Indian Journal of Agronomy **56** (1): 62–67 (March 2011)

Influence of nitrogen management on expression of cry protein in Bt-cotton (*Gossypium hirsutum*)

S.S. HALLIKERI*, H.L. HALEMANI, B.C. PATIL AND R.A. NANDAGAVI

Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, 580 007

Received: July, 2009

ABSTRACT

To assess the requirements of nitrogen (N), schedule of N application, detopping in Bt-cotton (*Gossypium hirsutum* L.) and their effects on cry protein concentration, other related parameters and yield were studied by conducting field experiment at Dharwad for 2 years (2005-06 and 2006-07) under irrigated conditions. Present study indicated that N fertilization significantly affected cry protein concentration in leaf at various growth stages. At 60 days after sowing (DAS) increased level of nitrogen from 120 and 160 kg N increased cry protein by 9.3 and 14.8 % over 80 kg/ha. Leaf nitrogen concentration and SPAD readings followed same pattern as that of cry protein concentration. Application of N in 7 splits significantly improved the cry protein level (5043 ng/g) over either N applied in 4 splits (4066 ng/g) or recommended practice of three splits (3583 ng/g). Studies also indicated that increasing level of nitrogen from 80 to 120 and further to 160 kg/ha significantly increased seed cotton yield by 12 and 19%, respectively. Application of nitrogen with recommended method of 3 splits (2.27 t/ha) and N applied in 7 splits at 15 days interval (2.24 t/ha) produced on par yields. There was no beneficial and significant effect of detopping in Bt-cotton at different growth stages.

Key words: Bt. Cotton, Cry protein, Leaf nitrogen, Nitrogen levels, SPAD readings, Split application

An important milestone that helped to break the problem of bollworm incidence in cotton was the release of Bt-cotton in the country. Though the release of Bt-cotton did not become foolproof pest control measure, yet it is a sound tool to act as a component of integrated pest management. Until then cultivation of cotton was totally under critical conditions, due to severe incidence of bollworms, higher cost of cultivation and lower productivity. Hence there was strong need to develop Bt-cotton, which offers resistance against bollworms. Bt-cotton not only offers resistance against bollworms, but also it helps in boosting the productivity and income level of the farmers.

Cry protein synthesis in the plant was though genetic core trait, still it was known to change its level with nitrogen supply pattern (Arnold Bruns and Craig Abel, 2003). Possible alteration of cry protein level with manipulation of management practices could be a sound practice to increase production, so also helps for further extension of cry protein level further at later stages of crop cycle when, the natural level in the plant will decline (Adamczyk *et al.,* 2000). Studies made worldwide on transgenic crops as reported by Arnold Bruns and Craig Abel (2003) in maize and by Rochester Ian (2006) in cotton indicated that nitrogen enhances the cry protein concentration in the plant. This gives more and more protection to the crop from pest incidence. Nitrogen management in Bt-cotton is a better challenge to boost production and protecting the crop from bollworm incidence. Nitrogen supplement pattern by split application becomes important as it is supplied ideally in a time when crop critically requires. Bt-cotton may differ in its requirement either by total or part of it in the different stages of crop. Thus, nitrogen use efficiency can be increased and better used to attain the objective of higher production. Cotton is long duration crop with an indeterminate growth habit. The N supplementation period can be increased with number of splits, which provides long time from square formation to boll development. Hence nitrogen requirement during critical stages can be better met with split application pattern.

Study was undertaken with N management strategies by doses and split applications to manage the higher productivity levels and increasing the cry protein levels so that the crop has the advantage in pest control. Cry protein which has altered due to N management has also included with spad meter observations and leaf N concentrations.

MATERIALS AND METHODS

Field experiment was conducted for 2 years (2005-06

***Corresponding author E mail:** hallsuasd@rediffmail.com

and 2006-07) at Agriculture Research Station Farm, University of Agricultural Sciences, Dharwad, Karnataka. Soil of experiment was medium deep black and neutral in its pH (7.6). Available N, P and K were 223,13 and 309 kg/ha respectively. Experiment was laid out in split-split plot design with three replications. Detopping and no detopping treatments were in the main plot and three levels of nitrogen (80, 120 and 160 kg/ha) were in sub-plots. Scheduling of nitrogen applications with 50% N applied as basal and remaining 50% N applied in 3 splits at 50,80 and 110 DAS (recommended method), four splits at 30, 60, 90 and 120 DAS (30 days intervals) and seven splits at 30, 45, 60, 75, 90, 105 and 120 DAS (at 15 days intervals) were in sub-sub plots. Recommended dose of fertilizer to irrigated intrahirsutum hybrid cotton (Bunny NCS 145 BG-1) for Karnataka is 120: 60: 60 kg N, P_2O_5 and K_2O/ha. At sowing, 100% of recommended P and K were applied to all the treatments and N was applied as per treatments. Experiment was sown during third week of June in both the years. Irrigations were made based on the 0.8 IW/CPE ratio recommended for cotton. Each time 60 mm depth of water used for irrigation. Leaf samples were used for estimation of leaf N concentration by following the 'Micro Kjeldahl digestion and distillation method'. Leaf N concentration was expressed in per cent. The Enzyme-Linked Immuno Sorbent Assay (ELISA) procedure was followed for estimation of Cry protein concentration in cotton leaf. The desi Gen Cry 1Ac plate was designed for quantitative laboratory estimation of Cry 1Ac protein in cotton leaf samples. Fully opened fresh 4th leaf from the top was collected in icebox in the morning. From the defined portion of the leaf blade (for every time estimation) only 20 mg of fresh leaf was taken in 1.5 ml microfuge tube and added 500 ml of 1x sample extraction buffer. Tissue was macerated at 3,000 rpm using a motor driven pestle till it gets extracted in to the buffer. The supernatant solution was used to load the plate. Positive and negative QC seed extracts of this supernatant solution of 100ml was used per well while plate loading. Cry protein standard and standard curves were generated from the 1mg/ml Cry 1Ac stock solution provided, in 1x diluent buffer (1:50 dilution). The other quantitation standards were generated.

The 100 ml of the buffer blank, standards (No. 1 to 6), positive controls, negative controls and samples diluted in 1x diluents buffer added to a standard ELISA plate provided. After loading all samples, plate was incubated at 37^{0}C for 90 min. in a humid environment. Samples were discarded and washed the plate with 1x wash buffer (prepared from 10x buffer-A) twice, allowing the plate to stand 5 min. with wash buffer in the wells. The 150 µl of antibody (Ab_2) to each well added and incubated the plate at 37^{0}C for 30 min. in a humid environment. Samples were discarded and plate was washed with 1x wash buffer twice. 250 µl of Ab3 to each well was added, mixed and incubated for 37^{0}C for 45 min. in a humid environment. After incubation samples discarded and washed similarly with wash buffer. Prepared 1x substrate buffer of 25ml by adding 20ml de ionized water to 5ml of 5x substrate buffer provided. 250 ml of prepared substrate buffer added to each well and incubated at room temperature in the dark for 30 min. Color development was observed. Read the absorbance of the plate at 405 nm. Standard curve (linear) was plotted with standard protein concentrations on the X-axis and OD values on the Y- axis. Determined the slope of the curve and estimated the Cry protein concentration. Chlorophyll meter observations (SPAD readings) were taken by light absorbance in the red and infrared bands with a chlorophyll meter (SPAD meter or SPAD-502) at 120 and 150 DAS. These observations were expressed as SPAD readings. Data was statistically analyzed using MSTAT-C programme. The level of significance used in 'F' test was at P=0.05.

RESULTS AND DISCUSSION

Leaf cry protein expression, leaf N content and spad readings

The present investigation on the nitrogen fertilization effect on the expression of cry protein indicated the significant changes in the levels of cry protein at various stages of crop growth. At 60 DAS, application of 80 to 120 kg N and further to 160 kg/ha increased cry protein concentration significantly (Table 1) by 9.3 and 14.8%, respectively. Similar trend was observed at 90, 120 and 150 DAS. Increased cry protein synthesis due to increased N application may be due to the synthesis of mRNA to produce more cry protein (Arnold Bruns and Craig Abel, 2003). Similarly, Pettigrew and Adamczyk (2006) and Rochester Ian (2006) reported the increase in cry protein concentration with increased N levels.

Leaf N concentration was significantly affected by application of different levels of nitrogen at various stages of crop growth (Table 1). Leaf N concentration was significantly high at 160 kg N/ha at 60 (2.05%), 90 (1.78%), 120 (1.72%) and 150 (1.63%) DAS when compared with 80 kg N/ha. But it was on par with 120 kg N/ha at 90 and 150 DAS. The leaf N concentration declined with maturity. In the present investigation correlation studies indicated the positive relation between cry protein and leaf nitrogen concentration. Leaf cry protein has a positive correlation with leaf nitrogen concentration at 60 and 150 DAS ($r = 0.468$ $P < 0.05$), (Table 3).

The SPAD observations made during 120 and 150 DAS showed significant effect due to application of nitrogen. The SPAD readings were significantly high with ap-

plication of 120 kg (38.1) when compared with 80 kg (36.3) N/ha at 120 DAS. Similar trend was followed at 150 DAS (Table 1). Status of chlorophyll in leaf was affected by application of nitrogen. Similarly, increase in the level of chlorophyll was recorded by Brar *et al.* (2002) with increased level of nitrogen application. Studies of Veeraputhiran *et al.* (2002) on chlorophyll meter readings also revealed that the mean SPAD readings with various levels of nitrogen associated with higher SPAD readings at higher N levels. In the present investigation, leaf N content had a significant positive correlation with SPAD readings both at 120 and 150 DAS (Table 3). This indicated that the chlorophyll meter readings could be effectively used for instantaneous determination of leaf N content. A significant positive correlation between SPAD values and leaf N concentration in cotton was reported by Wu Feibo *et al.* (1998).

Application of N in 7 splits (S_3) significantly increased cry protein level (5,043 ng/g) over either N applied in 4 splits (4,066 ng/g) or recommended practice (3,583 ng/g)

Table 1. Effect of nitrogen levels, split application of nitrogen and detopping on cry protein, chlorophyll meter observation and leaf nitrogen concentration in Bt-cotton.

Treatment	Cry protein concentration (ng/g of fresh leaf wt.)				Leaf nitrogen concentration (%)*				Chlorophyll meter readings (SPAD Meter readings)	
	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS	120 DAS	150 DAS
Detopping (D)										
D_0 No Detopping	4484	1583	901	454	1.89	1.76	1.59	1.56	40.56	37.33
D_t Detopping	4228	1613	936	436	1.91	1.69	1.64	1.57	41.63	37.89
SEm ±	42	18	9	12	0.03	0.03	0.01	0.03	0.32	0.29
CD (P=0.05)	NS	NS	27	NS	NS	NS	NS	NS	0.96	NS
Nitrogen levels (kg /ha)										
N_1 80	3917	1262	801	396	1.79	1.68	1.51	1.47	40.06	36.3
N_2 120	4281	1675	975	442	1.86	1.72	1.62	1.59	41.22	38.1
N_3 160	4495	1857	979	497	2.05	1.78	1.72	1.63	41.99	38.5
SEm ±	57	15	14	13	0.03	0.02	0.03	0.02	0.43	0.37
CD (P=0.05)	141	37	35	32	0.08	0.06	0.08	0.05	1.06	0.91
Split application of N (S)										
S_1 Recommended method	3583	1318	803	431	1.89	1.75	1.61	1.52	40.46	37.7
S_2 4 splits at 30 days interval	4066	1577	895	458	1.88	1.75	1.60	1.59	41.38	37.2
S_3 7 splits at 15 days interval	5043	1900	1058	445	1.94	1.68	1.64	1.58	41.44	37.9
SEm ±	74	19	13	11	0.03	0.02	0.02	0.02	0.39	0.26
CD (P=0.05)	175	45	31	NS	NS	NS	NS	0.05	NS	NS
Interactions										
$D_0 N_1 S_1$	3948	992	594	421	1.72	1.72	1.48	1.47	38.75	36.38
$D_0 N_1 S_2$	4853	1209	682	451	1.72	1.78	1.53	1.51	40.17	35.25
$D_0 N_1 S_3$	4472	1816	1112	392	1.93	1.66	1.45	1.49	39.03	37.07
$D_0 N_2 S_1$	3504	1302	949	443	1.90	1.74	1.63	1.46	39.63	37.58
$D_0 N_2 S_2$	4167	1620	878	437	1.83	1.83	1.54	1.69	40.48	36.83
$D_0 N_2 S_3$	5541	1955	994	463	1.85	1.75	1.63	1.55	40.42	38.77
$D_0 N_3 S_1$	3748	1444	834	407	2.01	1.79	1.72	1.58	41.58	38.30
$D_0 N_3 S_2$	4319	1720	1152	611	2.03	1.80	1.73	1.70	41.62	37.57
$D_0 N_3 S_3$	5800	2192	912	465	2.01	1.79	1.65	1.59	43.32	38.27
$D_t N_1 S_1$	2996	1052	625	338	1.87	1.68	1.52	1.41	40.13	36.83
$D_1 N_1 S_2$	3274	1238	870	358	1.67	1.60	1.51	1.44	41.35	36.33
$D_1 N_1 S_3$	3956	1267	922	416	1.84	1.61	1.59	1.52	40.93	35.73
$D_t N_2 S_1$	3362	1424	864	478	1.86	1.71	1.61	1.61	41.37	38.52
$D_1 N_2 S_2$	3747	1747	831	414	1.90	1.69	1.65	1.58	42.75	38.72
$D_1 N_2 S_3$	5365	2005	1335	417	1.84	1.61	1.65	1.62	42.68	38.12
$D_t N_3 S_1$	3939	1693	948	497	2.00	1.85	1.68	1.59	41.27	38.55
$D_1 N_3 S_2$	4038	1930	957	482	2.11	1.78	1.66	1.62	41.92	38.55
$D_1 N_3 S_3$	5125	2165	1072	519	2.13	1.63	1.85	1.72	42.27	39.63
SEm ±	180	47	33	27	0.07	0.05	0.06	0.05	0.96	0.64
CD (P=0.05)	427	111	78	64	0.17	0.13	0.15	0.13	2.27	1.52

*Days after sowing, *Fully opened 4th leaf from top

at 60 DAS (Table 1). However, cry protein levels were not significantly affected due to split application methods at 150 DAS. Enhancing the level of cry protein due to split application may be because of increased availability of nitrogen at all the stages for protein synthesis. At 150 DAS on par results may be due to that there was no further application of nitrogen after 120 days of sowing. This supports the theory of readily available state of N due to split application. Pettigrew and Adamczyk (2006) reported similar results and they found that split application of N exhibited 14% higher *Cry 1Ac* concentration over other treatments. Readily available state of nitrogen enhances the growth by activating protein synthesis in the plant. Anything that alters protein synthesis or metabolism potentially alters *Cry 1Ac* expression (Arnold Burns and Craig Abel, 2003).

Studies on interaction effects on cry protein concentration for nitrogen levels and split application reveals (NxS) that cry protein was significantly improved with nitrogen applied in seven splits either at 120 (N_2S_3) (5,453 ng/g) or at160 (N_3S_3) kg N/ha (5,462 ng/g) when compared with all other interactions at 60 DAS (Table 1). Similar trend was followed at 90 and 120 DAS. It shows that increase in split-levels with increased nitrogen improved the cry protein from 60 to 120 DAS. Similar trend was followed for leaf nitrogen concentration and leaf chlorophyll meter

Table 2. Effect of nitrogen levels, split application of nitrogen and detopping on yield and yield parameters of Bt-cotton

Treatment	No of good bolls/ plant	No of bad bolls/ plant	Total No. of bolls/ plant	Boll weight (g)	Yield per plant (g)	Seed cotton yield (t/ha)
Detopping (D)						
D_0 No Detopping	22.8	5.1	27.9	5.08	128.5	2.272
D_t Detopping	22.9	5.5	28.5	5.08	120.6	2.213
S.Em ±	0.28	0.06	0.26	0.06	2.56	0.023
CD (P=0.05)	NS	0.23	NS	NS	NS	NS
Nitrogen levels (Kg /ha)						
N_1 80	20.7	4.8	25.5	4.82	111.7	2.034
N_2 120	23.3	5.3	28.6	5.16	125.5	2.279
N_3 160	24.7	5.8	30.5	5.26	136.5	2.412
S.Em ±	0.28	0.12	0.33	0.04	1.76	0.023
CD (P=0.05)	0.64	0.36	0.99	0.12	5.28	0.069
Split application of N (S)						
S_1 Recommended method	23.0	5.4	28.5	5.12	127.7	2.267
S_2 4 splits at 30days intervals	22.0	5.4	27.5	4.96	120.3	2.222
S_3 7 splits at 15 days intervals	23.5	5.0	28.6	5.15	125.7	2.237
S.Em ±	0.34	0.13	0.40	0.06	1.96	0.026
CD (P=0.05)	0.94	NS	NS	0.17	5.43	NS
Interactions						
$D_0 N_1 S_1$	21.4	4.9	26.3	4.82	120.4	2.078
$D_0N_1 S_2$	19.9	4.2	24.1	4.51	108.2	2.083
$D_0N_1 S_3$	20.8	4.3	25.0	5.08	120.5	2.077
$D_0 N_2 S_1$	23.0	4.4	27.4	5.13	130.1	2.406
$D_0N_2 S_2$	23.3	5.4	28.7	5.23	126.7	2.306
$D_0N_2 S_3$	25.3	5.1	30.4	5.30	133.5	2.228
$D_0 N_3 S_1$	23.2	5.8	29.0	5.32	147.1	2.408
$D_1N_3 S_2$	24.7	6.5	31.2	5.10	136.6	2.429
$D_1N_3 S_3$	24.0	5.1	29.0	5.18	133.7	2.429
$D_t N_1 S_1$	21.6	6.0	27.6	4.88	118.7	2.167
$D_1N_1 S_2$	19.8	4.8	24.5	4.67	100.3	1.883
$D_1N_1 S_3$	20.5	4.8	25.3	4.92	101.8	1.918
$D_t N_2 S_1$	23.7	6.1	29.8	5.18	122.8	2.195
$D_1N_2 S_2$	20.4	5.7	26.1	4.90	108.7	2.208
$D_1N_2 S_3$	23.9	5.2	29.1	5.19	131.2	2.332
$D_t N_3 S_1$	25.4	5.3	30.6	5.37	127.0	2.348
$D_1N_3 S_2$	24.3	6.1	30.4	5.35	141.0	2.425
$D_1N_3 S_3$	26.8	5.9	32.8	5.22	133.6	2.437
SEm ±	0.83	0.3 3	0.97	0.15	4.80	0.064
CD (P=0.05)	2.30	0.91	2.69	0.41	13.30	0.177

Table 3. Correlations between cry protein concentrations and dependent variables

Variables	15	14	13	12	11	10	9	8	7	6	5
Cry protein at 60 DAS	-	-	-	-	-	0.530*	-	-	-0.345	-	-
Cry protein at 90 DAS	-	-	-	-	0.391	-	-	-0.249	-	-	-0.807*
Cry protein at 120 DAS	-	0.438	-	0.233	-	-	-0.226	-	-	-0.233	-
Cry protein at 150 DAS	0.295	-	0.737*	-	-	-	-	-	-	-	-
Number of larvae per plant at 90 DAS	-	-	-	-	0.126	-	-	0.253	-	-	1.000
Number of larvae per plant at 120 DAS	-	0.255	-	0.427	-	-	0.101	-	-	1.000	
Per cent fruiting damage 60 DAS	-	-	-	-	-	0.354	-	-	1.000		
Per cent fruiting damage 90 DAS	-	-	-	-	0.030	-	-	1.000			
Per cent fruiting damage 120 DAS	-	0.717*	-	0.045	-	-	1.000				
N concentration. in leaf at 60 DAS	-	-	-	-	-	1.000					
N concentration. in leaf at 90 DAS	-	-	-	-	1.000						
N concentration. in leaf at 120 DAS	-	0.753*	-	1.000							
N concentration in leaf at 150 DAS	0.598*	-	1.000								
SPAD readings at 120 DAS	0.567*	1.000									
SPAD at 150 DAS	1.000										

P< 0.05; r = 0.468, DAS: Days after sowing

observations at 120 and 150 DAS. Positive correlations between leaf nitrogen and spad readings have indicated their direct relationship. Further, leaf nitrogen contents at 60 and 150 DAS were positively correlated with cry protein concentrations.

Interaction of D X N for cry protein concentration at 90 DAS indicated that application of 160 kg N/ha at both detopping (D_tN_3) (1,929 ng/g) and no detopping (D_0N_3) (1,785 ng/g) recorded highest concentration (Table 1). Similar trend was followed at 150 DAS. It showed that irrespective of detopping which had minimum effect on cry protein expression has improved with increasing level of nitrogen. This indicated the effect of nitrogen levels rather than detopping to cause for change in cry protein levels. Similar trend was observed for leaf nitrogen concentration at 60, 90 and 120 DAS and chlorophyll meter readings at 120 and 150 DAS.

Seed cotton yield

Nitrogen fertilization studies made in present investigation on Bt-cotton indicated that as a mean of two years seed cotton yield differed significantly due to application of different levels of nitrogen. Increasing level of nitrogen from 80 to 120 and further to 160 kg/ha significantly increased the seed cotton yield by 12.1 and 18.6%. Thus application of 160 kg nitrogen/ha is required to produce higher yields of Bt-cotton (Table 2). Similar results with increasing levels of nitrogen application were reported by Yasar kasap and Fatih Killi (2004).

Results indicated that seed cotton yield was not significantly affected due to more number of splits. Pooled data indicated that application of N by recommended method with three splits (2.27 t/ha), N applied in 4 splits at 30 days intervals (2.22 t/ha) and N in seven splits at 15 days intervals (2.24 t/ha) produced on par yields (Table 2). Same trend was followed with total number of bolls harvested/plant and number of bad bolls/plant. Current recommended practice of top dressing with 50 per cent of nitrogen applied in 3 equal splits at 50, 80 and 110 DAS was coincided with the square, flowering and boll development stages. Therefore, the supplementation of N at these stages may be ideal to meet the requirements even for Bt-cotton (Srinivasan, 2003). Hence, the present recommended practice of split application of N to irrigated Bt-cotton could be followed to obtain high yields.

Canopy modification by detopping in Bt-cotton did not influence significantly on seed cotton yield per ha when compared with no detopping. Even though the plant was condensed vertically and reduced sympodial branches the productivity was not affected due to detopping. Similar results were recorded by Brar *et al.* (2002).

It was concluded that in Bt. Cotton application of 120 kg N/ha is required to keep the crop condition in a higher productivity with better management of cry protein level at all stages of crop. Split application of N increased the cry protein levels in cotton. SPAD meter readings can be used for relating with the status of leaf cry protein concentration in Bt-cotton.

REFERENCES

Adamczyk, J.J., Adams, L.C. and Hardee, D.D. 2000. Quantification of Cry 1Ac delta endotoxin in transgenic Bt-cotton: Correlating insect survival to different protein levels among plant parts and varieties. (In:) *Proceedings of Beltwide Cotton*

Conferences, held at San Antonio, USA, 4-8, January, 2000 **2**: 929–932.

Arnold Bruns, H. and Craig A. Abel, 2003. Nitrogen fertility effects on Bt ä-Endotoxin and nitrogen concentrations of maize during early growth. *Agronomy Journal* **95**: 207–211.

Brar, A.S., Novtej Singh and Deol, J.S., 2002. Influence of plant spacing and growth modification practices in yield and its attributing characters of two cotton cultivars (*G. hirsutum*). *Journal of Research, Punjab Agriculture University* **39**(2): 181–183.

Pettigrew, W.T. and Adamczyk, J.J. 2006. Nitrogen fertility and planting date effects on lint yield and *Cry 1 Ac* (Bt) Endotoxin production. *Agronomy Journal **98*:** 691–697.

Rochester Ian, J. 2006. Effect of genotype, edaphic, environmental conditions and Agronomic practices on *Cry 1AC* protein expression in transgenic cotton. *The Journal of Cotton Science* **10**: 252–262.

Srinivasan, G. 2003. Response of cotton (*Gossypium hirsutum*) to split application of major nutrients. *Indian Journal of Agronomy* **48**(1): 59–61.

Veeraputhiran, R., Kandaswamy, O.S. and Chandrasekhar, C.N. 2002. Standardization of Chlorophyll meter readings for summer irrigated hybrid cotton. *Madras Agricultural Journal* **89**: 144–146.

Wu feibo, Wu lianghuan and Wu fuhua. 1998. Chlorophyll meter to predict nitrogen side dress requirements for short season cotton (*Gossypium hirsutum* L.) *Field Crops Research* **56:** 309–314.

Yasar, Kasap. and Killi, Fatih. 2004. Effect of row spacing and nitrogen interaction in seed cotton yield under irrigated conditions of Turkey. *Indian Journal of Agronomy* **49**(1): 64–67.