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## Assessing the Out-crossing Ratio, Isolation Distance and Pollinator Insects in Cotton (*Gossypium hirsutum* L.)

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**Abstract:** The extent of out-crossing ratio, isolation distance and pollinator insects in cotton (*Gossypium hirsutum* L.) was studied in a two-year study in Turkey. Plant materials were Maraş-92 (a standard variety), Mc Namara (red plant) and NGF-63 (a glandless variety). The out-crossing ratio was determined in plot combinations where Maraş-92 with Mc Namara and Maraş-92 with NGF-63 were planted separately as alternated rows and plants. The out-crossing ratio in between alternated plants was 3.75 and 4.6%, while it was 3.25 and 3.84% in between alternated rows for Mc Namara and Maraş-92 in 2001 and 2002, respectively. The same ratio was 7.5 and 13.58% in alternated plants and 5.5% and 12.61% in alternated rows for NGF-63 and Maraş-92 in both years, respectively. The out-crossing ratio for isolation distance was 3.75 and 0.25% at 1st and 12th rows, respectively, in 2001, while it was 5.6 and 0.10% in rows a<sub>1</sub> and a<sub>13</sub>; 5.27%, 0.32% and 0.0% in rows b<sub>1</sub>, b<sub>8</sub> and b<sub>9</sub>, respectively, in 2002. It can be concluded that 12 to 13 rows (8 to 9 m) can provide good separation for isolation distance in cotton. In insect scouting, 0.046 wild bees and 0.005 honeybees in 2001 and 0.062 wild bees and 0.0077 honeybees were counted for each flower inspected in 2002.

**Key words:** Cotton, out-crossing, isolation distance, pollinator insect, breeding

### INTRODUCTION

The mating in cotton plant (*Gossypium hirsutum* L.) shows both out-crossing and self-pollinating (facultative) mechanism. The out-crossing ratio can be altered by genotype, preference to own pollens, flower color, nectar content of the flower, planting systems in the area, population of natural pollinators (honeybees and wild bees) nectarines and the amount of sugar within nectar during flowering period<sup>[1]</sup>. Since cotton pollens are big (90-140 μ diameter) and having ichnite structure, they can't be easily carried by wind from flower to flower<sup>[2,3]</sup>.

Out-crossing ratio was studied in different ecological conditions and it was found 5 to 50% in the USA<sup>[4]</sup>, 1.67-2.67% in Greece<sup>[5]</sup>, 0.58-1.21% in Adana, located in south of Turkey<sup>[6]</sup>, 0.33% in Aegean region located in west of Turkey<sup>[2]</sup>, 0.87-2.51% in Harran located in southeast of Turkey<sup>[7]</sup>. It was suggested that isolation distance between cotton plants should be 10 rows (8 m) or 20 rows (16 m) apart for Aegean region<sup>[2]</sup> and 10 m for Greece conditions for minimum pollination<sup>[5]</sup>.

In different studies for pollinator insects, 35 bee species were found on the cotton field by Moffet *et al.*<sup>[8]</sup> wild bees are known as real pollinators<sup>[9]</sup> and honeybees are secondary pollinators because they generally do not collect pollens or do not carry them far away<sup>[3,10]</sup>.

Additionally, wild bees mostly prefer nectar on the flower and carry the pollens that are adhered on their body from one flower to others and cause out-crossing. While honeybees visiting flowers, they follow specific directions and some times, they clean their body from the pollens that are getting heavy.

Researchers usually use the same genetic markers in their experiments such as red (R) and green (r) color, glandless (gl) and glanded (Gl) varieties with different planting schemes.

It is important to determine out-crossing ratio and isolation distance to ensure genetic integrity in growing seed and use the information for different plant breeding applications like in introduction, crossing or selection procedure. Also, determination of pollinator insect populations will help to find their effects in pollination and to make producers decide whether to produce hybrid seed or not in their region.

This study was conducted to determine out-crossing ratio, isolation distance and pollinator insects in cotton plant under Mediterranean climatic conditions in Turkey.

### MATERIALS AND METHODS

This experiment was conducted at Kahramanmaraş Agricultural Research Institute located in Kahramanmaraş,

Mediterranean region of Turkey, in 2001 and 2002. Plant materials were Maraş-92 (a standard variety of the region), Mc Namara (having red stem and leaves) and NGF-63 (a glandless variety). All are belong to *Gossypium hirsutum* L. species. No insecticide applied during cotton growing period in the region.

Plots were 20 rows with 65cm spacing and 10m long. Maraş-92 with Mc Namara and Maraş-92 with NGF-63 were planted as alternated rows (Fig. 1A) and alternated plants (Fig. 1B) in different plots. Row spacing between alternated plants was 20 cm. Isolation distance was determined in plots where Mc Namara variety was planted in 8 rows (10 m length) in center and surrounding plots where Maraş-92 variety, planted in 20 rows (13 m length). In this planting design, rows planted in the same direction with Mc Namara were called “a” rows and the rows planted parallel to Mc Namara were called “b” rows (Fig. 2).

Pollinator insects were counted on 50 m long rows, in early hours of the day during flowering period from 100 flowers in 2001. In 2002, during flowering period, once a week for 6 weeks, in alternated rows and alternated plants, 25 flowers in every plot, 150 flowers in total, were inspected. Insects found on the flowers were sent to Plant Protection Department in Atatürk University for identification. In harvest, boll samples were taken from the bottom, middle and top of the plants of Maraş-92 and NGF-63 varieties and the seeds were planted in the growth chamber. Germination test was replicated 10 times with 100 seed in each trial. Number of red color seedlings or the seedlings carrying gossypol glands were counted and recorded.

Also, considering the way of the wind in the area, some glass plates having adherent material on them, were located within the plots to see if there is wind affect on pollination.

## RESULTS AND DISCUSSION

**Outcrossing ratio (%):** Results from the alternated rows and plants consisted of Maraş-92 and Mc Namara; Maraş-92 and NGF-63 combinations are presented in Table 1 in which the out-crossing ratio in rows between red and green plants (alternated plants and rows) was 3.25-4.60%. It was 5.50-13.58% for alternated rows and plants in between green and glandless plants. It is also observed from the same Table 1 that out-crossing ratio was higher in neighboring plants (alternated plants) than neighboring rows (alternated rows). This condition shows that pollinator insects (honeybees and wild bees) usually move on the same rows during pollination.

In our observation, honey bees usually prefer nectars in outside of the flower and leaves and moves on the

Table 1: Planting combinations for neighboring rows (alternated rows) and plants (alternated plants) consisting of Maraş-92 with Mc Namara (red), and Maraş-92 (green) with NGF-63 (glandless) in 2001 and 2002

	Outcrossing Ratio (%)		Average of two years
	2001	2002	
Alternated plants (Mc Namara/Maraş-92)	3.75	4.60	4.18
Alternated rows (Mc Namara/Maraş-92)	3.25	3.84	3.55
Average	3.50	4.22	3.86
Alternated plants (NGF-63/Maraş-92)	7.50	13.58	10.54
Alternated rows (NGF-63/Maraş-92)	5.50	12.61	9.06
Average	6.50	13.10	9.80
Over all average	5.00	8.66	6.83

Table 2: Outcrossing ratio from central block (Mc Namara) to the outside rows (%) in 2001 and 2002

Distance from the center	Outcrossing ratio (%)			Average
	2002			
	2001 *	"a" rows	"b" rows	
1st row	3.75	5.60a	5.27a	4.87
2nd row	2.00	3.87b	3.07b	2.98
3rd row	2.00	3.32b	1.37cd	2.23
4th row	0.50	1.67c	1.52c	1.23
5th row	0.50	1.27cd	0.77de	0.85
6th row	1.00	1.00d	0.62e	0.87
7th row	0.50	0.80de	0.45ef	0.58
8th row	0.00	0.40ef	0.32ef	0.24
9th row	0.25	0.35ef	0.00f	0.20
10th row	0.50	0.27ef	0.00f	0.26
11th row	0.00	0.25ef	0.00f	0.08
12th row	0.25	0.20f	0.00f	0.15
13th row	0.00	0.10f	0.00f	0.03
LSD (0.05)	-	0.55	0.62	-
CV %	-	40.90	0.65	-

\*, rows were not separated as “a” and “b”, instead they were averaged

same rows, so very rarely go to the inside of the flower. On the other hand, wild bees (*Tetrolonia acutangula* Mor. and *Andrena* ssp.) generally prefer to get nectars at the base of the flowers (inside the calyx), doing so, pollens are attached their body and carried with them. These insects will cause out-crossing as well as self-pollination when they touch on the anthers, they outburst the pollens. The ratio of 4-13% out-crossing and 87-94% selfing shows that varieties generally prefer their own pollens for pollination. This situation was postulated several researchers that flowers mostly prefer their own pollens<sup>[9]</sup> and ovules were pollinated 75% by their own pollens<sup>[11]</sup>.

In varieties of green (Maraş-92) and glandless cotton (NGF-63) planting combinations, out-crossing ratio was 5.50-13.58% favoring the rows close to each other about 6-7% more than neighboring rows (Table 1). Also, red-green combination had more outcrossing (3-8%) than green-glandless combination. These indicate that

R	G	R	G	R	G
R	G	R	G	R	G
R	G	R	G	R	G
R	G	R	G	R	G
R	G	R	G	R	G
R	G	R	G	R	G
R	G	R	G	R	G

A

R	G	R	G	R	G
G	R	G	R	G	R
R	G	R	G	R	G
G	R	G	R	G	R
R	G	R	G	R	G
G	R	G	R	G	R
R	G	R	G	R	G

B

Fig. 1: Planting designs for cotton varieties of Maras-92 and Mc Namara as alternated rows (A) and alternated plants (B). R=Red plant (Mc Namara), G=Green plant (Maras-92). The same planting design was also used for Maras-92 and NGF-63

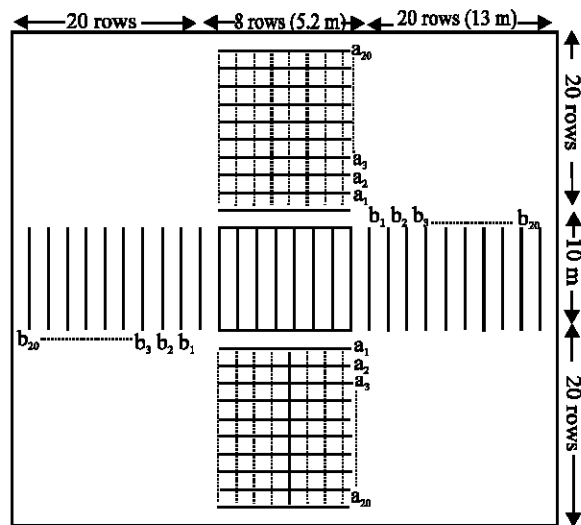


Fig. 2: One of the experimental blocks of four to determine isolation distance in cotton.

$a_1, \dots, a_{20}$  = "a" rows,  $b_1, \dots, b_{20}$  = "b" rows

whether red plant and red flower color repel insects when compared to cream-white colors or insects had preference on glandless plants as described by Mulrooney *et al.*<sup>[12]</sup>. Other explanation is that there might be some segregation in the glandless variety, NGF-63.

**Isolation distance:** From Table 2, out-crossing ratio decreased from the central block to outside. It was 4.87, 0.15 and 0.03 at first, twelfth and thirteenth rows, respectively and generally decreased significantly after the third row. In the "a" rows, planted in the same direction with red plants, until the thirteenth row and in the "b" rows, until the eighth row the out-crossing ratio was inspected. This indicates that cotton pollens are not carried far by pollinator insects and mostly carried in the same direction (row).

The proper distance for isolation is 15 rows (10 m). In the experiments, it is advisable to leave 4 rows isolation lines in between the breeding lines or to plant some other crop species (corn, flax, etc.) for better isolation. For seed production, it is better to ignore the rows within 10 m

distance between the breeding lines that will be used to produce seeds.

**Pollinator insect species and flower visits:** After the inspections and defining the insects, some wild bee species, *Tetralonia acutangula* Mor. (Anthophoridae) *andreaea* ssp. (Andrenediae) and *Aphis mellifera* (Aphidae) were found on the flowers. In 2001, wild bee population was 88.9% and honeybee population was 11.1%. In 2002, they were 90.2 and 9.8%, respectively.

The ratio of pollinator insects on red color plants and flowers for 100 flowers were 43% and it was 57% on flowers of glandless plants. This indicates that redness is less preferable than glandless. Pollinator insects per flower were 0.046 wild bees and 0.005 honeybees in 2001 and 0.062 wild bees and 0.0077 honeybees in 2002. This finding shows that the honey bees are not the primary pollinators, as also indicated by Moffet *et al.*<sup>[8]</sup>

In our region, bee populations are high due to less insecticide usage. So the outcrossing ratio in Kahramanmaraş ecological conditions changed from 3% to 13%. Hybrid seeds can be produced in natural conditions. Also, yield could be increased by locating honeybee boxes in the sides of the field. But to produce homogenous breeding materials, blocks have to be separated by 15 rows (10 m).

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