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IMPACT OF MOISTURE STRESS AND NITROGEN ON CROP GROWTH RATE, NITROGEN USE EFFICIENCY, AND HARVEST INDEX OF COTTON (*Gossypium hirsutum* L.)

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Nitrogen and moisture stress generally modify growth and development of cotton like several other field crops. Field experiments were conducted for two years to elucidate the interactive effect of moisture stress (I), nitrogen (N), and genotype (V) on agronomic traits, nitrogen use efficiency (NUE) and harvest index (HI) of cotton by using RCBD split-split plot experimental design. Four moisture stress treatments (I_1: no stress, I_2: moisture stress at inter-node elongation stage, I_3: moisture stress at vegetative growth stage, and I_4: moisture stress at inter-node elongation and vegetative growth stages), three nitrogen levels (50-N_1, 100-N_2, and 150 kg ha^{-1}-N_3), and three genotypes of cotton (NIAB-846-V_1, NIAB-824-V_2, and CIM-496) were used in this study. Moisture stress, nitrogen, and cotton genotypic interaction exhibited significant effect on number of bolls per plant, leaf area duration (LAI), crop growth rate (CGR), net assimilation rate (NAI), NUE, and HI. In all genotypes of cotton, highest CGR, NAI, NUE, and HI was recorded where irrigation was applied at all growth stages (in no stress treatment) and moisture stress at vegetative growth stage (in treatment where irrigation was withheld at vegetative growth stage) with N dose of 50 kg ha^{-1} expect in few treatments where 150 kg N ha^{-1} exhibited greater crop growth rate. During 1st year field experiment highest increase in HI of; NIAB-846 was 10.46% by $I_3 \times N_1 \times V_1$, NIAB-824 was 80.62% by $I_3 \times N_3 \times V_2$, and CIM-496 was 33.91% by $I_3 \times N_3 \times V_3$. During 2nd year, highest increase in HI was 31.60% in NIAB-846 (by $I_3 \times N_3 \times V_1$), 20.98% in NIAB-824 (by $I_1 \times N_1 \times V_2$), and 20.10% in CIM-496 (by $I_1 \times N_2 \times V_3$). Nevertheless, highest NUE was achieved in no stress treatment but moisture stress at vegetative growth stage didn't significantly reduce harvest index and nitrogen use efficiency as compared to no stress (well water) treatment. Thus it may be concluded that moisture stress at vegetative growth stage in these cotton genotypes could be applied for saving of irrigation water and nitrogen fertilizer.
Keywords: Cotton, crop growth rate, harvest index, moisture stress, nitrogen use efficiency.

INTRODUCTION

Nitrogenous fertilizers and irrigation water are essential inputs for sustainable production of cotton. Water scarcity due to global environmental changes, sharply increasing costs of fertilizers, and intensive cropping demands for judicious use of these inputs by use of improved management practices. Field crops' yield including cotton has been adversely affected by shortage of irrigation water in recent years (Dagdelen *et al.*, 2009; Singh *et al.*, 2010). Wastage of irrigation water and mismanagement in nitrogenous fertilizers application in cotton has also increased its cost of production (Devkota *et al.*, 2013).

In Pakistan, importance of water saving technologies has been recognized by many scientist for judicious use of limited river water supplies (Buttar *et al.*, 2007) that are further depleting day by day. It is great challenge for the scientists to generate new knowledge for better understanding of changes in agronomic and physiological traits of cotton under multiple stresses especially moisture and nutrients stress in the field. Environmental variations and drought has caused greater changes in genotype x year and/or genotype x location interactions of cotton (Islam *et al.*, 2013; Gul *et al.*, 2014). Even under favorable environment with higher nitrogen, the yield progress was not up to the mark (Howard *et al.*, 2001). A shift in production technologies is required for sustainable yield improvement under both the conditions. Injudicious use of irrigation water and nitrogenous fertilizers are major causes of lower average yield of seed cotton in Pakistan. Depletion of irrigation resources and ever increasing costs of fertilizers have further enhanced the importance of improvement in management practices for saving of irrigation water and fertilizers. The problem of irrigation water shortage has become a global (Clay *et al.*, 2001). In spite of the importance of water shortage, the research output of water saving technologies is negligible. There are several reasons of this shortcoming. Firstly, the traditional diagnostic tools that are being used for measurement of water use (e.g. plant transpiration and stomatal conductance) are point measurements. Secondly, the experimental approaches for direct measurement of water (i.e., weighing lysimeters) are expensive to build, operate, and maintain. Thirdly, it is general assumption by several scientists that management recommendations should not depend on unpredictable climatic conditions. So it is very important to develop water saving technologies. Over-irrigation and insufficient irrigation both may be drastic for crops (Fereres *et al*., 2007). Over-irrigation in cotton causes more vegetative growth, nutrients leaching, and contamination of ground water. On the other hand insufficient irrigation may affect plant growth, less fruiting and lower yield. It is a matter of great concern to increase the nutrients and water use efficiency of cotton under diminishing water resources. Although several components of soil-plant system can be managed but the effects on water use efficiency is not consistent across locations and experiments (Hatfield *et al*., 2001). The main reason may be the lack of information addressing the response of cotton plants to varietal-specific multiple stresses in the field. Due to important cash crop, several scientists have made efforts to increase water use efficiency of cotton (Tennakoon and Milroy, 2003; Tang *et al*., 2005); however, the knowledge about genotypic-specific response of cotton on alkaline calcareous soils under moisture stress and nitrogen interaction effects is rare. In Pakistan cotton is grown on >3 million hectares under extreme climatic conditions of high temperature ranging 28-50°C on soils which are alkaline calcareous in nature with problems of high nutrients losses coupled with brackish under-ground water that is not fit for irrigation. In this view it was imperative to investigate the changes in plant growth, nitrogen use efficiency, and harvest index of cotton by moisture stress, nitrogen, and genotypic interaction; to test the hypothesis that whether we can save irrigation water and nitrogen by imposing moisture stress at certain growth stages without any significant yield loss.

MATERIALS AND METHODS

***Experimental sites and treatments detail*:** Field experiments were conducted for two consecutive years (2008 to 2010) at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan situated in mixed cropping zone of country. Soil properties were examined prior to field experiment each year. Experimental fields were medium loam with greater proportion of sand and silt, alkaline calcareous in nature with low organic matter. Meteorological data of the experimental fields were also recorded at the nearest observatory (situated about 500 m away from experimental plots). Randomized complete block design with split-split plot arrangements in triplicate was used in these experiments. Plot size was kept 3.6 × 3.0 m. Three factors; moisture stress, nitrogen, and genotype were used in this study. Moisture stress treatments used were: no stress = eight irrigations as recommended by Government Agricultural Extension Departments, all irrigations were applied at 50% available soil moisture depletion level (ASMDL)-I_1; moisture stress at inter-node elongation stage = withholding irrigation in July up to 80% ASMDL-I_2; moisture stress at vegetative growth stage = withholding irrigation in September up to 80% ASMDL-I_3; moisture stress at inter-node elongation and vegetative growth stage = withholding irrigation in July and September at 80% ASMDL-I_4. Nitrogen treatments were: 50-N_1, 100-N_2, and 150 kg ha^{-1} -N_3 (recommended dose for farmers). Urea (46 % N) was used as a source of nitrogen in all the treatments. Cotton genotypes grown in these field experiments were; NIAB-846 (V1), NIAB-824 (V2), and CIM-496 (V3).

Table 1. The meteorological data during cotton growing seasons.

Climatic factor/growing season		May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean Max. Temperature									
Mean Max. Temp.	2008-09	38.40	37.80	37.70	35.00	35.30	33.70	28.30	22.90
	2009-10	40.10	40.90	37.90	36.50	36.10	33.40	26.30	22.90
Highest Max. Temp.	2008-09	44.50	41.60	41.00	39.40	38.30	37.30	33.60	27.40
	2009-10	44.90	45.00	43.60	40.60	38.60	37.50	32.50	27.00
Lowest Temp.	2008-09	30.50	30.60	32.20	27.70	29.00	28.00	23.60	24.00
	2009-10	31.80	34.20	32.00	26.30	25.70	28.50	23.50	19.60
Mean Min. Temperature									
Mean Min. Temp.	2008-09	23.40	26.90	27.70	26.00	23.20	19.90	10.80	7.80
	2009-10	24.30	25.60	26.50	26.40	23.40	16.20	10.00	4.60
Highest Min. Temp.	2008-09	26.70	30.00	30.40	28.40	26.20	25.50	16.00	15.00
	2009-10	29.50	29.50	30.00	29.70	26.30	25.10	16.80	7.80
Lowest Min. Temp.	2008-09	19.00	21.50	23.00	21.00	17.00	15.80	6.00	5.00
	2009-10	18.90	21.80	21.40	20.00	21.00	11.00	4.00	2.30
Rainfall Total	2008-09	53.90	118.20	63.40	273.00	37.00	-	-	14.50
	2009-10	10.00	5.80	52.50	137.40	30.20	14.80	0.40	0.00

From every plot, one half area was used for recording of growth parameters and destructive use, the remaining was allocated for the final harvest data. Soil water content in root zone was measured by using the gravimetric procedure of direct soil water measurement. The moisture content in root zone was computed by using the method as described by (Penman, 1970; French and Legg, 1979). Soil sampling from experimental plots (0-60 cm depth) was done on regular basis on alternate days with the help of soil sampling probe throughout cropping period (May-December) for determination of soil moisture. Soil sampling was done from inter-row spacing to avoid excavation of whole field. Effective root zone depth for moisture extraction in the soil was taken as 150 cm with exception of 15 cm surface layer. Oven drying of collected soil samples was made till constant weight at 100°C. When moisture content in the root zone was reached at desired level, irrigation water was applied to the treatments in measured quantity up to the field capacity. Rainfall received by the experimental plots was recorded and the remaining amount of water in a measured quantity was applied to the treatments at the achieving of desired moisture contents in the root zone as described for different moisture stress treatments. The total amounts of water (irrigation + rainfall) received by different treatments during 2008-09 growing seasons was 873.40 mm in I_1, 773.40 mm in I_2, 773.40 mm in I_3, and 673.40 mm in I_4. The same amount of water was applied in these treatments during 2009-10 growing season.

***Procedure for recording observations*:** Agronomic traits and physiological data were recorded from ten randomly selected guarded plants from every treatment. Portable laser leaf area meter (model CI-202, CID Bio-Science Inc.) was used in the field for measurement of leaf area. Leaf area of selected plants was measured, without detaching the leaves from plants and then averaged to get leaf area per plant.

The leaf area index was calculated by using the equation as given below:

$$\text{Leaf area index} = \frac{\text{Leaf area per plant}}{\text{Land area per plant}}$$

Leaf area duration is the relationship of leaf area index recorded at different time during growth period. Leaf area duration (LAD) was estimated according to Hunt (1978), as given:

$$LAD = \frac{LAI_1 + LAI_2}{2} \times t_2 - t_1$$

LAI_1 and LAI_2 are the leaf area indices at time t_2 (October) and t_1 (September) respectively.

A sample of three plants from every plot was harvested at ground level at 120 days (t_1) crop (peak fruiting stage) and at 150 days (t_2) crop (boll maturity stage). The plants components (leaves, fruits, stem, and branches) were separated and oven dried at 70°C in air forced oven till constant weight. Total dry matter per plant was obtained by adding weight of all components and average dry weight was calculated. Crop growth rate (g m^{-2} day^{-1}) was calculated as proposed by Hunt (1978):

$$CGR = \frac{W_2 - W_1}{t_2 - t_1}$$

Here W_1 and W_2 are the final dry matter at times t_1 and t_2, respectively.

Net assimilation rate (g m^{-2} day^{-1}) was calculated as proposed by Hunt (1978):

$$NAR = \frac{TDM}{LAD}$$

Here TDM and LAD are the total dry matter per plant and leaf area duration per plant, respectively.

For determination of seed cotton yield $plot^{-1}$, bolls from individual experimental plots were picked manually at 8-10% moisture contents in seed cotton and then weighed by using electronic balance, Then seed cotton yield was converted to yield ha^{-1} Total number of bolls on ten selected plants were counted and then averaged to calculate number of bolls per plant. Then plants were harvested at ground level and sun-dried for one week till 10±2% moisture and weighed and averaged to calculate Shoot dry weight per plant Harvest index was calculated by using the following formula:

$$\text{H.I. (\%)} = \frac{\text{Seed cotton yield kg per plant}}{\text{Total shoot dry weight kg per plant}} \times 100$$

Nitrogen use efficiency (NUE) was calculated as:

$$NEU = \frac{\text{Seed cotton yield kg per hectare}}{\text{Nitrogen applied kg per hectare}}$$

(Thind *et al*., 2008)

The NUE (kg seed cotton per kg N applied) of different treatments was determined by above mentioned method.

***Meteorological data*:** Meteorological data recorded during the crop growing period (Table 1) presents mean monthly values of the temperature and rainfall. Mean values of maximum and minimum temperature during 2008-09 growing season were recorded as 38.40°C and 22.90°C in the months of May and December, respectively. Whereas mean values of maximum and minimum temperature were found in the months of June and December as 40.90°C and 22.90°C, respectively. Highest maximum temperature ranges were 44.50°C during May 2008, 45°C in June 2009; and lowest, temperature ranges were 24°C and 19.6°C during 2008 and 2009, respectively. Total rainfall during growing period was 560 mm during 2008 and 251 mm during 2009. Both the year maximum rainfall of 273 mm and 137.4 mm was recorded in the month of August. Total rainfall during last four months of growth period from September to December was 51.5 mm in 2008 and 45.10 mm in 2009.

***Statistical analysis*:** Data collected were tabulated and analyzed by Randomized Complete Block 3-factors split-split plot design ANOVA process by using Fisher's analysis of variance technique. Factor effects were considered significant at the $P \leq 0.05$. Where F values were significant, means were separated by least significant difference (LSD) at 5% probability level (Steel and Torrie, 1984). Computer software packages Minitab-15 and Statistix version 8.1 were used for data analysis whereas. Microsoft excel version 2010 was used for preparation of graphs.

RESULTS

***Number of bolls per plant at maturity*:** The number of bolls per plant varied widely under different N levels (Table 2) during cropping season 2008-09 but remained non-significant during 2009-10 growing season. Treatments (I×N×V) exhibited significant variation in number of bolls per plant during two years study. Highest number of bolls per plant (during 2008-09) was observed in treatment $I_1 \times N_2 \times V_1$ (no stress × 100 kg N ha^{-1} × NIAB-846) with 47.67 bolls per plant, followed by $I_3 \times N_3 \times V_1$ (moisture stress at vegetative growth stage × 150 kg ha^{-1} N × NIAB-846) with 44.27 bolls per plant (Fig. 1a). Similar trend was observed during 2009-10 (Fig. 1b) where maximum number of bolls per plant; 43.17 and 39.73 were recorded in treatments $I_1 \times N_2 \times V_2$ (no stress × 100 kg ha^{-1} N × NIAB-824) and $I_3 \times N_1 \times V_1$ (moisture stress at vegetative growth stage × 50 kg N ha^{-1} × NIAB-846), respectively.

***Leaf area duration*:** Leaf area duration is the relationship of leaf area index recorded at different time during growth period. Nitrogen and moisture stress markedly affected leaf area duration (LAD). Highest LAD in cotton genotypes was attained by I_1 (no stress) and N_3 (150 kg N ha^{-1}) during both the years. Among genotypes, highest LAD during 2008-09 was observed in NIAB-824 whereas during 2009-10, NIAB-846 showed greater LAD. Interaction (I×N×V) significantly affected LAD as shown in Fig. 1c,d. During 2008-09 (Fig. 1c), highest LAD (216 d) was recorded by $I_1 \times N_3 \times V_2$ (no stress × 150 kg N ha^{-1} × NIAB-824) followed by 213 d LAD by $I_1 \times N_3 \times V_1$ (no stress × 150 kg N ha^{-1} × NIAB-846) and 210 days LAD by $I_1 \times N_3 \times V_3$ (no stress × 150 kg N ha^{-1} × CIM-496). During 2009-10 (Fig. 1d), highest LAD of 235 and 234 d remained in $I_1 \times N_3 \times V_1$ (no stress × 150 kg N ha^{-1} x NIAB-846) and $I_2 \times N_1 \times V_3$ (stress at internodes elongation stage × 50 kg N ha^{-1} x CIM-496), respectively. Lowest LAD was found in treatments where moisture stress at two stages (i.e. internodes elongation and at vegetative growth stages) was imposed (Fig. 1 c,d). Lowest LAD of 87 and 94 d was noted

Table 2. Moisture stress, nitrogen, and genotypes interaction effect on number of bolls per plant, leaf area duration, crop growth rate, net assimilation rate, nitrogen use efficiency, and harvest index of cotton.

Factors	Bolls per plant		LAD (d)		CGR (g m^{-2} d^{-1})		NAR (g m^{-2} d^{-1})		NUE (kg SCY kg^{-1} N applied)		HI (%)	
	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10	2008-10	2009-10	2008-09	2009-10
Moisture stress (I)												
I_1	37.70a	35.58a	184a	167a	5.26a	4.81b	3.06a	3.16b	51.56a	53.01a	18.33b	19.14a
I_2	33.67b	32.28b	158c	164a	1.78c	1.49c	2.97b	3.28b	36.46c	48.37b	17.64b	17.98b
I_3	33.78b	35.96a	168b	155b	4.20b	5.63a	2.70b	3.61a	47.93b	52.30a	23.32a	19.68a
I_4	32.04b	29.47c	111d	143c	1.51d	1.30c	4.11a	3.75a	32.78d	44.21c	14.36c	17.90b
LSD P<0.05)	2.68*	2.20*	7.83*	11.43*	0.17*	0.20*	0.22*	0.09*	1.88*	1.63*	2.63*	1.03*
Nitrogen levels (N)												
N_1	31.92b	32.39	142b	168a	3.35a	3.40a	3.49a	3.24a	64.65a	79.98a	18.61	18.99
N_2	32.53b	34.32	161a	161b	3.26a	3.42a	2.98b	3.39b	39.27b	41.07b	18.51	18.80
N_3	38.44a	33.27	163a	142c	2.96b	3.10b	3.16b	3.72b	22.62c	27.38c	18.11	18.24
LSD (P<0.05)	2.32*	NS	6.78*	9.90*	0.15*	0.17*	0.22*	0.162*	1.63*	1.41	NS	NS
Genotypes (V)												
V_1	37.96a	35.18a	163a	178a	3.60a	3.76a	3.08b	3.03b	42.13	49.64a	20.20a	19.69a
V_2	31.37b	33.44a	168a	163b	2.91b	2.96c	2.99b	3.24b	42.12	51.00a	17.54ab	17.95b
V_3	33.56b	31.35b	134b	130c	3.06b	3.21b	3.57a	4.07a	42.29	47.79b	17.48b	18.38b
LSD (P<0.05)	2.32*	1.90*	6.78*	9.90*	0.15*	0.17*	0.20*	0.162*	NS	1.41	2.07*	0.89*
Interaction^^												
I × N	NS	2.61*	27.10	9.80*	0.30*	0.35*	0.20*	0.16*	3.26*	2.82*	5.84*	1.78*
I × V	NS	2.19*	27.10*	9.80*	0.30*	0.35*	0.20*	0.16*	3.26*	2.83*	5.84*	1.78*
N × V	NS	NS	23.47*	7.15*	0.26*	0.30*	0.17*	0.14*	2.82*	2.45*	4.79*	1.54*
I × N × V	8.04*	6.59*	6.94*	4.30*	0.51*	0.61*	0.34*	0.28*	5.64*	4.89*	11.99*	3.08*
Grand mean	34.29	33.32	155.3	57.00	3.19	3.31	3.21	3.45	42.18*	49.48	18.41	18.67
CV	14.39	12.14	9.29	6.73	9.89	11.25	13.08	10.00	8.22	6.07	19.93	10.16

*Factor/treatment effect is significant at $P \leq 0.05$, NS = not-significant at $P \leq 0.05$; Values within a column (factor wise) followed by the same letter are not statistically different at $P \leq 0.05$; ^^LSD values at $P \leq 0.05$; d = days during 2008-09 in $I_4 \times N_3 \times V_3$ and $I_4 \times N_3 \times V_1$ treatments, respectively. Similar trend was observed during 2009-10 when lowest LAD was found as 79 and 83 by $I_4 \times N_3 \times V_3$ and $I_4 \times N_1 \times V_3$, respectively (as shown in Fig. 1 c,d).

***Crop growth rate*:** Both the year crop growth rate (CGR) varied significantly by nitrogen and moisture stress treatments as shown in Table 2. During 2008-09, highest CGR of 9.05 g m^{-2} d^{-1} was recorded (Fig. 1e) in treatment $I_1 \times N_3 \times V_2$ (no stress × 150 kg ha^{-1} N × NIAB-824), followed by CGR of 6.94 and

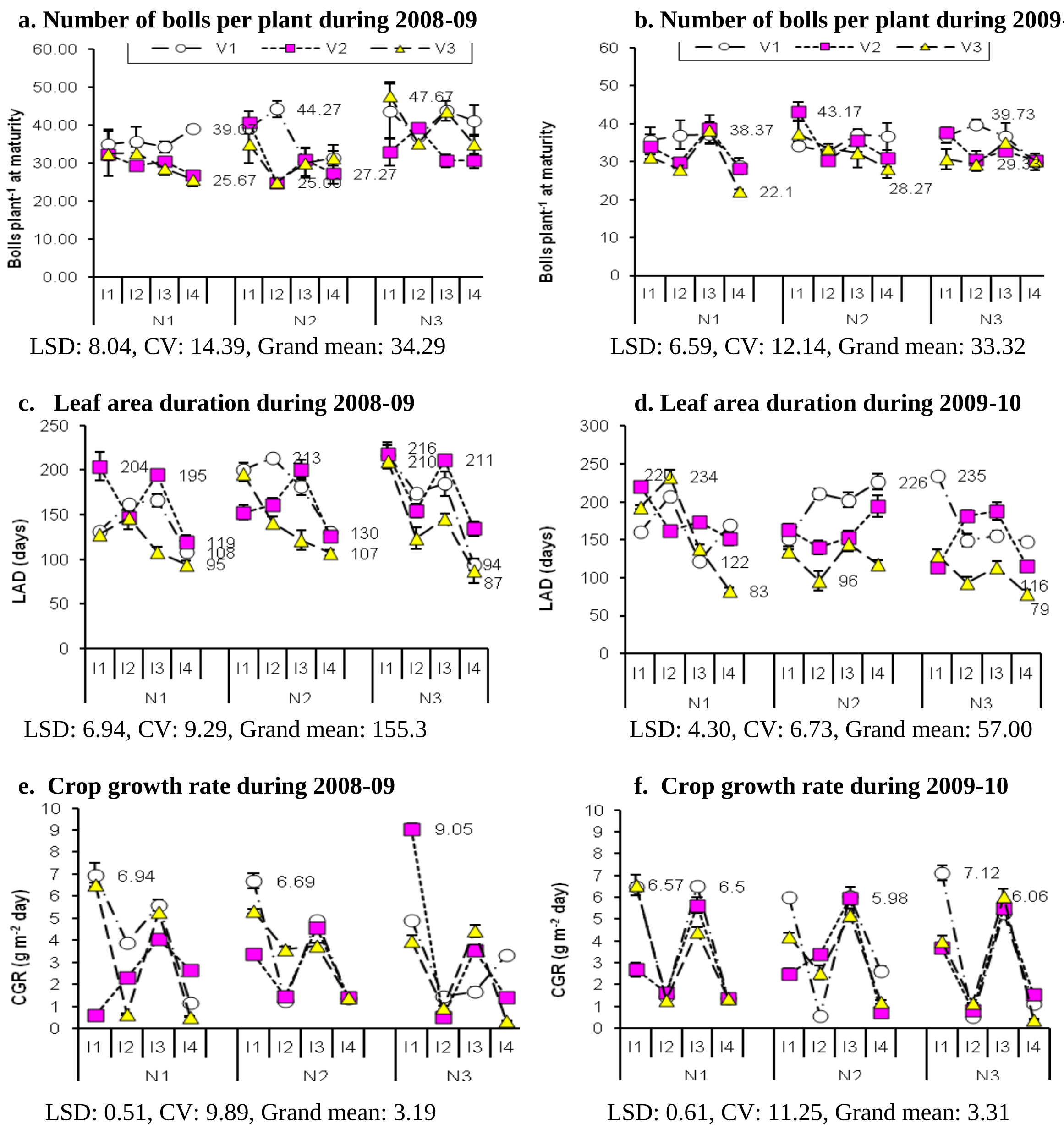

Figure 1. Interaction effect of moisture stress, nitrogen, and genotypes on; number of bolls per plant (a, b), leaf area duration (c, d), and crop growth rate (e, f). Factors are; moisture stress (I), nitrogen (N), and genotypes (V). Whereas moisture stress levels are; I_1 = no stress = water application at 50% available soil moisture depletion level (eight irrigations); I_2 = moisture stress at inter-node elongation stage at 80% ASMDL; I_3 = moisture stress at vegetative growth stage at 80% ASMDL; I_4 = moisture stress at inter-node elongation and vegetative growth stage at 80% ASMDL; nitrogen levels are; N_1 = 50 kg N ha^{-1}, N_2 = 100 kg N ha^{-1}, and N_3 = 150 kg N ha^{-1}; genotypes are; V_1 = NIAB-846, V_2 = NIAB-824, V_3 = CIM-496.

6.69 g m^{-2} d^{-1} in treatments $I_1 \times N_1 \times V_1$ (no stress × 50 kg ha^{-1} N × NIAB-846) and $I_1 \times N_2 \times V_1$ (no stress × 100 kg ha^{-1} N × NIAB-846), respectively. Lowest CGR of 0.33 g m^{-2} d^{-1} and 0.50 g m^{-2} d^{-1} in treatments $I_4 \times N_3 \times V_3$ (moisture stress at internodes elongation and vegetative growth stages × 150 kg ha^{-1} N × CIM-496) and $I_2 \times N_3 \times V_2$ (moisture stress at internodes elongation stage × 50 kg ha^{-1} N × NIAB-824), respectively. In all genotypes, highest crop growth rate was recorded in treatments of where no stress and moisture stress at vegetative growth stage with N dose of 50 kg ha^{-1} was used expect in few treatments where 150 kg N ha^{-1} exhibited greater crop growth rate (Fig. 1e,f). Lowest CGR, 0.40 g m^{-2} d^{-1} and 0.54 g m^{-2} d^{-1} was observed in $I_4 \times N_3 \times V_3$ (moisture stress at internodes elongation and vegetative growth stages × 150 kg N ha^{-1} × CIM-496) and $I_2 \times N_3 \times V_1$ (MSI × 150 kg N ha^{-1} × NIAB-846), respectively.

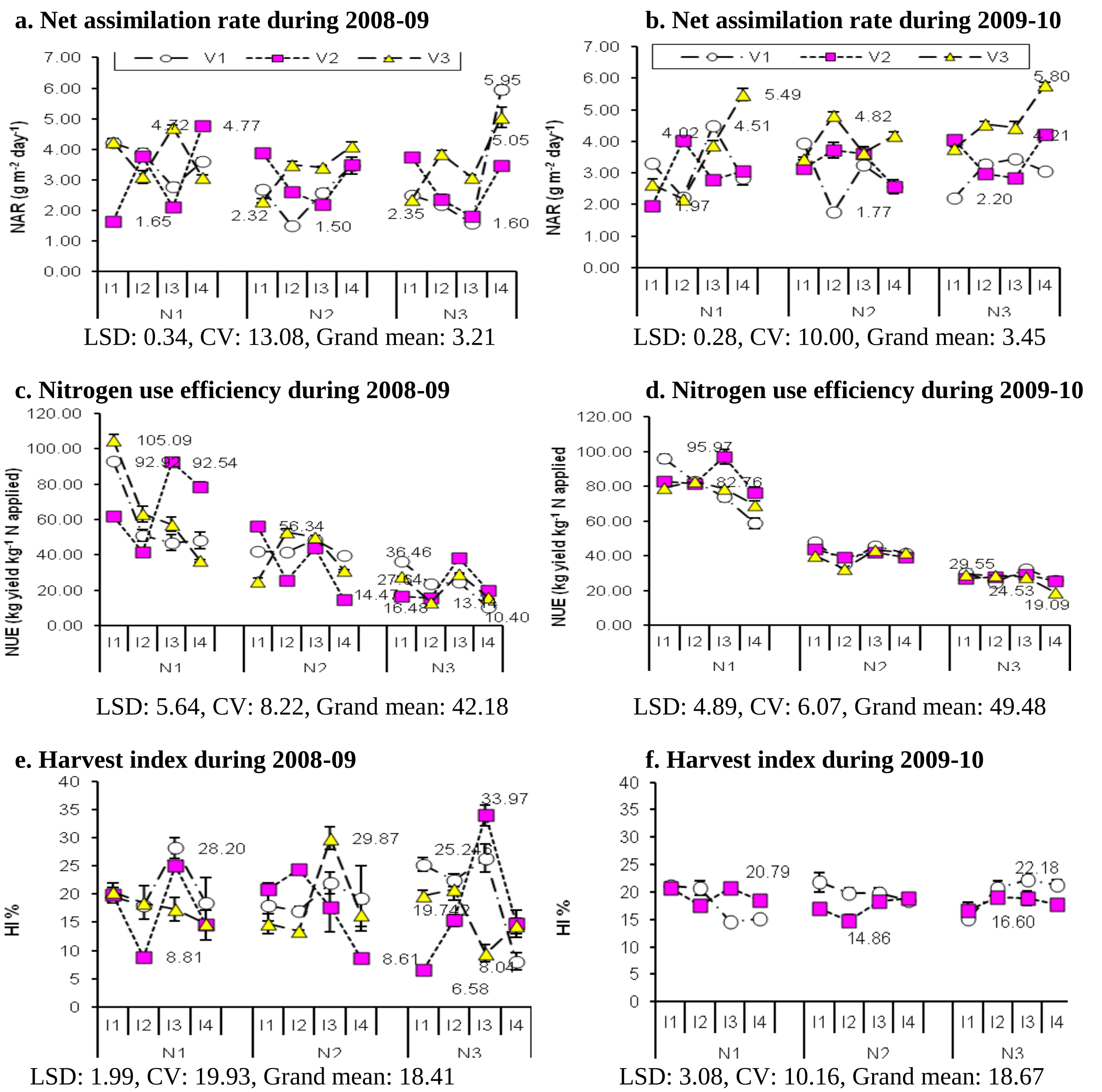

Figure 2. Interaction effect of moisture stress, nitrogen, and genotypes on; net assimilation rate (a, b), nitrogen use efficiency (c, d), and harvest index (e, f) of cotton. Factors are; moisture stress (I), nitrogen (N), and genotypes (V). Whereas moisture stress levels are; I_1 = no stress = water application at 50% available soil moisture depletion level (eight irrigations); I_2 = moisture stress at inter-node elongation stage at 80% ASMDL; I_3 = moisture stress at vegetative growth stage at 80% ASMDL; I_4 = moisture stress at inter-node elongation and vegetative growth stage at 80% ASMDL; nitrogen levels are; N_1 = 50 kg N ha^{-1}, N_2 = 100 kg N ha^{-1}, and N_3 = 150 kg N ha^{-1}; genotypes are; V_1 = NIAB-846, V_2 = NIAB-824, V_3 = CIM-496.

***Net assimilation rate*:** Factors and treatments affected net assimilation rate (NAR) and it has been shown in Table 2 and Figure 2a,b. As shown in Fig. 2a, during 2008-09 highest NAR was recorded by $I_4 \times N_3 \times V_3$ (moisture stress at internodes elongation and vegetative growth stage × 150 kg N ha^{-1} × NIAB-846) with 5.95 and 5.05 NAR in $I_4 \times N_3 \times V_3$ and $I_4 \times N_1 \times V_2$, treatments, respectively. Lowest NAR of 1.50 and 1.60 g m^{-2} day^{-1} was recorded in treatments $I_2 \times N_2 \times V_1$ (moisture stress at internodes elongation stage × 100 kg N ha^{-1} × NIAB-846), and $I_3 \times N_3 \times V_1$ (moisture stress at vegetative growth stage × 150 kg N ha^{-1} × NIAB-846), respectively. Fig. 2b shows treatments effect on NAR during 2009-10, highest NAR 5.80, 5.49, and 4.82 g m^{-2} day^{-1} were recorded by $I_4 \times N_3 \times V_3$ (moisture stress at internodes elongation and vegetative growth stages × 150 kg ha^{-1} N × CIM-496), $I_4 \times N_1 \times V_3$ (MSI+MSV × 50 kg ha^{-1} N × CIM-496), and $I_2 \times N_2 \times V_3$ (MSI × 100 kg ha^{-1} N × CIM-496) treatments, respectively. Lowest, 1.77, 1.97, and 2.20 g m^{-2} day^{-1} NAR were observed in $I_2 \times N_2 \times V_1$ (moisture stress at internodes elongation stage × 100 kg ha^{-1} N × NIAB-846), $I_1 \times N_1 \times V_2$ (no stress × 50 kg ha^{-1} N × NIAB-824), and $I_1 \times N_3 \times V_1$ (no stress × 150 kg ha^{-1} N × NIAB-846), respectively.

***Nitrogen use efficiency*:** Nitrogen use efficiency (NUE) in cotton genotypes as influenced by factors and treatments are shown in Table 2. Main effects results show higher NUE by no stress, and moisture stress at vegetative growth stage. Nitrogen dose 150 kg ha^{-1} showed higher NUE. As shown in Figure 2c, interaction of moisture stress, N levels, and genotypes (I×N×V) significantly affected NUE during 2008-09, with highest NUE of 105.09, 92.92, and 92.54 kg seed cotton yield (SCY) kg^{-1} N by $I_1 \times N_1 \times V_3$ (no stress × 50 kg ha^{-1} N × CIM-496), $I_1 \times N_1 \times V_1$ (no stress × 50 kg ha^{-1} N × NIAB-846), and $I_3 \times N_1 \times V_2$ (moisture stress at vegetative growth stage × 50 kg ha^{-1} N × NIAB-824) treatments, respectively. Lowest NUE, 10.40 and 13.14 kg SCY kg^{-1} N was recorded in $I_4 \times N_3 \times V_1$ (moisture stress at internodes elongation and vegetative growth stage × 150 kg ha^{-1} N × NIAB-846), and $I_2 \times N_3 \times V_3$ (moisture stress at internodes elongation stage × 150 kg ha^{-1} N × CIM-496) treatments, respectively. As shown in Fig. 2d, during 2009-10 highest NUE 97.06 and 95.97 kg SCY kg^{-1} N were recorded by $I_3 \times N_1 \times V_2$ (moisture stress at vegetative growth stage × 50 kg ha^{-1} N × NIAB-824) and $I_1 \times N_1 \times V_1$ (no stress × 50 kg ha^{-1} N × NIAB-846), respectively.

***Harvest index*:** Results showed (Table 2) that moisture stress, nitrogen, and genotypic interaction significantly affected harvest index (HI). During 2008-09 and 2009-10, highest HI of 23.32% and 19.68% was recorded in the moisture stress treatment at vegetative stage (I_3). NIAB-846 showed highest HI of 20.20% during 2008-09 and during 2009-10 highest HI of 17.95% was recorded NIAB-824. Minimum HI of 14.35 and 17.90 % was observed in I_4 (moisture stress at internodes elongation and vegetative growth stage). Treatments (I×N×V) results shown in Fig. 2e,f indicate a significant effect on HI by interaction effect of factors. Highest HI of 33.97% during 2008-09 (Fig. 2e) was recorded in $I_3 \times N_3 \times V_2$ (moisture stress at vegetative growth stage × 150 kg N ha^{-1} × NIAB-824) followed by 28.87% HI in $I_3 \times N_2 \times V_3$ (moisture stress at vegetative growth stage × 100 kg N ha^{-1} × CIM-496). During 2009-10 (Fig. 2f), treatment $I_1 \times N_2 \times V_3$ (no stress × 100 kg N ha^{-1} × CIM-496) resulted with 25.07% HI followed by 22.18% HI in $I_3 \times N_3 \times V_1$ (moisture stress at vegetative growth stage × 150 kg N ha^{-1} × NIAB-846) treatment. Minimum HI (during 2008-09) of 6.58% and 8.05% was observed by treatments $I_1 \times N_3 \times V_2$ (no stress × 150 kg N ha^{-1} × NIAB-824) and $I_4 \times N_3 \times V_1$ (moisture stress at internodes elongation and vegetative growth stages × 150 kg N ha^{-1} × NIAB-846), respectively. During 2009-10, lowest HI of 14.86%, 15.17%, and 15.59% was recorded in treatments $I_2 \times N_2 \times V_2$ (moisture stress at internodes elongation stage × 100 kg N ha^{-1} × NIAB-824), $I_1 \times N_3 \times V_1$ (no stress × 150 kg N ha^{-1} × NIAB-846), and $I_4 \times N_3 \times V_3$ (moisture stress at internodes elongation and vegetative growth stages × 150 kg N ha^{-1} × CIM-496), respectively.

DISCUSSION

Water scarcity due to global environmental changes is drastically affecting the seed cotton production. This scenario has opened up a challenge for agronomists to develop new technologies. Moisture stress along with nitrogen management at certain growth stages of cotton is found a promising approach for saving of irrigation water and urea fertilizer. Plant growth, nitrogen use efficiency and harvest index of cotton is improved by imposing moisture stress at vegetative growth stage coupled with lower N. Prior to this study, Meredith *et al.* (1997) noted highest yield in cotton genotypes by lower N application as compared to high N dose.

Field experiments were conducted (during 2008-2010) to investigate the impact of interaction of moisture stress, nitrogen, and genotypes on growth, nitrogen use efficiency, and harvest index of cotton. Higher number of bolls per plant was observed in treatments of; no moisture stress, and moisture stress at vegetative growth stage. Number of bolls per plant were significantly reduced in cotton genotypes by I_4 and I_2. Results showed that NIAB-846 got sustainable fruiting by different N doses used exhibiting more stability towards multiple stresses where as other two genotypes showed inconsistent trend in fruit bearing by application of different N doses. Mixed response of cotton genotypes towards fruit bearing by N application indicates that deteriorating and/or adaptive changes in cotton plants under multiple interaction effects in the field, is genotypic-specific which depends upon morphological response of specific genotypes. The results in the present study show that highest number of bolls per plant was recorded by lower application of N (100 kg N ha^{-1}) in NIAB-824 as compared to higher N (150 kg ha^{-1}) application during 2009-10 as shown in Fig. 1a. Highest number of bolls per plant (Fig. 1b), was recorded in CIM-496 by application of 150 kg ha^{-1}N in no stress treatment. In previous studies (Milroy and Bange, 2004), N deficiency had been reported as a limiting factor for vegetative growth and fruiting. In some other studies it had been reported that higher level of N application cause more vegetative growth while shifting the balance between reproductive and vegetative growth phases and causing less fruiting (Howard *et al*., 2001). Sensitivity of cotton to N application is well established; excessive as well as lower N dose both may cause drastic effects causing higher vegetative growth, less fruiting, and ultimately lower seed cotton yield. Field experiments resembling the present investigation will help to sort-out best genotypic-specific N and moisture stress combinations for achievement of better fruiting and higher seed cotton yield.

The results of leaf area duration (LAD) are presented in Figure 1c,d. Lowest LAD was observed in high moisture stress treatments (i.e. moisture stress at internodes elongation and at vegetative growth stages). In all genotypes LAD was not affected by N levels in I_4 stress level. However, LAD was increased with higher N application in no stress treatment. Improvement in LAD was also note in treatments where moisture stress was imposed at vegetative growth stage. Higher seed cotton yield was observed in the treatments where more LAD was achieved due to increase in fruiting and crop growth rate. Wolfe *et al*. (1983) also studied nitrogen and moisture stress impact on LAD of maize where they found an inhibition in LAD, crop biomass, and ultimately grain yield by deficit irrigation and N application. In present study we have observed that deficit irrigation at vegetative growth stage of cotton didn't markedly inhibited LAD and seed cotton yield as compared to well water (no stress) treatment, even by application of lower N dose. This technique may be helpful in saving of irrigation water and N fertilizer.

Highest crop growth rate was achieved by well water (no stress) treatments as shown in Figure 1e,f. The cotton genotypes grown under no stress (well water) treatments showed inconsistent response towards different N doses. Higher CGR trend was observed in NIAB-846 by lower N dose (50 kg ha^{-1}), whereas higher CGR in other genotypes, CIM-496 and NIAB-824, was noted with higher N doses of 100, 150 kg N ha^{-1}, respectively. Lowest CGR was recorded in high stress treatments (moisture stress at internodes elongation and vegetative growth stages). In earlier studies, Monteith (1976) reported that radiation use efficiency and CGR in cotton was reduced under drought conditions. In another study made by McMichael and Hesketh (1982), it was observed that induced drought in cotton (*Gossypium hirsutum* L.) caused a reduction in photosynthesis rate, leaf expansion, vegetative nodes, and CGR. However, the information about the changes in CGR by multiple interaction effect of moisture stress at certain growth stages of cotton, N doses, and genotypes; was missing.

Net assimilation rate was significantly affected by moisture stress treatments as shown in Figure 2a,b. Highest NAR was observed in high moisture stress treatments (moisture stress at internodes and vegetative growth stages) along with higher N doses (100, 150 kg ha^{-1}). However, in no stress (well water) treatment highest NAR was noted when lower N dose (50 kg ha^{-1}) was applied. Kimball and Mauney (1993) studied the effect of moisture stress, N, and CO_2 levels on cotton and they observed 60-63% increase in cotton yield in moisture stress treatments as compared to control (no stress) treatment. They also found that under well managed moisture stress, CO_2 response was higher as compared to normal irrigation levels. In present study, higher NAR values were recorded in moisture stress treatments without any change in the CO_2 level and under no stress (normal irrigation); lower N dose resulted in higher NAR.

In cotton genotypes highest NUE was attained by N interaction with no stress (well water) and moisture stress at vegetative growth stage. In the treatments of vegetative growth stage stress, even lower N dose (50 kg ha^{-1}) gave higher NUE as shown in Figure 2c,d. Thind *et al*. (2008) investigated irrigation and N effect on cotton and found significant effect of treatments on NUE of cotton but he didn't evaluated the comparative NUE in different genotypes of cotton. While comparing highest NUE in three genotypes over control treatments ($I_1 \times N_3 \times V_1$, $I_1 \times N_3 \times V_2$, $I_1 \times N_3 \times V_3$), during 2008-09 (Fig. 2c), 60.76% higher NUE was noted in NIAB-846 in treatment $I_1 \times N_1 \times V_1$ (no stress × 50 kg N ha^{-1} × NIAB-846, 82.19% higher NUE in NIAB-824 in treatment $I_3 \times N_1 \times V_2$ (moisture stress at vegetative growth stage × 50 kg N ha^{-1}× NIAB-824) , and 73.86% higher NUE in CIM-496 in treatment $I_1 \times N_1 \times V_3$ (no stress × 50 kg N ha^{-1}× CIM-496). Similar trend was observed during 2009-10 (Fig. 2d) where 69.20% higher NUE was noted in NIAB-846 in $I_1 \times N_1 \times V_1$ treatment, 72.06% higher NUE in NIAB-824 in $I_3 \times N_1 \times V_2$ treatment, and 64.29% higher NUE in CIM-496 in $I_2 \times N_1 \times V_3$ treatment. In earlier experiments Meredith *et al*. (1997) also observed effect of N × genotypic interaction on NUE of cotton cultivars and they found lower N dose better as compared to higher N application. Higher N application is yield limiting factor in cotton due to shifting of plant balance towards more vegetative growth that delays maturity and lowers seed cotton yield (Howard *et al*., 2001). In present study we also observed highest NUE in cotton genotypes by lower N dose (50 kg N ha^{-1}) as compared to higher level of N (150 kg N ha^{-1}). These results are in line with the previous studies made by Nicholos *et al*. (2004) who elucidated that excessive use of N in cotton may be a limiting factor for the growth, yield and radiation use efficiency.

Treatments significantly affected harvest index (HI) of cotton genotypes as shown in Figure 2e,f. During 2008-09 (Fig. 2e), highest HI was noted in treatments where moisture stress was imposed at vegetative growth stage. Highest HI of 33.97%, 29.87%, and 28.20% was observed in treatments $I_3 \times N_3 \times V_2$

(moisture stress at vegetative growth stage × 150 kg N ha^{-1} × NIAB-824), $I_3 \times N_2 \times V_3$ (moisture stress at vegetative growth stage × 100 kg N ha^{-1} × CIM-496), and $I_3 \times N_1 \times V_1$ (moisture stress at vegetative growth stage × 50 kg N ha^{-1} × NIAB-846), respectively. In comparison to control 10.46% higher HI was noted in $I_3 \times N_1 \times V_1$ (moisture stress at vegetative growth stage × 50 kg N ha^{-1} × NIAB-846), 80.62% in $I_3 \times N_3 \times V_2$ (moisture stress at vegetative growth stage × 50 kg N ha^{-1} × NIAB-846), higher HI in NIAB-824, and 33.91% higher HI in CIM-496 was noted. During 2009-10, the increase in HI values over control treatments were, 31.60% in NIAB-846 (in treatment $I_3 \times N_3 \times V_1$), 20.98% in NIAB-824 (in treatment $I_1 \times N_1 \times V_2$), and 20.10% higher in CIM-496 (in treatment $I_1 \times N_2 \times V_3$). In other stress treatments (I_2: moisture stress at internodes elongation stage, and I_4: moisture stress at two stages-internodes elongation and vegetative growth stages), lower HI values were observed as compared to stress at vegetative growth stage (I_3). In previous studies Steduto *et al*. (2009) reported that timing of moisture stress leads towards biomass partition and HI and it may increase or decrease HI depending upon crop growth stage but the information about the extent of change in HI by moisture stress and the identification of specific growth stages of genotypes at which moisture stress is useful, was missing in previous studies. In this study we have identified specific growth stages of cotton as well as genotypic-specific N dose for getting better crop growth rate, improvement in nitrogen use efficiency, and higher harvest index; leading towards cotton cultivation economically under limited irrigation application.

Conclusions**:** In cotton, moisture stress at vegetative growth stage coupled with genotypic-specific N management may be used for saving of irrigation water and urea fertilizer without compromising the harvest index and nitrogen use efficiency as compared to farmers' practice.

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