Photosynthesis and plant growth response of transgenic Bt cotton (*Gossypium hirsutum* L.) hybrids under field condition

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Abstract

Field experiments were conducted under rain-fed conditions to study the growth and photosynthetic efficiency of transgenic Bt cotton hybrids during 2002–03 and 2003–04 seasons. Three Bt cotton hybrids (Bollgard 1) and their non-Bt (NBt) counterparts viz. MECH 12, MECH 162, and MECH 184 were grown along with a local hybrid NHH44. Growth parameters such as plant height, main-stem nodes, biomass accumulation, and physiological processes like stomatal conductance (g_s), and rates of transpiration (E) and photosynthesis (P_N) did not differ significantly between Bt and NBt hybrids up to 80 DAS (d after sowing). Squaring commenced at 50 DAS both in Bt and NBt. The loss of young fruiting forms by the entomological factors was three times less in Bt than NBt. As a consequence, Bt had more early formed bolls on the lower canopy which contributed to higher biomass and seed cotton yield. On the other hand, bolls distributed intermittently in NBt. Heavy boll load altered the growth and physiological processes, and as a result Bt had higher g_s, E, and P_N than NBt. Since developing bolls (sink) divert the saccharides and nutrients from other organs, Bt plants with heavy boll load senesced early and stopped the production of new squares and bolls. Thus, the boll load influenced the change in growth and physiological processes of Bt from NBt.

Additional key words: boll load; dry matter; *Gossypium*; node number; plant height; senescence; square and boll shedding; stomatal conductance; transpiration; yield.

Introduction

Realizing the importance of severity of the loss of cotton crop due to insect pests, Bt cotton (Bollgard 1) hybrids have been approved for commercial cultivation in India, since March 2002. In most of the experimental trials as well as in farmers' fields, Bt hybrids out-yielded NBt hybrids (Mohan and Manjunath 2002, Quaim and Zilbermann 2003, Ramasundaram 2005). It is expected that Bt hybrids retain more bolls and yield higher because of better insect control over their NBt counterparts. Also the growth rate of cotton has changed with the introduction of Bt gene. Sahai and Rahman (2003) reported Bt plants with less vigorous growth, fewer branches, smaller leaves, and small bolls as compared to NBt plants. On the contrary, Godoy *et al*. (1988) observed a significant increase in biomass (crop growth rate) during 84–105 d after planting while Dong *et al.* (2006) reported an increase in growth and yield of Bt plants compared with conventional cultivars. At harvest, Bt plants had a better apportioning of biomass towards yield (Singh *et al.* 2003). The growth, temporal variation in fruit load, and the associated physiological processes of Bt cotton hybrids especially with their NBt counterparts are not well known. In this study the fruit load on the plant was monitored at regular intervals and the associated growth and photosynthesis of Bt hybrids and their NBt counterparts were quantified at 50, 80, and 110 d after sowing (DAS).

Materials and methods

Experimental site: Field experiments were conducted under rain fed conditions at Central Institute for Cotton Research Farm, Nagpur (21°N and 79°E) in Central India, during 2002–03 and 2003–04 seasons. Rainy season in this region generally commences in the month of June and lasts till September–October (Fig. 1). Total rainfall received during the cropping season ranges between 600 and 700 mm. Temperature, solar radiation, rainfall, and its distribution pattern (data recorded at the experimental site) are presented in Fig. 1.

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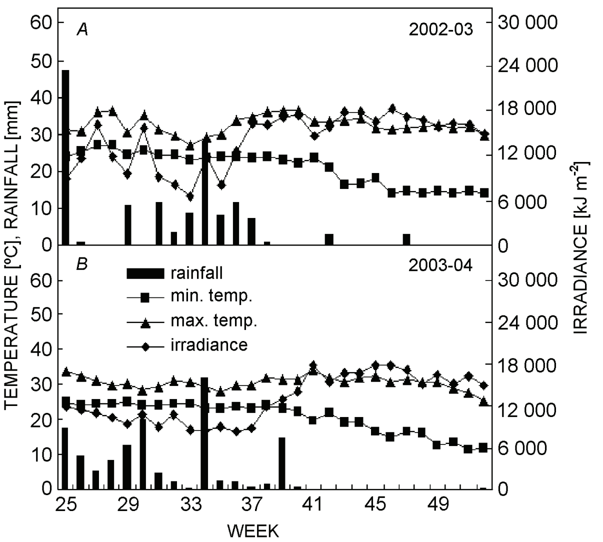

Fig.1. Maximum and minimum temperature [°C], mean rainfall [mm d^{-1}], and solar radiation [kJ m^{-2}] of the experimental site during cropping seasons of 2002–03 (*A*) and 2003-04 (*B*). Data are presented at weekly intervals.

Plants: Seven intra-hirsutum cotton hybrids, three of them Bt cotton (Bollgard 1) MECH 12, MECH 162, and MECH 184 and their NBt counterparts were grown along with a popularly cultivated intra-hirsutum hybrid NHH44. A split plot design was used with Bt and NBT as main plot treatment and different hybrids as subplot treatments. Each genotype was grown in plots of size 12×12 m and replicated thrice. Sowing was done on 19th June 2002 in the 1st experiment and 25th June 2003 in the 2nd experiment. As per the common practice of this region, a spacing of 0.6 m was maintained between the rows as well as between the plants, which accommodated a plant population of 27 777 plants ha^{-1}. Two weeks after emergence, the plant population was thinned and single plant was maintained per hill. Fertilizer N (90 kg ha^{-1}) was applied in three splits at 20, 45, and 70 DAS. Phosphorus and potassium at the rate of 45 : 45 kg P_2O_5 and K_2O or 19 : 37 kg P : K ha^{-1} were applied basally. Plant protection measures were taken up as recommended by entomologists. Both the years methyl demeton (*metasystox*) 300 cm^3 per ha was sprayed at 30 DAS to control sucking pests in both Bt and NBt hybrids. NBt hybrids were sprayed with endosulfan (875 cm^3 per ha) twice at 70 and 90 DAS in the 1st experiment and once at 90 DAS in the 2nd experiment to control bollworm (*Heliothis armigera*).

Time course measurement of squares, flowers, and open bolls and their abscission pattern: Plant height, date of successive node appearance, squares, flowers, and bolls from 10 plants of each genotype were measured every alternate day from the date of square initiation. The date of square appearance was recorded on a chart paper along with its position on sympodia or monopodia. Every alternate day the monopodia and sympodia were observed and the new squares that appeared on the successive positions from the previous date were recorded. Appearance of pinhead squares (approx. 3 mm size) was considered as new squares for counting. The fate of these squares was monitored as long as they were on the plant. The date of flowering and boll opening of each square, which was retained on the plant, was noted. The abscised squares on the ground were collected and carefully observed to ascertain the causal factor for shedding (physiological or insect attack). Total squares produced, their distribution on the monopodia and sympodia, open flowers and open bolls developed from the squares produced at different stages, and shedding pattern of different genotypes were estimated.

Plant growth and physiological processes: At 50, 80, and 110 DAS, plants from 1 m^2 were cut at the soil surface and used for assessing total above ground dry matter. At the end of the boll opening, the seed cotton from the central 4 rows was manually harvested and weighed.

Along with the above sampling, at 50, 80, and 110 DAS, the stomatal conductance (g_s), and rates of transpiration (E) and net photosynthesis (P_N) of the youngest fully expanded leaf (3rd leaf from top) were measured with a Portable Photosynthesis System (model *CIRAS-1*, *PP Systems*, UK). Measurements were taken in each plot at least twice under conditions of full sunlight, between 1 200 and 1 500 µmol m^{-2} s^{-1}. The data was analyzed using split plot design.

Results

Plant growth and seed cotton yield: Plant height, main stem node number, and the dry matter accumulation did not vary significantly between Bt and NBt hybrids up to 80 DAS (Table 1). At 110 DAS, Bt hybrids, however, accumulated 10 % more biomass than NBt hybrids and the differences were significant. Among Bt hybrids, MECH 184 had the highest biomass while MECH 184 NBt had the least. On the other hand, NBt plants produced more main stem nodes and they were significantly taller than Bt plants. Maximum plant height and main stem nodes were recorded in MECH 162 NBt, while they were the least in MECH 12 Bt. Seed cotton yield was 19 % more in Bt hybrids compared to NBt hybrids (Table 2). Bt hybrids MECH 184, MECH 162, and MECH 12 produced 39, 16, and 13 % higher yield compared to NBt counterparts, respectively. Seed cotton yield of NBt hybrids was comparable to that of the local check NHH44.

Table 1. Plant growth and physiological processes: transpiration rate (E), stomatal conductance (g_s), and net photosynthetic rate (P_N) of field grown Bt and NBt hybrids measured at 50, 80, and 110 DAS. Means of three hybrids.

DAS	Treatment	Main stem node number	Plant height [cm]	Dry matter per plant [g]	E [mol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	P_N [μmol m^{-2} s^{-1}]
50	Bt	11	23	55	5.76	474	26.8
	NBt	11	20	57	6.56	466	26.7
	SEM	0.20	0.41	0.61	0.03	0.35	0.20
	LSD (p<0.05)	NS	NS	NS	NS	NS	NS
90	Bt	20	57	87.80	6.56	488	31.2
	NBt	20	56	83.42	6.59	487	31.8
	SEM	0.37	0.13	1.94	0.12	45.72	0.16
	LSD (p<0.05)	NS	NS	NS	NS	NS	NS
110	Bt	27	66	107.46	7.23	673	33.6
	NBt	29	78	94.6	6.63	493	29.2
	SEM	0.14	1.48	0.65	0.02	0.35	0.20
	LSD (p<0.05)	NS	9.17	3.96	0.62	6.40	2.56

Table 2. Comparison of plant growth and physiological processes at 110 DAS and seed cotton yield at harvest of three Bt hybrids MECH 12, MECH 162, and MECH 184 along with their NBt counterparts and a local conventional hybrid NHH44. [a]Values of conventional hybrid NHH44 NBt were not used for statistical analysis. Means of three replications.

	Treatment	MECH 12	MECH 162	MECH 184	Mean	NHH44[a]	LSD G	at 5 % T	G×T
Main stem node number	Bt	24	29	28	27	–		NS	NS
	NBt	25	31	30	29	29	1.93	NS	NS
	Mean	25	30	29					
Plant height [cm]	Bt	57	70	70	66	–			
	NBt	63	95	73	78	75	5.21	9.17	7.37
	Mean	60	82	72					
Dry matter per plant [g]	Bt	108.9	103.8	109.6	107	–			
	NBt	103.0	97.4	83.3	94	84.5	4.78	3.96	6.77
	Mean	105.9	100.6	96.5					
Seed yield [kg ha^{-1}]	Bt	939	1595	1283	1273	–			
	NBt	819	1371	783	991	1061	230	160	270
	Mean	879	1483	1033					
E [mol m^{-2} s^{-1}]	Bt	7.4	7.3	6.9	7.2	–			
	NBt	7.1	6.7	5.9	6.6	6.9	0.21	0.62	NS
	Mean	7.3	7.0	6.4					
g_s [mol m^{-2} s^{-1}]	Bt	720	740	560	673	–			
	NBt	503	494	483	493	561	28.42	6.40	40.21
	Mean	612	617	522					
P_N [μmol m^{-2} s^{-1}]	Bt	34.6	33.8	33.1	33.8	–			
	NBt	28.3	29.9	29.5	29.2	30.2	1.40	2.56	1.96
	Mean	31.4	31.8	31.3					

Distribution of squares, flowers, and open bolls: Square production started almost at the same time in both Bt and NBt hybrids, *i.e.* 50 DAS. During the initial few weeks (6 to 7 weeks) there was no difference in square production between Bt and NBt hybrids (Fig. 2*A*). Beyond 8th week, square production started declining in Bt and by 11th week it ceased. On the other hand, NBt plants continued to produce squares up to 18 weeks. On an average, Bt hybrids produced 145 squares as against 217 in NBt hybrids. Most of the early formed squares were shed in NBt (>60 %) while in Bt most of them were retained, flowered, and opened as mature bolls (Fig. 2*B,C*). In Bt, squares formed in the first 3 weeks contributed to nearly 50 % of the open bolls while the corresponding contribution of squares to open bolls was only 20 % in NBt plants. In NBt, those squares formed between 8 and 9 weeks from the square initiation contributed maximum to open flowers and bolls. Thus, the peak boll development in Bt plants coincided with squaring and flowering of NBt plants.

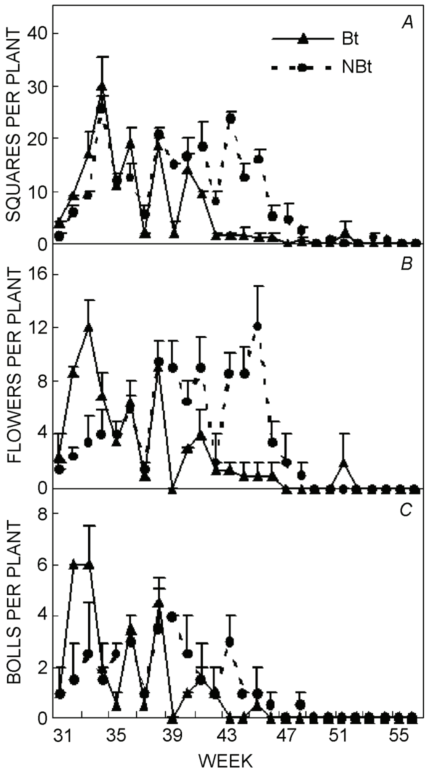

Fig. 2. Temporal variation in square production per plant at weekly intervals (*A*) and their contribution to open flower (*B*) and open boll (*C*) in Bt and NBt hybrids. Means of three hybrids. *Vertical bars* indicate standard error.

Abscission of fruiting forms: Shedding of squares and young bolls ranged from 75 to 80 % across the cultivars. Shedding was marginally higher in NBt compared to Bt (Fig. 3). At early squaring, shedding due to physiological factors was more in Bt plants (Fig. 3*A*). During the same period, the loss due to entomological factors was very high in NBT (Fig. 3*B*). Fruiting forms shed due to entomological factors accounted for 20 and 50 % in Bt and NBT, respectively. Total loss of fruiting forms by the physiological factors was almost the same between Bt and NBt hybrids while the entomological loss in NBt was three times that of Bt plants.

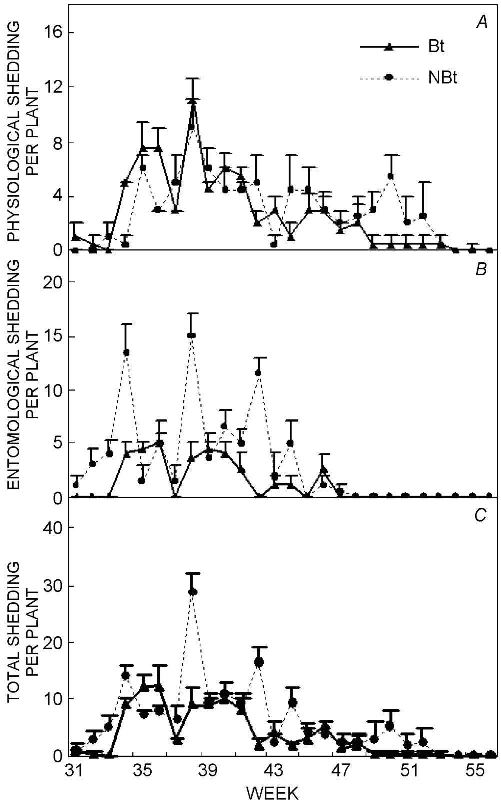

Fig. 3. Time course recording of physiological (*A*), entomological (*B*), and total shedding (*C*) of fruiting forms per plant in Bt and NBt hybrids. Means of three hybrids presented at a weekly interval. *Vertical bars* indicate standard error.

Process parameters: E, g_s, and P_N increased with age of the plant (Table 2). All through the measurements at 50, 80, and 110 DAS, Bt plants showed an increase in these parameters while in NBt the increase was recorded up to 80 DAS. Between Bt and NBt hybrids significant differences were not seen in E, g_s, and P_N at 50 and 80 DAS. At 110 DAS Bt plants had significantly higher P_N, g_s, and E. The highest increase was recorded in MECH 12 Bt (22 %) followed by MECH 162 BT (13 %) and MECH 184 Bt (12 %). P_N of NHH44 hybrid was comparable to those of NBt hybrids.

Discussion

In the rainfed tracts of central India, rain commences in the 2nd fortnight of June and lasts till September end (Fig. 1). Cotton crop, which is sown with the onset of monsoon, experiences intermittent drought or waterlogging owing to uneven distribution of rainfall or terminal drought because of its early cessation. These environmental adversities limit the availability of photosynthate to the developing organs and leads to the shedding of fruiting forms (Guinn 1985). This in addition to the loss due to entomological factors causes shedding of fruiting forms to the tune of 75 to 80 % in rain-fed cotton (Bhatt *et al.* 1972). Transgenic Bt cotton has been commercialized in order to protect the losses of fruiting forms by the entomological factors. As expected, Bt had better retention of early-formed squares and bolls due to better insect control. On the other hand, in NBt there was three times greater loss of fruiting forms by the entomological factors. As a consequence, at 110 DAS, Bt plants were full of developing bolls on the lower canopy, while NBt plants had few squares, flowers, and developing bolls spread intermittently throughout the canopy. This resulted in yield improvement with Bt cotton cultivation as shown by earlier studies (Quaim and Zilbermann 2003). But for this difference in boll load, this study had clearly shown that there was no difference in growth, physiological processes, and commencement of squaring of Bt and NBt at 50 and 80 DAS. Plants carrying heavy boll load as in Bt had altered the growth and physiological processes.

At 110 DAS, Bt plants had higher g_s and P_N. Several studies have emphasized that leaf photosynthesis can be influenced by the presence of developing fruits. A positive effect of crop load on P_N has been reported in many plant species, including *Citrus unshiu* (Iglesias *et al*. 2002), *Prunus cerasus* (Layne and Flore 1995), *Vitis vinifera* (Edson *et al*. 1995, Naor *et al*. 1997), soybean (Setter *et al*. 1980), cotton (Jasoni *et al*. 2000). On the other hand, NBt plants had lower g_s and P_N during the same period. Saccharide accumulation has an inhibitory effect on photosynthesis (Foyer 1988, Goldschmidt and Huber 1992, Martinez *et al*. 1993), leading to the assumption that a lack of sink activity in NBt, resulting from the abscission of fruiting forms, led to saccharide accumulation and feed-back inhibition of net photosynthesis (Wünsche *et al*. 2000, Iglesias *et al*. 2002). The indeterminate growth nature of cotton allowed it to utilize this photosynthate for the production of new leaves, stems, roots, and squares (Pettigrew *et al.* 1992, Sadras 1995) as in NBt, which had more of main stem nodes and higher plant height.

During boll growth the supply of saccharides and nutrients will be shifted from leaves to the developing bolls. This imbalance of source and sink leads to the premature senescence of cotton (Wright 2004). Bt hybrids carrying much higher early boll load than conventional hybrids were found to be more prone to senescence and early maturity, which led to early cessation of squaring.

Thus, our study shows that there was no marked difference in growth, physiological processes, and square production of Bt and NBt hybrids up to 80 DAS. Because of better bollworm control in Bt, there was greater retention of early-formed bolls on the lower canopy, while they were intermittently distributed in NBT. Photosynthesis and growth during boll development have a positive association with boll load in Bt. The higher sink activity leading to lowered source to sink ratio of Bt led to faster senescence and crop maturity compared to NBt.

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