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Out Crossing, Heterozygosis and Inbreeding with Environments Interaction in Recurrent Selection Sorghum Population's Progenies

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Abstract: **The progenies of five sorghum heterozygous populations' cycles were tested under main and off-season on two different environments irrigated and rainfall conditions for their outcrossing, heterozygosity and inbreeding coefficient using SSR markers, the marker combinations were optimized according to their fragment size. Multi-locus outcrossing rate (tm) and average single-locus (t_s) outcrossing rates were estimated using the MLTR software, and TFPGA computer program. The outcrossing rate effected directly by the temperature and relative humidity (RH) during the initial flowering period, which the low temperature with high RH under main season is revealed positive increased in outcrossing than off-season. Progenies outcrossing rate revealed same trend with main population outcrossing and the same trend was observed heterozygosity with decreased in inbreeding coefficient. Higher levels in outcrossing rate and heterozygosity was detected under rainfall environment in two based population progenies, but in three advanced population cycles the outcrossing rate was higher under irrigated than rainfed environment. Inbreeding coefficient revealed negative relation with outcrossing rate and heterozygosity in different population's progenies.**

Keywords: **environment, recurrent selection, outcrossing, sorghum, progenies.**

I. INTRODUCTION

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 . Ollitrault *et al*. (1997) found a mean value of t=0.19 for sorghum landraces from Burkina Faso belonging to the guinea race, based on progeny analysis. Using an indirect method based on the value of the inbreeding coefficient, the mean value of t=0.18 under field conditions in landraces from North-western Morocco grown according to traditional practices (Dje *et al*. 1999).Haussmann,(1995) investigate the effect of heterozygosity, heterogeneity and their interaction on the a daptedness of sorghum to a semi-arid area of Kenya and studied the relationships between various traits and between lines and hybrids, across environments, hybrid superiority over lines was 54% for grain yield, 1.5 units for the drought response index, 35% for above-ground dry matter, 3% for the harvest index, 38% for plant height, 6-19% for the grain yield components, -3 days for both days to an thesis and days until the four lowest leave were dry (stay-green), and -0.4 units for the leaf rolling score (scale from 1 to 9). Heterozygosity has a strong positive effect on yield stability, in other words, inbred lines of outcrossing are unstable . Heterozygosity may improve yield stability, but to a lower extent than in purely outcrossing species .Various methods have been proposed to estimate outcrossing rates in mixed mating populations employing genetic markers. Ritland and Jain (1981) as well as Brown *et al*. (1978) used progeny arrays (families) to estimate the outcrossing rate of maternal parents in barley. Enjalbert and David (2002) described a maximum-likelihood procedure to estimate the season-specific outcrossing rate in the parent generation and the average outcrossing rate in the parent and previous generations based on the analysis of multilocus heterozygosity in a single generation assumed to be in inbreeding equilibrium (Brown and Allard 1970). Male fertility of cereal crops is a function of pollen production and viability and can be strongly influenced

by the environment . Problems with male fertility can result in reduced seed set and yield potential in grain and seed production (.Tuinstra M.R and Wedel J. 2000). Sorghum flowering begins within 3 days of emergence of the panicle from the booting stage i.e. it start at or near the apex and proceeds towards the proximal end of the panicle, being complete in 4 to 7 days. Schertz and Dalton (1980) reported that stigmas are receptive up to 2 days before blooming and can remain receptive for up to 16 days in the absence of pollination. Anthesis usually occurs after sunrise but has been noted during the night hours, even as early as 10 P.M. (Stephens and Quinby, 1934). Viable pollen, which is wind blown, is shed until about noon. Gieger *et al*. (1994) suggested recurrent selection for outcrossing related floral traits such anther extrusion and anther and stigma size as a possible approach. Male fertility of sorghum varies from day to day under normal field conditions. Adverse environmental conditions can result in reduced pollen fertility (Bandy-opadhyay *et al*. 1998). Research has indicated that variation in temperature, humidity, and cloud cover can influence pollen production and viability (Artschwager and McGuire, 1949). The study of variability within species is important to all biologists who use genetic markers. Since the discovery of molecular variability among normal individuals, data have been collected from a wide range of organisms, and it is important to understand the major factors affecting diversity levels and patterns. Comparisons of inbreeding and outcrossing populations can contribute to this understanding, and therefore studying plant populations is important, because related species often have different breeding systems. DNA sequence data are now starting to become available from suitable plant and animal populations, to measure and compare variability levels and test predictions (Deborah Charlesworth., 2003). Breeding system differences, such as differences in self-fertilization rates, are probably among the factors with major effects on genetic variability; clear enough to be discernible even in the presence of other factors. Baker (1953) was one of the first to recognize that inbreeders often have lower genetic variability than outcrossing species, and that variability tends to be chiefly found between populations in inbreeding species rather than within them (Baker., 1953). The amount of diversity and differences in diversity patterns (the distribution of variants between individuals and populations), and this helps an understanding of the effects of inbreeding on diversity patterns only. The frequency of homozygotes is increased, but initially neutral variants should not be lost. However, the rarity of heterozygotes means that variants are less likely to be maintained by over dominance (heterozygote advantage) in inbred populations, and these would be expected to be lost within relative small number of generations of inbreeding (Kimura & Ohta., 1971; Charlesworth *et al*., 1995). In longer term, inbreeding represents an important deviation from panmixia, and has several effects that can strongly affect amounts of diversity. First high homozygosity reduces effective size relative in to or out breeding populations with the same number of individuals. Second, inbreeding reduces the effective frequency of recombination throughout the genome. Finally, inbreeding increases isolation between individuals and populations (Charlesworth 2003). High rates of self-fertilization are often found in geographical marginal Rick *et al*. 1977; Schoen, 1982) or ecologically marginal populations (Allard *et al*. 1970; Brown *et al*). In several studies, adult plants were significantly less inbred than their seeds, implying heterotic selection during the course of development.

The main objective of this study to estimates the progenies out crossing in recurrent selection (RS) sorghum population, and the change in inbreeding with change in outcrossing under different environments.

II. MATERIAL AND METHOD

One hundred preferred feterita type sorghum landraces (PFSLR) of early maturity taken from Agricultural Research Corporation (ARC) gene bank, mainly chosen from the Sudanese/Sahelian zone in Sudan and ecological similar neighboring regions. All field work conducted at the Gezira Research Station (GRS) of ARC at Wad-Medani, Sudan, for main cycles. Seeds planted in small pots filled with soil in July for main-season and in January or December for off-season. All established plants sampled in the two-leaf stage for DNA extraction and 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 recurrent selection (RS) cycles. 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. A total of 15 genomic SSR markers (Brown et al., 1996) were used, the markers were combined 29 times in deferent triplex and duplex combination to reduce the number of PCR reaction needed for individual SSR analysis. And the marker combinations were optimized according to their fragment size. Multi-locus outcrossing rate (tm) and average single-locus (t_s) outcrossing rates were estimated using the MLTR- version 3.2 software (Ritland,2002). Five random-mating sorghum populations (total of 500 individual

line per population) derived from four recurrent selection cycles from (C_0 to C_4 populations) with different levels of heterozygosity and outcrossing rate tested under two different environment in Sudan in two different locations during rainy season 2009/2010, one location under completely irrigated condition (Wad Medani) and the other one under completely rainfed condition (Damazin).

III. RESULTS

Multi-locus outcrossing rate (tm) was estimated in four RS based cycles using 3200 individual progenies employing the MLTR software (Ritland and Jain 1981; Ritland 2002, 2008). Considerable changes in outcrossing rate were obtained by repeated cycles of selection. The based cycles estimated levels of outcrossing were 0.08, 0.09, 0.19, 0.34 and 0.48 for cycles C_0, C_1, C_2, C_3 and C_4 respectively. Moreover, decreased in families maternal inbreeding coefficient (F_f) led to increase in heterozygosity level (i.e. heterozygosity was increased) by repeat cycles of selection, table (1). Temperature and relative humidity revealed considerable differences during the flowering initial period in main- season and off-season during based cycles. Generally, in main-season the mean temperature was lower than off-season, and on the other hand the percentage of relative humidity was higher in main-season, Table (2). The mean of temperature at initial flowering period recorded was 29C° with relative humidity 62.8% in main-season, 2006 for C_0 population.

Table (1): Outcrossing rate, observed heterozygosity and inbreeding coefficient (F_f) in four RS based cycles

Cycles	tm (SD)	Ho	F_f (SE)
C_0	0.08 (0.019)	0.0683	0.916 (0.035)
C_1	0.09 (0.016)	0.0625	0.895 (0.019)
C_2	0.19 (0.015)	0.1580	0.895 (0.019)
C_3	0.34 (0.010)	0.2810	0.663 (0.026)
C_4	0.48 (0.009)	0.4225	0.523 (0.022)

Next cycle (C_1) was conducted in off-season, February, 2007 and the mean of temperature recorded during flowering period between April and May was very high (35.1C°) with low relative humidity percentage 22.8%. To escape this period of high temperature in off-season at flowering time in next advanced cycles, we planting our material under net house early in late December to initiate the flowering period in March for C_2, C_3 and C_4 populations, Table (2). Progenies outcrossing rate was changed from 0.001 in P_0 to 0.266 in P_4 and from 0.001 to 0.260 under rainfed and irrigated environment, respectively. In consequence, heterozygosity (Ho) level elevated from 0.1 in P_0 to 0.2467 in P4 and from 0.0367 to 0.2767 in rainfed and irrigated environment, respectively Table (4). In general, higher levels of outcrossing rate and

Table (2): Temperature and relative humidity (RH %) during RS cycles.

Cycles	Season	Sowing date	Initial flowering month	Mean of temp. C°	Max. of temp. C°	Mini. of temp. C°	RH%
C_0	Main-2006	Jul – 2006	September	29.0	36.0	23.5	62.8
C_1	Off – 2007	Feb.-2007	April-May	35.1	42.9	26.0	22.8
C_2	Off – 2008	Des.-2007	March	30.8	40.8	20.5	22.1
C_3	Main–2008	July- 2008	September	28.9	36.6	22.9	55.6
C_4	Off - 2009	Des- 2008	March	29.5	39.4	20.1	20.6

Table (3): Number of families and progenies per cycle (F,P), SSR markers used for analysis, observed heterozygosity (Ho) and inbreeding coefficient per locus (F_L).

C	F,P	SSRm	Ho	F_L
C_0	100,	Sb 1-10	0.0400	0.8871
	2538	Sb 6-36	0.0300	0.9080
		Sb 4-15	0.0000	1.0000
		Sb 6-84	0.0000	1.0000
C_1	200,	Sb 1-10	0.0300	0.6484
	3200	Sb 6-36	0.0400	0.8984
		Sb 4-15	0.0000	1.0000
		Sb 6-84	0.0100	0.9570
C_2	200,	Sb 6-342	0.0200	0.7570
	3200	Sb 6-34	0.0250	0.7556
		Sb 5-206	0.0150	0.8031
		Sb 1-10	0.1000	0.8401
		Sb 6-36	0.0200	0.9040
C_3	200,	Sb 6-342	0.1300	0.6000
	3200	Sb 6-34	0.1200	0.8433
		Sb 5-206	0.0100	0.6910
		Sb 1-10	0.0150	0.6910
		Sb 6-36	0.0200	0.7188
C_4	200,	Sb 6-342	0.2100	0.5900
	3200	Sb 6-34	0.0700	0.5593
		Sb 5-206	0.1200	0.5334
		Sb 1-10	0.0600	0.5514
		Sb 6-36	0.0350	0.6117

C= Cycles.

heterozygosis were detected in rainfed environment compared with irrigated environment in P_0 and P_1, while in P_2, P_3 and P_4 the outcrossing were higher under irrigated than rainfed environment (figure 1). Similar trend was detected for observed heterozygosis in both environments. Under rainfed environment the base and first Cycle of RS showed higher heterozygosis than irrigated. Inbreeding coefficient estimates per locus (FL) (Table 3), and inbreeding coefficient estimates for progenies generations (Fp) (Table 4), and for families, generally similar and had same changed sequences trend from C_0 to C_4 populations. Over the three part of inbreeding coefficient above, the inbreeding coefficient decreased from C_0 toC_4.

Inbreeding coefficient estimates for progenies generations (*F*p) (Table 4), inbreeding coefficient per locus (F_L) (Table 3) and for families (*F*m) (Table 2), generally similar and had same changed sequences trend from C_0 to C_4 populations. Over the three part of inbreeding coefficient above, the inbreeding coefficient is very high in base families for C_0population over cycles, locus and progenies within populations which the F-statistic value revealed 0.916, 1.000, and 0.918 respectively, which revealed that the sorghum germplasm collection is highly inbreeding coefficient over all accessions, in same time the average proportion of observed heterozygosity is very low in base material of cycles which is revealed 0.0683 in C_0population and 0.000 in mean of observed heterozygosity per locus Table (3).

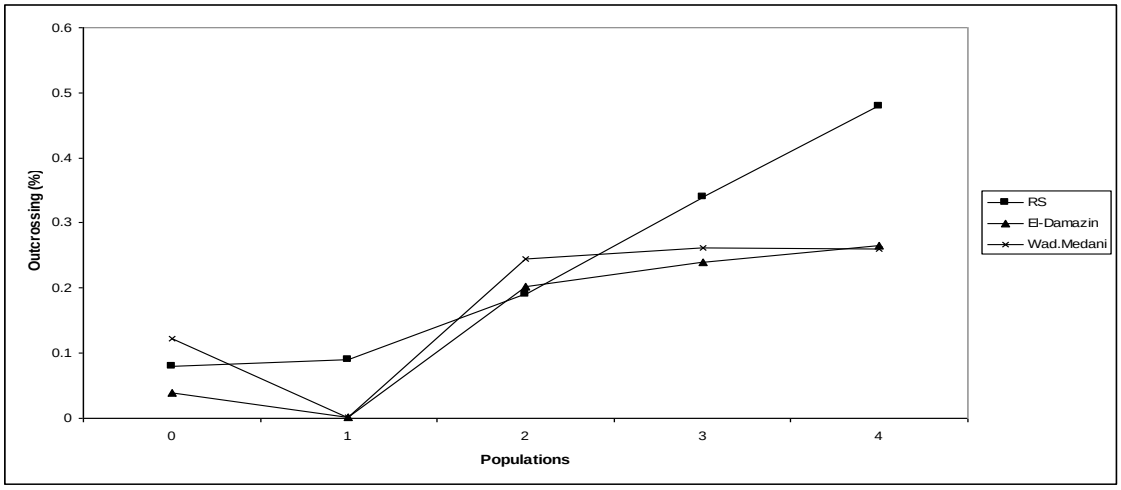

Figure (1): The outcrossing rate during (RS) and evaluation of all RS populations in two environments El-Damazin and Wad Medani in 2009.

Table (4): Estimation of multi-locus outcrossing rate (tm) and single locus outcrossing rate (ts), average of observed heterozygosity (Ho) and progenies inbreeding coefficient (F_p) among five sorghum RS populations:

Population	environment	tm	ts	tm-ts [a]	Ho	F_p
P_0	Rainfed	0.0390	0.022	0.017	0.1000	0.844
P_0	Irrigated	0.1230	0.077	0.045	0.0367	0.918
P_1	Rainfed	0.0010	0.001	0.000	0.1533	0.844
P_1	Irrigated	0.0010	0.001	0.000	0.1080	0.782
P_2	Rainfed	0.2030	0.129	0.073	0.2167	0.713
P_2	Irrigated	0.2440	0.137	0.107	0.2100	0.680
P_3	Rainfed	0.2400	0.135	0.105	0.2167	0.669
P_3	Irrigated	0.2620	0.152	0.111	0.2400	0.638
P_4	Rainfed	0.2660	0.154	0.112	0.2467	0.628
P_4	Irrigated	0.2600	0.150	0.110	0.2767	0.581

[a] the differences between multilocus and single locus outcrossing rate.

IV. DISCUSSION

Reduction in outcrossing rate in P_1progenies population compared with C_1population in both environment, (Figure 1), and also reduction in outcrossing families with C_1population due to different temperature between main and off season, and relative humidity at flowering period, (Table 2). It's revealed there was increase in mean, maximum and minimum temperature with decrease in relative humidity in off season compared with main season which is revealed lower temperature with a high relative humidity. In C_1 population the later sowing date in February lead to facing a higher temperature with low relative humidity at the initial flowering period between April and May effected directly on pollen grain viability and of course in the magnitude of outcrossing rate. To escape this period in next off-season (C_2population) the material grown early in December and keep seedling under Net house for one month to reduce the long day period and synchronize the lower temperature in flowering period in March. Off season delayed flowering and duration of maturity by about a month compared to the main season, also the off season resulted in relatively shorter plants with lower

productivity. Environment usually influenced on the male fertility and pollen viability and production of cereal crops. Environments can result in positive or negative in seed setting and yield potential in grain and seed production fields. Male fertility of sorghum varies from day to day under normal field condition can result in reduced pollen fertility and predispose sorghum to ergot infection (Bandy-opadhyay *et al*. 1998). The results of research have indicated that variations in temperature, humidity can influence pollen production and viability. Stress-induced aberrant starch deposition profiles in microspores resulted in reduced male fertility across various crops including rice (Sheoran and Saini.1996), wheat (Dorion *et al*. 1996), barley (Sakata *et al*. 2000), sorghum (Jain and Aloni. 2002). In rice the impact of increased air temperature is critical to reproductive development and the most sensitive stage is at heading especially at the time of anthesis (Weerakoon *et al*. 2008). Increased air temperature causes grain sterility. Cold temperature stress in sorghum prior to flowering appears to reduce pollen viability during anthesis by disrupting meiosis during early stages of microsporogenesis (Brooking, 1979). The physiological effects of high temperature stress, humidity, and cloud cover on fertility of sorghum are not as well characterized, and pollen viability can be quantified by several different procedures (M.R.Tuinstra., and J.Wedel, 2000). The observed heterozygosity among loci ranged from 0.00 to 0.21 and the expected heterozygosity changed from C_0 to C_4population and effect on the percentage values of polymorphic loci. (Holtsford & Ellstrand., 1989) the outcrossing rate for eight accessions of *Clarkia tembloriensis* indicate that this annual plant species has a wide inter-population range of outcrossing rate (tm = 0.3 – 0.87). Populations outcrossing rate estimates were significantly correlated with observed heterozygosity estimated fixation, *F*, for most populations were very close to their expected values, F_{eq}, given outcrossing rate. The independent segregation of alleles at the marker loci is an important assumption for the unbiased estimation of multi-locus outcrossing rates (Ritland and Jain., 1981), sample variance of observed heterozygosity (Archie, 1985) and sample variances of multi-locus co ancestry coefficient .The increase in average polymorphism per locus due to increase in the observed heterozygosity per locus and number of alleles per locus. Inbreeding was decreased gradually from C_0populalation to C_4populations per cycles and locus. Population heterozygosity level effect by increased in inbreeding and decreased frequency of heterozygote in population. Selfing affects heterozygosity directly, the relationship between outcrossing and observed heterozygosity stronger than that of outcrossing with number of alleles per cycles. The direct effect of outcrossing rate on average heterozygosity is clear; one should interpret the correlation between outcrossing and number of alleles. Sorghum is a primarily inbreeding, genetically homogeneous species comprising a relatively small number of alleles [up to 12 alleles per SSR marker, including rare alleles (frequency less than 5%)]. The high level of allelic variability but low levels of heterozygosity observed in study correspond with a previous SSR marker study of five Guinea-race accessions revealed an observed heterozygosity (Ho) of 0.089, with average expected gene diversity (He) of 0.224. These figures nicely comply with the observations among the 100 Guinea-race sorghum accessions (Rolf *et al.*, 2005). In our study the values mean of heterozygosity per locus and heterozygosity per cycles revealed increase by increase in outcrossing, this fact that in Caudatum-race sorghum the inbreeding decreased with high level of observed heterozygosity and high level of allelic variation and increase in outcrossing of populations lead to have greater allelic diversity and more heterozygosity which lead to expect variation between recurrent selection populations. The observed heterozygosis (Ho) in four base recurrent selection cycles ranged from 0.0625 to 0.4225 in C_0 and C_4population respectively and significantly correlated with outcrossing rate. Change in observed heterozygosity affected by change in outcrossing in C_0 to C_4populations. Outcrossing populations are expected to have high allelic diversity, higher levels heterozygosis, and low differentiation within populations compared with self-populations.

V. CONCLUSIONS

Outcrossing rate in sorghum influenced by environmental factors spatially temperature and relative humidity, lower temperature with high relative humidity usual effected in increased outcrossing but the increased in temperature with low relative humidity in long day light period usually reduced outcrossing and delayed flowering time by one month.

The increased outcrossing and heterozygosity lead to decreased population inbreeding coefficient per locus in progenies and families within population under both rainfed and irrigate environment. Inbreeding coefficient estimates per locus, and for progenies generations, and families, generally similar and had same changed sequences trend among populations. Over the three part of inbreeding coefficient above, the inbreeding coefficient is very high in based families, locus and progenies within populations.

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