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Biostimulants based nutrient management on growth and yield of spring maize (*Zea mays*) under legume based cropping sequence

B R PRAVEEN[1,2], MAGAN SINGH[2]*, R T CHETHAN BABU[3], SANJEEV KUMAR[2], RUXANABI NARAGUND[4], K S SACHIN[5], PRASANNA S PYATI[6], RAKESH KUMAR[2] and KASHINATH GURUSIDDAPPA TELI[7]

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001, India

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ABSTRACT

The study was carried out during 2020–21 and 2021–22 at ICAR-National Dairy Research Institute, Karnal, Haryana to evaluate biostimulants based nutrient management practices in spring maize (*Zea mays* L.) under legume based cropping sequence. Experiment was conducted in a randomized block design (RBD) with 9 treatments of biostimulant based nutrient management, viz. T_1, Absolute control; T_2, 100% RDF (recommended dose of fertilizer); T_3, 75% RDF + *Azotobacter*; T_4, 50% RDF + *Azotobacter* + PGPR (Plant growth promoting rhizobacteria); T_5, 75% RDF + *Azotobacter* + PGPR; T_6, 50% RDF + *Azotobacter* + PGPR + Humic acid (HA); T_7, 75% RDF + *Azotobacter* + PGPR + HA; T_8, 50% RDF + *Azotobacter* + PGPR + HA + Seaweed extract (SWE); and T_9, 75% RDF + *Azotobacter* + PGPR + HA + SWE, replicated thrice. Results showed that the growth parameters, viz. plant height, leaf length, leaf width and number of leaves/plant had no significant response at 30 DAS (days after sowing) during both the studied year. However, at 60 DAS, these parameters were significantly higher in 100% RDF which was statistically on par with 75% RDF + *Azotobacter* + PGPR + Humic acid (HA) + Seaweed extract (SWE) and 75% RDF + *Azotobacter* + PGPR + HA. Whereas at harvest, growth attributes were significantly higher in 75% RDF + *Azotobacter* + PGPR + HA + SWE which was statistically on par with RDF + *Azotobacter* + PGPR + HA and 100% RDF. Similarly, grain (7.81 and 8.00 t/ha), stover (12.18 and 12.48 t/ha) and biological yield (22.47 and 22.92 t/ha) were significantly higher in 75% RDF + *Azotobacter* + PGPR + HA + SWE which was statistically on par to RDF + *Azotobacter* + PGPR + HA and 100% RDF during 2020–21 and 2021–22, respectively. Hence, the treatment 75% RDF + *Azotobacter* + PGPR + HA + SWE found better and can replace up to 25% RDF as comparable to conventional practice without compromising the crop yield.

Keywords: Biostimulants, Biological yield, Humic acid, Seaweed extract, Spring maize

Maize (*Zea mays* L.) is the most adaptable and high yield potential crop grows in a variety of agro-climatic situations (Chaudhary *et al.* 2012). It is generally grown during rainy (*kharif*) season across the country. However, it is grown as a spring or summer crop in north-western parts of India particularly in Haryana, Punjab and western Uttar Pradesh. Introducing legume into cereal based cropping sequence enhance the productivity of cereals as legume is a field-based mini-nitrogen manufacturer. Legume aids in promoting microbial activity, replenishing organic matter and solubilizing insoluble nutrients in the soil. In particular, fodder legumes are typically more effective at raising the yield of cereals (Tufail *et al.* 2018). Unlimited and imbalance use of chemical fertilizers deteriorated the soil health, crop productivity and food quality and amplified the water and soil pollution over the period (Chakraborti and Singh 2004). In respect, organic and inorganic nutrient management together is the effective approach towards minimizing the risk of chemical hazards.

Among biostimulants, humic acid (HA) positively affects the crop productivity, root growth and soil quality upon foliar or soil application along with improving physico-chemical and biological attribute of the soil (Jardin 2015). The next emerging biostimulant is seaweed extract (SWE), which is a purified compound of fresh seaweeds and their polysaccharides that partially replace the need for artificial fertilizers if used in appropriate quantities (Craigie 2011,

[1]Tea Research Association, North Bengal Regional Research and Development Centre, Nagrakata, Jalpaiguri, West Bengal; [2]ICAR-National Dairy Research Institute, Karnal, Haryana; [3]S. V. Agricultural College (Acharya N. G. Ranga Agricultural University, Guntur, Andhra Pradesh), Tirupati, Andhra Pradesh; [4]University of Horticultural Sciences, Bagalkot, Karnataka; [5]The Rubber Board of India, Kolasib, Mizoram; [6]Tocklai Tea Research Institute, Tea Research Association, Jorhat, Assam; [7]ICAR-Indian Agricultural Research Institute, Hazaribagh, Jharkhand. *Corresponding author email: magansingh07@gmail.com

Hernandez-Herrera *et al.* 2014). Biofertilizers are another category of biostimulants that contains living cells of various microbes colonizes the rhizosphere and stimulate growth by transforming unavailable mineral nutrients to available form through a biological process (Rokhzadi *et al.* 2008). Rhizosphere bacteria namely *Acetobacter*, *Achromobacter*, *Agrobacterium*, *Azotobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Rhizobium* etc. can saturate insoluble nutrients and helps in their availability to plants which are together referred as plant growth promoting rhizobacteria (PGPR). Particularly *Azotobacter* which is not only helpful for biological nitrogen fixation in cereal crops but also releases plant hormones namely naphthalene acetic acid and gibberellins and vitamin-B complex that act as root growth promoters by inhibiting specific root-acting pathogens (Mathivanan *et al.* 2015). Keeping these points in view, an experiment was planned to study the effect of biostimulants based nutrient management on growth and yield of spring maize under legume based cropping sequence.

MATERIALS AND METHODS

Experimental site: The study was carried out during 2020–21 and 2021–22 at ICAR-National Dairy Research Institute, Karnal (Latitude of 29°43' N and Longitude of 76°58' E; 245 m amsl) Haryana. The experimental field found to be clay loam in texture with 44.32% sand, 20.97% silt and 34.71% clay. Medium organic carbon, low available nitrogen, medium available phosphorus and high available potassium with neutral to alkaline in reaction were documented from the experimental field.

Experimental design, treatments and crop management: The experiment was conducted under randomized block design (RBD) with three replications and nine treatments of biostimulant based nutrient management, viz. T_1, Absolute control; T_2, 100% RDF (recommended dose of fertilizer); T_3, 75% RDF + *Azotobacter*; T_4, 50% RDF + *Azotobacter* + PGPR (Plant growth promoting rhizobacteria); T_5, 75% RDF + *Azotobacter* + PGPR; T_6, 50% RDF + *Azotobacter* + PGPR + Humic acid (HA); T_7, 75% RDF + *Azotobacter* + PGPR + HA; T_8, 50% RDF + *Azotobacter* + PGPR + HA + Seaweed extract (SWE); and T_9, 75% RDF + *Azotobacter* + PGPR + HA + SWE. The maize hybrid Dekalb 9108 Plus was sown in late spring season after third cut of berseem using seed rate of 20 kg/ha with 60 cm × 25 cm spacing. The seeds were treated with *Azotobacter* and PGPR at the rate of 50 ml/10 kg seeds or 125 ml/ha as per treatment specifications before sowing. The fertilizers were used at the rate of 120:60:30:25 kg/ha ($N:P_2O_5:K_2O:ZnSO_4$) through urea, Diammonium phosphate (DAP), Muriate of potash (MOP) and $ZnSO_4$. Monohydrate as per the requirement being 1/3rd of N and full of P, K and $ZnSO_4$ were basally applied, 1/3rd N was top-dressed at knee high stage and remaining 1/3rd at pre-tasselling stage. Two sprays of humic acid and seaweed extract at the rate of 3 ml/litre and 2 ml/litre of water, respectively were given at knee high and pre-tasselling stage as per the treatments. Pre-emergent application of Atrazine @1.0 kg a.i./ha by 2–3 days after sowing and post-emergent application of Tembotrione 42% SC @115 ml a.i./acre at 25–30 DAS were done to maintain weed free condition. Around eight irrigations were given in total from which first at soon after germination and later during critical growth stages at an interval of 10–12 days.

Measurement of growth attributes and yield: The plant height, leaf length, leaf width and number of leaves/plant were recorded from 10 randomly selected plants of maize at 30, 60 DAS and harvest and then worked out the average. After harvesting of cobs from each of net plots, the cobs were allowed to dry for few days in the yard to reduce moisture content and facilitate shelling process. Immediately after shelling of cobs, the grains were dried to get the moisture content of around 14% and the grain yield from each treatment was recorded and expressed in t/ha. Thereafter, maize stover was harvested and the weight of stover from each net plot was recorded after few days of sun drying and expressed in t/ha. Similarly, the biological yield was recorded by adding grain and stover yield from each treatment along with weight of shelled cobs after shelling.

Statistical analysis: Experimental data were processed in Microsoft Excel-2019 and analyzed by using Analysis of Variance (ANOVA) technique as per randomized block design (Gomez and Gomez 1984). The significance of the treatments was tested using an F test with a 5% level of significance ($P \leq 0.05$) and means were compared using the least significant difference (LSD) test at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Plant height: Biostimulants based nutrient management had no effect on plant height significantly at 30 DAS but influenced significantly at 60 DAS and harvest stages during both the studied years (Table 1). Plant height was significantly higher in T_2 (187.1 and 190.3 cm) at 60 DAS over remaining treatments except T_9, T_7 and T_5 where in it showed on par results during both 2020–21 and 2021–22, respectively. However at harvest, it was significantly higher in T_9 (242.7 and 246.8 cm, respectively) which was on par with T_2 and T_7 over remaining treatments during successive years.

Leaf length: Results revealed that the leaf length was influenced by various treatments of biostimulants based nutrient management significantly during both the studied years at 60 DAS and harvest stage (Table 1). However, no significant effect was observed at 30 DAS. Leaf length was significantly higher in T_2 (92.42 and 93.46 cm) over remaining treatments except T_9, T_7 and T_5 where in it showed on par results at 60 DAS during 2020–21 and 2021–22, respectively. However at harvest stage, it was significantly higher in T_9 (107.86 and 109.56 cm) which was on par with T_2 and T_7 over other treatments of interest during 2020–21 and 2021–22, respectively.

Leaf width: Similar to leaf length, leaf width influenced significantly by biostimulants based nutrient management during both 2020–21 and 2021–22 at 60 DAS and harvest stage with no significant difference at 30 DAS (Table 1). The leaf width was significantly higher in T_2 (10.25 and 10.32 cm) over remaining treatments except T_9, T_7, T_5 and T_3

Table 1 Effect of biostimulants based nutrient management on plant height, leaf length and leaf width of spring maize

Treatment	Plant height (cm)						Leaf length (cm)						Leaf width (cm)					
	30 DAS		60 DAS		At harvest		30 DAS		60 DAS		At harvest		30 DAS		60 DAS		At harvest	
	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22
T_1	49.7	47.7	128.6	122.7	167.8	163.3	36.32	35.62	70.85	68.69	78.35	77.69	5.31	5.42	6.59	6.48	7.65	7.45
T_2	65.3	65.2	187.1	190.3	231.4	235.4	51.37	50.23	92.42	93.46	104.75	104.67	7.18	7.25	10.25	10.32	11.86	11.89
T_3	55.5	54.4	163.4	165.3	201.7	203.8	45.56	44.38	81.28	81.95	90.72	91.34	6.08	6.05	8.66	8.65	9.86	9.71
T_4	53.2	54.6	148.7	149.3	175.9	176.9	42.53	42.86	76.59	77.62	82.29	84.65	5.53	5.45	7.39	7.35	8.11	8.04
T_5	58.2	59.0	168.5	170.3	209.3	212.3	46.55	47.01	82.13	82.31	93.28	93.95	6.17	6.25	8.72	8.86	10.04	10.16
T_6	53.5	54.1	156.4	157.3	189.7	191.4	42.76	43.13	79.36	82.05	87.96	88.96	5.47	5.62	7.42	7.48	8.53	8.71
T_7	58.3	58.1	176.3	179.6	225.7	228.2	46.72	47.62	84.67	86.48	102.74	103.45	6.35	6.32	9.15	9.23	11.59	11.68
T_8	54.9	55.2	160.8	161.8	200.5	203.4	43.22	43.68	80.76	81.71	92.42	94.26	5.62	5.76	7.68	7.79	8.96	9.46
T_9	58.7	59.3	181.8	183.7	242.7	246.8	46.46	47.09	87.78	88.74	107.86	109.56	6.22	6.38	9.39	9.45	12.28	12.39
SEm ±	2.96	3.31	7.01	7.33	9.61	10.22	2.75	2.75	3.58	3.75	4.33	4.71	0.42	0.42	0.6	0.61	0.64	0.58
LSD ($P \leq 0.05$)	NS	NS	21.01	21.96	28.81	30.64	NS	NS	10.74	11.24	12.99	14.13	NS	NS	1.79	1.84	1.93	1.74

DAS, Days after sowing. T_1, Absolute control; T_2, 100% RDF (recommended dose of fertilizer); T_3, 75% RDF + *Azotobacter*; T_4, 50% RDF + *Azotobacter* + PGPR (Plant growth promoting rhizobacteria); T_5, 75% RDF + *Azotobacter* + PGPR; T_6, 50% RDF + *Azotobacter* + PGPR + Humic acid (HA); T_7, 75% RDF + *Azotobacter* + PGPR + HA; T_8, 50% RDF + *Azotobacter* + PGPR + HA + Seaweed extract (SWE); T_9, 75% RDF + *Azotobacter* + PGPR + HA + SWE.

where in it showed on par results at 60 DAS during 2020–21 and 2021–22, respectively. However at harvest stage, it was higher in T_9 (12.28 and 12.39 cm) significantly which was on par to T_2 and T_7 over other treatments of interest.

Number of leaves/plant: Results (Table 2) revealed that no significant effect was observed in the number of leaves/plant of maize at 30 DAS. However, significant dissimilarity was recorded at 60 DAS and harvest stage by biostimulant based nutrient management during both the studied years. The treatment T_2 (15.32 and 15.52) witnessed higher number of leaves significantly and found on par results with T_9, T_7, T_5 and T_3 at 60 DAS over remaining treatments during 2020–21 and 2021–22, respectively. However, the number of leaves were significantly higher in T_9 (17.38 and 17.59) at harvest which were on par with T_2 and T_7 over remaining treatments during 2020–21 and 2021–22, respectively.

Grain yield: The grain yield data (Table 2) revealed a significant variation among the treatments during both the studied years. During 2020–21 and 2021–22, the grain yield was maximum in T_9 (7.81 and 8.00 t/ha) which was significantly superior over remaining treatments except T_7 (7.25 and 7.30 t/ha) and T_2 (7.11 and 7.20 t/ha), respectively where in statistically similar results were observed.

Stover yield: Similar to grain yield, the stover yield also affected significantly during 2020–21 and 2021–22 by biostimulants based nutrient management (Table 2). Significantly, it was higher in T_9 (12.18 and 12.48 t/ha) which was statistically similar to T_7 (11.28 and 11.36 t/ha) and T_2 (11.06 and 11.19 t/ha) over remaining treatments during both the successive years, respectively.

Biological yield: Significant dissimilarity was noticed among the treatments of biostimulants based nutrient management during the years 2020–21 and 2021–22 (Table 2). Similar to grain and stover yield, maximum biological yield was witnessed in T_9 (22.47 and 22.92 t/ha) which was significantly superior to remaining treatments except T_7 (20.95 and 21.06 t/ha) and T_2 (20.78 and 20.70 t/ha) where in statistically on par results were observed during the successive years, respectively. However, absolute control recorded biological yield (12.99 and 12.20 t/ha) which was minimum in comparative to remaining treatments.

Inoculation of *Azotobacter* and PGPR to maize seeds with basal application of chemical fertilizers enhanced the nutrient availability and uptake which improves plant growth and yield. These microbial biostimulants might have produced vitamins, amino acids and growth promoting substances namely IAA and GA could have improved nutrient availability, uptake and translocation and also synthesise photosynthetic assimilates which in turn enhanced the crop growth and yield. These results were in accordance with the findings of Beyranvand *et al.* (2013) and Tiwari *et al.* (2017). Additionally, humic acid foliar application had positive relation with the growth as cited by Celik *et al.* (2011) in maize that growth enhancement was due to its stimulating effect on respiration, photosynthesis, protein and nucleic acid synthesis and enzyme activity modulation. Thereby, it regulated the plant hormone level, increased

Table 2 Effect of biostimulants based nutrient management on number of leaves/plant, grain, stover and biological yield of spring maize

Treatment	Number of leaves/plant						Grain yield (t/ha)		Stover yield (t/ha)		Biological yield (t/ha)	
	30 DAS		60 DAS		At harvest							
	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22
T_1	5.13	5.09	9.26	9.23	10.39	10.24	4.26	3.97	6.49	6.03	12.99	12.20
T_2	7.25	7.22	15.32	15.52	16.39	16.45	7.11	7.20	11.06	11.19	20.61	20.78
T_3	6.27	6.29	13.12	13.15	14.16	14.29	6.23	6.28	9.65	9.73	18.20	18.30
T_4	5.38	5.42	11.86	11.92	12.79	12.86	5.63	5.66	8.69	8.74	16.58	16.65
T_5	6.32	6.35	13.56	13.68	14.69	14.78	6.57	6.62	10.19	10.27	19.12	19.21
T_6	5.42	5.45	12.08	12.27	13.27	13.45	6.04	6.09	9.35	9.43	17.72	17.84
T_7	6.35	6.41	14.18	14.29	15.95	16.23	7.25	7.30	11.28	11.36	20.95	21.06
T_8	5.43	5.49	12.55	12.63	13.96	14.14	6.35	6.42	9.85	9.95	18.58	18.71
T_9	6.38	6.45	14.64	14.76	17.38	17.59	7.81	8.00	12.18	12.48	22.47	22.92
SEm ±	0.46	0.46	0.82	0.85	0.72	0.80	0.25	0.28	0.42	0.44	0.81	0.86
LSD ($P \leq 0.05$)	NS	NS	2.47	2.56	2.14	2.39	0.76	0.83	1.25	1.32	2.43	2.56

DAS, Days after sowing. T_1, Absolute control; T_2, 100% RDF (recommended dose of fertilizer); T_3, 75% RDF + *Azotobacter*; T_4, 50% RDF + *Azotobacter* + PGPR (Plant growth promoting rhizobacteria); T_5, 75% RDF + *Azotobacter* + PGPR; T_6, 50% RDF + *Azotobacter* + PGPR + Humic acid (HA); T_7, 75% RDF + *Azotobacter* + PGPR + HA; T_8, 50% RDF + *Azotobacter* + PGPR + HA + Seaweed extract (SWE); T_9, 75% RDF + *Azotobacter* + PGPR + HA + SWE.

leaf water retention, enhanced plant stress tolerance and antioxidant metabolism that contributed to better plant growth response which ultimately enhances the economic crop yield (Tejada and Gonzalez 2003). Similarly, foliar supply of seaweed extract also contributed in growth enhancement of maize, in turn improving the yield might be owing to good nutrient availability (Pramanick *et al.* 2013), presence of micronutrients (Sridhar and Rengasamy 2011) and some growth promoting substances lead to enhance growth attributes of the plant (Layek *et al.* 2017). Identical outcome was in agreement with the present investigation as highlighted by Pal *et al.* (2015) in sweet corn that seaweed extract foliar spray with reduced RDF increased the plant metabolic activity and act as stimulator for growth and development. Thus, combination of microbial and non-microbial biostimulants comprising inoculation of *Azotobacter* and PGPR at sowing time and humic acid and seaweed extract foliar application at later crop growth stages along with reduced RDF resulted in improved growth of maize which in turn enhanced the yield as witnessed in present investigation.

Correlation studies: The correlation between growth attributes (plant height, number of leaves/plant, leaf length and leaf width) and biological yield of spring maize (Table 3) revealed that the plant height (r = 0.956 and 0.956), number of leaves/plant (r = 0.990 and 0.990), leaf length (r = 0.956 and 0.963) and leaf width (r = 0.915 and 0.928) showed higher positive correlation significantly with biological yield during 2020–21 and 2021–22, respectively in increasing trend of biological yield with growth attributes of maize.

Regression studies: The regression analysis exhibited a significant polynomial relation between growth attributes and biological yield of spring maize during both the studied year 2020–21 and 2021–22 (Fig. 1 and 2). The R^2 value between biological yield and plant height, number of leaves/plant, leaf length and leaf width were 0.934, 0.986, 0.947

Table 3 Correlation coefficient (r) between growth attributes and biological yield of spring maize under biostimulants based nutrient management during 2020–21 and 2021–22

Pearson correlation	2020–21					2021–22				
	BY	PH	NL	LL	LW	BY	PH	NL	LL	LW
BY	1					1				
PH	0.956**	1				0.956**	1			
NL	0.990**	0.978**	1			0.990**	0.982**	1		
LL	0.956**	0.995**	0.975**	1		0.963**	0.991**	0.984**	1	
LW	0.915**	0.982**	0.955**	0.976**	1	0.928**	0.992**	0.967**	0.985**	1

**, Correlation is significant at the 0.01 level (2-tailed). BY, Biological yield; PH, Plant height; NL, Number of leaves/plant; LL, Leaf length; LW, Leaf width.

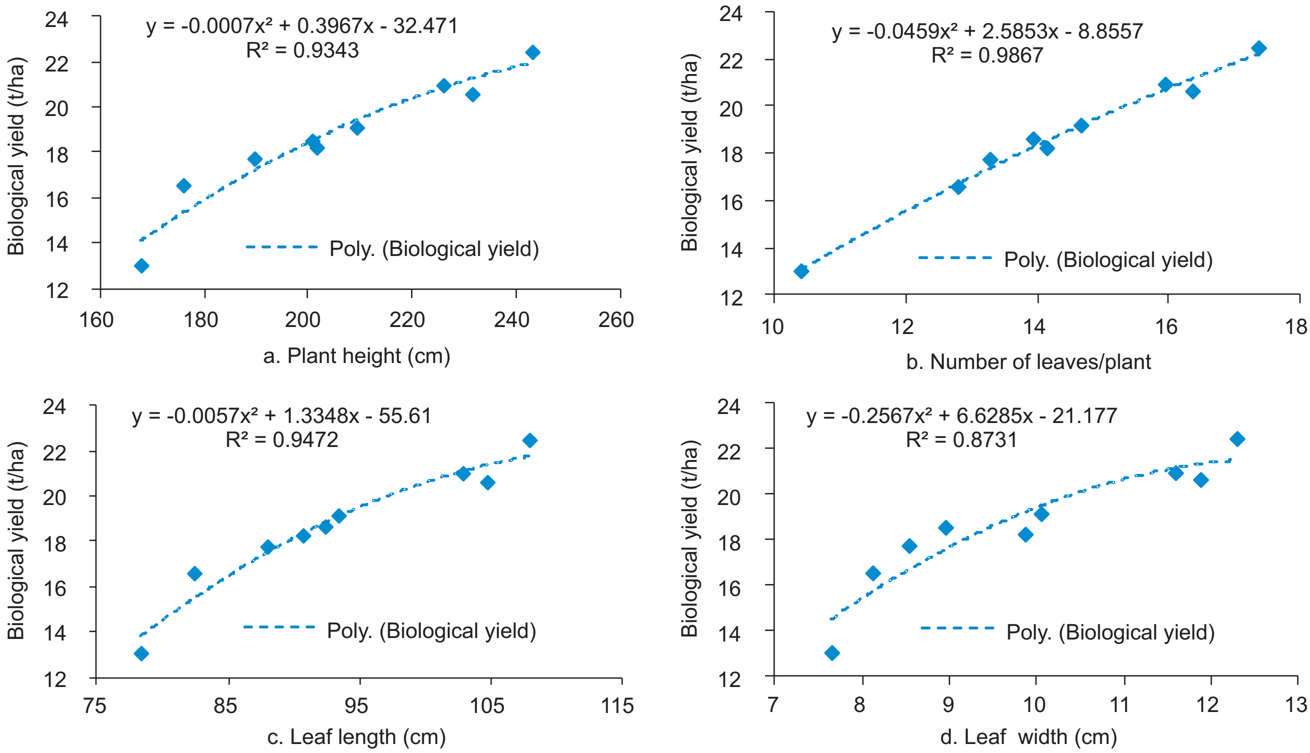

Fig. 1 Relationship between growth attributes and biological yield of spring maize under biostimulants based nutrient management during 2020–21.

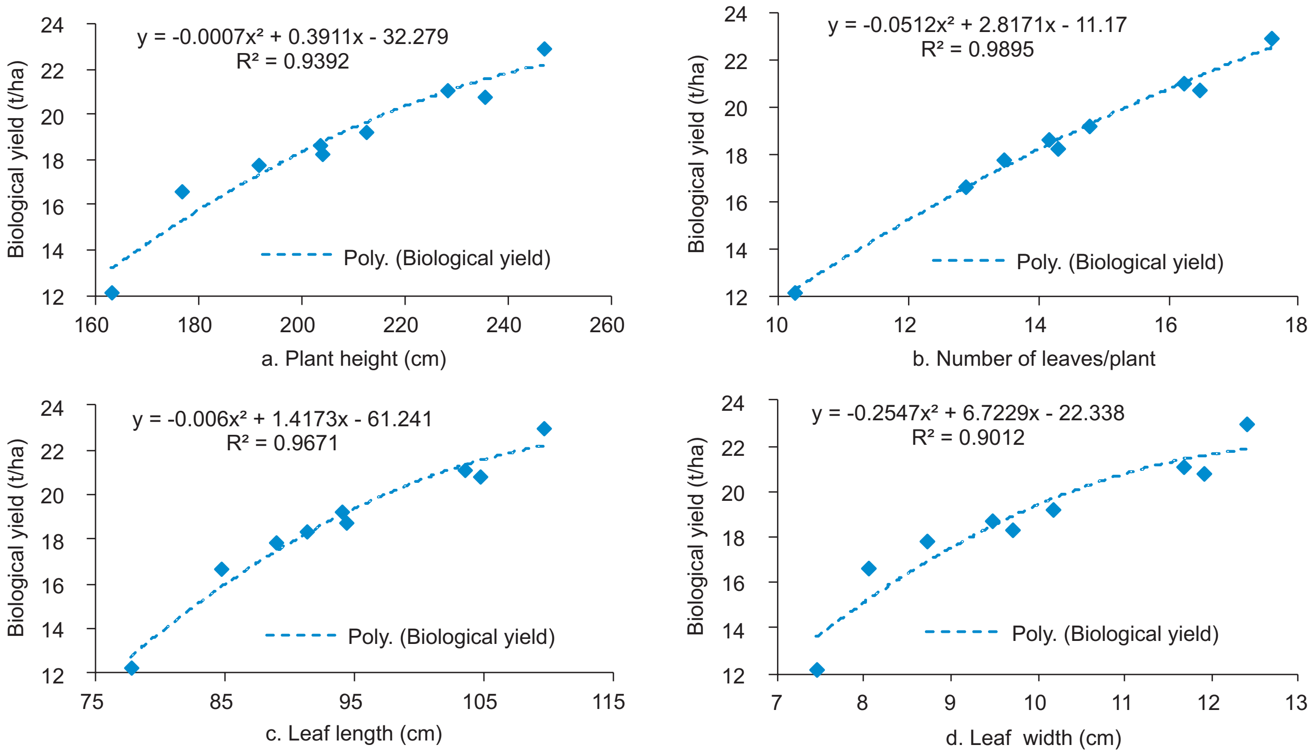

Fig. 2 Relationship between growth attributes and biological yield of spring maize under biostimulants based nutrient management during 2021–22.

and 0.873 during 2020–21 and 0.939, 0.989, 0.967 and 0.901 during 2021–22, respectively. This indicated that that plant height, number of leaves/plant, leaf length and leaf width of spring maize accounted 93.4, 98.6, 94.7 and 87.3% of variation in biological yield during 2020–21 and 93.9, 98.9, 96.7 and 90.1% during 2021–22, respectively.

Based on findings of two-year investigation, the treatment comprising 75% RDF + *Azotobacter* + PGPR + HA + SWE was found better as growth and yield of spring maize was concerned over 100% RDF. So, these biostimulants combination can replace up to 25% RDF without compromising the yield. Hence, the treatment 75% RDF + *Azotobacter* + PGPR + HA + SWE can be recommended as an alternative to conventional fertilizer application in spring maize under legume based cropping sequence.

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