

Full Length Research Paper

Sorghum populations' improvement

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The objective of this study was to increase the outcrossing rate and yield potential for improve advance heterotic *Sorghum bicolor* (Linn.) Moench, Caudatum-race populations with high heterozygosity using recurrent selection (RS) and SSR markers as a tool for improve new sorghum breeding material. One hundred accession chosen for their early flowering and maturity were intermitted using random-mating system in an isolated crossing block for duration period of four recurrent selection cycles over main and off-season respectively. The selections were based on detection of polymorphism at seedling stage among populations. All established plants were sampled at the two-leaf stage for DNA extraction. Leaf tissue taken from 3200 plants (32 plants/accession) was genotyped using SSR markers and the highest 200 polymorphic plants were selected as parents for RS cycle₁. This procedure was repeated in three subsequent cycles. Fifteen genomic SSR markers were used in different combinations of duplex and triplex PCR. Genotyping data from five microsatellites loci were used to estimate outcrossing rate in each cycle by MLTR program. The outcrossing rate increased after four cycles of recurrent selection from 8% to 9%, 19%, 34% and 48% from C₀ to C₄ populations respectively. The progenies from recurrent selection were evaluated for genotypic and phenotypic variance under rainfed and irrigated different environments. The results revealed high significant change in observed heterozygosity which is confirmed superior and positive effect of heterozygosity on grain yield and some agronomic traits. Broad sense heritability was high for most of traits under study. High significant and positive correlation of outcrossing observed with some traits especially head diameter and head shape, reflects the direct effect of outcrossing through RS cycles and the increased outcrossing confirms that selection for grain yield and yield components could be achieved through selection of multi-locus individuals within populations. The study revealed the usefulness of the markers in accelerating the selection process and increasing the efficiency and accuracy of constructing heterotic populations that are expected to have more breeding advantage over the base population.

Key words: Heterotic, sorghum, population, improvement.

INTRODUCTION

The sorghum greatest variability of the wild and crop plants occurs in the north-east quadrant of Africa (Dogget, 1988). Sorghum is the most important cereal crop in terms of acreage and production in the Sudan, grain yields per unit area are extremely low both in the rainfed sector (0.7 t/ha) and in the irrigated sector (1.4 t/ha) compared with yields obtained in Developed countries where sorghum is grown, this situation can be attributed to the use of late, tall and physiologically inefficient traditional varieties (with low yield potential) (Mohamed et al., 1999). Genetic improvement in self-

pollinated species has been largely confined to varietals improvement methods based on pedigree, bulk and backcross methods. These methods have limitation such as limited use of available genetic variability resulting in the development of varieties with narrow genetic base, successive loss of genes in the segregating generations with no chance of recombination (Jensen, 1970).

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Population improvement involves the use of recurrent selection to increase the frequency of genes for quantitatively inherited characters. Initially, it is necessary to synthesize a sorghum random-mating population, using cultivars or breeding lines that possess genes that will contribute the character to be improved. Sorghum is an annual and wind-pollinated cereal that is known to be predominantly selfing (Doggett, 1988). Under experimental conditions, Ellstrand and Foster (1983) showed that the outcrossing rate (t) was influenced by population structure and obtained an average value of $t = 0.30$. Recurrent selection is a cyclic breeding procedure designated to improve trait means of populations under selection. This can be accomplished by a gradual increase in the frequency of favorable alleles with the simultaneous maintenance of genetic variability. Mass selection is the oldest and simplest form of recurrent selection. Its simplicity and the completion of cycle in the course of one year are its greatest advantages over other methods (Milorad et al., 2002). Outcrossing rates in plants are genetically controlled but are also influenced by environmental conditions. High rainfall and cool environmental conditions can promote outcrossing in barley, higher outcrossing rates under fluctuating rainfall conditions, Parzies et al. (2000). SSR markers are generally highly discriminative and are often used to distinguish varieties. The use of SSR markers has been proved as a powerful technique for studying the diversity of sorghum (Ghebru et al., 2002). Some reports have documented the positive effect of heterogeneity and heterozygosity on yield stability in sorghum (Reich and Atkins, 1970), maize (Schnell and Becker, 1986), rapeseed and faba bean. In sorghum, Haussmann et al. (1998) found that heterozygous entries were highly superior over homozygous entries for grain yield, Stover yield, and aboveground dry matter, and slightly superior for harvest index in a semi-arid region of Kenya. Bhola (1982) reported that the most striking change occurred between yield and maturity, where positive correlations in the original populations were changed to significant negative values. The correlation between plant height and yield continued to be significantly positive, though much lower values were observed in the improved populations. It appears possible to reduce the strength of the correlation after a few more cycles of selection. The results of the study confirm the experience in other crops that recurrent selection techniques are effective in improving populations in the desired direction. The genetic variability for grain yield in improved cycles was not affected significantly. There was slight reduction in variability after two cycles. However variability was increased in the third cycle, during which some additional elite lines were introduced in the populations. The variability for maturity and plant height was reduced. The means and variances of the traits for which deliberate selection was not practiced remained unchanged. The trend is encouraging, as one would like the improved

populations to be more uniform for height and maturity but still maintain variability for economic traits. The key concept of this experiment was that only plants which are likely to originate from outcrossing advanced to successive selection cycles. Such were plants determined by screening for heterozygosity at co-dominant DNA marker loci. These selected plants are expected to carry parental alleles favoring outcrossing. Open pollination of the selected plants in a crossing block will recombine superior genes from different source and enable further progress in the subsequent RS cycles.

The main objective of this project was to increase the outcrossing level and yield potential in *Sorghum bicolor* (Linn.) Moench, Caudatum-race population from Sudan, using the variation in partial outcrossing rate in wild landraces, through RS procedure. The specific objectives of this study were to: (1) prove the concept of a new marker-based recurrent selection for increase heterozygosity in sorghum. (2) Estimate population parameters which determine the progress from selection. (3) Provide sorghum material with increased out crossing rate for practical plant breeding programs in Sudan and ecologically similar region of sub-Saharan Africa.

MATERIALS AND METHODS

Plant materials

One hundred feterita type sorghum landraces (FSLR) of early maturity taken from gene bank of Agricultural Research Corporation (ARC), Sudan, mainly chosen from the Sudanese/Sahelian zone in Sudan and ecological similar neighboring regions. Seeds planted in small pots filled with soil in July for main-season and in January for off-season. All established plants sampled in the two-leaf stage for DNA extraction. After the marker genotypes have been assessed, selected plants were transplanted to a pollen-isolated, randomized crossing block and cultivated there until maturity. All seeds were harvested plant-wise and were stored in a cold store. The seedlings before transplanting already spend one month alight-save in plastic net house for a short day treatment to flowering initiation.

Recurrent selection procedure

The base population (cycle C_0) composed 100 FPSLR s and each accession grown in 32 replicates. In the seedling stage, leaf tissue is taken from 32 random plants per accession for marker analysis and then genotyped plant-wise. Four random bulks are grouped from each accession (400 bulks in total). Bulks with the highest degree of marker polymorphism were selected (1 bulk per accession) (800 plants in total). The two most heterozygous plants per accession are finally selected as parents of RS cycle C_1 (200 parent plants in total). To initiate cycle C_1 , 16 seedlings per parent plant are raised

Table 1. Outcrossing rate (t_m), average observed heterozygosity (H_o), percentage of polymorphic loci (PL), heterozygous loci (HL), families inbreeding coefficient (F_I) and its standard error per cycle.

Cycles	t_m (SD)	H_o	PL%	HL%	F_I (SE)
C ₀	0.08 (0.019)	0.0603	2.0	0.9	0.916 (0.035)
C ₁	0.09 (0.016)	0.0625	20.9	1.6	0.895 (0.019)
C ₂	0.19 (0.015)	0.1580	24.8	9.9	0.895 (0.019)
C ₃	0.34 (0.010)	0.2810	24.8	17.5	0.663 (0.026)
C ₄	0.48 (0.009)	0.4225	30.2	29.9	0.523 (0.022)

SD & SE = standard deviation and standard error respectively according to MLTR program.

for plant-wise genotyping. They are randomly grouped to two 8-plant bulks (400 bulks in total). As in C₀, 100 highly polymorphic bulks are selected, and 200 plants are selected as parents for cycle C₂. This procedure will be repeated in the two subsequent cycles (C₃ and C₄). Throughout the cycles, selection on the seed-parent side will be practiced within accessions only, i.e. each accession will be represented by 2 plants in the finally selected fraction of 200 parents. This restriction is taken to avoid a rapid decline of genetic diversity. Finally, a comparative evaluation of cycles C₀-C₄ will be conducted at two different locations in Sudan under rain-fed and irrigated conditions. Out crossing rates will be estimated from samples of 100 single plant progenies (3 seedlings/prog.) per RS cycle amounting to 5 × 100 × 3 = 1500 seedlings per location (= 3000 in total) which will be genotyped at ARC Wad Medani.

Experimental approach

The key concept of this experiment was that only plants which are likely to originate from outcrossing advanced to successive selection cycles. Such were plants determined by screening for heterozygosity at co-dominant DNA marker loci. These selected plants are expected to carry parental alleles favoring outcrossing. Open pollination of the selected plants in a crossing block will recombine superior genes from different source and enable further progress in the subsequent RS cycles.

Estimation of outcrossing rates

Multi-locus outcrossing rate (t_m) and average single-locus (t_s) outcrossing rates were estimated using the MLTR- version 3.2 software (Ritland, 2002). Estimation is based on a multilocus mixed mating (that is mixture of outcrossing and self-fertilization) model (Ritland and Jain 1981; Ritland 1990), assuming self-fertilization at a rate of s and random mating (outcrossing) at a rate of $t=1-s$. MLTR is applicable to highly polymorphic markers such as microsatellites, where an arbitrary number of alleles per locus may occur. The program uses the method of Brown and Allard (1970) first to infer maternal genotypes from expected if the maternal genotype was selfed.

Multilocus estimates of outcrossing rate improve the chance of detecting outcrosses when parents are related, so that the difference between t_m and t_s provides a measure of inbreeding.

Population's experimental procedure

Five RS sorghum populations (total of 500 individual lines) grown together in square crossing block 10 × 10 m using Randomized complete block design (RCBD), and the spacing between plants 0.23 and 0.80 m between rows, 12 rows with 42 individual per one row, two replications of crossing block was conducted in each location.

Statistical analysis

The computer program PLABSTAT (uz, 2000) was used for statistical analysis to measure the variance, minimum, maximum and the mean of traits, coefficient of variation and phenotypic correlation between traits. Tests for out layers were conducted according to Anscombe and Tukey (1963) and significant out layers were considered as missing values in the analysis. The estimates of broad-sense heritability (h^2) for different traits were computed using the variance components method based on the combined analysis over the two test locations for each population and based on results of variance analysis made separately for each environment. Traits means were used in the combined analysis across environment.

RESULTS

The results of data analysis using repeated cycles of selection are shown in Table 1. Considerable changes in outcrossing rate were obtained by repeated cycles of selection. The estimated rates of outcrossing were 0.08, 0.09, 0.19, 0.34 and 0.48 for cycles C₀, C₁, C₂, C₃ and C₄ respectively and the results obtained by TFGA computer program revealed that change in outcrossing rate, led to change in observed heterozygosity per cycles, observed heterozygosity per locus and the percentage of polymorphic loci within cycles. Moreover, decreased in

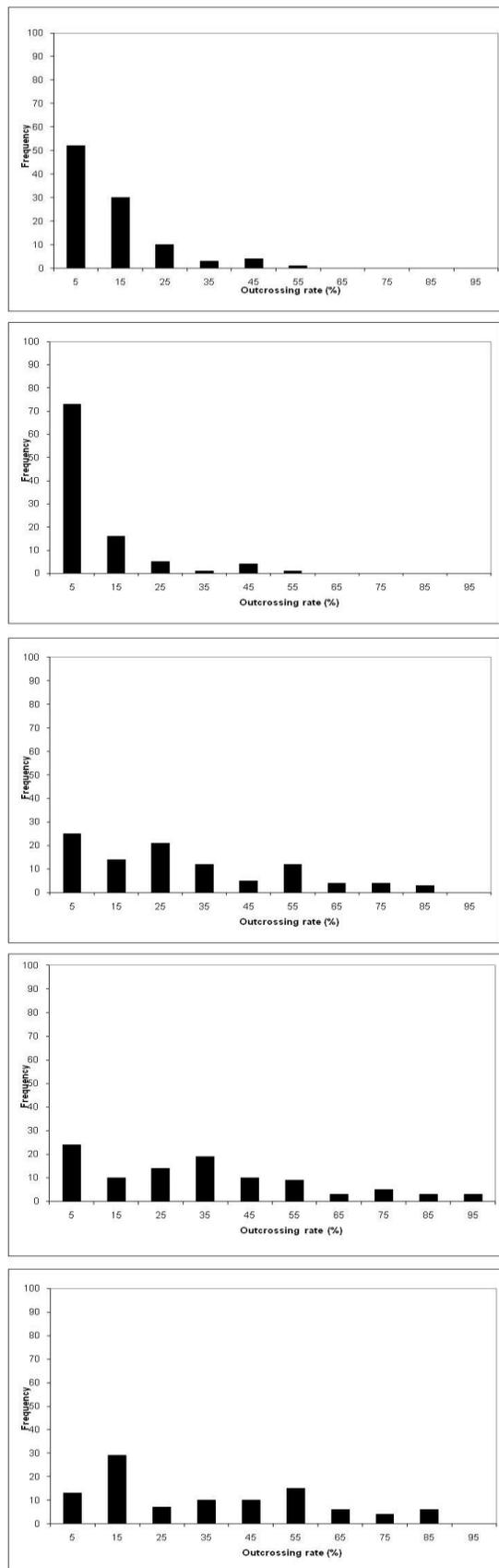


Figure 1. Change in the level of outcrossing by.

families maternal inbreeding coefficient (F_i) led to increase in heterozygosity level (i.e. heterozygosity was increased) by repeat cycles of selection.

The out crossing rate among families ranged from 5 to 55% in the original population (C_0) and in the first RS cycle (C_1), and ranged from 5 to 85% in the second and fourth cycle of RS, while wider ranges occurred in outcrossing rate among families in the third cycle ranging from 5 to 95%. And 90% of families in C_1 had outcrossing rate ranging from 5 – 55%, while families with wider ranges of outcrossing (5 to 85%) were detected in the advanced cycles of selection (C_2 , C_3 and C_4 populations). In C_2 , C_3 and C_4 cycles, more than 10% of the families showed outcrossing rate between 65 and 85%, Figure 1. Observed grain yield per cycles ranged from 5 to 55, from 25 to 75, from 55 to 95, from 55 to 105 and 85 - 135 g. per plant in C_0 , C_1 , C_2 , C_3 and C_4 , respectively. The frequencies of high yielding plants are increased by repeated cycles of selection Figure 2. The inbreeding F-statistic value revealed 0.916, 0.895, 0.895, 0.663 and 0.523 from C_0 to C_4 respectively, reflect the highly inbreeding coefficient inbase sorghum germplasm collection which was reduced later gradually through cycles of selection. Similar trend of gradually outcrossing increase was obtained after RS cycles (Figure 3).

Evaluated ofobserved heterozygosity for RSpopulations cycles under two environments revealed that (C_3 and C_4) in irrigated environment showed higher than rainfed environment (Figure 4).

Percentage of populations grain yield change over base population in improved cycles affected by the change in observed heterozygosity per populations, however the increase in observed heterozygosity lead to increased yield gradually from C_0 to C_4 population.

Yield potential of populations has been increased over base population by cycles especially under rainfed condition. Which is confirmed the highly efficiency of heterozygosity in yield potential under rained condition. Outcrossing rate, expected and observed heterozygosity revealed highly significant by nearly 384.40% under rained condition (Table 2) and 353.49% under irrigated condition, after four cycles of RS which is confirmed the highly efficiency of heterozygosity in yield potential. ($P < 0.01$) differences between populations in combined environment, Table 3. The families within P_2 , P_3 and P_4 revealed higher significant differences than families within P_0 and P_1 population for (tm), and (He) respectively. There was a highly significant differences ($P < 0.01$) between families in all populations for observed heterozygosity. So the (tm), (He) and (Ho) changed within families over population from P_0 to P_4 population.

DISCUSSION

The multi-locus outcrossing rate (tm) calculated from base cycles, progenies of population per cycle and families per cycles varied greatly. The estimates of

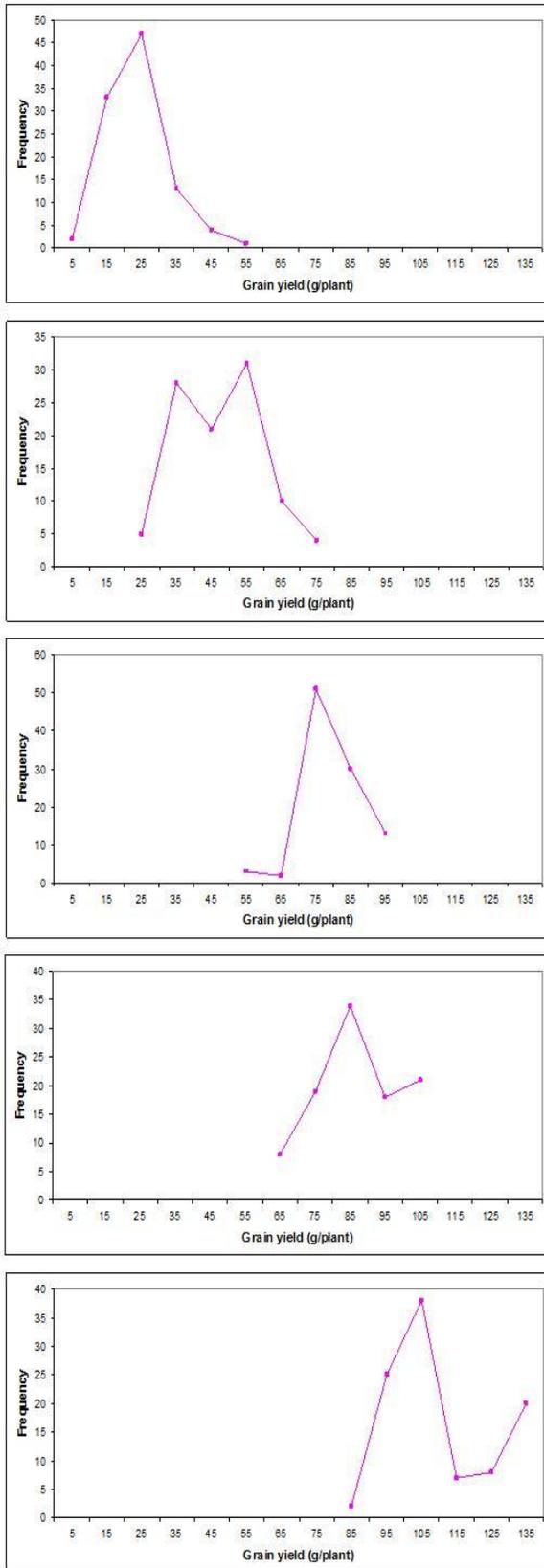


Figure 2. Change in grain yield across environments. Repeated cycles of RS (C_0 - C_4) in populations after four repeated cycles of RS (P_0 - P_4)

outcrossing rate (t_m) in four recurrent selection cycles, revealed 8, 9, 19, 34 and 48% for C_0 , C_1 , C_2 , C_3 and C_4 population respectively. On natural outcrossing of sorghum is limited in geographical reference varietal sorghum is no longer of genetic resource a viable to the sorghum. Evidence of natural outcrossing in sorghum is anecdotal rather than experimental base. In literature 18 to 77% outcrossing in an experimental line of Sudan grass produced in Pennsylvania, with an increase in cross-fertilization. In Cameroon, they grow numerous land races mixed in field, and calculated the multi-locus outcrossing rate from all progenies was 18% and ranged from 0 to 73% among progenies, outcrossing rate varied greatly among landraces from 5 to 40%. Previous studies, conducted under experimentally controlled conditions have shown that outcrossing rate (t_m) varied from 1 to 30%. Dje et al. (2004) calculated outcrossing rate in two farmers' fields in Morocco, estimating an outcrossing rate two times as high in one field (landraces of durra-bicolor intermediate type, $t_m = 16\%$) as in other (durra landraces $t_m = 7\%$). The change in yield increased as a percentage under rainfed environment presented (104.78, 254.81, 306.67 and 384.4%) from C_1 to C_4 populations respectively, and under irrigated environment presented (75.70, 226.62, 262.35 and 353.49%) from C_1 to C_4 populations respectively. Haussmann *et al.*, 1998, estimates the relative hybrid superiority for sorghum grain yield ranged from 32 to 36% with the highest estimate obtained under moderate stress conditions. An increasing relative superiority of sorghum hybrids over mid-parent values and varieties with increasing environmental stress). Kapran et al. (1997) reported heterosis values of 45% under irrigation and 66% in rainfed conditions. In the same test, hybrids out-yielded local controls by 61% with irrigation and by 49% under rain-fed conditions. Obi Lana and El-Rouby (1980) in Nigeria reported 38.4 and 40.4% increased grain yield in two populations over three cycles of mass selection. The selection response per cycle was 12.8 and 13.5% in these populations. They did not observe a significant associated response for maturity in their populations. Doggett (1968) proposed modified mass selection with alternating male-sterile (female) and male-fertile (normal) plant selection in successive generations to increase selection response by increasing parental control. Grain yield ranged from 5 to 55 g/plant in C_0 population, and from 25 to 75, from 55 to 95, to 105 and from 85 to 135 g/plant in C_1 , C_2 , C_3 and C_4 population respectively (Figure 4). Recurrent selection is applicable to both outcrossing and self-pollinated species and is a powerful procedure to accumulate desirable genes and facilitates breaking of linkage. The results of yield indicated that there is agreement with the predicted and observes response to recurrent selection for yield per plant and effected of outcrossing rate in increase heterozygosity. Until a method of producing hybrid sorghum theoretically the plant breeding had not increased yielding ability in

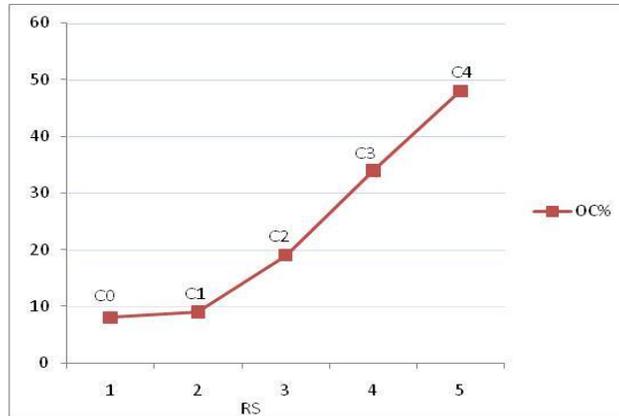


Figure 3. The percentage of outcrossing rate and the four (RS) cycles.

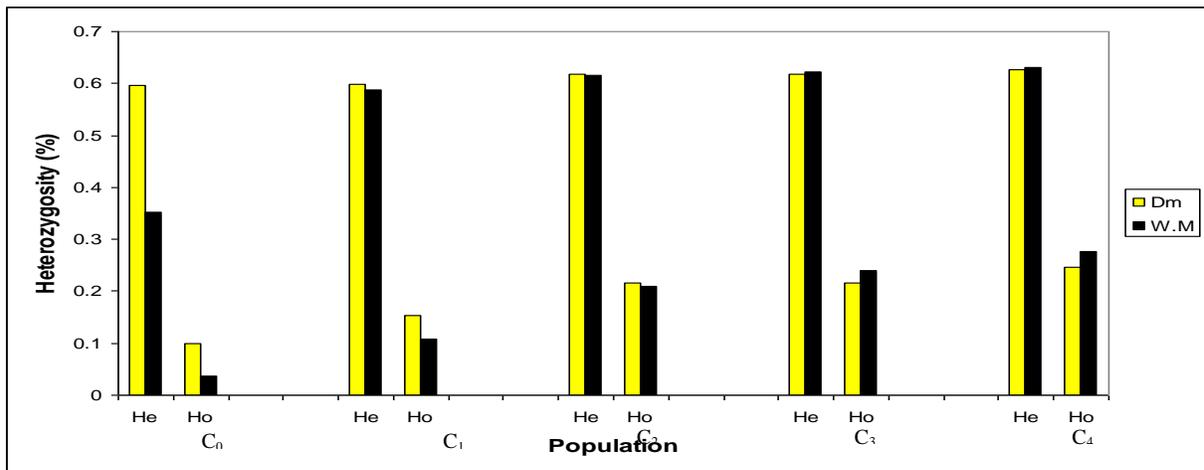


Figure 4. Estimated of (He) and (Ho) for four RS cycles in El-Damazin (Dm) and Wad Medani (W.M).

Table 2. The yield potential of four improved populations over base population under tow different irrigated and rained environments.

Environments	Populations			
	P1	P2	P3	P4
Irrigated	75.70	226.62	262.35	353.49
Rainfed	104.78	254.81	306.67	384.40

Table 3. Outcrossing rate (tm), expected (He) and observed heterozygosity (Ho), in five RS populations (P), across environments based on families per population.

Populations	tm	He	Ho
P ₀	0.152 b	0.474 c	0.068 e
P ₁	0.053 c	0.593 b	0.131 d
P ₂	0.280 a	0.617 a	0.213 c
P ₃	0.323 a	0.620 a	0.228 b
P ₄	0.351 a	0.629 a	0.262

sorghum and the recurrent selection methods utilizing both hand crossing and outcrossing mechanism like genetic male- sterility which have been proposed for selfing crops random-matingsorghum population production new genetic recombination's in large numbers with a minimum labor requirement. Highly significant in outcrossing rate, expected heterozygosity and observed heterozygosity between populations due to accumulation of allelic diversity through recurrent selection procedure. The population heterozygous is a function of two or more genotypes that, when grown together, have morphological or agronomic qualities that compensate for inadequacies found in each, this compensation effect when the heterozygous population is grown in diverse environmental conditions.

CONCLUSION

Significant higher outcrossing rates were found among improved sorghum populations ranging from 8 to 48% and increase in outcrossing rate led to significantly change in heterozygosity between populations and individuals depend upon genetic constitution of the base population for improving single trait for selecting several traits in population .The study revealed the usefulness of the markers in accelerating the selection process and increasing the efficiency and accuracy of constructing Heterotic population that are expected to have breeding advantage over the base line population. Molecular markers are suitable to specific monogenic or polygenic trait in germplasm and varieties within the breeding process. Promising material is generated for development of high yielding sorghum varieties. Improved sorghum populations have variability in heterozygosity could be used as material in sorghum breeding programs and recurrent selection in sorghum increased the frequency of favorable alleles for outcrossing in population and selection of suitable parents for developing new population is very important steps. Increase in hybridization percentage lead to clear increase in yield potential in sorghum.

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