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BIO-STIMULATION AND YIELD RESPONSIVENESS IN MAIZE

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Abstract

Since green revolution, synthetic fertilizers have been used extensively to enhance the crop productivity which posed harmful effects in various ecosystems. It is therefore needed to substitute or supplement the application of synthetic nutrients with organic ones for sustainable agricultural productivity. More recently, there is an increasing interest to organic bio-stimulants and if they are combined with synthetic nutrients, they have been reported to increase the crop productivity. Current investigation was planned to evaluate responsiveness of eleven commercial hybrids of maize against different combinations of a bio-stimulant (Plant Protector^{TM}) and synthetic fertilizer. Application of bio-stimulant increased plant biomass and chlorophyll contents, and reduced leaf temperature of the plants making them able to withstand high atmospheric temperratures. Treatment of bio-stimulant combined with 50% of prescribed synthetic fertilizer at 7 leaf stage showed 73.53% and 68.58% increase in the yield as compared to control and recommended fertilizer dose, respectively. Treatments, genotypes and their interactions contributed 35.97%, 26.23% and 27.79% of total variation for cob yield, respectively. Among the studied genotypes, FH-963, FH-985 and FH-988 were highest yielding. Results suggested that maize hybrids were highly responsive to nutrients at 7-leaf stage. It is recommended that for highest yield, dose of synthetic fertilizer could be reduced to half and supplemented with bio-stimulant at 7-leaf stage.

Keywords: Plant Protector, Bio-stimulant, NPK, Maize, Contour plot, AMMI biplot

Introduction

Almost 18.5% Gross Domestic Product (GDP) of Pakistan is contributed from agricultural sector which employs about 38.5% of rural population and supports the whole population either directly or indirectly (Anon., 2019). Contributing as food and feed, maize has worldwide significance. Apart from usage as staple food in a number of countries, it is also used for the production of numerous industrial products including starch, oil, wax, syrup, dextrose and some cosmetics. One way or another, every part of maize plant is utilized in different industries (Haddadi *et al*., 2012; Aysin *et al*., 2020; Nawaz *et al*., 2020). This “Queen of Cereals” is cultivated in almost all altitudes of the country and throughout the year (Baloch *et al*., 2015). Maize is the third most cultivated crop worldwide covering an area of about 191.72 million hectares, yielding 5.86 tons per hectare (Anon., 2020).

Maize is fourth most cultivated crop and third most cultivated cereal in Pakistan, which adds about 2.6% value in agriculture and 0.5% to the GDP. It is cultivated on about 1318 thousand hectares producing 6309 thousand tons of yield (Anon., 2019). Pakistan is ranked 19 according to maize production (Anon., 2019). Decrease in area under cultivation coupled with other biotic and abiotic factors contributed to the low production of maize in Pakistan (Dhungana *et al*., 2006; Walker & Schulze, 2008). Furthermore, Pakistani soils are generally calcareous and poor in nutrients and organic matter concentration. High temperature during kharif season makes situations even tougher for ideal crop yields (Sadiq *et al*., 2014).

The primary strategy for increasing the production has always been the application of synthetic fertilizers which mainly comprise of nitrogen, phosphorus and potassium (NPK), and amounts of heavy metals including mercury, cadmium, arsenic, lead, copper and nickel accompanied by various radioactive isotopes of thorium, polonium or uranium etc. (Sönmez *et al*., 2007). The large scale application of synthetic fertilizers without understanding the crop requirements and soil fertility status have caused the degradation of soil profile, and pollution of surface and underground water bodies. In addition, deprived administration of organic matter causes loss in soil fertility causing disturbance in the activities of valuable microbes (Shah *et al*., 2009). Moreover, excessive nitrogen in fertilizers leaches and percolates contaminating the underground water which causes serious health issues (Cheema *et al*., 2010). On the other hand, accumulation of heavy metals and radioactive elements in the soil become part of the plant, which finally enter our food chain causing problems which might take generations to solve (Savci, 2012).

Considering the above mentioned facts, it is high time we need to substitute the use of synthetic nutrients or supplement them with organic ones. Fageria *et al*., (2008) described that foliar application of bio-active nutrients could cut down the fertilizer requirement of the crop. Use of bio-stimulants can therefore be considered as a dependable option. Bio-stimulants have been studied to influence the growth and development of the plants by enhancing the nutrient uptake and efficienct use (Ertani *et al*., 2009). Various organic bio-stimulants have been used in recent studies like plant debris, enzymes, vitamins, humic acids, carbohydrates, seaweed filtrates, hormones, various types of manures, green manures and bio-fertilizers etc. (Du-Jardin, 2015).

To evaluate the responsiveness of maize against various combinations of synthetic fertilizers and organic bio-stimulant named Plant Protector™, research was conducted with the objectives of comparing synthetic fertilizer with bio-stimulant, estimating the doses of bio-stimulant and synthetic fertilizer for yield optimization, and evaluating the most responsive maize genotypes against the best combination of fertilizers for future breeding programs.

Materials and Methods

Experimental material: The research was performed at Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (31.4336 latitude and 73.0683 longitude) during the spring seasons of year 2015 and 2016. The meteorological data of both the seasons including minimum, maximum and average temperature along with humidity and precipitation is given in (Fig. 1). The experimental material comprised of 11 commercial maize hybrids of distant origin (P-1543, TG-4265, TG-4557, TG-4560, TG-46B90, 15BJSF6, DK-6103, FH-985, FH-988, FH-963 and FH-1046). The genotypes were sown using split-split plot under randomized complete block design. Distances among rows and plants were maintained 75 cm and 20 cm respectively. Recommended agronomic and cultural practices were carried out throughout the entire study to ensure the provision of same environment to all the experimental material.

Treatments and parameters: Total eight different fertilizer treatments (T1-T8) were applied to the genotypes under study. Control was taken as "T1". Application of bio-stimulant at 5 leaf stage was "T2", application of bio-stimulant at 7-leaf stage was "T3", and application of bio-stimulant at 9-leaf stage was taken as "T4". Whereas, NPK application with the ratio of 120:60:60 was taken as "T5". On the other hand, NPK application with ratio of 60:30:30 along with bio-stimulant at 5- leaf stage was "T6", NPK application following 60:30:30 along with bio-stimulant at 7-leaf stage was "T7", and NPK of 60:30:30 along with bio-stimulant at 9-leaf stage was taken as "T8". Plant Protector™ (bio-stimulant) was applied as foliar spray at 5, 7, and 9-leaf stages of the crop when average numbers of leaves per plant including cotyledonary leaf were 5, 7 and 9, respectively. Plant Protector™ is actually an organic bio-stimulant having main constituent as poly-potassium benzoic acid that was applied with the rate of 100 ml per 20 liters of water. One application per treatment during year 2015 and two applications per treatment during 2016 were applied, while synthetic fertilizer was applied following general recommendations. Normal and ½ of synthetic fertilizer was applied as a ratio of 120:60:60 and 60:30:30 Kg ha^{-1} of Nitrogen (N), Phosphorus (P) and Potassium (K) respectively. The studied parameters were morphological i.e., plant height (cm), ear height (cm), cobs per plant, leaves per plant, stem diameter (cm), days to tassel, days to silk; physiological i.e., leaf temperature (ºC) and chlorophyll contents (atLEAF value); and yield contributing traits i.e., cob length (cm), cob diameter (cm), cob weight (g), number of grain rows per cob, grains per row, grains per cob and 100 grain weight (g). The process of data collection began two weeks after the application of all the treatments.

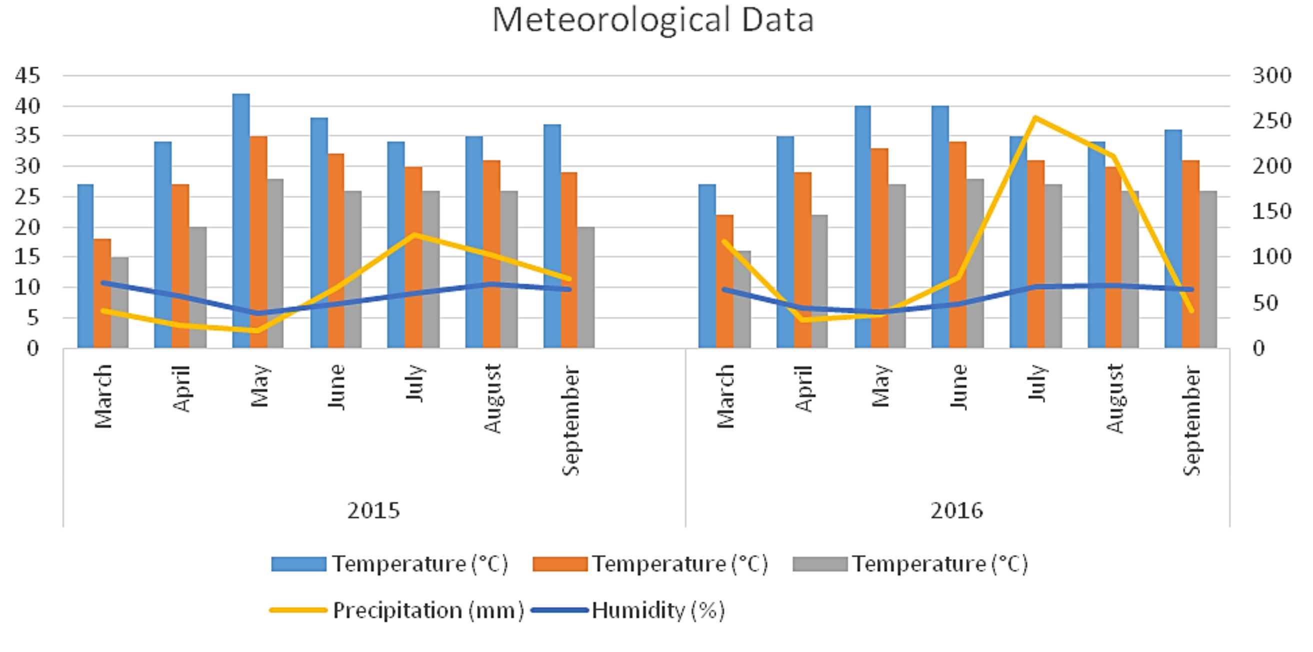

Fig. 1. Meteorological data of University of Agriculture (Faisalabad, Pakistan).

Statistical analyses

Analysis of variance for all the parameters was carried out as given by Steel *et al*., (1997) using Statistix 8.1 software to estimate the level of differences among the genotypes studied and treatments applied. Means of all the genotypes and treatments were compared separately through Bonferroni test as given by Dunn (1961) using Statistix 8.1 software in order to estimate the most and least responsive genotypes and treatments for each trait. Percent changes in all the studied parameters were also compared using Microsoft Excel in order to conclude maximum incline and decline in the traits under study after the application of treatments. Firstly, % change was calculated for all the genotypes under each treatment by the following formula:

$$\text{\% Change for genotype} = \frac{\text{Mean of a treatment} - \text{Mean of control}}{\text{Mean of control}} \times 100$$

Then % change was calculated for each treatment by computing average of the % changes of all the genotypes under that specific treatment to measure the overall change caused by a certain treatment.

$$\text{\% Change for treatment} = \frac{\text{Sum of \% change of all genotypes under a treatment}}{\text{Number of genotypes under that treatment}} \times 100$$

The data was prepared by pooling the information from all the replications and both seasons to make a single value for each entry. Then diagrammatical representation of the complete performance, and trend of responses of all the genotypes under all the treatments for each trait was done by Contour Plot Analysis (Watson, 1992) using Minitab-17 software (developed by Pennsylvania State University) in order to find out the best treatment for the best genotype under a specific trait. Further, AMMI biplot analysis (Gaugh, 1988) was done using Genstat software (developed by Rothamsted Research) for yield related traits to find out the genotype-environmental interactions of the study.

Results and Discussion

Morphological parameters: Analysis of variance revealed significant variation ($p<0.01$) in the studied genotypes, treatments and their interactions for all the studied parameters except days to silk and days to tassel where only genotypes were significantly different (Table 1). Year-treatment interactions were also significant for leaves per plant. Contour plots showed an increment in plant height after bio-stimulant application of at 7-leaf stage (185.92 cm) and 9-leaf stage (187.71 cm) of the crop (T2 and T4 respectively) as compared to control (182.02cm) or recommended inorganic fertilizer (176.16 cm) that could be the result of improved nutrient use efficiency (Fig. 2). Same work done by Shah *et al*., (2009) and Haggag *et al*., (2014) who also noticed increase in height and leaves of maize and olive genotypes respectively due to improved nitrogen use efficiency after the application of certain bio-stimulant. Even higher plant height was observed (195.75 cm) after application of Plant Protector™ along with half of recommended synthetic fertilizer i.e., 60:30:30 of NPK (T7). Same was the case with leaves per plant as T2 and T4 produced more leaves (12.48 and 12.01 respectively) compared to control (11.39), but this increase was not enough as compared to the findings of T5 (13.36). Significant increase as compared to recommended dose was observed after the application of T7 (14.45). Cobs per plant were maximum (2.09) under T5 contrary to Shah *et al*., (2009). An average increase of 5.75 and 30.02 percent in plant height and leaves per plant respectively, and decrease of 80.30 percent in cobs per plants was observed compared to control (Table 2). Increment in plant height and leaves is a good indication of increased plant biomass that can be used for silage purposes. In case of stem diameter, there was no significant impact of any treatment, but contour plot showed that average stem diameter under T5 was a bit higher than other treatments. This suggested that under recommended synthetic fertilizer application, plants utilized most of their nutrients in stems and produced more than one cobs, thereby, reducing biomass production (Fig. 2). Ear height, days to silk and days to tassel were not affected satisfactorily with variable treatments application.

Physiological parameters: As Plant Protector™ was applied as foliar spray on the leaf surface, a significant increment in leaf chlorophyll contents was noticed after the application of bio-stimulant (Table 1). However, maximum values were observed under T7 (92.60) compared to control i.e., T1 (80.03) and recommended synthetic fertilizer i.e., T5 (80.80), shown by contour plot (Fig. 3). These findings were in accordance with the studies previously conducted by other researchers (Ertani *et al*., 2010; Kazemi, 2014; and Lucini *et al*., 2015) who noticed increased photosynthetic activity in terms of leaf chlorophyll contents in bio-stimulant applied plants. A significant mitigation in the environmental impact in terms of heat was also observed by a decrease in leaf temperature of the genotypes treated with bio-stimulant as found in earlier studies (Azimi *et al*., 2013; Moghadam *et al*., 2014; and Du-Jardin, 2015) where mitigation of heat and drought stresses was noticed. Least leaf temperature was noticed under T7 (25.34ºC) whereas most temperature was noticed under T5 (32.92ºC) suggesting that Plant Protector™ increased transpiration rates in the plants that resulted in decrease in leaf temperature. This might have improved the root structure and brought about increased nutrient and water uptake by the plant. Year-treatment interactions were also significant in addition to treatment, genotype and treatment-genotype interactions suggesting that there was a significant role of increased bio-stimulant application (second year) in reduction of leaf temperature (Table 1). Average decrease of 12.88 percent in leaf temperature and increase of 17.02 percent in chlorophyll contents were observed compared to control (Table 2).

Table 1. Split-split plot analysis of variance under RCBD.

Source	DF	PH	EH	CPP	LPP	SD	DT	DS	LT
Replication	1	11.28	156.89	0.1	0	0.01	1.37	19.57	123.32
Year	1	0.07	101.48	0.18	121.73	0.08	1.92	10.57	107.61
Error R*Y	1	11.28	30.14	0	1.78	1.40E-04	1.14	1.25	0.04
Treatment	7	1724.25**	403.50**	3.29**	60.39**	0.37**	19.65	14.76	215.70**
Y*T	7	41.83	78.42	0.09	11.96**	0.32	1.26	6.35	11.06**
Error R*Y*T	14	144.77	34.12	0.17	1.85	0.11	6.07	11.7	1.84
Genotype	10	1020.88**	5619.55**	1.86**	21.20**	0.85**	264.35**	360.72**	11.48**
Y*G	10	1.11	32.5	0.25	0.55	2.50E-32	0.15	0.06	0.09
T*G	70	725.59**	319.68**	0.38**	5.25**	0.15**	3.55	3.43	4.56**
Y*T*G	70	1.24	28.23	0.14	0.44	2.10E-32	0.25	0.35	0.21
Error R*Y*T*G	160	79.08	70.41	0.17	1.22	0.03	1.76	2.87	0.72
Total	351								
Source	**DF**	**CC**	**CL**	**CD**	**CW**	**RPC**	**GPR**	**GPC**	**100GW**
Replication	1	2.84	7.02	0.87	36795.9	0.74	236.64	24734	223.97
Year	1	386.4	184.44	0.79	5729.2	5.09	74.83	16206	161.06
Error R*Y	1	0.03	0.39	0	30.8	0	3.15	42	0.07
Treatment	7	1985.65**	81.72**	1.73**	45517.1**	11.58**	388.09**	131535**	188.18**
Y*T	7	30.92	17.48	0.21	820.9	0.31	22.1	1334	24.09
Error R*Y*T	14	115.71	7.32	0.09	2372.3	0.99	18.45	4232	36.26
Genotype	10	626.70**	40.37**	1.43**	32081.2**	23.61**	452.85**	164121**	168.22**
Y*G	10	0.42	0.09	0.01	36.5	0.02	0.59	5	0.21
T*G	70	314.46**	7.28**	0.13**	3516.1**	2.28**	37.83**	10942**	70.15**
Y*T*G	70	0.24	0.09	0.01	37	0.02	0.58	4	0.22
Error R*Y*T*G	160	52.34	2.38	0.06	1605	1.04	24.08	6100	26.23
Total	351								

PH: Plant height; **EH**: Ear height; **CPP**: Cobs per plant; **LPP**: Leaves per plant; **SD**: Stem diameter; **DT**: Days to tassel; **DS**: Days to silk; **LT**: Leaf temperature; **CC**: Chlorophyll contents; **CL**: Cob length; **CD**: Cob diameter; **CW**: Cob weight; **RPC**: Rows per cob; **GPR**: Grains per row; **GPC**; Grains per cob; **100GW**: 100 Grain weight

Table 2. Percent change in studied traits as compared to control (T1).

Traits		Treatments						
		T2	T3	T4	T5	T6	T7	T8
PH	Season 1	-0.29 (5)	-2.64 (6)	2.04 (3)	-3.75 (7)	**5.33 (1)**	5.13 (2)	1.23 (4)
	Season 2	-1.73 (5)	-3.77 (7)	2.87 (3)	-2.52 (6)	**6.16 (1)**	5.95 (2)	0.13 (4)
EH	Season 1	0.32 (7)	0.87 (6)	6.16 (3)	1.45 (5)	8.57 (2)	**8.76 (1)**	1.95 (4)
	Season 2	4.16 (5)	4.02 (6)	6.16 (3)	6.02 (4)	8.76 (2)	**10.88 (1)**	-1.15 (7)
CPP	Season 1	33.33 (2)	10.61 (5)	21.21 (4)	**89.39 (1)**	10.61 (5)	33.33 (2)	25.76 (3)
	Season 2	21.21 (4)	19.70 (5)	13.64 (7)	**71.21 (1)**	15.15 (6)	28.79 (3)	33.33 (2)
LPP	Season 1	1.47 (5)	-10.18 (7)	-1.01 (6)	13.07 (2)	5.95 (4)	**13.22 (1)**	8.20 (3)
	Season 2	21.87 (5)	6.89 (7)	13.26 (6)	26.96 (4)	32.05 (2)	**46.82 (1)**	30.74 (3)
SD	Season 1	1.53 (4)	-1.86 (6)	1.36 (5)	2.82 (2)	**4.94 (1)**	-2.44 (7)	2.70 (3)
	Season 2	-4.67 (3)	-10.37 (4)	1.78 (2)	**8.52 (1)**	-11.76 (5)	-16.28 (6)	-20.48 (7)
DT	Season 1	0.40 (2)	1.01 (1)	-0.94 (5)	-1.79 (6)	0.21 (3)	-2.23 (7)	0.03 (4)
	Season 2	1.67 (2)	2.04 (1)	0.24 (4)	-1.31 (6)	-0.34 (5)	-1.81 (7)	0.54 (3)
DS	Season 1	-0.01 (1)	-0.09 (2)	-1.55 (5)	-2.20 (7)	-0.09 (3)	-2.00 (6)	-0.24 (4)
	Season 2	-2.01 (4)	-0.95 (2)	-2.26 (5)	-0.75 (1)	-1.16 (3)	-4.08 (7)	-2.60 (6)
LT	Season 1	-3.78 (6)	-1.45 (4)	1.92 (3)	**12.22 (1)**	-1.46 (5)	-8.98 (7)	4.83 (2)
	Season 2	-9.81 (6)	-7.51 (5)	-4.04 (3)	**14.39 (1)**	-7.48 (4)	-16.79 (7)	-0.80 (2)
CC	Season 1	-5.09 (7)	10.34 (3)	**14.56 (1)**	2.26 (5)	-4.64 (6)	14.39 (2)	7.40 (4)
	Season 2	-0.45 (7)	15.15 (3)	**19.48 (1)**	2.57 (5)	0.02 (6)	19.22 (2)	12.21 (4)
CL	Season 1	11.95 (3)	0.44 (7)	4.54 (6)	12.23 (2)	11.63 (4)	**13.23 (1)**	11.08 (5)
	Season 2	25.53 (3)	13.25 (7)	16.48 (6)	16.88 (5)	25.76 (2)	**39.36 (1)**	25.23 (4)
CD	Season 1	5.96 (5)	2.15 (7)	5.82 (6)	10.00 (3)	**10.33 (1)**	10.10 (2)	9.88 (4)
	Season 2	11.80 (4)	7.93 (7)	11.71 (5)	10.61 (6)	16.34 (2)	**20.87 (1)**	15.88 (3)
CW	Season 1	38.12 (5)	7.99 (7)	26.64 (6)	**70.37 (1)**	51.47 (4)	64.19 (2)	62.21 (3)
	Season 2	50.52 (5)	18.71 (7)	38.16 (6)	66.80 (3)	62.95 (4)	**82.87 (1)**	73.22 (2)
RPC	Season 1	-1.08 (2)	-13.07 (6)	**1.30 (1)**	-2.47 (3)	-3.19 (4)	-14.77 (7)	-4.26 (5)
	Season 2	10.38 (2)	2.84 (7)	**10.57 (1)**	5.15 (5)	8.00 (3)	3.02 (6)	7.52 (4)
GPR	Season 1	16.96 (5)	4.96 (7)	9.26 (6)	**29.12 (1)**	17.14 (4)	25.77 (2)	24.35 (3)
	Season 2	24.42 (5)	12.41 (7)	16.69 (6)	26.36 (3)	24.62 (4)	**39.62 (1)**	32.08 (2)
GPC	Season 1	18.17 (5)	5.21 (7)	17.76 (6)	**37.39 (1)**	23.67 (4)	36.46 (2)	30.72 (3)
	Season 2	23.26 (5)	10.20 (7)	22.86 (6)	37.66 (2)	28.83 (4)	**44.83 (1)**	35.93 (3)
100GW	Season 1	12.19 (4)	3.79 (7)	8.84 (6)	16.14 (2)	12.15 (5)	**16.68 (1)**	14.47 (3)
	Season 2	20.32 (4)	11.80 (7)	16.74 (5)	14.77 (6)	21.67 (3)	**30.52 (1)**	22.56 (2)

PH: Plant height; **EH**: Ear height; **CPP**: Cobs per plant; **LPP**: Leaves per plant; **SD**: Stem diameter; **DT**: Days to tassel; **DS**: Days to silk; **LT**: Leaf temperature; **CC**: Chlorophyll contents; **CL**: Cob length; **CD**: Cob diameter; **CW**: Cob weight; **RPC**: Rows per cob; **GPR**: Grains per row; **GPC**; Grains per cob; **100GW**: 100 Grain weight

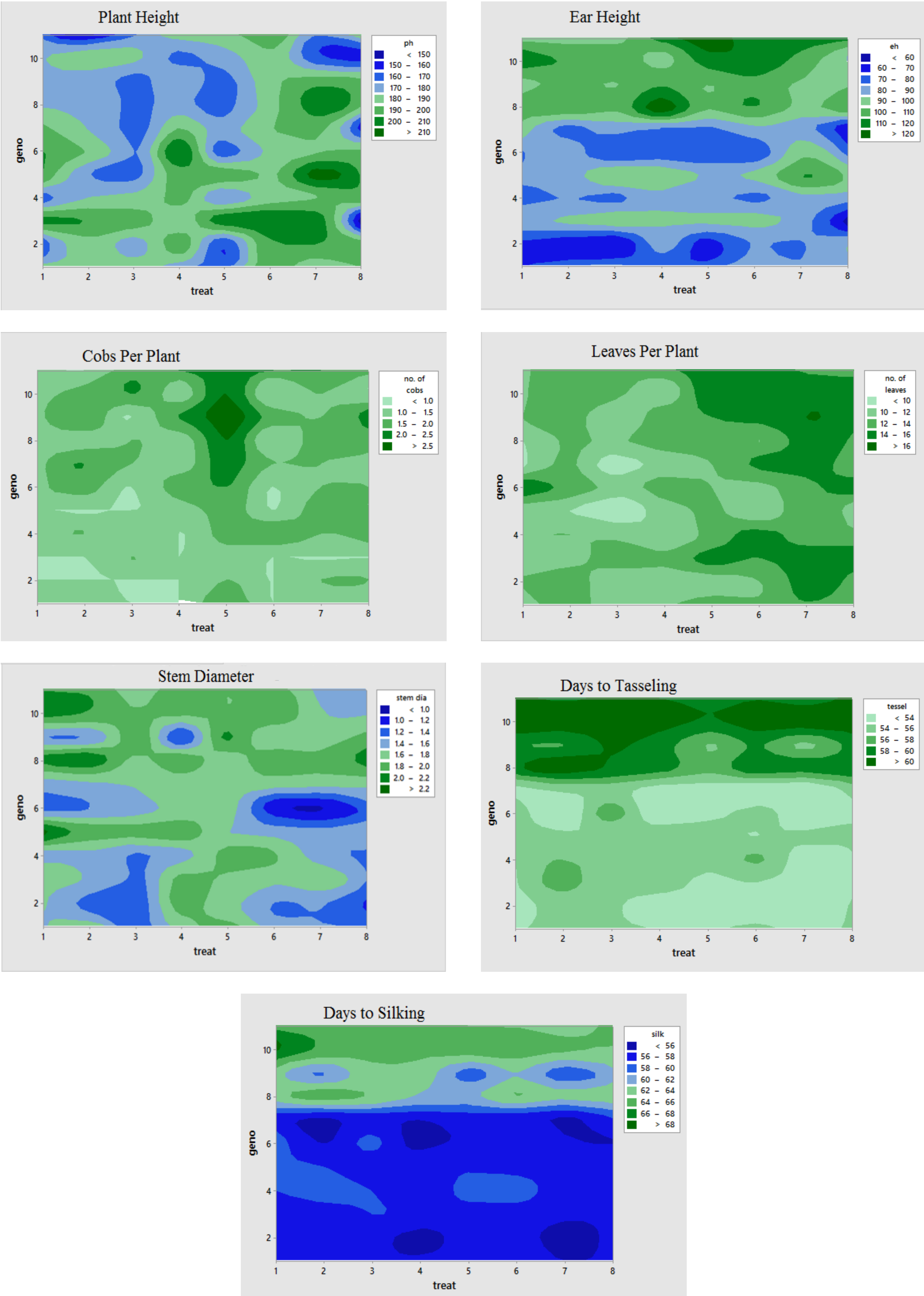

Fig. 2. Contour plots for morphological parameters.

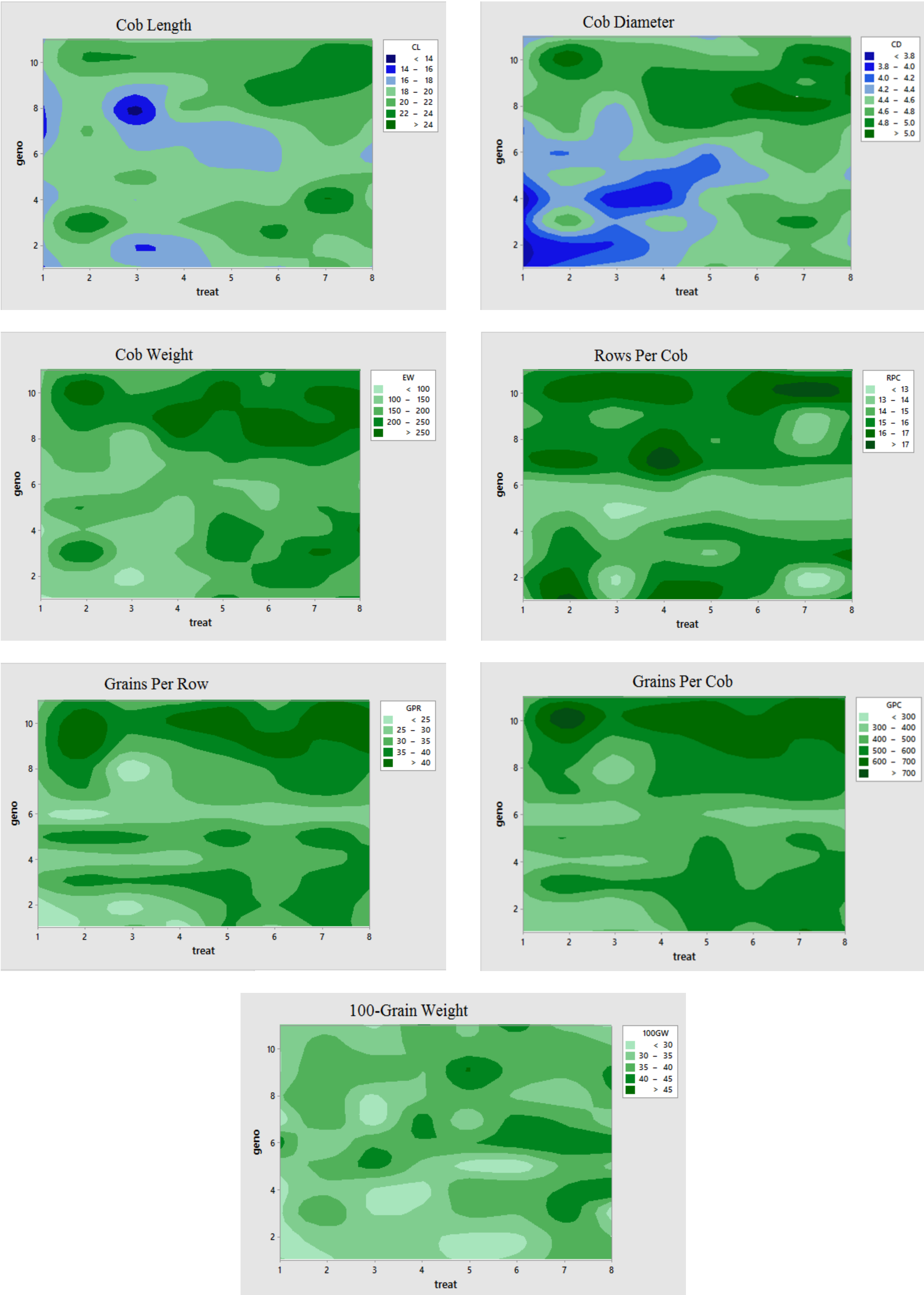

Fig. 4. Contour plots for yield contributing parameters.

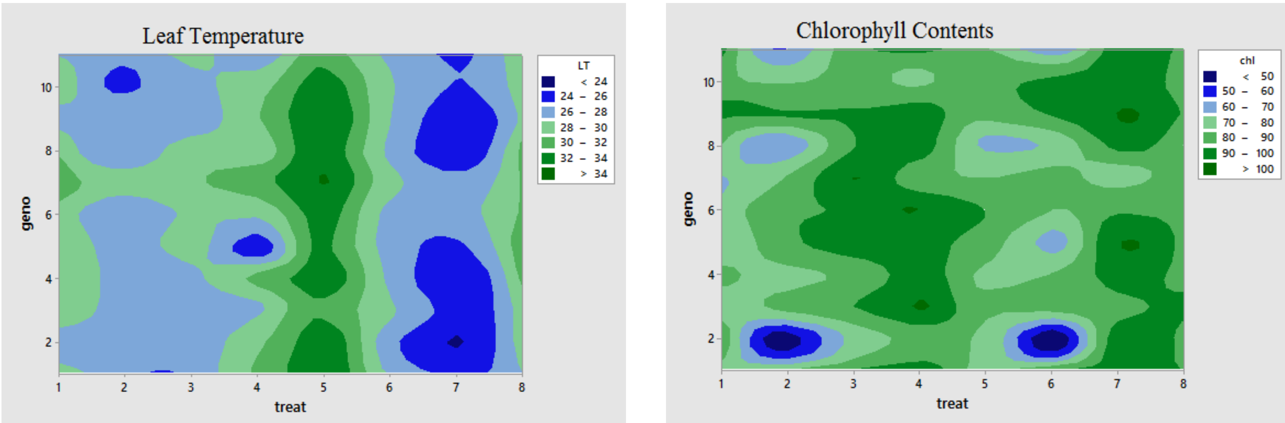

Fig. 3. Contour plots for physiological parameters.

Yield contributing parameters: More or less, similar results were observed in yield related traits i.e., cob diameter, cob length, cob weight, rows per cob, grains per row, grains per cob and 100 grain weight, and significant mean squares for treatments, genotypes and interactions were noticed for all the traits under study (Table 1). Again extreme values for yield contributing traits were observed under T7 except rows per cob, which were maximum in T4 (Fig. 4). Further analysis of grains per row, grains per cob, cob weight and 100 grain weight by AMMI biplot revealed that T7 produced highest yield than other treatments (Fig. 5). On the other hand, AMMI biplot also suggested that genotypes FH-963, FH-985 and FH-988 proved as the highest yielding of all the studied genotypes. There findings were similar to the studies of the researchers who sustained the productivity of crops by reducing the application of synthetic nutrients by supplementing them with natural ones, and came up with similar findings (Braccini *et al.*, 2012; Ahmad *et al.*, 2013; and Delkhoshi & Jalilian, 2012). Average increase of 15.48, 26.30, 73.53, 5.94, 32.70, 40.64 and 23.60 percent was observed under cob diameter, cob length, cob weight, rows per cob, grains per row, grains per cob and 100 grain weight respectively compared to control (Table 2).

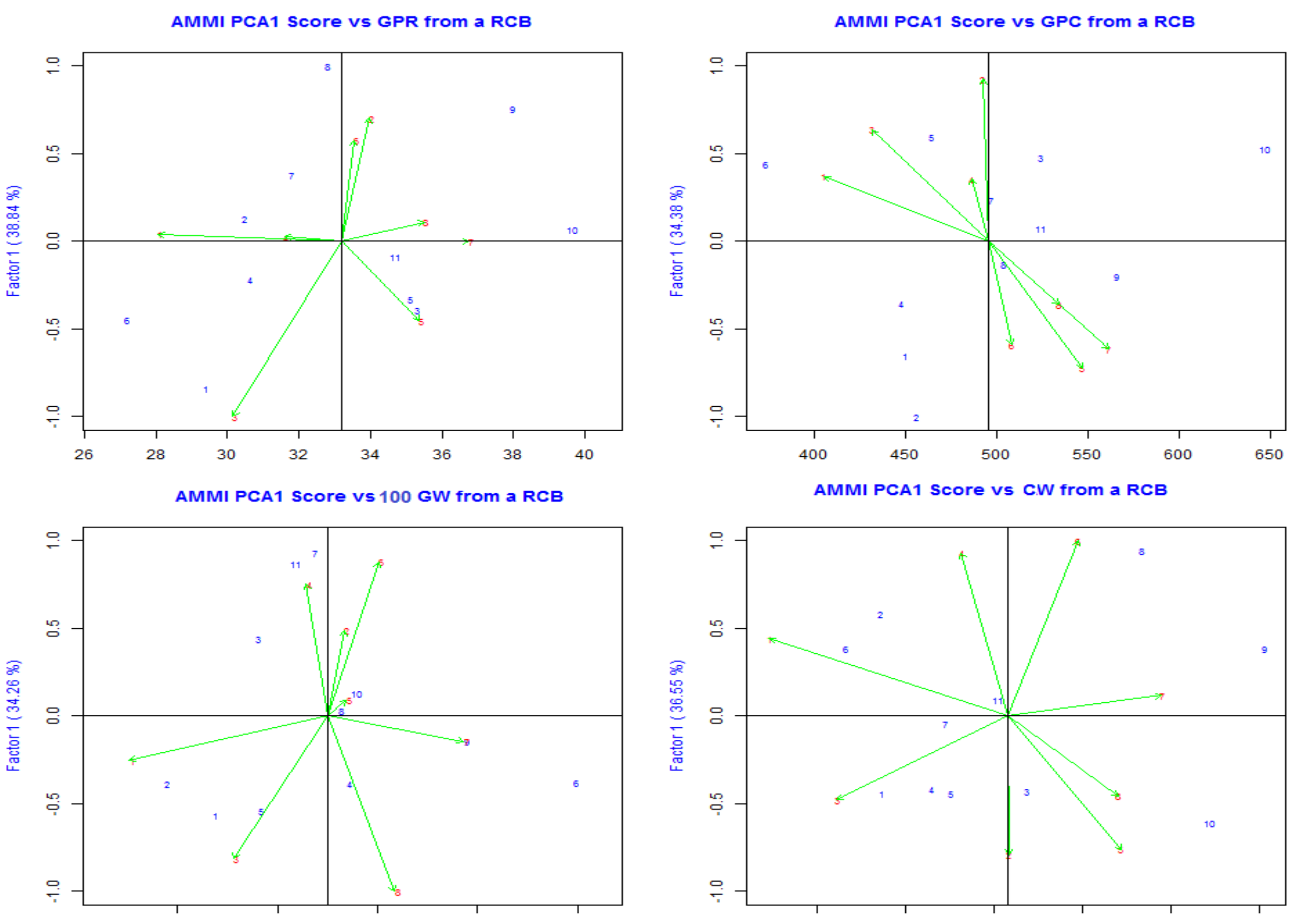

Fig. 5.AMMI PCA1 scores of GPR (Grains per Row), GPC (Grains per Cob), 100 GW (100 Grain Weight) and CW (Cob Weight).

Conclusion

From the above study, it was found that bio-stimulant (Plant Protector™) increased the yield by reducing leaf temperature and increasing plant biomass and chlorophyll contents of the studied genotypes compared to control. It means that increased plant biomass and chlorophyll contents along with decrease in stem diameter and leaf temperature are essential parameters for increase in the grain productivity of maize and, therefore, can be used as selection standards against breeding for higher yields. Maximum yield was obtained when Plant Protector™ was applied at 7-leaf stage of the crop with 60:30:30 of NPK fertilizer. It suggested that most critical stage of the maize crop is 7 leaf stage at which it is most responsive to the nutrient applications. Hence application of right nutrients i.e., 60:30:30 Kg/ha of NPK plus bio-stimulant (Plant Protector™) at right time (7-leaf stage) not only sustains the production but can also increase it while minimizing environmental impact on the other hand. Finally, FH-963, FH-985 and FH-988 were high yielding genotypes to the best combination of fertilizers compared to all other studied genotypes proving their worth to be used to maximize the yield.

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