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COMPARISON OF Bt AND NON-Bt COTTON CULTIVARS FOR THE INCIDENCE OF COTTON LEAF CURL VIRUS WHEN SOWN AT DIFFERENT TIMES AND SUPPLIED WITH VARYING DOSES OF NITROGEN

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ABSTRACT

Background Cotton leaf curl virus (CLCuV) is a serious threat to cotton crop. This virus has an increased ability to mutate under favorable conditions and damages the cultivar resistance. The occurrence of CLCuV on Bt and non-Bt cotton was investigated at various sowing dates and nitrogen (N) application levels.

Methodology Experiment plan consisted of two sowing dates i.e. mid-March and mid-May kept in main plots, two cultivars viz. MNH-786 (Non Bt) and IR-1524 (Bt) kept in sub plots, and three N levels viz. 115, 145 and 175 kg ha^{-1} were kept in sub-sub plots.

Results CLCuV occurrence was greater in mid-May sowing (65.2%) than the mid-March sowing (11.7%). Number of sympodial branches, number of opened bolls per plant and plant height was greater in mid-March sowing than mid-May sowing. Low level of N (115 kg ha^{-1}) produced less number of CLCuV infected plants (34.6%) and it was statistically similar (39.6%) with the medium N level (145 kg ha^{-1}), while high N level (175 kg ha^{-1}) resulted in higher number of CLCuV infected plants (41.4%). Number of sympodial branches per plant and number of opened bolls per plant were less at 115 kg ha^{-1} than 145 and 175 kg ha^{-1}. Plant height (cm) was also minimum at 115 kg ha^{-1} which was statistically similar with 145 kg N ha^{-1}, while higher N level produced taller plants. Cultivars did not differ significantly in CLCuV susceptibility; Bt cultivar showed 39.6% attack while non-Bt showed 37.3% attack of CLCuV.

Conclusion The incidence of CLCuV was lowest in early sowing, non-Bt cotton supplied with low N level.

INTRODUCTION

Cotton is the white gold of Indo-Pak, and it plays an important role in the economy of Pakistan by contributing 10% to gross domestic product and 55% to foreign exchange earnings (Rehman et al. 2019). However, seed cotton yield in Pakistan is far below than major cotton producing countries (Khan et al. 2016). There are many factors responsible for low seed cotton yield, however, the major one is cotton leaf curl virus (CLCuV) (Hassan et al. 2016). Both biotic and abiotic factors are considered important to pose serious threat to agriculture and food security throughout world. Among abiotic factors, viruses are responsible for huge losses in yield and quality of cotton crop throughout the world and also in Pakistan. Cotton leaf curl disease is a serious problem to genus *Gossypium* found mainly in Africa and South Asia (Sattar et al. 2013). CLCuV was first discovered in Nigeria in 1912 (Farquharson 1912), then in Sudan (Golding 1930), followed by Tanzania (Kirkpatrick 1931) and Philippines in 1959. In Pakistan, CLCuV started as a destructive disease in 1987, appeared as an epidemic with a yield reduction of 9.05 million bales in 1990-93 while 8.04 million bales in 1993-94 (Mahmood et al. 2005).

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Cultivars that were resistant to CLCuV became susceptible to CLCuV Burewala strain which appeared in 2001 (Amrao et al. 2010).

CLCuV is transmitted through two whitefly species, *Bemisia tabaci* and *B. argentifo1ii*, and through petiole grafting (Monga et al. 2011). The typical symptoms appeared due to CLCuV attack are leaves upward curling, vein thickening and appearance of small leaf like outgrowths on lower side of the leaf called "enation"(Akhtar and Khan 2002; Sattar et al. 2013). Appropriate maximum and minimum air temperature for CLCuV development is 33-45°C and 25-30°C, respectively, while relative humidity is 70-80% and wind velocity is 6-12 Km h^{-1} (Khan et al. 1998). These favorable conditions prevail during July-August, mostly at the end of July and in the beginning of August (Arshad et al. 2007). Losses due to CLCuV varies greatly depending on plant age, plant vigour and time of infection of the virus (Akhtar et al. 2003). Early planting had low infestation than the late planted crop (Iqbal and Khan 2010). The occurrence of CLCuV is greater in mid-June planting crop than in mid-May planting crop (Tahir et al. 2004).

Plant nutrition has a key role to resist the attack of insects and diseases. Among plant nutrients, nitrogen is particularly important to affect plant resistance to various biotic and abiotic stress factors (Khan et al. 2001; Khan et al. 2016). However, anatomy, morphology and biochemical composition of the plants may increase or decrease the resistance or tolerance to pests and diseases (Marschner 1995). Virus multiplication is occurred in living cells and they require amino acids and nucleotides for their development. Nutrition helps in the growth of the host plant and is vital for virus multiplication. Cultivars differ in the accumulation of nutrients in their leaves (Tahir et al. 1994). Leaf epidermal hairs provides defense against insects, therefore, the cultivar with much hairs has less insect and CLCuV attack (Rashid et al. 1995). About 10-20% disease incidence depends on variety (Khan and Khan 1995).The present study was conducted with the objective to compare Bt and non Bt cultivars for CLCuV disease incidence when sown at different times and supplied with varying doses of nitrogen.

MATERIALS AND METHODS

A field experiment was carried out to determine the effect of different sowing dates and nitrogen levels on the incidence of CLCuV in Bt and Non-Bt cultivars at Agronomic Research Area, University of Agriculture, Faisalabad. The soil of the experimental site was sandy clay loam with 7.4 pH, 0.65% organic matter, 0.039% N, 7.85 ppm available phosphorus and 220 ppm available potassium. The experiment was laid out in randomized complete block design (RCBD) with split split plot arrangement and replicated thrice. Experimental treatments comprised of two sowing dates viz., mid-March (S_1) and mid-May (S_2), three nitrogen levels viz. 115 (N_1), 145 (N_2) and 175 (N_3) kg ha^{-1} and two cotton cultivars viz., MNH-786 (V_1) and IR-1524 (V_2). The plot size was 6×4.5 m^2. Each plot contained six rows of cotton crop. Seedbed was prepared by cultivating one time with rotavator and two times with tractor mounted cultivator, each followed by planking. The crop was sown with the help of dibbler maintaining 0.75 m row spacing and 0.30 m plant to plant distance. Whole of phosphorus and potassium were applied at the time of sowing, while nitrogen was applied in three equal splits viz., at sowing, 35 days after sowing (squaring stage) and 65 days after sowing (flowering stage). Nine irrigations were applied throughout crop growth period. Weeds were controlled by two hoeings viz., 35 and 65 days after planting. Both, sucking insects (Aphid, Jassid, Whitefly, Thrips and Mites) and bollworms (American bollworm, Pink bollworm and Spotted bollworm) were controlled with insecticides. When seedlings were established, ten true representative plants were selected randomly from each plot and tagged for recording following data:

Number of plants infected by CLCuV per plot

Infected plants were counted at the first and second picking by the visible symptoms of the plant from each plot of the ten selected plants and percentage of infected plants was calculated by following formula:

$$\frac{\text{Number of plants infected by CLCuV per plot}}{\text{Total number of plants per plot}} \times 100$$

Number of sympodial branches per plant

Sympodial branches were recorded by counting the selected plants of each plot and taken the average.

Number of opened bolls per plant

Opened bolls were recorded by counting the opened bolls at first and at second picking of the selected plants and taken the average.

Plant height

Plant height was measured at the time of last picking with the help of meter rod and average of the ten selected plants was taken.

Statistical analysis

Data collected for different parameters were analyzed statistically by using MSTAT-C program (Anonymous 1986) for analysis of variance and

means were separated using Fisher's protected least significant difference (LSD) test at 5% probability level (Steel et al. 1997).

RESULTS

CLCuV infected plants were markedly affected by different sowing dates and nitrogen levels while cultivars and all other interactions were non-significant (Table 1). Mid-May sowing resulted in higher number of CLCuV infected plants (65.2%) than mid-March sowing (11.7%). Higher N level (175 kg ha^{-1}) showed greater number of infected plants (41.4%) and it was statistically similar with medium level of nitrogen (39.3%). Low level of N produced less number of infected plants (34.6%) and it was statistically similar with the medium level of nitrogen.

Number of sympodial branches per plant was affected by sowing dates, cultivars, nitrogen levels and C x N interaction while all other interactions were non-significant. Mid-March sowing resulted in more sympodial branches (23.1) than the mid-May sowing (19.1). The interaction between cultivars and nitrogen levels was significant. Non-Bt cultivar (MNH-786) performed better at medium level of nitrogen (145 kg ha^{-1}) in producing sympodial branches while Bt cultivar (IR-1524) produced less number of sympodial branches (19.9) at low level of nitrogen than medium (23.9) and high level (25.30).

Sowing dates, cultivars, nitrogen levels and C x N interaction affected the number of opened bolls per plant. Mid-March sowing produced higher number of bolls (36.1) than mid-May sowing (28.9). The interaction between cultivars and N levels was significant indicating that Non-Bt cultivar (MNH-786) showed less number of opened bolls (20.5) at higher level of N than the medium (26.4) and low level (25.7).

Plant height was also influenced by sowing dates, cultivars and nitrogen levels while all their interactions were non-significant with respect to CLCuV (Table 1). Mid-March sowing showed more plant height (125.7 cm) than Mid-May sowing (109.6 cm). The cultivars were significantly different from each other in plant height. Non-Bt cultivar (MNH-786) gave more plant height (123.2 cm) than Bt cultivar (IR-1524) (112.0 cm). Nitrogen level of 175 kg ha^{-1} resulted in maximum plant height (125.7 cm) and it was statistically similar with 145 kg N ha^{-1} (117.1 cm) while 115 N kg ha^{-1} showed lowest plant height.

DISCUSSION

Number of plants infected by CLCuV was affected by different temperatures, relative humidity and wind speeds at mid-March and mid-May sowing dates and nitrogen levels. Cultivars were non-significant with respect to number of plants infected by CLCuV,

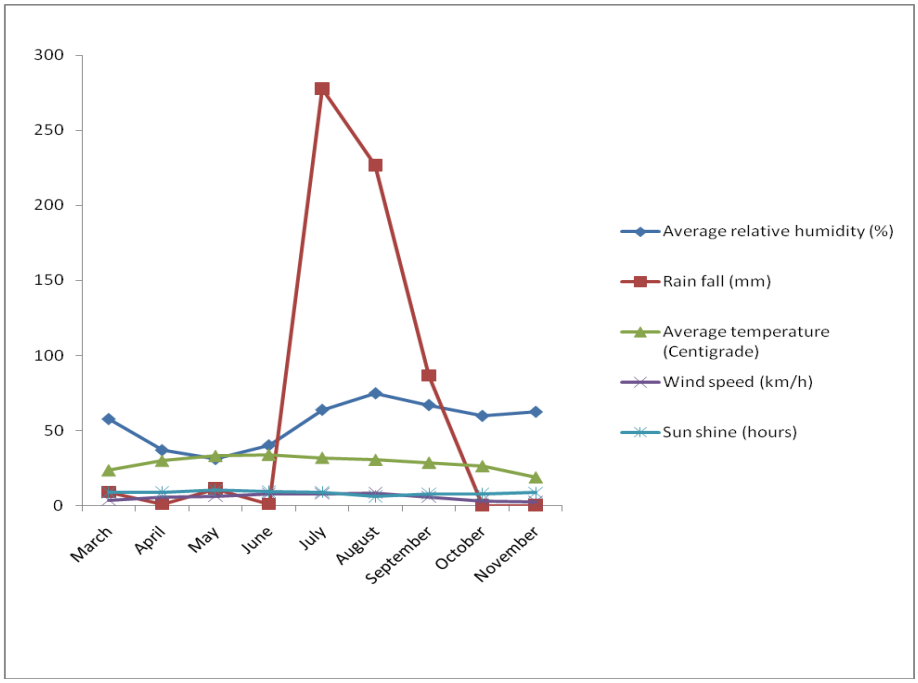

Figure 1 Weather conditions during cotton growing period

Table 1 Effect of sowing dates, cotton cultivars and nitrogen rates on growth and CLCuV incidence

Treatment	CLCuV(IP)	SB	OB	PH
Sowing dates (S)				
Mid-March (S_1)	11.7 b	23.1 a	36.1 a	125.7 a
Mid-May (S_2)	65.2a	19.1 b	28.9 b	109.6 b
LSD (p=0.05)	9.83	3.16	2.65	11.31
Cultivar (C)				
MNH-786 (V_1=Non Bt)	37.3	19.2 b	24.2 b	123.2 a
IR-1524 (V_2 = Bt)	39.6	23.0	40.8 a	112.0 b
LSD (p=0.05)	Ns	3.35	2.05	3.26
N rate (kg ha^{-1})				
115 kg ha^{-1}	34.6 b	19.5 b	30.8 b	110.1b
145 kg ha^{-1}	39.3 ab	22.7 a	34.3 a	117.1 ab
175 kg ha^{-1}	41.4 a	21.1 ab	32.4 ab	125.7 a
LSD (p=0.05)	4.87	2.21	2.71	11.63
C × N interaction				
C1 x N1	33.7	19.1 bc	25.7 c	115.6
C1 x N2	37.7	21.6 ab	26.4 c	120.8
C1 x N3	40.4	17.0 c	20.5 d	133.4
C2 x N1	35.5	19.9 bc	35.9 b	104.6
C2 x N2	40.9	23.9 a	42.2 a	113.4
C2 x N3	42.4	25.3 a	44.4 a	118.0
LSD (p=0.05)	Ns	3.13	3.84	Ns

Means not sharing a letter in common within a column differ significantly at 5% probability level. ns= Non-significant, CLCuV (IP) = Cotton leaf curl virus (infected plants% per plot), SB= Number of sympodial branches per plant, (OB)= Number of opened bolls per plant, PH= Plant height (cm)

with relatively more infected plants in Bt cultivar than Non-Bt cultivar. All other interactions were non-significant for number of plants infected by CLCuV. The incidence of CLCuV occurred on all the cultivars but the resistant cultivar showed fewer symptoms than the susceptible cultivar because the resistant cultivar has developed internal mechanism by providing unfavorable conditions to CLCuV, and it did not allow virus to use its proteins (Tahir et al. 2004). Mid-May sowing resulted in higher number of CLCuV infected plants because in mid-May sowing there was more rainfall and humidity and thus more sucking insects attack occurred than in mid-March sowing. The appropriate temperature and relative humidity during July-August favored CLCuV activation, CLCuV attack was maximum at the end of July. Virus may be present in mid-March sown crop but its activation occurred at the end of July when the crop was fully matured and CLCuV could infect only a few plants. Arshad et al. (2007) also reported the effect of sowing dates on CLCuV incidence in cotton. CLCuV disease appeared in all the sowing dates but the symptoms that appeared late were due to environmental differences. The occurrence of CLCuV was greater in mid-June planting cotton as compared to mid-May planting cotton (Tahir et al. 2004; Tahir and Mehmood 2005). Virus was more virulent at early stages of growth than at the maturity of the crop (Brown et al. 1987; Akhtar et al. 2003; Arshad et al. 2006).

Plants nutrients are helpful for inducing physiological changes within plants helping plants to bear biotic and abiotic stresses (Zafar et al. 2010; Athar et al. 2011; Huber et al. 2012), thus mitigating the adverse effects of diseases. Nitrogen is important for crop growth (Chandra and Mishra 1991). High nitrogen level application increased nitrogen contents and amino acids in plants, leading to increased CLCuV infected plants (Zafar et al. 2010). CLCuV susceptible cultivars could be managed by optimizing nitrogen fertilization (Iqbal et al. 2008). Athar et al. (2011) reported that higher levels of nitrogen increased whitefly in okra plants. Early planted cotton produced higher number of sympodial branches per plant (Arain et al. 2001). Early planting from March to April in Punjab produced more sympodial branches than May planted cotton (Buttar et al. 2005). Cotton leaf curl disease at initial stages of growth caused serious reduction in number of monopodial and sympodial branches per plant, number of bolls per plant, boll weight and ultimately reduction in seed cotton yield (Tahir and Mehmood 2005; Iqbal and Khan 2010). Number of sympodial branches per plant increased with higher levels of nitrogen (Boquet et al. 1993; Brar et al. 1993). With increasing nitrogen level upto150 kg ha^{-1} number of sympodial branches per plant increased. Nitrogen application higher than the optimum dose caused a

delay in maturity, increased vegetative growth and resulted in lower yield (McConnell et al. 1996). Number of opened bolls per plant increased when nitrogen level of 100 kg ha^{-1} was used (Ram and Prasad 2001). Nitrogen application at 150 kg ha^{-1} enhanced opened bolls per plant. Sawan et al. (2006) also reported an increase in number of opened bolls per plant when 143 kg nitrogen ha^{-1} was used compared to 95 kg ha^{-1}.

CONCLUSIONS

Mid-March sowing showed less number of CLCuV infected plants and more number of sympodial branches, opened bolls per plant and plant height than the mid-May sowing. Non-Bt cultivar produced less number of sympodial branches, less opened bolls per plant and more plant height than the Bt cultivar. Nitrogen level of 115 kg ha^{-1} produced less CLCuV infected plants closely followed by 145 kg ha^{-1} than by 175 kg ha^{-1}. Number of sympodial branches, opened bolls per plant and plant height were lower at low level of nitrogen than medium and high level of nitrogen.

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