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Hybrid Seed Production Using Cytoplasmic Male Sterile Lines in Cotton (*Gossypium hirsutum* L.)

WANG XUEDE, YAGYA DUTT[1] AND ZHAO XIANGQIAN

Department of Agronomy, College of Agriculture & Biotechnology, Zhejiang University, Hangzhou 310029, China
dutta_yagya@yahoo.co.in

ABSTRACT The production of hybrid seed in cotton using CMS system requires adequate movement of fertile pollen from flower of restorer plant to flower of sterile plant. Natural transfer of pollen in cotton is not possible because pollen is heavy and sticky and not easy to be transferred by wind. Therefore, study on bee visiting and factors affecting the bee visit during pollination are essential in hybrid seeds production in cotton. The present study was conducted to find out the possible effect of total soluble sugar, sucrose and fructose content of different flower parts on the visiting frequency of honeybees and to analyze the relationship between the visit frequency of honeybee and yield of hybrid seed. The results shows that sucrose content in flowers especially in CMS flowers affected significantly to the honeybee population density and might be the key factor for higher hybrid seed production in some CMS (Kang-A and Y-11) and fertile lines. Thus, breeding male sterile lines with higher sugar content in flowers was propitious to the attraction of bee visiting, which could increase the rate of effective bolls per plant and decrease the rate of aborted seed and result higher yield of hybrid seed. Secondly, the mixed fashion method of seed production could be used to improve the bee visiting frequency. The mixed sowing fashion could shorten the distance of honeybee visiting flowers between restorer and sterile plants. Using indicative trait as marker, such as fiber or fuzz colour could make the mixed sowing fashion possible and effective, because hybrid seed could easily be picked out according to the indicative trait of the male sterile plants which will be different from restorer plants. Thus, cytoplasmic male sterility can be used effectively in cotton hybrid seed production, through developing a satisfactory restorer and a sterile line with higher sucrose content, and then application of a mixed planting method.

Key words: Cotton, CMS line, Sugar content, planting fashion of hybrid seed production

Cotton is the world's leading natural fiber crop and it is the cornerstone of textile industry. In China, it is an important cash crop. It is the largest producer of cotton in the world. The demand of cotton fibre is continuously increasing all over the world especially in countries like China and India, where largest human population of the world is living. To meet this increasing demand of cotton fiber, increase in productivity of cotton cultivars is essential. Hybrids are generally more productive than varieties and are successfully used to increase the productivity of many crops especially in maize, rice and pearl millet.

Two pre-requisites for application of heterosis in crop production are high level of heterotic performance in hybrids and low cost of hybrid seed production. Mell [1] first reported the phenomenon of heterosis in cotton and later on numerous workers have reported significantly desirable level of heterosis in cotton. But even then heterosis could not be used very effectively in cotton as compared to cereal crops. The major limiting factor of not utilizing cotton heterosis at commercial level is the high expense on hybrid seed production. In most of hybrid growing countries like India and China, the hybrid seed

[1]Present address: CCSHAU, Cotton Research Station, Hisar

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production has been done mainly either through hand emasculation and/or through genetic male sterility approach due to sufficient availability of cheap labour in these countries. The most economic and easy method of hybrid seed production in cotton is the CMS approach. After the development of the most acceptable CMS line based on *G. harknessii* cytoplasm and its restorer for fertility restoration [2], it was thought that CMS approach will be widely used in hybrid seed production programme in cotton. But there are problems in the production of hybrid seed through CMS approach, such as weak restorability of restorers and insufficient pollen transfer. In view of the economic importance of cotton in the world, these aspects deserve more investigation. Thus, the major objective of this study was to generate data on hybrid seed production in cotton and suggest the suitable methodology to solve the problems faced during hybrid seed production through CMS approach. The present study was conducted (i) to study the correlation between different sugar components of cotton flowers and honeybee visit frequency and also with different seed yield components, and (ii) to compare the two fashions of planting i.e. mixed planting fashion vs. row spaced planting fashion for hybrid seed production.

MATERIALS AND METHODS

All experiments were conducted during 2000-2003 at experimental farm of College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China. The material for 1st experiment involved five CMS (Kang-A, Y-1, Y-11, Y-21 and Y-7), one maintainer and one restorer line. These seven genotypes were grown in a randomized block design with three replications. Each entry was accommodated in two rows of twenty meter length. The data for different seed yield traits like bolls/plant; boll setting percentage, seeds per boll and aborted seeds per boll were recorded on twenty randomly selected plants in each genotype for each replication. Data on different sugar traits like total sugar content, sucrose content and fructose content in flowers of CMS, maintainer and restorer lines were recorded for all the seven genotypes. Data on honeybee visitation were taken at the peak flowering (last week of July up to the end of August). The honeybee counts were made between 08.00 to 09.00 hr in the clear weather. Ten thousand flowers in one hundred plants were used for recording data on honeybee visitation in each genotype. The simple correlations between seed yield and sugar components of flowers and honeybee visit frequency have been calculated.

The material for the 2nd experiment involved the two CMS lines (ZO16 and ZO35), and two restorers (SO13 and SO29). The CMS and restorer lines represented brown and white fiber cottons, respectively. These CMS and restorer lines were used in hybrid seed production of two F_1 combinations i.e. ZO16 x SO29 and ZO35 x SO13. For production of hybrid seeds of these two hybrids, two planting fashions/methods were used. In the 1st method, (mixed fashion method) seeds of respective CMS and restorer lines were mixed in 3:1 ratio and then grown in the field. The brown fiber/fuzz color (controlled by a dominant gene) CMS lines used as indicator marker in this method of hybrid seed production so that at the time of harvesting, the hybrid bolls could be separated easily from restorer. In the second method, (row spaced planting method), CMS and restorer lines sown in rows in the 3:1 ratio i.e. after every four rows of CMS line, grown one row of restorer line. Two planting fashions were located at different fields isolated by a corn field. Each sowing fashion accommodated a plot size of 667 m^2. As there were many natural bees in the farm, honeybee boxes were not needed. The data were recorded for seed yield and its components like boll setting percentage, number of seeds/boll and aborted seed percentage.

In both experiments, row-to-row and plant-to-plant distances were maintained 0.6 M and 0.30 M, respectively. All the recommended cultural practices for cotton crop were followed. The data on lint and seed yield was measured on per plot basis and then converted into per hectares. The other yield components such as number of bolls per plant, boll weight, boll setting percentage, number of seeds per boll, aborted seed percentage and lint percentage were recorded on 20 randomly selected plants in each plot and mean of 20 plants were taken for further analysis.

RESULTS AND DISCUSSION

Pollen of cotton is relatively heavy and sticky, and their transfer between the flowers is not easy by wind and mainly depends on insects for the pollination. The major aim of present study was to investigate the factors affecting the pollen transfer in hybrid seed production of cotton through CMS approach and to suggest the appropriate strategy to solve those problems. The results of sugar components revealed that sucrose content in flowers of CMS, maintainer and restorer lines might be a key factor that affects the frequency of bee visit (Table 1). Mofett *et al.* [3] also reported that honeybee visits were positively correlated with the sugar concentration of cotton nectar. Thus, breeding lines with higher sucrose content in flower is propitious to the attraction of bee visiting, which can increase the rate of effective bolls per plant and decrease the rate of aborted seed and result in higher yield of hybrid seed.

The knowledge of the correlation between different traits is important in a breeding programme because they may affect selection method. Plant breeders must be concerned with the total array of economic characters in their effort to develop a crop variety acceptable to farmers and also to the seed producers. The results of correlation analysis (Table 2) showed that among the sugar components of flowers, only sucrose showed highly positive and significant correlation (0.987) with the visiting honeybee frequency indicating that sucrose content in the flower parts is the key factor that affects the visiting honeybee frequency. Further, the visiting honeybee frequency showed highly positive and significant correlation with boll setting percentage (0.945) and negatively significant correlation with aborted seeds percentage (–0.967). This indicated that for increasing boll setting percentage and reducing the aborted seeds percentage in cotton CMS lines, the visiting honeybee frequency should be high. The results of correlation analysis suggested that among the sugar components of flower, breeders major emphasis should be on the selection of lines with flowers having higher sucrose content.

The second approach of increasing honeybee visits in CMS lines is mixed planting fashion for hybrid seed production. The behavior characteristics of honeybees on cotton are rather unique that may also be detrimental to high seed yield. The bees generally visits several flowers in near distance and before returning to the hives, they meticulously combs off (grooms) the pollen from their body [4]. This grooming behavior reduces the rate and quantity of pollen movement from

Table 1. Sugar components of sterile and fertile flower, honeybee visit frequency and component traits of hybrid seed yield

Lines		Total soluble sugar (%)	Sucrose content (%)	Fructose content (%)	Visiting bees/10,000 flowers	Bolls/ plant	Boll setting percentage	Seeds/ boll	Aborted seeds (%)
Sterile	Kang-A	13.387	1.102	4.723	125	27.1	39.12	32.1	6.97
(CMS)	Y-1	12.110	0.891	4.168	93	26.2	34.34	30.8	12.06
lines	Y-11	12.505	1.098	4.064	119	27.5	40.18	32.1	7.01
	Y-21	12.930	0.919	3.782	101	23.1	36.21	29.9	7.61
	Y-7	12.865	1.003	3.969	107	22.5	36.92	27.6	10.25
Average (S)		12.759	1.003	4.141	109.00	25.31	37.35	30.50	8.78
Fertile	Restorer	9.901	2.730	3.841	121	21.3	41.20	33.2	6.02
lines	Maintainer	9.091	2.862	4.225	118	26.1	37.60	31.8	6.81
Average (F)		9.496	2.796	4.033	119.50	23.70	39.40	32.50	6.42
F-S		-3.263	1.793	-0.108	10.50	-1.58	2.05	2.00	-2.31
F > or < S (%)		-25.55**	178.76**	-2.61	9.63*	-6.32	5.49	6.56	-26.88**

*,**indicate significant at 0.05 and 0.01 level of probability, respectively.

Table 2. Correlation coefficient between different sugar components of flowers, honey bee visit and and hybrid seed yield

Lines	Total soluble sugar (%)	Sucrose content (%)	Fructose content (%)	Visiting bees/10,000 flowers	Bolls/ plant	Boll setting percentage	Seeds/ boll	Aborted seeds (%)
	1	2	3	4	5	6	7	8
1	-	0.512	0.423	0.652	-0.154	0.471	-0.002	-0.666
2		-	0.557	0.987**	0.518	0.961**	0.446	-0.918*
3			-	0.612	0.659	0.367	0.577	-0.511
4				-	0.492	0.945*	0.486	-0.967**
5					-	0.482	0.928*	-0.48
6						-	0.482	-0.947
7							-	-0.561
8								-

*,**indicate significant at 0.05 and 0.01 level of probability, respectively.

pollen parent to flower of seed parent and that consequently reduce the hybrid seed yield in cotton. Thus, any effort that could reduce the distance between CMS and restorer lines in the seed production field, might be a problem of pollen transfer between CMS and restorer lines. The idea of mixed fashion planting of seeds of A and R lines might help to overcome this problem. Keeping above views in mind, the two methods of seed production i.e. mixed fashion planting and spaced row planting methods were compared (Table 3) in the present study. The comparison of seed yield of mixed fashion model with the spaced row planting model showed that mixed fashion model recorded 14. 90 per cent more number of bolls per plant, 1.03 per cent more number of seeds per bolls and 3.01 per cent reduced aborted boll and finally 13.03 per cent higher seed yield as compared to spaced row planting method. The results of present study are in close agreement with the earlier studies [5,6], who reported that mixed fashion of plantings produced more hybrid seed per acre than the other planting methods. The low yield in case of spaced planting as compared to mixed planting may be due to fewer visitations of bees in row spaced planting method than mixed planting. The mixed sowing fashion could shorten the distance of the honeybee as bees has to travel between restorer and sterile plants and also increased the pollen amount for a sterile flower stigma. Finally this increased the hybrid seed yield due to higher boll setting percentage and lowered the rate of aborted seeds of sterile plants in the mixed showing fashion. Weaver [5] conducted mixed planting studies and provided evidence that mixed plantings might enhance bee visitation and pollen transfer. The use of indicative marker in the hybrid seed production of colored fiber cotton have many advantages, for example, no difficulty was faced at the time of harvesting because hybrid seed can easily be picked out based on fiber color of the male sterile plants which is different from restorer plants. Thus, fiber color can be used as indicator trait for hybrid seed production in mixed fashion of planting in cotton. Secondly, white fiber restorer increased the seed yield of colored fiber cotton because our personnel experience showed that colored (A) x white (R) combination produces more seed yield than colored (A) x colored (R) combination.

In conclusion, among the different sugar components of flower, sucrose content is the most important factor influencing the honeybee visit

Table 3. Comparison of seed yield components in mixed planting fashion (MPF) and spaced row planting fashion (RPF)

Genotype		Bolls/plant	Seeds/boll	Aborted seeds (%)	Seed yield (kg/ha)
MPF	ZO16 x SO29	24.74	28.68	17.4	2260.8
	ZO35 x SO13	26.28	26.24	20.52	2191.2
	Average	25.51	27.46	18.96	2226.0
RPF	ZO16 x SO29	19.63	28.11	17.17	1852.5
	ZO35 x SO13	18.7	25.46	24.03	1537.2
	Average	19.17	26.79	20.6	1694.85
MPF > RPF (%)		33.07**	2.50	-7.96*	31.34**

*,**indicate significant at 0.05 and 0.01 level of probability, respectively.

frequency. Against some previous reports, the results of present investigation showed that honeybee visit was higher on fertile (restorer and maintainer) lines as compared to sterile (CMS) lines. Low pollen availability combined with low honeybee visits in CMS lines is one of reasons for lower seed yield in CMS based cotton hybrids and this problem can be solved in two ways (i) breeding CMS lines with high sucrose content, and (ii) using mixed planting fashion of seed production. Breeding A and R lines having flowers with high sucrose content could increase the honeybee visit frequency. Honeybee visit frequency influenced very much to the seed yield i.e. higher the honeybee visit frequency the higher the seed yield and *vice-versa*. Secondly, mixed planting fashion of seed production shortened the distance between flowers of CMS and restorer plants and thus provided more pollen for pollination of CMS line flowers and resulted in higher seed yield as compared to row spaced planting of seed production.

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