

Natural Outcrossing and Distance of Isolation in Cotton (*G. hirsutum*) under Gezira Conditions

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Abstract

The extent of the natural crossing in cotton (*Gossypium hirsutum* L.) was studied in two experiments conducted at the Gezira Research Farm during 2001-2003 using an okra leaf-shape as a genetical marker. Varieties Sudac- k (okra leaf- shape) and Barac (67)B (normal leaf-shape) were used as a marker and test variety, respectively. In both experiments, the marker variety, Sudac-K was sown in form of a block of 10 m² in the middle, while the test variety, Barac (67)B was sown in form of a block and strips in the first and second experiments, respectively. The effects of six distances from the marker variety, four directions and three positions of bolls on the plant, on cross-pollination were investigated. The main effects of the three factors on cross-pollination were significant. The ranges of outcrossing throughout the treatments were 0 - 1.33% and 0 - 3.32% for the first and second experiments, respectively. Natural crossing percentages were higher when the test variety was sown in strips than when sown in full blocks. In both experiments, the mean percentage of natural crossing reached zero value at the distance of 15 m from the marker across directions with the exception of the East where it reached zero value at 30 m. Bolls harvested from the lowest part of the plant gave the highest mean percentages of outcrossing. It is quite evident from the current study that distances greater than 30 m can effectively prevent natural crossing and preserve cultivar purity.

Introduction

Cotton (*Gossypium spp.*) is a self - pollinated crop and the pollen is directly shed on the stigma but sometimes carried by insects to cause some natural cross pollination. Insects are the major cross - pollinators of cotton since its pollen is heavy and sticky. Pollen dispersion by wind is almost zero (Niles and Feaster, 1984).

Bees are the main insect pollinators of cotton. The activity of these pollinators is greatly affected by the use of insecticides. So the species, cultivar, population, environment, the activity and flying pattern of the insect pollinators influence the percentage of natural cotton outcrossing (Poehlman and Sleper, 1995; Stewart, 1995). The examination of the literature revealed that the extent of natural crossing in cotton varied from zero to 50 % (Poehlman and Sleper, 1995; Xanthopoulos and Kechagia, 2000). Afzal and Khan (1950) reported the occurrence of about 2 percent natural crossing within each of two species of cotton grown in Pakistan, namely, *G. arboreum* and *G. hirsutum*. In Egypt, Galal *et al.* (1972) reported an average of 3.0%. In USA, Simpson and Duncan (1956) reported 47% outcrossing in all sampling points at

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Tennessee in 1954. Meredith and Bridge (1973) reported that outcrossing ranged from 0 to 5.9 % at 5 sites in the central Mississippi River Delta. Umbeck *et al.* (1991), using a kanamycin resistance marker gene to test for seeds resulting from pollen movement out of the central transgenic plot, found 5.0 - 5.7 % outcrossing in subsequent rows, and as the distance progressively increased the percentage dropped rapidly. In Australia, Liewellyn and Fitt (1996) found that outcrossing in 1m distance among transgenic and non – transgenic cottons was 0.15 – 0.4 % and a 20m buffer zone could serve to limit dispersal of transgenic pollen from small scale field tests. In Greece, Christidis (1965) reported 4.7 – 9.7 % outcrossing, which was greatly affected by the distance; from about 7.5 % in adjacent rows; it decreased to 1.4 % after 4 rows spacing (4m), and after 20m it fell almost to zero.

Xanthopoulos and Kechagia (2000), in Greece, conducted two experiments using glandless and red – leaf traits as genetical markers to determine the extent of outcrossing in cotton. They found that, in the first experiment where estimation of natural crossing was based upon gland status, the percentage ranged from 1.67 % to 2.67 % in adjacent rows, dropped to 1.42 % in plants 2m apart, and declined to almost zero after 10m. In the second experiment, where the red leaf marker gene was used, the mean of natural crossing was 3.85 % in adjacent rows, dropped to 2.79 % in plants 2m apart, and progressively diminished to 0.31% after 10m. They concluded that distances greater than 10m among cottons were sufficient to minimize outcrossing ranges.

Contamination of seed crops resulted from mechanical admixture and cross – pollination. Contamination resulting from natural out crossing is important in plant breeding, and seed production and multiplication programs. Its extent should be determined at specific locations and seasons periodically because it is greatly affected by species, variety, weather, and insect population, i.e. the environment.

Natural outcrossing necessitates isolation requirements for seed multiplication of different crops. Galal *et.al.*(1972) stated that since the percentage of natural outcrossing of cotton in Egypt was low (3%), the expected degree of contaminated seed will be low. Hence, cross pollination need not be a serious source of impurity in elite breeding material if proper control of the nursery and of the seed is observed. Furthermore, it is believed that the isolation distance of 1km adopted in Egypt can be drastically reduced without fear of contamination.

Xanthopoulos and Kechagia (2000) reported that the cotton isolation distances of 8.05 to 16.09 km have become progressively smaller in most cotton seed production countries. In Europe, the isolation requirements are 400m for certified cotton seed production. This is also considered to be improper because of changes in ecological conditions, advances in cotton culture, heavy use of insecticides and the large increase in the number of cultivars released.

Varietal mixing of cotton cultivars in the Sudan is a complaint of cotton growers as well as cotton breeders. Information on the extent of natural outcrossing of cotton is meager under Gezira conditions where most of the released crop varieties were bred. Such a fact necessitates conducting the current study to estimate the extent of natural outcrossing in cotton under the conditions of the Gezira Scheme, the Sudan main cotton producing area and recommend distance of isolation.

Material and Methods

To determine the extent of natural crossing in cotton under Gezira conditions, two experiments were carried out at Gezira Research Station (14° 24' N, 33° 29' E and 407 masl), Agricultural Research and Technology Corporation (ARTC), Wad Medani, Sudan. The experiments were sown during the second week of July. Two local varieties, Barac (67)B (normal leaf-shape) and Sudac-K (okra leaf-shape), were used to estimate the percentage of natural cross-pollination. Both varieties belong to *Gossypium hirsutum*, Acala group and are medium staple varieties. The okra leaf-shape of Sudac-k was used as a morphological marker to assess the percentage of natural outcrossing in Barac (67)B which has normal leaf-shape. F1 crosses were made between Sudac-K and Barac (67)B to confirm the validity of the marker. The 50% flowering time of both varieties coincide and okra leaf-shape trait is dominant to normal leaf-shape character.

In 2001-02 season an area of 10m² of each variety was sown in the middle of the other variety, i.e. 10m² of Barac (67)B was surrounded from all directions by Sudac - k and vice versa. In each direction (north, south, east and west), three samples of three bolls each, were taken randomly at maturity, at six different distances away from the marker variety, namely at 5, 10, 15, 20, 25 and 30m. Seeds were collected after hand ginning and sown in 2002-03 to score natural outcrossing as indicated by plants with okra leaf shape in Barac (67)B.

In 2002-03, a second experiment was carried out with the same objective. In this experiment, Sudac - k was planted in the middle of the test variety, Barac(67)B, in an area of 10m². Barac (67)B was sown at six different distances (5, 10, 15, 20, 25 and 30 m) in four different directions (north, south, east and west). At maturity, four plants were selected randomly from each distance for examination (Fig 1). Each plant was divided into lower, middle and upper regions. From each region, three bolls were harvested randomly and ginned for seeds collection. These seeds were grown in 2003/2004 to estimate the cross-pollination percentages in Barac (67)B. The percentage of cross-pollination was affected by three factors, namely, direction (north, south, east and west), distance from the marker (5, 10, 15, 20, 25 and 30m) as well as position of the bolls on the mother plant (lower, middle and upper plant regions).

During the time span of these experiments, meteorological information was recorded on wind direction and speed, maximum and minimum temperature, relative humidity and rainfall. (Table 1).

Insect counts were made just before and after spraying with endosulfan (Table 2). Spraying started on 30.9.2001 and 27.8.2002, in 2001-02 and 2002-03 seasons, respectively.

The data was transformed using the square root transformation ($\sqrt{y + 0.5}$) since the data was less than 10 and contained a lot of zeroes. The transformed data was statistically analyzed using a completely randomized design.

Results

The percentage of natural cross-pollination must be determined in self-pollinated crops like cotton to prevent genetic mixing. Genetical markers are used for this purpose. In the present study the okra leaf-shape of the cotton variety Sudac - k

was used as a morphological marker to assess the percentage of natural crossing in Barac (67)B which has normal leaf-shape.

In the 2001-02 experiment where the two varieties were sown in such a way that each variety was surrounded from all directions by the other variety, measuring the 50% flowering time showed that their flowering curves coincide. Their mean 50% flowering time was 60 days. Misaka (2003) found that the mean 50% flowering time for these varieties was 61 days and designated them as early maturing varieties.

In 2001-02 manual crossing was made between Sudac – k and Barac (67) B. All the resulting F₁ plants showed okra leaf-shape indicating the fact that okra leaf-shape is at least partially dominant over normal leaf-shape. The F₁ plant was clearly differentiated from the parents with the deeply five-lobed okra leaf.

Percentage of plants, grown from natural seeds collected from Barac (67)B, with okra leaf-shape were given in Table 3, as affected by direction (north, south, east and west) as well as distance (5, 10, 15, 20, 25 and 30m) from the marker variety Sudac-K.

Mean natural crossing percentage ranged from zero to 1.33% (Table 3). Outcrossing percentage reached almost zero at the distance of 15m in all directions except the east. In the eastern direction outcrossing continued up to the distance of 25m with a low percentage of 0.33. The highest mean percentage of outcrossing according to direction across distance was shown in the east (0.88%) while the lowest was depicted in the south and west (0.23% and 0.24%, respectively). Over all directions, cross – pollination decreased as the distance from the marker variety increased, i.e. 0.63% at 5m and 0.08% at 25 m.

In 2002-03 experiment, the main effects, on natural crossing, of direction, distance from the marker variety and position of the harvested boll on the mother plant were highly significant (Table 4). Only the interaction effect of distance × position on natural pollination percentage was significant. In general, the mean natural crossing percentage ranged from zero up to 3.32 (Table 5). At the distance of 15m from the marker, natural crossing decreased to zero but increased slightly to 0.83% and 0.33% at 20 and 25m, respectively, to return to zero% again at the distance of 30m (Table 5).

When bolls were harvested randomly from three positions on the plant (low, medium and high) those harvested from the low position gave significantly higher percentages of outcrossing irrespective of the distances from the marker (Table 5).

At closer distances of 5 and 10m, the low boll position gave higher values of 2.78% and 3.36% of outcrossing compared to 0.30% and 0.15% outcrossing at medium position.

The eastern direction gave the highest mean percentages of crossing across position and distance (Table 6). Its mean was more than double of those at other directions (1.45%). Even within the eastern direction, the highest mean of outcrossing (2.62%) was at low boll position.

The interaction effects of direction and distance on mean percentages of outcrossing occurred mainly at the distances of 5 and 10m except in case of the eastern direction where it continued up to the distance of 25m (Table 7). At the northern, southern and western directions, there was no outcrossing after the distance of 10m from the marker but it continued up to 25m in case of the eastern direction.

Discussion

In 2001-02 experiment outcrossing range was 0 – 1.33% while it was 0 – 3.32 in 2002-03 experiment. Generally the values of mean percentage of natural crossing were higher when the test variety was grown in strips (Tables 3 and 4). This is in accordance with the results of Xanthopoulos and Kechagia (2000) in Greece who found that mean natural crossing of cotton was 3.24 at the corners and 2.21% at the center of the same field. This is expected since insect pollinators and wind movements are higher at the corners than in the field center.

In both experiments, the mean percentage of natural crossing reached a zero value at the distance of 15m from the marker across directions with the exception of the east where it reached that value at 30m. It decreased progressively with increasing distance from the marker. Many researchers, e.g. Christidis (1965) in Greece, Galal (1972) in Egypt, Liewellyn and Fitt (1996) in Australia, Xanthopoulos and Kechagia (2000) in Greece stated that mean percentages of natural crossing of cotton decreased with increasing distance from the marker and fell almost to zero at 20m distance. The variation in their findings was attributed to differences in environment, markers used, test varieties, populations as well as the activity of pollinating insects.

The fact that the eastern direction gave the highest mean percentages of crossing and at almost all distances from the marker is very difficult to explain. This is because the pollen of cotton is heavy and sticky and pollen dispersion by wind is almost zero (Niles and Feaster, 1984). Even if the wind is a pollinating agent, its direction was S/SW during August and September and N/NE during October – January (Table 1). The major cross- pollinators of cotton are insects, particularly bees. Our close observation and insect count (Table 2) during the time span of these experiments showed that the insects count and activity was very low due to the use of insecticides. It reached zero values immediately after spraying. Even if the cotton pollen vectors activity is normal, why the eastern direction only? The experiments were sown far away from trees (>30m) and/ or bee colonies.

In general the relatively low values of natural outcrossing attained under the conditions of these experiments compared to that reported by Galal (1972) of zero to 11.9% or Umbeck *et al.* (1991) of zero to 5.7%, were attributed to low insect activity. However, these values are quite comparable to those reported by Xanthopoulos and Kechagia (2000) of zero – 2.79%.

Bolls harvested from the lowest part of the plant gave the highest mean percentages of out crossing irrespective of distance or direction. A fact that can be explained on the basis of the idea that such bolls open and mature early and have a longer time span for exposure to introduced pollens compared to those produced at medium or upper part of the plant.

The current study depicted quite clearly that distance from the marker is the main determinant factor of outcrossing. Practically a distance of 15m from the marker is quite enough to prevent outcrossing when the foreign pollen is going to north, south or west. But if the test variety is lying east of the source of the foreign pollen, an isolation distance of 30m is needed under the conditions of the Gezira Research Station, Wad Medani, Sudan, where almost all the Sudanese cotton varieties had been released.

Recommendation

Based on the presented results, a minimum distance of 30 m is recommended for isolation between different cotton varieties or breeding lines to prevent natural out crossing in breeding and seed propagation fields.

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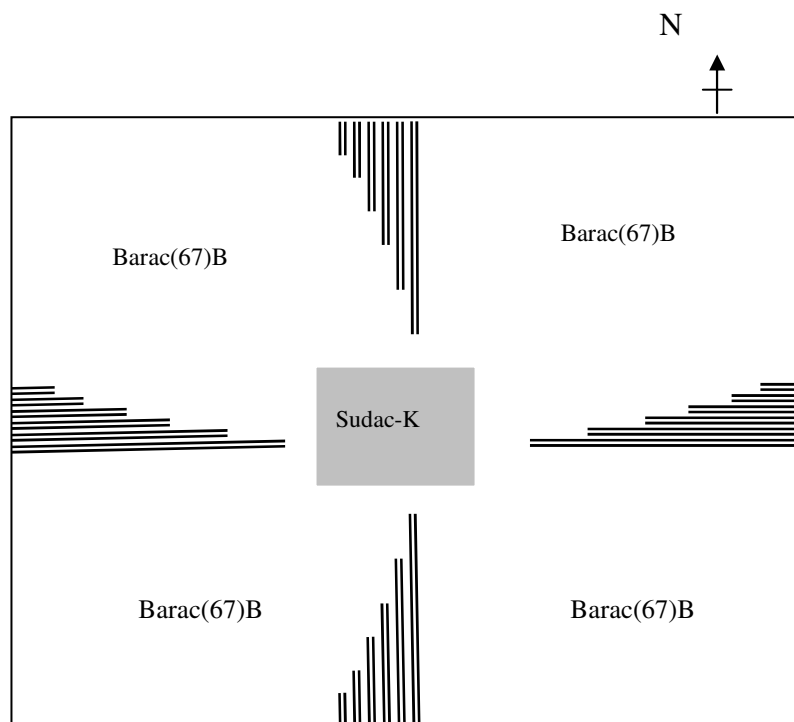


Fig.1. Layout of outcrossing experiment. Bars indicate sampling positions at 5, 10, 15, 20, 25 and 30 m distances from the marker genotypes (Sudac-K).

Table 1. Monthly means of selected meteorological data during 2001-02 and 2002-03 seasons at Wad Medani meteorological station.

Month	Temp. (C°)		Relative humidity (%)	Rainfall (mm)	Wind direction	Wind speed (knot)
	Max.	Min.				
<u>2001-02</u>						
August	34.1	22.7	82	33.3	S/SW	14
September	36.7	22.6	76	50.1	S/SW	6
October	38.9	22.0	59	05.7	N/NE	6
November	37.7	18.8	45	0.4	N/NE	4
December	35.7	17.5	47	Nil	N/NE	3
January	31.5	13.2	43	Nil	N/NE	3
<u>2002-03</u>						
August	35.9	22.8	76	84.3	S/SW	4
September	35.5	25.7	75	92.1	S/SW	3
October	38.9	27.9	54	16.1	N/NE	4
November	38.1	20.1	43	Nil	N/NE	5
December	33.4	14.6	39	Nil	N/NE	4
January	34.8	15.5	41	Nil	N/NE	3

Table 2. Count of insect (insect /100 plants) on 27.8.2002 at Gezira Research Station.

Insect	Direction			
	North	South	East	West
White fly	50	87	82	87
Jassid	177	187	137	119
American boll worm	7.5	15	0	24
Aphids	0	0	15	7.5

Table 3. Effect of direction and distance on mean natural crossing percentages in Barac(67)B using Sudac-K as a marker at Gezira Research Station, season 2001-02.

Distance (m)	Direction				Mean
	North	South	East	West	
5	1.20	0.00	1.33	0.00	0.63
10	0.70	0.90	0.76	0.96	0.83
15	0.00	0.00	1.10	0.00	0.28
20	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.33	0.00	0.08
30	0.00	0.00	0.00	0.00	0.00
Mean	0.32	0.23	0.88	0.24	

S.E.(±) 0.07, 0.06 and 0.14 for distance, direction and distance × direction, respectively.

Table 4. Mean squares of natural crossing percentages as affected by direction, distance and position for cotton grown at the Gezira Research Station (GRS), season 2002-03.

Source	Degrees of freedom	Ms
Direction	3	1.105**
Distance	5	1.431***
Position	2	3.341***
Direction × Distance	15	0.204
Direction × Position	6	0.177
Distance × Position	10	0.586*
Direction × Distance × Position	30	0.402

*,**,*** Significant at the probability levels of 0.05, 0.01 and 0.001, respectively.

Table 5. Percentages of cotton natural crossing as affected by distance and position for a crop grown at Gezira Research Station (GRS), season 2002-03.

Position	Distance (m)						Mean
	5	10	15	20	25	30	
High	0.16	0.85	0.00	0.00	0.40	0.00	0.31
Medium	0.30	0.15	0.00	0.00	0.00	0.00	0.08
Low	2.78	3.36	0.00	2.48	0.58	0.00	1.53
Mean	1.23	1.45	0.00	0.83	0.33	0.00	

S.E (\pm) of 0.09, 0.07 and 0.16 for distance, position and distance \times position, respectively.

Table 6. Percentages of cotton natural crossing as affected by direction and position for a crop grown at Gezira Research Station (GRS), season 2002-03.

Position	Direction			
	North	South	East	West
High	0.00	0.00	1.13	0.11
Medium	0.00	0.00	0.61	0.00
Low	1.80	1.10	2.62	0.61
Mean	0.60	0.37	1.45	0.24

S.E (\pm) of 0.08, 0.13 for direction and direction \times position, respectively.

Table 7. Percentages of cotton natural crossing as affected by direction and distance for a crop grown at Gezira Research Station (GRS), season 2002-03.

Distance (m)	Direction			
	North	South	East	West
5	1.26	1.10	1.91	0.44
10	2.11	1.13	1.60	0.99
15	0.00	0.00	0.00	0.00
20	0.00	0.00	3.32	0.00
25	0.00	0.00	1.31	0.00
30	0.00	0.00	0.00	0.00

S.E (\pm) of 0.19 for direction \times distance.