INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 5, p. 55-63, 2018

RESEARCH PAPER OPEN ACCESS

Stability analysis of candidate bollgard bt cotton (*Gossypium hirsutum* L.) genotypes for yield traits

Muhammad Zaffar Iqbal, Shahid Nazir*, Sajid-ur-Rahman, Muhammad Younas

Agricultural Biotechnology Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad-Pakistan

Key words: Bollgard Cotton, Yield, Stability, Adaptability, GGE, Mega environment.

http://dx.doi.org/10.12692/ijb/13.5.55-63 Article published on November 01, 2018

Abstract

During varietal development process, multi-location trials are conducted to evaluate the performance of new cotton lines for yield potential and stability. Multi-location trials consisting of 89 candidate cotton genotypes were carried out at 10 locations under different agro-climatic zones. Presence of *Cry1Ac* gene of Mon-531 event was verified using isolated DNA and event-specific primers in PCR. Toxic cry protein was identified using qualitative strip test from ten randomly selected plants. To assess genotype by environment interaction and to evaluate the stability and adaptability, data were analyzed using GGE-biplot approach. Two mega environments were found and Ghotki (SG) was ideal location with maximum discriminative and representative properties. Genotype, MNH-1026 (1) performed best in all locations and proved to be an ideal genotype with maximum stability and adoptability followed by GH-Deebal (2). Hence, this information will be very useful for cotton breeders who intend to develop high yielding, widely adopted and stable genotypes, and be helpful for variety registration/approval departments for giving general and specific recommendations.

* **Corresponding Author:** Shahid Nazir ✉ shahidnazir97@yahoo.com

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the main cash crops and occupies the major area as compared with other crops grown in Pakistan. Primarily, this crop is grown as a source of fiber. However, cotton seed is used as a major source of food for human being as oil and feed for animal as seed cake. Cotton crop earns largest export revenues for the country and in addition to lint, cottonseed accounts for 80 percent of the national production of oilseed. Currently, Pakistan stood at 4th position in case of cotton production with 1.68 million tonnes and 3rd for consumption with 2.23 million tonnes (Pakistan Economic Survey). The cotton sector in the country is normally characterized by low yields as compared with other cotton producing nations of the world. Low yielding and less adaptable varieties are the major cotton yield limiting factors in country. Cotton research and improvement programs throughout the country are developing high yielding and more adaptive cultivars regularly. Rapidly changing and unpredictable climatic conditions demand to identify and introduce more stable cotton genotypes with specific adaptation to specific environments. This factor has given the great variation to the performance of the same variety in different locations (Pretorius *et al.*, 2015; Baloch *et al.*, 2015). Every year a huge number of newly developed genotypes are being tested for their yield stability and suitability for particular environmental conditions. This is the pre-requisite activity to approve and recommend the new cotton varieties for general cultivation in the country. However, recommendation of varieties for specific areas has been a challenge, as it depends largely on the variety adaptability to the soil and climatic conditions of the region (Maleia *et al.*, 2017). Evaluation and testing of newly developed cotton genotypes in wide agro-ecologies is of paramount importance as it shows the stability and adaptability for yield and other desirable traits. The genotype and environment (G x E) interaction tends to limit the selection index and the progress in breeding program for genetic improvement, particularly for quantitative traits like yield because it confuses the explanation of trials involving various locations. G x E component requires multiple locations trial for performance evaluation tests in breeding program, whereas the extent of genotypic effect relative to G x E component might reduce the number of locations essential for performance tests (Zeng *et al.*, 2014). This is very significant especially when we are working with advanced generations, which have not been tested yet, for yield stability and adaptability under various environmental conditions (Tukamuhabwa *et al.*, 2012). GGE biplot analysis is the most significant type for the identification of mega environments and for the selection of ideal genotypes (Yan *et al.*, 2007).

This method also helps the breeders to make conclusion about the stability and adaptation of breeding genotypes in several locations. Normally, breeding programs are prepared to fulfill the requirements of different stakeholders in the cotton value chain. In this way, the farmers demand varieties that are high yielding while the ginners and spinners require high lint yield with good fiber quality. Hence, prior to approval and recommendation of any new variety, it should be assessed and evaluated for yield stability and adaptability across the different environments. Therefore, the present work was designed to identify the yield stability and adaptability of 89 candidate varieties at different locations throughout the country.

Material and methods

Breeding material and sowing procedure

A total of 89 candidate upland cotton varieties developed by various cotton research institutes and private sector were grown at ten different locations under National Cotton Varietal Trial. Experiment was evaluated in normal growing season i.e. month of June across the ten environments. Each cotton genotype was planted with five seeds per hill at about 4 cm depth in a plot having four rows of five meter length and spacing was kept 75 cm between rows and 30 cm between plants in a randomized complete block design with three replications. Thinning was carried out keeping one plant per hill after about three weeks of crop emergence in the respective locations (Orawu *et al.*, 2017; Ali *et al.*, 2017).

Agronomic practices

All the agronomic field management practices i.e. irrigation, weeding, fertilizer application and pesticide applications etc. were done as and when required. Weeding was done manually to remove any weeds from the trials when necessary. Weeding at all sites was done three times for the whole season. The cotton pests were controlled following the recommended cotton pest scouting and insecticide application instructions (Maleia *et al.*, 2017).

DNA isolation and PCR

Total genomic DNA was isolated from the leaves of cotton varieties using CTAB methods with modifications (Rogers and Bendich, 1985). Quality and quantity of isolated DNA was verified using Nanodrop spectrophotometer (Nano Drop Technologies, USA). The presence of *Cry1Ac* gene was confirmed in PCR using Mon-531 event specific primers (Yang *et al.*, 2005). The PCR programme was consisted of 35 cycles of 94°C for 30 seconds, 56°C for 45 seconds and 72°C for 1.0 minute. The amplified PCR product was resolved in 1.5% agarose gel and visualized under UV gel documentation system (Photonyx Ultra, UK).

Picking of bolls

Picking was carried out in the last week of November, from central two rows, ten plants were randomly selected in each sub-plot/replication to record the data pertaining to seed cotton yield of each plant. Data on seed cotton yield of all picks was measured using an analytical balance (Hicks, 1982; Gomez and Gomez, 1984). Total seed cotton yield for each cotton genotype from each plot was weighed in kilogram and converted into kilogram per hectare. The total yield was computed from the sum of the weight of boll samples together with the seed cotton weights at different pickings.

Statistical analysis

The analysis of data for yield stability and adaptability of cotton was carried out using R software (Farias *et al.*, 2016). The seed cotton yield across all locations was analyzed using the application of the genotype and genotype by environment (GGE) biplots. Suitability and stability analysis for each genotype in respective environment require the use of GGE biplot (Blanche *et al.*, 2007; Yan, 2001). Moreover, the GGE biplot is generally considered the type of biplots for mega-environment investigation, genotype and test location evaluation, thus performs data by graphic approach (Xu *et al.*, 2014; Zeng *et al.*, 2014). The GGE biplot was constructed by considering the principal components (PC1 and PC2).

Results and discussion

Molecular identification of Bollgard cotton

For the verification of the presence of Bt Cry protein in the upcoming cotton varieties, a qualitative strip test specific for Cy1Ac protein was performed on 10 randomly selected plants from each variety.

Table 1. Tested genotypes with their codes used in the study for stability analysis.

Genotypes	Code	Genotypes	Code	Genotypes	Code
MNH-1026	1	TJ-MAX(CEMB 2)	31	FH-142 (St-2)	61
GH-Deebal	2	RH-668	32	KZ-125	62
GH-Hadi	3	NIAB-545	33	Shahab-7	63
D-19	4	ICI-2121	34	SLH-19	64
GH-Mubarak	5	Cyto-515	5	Suncrop-6	65
Crystal-12	6	CIM-663	36	Sahara-210	66
IUB-65	7	Tahfuz-10 (CEMB 2)	37	FH-142 (St-2)	67
Eagle-2	8	CEMB-88(DG)	38	CEMB-55(DG)	68
B-2	9	Sahara-2020 (CEMB-2)	39	NIAB-1048	69
CIM-343	10	MNH-1020	40	Sitara-16	70
Shaheen-16	11	IUB-69	41	SASUI-2018	71
CIM-625	12	Weal-Ag-1606	42	Bahar-2017	72

Weal-AG-6	13	NIAB-898	43	Tipu-1	73
FH-444	14	Shaheen-1	44	Tarzan-5	74
RH-662	15	CIM-602 (Std-1)	45	NS-191	75
BH-221	16	FH-142 (St-2)	46	Suncrop-5	76
CIM-602 (Std-1)	17	Cyto-313	47	SAU-1	77
Bh-201	18	FH-152	48	Tipu-9	78
AGC-Nazeer-1	19	RH-Manthar	49	VH-383	79
CEMB-100(DG)	20	CIM-602 (Std-1)	50	Sikandar-1	80
Badar-1(CEMB 2)	21	Evyol-148	51	SLH-6	81
CEMB-101 (DG)	22	Thakkar-808	52	NIAB-Bt-2	82
VH-189	23	CIM-632	53	NS-181	83
Bahar-07	24	Tassco-902	54	D-12	84
FH-490	25	NU-21 (CEMB-2)	55	AA-933	85
BS-18	26	Sitara-15	56	CRIS-600	86
CIM-602 (Std-1)	27	Bakhtawar-1	57	BS-80	87
RH-Afnan	28	Auriga-216	58	VH-Gulzar	88
BZU-05	29	FH-142 (St-2)	59	NIA-85	89
Weal-AG-5	30	MNH-1016	60		

It was observed that all the plants were positive for *Cry1Ac* protein. Furthermore, genetic transformation event and responsible gene for Cry protein was investigated using PCR which successfully verified that all the candidate cotton varieties have Mon-531 event of Bollgard cotton. This event has *Cry1Ac* gene of soil born bacterium, i.e. *Bacillus thuringiensis* (Bt) which was introduced into cotton by Monsanto Company to control the lepidopteron insects. In PCR amplification of 346bp fragment successfully verified that all cotton genotypes have Bt*Cry1Ac* gene of Mon-531 event as shown in representative gel image of Figure-1. This is the only approved GM cotton event for commercial cultivation in the country.

Table 2. Tested locations/environments with code used in the study for stability analysis.

Sr. #	Location	Code
1	Multan	PM
2	Bahawalpur	PB
3	Sahiwal	PS
4	Khanpur	PK
5	Vehari	PV
6	Sakrad	SS
7	Ghotki	SG
8	Tandojam	ST
9	Lasbella	BL
10	Dera Ismail Khan	KD

The approved Bt cotton event Mon-531 was verified using PCR technology and toxic protein was investigated by qualitative strip test. These are very basic, easy and quick methods for the verification of GM cotton event in upcoming varieties and all were positive for approved Bt cotton event.

GGE biplot analysis

Genotype by environment study was carried out for 89 candidate upland cotton varieties (Table-1) by growing in normal cotton growing season at ten different locations throughout the country (Table-2). The GGE biplots were conducted for the mega-environments and both principal components of PC1 and PC2 when plotted, contributed 53.87% of the total variations of GGE for the seed cotton yield (Figure 2). In developing adapted cotton varieties, the

concept of mega-environment has been proposed and use of GGE biplot resulted in identifying two distinct mega-environments where cotton trials were evaluated. The use of GGE in explaining the principal components of PC1 and PC2 clearly provided an indication of their suitability for analysis of environments in the trials. The GGE biplot provide an effective statistical analysis approach for analyzing the effects of genotype by environment interaction in crop test locations (Yan *et al.*, 2000; Yan, 2001).

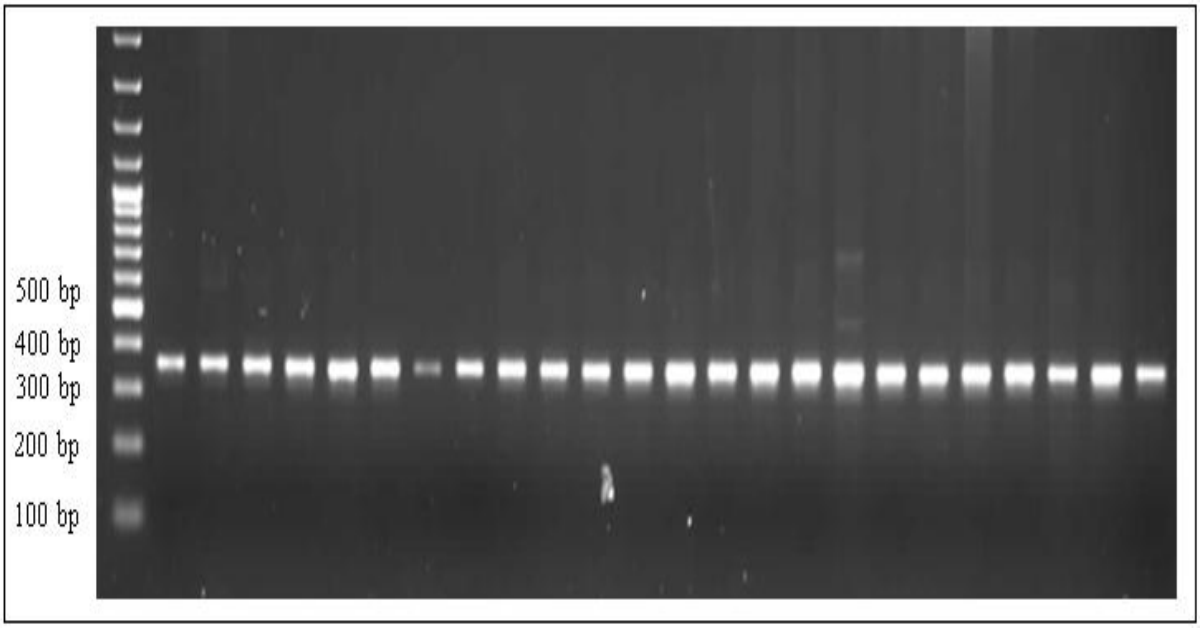

Fig. 1. Representative PCR gel image of new Bollgard cotton lines using Mon-531 event specific primers.

However, test environments are dynamic factors that fluctuate considerably between years. When using GGE biplot for genotype by environment interaction and define ecological locations for planting genotypes, it is necessary to perform analysis based on test data from multi-years and locations (Yan, 2015).

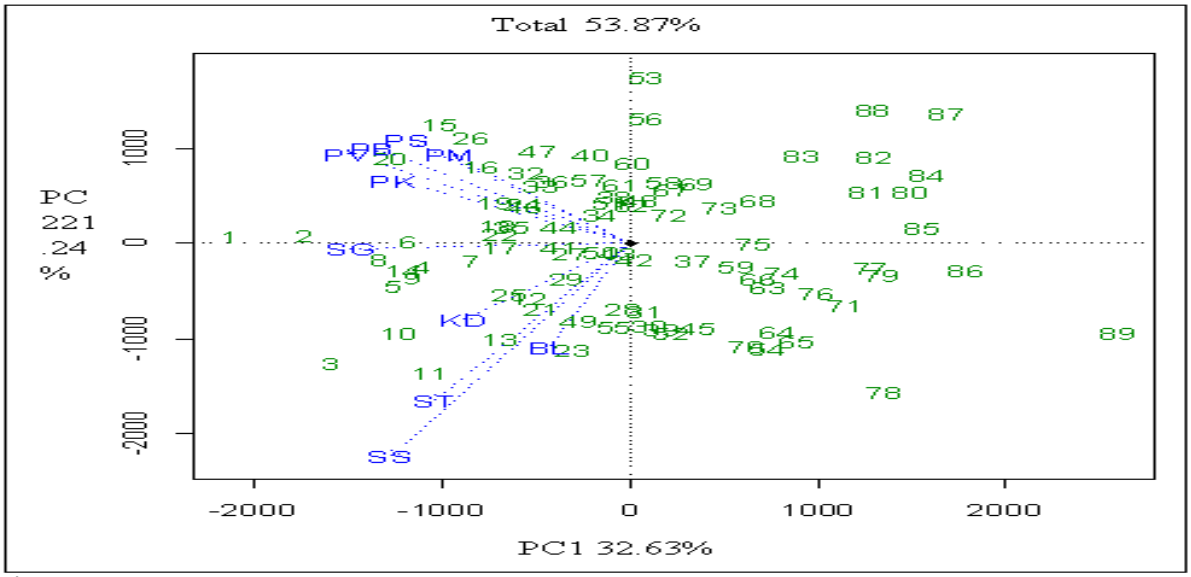

Fig. 2. GGE biplot analysis for stability and adaptability of candidate's cotton genotypes under 10 different locations with total underline structure.

Which Won Where

Ten environments were grouped into two mega-environments and named as ME1 and ME2. ME1 was comprised on 06 locations i.e. Ghotki (SG), Khanpur (PK), Vehari (PV), Multan (PM), PS (Sahiwal), and Bahawalpur (PB) while 04 locations i.e. Dera Ismail Khan (KD), Lasbela (BL), Tandojam (ST) and Sakrand (SS) were grouped under second mega environment i.e. ME2 (Figure 2). The winning genotypes for ME1 is genotype MNH-1026 (1) followed by GH-Deebal (2). On the other hand, in ME2, winning genotypes are GH-Hadi (3) followed by No. Shaheen-16 (11) and No. 10 (CIM-343). Genotypes (Tipu-9 (78), BS-80 (87), VH-Gulzar (88) and NIA-85 (89) are in opposite direction to mega environments which shows that their performance is not stable and ultimately is unsatisfactory.

From Figure 3 it could also be concluded that genotype MNH-1026 (1) should be recommended for environments present in ME1 and GH-Hadi (3) for environments in ME2.

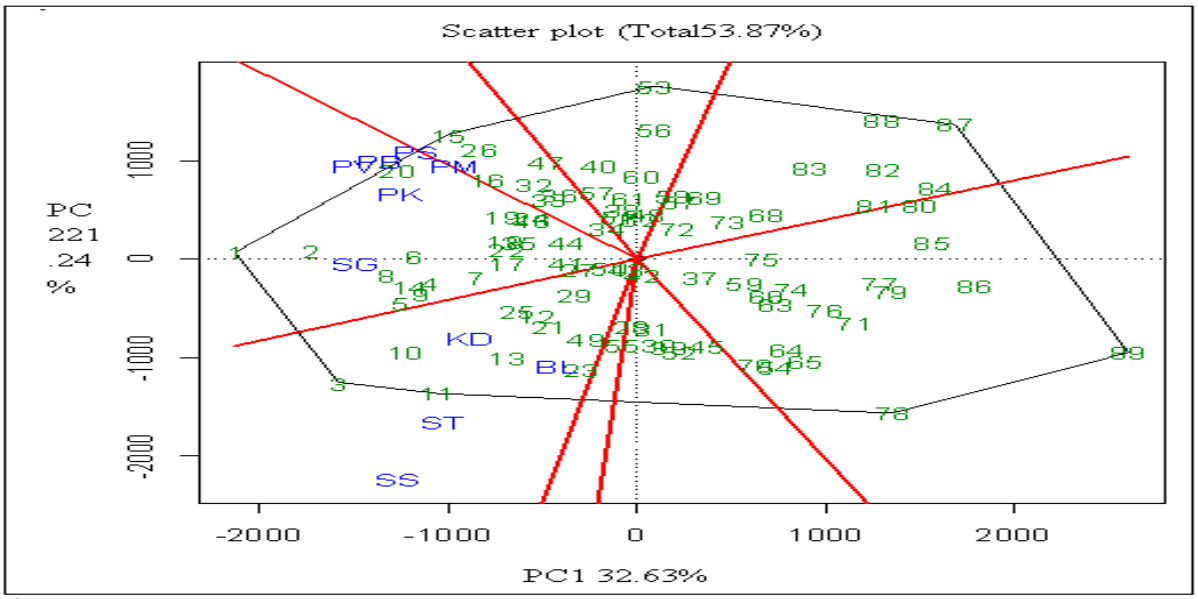

Fig. 3. Polygon view of GGE biplot based on environment scaling for the "which-won where" pattern of genotypes and environments.

Identification of ideal environment

The environments that tend to be close to the center are considered as ideal test environments. There are normally two major qualities of ideal environments i.e. firstly it should be representative and secondly it should have maximum discriminative ability. From these result we can see in figure 4 that there is no location or environment which is ideal as no one is close to the center. But Ghotki (SG) can be taken as somewhat ideal environment and can be used for further experimentation work because it is very near to the innermost concentric rings. After that, Dera Ismail Khan (KD), Khanpur (PK), PV (Vehari) and Bahawalpur (PB) are fairly good and could also be used in case of unavailability of Ghotki (SG) for experimentation. The environments Sakrand (SS) and Lasbela (BL) are far away from the center and are considered as diverse environments because they are far away from each other. These are very unpredictable environments, hence should be avoided if only one location is used in experiment. These results showed that Ghotki (SG) is the ideal test environment in discriminating and representativeness manner. The ideal test locations demonstrate high efficiency in selecting genotypes with a wide adaptability and genotypes selected from ideal environments have an outstanding average performance with wide adaptation. Discriminating test environments, accurately resolve genotype differences; thus providing the necessary information for selection by plant breeders (Tukamuhabwa *et al.*, 2012; Mukoyi *et al.*, 2015) have shown similar

findings for ideal test environment as one which could be discriminating of the genotypes and representative of the mega-environment because such sites can be used for early generation screening of the experimental lines while discriminating sites can be used for selecting specifically adapted varieties in the mega-environment. Considering the test location at SG is highly discriminating but not representative and therefore, it can be used as a culling environment to quickly eliminate unstable genotypes in regard to performance during the selection stages of evaluation (Yan and Kang, 2003).

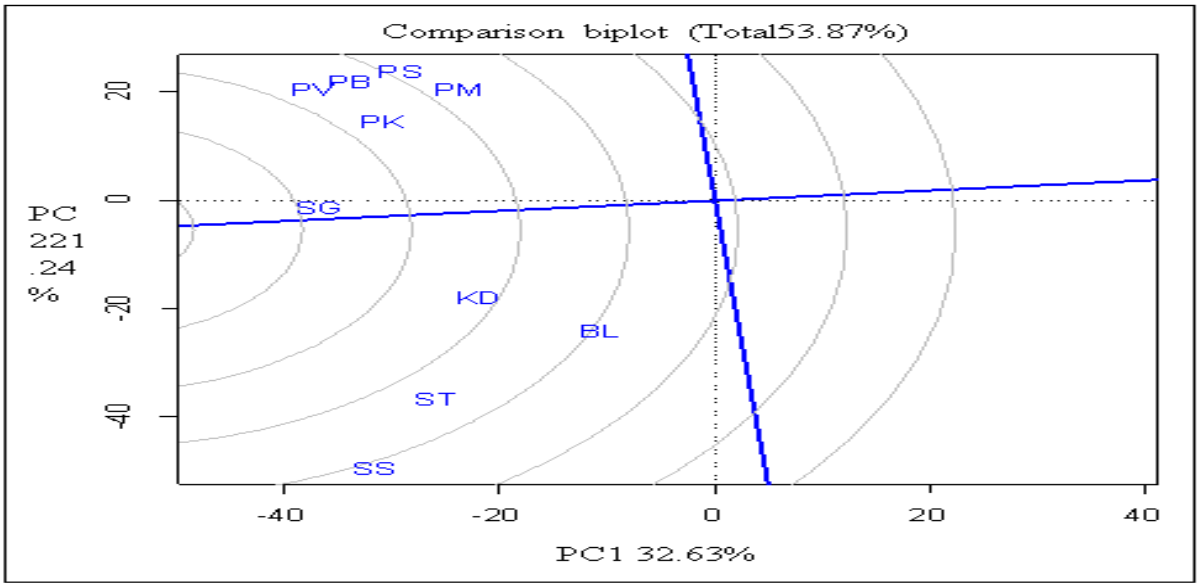

Fig. 4. GGE biplot showing environment comparison of the average environment for seed cotton yield of 89 candidate's cotton genotypes at 10 testing locations.

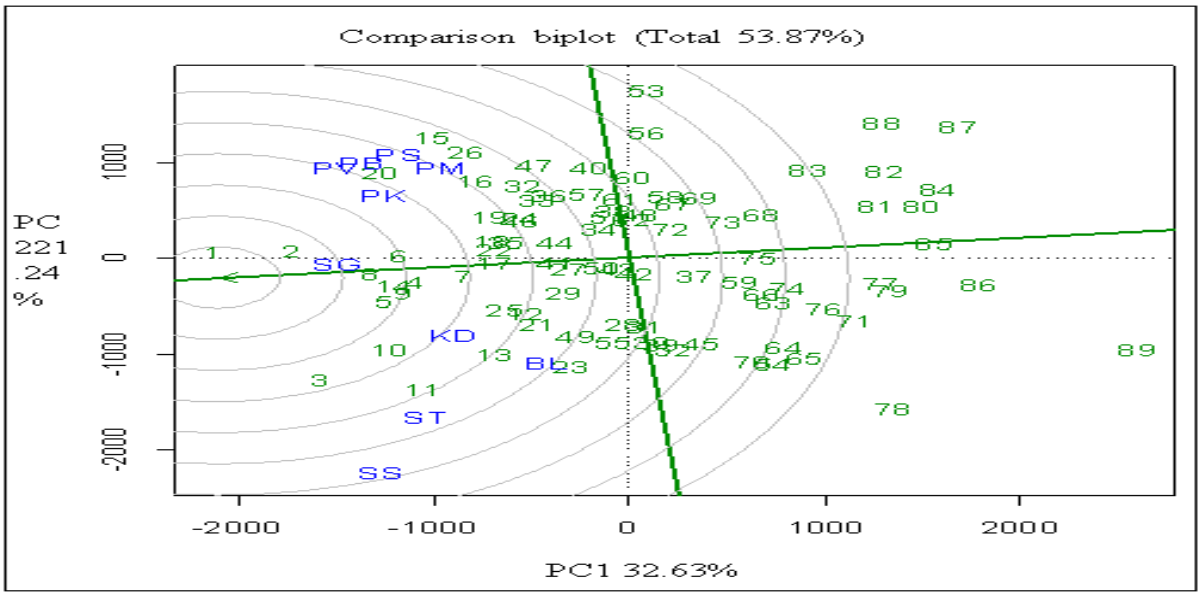

Fig. 5. GGE biplot based genotype focusing scaling for the mean performance ranking and stability of 89 candidate's cotton genotypes at 10 testing locations.

Identification of ideal genotype

Similar to ideal environment, the genotype close to the centric ring is considered as the ideal genotype. Likewise to environment, the ideal genotype should also have two main abilities. Firstly it should be high yielding and secondly it should be stable across the environments. Keeping in view the mentioned qualities, the genotype MNH-1026(1) proved the ideal

one as it falls in the centric ring (Figure 5) and could be used for general adaptation. The second ideal genotype is GH-Deebal (2) which is also very near to the centric ring. Genotypes Tipu-9(78), VH-383 (79), NIAB-Bt-2 (82), D-12 (84), CRIS-600 (86), BS-80 (87), VH-Gulzar (88) and NIA-85 (89) showed poor performance. These genotypes could be eliminated/discarded from further experimentation work. This information is relevant to plant breeders intending to evaluate the advanced experimental materials in several multi-location trials as some may give inaccurate results because of their low discriminating capability and lack of representativeness considering the costs in terms of time and resources likely to be incurred (Zeng *et al*., 2014; Mukoyi *et al*., 2015).

Conclusion

The stability and adaptability analysis of cotton for yield was assessed in newly developed up-coming varieties. The trial was comprised on 89 genotypes and 10 locations were used for stability analysis. The presence of Bt*Cry1Ac* gene in all genotypes was confirmed using PCR technology and developed toxic protein was identified using qualitative strip test. To assess the genotype by environment interaction and to evaluate the stability and adaptability, the genotypes were tested using GGE-biplot approach. Two mega environments were found and Ghotki was the ideal location with maximum discriminative and representative properties. Genotype MNH-1026 performed best in all locations and was selected as ideal genotype with maximum stability and adoptability followed by GH-Deebal. This information could be very useful for breeder and other departments involved in variety registration and approval.

References

Ali I, Naqib UK, Fida M, Muhammad AI, Ammad A, Farhatullah, ZB, Sardar A, Ibni AK, Sheraz A, Mehboob R. 2017. Genotype by environment and GGE-biplot analyses for seed cotton yield in upland cotton. Pakistan Journal of Botany**49,** 2273-2283.

Baloch M, Baloch W, Baloch MK, Mallano A, Baloch M, Baloch NJ, Abro S. 2015. Association and heritability analysis for yield and fibre traits in promising genotypes of cotton (Gossypium hirsutum L.). Sindh University Research Journal **47,** 303-306.

Blanche SB, Myers GO, Zumba JZ, Caldwell D, Hayes J. 2006. Stability comparisons between conventional and near-isogenic transgenic cotton cultivars. Journal of Cotton Science **10,** 17-28.

Farias FJC, Carvalho LP, Silva Filho JL, Teodoro PE. 2016. Biplot analysis of phenotypic stability in upland cotton genotypes in Mato Grosso. Genetics and Molecular Research **15,** gmr15028009. https://doi.org/10.4238/gmr.15028009

Gomez KA, Gomez AA. 1984. Statistical Procedures for Agricultural Research (2nd ed.). John Wiley & Sons Inc. New York. USA.

Hicks CR. 1982.Fundamental concepts in the design of experiments. CBS College Publ. New York.

Maleia MP, Afonso R, Leonel DM, Jaime OT, Fátima C, Edson J, Joaquim ND, Badrodine AA. 2017. Stability and adaptability of cotton (Gossypium hirsutum L.) genotypes based on AMMI analysis. Australian Journal of Crops Science **11,** 367-372. https://doi.org/10.21475/ajcs.17.11.04.pne60

Mukoyi F, Mubvekeri W, Kutywayo D, Muripira V, Mudada N. 2015. Development of elite medium staple cotton (G. hirsutum L.) genotypes for production in middle veld upland ecologies. African Journal of Plant Science **9,** 1-7. https://doi.org/10.5897/AJPS2014.1236

Orawu M, Gladys A, Lastus S, George O, Chris O. 2017. Yield stability of cotton genotypes at three diverse agro-ecologies of Uganda. Journal of Plant Breeding and Genetics **5,** 101-114.

Pretorius MM Allemann J, Smith MF. 2015. Use of the AMMI model to analyse cultivar-environment interaction in cotton under irrigation in South Africa. African Journal of Agriculture**2,** 76-80.

Rogers SO, Bendich AJ. 1985. Extraction of DNA from milli-gram amounts of fresh, herbarium and mummified plant tissues. Plant Molecular Biology**5,** 69–76.
https://doi.org/10.1007/BF00020088

Tukamuhabwa P, Asiimwe M, Nabasirye M, Kabayi P, Maphosa M. 2012. Genotype by environment interaction of advanced generation soybean lines for grain yield in Uganda. African Crop Science Journal**20,** 107-115.

Xu N, Fok M, Zhang G, Li J, Zhou Z. 2014. The application of GGE Bi-plot analysis for evaluating test locations and mega-environment investigation of cotton regional trials. Journal of Integrative Agriculture **13,** 1921-1933.
https://doi.org/10.1016/S2095-3119(13)60656-5

Yan W. 2015. Mega-environment analysis and test location evaluation based on unbalanced multiyear data. Crop Science **55,** 113-122.
https://doi.org/10.2135/cropsci2014.03.0203

Yan W, Kang MS. 2003. GGE Bi-plot analysis: A graphical tool for Breeders, Geneticists and Agronomists. Florida, USA: CRC, Press.

Yan W, Hunt LA, Sheng QL, Szlavnics Z. 2000. Cultivar evaluation and mega-environment investigation based on GGE bi-plot. Crop Science **40,** 596-605.
https://doi.org/10.2135/cropsci2000.403597x

Yan W, Kang MS, Ma B, Woods S, Cornelius PL. 2007.GGE Bi-plot vs. AMMI Analysis of Genotype-by-Environment Data. Crop Science **47,** 643-655.
https://doi.org/10.2135/cropsci2006.06.0374

Yan WK. 2001. GGE biplot-A windows application for graphical analysis of multi-environment trial data other types of two-way data. Agronomy Journal **93,** 1111-1118.
https://doi.org/10.2134/agronj2001.9351111x

Yang L, Pan A, Zhang K, Yin C, Qian B, Chen J, Huang C, Zhang D. 2005. Qualitative and quantitative PCR methods for event-specific detection of genetically modified cotton Mon1445 and Mon531.Transgenic Research **14,** 817-3.
https://doi.org/10.1007/s11248-005-0010-z

Zeng L, Meredith WR, Campbell BT, Dever JK, Zhang J, Glass KM, Jones AS, Myers GO, Bourland FM. 2014. Genotype-by-Environment Interaction Effects on Lint Yield of Cotton Cultivars across Major Regions in the U.S. Cotton Belt. The Journal of Cotton Science**18,** 75-84.