35
SOMTUX_P17	14.43ab	10.11	14	10	459.70	330.71	117.75c	86.09	4.41ab	3.99	3.75
PAN6P_110_P4	14.91ab	10.20	14	12	510.50	333.49	131.85b	87.62	4.44ab	4.00	6.25
PAN12_P6	14.89ab	10.12	16	10	452.30	331.64	130.75b	87.64	4.55ab	4.11	3.70
MENA_P9	16.105a	10.05	14	10	468.40	334.54	142.20a	88.44	4.61a	4.11	5.05
MENA_P7	14.02ab	10.00	14	12	502.50	328.23	116.70c	85.26	4.23ab	4.01	4.15
MENA_P11	13.78ab	10.20	16	10	511.40	330.31	127.45b	87.50	4.70a	4.06	4.45
MENA_P10	15.52ab	10.04	14	12	455.20	333.05	116.85c	85.87	4.18ab	3.95	5.45
MAS_47D_P6	15.04ab	10.21	16	12	510.20	332.20	139.00a	87.45	4.48ab	3.97	7.20
MAS_38D_P4	14.60ab	10.16	14	10	419.50	329.79	110.10d	86.21	4.21ab	3.91	4.00
GASTI_P6	14.98ab	10.22	14	12	485.50	332.12	143.30a	88.86	4.49ab	4.08	6.30
FRONTAL_P4	15.38ab	10.29	14	10	509.30	333.43	142.85a	88.81	4.48ab	4.09	5.95
FOTSY_P9	14.98ab	10.43	14	10	467.70	331.19	128.8b	88.74	4.51ab	4.09	3.20
FOTSY_P7	13.63ab	9.99	14	10	481.40	333.54	112.9cd	87.14	4.16ab	4.15	4.05
FOTSY_P6	13.59ab	10.19	14	10	447.80	330.69	112.7cd	85.80	4.20ab	3.91	3.05
FOTSY_P12	14.77ab	9.86	14	10	471.90	330.79	123.55bc	85.19	4.54ab	3.99	5.15

Note: means followed by a common letter within the same column are not significantly different ($p > 0.05$).

For the REML results, the partition of phenotypic variance into genetic, environmental, and G × E interaction variance (Figure 2) underlines that, for any traits, genotypic variance was greater than environmental and G × E variances. Ear length and ear diameter traits showed the highest genotypic variance, explaining more than 30% of the phenotypic variance, while for all other traits, the environment and its interactions with genotype explained nearly 80% of the variation, underlining how all these traits depend more on environmental factors than genotype.

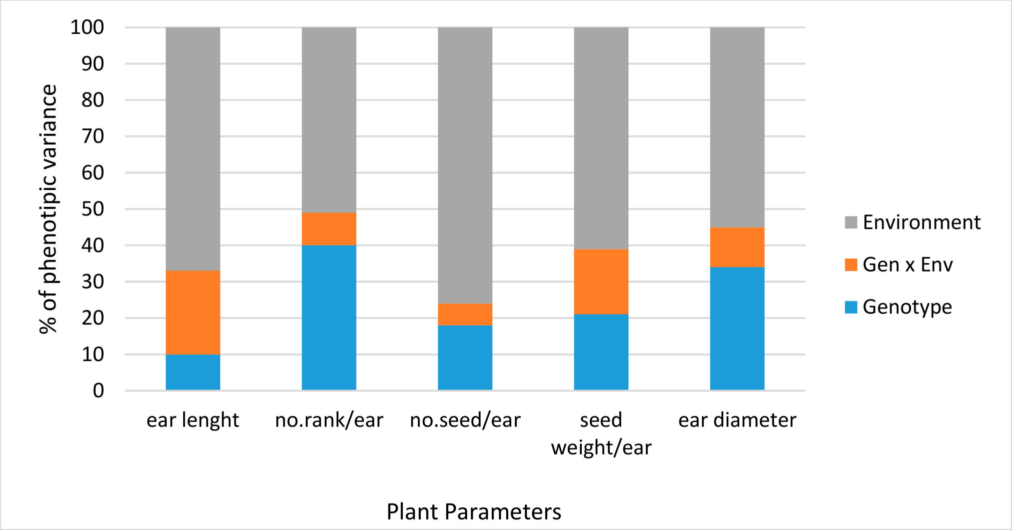


Figure 2. Partition of the phenotypic variance of the different plant traits.

BLUP predictors were calculated for each half-sib line across both environments (Tables S2 and S3) and used with the values of the yield to carry out the PCA analysis. Results from the PCA analysis (Figure 3) show that the first two components explained 85% of the data's variability. The number of seeds per ear and seed weight per ear, together with yields, were the most important variables in the first component (0.918, 0.963, 0.860, respectively), while the rank number per ear and ear length were the most important in the second component (0.777, −0.612, respectively). The cultivation conditions clearly affect the agronomic performance of the half-sib lines that were divided into two different areas of the graph according to the cultivation sites.

The results from both the GLMM and PCA analyses explain that the half-sib lines that showed the best performance for both cultivation areas and were combined for the two CCP populations were MENA_P10, GASTI_P6, PAN6P_110_P4, MENA_P7, and MAS_47D_P6 for the yellow population, and SOMTUX_P20, SOMTUX_P40, SOMTUX_P27, SOMTUX_P25, and FOTSY_P12 for the white one.

Concerning the two years of CCP open field trials performed in Satrokala (AS 2016–2017 and 2017–2018) without fertilization and irrigation, the soil condition (block factor) and cultivation years did not significantly affect the varieties' productivity (F = 52.8; $p > 0.05$ and F = 68; $p > 0.05$ respectively). The yields significantly varied according to their different accessions (F = 35; $p < 0.05$). Hybrids exhibited the best performances in both years, with Gasti producing 4.8 t/ha and Ranki 4.1 t/ha (Figure 4). The yellow CCP population's yield was significantly lower than the hybrid productions' yield but did not differ from that of the Somtux populations and was significantly higher than the landrace yields. The white CCP population produced more than the local landraces but less than the other populations and the hybrid varieties.

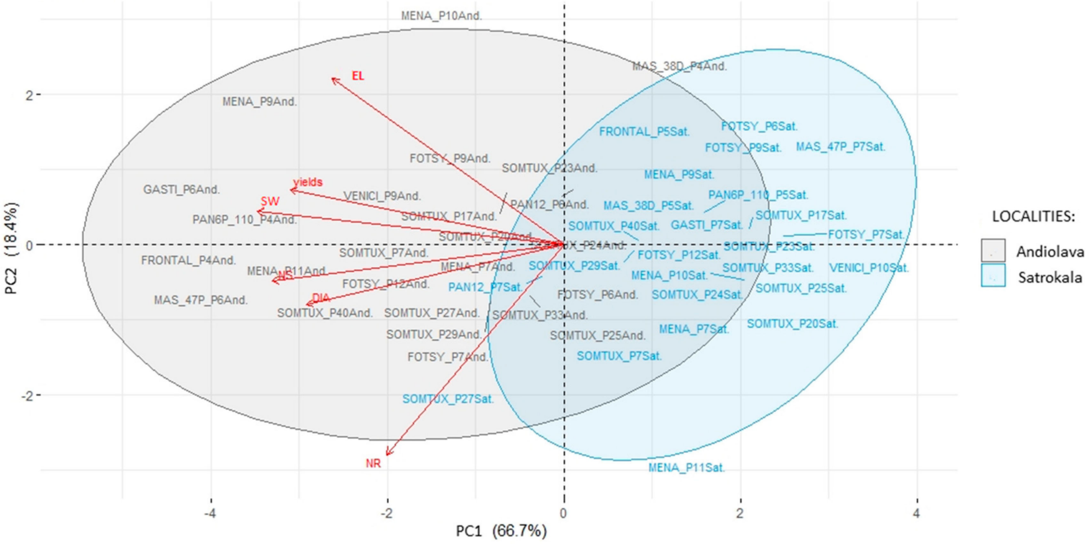


Figure 3. Principal component graph of the half-sib progeny vegetative and productive traits in the two localities. And. = Andiolava; Sat. = Satrokala; NR = number of rank/ear; DIA = ear diameter; NS = number of seed/ear; SW = seed weight/ear; EL = ear length.

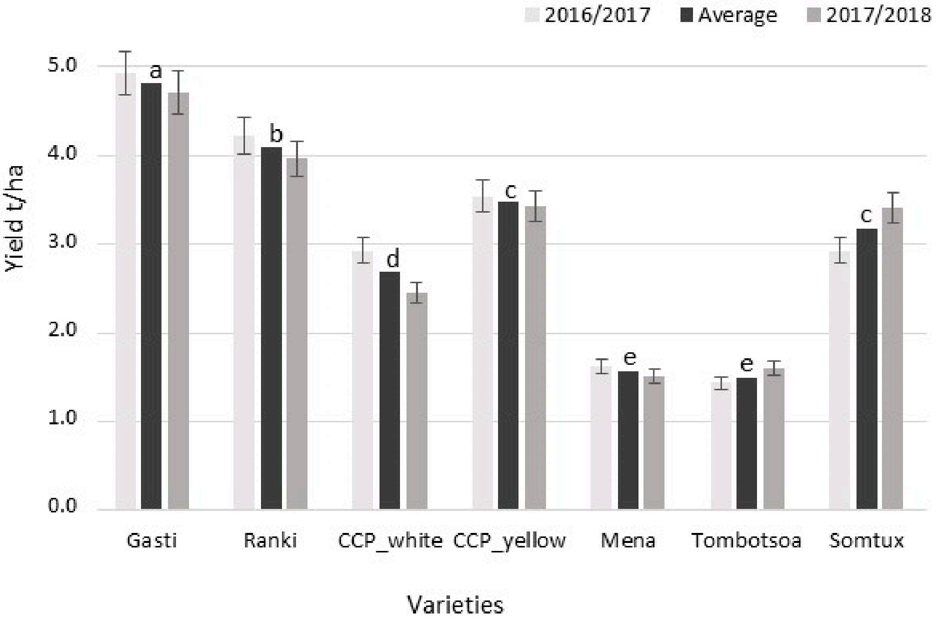


Figure 4. Comparison of yields production in two cultivation cycles between the new CCP populations, the hybrids, local varieties and Somtux (Somalian Population). Common letters between mean columns are not significantly different ($p > 0.05$).

4. Discussion

In this study, starting from maize inbred lines, 30 half-sib lines were selected, tested, and crossed to constitute two CCPs of different kernel colors. Grain color is a very important commercial and social trait in maize, as yellow kernel varieties are generally required by the European market both for human and animal feeding, while the white varieties are particularly requested as human food in the Malagasy and African markets [47]. These populations were multiplied and evaluated for two years and then distributed among smallholder farmers to start a participative breeding program.

Among the 30 inbred lines originally selected as possible maternal lines for half-sib progeny production (AS 2013–2014), 5 lines produced less than 1 kg of seed, underlining the reluctance to combine different genotypes, and were removed from the half-sib field evaluation in the AS 2014–2015.

During these trials, a remarkable difference was found in the yield and crop performance among the half-sib progeny for the two cultivation areas. These differences were likely contributed to by soil properties and the different pedoclimatic conditions that characterize the areas of Andiolava and Satrokala, as demonstrated in other studies carried out in these areas [47,57]. The highest yields were found, on average, in Andiolava, where the soil was characterized by a higher organic matter content (1.33%) and a less acidic pH (5.8) compared to Satrokala.

Half-sib lines belonging to the four maternal inbred lines from hybrid varieties exhibited the highest yields and performance, despite the absence of any fertilization event during the cultivation period. These lines were selected with the best four half-sib lines selected from the Somtux population and two lines from local varieties as parental clusters to produce the two CCPs. Thus, establishing CCPs from smaller sets of high-performing parent lines might optimize their yielding ability, as assessed by Döring et al. [58], with winter wheat CCPs. The optimal number of genotypes to be intercrossed to create these populations is still debated and requires further investigation; however, more than five pure lines should be chosen to create a parental cluster [59]. In our work, we tried to overcome the problem creating CCPs from half-sib lines, instead of pure lines, for two reasons: (i) to provide each CCP with wide within-population genetic variability contributed by thousands of different genotypes; and (ii) to transfer a good amount of favourable alleles supported by the selection of the best-inbred lines used as maternal lines to each population.

All the half-sib lines that we selected as parental lines were evaluated according to the morphological and productive BLUPs values that allowed us to identify promising genotypes through information revealing their true genetic value and minimizing estimate distortions due to environmental effects [38]. The need for robust and accurate models in evaluating complex experiments had led to the development of mixed models based on restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) which were very useful in plant breeding programs for both annual and perennial plants, for a long time [60]. For maize, these methods were effective in assessing the performance of the lines and accurately predicting their variance components and breeding values [61].

The two created CCPs have shown promising yields during the two years of open field trials, considering that both populations were not yet in equilibrium (based on Hardy–Weinberg) and under low input cultivation management. The CCP yields in both years were higher than the local varieties of Tombotsoa and Mena, which, despite low yield performance, were found to be closer to the national maize average yield (i.e., 1.7 t/ha) [11]. This proves that despite local varieties are adapted to the Malagasy environment, their agronomic performance is very low [25]. This suggests that our CCPs under typical low-cost Malagasy farming systems may represent (also at the beginning of their evolution) a beneficial solution pertaining to cost since the self-production of seeds would reduce farming costs, while an improved yield will increase income. During the field trials, Gasti and Ranki hybrids, characterized by an intermediate growing cycle, showed the best production in both years—nearly 5 t/ha and 4 t/ha, respectively. This performance was affected by the favourable growing cycle length of these genotypes [62], since the most productive hybrids for the experimental area of Satrokala were those characterized by an intermediate growing cycle (i.e., FAO 300–400, maize class index for growth cycle duration), such as Maggi, Joliet, and Drasti, while those characterized by either shorter or longer growing cycles exhibited bad performance [47]. Hybrids with shorter growing cycles may easily avoid drought stress due to their shorter and faster grain-filling periods [63], while late genotypes (i.e., FAO 700) may encounter abiotic stresses as reduced water and nutrient supply during the grain-filling period, thereby reducing their potential production. However, a short-intermediate growing cycle did not guarantee a high production [47,62]; as it happened for our CCPs, which were characterized both by a short cycle of nearly 100 days for the yellow varieties and an intermediate cycle of 120 days for the white ones. Also, the improved Somtux population had a short-intermediate growing cycle of 110 days and showed nearly the same yields as the yellow CCP in both years of the test, while the production increased in the second year compared to the white CCP. This higher production of the Somtux population compared to white CCP was probably due to the shorter (less than 10 days) growing cycle of the Somalian genotypes that favored their yields production at the end of the maize growing season of 2018. The reduced yields of the white CCP compared to the yellow CCP was probably due to the growing cycle, again, which was longer than 20 days, thereby responding to possible drought stress during the grain-filling period. Another reason may be the lines used as parental cluster; for the yellow CCP they were almost all selected from hybrids inbred lines, so they may transfer to the population a major amount of improved yields alleles compared to the parental group of the white CCP. On the whole, these results may suggest that using CCP with increased within-crop genetic diversity can produce maize crops with improved yield stability and good yield reliability across variable and unpredictable cropping environments.

The positive yield stability of both CCPs will be the basis for the next genotype selection that will be carried out in future participatory breeding processes led by local farmers through a mass selection approach. In the smallholder farm context of Madagascar, controlled pollination is not feasible. Consequently, the use of mass selection represents the only way for farmers to control the evolution of their crop populations [45,64]. In the Malagasy farm context, where low inputs are not able to offset environmental stresses, heterogeneous populations like CCPs are expected to be more stable compared to elite germplasms, especially from the perspective of climate change [21,25,37]. The achieved intra-specific genetic variability may act as a mechanism for the wider adaptation of these plants, thereby stabilizing their production [65–68]. These populations may suffer from low yields compared to improved hybrid and elite varieties [67], but also using lines selected from those varieties in the parental cluster may improve some yield traits, thereby increasing their agronomic performance and maintaining a high genetic diversity level [35,69]. For these reasons, we also selected and used inbred lines from modern hybrid genotypes as parental lines to constitute our populations.

Contrary to modern maize breeding programs that have selected genetically uniform high-yield commercial hybrids [70,71] the future breeding programs should be focused not only on selecting higher-yield plants but also on the development of stress-tolerant plants, allowing them to be resilient to different biotic stresses and climate change. The development of successful CCP populations for low-input farming systems should select parental lines from among local landraces in order to provide them with the ability to resist biotic and environmental stress and efficiently use organic nitrogen [72–74]. For this reason, we used inbred lines from both Malagasy local varieties and the Somtux population as parental lines.

5. Conclusions

In countries like Madagascar, where most of the farmers are self-subsistence smallholders that cultivate crops with limited agricultural management and use old landraces or obsolete hybrid varieties, the use of a composite cross population selected with a participatory breeding program within the farm may represent a possible inexpensive step to improve crop yields, thus ameliorating their life conditions. The two CCP populations produced in this study, which represent the base materials for a future evolutionary breeding program carried out by farmers, have shown promising agronomic performance. These evolving populations may be useful to increase food production in the Malagasy agricultural context, ensuring yield stability and resilience to climate change. Through a participatory breeding program started this year (AS 2018/2019), farmers, with our support, will contribute to driving the selection process of their own populations, applying their agronomic management and selecting the best genotypes that will adapt to their own cultivation areas and farming system. The expanded cultivation of maize over dry areas in Madagascar should be considered a very reliable alternative staple food-source other than rice, as maize may provide benefits in terms of food security and functional compounds for local diets with lower water demands.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/54/s1, Table S1: Andiolava and Satrokala soil characteristics, Table S2: half-sib comparison in Andiolava, Table S3: half-sib comparison in Satrokala.

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