Effect of climate change on Bt gene expression and yield related parameters

Thesis submitted to Quaid-i-Azam University, Islamabad in the partial fulfillment of the requirement for the degree of

MASTER OF PHILOSPHY

In

PLANT GENOMICS AND BIOTECHNOLOGY

By

Maryam Mubeen

Department of plant Genomics and Biotechnology

PARC Institute of Advanced Studies in Agriculture

National Agriculture Research Centre, Islamabad, Pakistan

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Maryam Mubeen

Dated: _______________

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LIST OF ABBREVIATIONS

Word	Abbreviation
µl	Microlittre
NARC	National Agriculture Research Centre
PCCC	Pakistan central cotton committee
APTMA	All Pakistan Textile Mills Association
GOP	Government of Pakistan
cm	Centimeter
GMO	Genetically modified organism
RCBD	Randomized complete Block Design
CEWRI	Climate, Energy and water Research Institute
DAS	Days after Sowing
GOT	Ginning out turn
rpm	Revolution per minute
min	Minute
ELISA	Enzyme Linked Immunosorbent assay
ppb	Parts per billion
ANOVA	Analysis of variance
nm	Nanometer
ha	Hectare
I.D	Internodal distance
G%	Germination percentage
PMD	Pakistan Meteorological Department
WRRI	Water Resources Research Institute
Bt	Bacillus thuringiensis
s	Seconds
CLCuV	Cotton leaf curl virus
IPM	Integrated pest management

ABSTRACT

Climate change is a global challenge that have significant potential to change crop yields worldwide. Thus, the measurement of crop yield in terms of climate change is significant in the prospect of food security for agro-economic regions like Pakistan especially in the upcoming decades. With its largest value chain in the textile industry cotton is the most important cash crop in developing countries like Pakistan. In the current study three Bt cotton varieties viz FH-142, IUB-2013 and NIAB-878 were selected and sown in three different ecological zones i.e., Islamabad, Multan and D.I Khan for comparative morphological, yield and Bt gene expression analysis. Meanwhile meteorological data (temperature, humidity and rainfall) for all three zones were also recorded for comparison. Maximum rainfall and humidity were recorded in Islamabad during the month August 2020 and temperature was noted during the month of June 2020 at Multan. Based on results, diverse morphological responses were obtained in cultivars and growing areas. Maximum germination percentage was secured in FH-142 cultivar at Islamabad zone followed by NIAB-887 in D.I. Khan. Similarly, maximum plant height, Boll weight, Bolls per plant and yield were reported in D.I Khan in NIAB-878 cultivar while number of seeds per boll and internode distance were excellent in IUB-2013 cultivar at Islamabad and D.I Khan respectively. Early flower initiation was reported in FH-142 at Multan as compared to other cultivars and zones. GOT was high in Multan area where there were no any significant differences observed in other varieties and zones as well. At 90 DAS, Cry1Ac gene expression was checked from the leaves of all cultivars grown under three ecological zones through ELISA. ELISA results of Cry1Ac protein exhibited that maximum expression was secured in NIAB-878 at Islamabad followed by IUB-2013 at D.I Khan respectively. It has been concluded that NIAB-878 performed well in yield and boll weight at D.I khan and Multan while Bt gene expression was higher at Islamabad.

INTRODUCTION

1.1 Importance of cotton

Cotton (*Gossypium hirsutum* L.) is considered to be the main plant fiber crop worldwide. More than 50 different countries grow this plant fiber crop with total coverage of 34 million hector (ha) and with contribution of 40% to the global market. Production of this crop have an impact on the life of 350 million people directly or indirectly (Shahrajabian *et al*., 2020). Countries where the climatic conditions are suitable for the growth of this crop includes USA (985 kilogram/hectare), Uzbekistan (832 kg/ha), China (1,266 kg/ha), India (565 kg/ha), Australia, Pakistan (600 kg/ha) and the Middle East because of the session of hot and dry weather and an optimum level of moisture (Ullah *et al*., 2019). In terms of cultivated area India is the top country and it occupies more than quarter of worlds cotton production followed by China (have highest level of productivity (1265kg/ha), USA and Pakistan (Arunkumar *et al*., 2019). About 27MMT (million metric tons) of cotton is produced throughout by these countries (Shahrajabian, *et al*., 2020). Cotton fulfills the fiber need of half of the world and considered to be highly valuable fiber crop and important industrial commodity (Ullah *et al*., 2019). Cotton seeds are also domestically used for cooking oil as well as residual oil cake to feed livestock (Ahmad *et al*., 2021).

Cotton act as perennial plant but it can be grown as annual crop in LB (longitudinal band) of 37°N and 32°S. In China it has been extended to 45°N.Commercially four different species of *Gossypium* named *G. hirsutum, G. arboreum, G. barbadense and G. herbaceum* are grown; *Gossypium hirsutum*, or upland cotton, produces the bulk of cotton worldwide. For high staple length *G. barbadense* is the second. Fifty of the cotton species are marked in the world and four among are cultivated. Two of species of cotton named *G. arboreum* and *G. herbaceum* are diploids, and two named *G. hirsutum* and *G. barbadense* are tetraploid. Tetraploid cotton covers more than 80% of worlds cotton area (Shahrajabian *et al*., 2020). *Gossypium hirsutum L*. is most important among all cotton varieties from economic point of view due to best quality of its fiber.

Globally Bt cotton is very important because of its adoption has risen dramatically from 1.9 million acres in 1996 to 19.40 million acres in 2010 as 55% of cotton crop grown in

USA, 77% in China and 81 % in Australia was Bt cotton (Arshad, *et al.*,2007). For pest control the most effective and the safe way is the adoption of Bt cotton; the first transgenic non-food product. Only in China the adoption of Bt cotton has increased to an area of 2.4 million hectares because of less insecticide exposure, reduced pesticide and the cost of labor. (Arshad, *et al*.,2007).

1.2 Cotton production in Pakistan

In Pakistan the average cotton yield is 571 kg/Hectare (ha) being the second huge country which export raw cotton and third huge country that consume cotton in the world (APTMA, 2016-17). Almost 55% of Country's domestic needs are fulfilled by cotton and it contributes 7.9% toward the better quality in agriculture. In Pakistan the area under cultivation is approximately 2.63 million hectares with net production of 10.98 million bales cotton (Ahsan, *et al*.,2019).

Being a major crop in Pakistan after wheat it holds the largest area in Pakistan in comparison with other crops. Largest export revenue is earned by cotton plant. In addition to fiber (lint) seeds hold 80% of national production of oil seeds. Cotton serves the raw material to local industries which comprises 396 textile mills,9.7 million spindle,960 ginning factories and over 2622 oil expelling units (PCCC, 2016). (Ullah *et al*., 2019). During the year of 2018-2019 the cultivation area of cotton crop has been increased in the past 30 years around 7.87 million acres (Rehman *et al*., 2019). Sindh (79%) and Punjab are the major provinces of Pakistan in terms of cotton cultivation. Cultivated areas of cotton in Punjab are in d zone which is the hot and dry zone of the country with average of high temperature being recorded. (Malik *et al*., 2016) Cotton growing season in Pakistan is from May until June when the temperature is high and harvesting time is from September until December (Shahbaz *et al*., 2019).

1.3 Factors affecting cotton production

Biotic and abiotic stresses effect the yield, quality and the fineness of fiber (Karar *et al*., 2020). Abiotic stresses and climate change affects the production of cotton to much extent by using some transgenic approaches we tackle some of the problems still the biggest challenge is to give permanent resistance to plant against insects and stresses.

No doubt transgenic cotton has proven effective but the effectiveness of it depends on many factors including seed purity, genotype back crossing and others. Cotton biotechnology started in 1992 in Pakistan for the sake of improving quality of the fiber as well as with focus on resistance against insects (Shahrajabian *et al*., 2020). Warm days and cool nights provide optimum growth to cotton plant. One of the major factors is temperature.

There is significant effect of temperature on elongation of nodes, biomass and the leaf expansion. Seedlings were sensitive to increase in temperature during the first two weeks and after that they become temperature sensitive as the decrease in biomass was noted as compared to optimum temperature for the growth which is 30/20°C (Sankaranarayanan *et al*., 2010).

1.4 Factors affecting Bt gene expression in cotton

In Pakistan bollworms are serious pest as they cause heavy damage (variable) to cotton plant but the average accounts 30-40% yield reduction and heavy amount of spray was needed but after the introduction of Bt cotton, the number of spray operations needed for optimum growth per crop has been reduced .(Arshad *et al*.,2007) Bt cotton contains specific type of proteins that when consumed by a specific type of insect larvae, damage the insect gut walls by creating holes in it, which causes larvae to stop feeding and eventually die. The Bt gene is inserted in plants by genetic alteration in which the source code of the DNA is changed to produce the proteins / toxins which reduces the need for the application of insecticides (Shahbaz *et al*., 2019).

Bt cotton was brought in Pakistan during 2005 illegally and formally approved in 2010 the productivity increase in Bt cotton remains yet to be seen. This is more surprising when in India and throughout the world, the productivity of Bt cotton increased manifolds. Bt cotton's main function is not to increase productivity but to check the role of the boll worms in decreasing productivity and through decreasing the pest attacks on the crops; Bt cotton serves to increase productivity by decreasing sub economic threshold levels damages and creating more reliable insect control in all weather conditions (Shahbaz *et al*., 2019).

Bt insect resistance is unstable and it was observed in China that during the cotton growth the insecticidal ability is higher in seedling stage and lower at flower initiation and boll formation. In terms of organ expression of gene leaves shows highest insecticidal ability especially the younger leaves. (Jun *et al*.,2015) Based on previous literature different genotype performed different in different environment due to their genetic makeup and interaction with the environment. Environment has a significant role in the performance of a particular variety (Khan and Hassan, 2011). Complex traits like seed cotton yield due to genotype × environment interaction hinder the identification of best genotype. G×E interaction for any cultivar lessen the usefulness of mean of genotype over all location for advancing and choosing superior genotypes. Stability of a particular genotype is very important. A genotype is thought to be stable if it shows less variation in different environment (Ahsan *et al*., 2019). Unfavorable environment affects the yield and the quality of cotton crop. Not only the environment, sowing date also has major impact on cotton yield, quality and insect pest management (IPM) (Ullah *et al*., 2019). Previous studies clearly indicates that the expression level of Bt protein varies among varieties, age of the plant, which part of the plant is under consideration, which type of Bt gene is present and on which position the Bt gene is present in the given genome (Ahsan *et al*., 2019).

In Pakistan many challenges have been faced by cotton crop including pest attack, climatic variation and the fugitive price. The introduction of Bt. (Bacillus thuringiensis) cotton has reduced the problems of pest attack but the climatic variation are independent of this new variation in cotton variety and these variations have serious effect on cotton production system (Raza *et al*.,2015) 20-30% decline in cotton plant production is due to climate change (GOP 2012). Pakistan is a country that is likely to suffer more as it is ranked fourth in world cotton production in 2010 (Luqman *et al*., 2020). Climate changes had badly affected food and cash crop productivity of Pakistan, which highlighted the issues of food and raw material security of the country.

1.5 Effect of climate changes on yield and Bt protein of cotton

Depending on the climatic zone 2-7mm of water in soil is needed by cotton plant. 500-750 mm is required for the plant to grow and develop fully (Top *et al*., 2011). Regions with average annual rainfall 701mm or more than this is best for the growth of rain- fed cotton in practice, since the inter-annual and intra-annual rainfall variability, and amount of resulting run-off, have to be taken into account (Top *et al*., 2011). Climate change is associated with changes in patterns of precipitation and water availability, hence, cotton plants in some regions may be subjected to plant water deficits.

Water deficit limits growth and productivity of cotton plants and severity of the problem may increase due to changing world climatic trends (Le Houérou, 1996). Climate change is likely to affect cotton production both positively and negatively. Temperature influences cotton growth and development by determining rates of fruit production, photosynthesis and respiration (Jans *et al*., 2021). Changing environments effects the cotton plant and induce a response depends on the planting conditions, development of plant in early season, flower initiation and boll formation and the condition of plant during harvesting stage at the end of season (Top *et al*., 2011). Growth of cotton plant differs at varying stages of plant development, so optimum temperature for plant growth cannot be marked. However, temperature greater than 40° effects the growth as well as yield of plant. Moreover, it increases the evaporative demand in cotton plant leading more intense water stress (Jans *et al*., 2021). Cotton becomes sensitive to temperature change when is in flowering or boll formation stage as depicted by finding of (Reddy *et al*. (Abbas *et al*.,2020) environmental factors are predictable and unpredictable variables which determine the growing season of a specific crop and also disclose the adoptive potential of a particular genotype. Documented rise in temperature due to global warming has been forecasted by different models that shows the direct or indirect effect of temperature on plant growth (Ullah *et al*., 2016). Not only the temperature, drought effects the insecticidal efficacy and reduces it.

The insecticidal ability of reproductive organ of the plant could be the indicative for insect resistance of Bt cotton However, earlier studies in cotton usually used the leaf insecticidal ability to represent insect resistance of Bt cotton. Studying the insect-resistant characteristics of the reproductive organs under extreme environment can assess the insecticidal efficiency more precisely, and ensure a successful resistance management strategies designed high temperature was the main environmental factor results in leaf senescence and shedding of squares and bolls, which eventually led to less cotton lint yield (Zhou *et al*. 1996; Zhou 1999).

The insecticidal efficacy reduced during high temperature growing seasons (Chen *et al*., 2012), and leaf insecticidal protein decomposed at 38°C noticeably which resulted in reduction of insecticidal ability in Bt cotton (Chen *et al*., 2005). Changes in the major independent variables, viz temperature, CO2 and availability of water to the extent that they actually occur may change plant growth rates, biomass reservoirs and plant community composition at local, regional as well as global scales (Sankaranarayanan *et al*., 2010). In case of cotton plant drier regions are more sensitive for climatic variability (Sultan *et al*., 2010). (Chandio *et al*.,2019) explored the effect of carbon dioxide emission, average temperature, cultivated areas and consumption of fertilizers on rice production in Pakistan from 1968 to 2015. The findings revealed a significant positive effect of temperature and CO2 emission along with the other variables on rice production. Rainfall significantly effects critical stages of plant growth. High rainfall could result as a result of on top of saturation and water logging that ultimately affects the cotton plant in negative way (Adre *et al*., 2016; Pandya *et al*., 2020). Excessive rainfall along with consecutive floods in 2010 to 2014 caused immense loss of summer crops especially cotton crop (Abid *et al*., 2015). These unfavorable environmental changes resulted in farmer's major economic and social losses and generated frustration among farmers (Ahmad & Afzal., 2020; Akbar *et al*., 2020).

1.6 Climate change scenario in Pakistan

Agriculture sector of Pakistan is more vulnerable for climatic conditions and almost 60% of the production of agriculture is affected by changes in climate (Luqman *et al*., 2020). In a report issued by World Bank, 2009 Pakistan is extremely exposed country to climatic changes. There is no notable effect on the productivity of wheat in Pakistan due to climatic changes (Janjua *et al*., 2010). Increase in temperature, higher absorption of carbon dioxide and decreased precipitation have positive affect on crop (Rashid, *et al*., 2020). Due to increase in temperature, transpiration rate increases, which ultimately increases water stress and lessen plant growth and production of cotton crop.

There are many studies that have been conducted on crops like wheat, rice and maize of Pakistan that check the effect of climate on growth of these crop however in case of cotton little is known about the damages caused by climate change. Hence its important and the need of time to face the potential harms of environment on production of cotton.

In Pakistan Punjab and Sindh are the areas for cotton growth because of high temperature and low precipitation (Iqbal, 2011). Due to dire climatic changes, cotton production is under threat. Cotton production is predicted to be decline by 20-30% due to change in climate (GOP, 2009). Review of the empirical literature has revealed that no study that has been done to explore the effect of climate change on cotton production, especially in Pakistan (Abbas *et al*., 2020).

The main aim of this study is to check the effect of variables of climate viz; rainfall, temperature and humidity on the expression of Bt gene and the growth of plant in terms of yield in three different climates of Pakistan. The study will also explore the impact of some other factors on cotton production and based on these finding the study will recommend some suitable policy framework to manage climate change impacts if any. Short turn relationship between climate and cotton production in terms of Pakistan will be addressed in this study.

1.7 Aims/Objectives

i. Sowing of cotton cultivars seeds at multi locations

ii. Comparative yield related parameters of cotton under different ecological zones

iii. Quantification of Bt protein under diverse conditions

iv. Climate wise comparison of selected cotton cultivars

MATERIALS AND METHODS

2.1. Experimental location

All the experiments were conducted under field conditions of Islamabad, Multan and D.I Khan Pakistan, during May 2020 to November 2020. Regular cultural practices i.e., weeding, watering and hoeing were uniformly provided to all plants.

2.2. Germplasm Acquisition

The seeds of three upland cotton varieties i.e., FH-142, IUB-2013 and NIAB-878 were obtained from GMO testing lab of NIGAB, NARC, Islamabad.

2.3. Sowing plan of cotton seeds

Cotton seeds of three varieties were sown at three different locations named NARC Islamabad, DI Khan and Multan. The layout of the experiment was randomized complete block design (RCBD). Sowing methods significantly affect the yield and growth of cotton crop. The method used for sowing include manual line sowing with row spacing of 70 cm and plant spacing of 30cm. Recommended doses of agronomic practices and plant protective measures were applied as per requirement of plant growth throughout the experiments.

Figure 2.1: Pakistan cotton (Source: Pakistan journal of Meteorology vol 6 and issue 12)

2.4. Recording of meteorological data

Complete meteorological data was taken from three regions on monthly basis for cotton growing to harvesting. Three climatic factors named temperature (both maximum and minimum), humidity and average rainfall have been recorded for comparison in this study.

2.5. Comparative analysis of multi zonal Data

All experiments were performed in three replications while recommended doses of agronomic practices and plant protective measures were applied as per requirement of plant growth. From seed germination till harvesting various morphological parameters were recorded for all the cultivars under specific locations.

2.6. Morphological characters of cotton

2.6.1. Plant Height Measurement (cm)

Plant height of all the three varieties was measured in centimeter (cm) when plant attained maximum height. It was measured from the base of the soil up to the plant top by using measuring tape and the average height was measured.

2.6.2. Inter nodal distance

Distance between the two middle nodes of ten randomly selected plants was measured in cm with the help of ruler and then average was measured.

2.6.3. Number of flowers per plant

Counting of the flower number was done manually and at the end average was taken.

2.6.4. Days to flower initiation

From randomly selected ten plants, days to flower initiation was counted for all cultivars plants. Mean was taken at the end for comparative analysis.

2.6.5. Boll weight Measurement

Ten opened bolls were collected from selected plants and their average weight was calculated with the help of an electric balance.

2.6.6. Seed cotton yield

The seed cotton yield of crop was calculated by weighing seed of each replication and then converted to kg/ha.

2.6.7. Ginning out turn

Ginning out turn (GOT) was determined by using the formula of Singh (2004).

2.7. Expression analysis of Bt (Cry1Ac) gene in cotton

Expression analysis of Cry1Ac gene in cotton cultivars under three environments was performed through ELISA kit for Cry1Ab/Ac of You long Biotech Company, Shanghai China. For this method, sample extract (blank control/standard/sample 100 µl was added to the corresponding micro wells and shaken gently (Reaction is allowed to happen for 45 min at 25 °in dark environment after covering the mouth of plate with aluminum foil). After shaking the liquids in wells, 250 µl of washing working solution (solution 1) was added used to fully wash the wells. Washing was done 4-5 times with an interval of 10 seconds (s) (pat dry with absorbent paper, bubbles that have not been removed after the pat dry were pierced with a clean pipette tip). After that 100 µl of enzyme labeled working solution was added in each well. Shaken and mixed to react for 30 min in 25 ° in dark environment. After washing and air drying the micro wells 100 µl of color developer was added and allowed to react for 15 min in a dark environment at 25 °. Final determination of color was carried out by adding 100 µl stop solution/well, gently shaken and mixed. After setting the microplate reader at 450nm (dual wavelength 450/630 was used). Readings were noted. Quantitative determination was carried out. The calculated value was multiplied by the sample dilution factor. Sample absorbance value was positively correlated with the amount of cry1Ab/Ac contained. Comparison of the average absorbance value of the sample with the standard value to get the calculated value range (ppb) was carried out. The final Bt protein contents were measured as µg/g.

2.8. Statistical Analysis

Ten random plants were selected from single replications and their means were compared by using excel software. Data of all the ecological zones and cultivars were taken separately by following RCBD design and their average data was analyzed on ANOVA by using statistics 8.1 software. The LSD test ($p<0.05$) was used in order to compare the significant treatments means by using the Statistic version 8.1 (Analytical Software ©, 1985-2005).

RESULTS

3.1 ANOVA results of morphological characters

The analysis of variance (ANOVA) results showed significant differences and confirmed the presence of variations among genotypes for many morphological traits namely plant height, number of bolls per plant, boll weight, GOT %, seed weight and yield of cotton. LSD test (P 0.05) was computed to determine the multiple comparison of all traits of varieties.

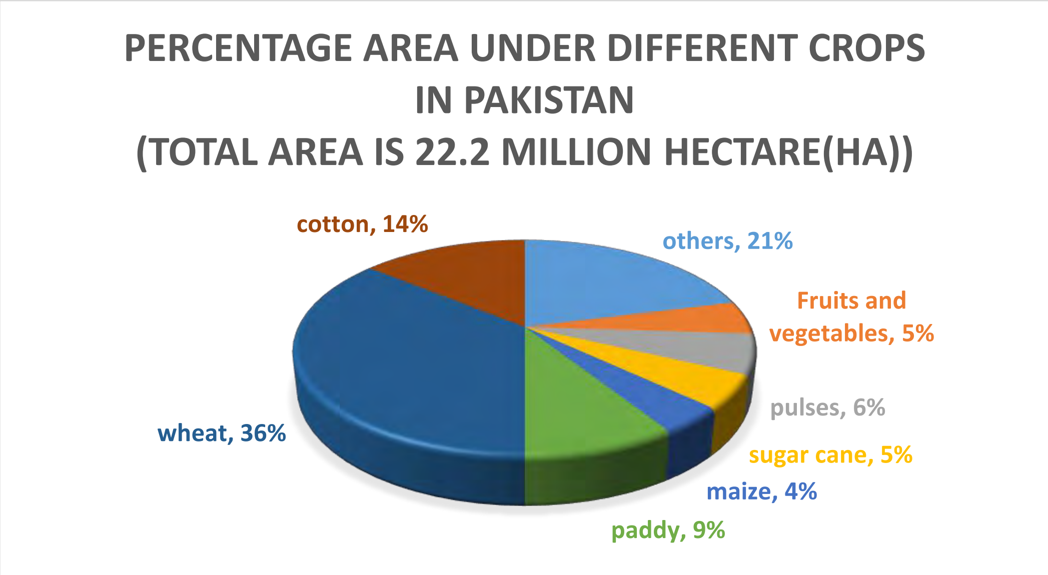

Figure 3.1: Percentage area under different crops in Pakistan

3.2 Plant height

Cultivars wise results of plant height illustrated that maximum plant height (190.67cm ±8.6) was secured by NIAB-878 cultivar in climate of D.I khan followed by cultivar IUB-2013 and FH-142 (184.00cm ± 9.53 and 157.00cm ± 5.3) respectively. While minimum plant height was recorded in IUB-2013 (117.33cm ±4.7). The results of plant height comparison at location wise revealed that best mean plant height was noted at Multan followed by D.I Khan and Islamabad respectively as shown in figure 2. ANOVA Results of plant height variety × variety interaction showed highly significant differences with $p < 0.01$. N-878 cultivar is statistically at par with IUB-2013.Variety × location results also showed high significant differences ($p < 0.01$). N-878 and IUB-2013 behave similarly in the climate of Islamabad while same is observed in the case of N-878 and FH-142 in the climate of Multan. In the climate of DI khan all the three cultivars used shows differences in terms of their plant height.

The effect of climate change on the basis of three locations further illustrated that high temperature zone like Multan directly influenced plant height of cotton cultivar as compared to lower temperature zones i.e., DI Khan and Islamabad. The variations in height might be due to genetic constitution of different varieties and environmental impact as well.

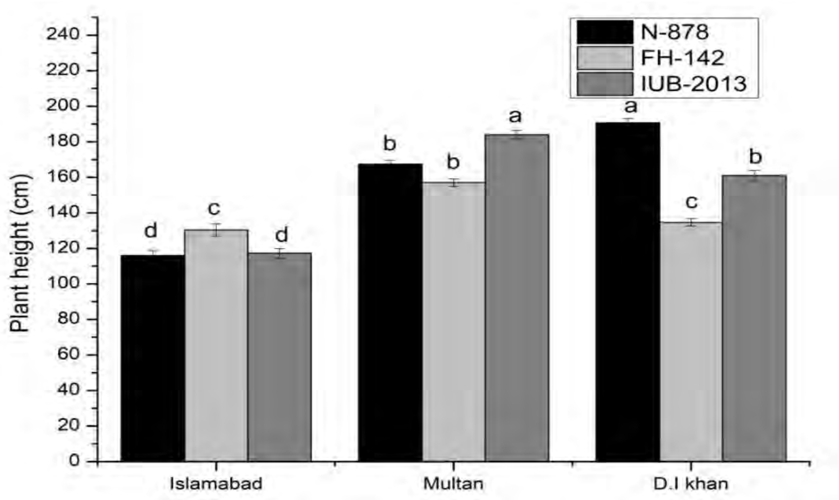

Figure 3.2: Variety wise comparison of cotton plant height at multi-locations

3.3 Inter nodal Distance (I.D) cm

ANOVA results of inter nodal distance for each cultivar were significant with $p < 0.05$. Further analysis by LSD All pairwise Comparison test showed that Variety × location interaction results have significant differences ($p < 0.05$) with grand mean value of 3.9. IUB-2013 cultivar showed maximum internodal distance value of 4.6cm in climate of Islamabad, 3.9 cm in climate of Multan and 3.5cm in climate of D.I. Khan. IUB-2013 cultivar and FH-142 cultivar are statistically at par in the climate of Islamabad while in the climate of D.I. Khan all the three cultivars showed similar value with no statistical differences. (Figure 3.3)

3.4 Days to flower Initiation

Results for flower initiation for all the cotton cultivar are not significant in terms of location ($p < 0.05$). ANOVA results for genotype were significant with $p <0.05$. Further comparison by LSD test showed that variety ×location interaction results were significant and means were divided into three groups. Niab-878 in Islamabad took 62 days which is statistically at par with IUB 2013 in Islamabad. In Multan N878 shows maximum days for flower initiation (64) whereas the IUB 2013 and FH 142 exhibit same days for flower initiation. In DI khan IUB-2013 significantly took more days for initiation than other cultivars (64 days).

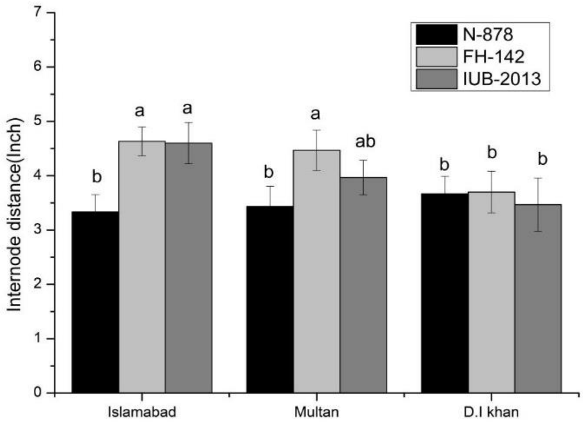

Figure 3.3: Variety wise comparison of internodal distance in cotton plant at multi-locations

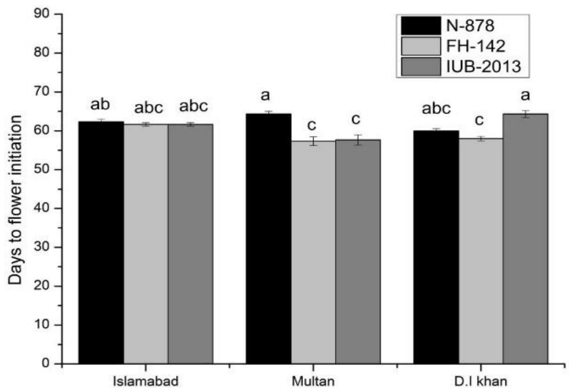

Figure 3.4: Variety wise comparison of cotton plant for flower initiation at multi-locations.

3.5. Number of bolls per plant:

The response of three different cultivars to number of bolls per plant under three different climate condition was found highly significant with $p < 0.01$. The maximum number of bolls per plant (92) were counted in N878 cultivar with average boll number value of (86) in all three locations. The minimum number of bolls per plant (47) was counted in IUB 2013 in the climate of Islamabad. Average value is 49. LSD All-Pairwise Comparisons Test of bolls per plant showed that all three means are significantly different from one another in terms of variety and location. For location× variety interaction there were no significant differences observed as shown in (Figure 3.5).

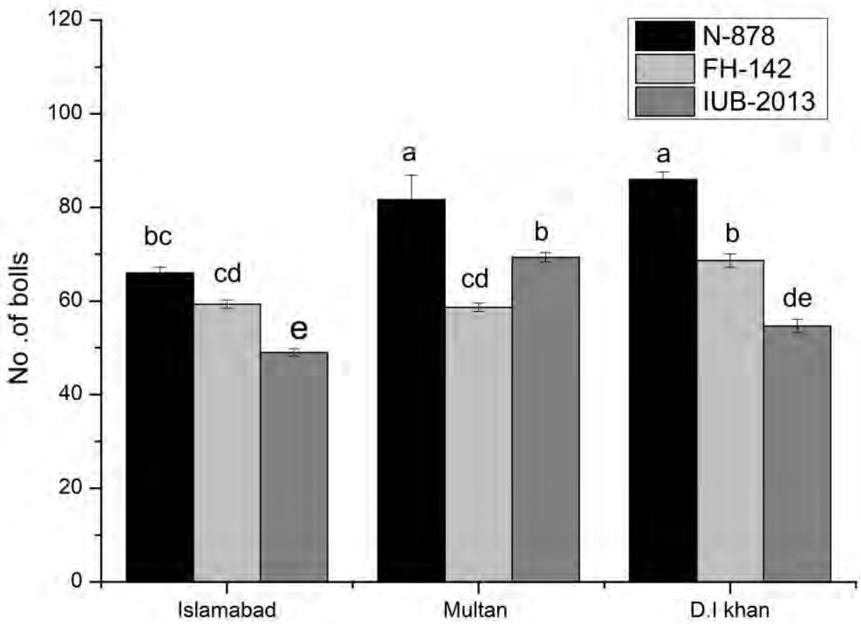

Figure 3.5: Variety wise comparison of Number of bolls of cotton per plant at multi-locations

3.6. Seed Cotton Yield

All the cultivars showed significant differences with $p < 0.01$ for yield which includes the number of bolls in each plant and boll weight. Variety mean results were also significant with $p < 0.05$.LSD results for Variety× Location was also significant ($p<0.05$). Five different groups were observed in which the means are not significantly different from one another. The highest seed cotton yield was shown by cultivar FH-142 in climate of

Islamabad (660 kg acre-1) and by N-878 (711kg acre-1) which is statistically at par with IUB 2013 (699 kg acre-1) in Multan and by N878 (729 kg acre-1 in DI khan. However, the lowest seed cotton yield is produced by cultivar IUB 2013 in Islamabad (617 kg acre-1) was also at par with cultivar N-878 (633 kg acre -1). In Multan the lowest cotton yield was observed in FH-142 (696 kg acre-1) and In DI khan it was observed in FH-142 (672 kg acre-1) and IUB 2013(674 kg acre-1) as shown in Figure 3.6.

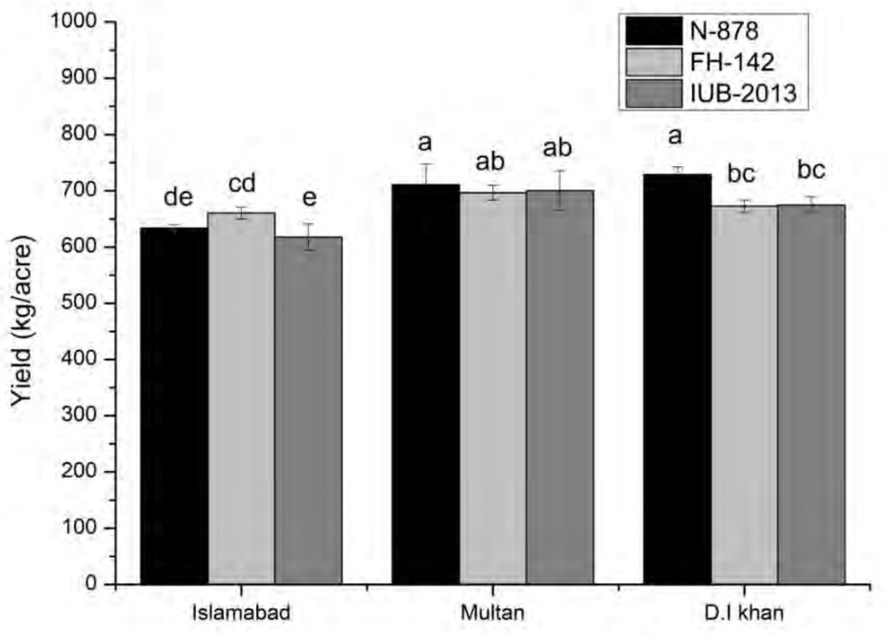

Figure 3.6: Variety wise comparison of cotton plant yield(kg/acre) at multi-locations

3.7. Number of seeds per boll

In terms of seed number per boll, ANOVA results were non-significant for location with p >0.01. LSD results for variety and Variety ×location was highly significant with p <0.01. IUB-2013 cultivar and FH-142 behave similarly in the climate of DI. Khan. Maximum number of seeds in each boll (34) was counted in cultivar IUB-2013 in climate of Islamabad and minimum number of seeds per boll (29) was counted in FH-142 in climate of Islamabad and N-878 in climate of DI. Khan (Figure 3.7). LSD results confirmed that there were two groups in which means are not significantly different from one another

3.8. Germination percentage (G%)

ANOVA results for germination percentage were highly significant with p <0.01 for variety ×location. LSD results showed there are five different groups in which the means are not significantly different from one another. All three means of cultivars are also significant. Maximum Germination % is shown by FH 142 (64%) in Islamabad while in Multan and DI khan cotton cultivar N-878 shows the maximum G% (68% in DI khan and 64% in Multan). (Figure 3.8)

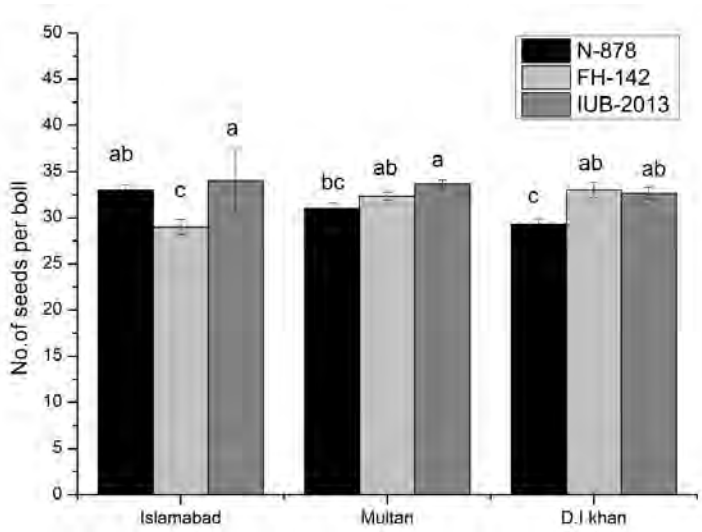

Figure 3.7: Variety wise comparison of cotton plant seeds/boll at multi-locations

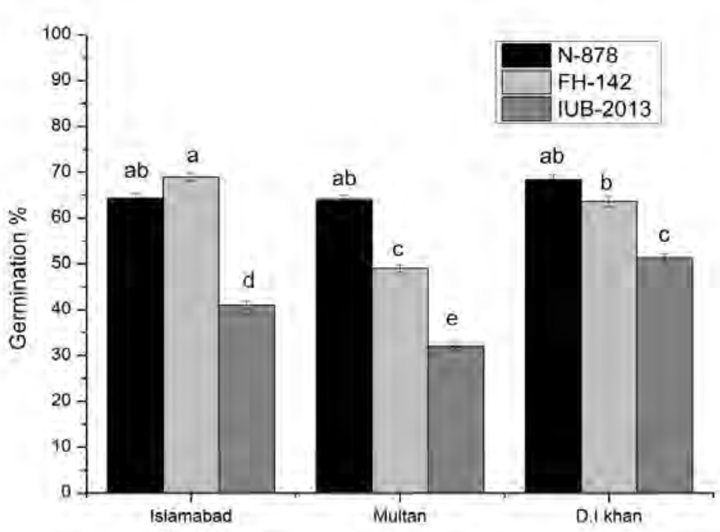

Figure 3.8: Variety wise comparison of cotton plant G% at multi-locations

3.9. Ginning out turn (GOT)

For GOT Significant results were observed in terms of three different location but results for variety and variety ×treatment was not significant LSD test showed three different groups were observed. All the cultivars were statistically at par with each other. Maximum GOT was obtained in relatively hot climate as shown by results were each cultivar obtained the same GOT (38) in Multan and in DI. Khan. Minimum value of GOT was in climate of Islamabad (37); similar value in each cultivar

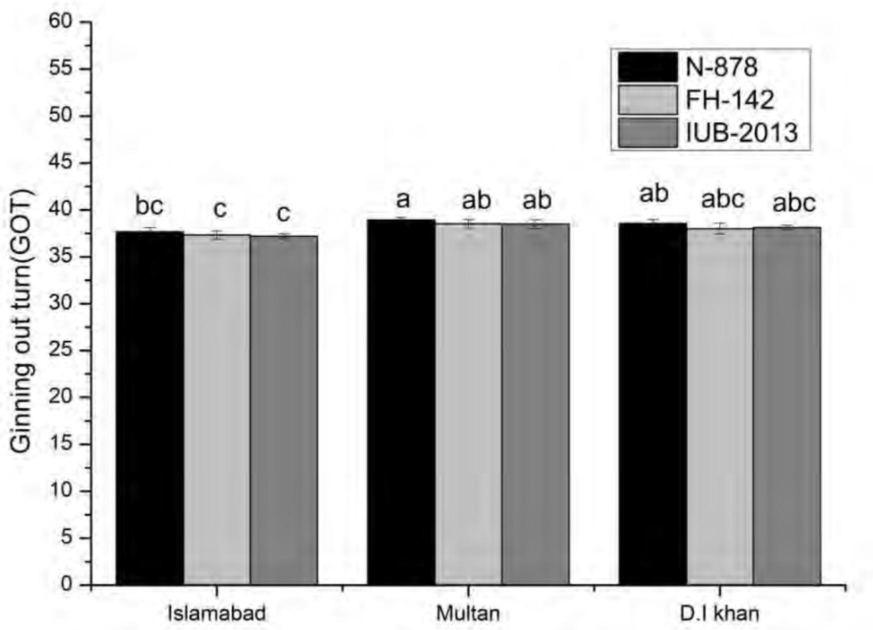

Figure 3.9: Variety wise comparison of cotton plant GOT at multi-locations

3.10. Boll weight

ANOVA test was carried out and results were carefully analyzed. Results of boll weight clearly depicts that all the cultivars in terms of boll weight are statistically at par with no significant difference (P >0.05). Further analysis by All-Pairwise Comparisons Test showed there was no significant pairwise differences among their means in terms of boll weight. Average boll weight secured by cultivar was in range of 4-5g. Variety× Treatment interaction results are also non -significant with p >0.05 which depicts that environment has no significant impact on the boll weight of cotton. Variety wise comparison of cultivars in terms of boll weight showed N-878 have the maximum mean boll weight in each location. FH-142 cultivar showed maximum boll weight in location of Islamabad which was highest noted boll weight in study (Fig. 3.10)

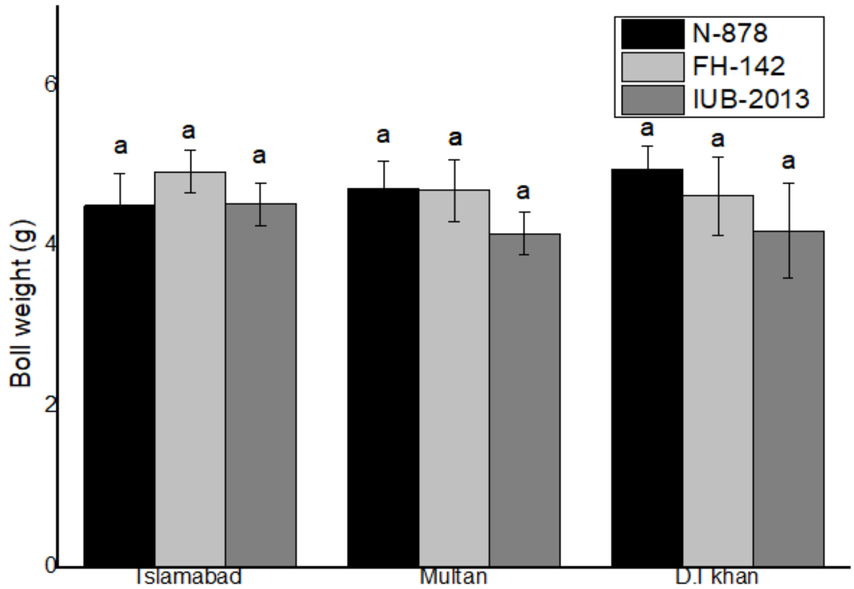

Figure 3.10: Variety wise comparison of cotton Boll weight at multi-locations

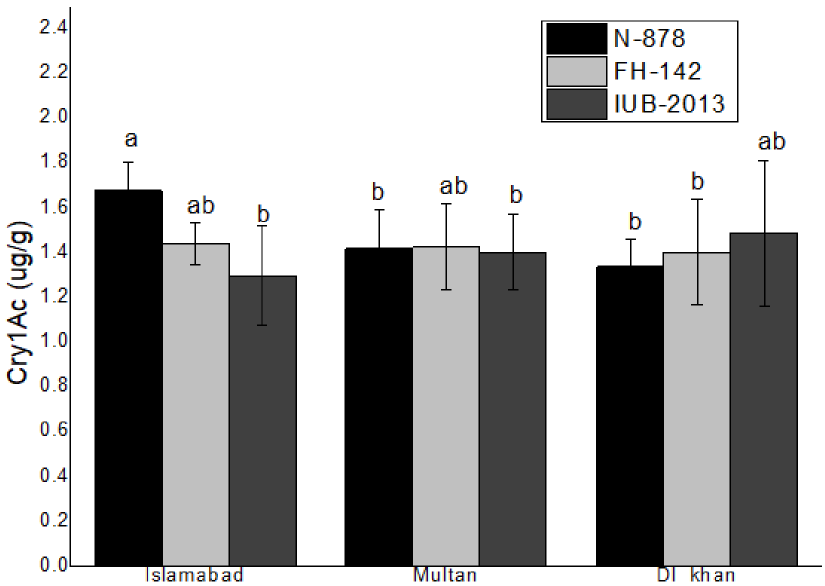

Figure 3.11: Variety wise comparison of cotton plant level of Cry1Ac at multi-locations

3.11. Cry1Ac level

High level of expression of cry1Ac are observed in N-878 cultivar in the climate of Islamabad (1.7µg/g) followed by FH-142 .Minimum level of Cry1Ac was observed in cultivar IUB-2013 (1.29µg/g).In the climate of Multan and DI .khan all the cultivars behave similar in terms of Cry1Ac level .Under the climate of Multan N-878 and FH-142 results are statistically at par .ANOVA and LSD result confirmed that variety ×location interaction results are non-significant with p =0.08.There are two groups in which means are not statistically different from each other.

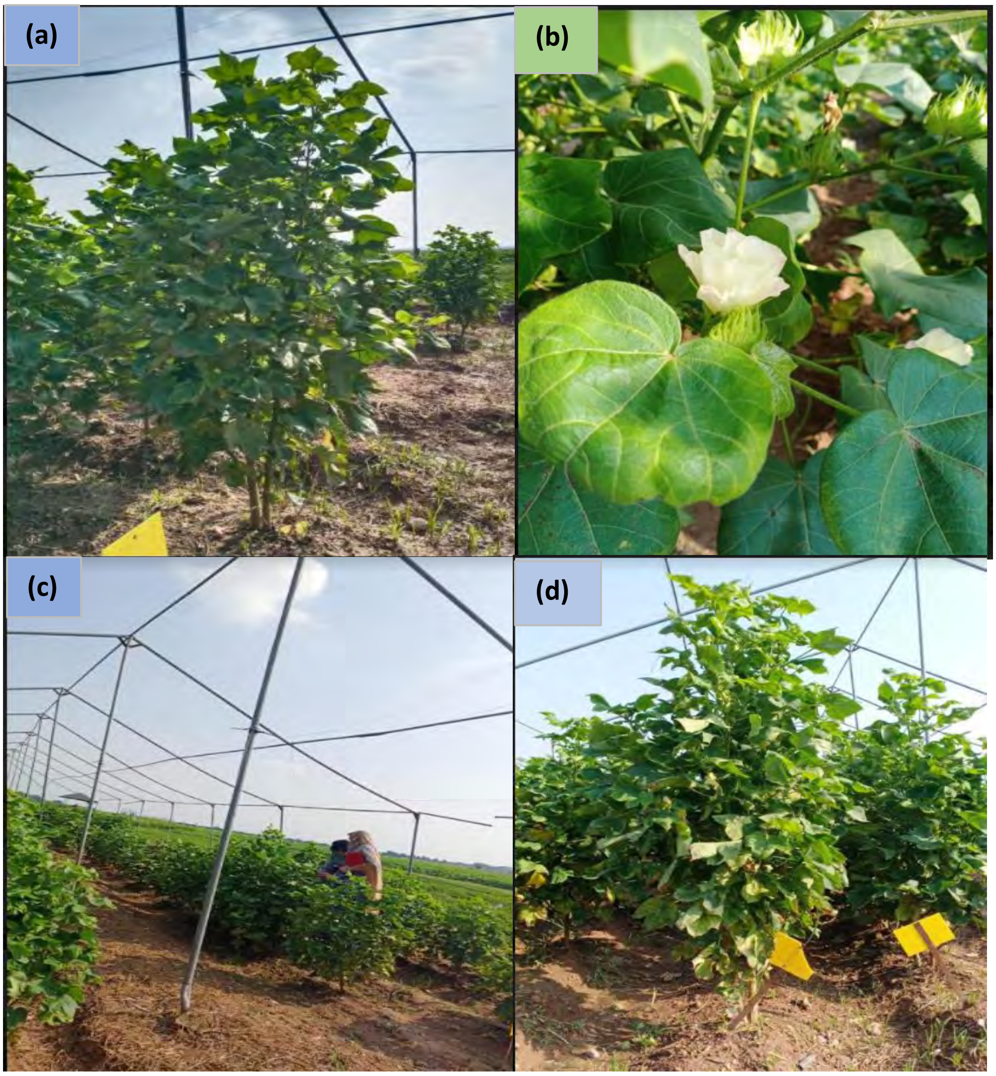

Figure 3.12.1: Different stages of field experiment (a) Crop at maturation (b) Flower initiation (c) Plant height measurement (d) Boll number measurement

Figure 3.12.2: Different stages of field experiment (a) Flowering stage (b) seeds counting (c) Measuring the weight of seeds (d) Drying the fiber

3.12. Climate data of multi-locations:

Average monthly climate data i.e., Temperature, humidity and rainfall of Islamabad, Multan and DI. Khan was obtained from Pakistan Meteorological Department (PMD) and Water resources Research Institute (WRRI) from the time of cotton sowing to harvesting and then the data was plotted in order to check the effect of change in climate on cotton yield and Bt gene expression.

3.13. Maximum temperature comparison of Three locations

In Multan the average mean of monthly maximum temperature was higher as compared to other climates. Highest temperature was recorded in the month of June for all locations For Minimum Temperature the average monthly minimum temperature was recorded in climate of DI. khan in the month of September.

3.14. Precipitation comparison of all locations

Highest precipitation (mm) was recorded in the month of July and August in the climate of Multan and DI. khan .and least one at the time of harvesting i.e., in the month of October.

3.15. Humidity % comparison

Climate data in terms of humidity was also compared in which the maximum % humidity was observed in the month of July in the climate of Multan. Graph below shows the month wise comparison for each location

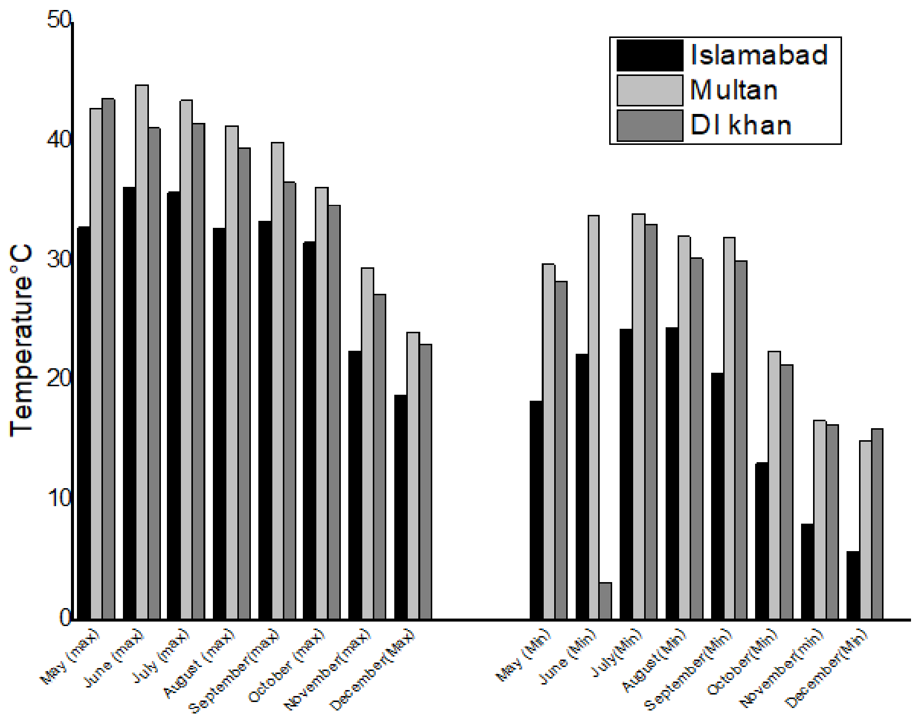

Figure 3.13: Comparison of maximum and minimum temperature of Islamabad, Multan and DI khan.

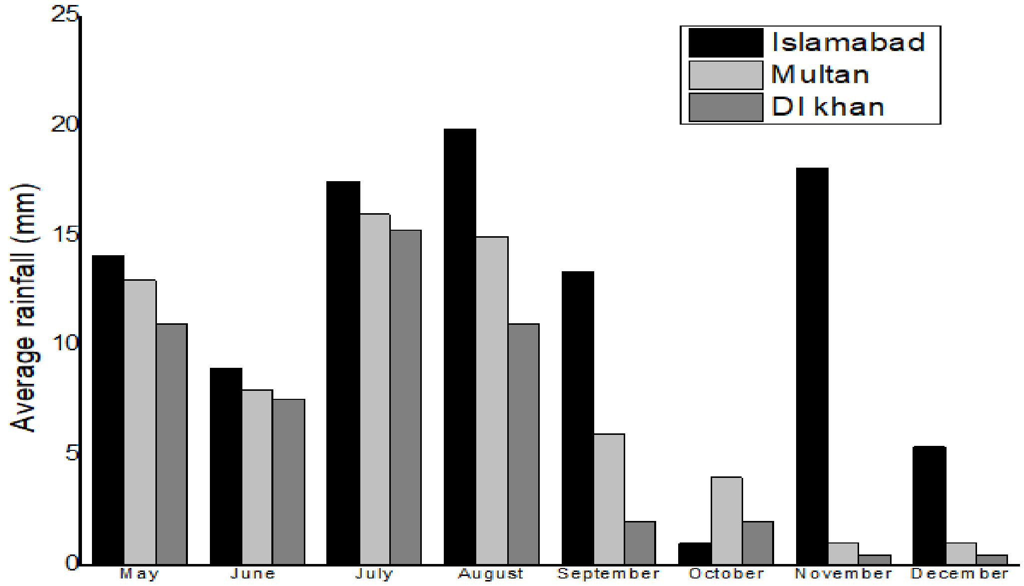

Figure 3.14: Monthly average rainfall comparison of Islamabad, Multan and DI khan

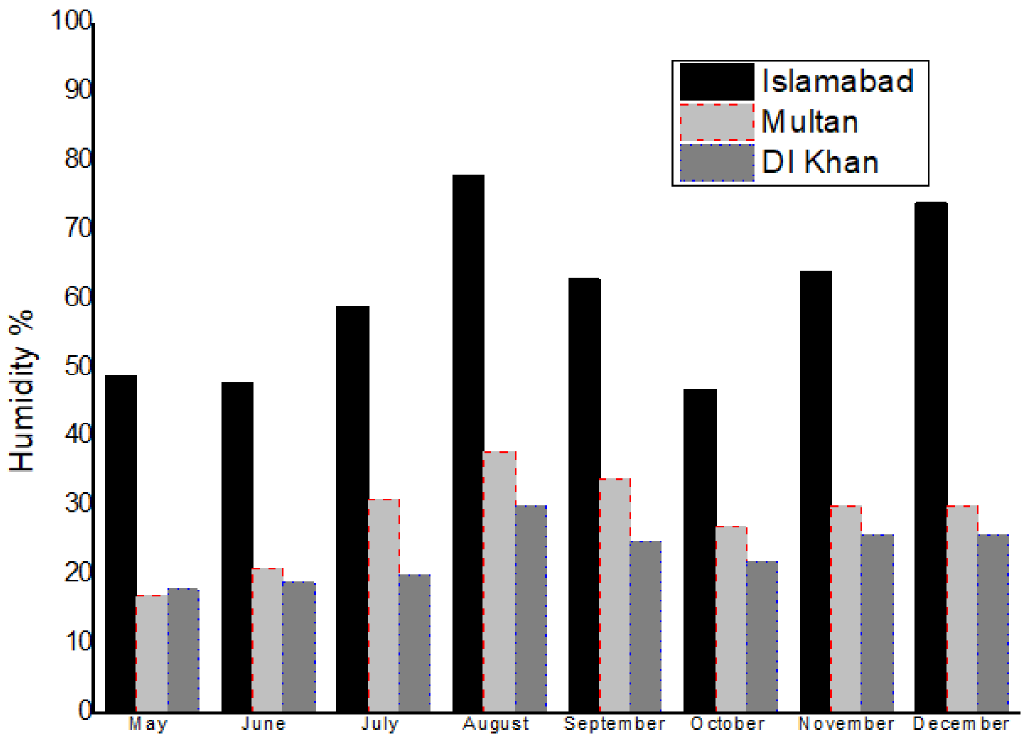

Figure 3.15: Monthly comparison of % humidity in Islamabad, Multan and DI khan

DISCUSSION

Narrow genetic difference in cotton cultivar is one of the major factors while climate change is the other major environmental stress that cost yield of seed cotton and quality of cotton. Cotton production plays an important role in environment change and also get effected by change in that climate. Water Resources are under threat due to increase in global warming and evapotranspiration which are the results of increase in earth temperature (Tariq *et al*. 2017). The effects of climate change could be seen in developing countries like Pakistan where the cotton production exhibit a reduction in yield during the last seven years. It is worth stating that changing climate is the most significant issue (Ahmad *et al*. 2017). The drastic impacts of climate change on cotton production are largely related to lessen availability of irrigation at specific growth stages of cotton plant like during germination, flower initiation and fertilization, boll formation and maturation of boll (Amin *et al*. 2017). The other most important impact on cotton is the rise in temperature specially in Pakistan, while in other cotton growing countries minimal rise in temperature will favor crop growth and development.

In this study, we have taken the data of temperature, rainfall and humidity of various zones. In our results of morphological traits i.e., plant height, no. of bolls per plant, boll weight that have showed diverse responses in cultivars and location wise under study. Similar to our results, maximum height showed a greater number of bolls and ultimately more yield as proposed in the findings of (Rauf *et al*. 2004); (Karademir *et al*.,2009) and (Saleem *et al*. 2015) also identified positive correlation of number of bolls per plant with plant height. Results of cultivars in terms of their plant height were significant. The produced results are in conformity with the work of (Sharma and Sharma.,1994) reported in his work that the difference in plant height is due to their genetic makeup. Boll weight is directly linked with yield in terms of seed. Genotypes that have higher boll weight would be high yielding. Similarly, good internode distance range was obtained in IUB-2013 in D.I. Khan. Diverse genotypes have different responses in terms of internode distance as reported by (Radin *et al*., 1992). Under rainfall areas, the internode distance become shortened due to severe shedding of square tissue (Oosterhuis and Hake 2001).

In the current study, maximum results of cotton yield were obtained in Multan and D.I. Khan that correlates that yield it is directly linked with increase in temperature i.e., Multan and D.I. Khan areas. These results are in conformity with that of (Bange 2007) study in which he showed that temperature effects the plant both in terms of negative and positive way. Rise in temperature increases the cotton yield however increased heat stress can damage plant tissue, resulting in parrot-beaked bolls, boll freeze, and cavitation, which reduces the yield. This result opposes that of (Chuang Zhao, 2017) in which decrease in yield with increase in temperature in four major crops (wheat, rice, maize, soybean). Higher temperatures create favorable conditions for the survival and reproduction of cotton sap-sucking pests like whiteflies, thrips, aphids, mealybugs and many others which cause serious yield losses in cotton (Bange,2007). Our results showed that average yield of cotton in different climate was different. The narrow genetic base of tetraploid cotton is one of the main factors that affects both the yield and quality of the crop (Wendel *et al*., 118 2015). Raza and Ahmad (2015) and Song *et al*. (2010) explained that maximum temperature has significantly positive impact on cotton productivity. (Reilly 2002) determined that cotton is well adopted to higher temperature.

In our results of germination percentage diverse responses were achieved in cultivars and location. Similarly, (Sattar *et al*.,2010) also stated that diverse germplasm of cotton has different germination response. Ginning out turn is an important character that was controlled by both additive and non-additive genes with interactions. Tuinstra *et al*. (1997) reported that yield and its related traits show changed behavior of gene expression under different environmental conditions. Days to first flower initiation in our study revealed that optimum results obtained in cotton variety FH-142 at Multan. In cotton plant, days to flower initiation is associated with earliness and diverse response in genotypes under different environmental conditions were reported by Anjum *et al*. (2001), Chaudhry and Guitchounts (2003).

In our results of Cry1Ac protein content, the high protein was noted in NIAB-878 cultivar followed by IUB-2013 in Islamabad and D.I Khan respectively. It means that high Bt protein was noted in Islamabad region as compared to D.I Khan and Multan. Similar to our findings, Huang *et al*. (2014) also reported that variable geographic conditions also affect *Cry1Ac* expression. IUB-13 showed diverse expression in various zones. It has been concluded that geographical conditions strongly affect Cry endotoxin expression.

Further justification to our results got evidence of (Zhang *et al*.,2012) that temperature increase can reduce Cry1Ac protein contents in cotton. Similar finding was also reported by (Chen *et al*.,2012) that extreme temperature reduces Cry1Ac protein in cotton plant. Very high or low level of Cry protein can cause cross resistance development in pests of cotton (Ahmad *et al*., 2019).

CONCLUSION AND FUTURE PERSPECTIVE

Determination of cotton yield related parameters in Pakistan ecological zones are important for food security. In this study, diverse responses in data parameters were recorded in the tested cultivars and ecological zones of Pakistan. Comparatively NIAB-878 variety of cotton performed well in terms of yield and bolls per plant and gene expression as compared to other two varieties i.e., IUB-2013 and FH-142. While based on climatic zone wise, D.I Khan has secured best performance in yield, bolls per plant and maximum gene expression was reported at Islamabad as compared to Multan and D.I Khan respectively. Meteorological data demonstrated that maximum rainfall and humidity were recorded in Islamabad during the month August 2020 and temperature was noted during the month of June at Multan. In future more cotton genotypes need to be tested under diverse cotton zones to evaluate the best suited genotype with respect to specific area to improve yield. New improved varieties of cotton are required that might be helpful to sustain climate change across the country.

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Appendix - I

Analysis of Variance Table for yield

Source	DF	SS	MS	F	P
Replication	2	1416.1	708.0		
Treatment	2	22141.4	11070.7	27.20	0.0000
Genotype	2	3287.2	1643.6	4.04	0.0380
Treatment*Genotype	4	6009.0	1502.3	3.69	0.0259
Error	16	6512.6	407.0		
Total	26	39366.3			

LSD All-Pairwise Comparisons Test of yield for Treatment

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	633.67 ± 6.350852961 de	660.67 ± 36.35014901 cd	617.67± 13.61371857 e	637.33 B
Multan(T2)	711.00 ± 10.53565375 a	696.67 ± 13.31665624 ab	700.33 ± 11.01514109 ab	702.67 A
D.I khan(T3)	729.00 ± 23.51595203 a	672.67 ± 35.24674926 bc	674.67 ± 674.6666667 bc	692.11 A
Grand Mean	691.22 A	676.67 AB	664.22 B	

Appendix-II

Analysis of Variance Table for ball weight

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	**2**	0.35130	0.17565		
Treatment	**2**	0.07352	0.03676	0.14	0.8721
Genotype	**2**	1.20130	0.60065	2.26	0.1371
Treatment*Genotype	**4**	0.62593	0.15648	0.59	0.6763
Error	**16**	4.26037	0.26627		
Total	**21**	6.51241			

LSD All-Pairwise Comparisons Test of ball for Treatment*Genotype

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	4.5167 ± 0.453688586 a	4.9333 ± 0.2081666 a	4.5333± 0.2081666 a	4.6611 ± A
Multan(T2)	4.7333 ± 0.321455025 a	4.7000 ± 0.435889894 a	4.1667 ± 0.2081666 a	4.5333 ± A
D.I khan(T3)	4.9667 ± 0.251661148 a	4.6333 ± 0.723417814 a	4.2000 ± 1.044030651 a	4.6000 ±A
Grand Mean	4.7389 ± A	4.7556 ± A	4.3000 ± A	

Appendix III

Analysis of Variance Table for PH

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	**2**	185.9	92.93		
Treatment	**2**	12153.2	6076.59	163.82	0.0000
Genotype	**2**	1489.0	744.48	20.07	0.0000
Treatment*Genotype	**4**	4710.4	1177.59	31.75	0.0000
Error	**16**	593.5	37.09		
Total	**21**	19131.9			

LSD All-Pairwise Comparisons Test of PH for Treatment*Genotypes

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	116.00 ± 5.567764363 d	130.33 ± 3.055050463 c	117.33± 4.725815626 d	121.22 ± C
Multan(T2)	167.33 5.507570547 ±b	157.00 ± 5.291502622 b	184.00 ± 9.539392014 a	169.44 ±A
D.I khan(T3)	190.67± 8.621678104 a	134.67± 5.507570547 c	161.00± 8.544003745 b	162.11 ± B
Grand Mean	158.00 ±A	140.67 ± B	154.11 ± A	

Appendix- IV

Analysis of Variance Table for number of bolls

Source	DF	SS	MS	F	P
Replication	**2**	58.96	29.48		
Treatment	**2**	824.52	412.26	20.94	0.0000
Genotype	**2**	2025.41	1012.70	51.43	0.0000
Treatment*Genotype	**4**	687.93	171.98	8.73	0.0006
Error	**16**	315.04	19.69		
Total	**21**	3911.85			

LSD All-Pairwise Comparisons Test of bolls for Treatment*Genotype

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	66.000 4.582575695 ± bc	59.333 ± 2.516611478 cd	49.000 ± 2 e	58.111 B
Multan(T2)	81.667 ± 3.055050463 a	58.667 ± 2.516611478 cd	69.333 ± 3.055050463 b	69.778 A
D.I khan(T3)	86.000 ± 7.211102551 a	68.667 ± 6.110100927 b	54.667 ± 6.429100507 de	69.778 A
Grand Mean	77.889 A	62.222 B	57.667 C	

Appendix-V

Analysis of Variance Table for cry1AC

Source	DF	SS	MS	F	P
Replication	**2**	0.00534	0.00267		
Treatment	**2**	0.02214	0.01107	0.50	0.6183
Genotype	**2**	0.03167	0.01584	0.71	0.5070
Treatment*Genotype	**4**	0.22417	0.05604	2.51	0.0531
Error	**16**	0.35746	0.02234		
Total	**21**	0.64079			

LSD All-Pairwise Comparisons Test of cry1AC for Treatment*Genotype

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	1.6767 ± 0.049328829 a	1.4400 ± 0.026457513 ab	1.2967 ± 0.149777613 b	1.4711 ± A
Multan(T2)	1.4167 ± 0.094516313 b	1.4233 ± 0.1106044 ab	1.4000 ± 0.085440037 b	1.4133 ± A
D.I khan(T3)	1.3367 ± 0.047258156 b	1.4000 ± 0.16643317 b	1.4867 ± 0.31214313 b	1.4078± A
Grand Mean	1.4767 ± A	1.4211 ± A	1.3944 ± A	

Appendix- VI

Analysis of Variance Table for DF

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	**2**	2.296	1.1481		
Treatment	**2**	20.074	10.0370	1.54	0.2448
Genotype	**2**	48.963	24.4815	3.75	0.0461
Treatment*Genotype	**4**	108.370	27.0926	4.15	0.0170
Error	**16**	104.370	6.5231		
Total	**21**	284.074			

LSD All-Pairwise Comparisons Test of DF for Treatment*Genotype

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	62.333 ± 1.527525232 ab	61.667 ± 0.577350269 abc	61.667 ± 0.577350269 abc	61.889 A
Multan(T2)	64.333 ± 1.527525232 a	57.333 ± 3.785938897 c	57.667 5.131601439 ± c	59.778 A
D.I khan(T3)	60.000 ±1 abc	58.000 ± 1 bc	64.333± 2.309401077 a	60.778 A
Grand Mean	62.222 A	59.000 B	61.222 AB	

Appendix- VII

Analysis of Variance Table for G

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	**2**	17.85	8.93		
Treatment	**2**	803.63	401.81	47.69	0.0000
Genotype	**2**	2914.74	1457.37	172.96	0.0000
Treatment*Genotype	**4**	436.37	109.09	12.95	0.0001
Error	**16**	134.81	8.43		
Total	**21**	4307.41			

LSD All-Pairwise Comparisons Test of G for Treatment*Genotypes

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	64.333 ± 3.785938897 ab	69.000 ± 2 a	41.000 ±2 d	58.111 ± B
Multan(T2)	64.000 ± 3 ab	49.000 ± 2 c	32.000 ± 3 e	48.333 ± C
D.I khan(T3)	68.333 ± 4.041451884 ab	63.667 ± 3.055050463 b	51.333 ± 2.516611478 c	61.111 ± A
Grand Mean	65.556 ± A	60.556 ± B	41.444 ± C	

Appendix-VIII

Analysis of Variance Table for GOT

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	**2**	0.9430	0.47148		
Treatment	**2**	7.3474	3.67370	12.41	0.0006
Genotype	**2**	1.1563	0.57815	1.95	0.1742
Treatment*Genotype	**4**	0.0659	0.01648	0.06	0.9936
Error	**16**	4.7370	0.29606		
Total	**21**	14.2496			

LSD All-Pairwise Comparisons Test of GOT for Treatment*Genotype

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	37.667 ± 0.577350269 bc	37.333 ± 0.577350269 c	37.200 ± 0.2 c	37.400 ± B
Multan(T2)	38.933 ± 0.2081666 a	38.533 ± 0.611010093 ab	38.500 ± 0.655743852 ab	38.656 ± A
D.I khan(T3)	38.567 ± 0.513160144 ab	38.000 ± 1 abc	38.133 ± 0.152752523 abc	38.233 ± A
Grand Mean	38.389 ± A	37.956 ± A	37.944 ± A	

Appendix-IX

Analysis of Variance Table for Internodal Distance (ID)

Source	DF	SS	MS	F	P
Replication	**2**	0.17852	0.08926		
Treatment	**2**	1.52074	0.76037	4.10	0.0365
Genotype	**2**	2.91630	1.45815	7.862	0.0042
Treatment*Genotype	**4**	2.07704	0.51926	2.80	0.0616
Error	**16**	2.96815	0.18551		
Total	**26**	9.66074			

LSD All-Pairwise Comparisons Test of ID for Treatment*Genotype

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	3.3333 ± 0.305505046 b	4.6333 ± 0.2081666 a	4.6000 ± 0.435889894 a	4.1889 ± A
Multan(T2)	3.4333 0.4163332 b	4.4667 ± 0.4163332 a	3.9667 ± 0.305505046 ab	3.9556 ± AB
D.I khan(T3)	3.6667 ± 0.305505046 b	3.7000 ± 0.435889894 b	3.4667 ± 0.723417814 b	3.6111 ± B
Grand Mean	3.4778 ± B	4.2667 ± A	4.0111± A	

Appendix- X

Analysis of Variance Table for Seeds

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	**2**	4.667	2.3333		
Treatment	**2**	2.000	1.0000	0.73	0.4985
Genotype	**2**	28.667	14.3333	10.42	0.0013
Treatment*Genotype	**4**	48.667	12.1667	8.85	0.0006
Error	**16**	22.000	1.3750		
Total	**26**	**106.000**			

LSD All-Pairwise Comparisons Test for Treatment * Genotype

	N-878 (G1)	FH-142(G2)	IUB 2013(G3)	Grand Mean
Islamabad(T1)	33.000 ±1 ab	29.000 ± 2 c	34.000 ±1 a	32.000 ±A
Multan(T2)	31.000 ± 1 bc	32.333 ± 0.577350269 ab	33.667 ± 0.577350269 a	32.333 ± A
D.I khan(T3)	29.333 ± 0.577350269 c	33.000 ±2 ab	32.667 ± 1.154700538 ab	31.667± A
Grand Mean	31.111 ± B	31.444 ± B	33.444 ± A	

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