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ROOTING PATTERN AND NITROGEN USE EFFICIENCY IN COTTON (*GOSSYPIUM HIRSUTUM* L.) UNDER MOISTURE STRESS CONDITIONS

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ABSTRACT

Roots play an important role in exploring the plant's ability to explore the available soil volume for water and nutrients. Among abiotic stresses, moisture stress is one of the most imperative factor that exerts substantial impact on root growth, nutrients use efficiency, and seed cotton yield. Field experiments were conducted during 2008-09, to elucidate the effect of moisture stress on rooting pattern, seed cotton yield, and nitrogen use efficiency (NUE) of different genotypes grown in alkaline calcareous soils. Three factors were: {three genotypesviz.V_1 = NIAB-846, V_2 = NIAB-824, and V_3 = CIM-496); four moisture stresses(viz.I_1 = local control-eight irrigations (all applied at 50% available soil moisture depletion level, ASMDL),I_2 = moisture stress at inter-node elongation stage- in July up to 80% ASMDL, I_3 = moisture stress at vegetative growth stage- in September up to 80% ASMDL,and I_4 = moisture stress at inter-node elongation and at vegetative growth stage-in July and September up to 80% ASMDL)); and three nitrogen doses (viz. N_1 = 50 kg ha^{-1}, N_2 = 100 kg ha^{-1}, and N_3 = 150 kg ha^{-1}.)}arranged in Randomized Completed Block Design with split-split plot arrangements replicated thrice while keeping genotypes in main plots, moisture stresses in split plots, and nitrogen levels in split-split plots. Interaction treatments (I×N×V) significantly affected root:shoot ratio, seed cotton yield, and NUE. During 2008, highest root:shoot ratio (0.413) was recorded in NIAB-824 by$I_2 \times N_1 \times V_2$ followed by 0.333 root:shoot ratio in NIAB-846 by$I_2 \times N_1 \times V_1$treatment.During 2009, highest root:shoot ratio (0.412) was recorded in CIM-496 by $I_3 \times N_1 \times V_3$treatment. Under higher N dose (150 kg N ha^{-1}), root:shoot ratio didn't vary significantly by different moisture stress treatments in all genotypes. Results showed that, highest seed cotton yield of 4871 and 4853 kg ha^{-1} was achieved in NIAB-846 and NIAB-824 by $I_3 \times N_3 \times V_2$ and $I_1 \times N_2 \times V_2$, respectively (during 2008);and 5709 and 5634 kg ha^{-1}in NIAB-824 by treatments $I_3 \times N_3 \times V_2$ and $I_1 \times N_2 \times V_2$,(during 2009), respectively. During 2008, highest NUE of 105.09, 92.92, and 92.54 kg yield kg^{-1} N applied was obtained in $I_1 \times N_1 \times V_1$, $I_1 \times N_1 \times V_2$, $I_3 \times N_1 \times V_3$, respectively. Similar trend was observed during 2009 with highest NUE in I_1 and I_3 treatments. Non-significant difference in highest NUE in no stress (control) and moisture stress at vegetative growth stage was observed. These results led towards the conclusion that N application @50 kg ha^{-1}under moisture stress either in July or September causes higher root:shoot ratio in cotton genotypes and highest NUE in these genotypes can be achieved by imposing moisture stress at vegetative growth stage in September (by skipping one irrigation)coupled with 50 kg N ha^{-1}.

Key words: Moisture stress, *Gossypium hirsutum* L., rooting pattern,nitrogen use efficiency.

INTRODUCTION

Plant's ability of root development under moisture stress helps in sustainable translocation of assimilates to the above ground plant's shoots. Genotypes significantly differ in root/shoot characteristics under moisture stress (Jamal *et al*., 2014). Plant's ability to sustain water stress under dry environment is attributed to its potential in maintenance of photosynthesis rate upto desired level (Niu *et al.*, 2005). Roots are main source of nutrients supply to shoots; therefore, these are interdependent to each other. Any adjustment in root due to moisture stress in the soil will change shoot physiological processes including photosynthesis.

In some crops like sorghum, wheat, maize and sunflower, moisture stress have shown drasticeffects on root/shoot dry weights (Bibi *et al.*, 2012),however, in case of cotton, moisture stress is beneficial in terms of improvement of water use efficiency without significant yield loss (Jamal *et al*., 2012). For evaluation of drought resistance, root vigour is a reliable and sensitive indicator (Wang *et al.*, 2006). Root vigour reflects the ability of plants for water and nutrients absorption under drought stress conditions. Alternate deficit irrigation may lead towards deeper roots in cotton as compared to shallow roots in well irrigated no stress field (Sampathkumar *et al.*, 2012). Deficit irrigation is more economical due to water saving (up to 22%) and more area under cultivation as compared to little yield loss due to deficit irrigation (Mustafa *et al.*, 2011). Under severe water stress, an increase in root growth and decrease in shoot growth was observed (Maranov *et al.*, 1998) due to utilization of limited available water by root and transportation of a small amount of water towards shoot. Drought also effects leaf expansion and root growth (Ball *et al.*, 1994).

Nitrogen application under moisture stress also influences cotton plants by decreasing soil relative water content, but leaf area, dry matter production, nitrogen accumulation, root:shoot ratio, and root-N:shoot-N ratios may be increased (Liu *et al.*, 2008). Nitrogen application enhances peroxidase (POD) and catalase (CAT) activities of cotton root under moisture stress, however, superoxide dismutase (SOD) activity is reduced during water stress and recovery period. Malondialdehyde (MDA) content significantly ($P<0.05$) increased. Nitrogen also affects rooting pattern of plants and have a significant impact on root-shoot relationship (Lioert *et al.*, 1999).Significance of N on plant responses under soil moisture stress is well documented (Shangguan *et al.*, 2000; Stroup *et al.*, 2003).

Seed cotton yield and nitrogen use efficiency is linked with moderate application of N (Dongmei *et al*., 2012).The information about impact of genotypic-specific nitrogen and moisture stress interaction on rooting pattern and nitrogen use efficiency of cotton grown on alkaline calcareous soils is missing. This research was conducted to explore the impact of moisture stress at certain growth stages of cotton plant on rooting pattern, nitrogen use efficiency and ultimately yield. The aim was also to see whether deficit irrigation at certain growth stages of cotton with optimum N level is beneficial and useful in saving of irrigation water and urea fertilizer.

MATERIALS AND METHODS

Experimental site, design and treatments detail: Field experiments were conducted (2008-09) on wheat fallow plots at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, located 31°, 23' North latitude and 73°, 2' East longitude.Soil (0-30 cm depth) of experimental fields was alkaline calcareous, with low organic matter, low saturation%, lower nitrogen and phosphorus, deficient in zinc and boron, and adequate in potash and iron (Table 1). The soil of selected field pertained to Lyallpur soil series, belonging to US Soil Taxonomy Subgroup Udic Ustochrept (Soil Survey of Pakistan, 1968).Experiments were designed by using three factors (moisture stress, nitrogen, and genotypes) in randomized complete block design with split plots arrangements in three replications. Moisture stress treatments used were: local control = eight irrigations as recommended by Govt. Agri. Extension Departments (I_1)- all irrigations were applied at 50% ASMDL, moisture stress at inter-node elongation stage (MSI) = withholding of irrigation in July up to 80 % ASMDL (I_2), moisture stress at vegetative growth stage (MSV) = withholding irrigation in September up to 80 % ASMDL (I_3), and moisture stress at inter-node elongation and vegetative growth stage (MSI+ MSV) = withholding irrigation in July and September at 80 % ASMDL (I_4). Three nitrogen levels N_1 = 50, N_2 = 100, and N_3 =150 kg ha^{-1}); and three genotypes of cotton V_1 = NIAB-846, V_2 = NIAB-824, and V_3 = CIM-496 were used in this field experiment.

Crop husbandry: During both the years wheat fallow plots were selected for field experiments. Prior to sowing, experimental field was disc ploughed and cultivated twice followed by planking with tractor for preparation of fine seed bed. Field layout was done according to experimental design by using adjustable marker. Fertilizers application was made as per treatments in every plot. At sowing P_2O_5 fertilizer was uniformly applied to all treatments @ 70 kg ha^{-1} in the form of Single Super Phosphate (18 % P_2O_5) and one-third amount of Urea (46% N) fertilizer as per treatments level, was mixed in upper soil 0-9 cm depth manually in different treatments. Urea application was made in three equal splits (at sowing, dense flowering, and at boll development stages). Experimental crop was planted on 20th May and 25th May during 2008 and2009, respectively, while using different fields both the years. Dibbler method was used by placing 4-5 seeds per hill and maintaining row×row and plant×plant spacing of 75 cm and 30 cm, respectively while keeping net plot size of 3.6 × 3.0 m, and total seed rate of 20 kg ha^{-1}. Canal water was used to apply irrigation treatments. Single plant per hill was maintained by thinning operation of experimental crop at three-leaf stage. Different pesticides for control of boll worms and sucking pests (mealy bug, jassids, thrips and white-fly insect-pests) used were; Confidor, Talstar, Curacuron, Acetamiprid and Tracer to control spotted bollworms, mealy bug, jassids, thrips and white-fly insect-pests. Weeds were controlled by using three-tined manually operated weeder. Weather data of experimental site was also recorded at nearest observatory of Agronomy Section of Ayub Agriculture Research Institute (located about 1000 m distance from experimental plots), Faisalabad.Mean maximum and mean minimum temperature of crop growing period is also given in Table 4. Measured quantity of irrigation water was applied by using plastic container of known volume. The calculation of the amount of water applied was made by multiplying plot area with irrigation depth. Total amount of rainfall received was recorded and the remaining amount of water, as specified in treatments details, was applied by using container of known volume to maintain uniform application of total water (irrigation+rainfall) to each treatment during both years. Detail of the rainfall and irrigation application is given in (Table 2 & 3).

Procedure for recording observations

Seed cotton yield: At 50% bolls opening stage in the month of October, first picking of central rows from every plot was done manually at 8-10% moisture contents in seed cotton. Border lines were picked separately and that produce was not used in per plot yield in order to avoid border effects. Second picking of central lines from every plot was made at crop maturity in the month of November, when 90% bolls were opened. At completion of second picking, five randomly selected plants from every plot were selected and tagged for their roots observations while remaining plants were harvested at ground level. The harvested plants were also tagged and kept outside the field for 2 weeks for opening. Then picking of leftover unopened bolls from all harvested and standing plants was made. Total quantity of picked seed cotton was weighed by electronic balance for recording of yield per plot and then seed cotton yield per hectare was calculated.

Root- shoot ratio: For cotton roots study methodology developed by Prior *et al.*, (1994) was followed. Five randomly selected standing plants in every plot for roots study were cut at 1st coteledonary nodeand roots were excavated upto 90 cm soil depth by drain spade.The taproots and attached lateral roots were retrieved after loosening the soil carefully with drain spade to avoid any damage to the roots. Individual plants (shoots) and their respective roots were labeled by permanent marker, washed with water to remove attached soil particles and placed in air forced oven at 60°C temperature for drying till constant weight. Root:shoot ratio was determined by using the procedure given below:

$$\textit{Root shoot ratio} = \frac{\text{Dry root weight}}{\text{Dry shoot weight}}$$

Nitrogen use efficiency: The nitrogen use efficiency (NUE) was calculated as:

$$NUE = \frac{\text{Seed cotton yield kg/ha}}{\text{Nitrogen applied kg/ha}}$$ (Thind *et al.*, 2008)

The NUE has been reported as kg seed cotton kg^{-1} N applied.

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, 1997). Computer software packages Minitab-15, Statistix version 8.1, and Microsoft excel version 2010 were used for data analysis and preparation of graphs.

Table 1. Soil properties of experimental fields (0-30 cm).

Year	Saturation (%)	pH	CaCO3 (%)	Organic matter (%)	Nitrogen (NO_2) (mg kg^{-1})	$NaHCO_3$-P (mg kg^{-1})	NH_4 OAc-K (mg kg^{-1})	DTPA-Zn (mg kg^{-1})	Boron (mg kg^{-1})	Iron (mg kg^{-1})
2008	26	8.10	12	0.41	7.70	4.00	180	0.70	0.48	5.80
2009	30	8.40	13	0.66	8.70	5.30	200	0.52	0.50	5.25

Table 2. Rainfall and irrigation received by different treatments during 2008.

Irrigation and rainfall detail	Treatments							
	I_1 (mm)		I_2 (mm)		I_3 (mm)		I_4 (mm)	
	Rainfall	Irrigation	Rainfall	Irrigation	Rainfall	Irrigation	Rainfall	Irrigation
July	63.40	200.00	63.40	100	63.40	200	63.40	100
August	273.00	-	273.00	-	273.00	-	273.00	-
September	37.00	200.00	37.00	200	37.00	100	37.00	100
October	-	100.00	-	100	-	100	-	100
Total	373.40	500.00	373.40	400	373.40	400	373.40	300
Irrigation + rainfall (mm)	873.40		773.40		773.40		673.40	

Table 3. Amount of water received by different treatments during 2009.

Irrigation and rainfall detail	Treatments							
	I_1 (mm)		I_2 (mm)		I_3 (mm)		I_4 (mm)	
	Rainfall	Irrigation	Rainfall	Irrigation	Rainfall	Irrigation	Rainfall	Irrigation
July	52.50	210.90	52.50	110.90	52.50	210.90	52.50	110.90
August	137.40	135.60	137.40	135.60	137.40	135.60	137.40	135.60
September	30.20	206.80	30.20	206.80	30.20	106.80	30.20	106.80
October	14.80	85.20	14.80	85.20	14.80	85.20	14.80	85.20
Total	234.90	638.90	234.90	538.90	234.90	538.90	234.90	438.90
Irrigation + rainfall (mm)	873.40		773.40		773.40		673.40	

Table 4. Mean maximum and mean minimum temperature during crop growing seasons 2008 and 2009

Climatic factor/growing season		May	June	July	August	September	October	November	December	Mean
Mean Max. Temp	2008	38.40	37.80	37.70	35.00	35.30	33.70	28.30	22.90	33.44
	2009	40.10	40.90	37.90	36.50	36.10	33.40	26.30	22.90	34.26
Mean Min. Temp.	2008	23.40	26.90	27.70	26.00	23.20	19.90	10.80	7.80	19.96
	2009	24.30	25.60	26.50	26.40	23.40	16.20	10.00	4.60	19.63

Source: Observatory of Agronomy Section of Ayub Agriculture Research Institute, Faisalabad.

RESULTS

Moisture stress, nitrogen, and genotypic interaction effect on root:shoot ratio: Analysis of variance revealed significant ($P \le 0.05$) effects of moisture stress (I) on root:shoot ratio (Table 5) with highest root:shoot ratios of 0.226 and 0.333 from $I_3$and I_4 treatments during 2008and 2009, respectively (Table 8). Similarly genotypic (V) main effect were significant during 2008, exhibiting highest root: shoot ratio of 0.248 in NIAB-846 (V_1), however during 2009 main effects of V were non-significant. Main effects of N were non-significant both the year. Two way interaction effects I×V, N×V and three way interaction I×N×V found significant (Table 8) during 2008-09. However, two way interaction I × N remained significant during 2008but non-significant during 2009.Three way interaction (I×N×V) effect on root: shoot ratio of cotton genotypes found significant during both years and is presented in Fig. 1.a,b. Highest root: shoot ratio of 0.413 during 2008 was achieved by treatment $I_2 \times N_1 \times V_2$ (MSI × 50 kg N ha^{-1} × NIAB-824) followed by 0.333 and 0.305 root: shoot ratios by treatments $I_2 \times N_1 \times V_1$ (MSI × 50 kg N ha^{-1} × NIAB-846) and $I_1 \times N_2 \times V_3$ (LC × 100 kg N ha^{-1} × CIM-496), respectively (Fig.1.a). During 2009, maximum root: shoot ratio of 0.412 was recorded by $I_3 \times N_1 \times V_3$ (MSV × 50 kg N ha^{-1} × CIM-496) and least root: shoot ratio (Fig. 1.b) was found from $I_2 \times N_2 \times V_2$ (MSI × 100 kg N ha^{-1} × NIAB-824) and $I_1 \times N_3 \times V_1$ (LC × 150 kg N ha^{-1} × NIAB-846) treatments.

Table 5. Analysis of variance of root:shoot ratio as affected by moisture stress, nitrogen, and genotypic interaction

Treatments	2008					2009			
	df	SS	MS	F value	P value	SS	MS	F value	P value
Replication	2	0.00071	0.00036			0.02887	0.01444		
Irrigation (I)	3	0.03236	0.01079	12.72	0.0000	0.03659	0.01220	6.26	0.0008
Nitrogen (N)	2	0.00591	0.00295	0.19	0.8311	0.00164	0.00082	0.42	0.6578
Genotypes (V)	2	0.01978	0.00989	5.42	0.0065	0.00259	0.00130	0.66	0.5178
I × N	6	0.08681	0.01447	8.67	0.0000	0.02208	0.00368	1.89	0.0950
I × V	6	0.02624	0.00437	1.30	0.2671	0.04953	0.00826	4.23	0.0011
N × V	4	0.02253	0.00563	5.82	0.0004	0.03642	0.00910	4.67	0.0021
I × N × V	12	0.16059	0.01338	2.64	0.0056	0.05156	0.00430	2.20	0.0205
Error	70	0.15097	0.00216			0.13648	0.00195		
Total	107	0.50590				0.36577			
Grand mean	0.2296					0.3100			
C.V	20.22					14.25			

Factor/treatment effect is significant at $P \le 0.05$

Table 6. Analysis of variance of seed cotton yield as affected by moisture stress, nitrogen, and genotypic interaction.

Treatments	2008					2009			
	df	SS	MS	F value	P-value	SS	MS	F- value	P- value
Replication	2	1216206	608103				47501		
Irrigation(I)	3	4.100E+07	1.366E+07	42.55	0.0000	7539326	2513109	21.12	0.0000
Nitrogen (N)	2	2135221	1067610	3.32	0.0418	56075.4	28038	0.24	0.7907
Genotypes (V)	2	6846677	3423339	10.66	0.0001	3571941	1785971	15.01	0.0000
I × N	6	4036520	672753	2.09	0.0647	3262051	543675	4.57	0.0006
I × V	6	8698811	1449802	4.51	0.0006	1827834	304639	2.56	0.0267
N × V	4	6799716	1699929	5.29	0.0009	133817	33454	0.28	0.8892
I × N × V	12	7.874E+07	6561723	20.43	0.0000	3698798	308233	2.59	0.0066
Error	70	2.249E+07	321220			8328151	118974		
Total	107	1.720E+08				2.851E+07			
Grand mean	3561.8	Grand mean	4040.90						
C.V	15.91	C.V	8.54						

Factor/treatment effect is significant at P≤0.05

Moisture stress, nitrogen, and genotypic interaction effect on seed cotton yield: For seed cotton yield analysis of variance (P≤0.05) revealed significant effect of moisture stress, and genotypes during both cropping seasons, as shown in Table 6. However, nitrogen main effects on seed cotton yield remained significant during 2008and non-significant during 2009 (Table 8). During 2008, highest seed cotton yield of 4158 kg ha^{-1} and 4087 kg ha^{-1} was achieved by I_1 and I_3, and highest seed cotton yield (during 2009) of 4305 kg ha^{-1} and 4284 kg ha^{-1} was

Table 7. Analysis of variance of nitrogen use efficiency as affected by moisture stress, nitrogen, and genotypes of cotton

Treatments	**2008**					**2009**			
	df	SS	MS	F value	P value	SS	MS	F value	P value
Replication	2	0.00071	0.00036			0.03112	0.01037		
Irrigation (I)	3	0.03236	0.01079	5.00	0.0034	0.02917	0.01459	5.32	0.0000
Nitrogen (N)	2	0.00591	0.00295	1.37	0.2609	0.01384	0.00692	7.48	0.0000
Genotypes (V)	2	0.01978	0.00989	4.59	0.0134	0.03178	0.00530	3.55	0.0001
I × N	6	0.08681	0.01447	6.71	0.0000	0.01428	0.00238	2.72	0.0000
I × V	6	0.02624	0.00437	2.03	0.0733	0.02830	0.00707	1.22	0.0000
N × V	4	0.02253	0.00563	2.61	0.0427	0.05193	0.00433	3.63	0.0000
I × N × V	12	0.16059	0.01338	6.20	0.0000	0.13648	0.00195	2.22	0.0000
Error	70	0.15097	0.00216			0.36577			
Total	107	0.50590				0.02887	0.01444		
Grand mean	0.2296					0.3100			
C.V	20.22					14.25			

Factor/treatment effect is significant at P≤0.05

Table 8.Effects of moisture stress, nitrogen, and genotypes on root: shoot ratio and nitrogen use efficiency in cotton.

Factors	**Root: shoot ratio**		**Seed cotton yield (kg ha^{-1})**		**NUE (kg seed cotton kg^{-1} N applied**	
	2008	**2009**	**2008**	**2009**	**2008**	**2009**
Moisture stress (I)						
I_1	0.189 c	0.302 bc	4087 a	4284 a	51.56 a	53.01 a
I_2	0.237 b	0.320 ab	3360 b	3891 b	36.46 c	48.37 b
I_3	0.266 a	0.284 c	4158 a	4305 a	47.93 b	52.30 a
I_4	0.226 b	0.333 a	2642 c	3683 c	32.78 d	44.21 c

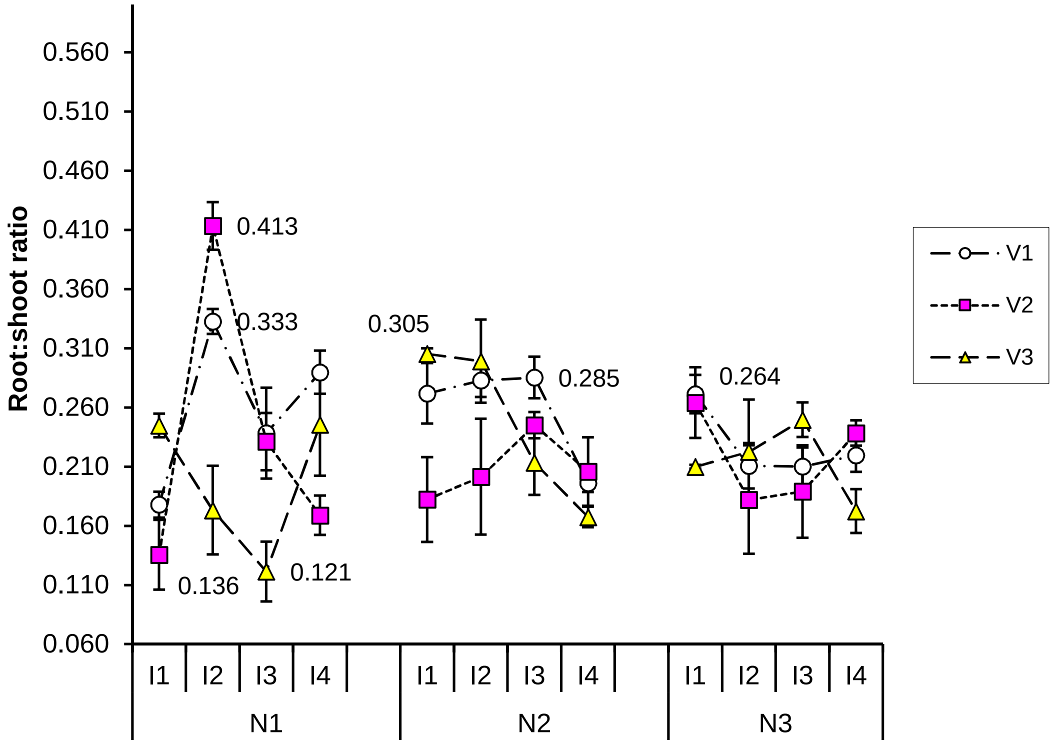
LSD (P<0.05)	0.025	0.024	307.65*	187.23*	1.88*	1.63*
			Nitrogen levels (N)			
N_1	0.230 a	0.305 a	3729 a	4017	64.65 a	79.98 a
N_2	0.226 a	0.310 a	3571 ab	4072	39.27 b	41.07 b
N_3	0.232 a	0.314 a	3385 b	4034	22.62 c	27.38 c
LSD (P <0.05)	NS	NS	266.43*	NS	1.63*	1.41
			Genotypes (V)			
V_1	0.248 a	0.304 a	3918 a	4232 a	42.13	49.64 a
V_2	0.228 ab	0.309 a	3380 b	4094 a	42.12	51.00 a
V_3	0.213 b	0.316 a	3387 b	3796 b	42.29	47.79 b
LSD (P <0.05)	0.022*	NS	266.43*	162.15*	NS	1.41
			Interaction^^			
I × N	0.044*	NS	NS	324.29*	3.26*	2.82*
I × V	0.044*	0.041*	532.86*	324.29*	3.26*	2.83*
N × V	0.038*	0.036*	461.47*	NS	2.82*	2.45*
I × N × V	0.076*	0.072*	922.95*	61.69*	5.64*	4.89*
Grand mean	0.230	0.310	3562	4041	42.18*	49.48
CV	20.22	14.25	15.91	8.54	8.22	6.07

*Factor/treatment effect is significant at P≤0.05, NS = not-significant at P≤0.05; Values within a column (factor wise) followed by the same letter are not statistically different at P≤0.05; ^^LSD values at P≤0.05

found by I_1 (LC) and I_3 (MSI) treatments, respectively(Table 8). Seed cotton yield was significantly affected by N levels during 2008 resulting highest yield of 3729 kg ha^{-1} by N_1 (50 kg N ha^{-1}), however during 2009, N didn't showed significant effect on yield. Interaction of moisture stress, nitrogen, and genotypes (I×N×V) significantly exhibited seed cotton yield during two years studies (Fig. 2.a,b). During 2009, highest seed cotton yield of 5709 kg ha^{-1} (Fig. 2.b) was achieved by $I_3 \times N_3 \times V_2$ (MSV × 150 kg N ha^{-1} × NIAB-824) treatment followed by 5634 kg ha^{-1} seed cotton yield of the same genotype from treatment $I_1 \times N_2 \times V_2$ (LC × 100 kg N ha^{-1} × NIAB-824). In NIAB-846, highest seed cotton yield of 5469 kg ha^{-1} was obtained from treatment $I_1 \times N_3 \times V_1$ (LC × 150 kgha^{-1}N × NIAB-846) followed by 4843 kg ha^{-1} seed cotton yield from $I_3 \times N_2 \times V_1$ (MSV × 100 kg ha^{-1} N × NIAB-846) treatment. In CIM-496 (V_3), highest seed cotton yields of 5296 kg ha^{-1} was recorded from $I_2 \times N_2 \times V_3$ (MSI × 100 kg ha^{-1} N × CIM-496) and 5255 kg ha^{-1} by $I_1 \times N_1 \times V_3$ (LC × 50 kg ha^{-1} N × CIM-496) followed by 5005 kg ha^{-1} seed cotton yield by $I_3 \times N_2 \times V_3$ (MSV × 100 kg ha^{-1} N × CIM-496). Lowest seed cotton yield of 1447, 1560, and 1830 kg ha^{-1} was recorded by treatments $I_4 \times N_2 \times V_2$ (MSI+MSV × 100 kg ha^{-1} N × NIAB-824) followed by 1560 kg ha^{-1} in treatment $I_4 \times N_3 \times V_1$ (MSI+MSV × 150 kg ha^{-1} N × NIAB-846) and 1830 kg ha^{-1} in $I_4 \times N_1 \times V_3$ (MSI+MSV × 50 kg ha^{-1} N × CIM-496). In all the genotypes lowest seed cotton yield was recorded from I_4 (MSI+MSV) treatments (Fig. 2.a,b).

Moisture stress, nitrogen, and genotypic interaction effect on nitrogen use efficiency: Treatments impact on nitrogen use efficiency (NUE) was significant during both the years (Table 6). During 2008, highest NUE (51.56 kg seed cotton yield kg^{-1} of N applied**)** was recorded from I_1 (LC) where as NUE, 53.01 and 52.30 kg seed cotton yield kg^{-1} of N applied found in I_1 and I_3 treatments, respectively. In case of N treatments, 50 kg N ha^{-1} (N_1) gave highest NUE during both cropping seasons. Among genotypes, NIAB-824 and NIAB-846 showed maximum NUE, 51.00 and 49.64 kg seed cotton yield kg^{-1} of N applied, respectively. (Table 7). During 2008 (Fig. 3.a), highest NUE of 105.09 kg SCY kg^{-1} N applied was noted from $I_1 \times N_1 \times V_3$ treatment followed by NUE values of 92.92 and 92.54 kg SCY kg^{-1} N applied by $I_1 \times N_1 \times V_1$ and $I_3 \times N_1 \times V_2$ treatments, respectively. Lowest NUE of 10.40, 13.14, and 14.47 kg SCY kg^{-1} N applied, were found in $I_4 \times N_3 \times V_1$ (MSI+MSV × 150 kg ha^{-1} N × NIAB-846), $I_2 \times N_3 \times V_3$ (MSI × 150 kg ha^{-1} N × CIM-496), and $I_4 \times N_2 \times V_2$ (MSI+MSV × 100 kg ha^{-1} N × NIAB-824) treatments, respectively (Fig. 3.a). During 2009, highest NUE values of 97.06 and 95.97 kg SCY kg^{-1} N applied were obtained by treatments, $I_3 \times N_1 \times V_2$ (MSV × 50 kg ha^{-1} N × NIAB-824) and $I_1 \times N_1 \times V_1$ (LC × 50 kg ha^{-1} N × NIAB-846), respectively (Fig. 3.b).

a) **2008**



b) **2009**

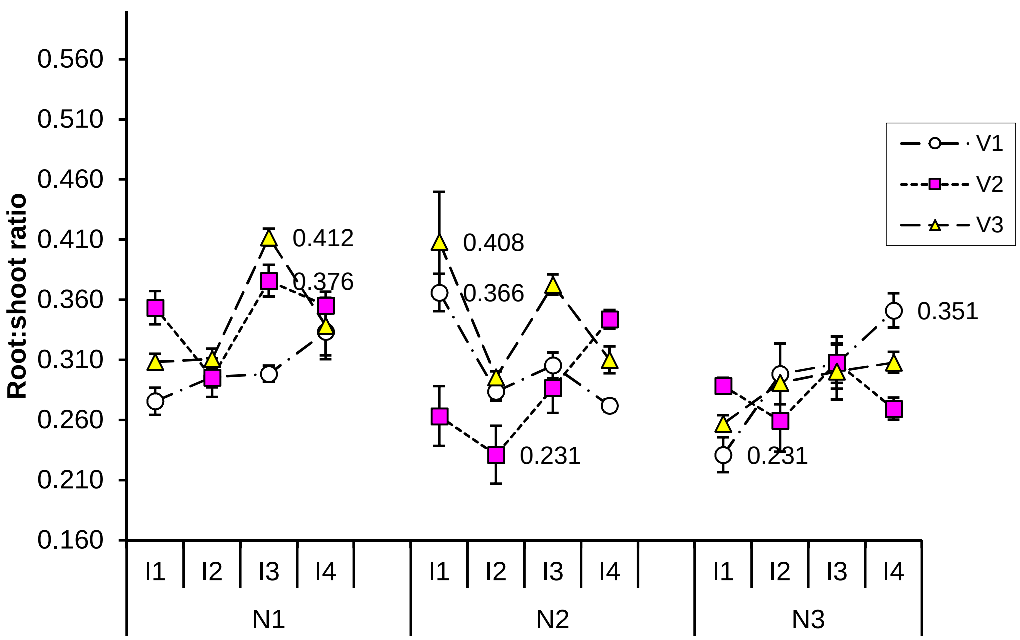
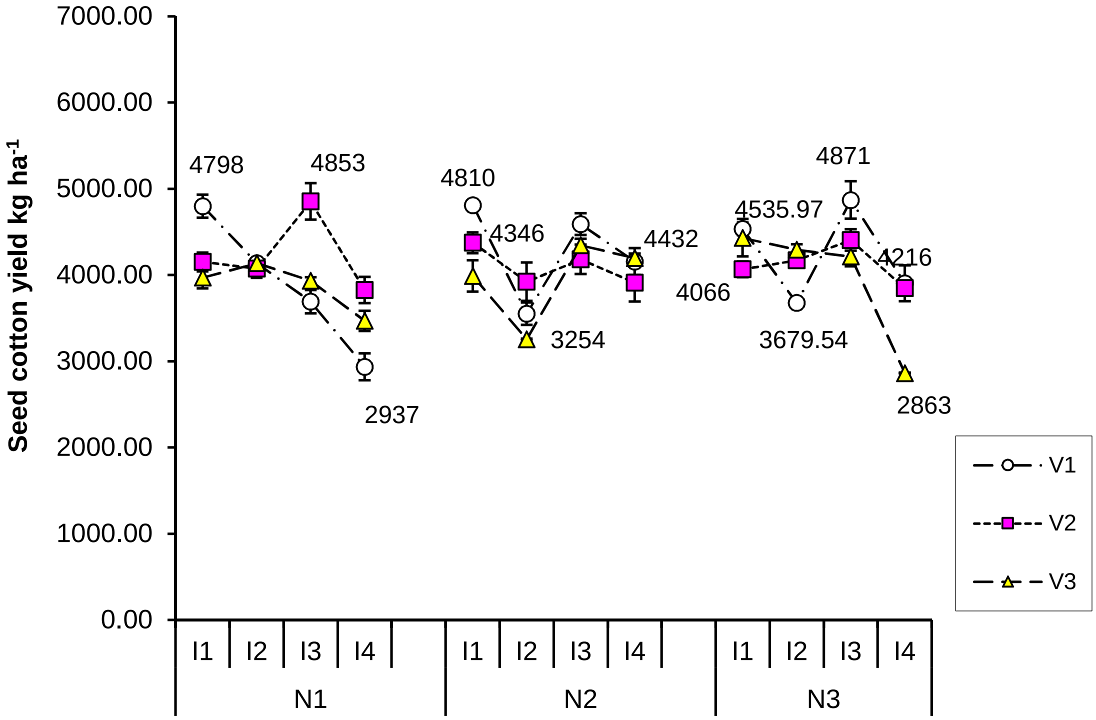


Fig. 1.Interaction effect of moisture stress, nitrogen, and genotypes on root:shoot ratio during 2008(a)and 2009(b).



a) **2009**

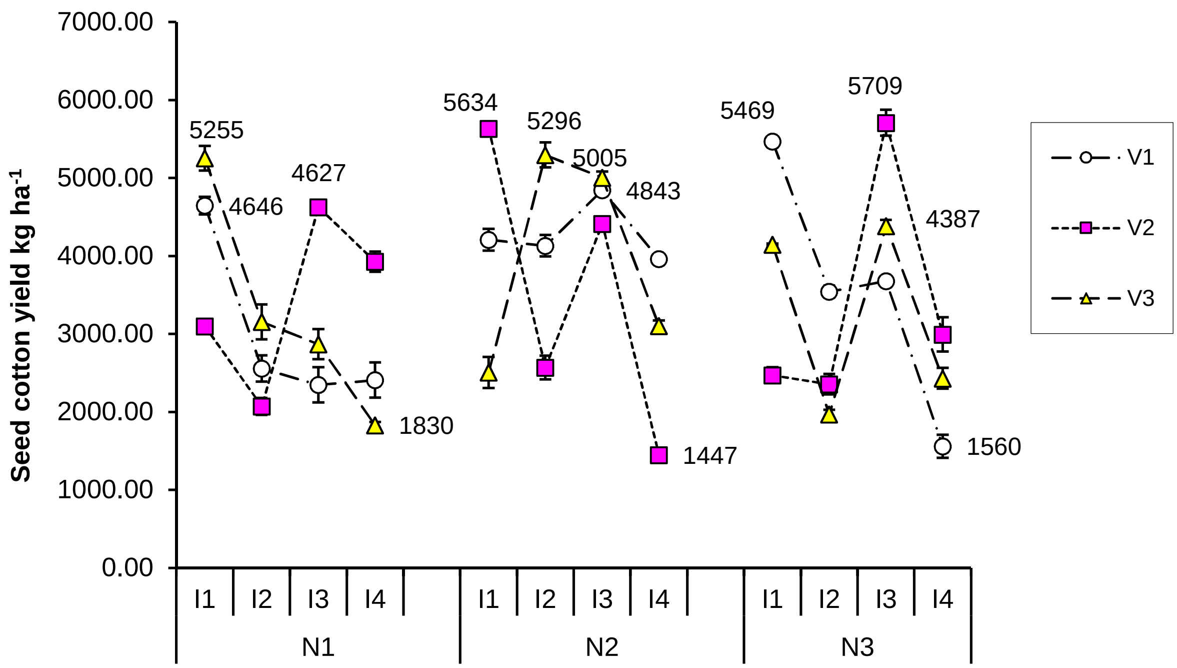
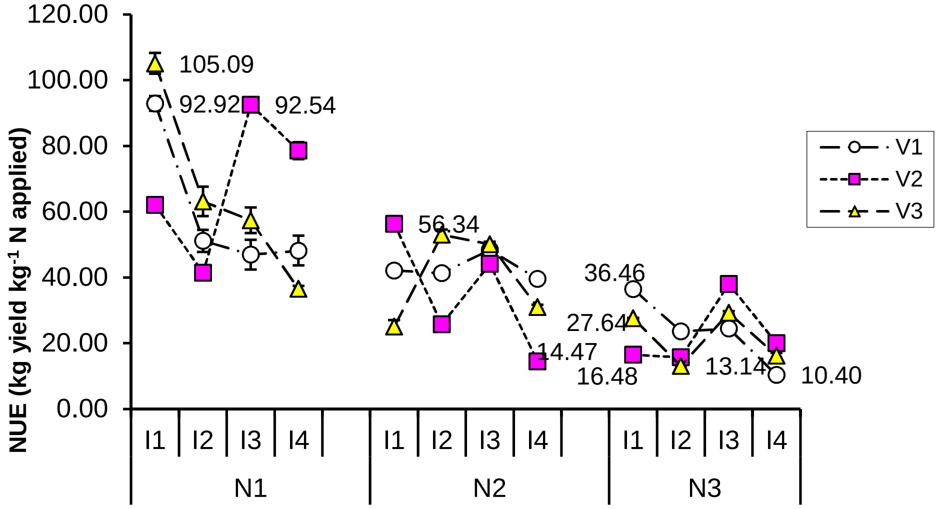


Fig. 2 Interaction effect of moisture stress, nitrogen, and genotypes on seed cotton yield during 2008(a) and 2009(b).

a) 2008



b) 2009

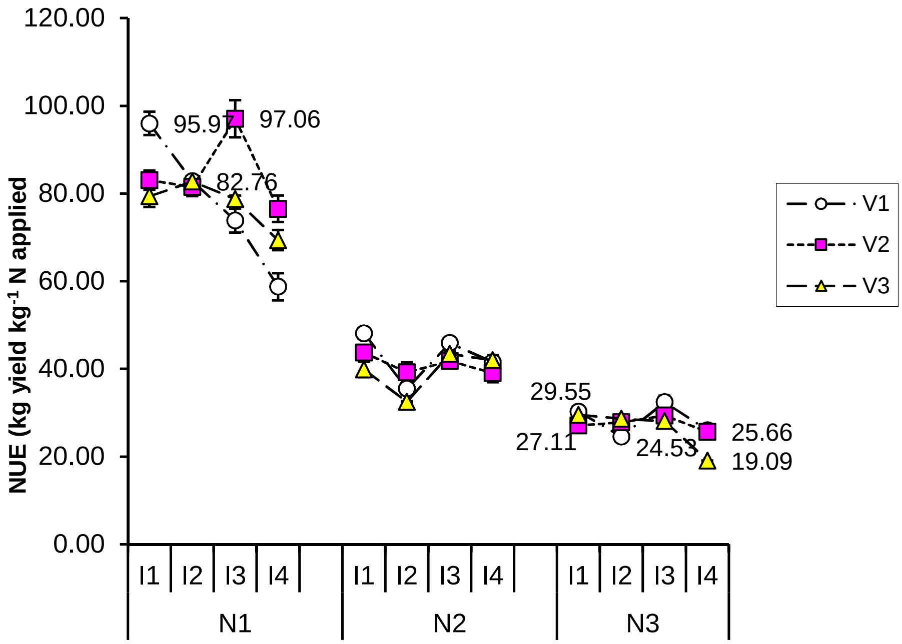


Fig. 3 Interaction effect of moisture stress, nitrogen, and genotypes on nitrogen use efficiency (NUE) during 2008(a) and 2009 (b).

Highest nitrogen use efficiency (NUE) was recorded in cotton genotypes in well water and moisture stress at vegetative growth stage treatments with lower N dose.

DISCUSSION

Roots growth under abiotic stresses is an important indicator of drought tolerance of any plant. The results obtained from the present field study clearly depicted divergent root: shoot ratio response of genotypes under moisture stress and nitrogen treatments (Fig. 1.a,b).During 2008, greater root: shoot ratio of NIAB-846 and NIAB-824 was observed when moisture stress was imposed at inter-node elongation stage in the month of July coupled with lower application of nitrogen @50 kg ha^{-1} (Fig 1.a). However, during 2009, these two genotypes exhibited higher root: shoot ratios in treatments where moisture was applied at vegetative growth stage in September with lower N levels (50 and 100 kg ha^{-1}).In CIM-496 different response was observed with highest root: shoot ratios of 0.305 and 0.408 in I_1 (LC) treatments during 2008 and 2009, respectively.

These results indicate diversity in rooting pattern among cotton genotypes due to their diverse genetic background as well as variation in soil fertility status on which these genotypes were grown. In previous studies rooting diversity in different cotton genotypes was observed under same set of environments (Kasperbauer and Busscher, 1990). Genotypes NIAB-846 and NIAB-824 exhibited higher root: shoot ratio by moisture stress at early growth stage in the month of July which depicts greater genetic potential of these genotypes towards more development of their rooting system for tolerance to moisture stress. These genotypes had shown ability to enhance root: shoot ratio under deficit moisture in the rooting zone leading towards an increase in transfer of assimilates from shoots to roots in establishing the root vigour to maintain the moisture and nutrients balance in the plant system. In previous it was observed that crop plants which have ability of drought tolerance, a greater portion of assimilates was transferred towards roots and caused an increase in root: shoot ratio (Jamal *et al.,* 2012, and Riaz *et al*., 2013).It has also been reported that impact of moisture stress on root; shoot ratio vary from crop to crop like cotton and maize with different response exhibiting a significant impact of moisture stress on root: shoot ratio in cotton (Jamal *et al.,* 2014).However in some cultivars of sorghum, wheat, maize and sunflower, drastic effects of moisture stress on root: shoot ratio may occur (Bibi *et al.,* 2012). Nitrogen application to cotton crop under moisture stress conditions is also very important. In this study, highest root: shoot ratios (Fig. 1.a,b) with lower N levels (50 and 100 kg N ha^{-1}) coupled with moisture stress as compared higher N application (@150 kg ha^{-1}) was observed. In cotton lower N application under drought is more beneficial as compared to higher N dose (Liu *et al*., 2008).An increase in N dose under moisture stress contributed in enhancing of root growth, but caused a decrease in shoots growth (Maranov *et al*., 1998). This was due to consumption of major portion of the limited water absorbed by roots in roots development and transportation of only a smaller fraction of absorbed water towards shoots. It is prevalent that genotypic response for changes in root: shoot ratios by moisture stress and nitrogen under field conditions depends upon varietal-specific morphological response that may vary in different genotypes of the same crop.

Higher seed cotton yield of any genotype grown in the field under different stresses depicts its tolerance for adverse conditions. Under moisture stress at vegetative growth stage (I_1), best seed cotton yield of NIAB-846 was obtained (Fig. 2.a,b) evenby the use of lower dose of N (50 kg N $^{-1}$) as compared to well water control (I_1, LC) treatment where higher dose of N (150 kg ha^{-1}) gave highest seed cotton yield. Because in both cases, the seed cotton yield was statistically at par, so nitrogen as well as irrigation water saving is possible by using NIAB-846 and imposing moisture stress at vegetative growth stage and N management by use of lower dose. However, the NIAB-824, at higher N levels (150 kg ha^{-1}) produced highest seed cotton yield with vegetative stage moisture stress, I_3 (MSV). The CIM-496 (Fig. 2.a,b) when put under stress at vegetative growth stage (I_3), gave the best yield with N_2 (100 kg N ha^{-1}). Both the years lowest seed cotton yields were achieved by treatments with I_4 (MSI+MSV). However, during 2009 overall higher seed cotton yield in all genotypes was achieved by different interaction treatments. Temperature variability significantly affects fruit setting and yield of cotton genotypes (Lou, 2011). However, mean maximum and mean minimum temperature didn't significantly differ during crop growing periods of 2008 and 2009 (Table 4). The higher seed cotton yield during 2009 may be attributed to the better fertility status of experimental plot (Table 2.The experimental plot selected for field experiment during 2009 was having higher organic matter and nitrogen, as well as with greater available P_2O_5 and K_2O.

Above mentioned results advocated that moisture stress at vegetative growth stage (I_3) has favourable impact on seed cotton yield of NIAB-846, NIAB-824, and CIM-496 genotypes due to non-significant yield loss as compared to well water (LC, I_1) treatment (Falkenberg *et al*., 2007)even in some cases higher yield as compared to well watered treatment. However, these genotypes differed in $N \times I_3$ interaction that may be due to diverse genetic background of these genotypes exhibiting different morphological responses of plants under moisture stress.

To enhance NUE under moisture stress was the main aim of this study. If we compare the highest NUE of three genotypes during 2008 (Fig. 3.a) with control treatments ($I_1 \times N_3 \times V_1$, $I_1 \times N_3 \times V_2$, $I_1 \times N_3 \times V_3$), 60.76% increase in NUE of NIAB-846 (by treatment $I_1 \times N_1 \times V_1$), 82.19% increase in NUE of NIAB-824 (by treatment $I_3 \times N_1 \times V_2$), and 73.86% increase in NUE in CIM-496 (by treatment $I_1 \times N_1 \times V_3$) over control was recorded. Similar trend was observed during 2009, where 69.20% higher NUE in NIAB-846 (by treatment $I_1 \times N \times V_1$), 72.06% higher NUE in NIAB-824 (by treatment ($I_3 \times N_1 \times V_2$), and 64.29% higher NUE in CIM-496 (by treatment $I_2 \times N_1 \times V_3$), was achieved over control (Fig. 3.b). During 2009, higher seed cotton yield was achieved by same N levels due to more favourable soil's environmental conditions (Table 4). Which resulted higher NUE of cotton genotypes. Cotton genotypes differ in NUE under moisture stress due to diversity in plant morphology and nitrogen × genotypic interaction. Above mentioned results indicate that higher NUE in all genotypes is achieved by lower N level (50 kg N ha^{-1}) as compared to higher level of N (150 kg N ha^{-1}). In these genotypes excessive use of N may be uneconomical and even in some cases yield limiting (Howard *et al*., 2001). High level of N use in cotton may cause in shifting of crop growth balance towards higher vegetative growth as compared to reproductive growth leading towards delayed maturity and a reduction in seed cotton yield (Nicholos *et al*., 2004).

Conclusion: In cotton, root:shoot ratio is sensitive to growth stage and nitrogen application which depends upon varietal-specific morphological response. Genotypes NIAB-846 and NIAB-824exhibited higher NUE by moisture stress at vegetative growth stage in September coupled with lower use of nitrogen (50 kg N ha^{-1}).

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