International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 1189-1193

Received: 03-09-2017
Accepted: 07-10-2017

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Identification of principal yield attributing traits through multivariate analysis of rainy season guava as influenced by varied micronutrient fertilization

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Abstract
The study is a synthesis of some yield attributing traits of rainy season guava through grown under seven micronutrient treatments (sole and combination of B, Zn and Cu) and a control (without micronutrient). Pooled analyses of two year experimental data are subjected to multivariate analysis (Principle Component Analysis) and biplot analysis to identify the principal yield attributes. Considerable variations were observed in yield attributing and leaf nutrient status of guava plant upon micronutrient fertilization (B, Zn and Cu). The highest fruit yield was obtained with combined foliar application of B and Zn. From Principle Component Analysis it was observed that leaf B followed by leaf Zn content has most significant role in regulating principal yield attributing traits and fruit yield of guava. From Biplot-analysis it was also clearly revealed that combined application of B and Zn were the most effective in improving yield attributing traits and ultimately the yield of guava.

Keywords: Guava, yield attributing traits, leaf nutrient content, multivariate analysis

Introduction

Guava (*Psidium guajava* L.), is one of the most important tropical as well as sub-tropical fruit, because of its high nutritive value and possibilities of cultivation even under adverse conditions (Yadav *et al.*, 2014) [1]. Guava is not only a delicious table fruit due to its excellent flavour; nutritive value and pectin content, but is also important fruit for processing industry for preparing many kinds of excellent products like jelly, jam, canned fruit products, fruit butter, toffee, cheese and guava nectar. It is a rich source of vitamin C and pectin (Rawat *et al.*, 2010) [2]. In guava, two distinct seasons of flowering, spring (March-April) and rains (June-July) occur from which fruits ripen during rainy and winter season respectively. In North Indian climate the rainy season crop of guava is poor in quality and nutritive value and is affected by many insect pests and diseases. On the contrary, the winter season fruits are superior in quality and fetches higher monetary return (Boora *et al.*, 2016) [3]. For this reason, most farmers regulate the cropping pattern with different techniques to get winter crop with avoiding rainy season crop. But now-a-days majority of the guava growers recently faced problem of yield stagnation and sub-optimal quality of the fruit by following conventional management practices. So, there is immense scope to augment yield and nutritional status of guava even in rainy season with proper nutrient management. For proper growth and development any plants needs an optimum availability of all macro and micronutrients (Sau *et al.*, 2016) [4]. Guava is such a crop that highly responsive to macro and micronutrient fertilization (Anjaneyulu and Raghupathi, 2009) [6]. Nutrients like nitrogen, phosphorus and potash play a pivotal role in promoting the plant vigour and productivity, whereas micronutrients like zinc, boron, copper and molybdenum perform a specific role in the growth and development of plant, quality produce and uptake of major nutrients (Rawat *et al.*, 2010) [2]. Foliar fertilization of nutrients to fruit plants has gained much importance in recent years which is quite economical and obviously an ideal way of evading the problems of nutrients availability and supplementing the fertilizers to the soil. A whole array of tools is used to provide nutritional support, such as tissue and soil analyses (leaf analysis being the most reliable for assessing the nutritional status of perennial plants) grounded on adequate sampling methods and on correct interpretation of analytical data (Bould *et al.*, 1960) [6].

The effects of modifying nutrient proportions due to interactions among the nutrients were first illustrated by Lagatu and Maume (1935) [7]. Plant tissue data convey relative information, as they are intrinsically multivariate, i.e., no one component can be interpreted in isolation; it must be related to other components (Tolosana-Delgado and van den Boogart, 2011) [8]. Hence, for compositional data (as in plant tissue nutrients), tools should be used that allow analysis of inter-component interactions for the sake of better understanding of plant nutritional status. Keeping all these facts in mind, we have tried to identify the principal yield attributing traits of rainy season guava through multivariate analysis grown under different micronutrient fertilization treatment.

Material and Methods

The experiment was conducted at the Horticulture Research Station, Mondouri, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India (22°56′ N, 88°31′ E, 9.75 m above mean sea level) during rainy season of 2013-14 and 2014-15. Five year old forty eight bearing guava trees (cv. Allahabad Safeda) of uniform vigour, size and maintained under uniform cultural schedule were selected for the present studies. The experiment consisted of eight treatment combinations of three micronutrients viz., zinc, copper and boron. The experiment was laid out in a complete randomized block design with eight treatments [$B_0Zn_0Cu_0$ = Control, B_1 = (H_3BO_3 @ 0.2%), Zn_1 = ($ZnSO_4$ @ 0.5 %), Cu_1 = ($CuSO_4$ @ 0.5%), B_1Zn_1 = (H_3BO_3 @ 0.2% + $ZnSO_4$ @ 0.5 %), B_1Cu_1 = (H_3BO_3 @ 0.2% + $CuSO_4$ @ 0.5%), Zn_1Cu_1 = ($ZnSO_4$ @ 0.5 %+ $CuSO_4$ @ 0.5%), $B_1Zn_1Cu_1$ = (H_3BO_3 @ 0.2%+ $ZnSO_4$ @ 0.5 %+ $CuSO_4$ @ 0.5%)] having three replications. Aqueous solutions of boron, zinc, copper were sprayed twice (five litres per tree) at the time of full bloom and one month after the first spray on rainy season (*Ambe Bahar*) in early morning with the help of a foot sprayer to ensure the maximum absorption of nutrients through the leaves. Observations on yield indicators of guava such as fruit set %, fruit drop% and fruit retention % were worked out using the formula given by Sau *et al.* (2016) [4].

Measurements of N (%), P (%), K(%), B (ppm), Zn (ppm) and Cu (ppm) in leaf were recorded from 30 fully mature leaves which were collected in August after fruit harvest on each treatment all around the trees. Total N was estimated by micro-Kjeldhal's method (Jackson, 1973) [9] on the basis of dry weight in leaves. Total P was determined as described by Jackson (1973) [9]. K content was estimated by Flame photometer. Total B was determined by Azomethine-H colorimetric method with the help of UV-VIS spectrophotometer (Gaines and Mitchell, 1979) [10] and estimation of total Zn and Cu was done through atomic absorption spectrophotometer (Jackson, 1973) [9].

Statistical Analysis

Statistical analysis was performed by the analysis of variance (ANOVA) for randomised block design (RBD) using SAS software version 9.2 applying analysis of variance (PROC GLM) with subsequent multiple comparisons of means for both of the experimental years. The ANOVA of the observed parameters of guava cultivation across the year's revealed a non-significant variant within the years as well as year × cultivar interaction at $p \leq 0.05$. The homogeneity of error variance was tested using Bartlett's χ^2 test (Zwick and Velicer, 1986) [11]. The significant difference between treatment means was tested by Tukey's Honest Significant Difference (HSD) test at $p \leq 0.05$.

Principle Component Analysis (PCA) is one of the most frequently used multivariate data analysis methods. PCA which also has been used as a method that transforms an original set of variables into a smaller set of uncorrelated linear variables retaining the most of the information in the original set of variables (Ray *et al.*, 2014) [12] was performed using the XLSTAT 2017 software (www.xlstat.com). The independent factors in the total data set which mostly contributed to the guava fruit yield were selected for PCA. The total variance is simply the sum of variances of these variables. As they have been standardized to have a variance of one, each observed variable contributes one unit of variance to the total variance in the dataset. The total nine independent yield attributing traits i.e. leaf nutrient (N, P, K, Zn, B and Cu) contents, fruit set %, fruit drop % and fruit retention % were selected for this purpose. The array of communality, the amount of the variance of a variable accounted by the common factors together, was estimated by the highest correlation coefficient in each array as suggested by Seiller and Stafford (1985) [13]. Factor loadings after varimax rotation along with Kaiser Normalization (Kaiser, 1974) [14] were estimated for determining the correlation of a variable with a factor. The highest value of the factor loading (where squared cosine is the largest) of a particular variable in a particular factor among the extracted factors plays the important role to churn out the factor.

After performing PCA, to represent both observations (micronutrient treatments) and variables (selected yield attributing traits) graphically in the factor space, a distance biplot analysis was performed (Gower, 1996 and Legendre, 1998) [15-16] using XLSTAT 2017 software. The biplot was used to interpret the distances between the observations (micronutrient treatments) as these are an approximation of their Euclidean distance in the p-dimensional variable (selected yield attributing traits) space. The position of two observations projected onto a variable vector was used to determine their relative level for this variable.

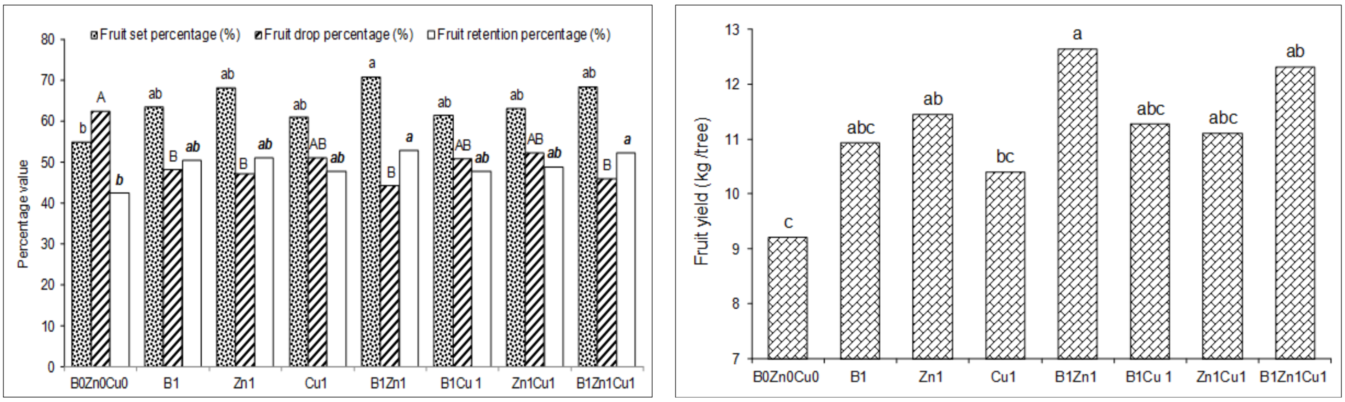
Results and Discussion

Fruit set percentage, Fruit drop percentage and Fruit retention percentage

Foliar Application of micronutrients significantly increased fruit set and fruit retention percent than the plant received no micronutrients i.e. control ($B_0Zn_0Cu_0$) (Fig. 1A). Amongst the treatment combinations, application of B_1Zn_1 recorded the maximum fruit set percent. Micronutrient fertilization was also effective for reduction of fruit drop percent of guava. Plant received B_1Zn_1 also recorded least fruit drop percent accounting 41.11% lower values than the plants receiving no micronutrient fertilization.

Fruit Yield (kg/tree)

Micronutrient application significantly ($p \leq 0.05$) increased fruit yield (kg/tree) over the control ($B_0Zn_0Cu_0$) (Fig. 1B). The highest fruit yield (12.63 kg/tree) was obtained with foliar application of B_1Zn_1 followed by $B_1Zn_1Cu_1$ and Zn_1.

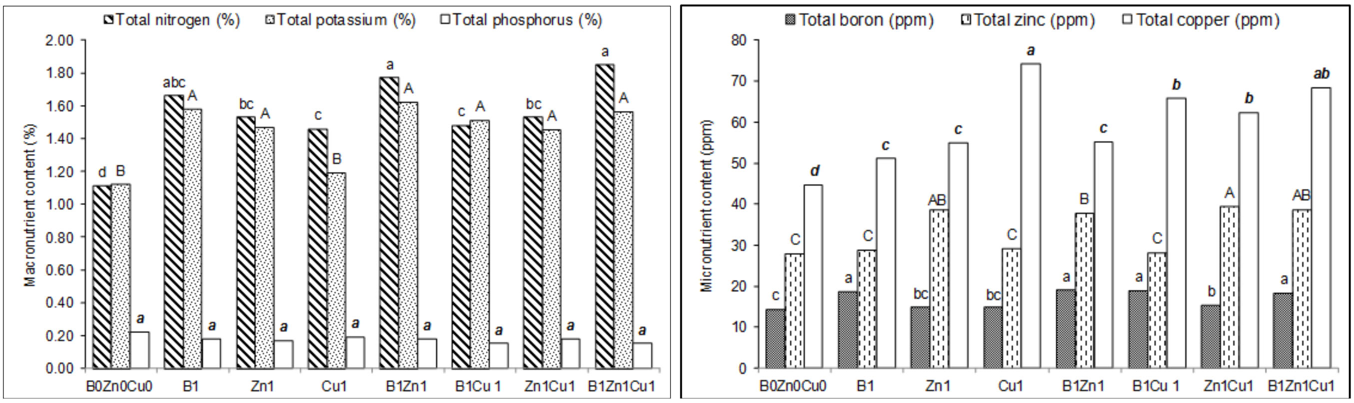


A B

Fig 1: Effect of micronutrients on [A] yield attributes and [B] yield of rainy season guava (Pooled data of 2013-14 and 2014-15). Vertical bar followed by different letters are significantly different (otherwise statistically at par) at p≤0.05 by Tukey's HSD (honest significant difference) test

Leaf nutrient content

The macronutrient (N, P and K) content in guava leaf was significantly ($p \leq 0.05$) altered with different foliar micronutrient fertilization (Fig. 2A). The highest N contents were found in plants treated with combined application of $B_1Zn_1Cu_1$ which was statistically at par with B_1Zn_1. Micronutrient fertilization (applied either alone or in combination) failed to bring any significant changes in leaf P content. As evident in Fig. 2A, micronutrient fertilization significantly changed leaf K concentration of guava. The maximum K uptake of guava leaf was observed in the plant fertilized with B_1Zn_1which was statistically at par ($p \leq 0.05$) with B_1, B_1Cu_1 and $B_1Zn_1Cu_1$. In harmony to the present study, researchers found that foliar application of zinc sulphate significantly increased N and K content and decreased P content in guava leaf (Rajkumar *et al.*, 2017) [17]. Foliar feeding of micronutrients significantly improved micronutrient contents (B, Zn and Cu) in guava leaves over control (Fig. 2B). Boron concentration in guava leaves were significantly ($p \leq 0.05$) increased with combined foliar application of B_1Zn_1, B_1Cu_1 and B_1. The highest B concentration (19.01 ppm) in guava leaf was recorded from the trees fertilized with B_1Zn_1. Similarly, highest Zn concentration was found in the leaves treated with Zn_1Cu_1 (39.36 ppm) which was statistically at par ($p \leq 0.05$) with $B_1Zn_1Cu_1$ and Zn_1. Unlike Zn concentration, highest Cu concentration in guava leaves was found from the tress received foliar fertilization of Cu_1 which was 65.70% higher than the trees received no micronutrients.



A B

Fig 2: Effect of micronutrients on leaf [A] macronutrient and [B] micronutrient content of rainy season guava (Pooled data of 2013-14 and 2014-15). Vertical bar followed by different letters are significantly different (otherwise statistically at par) at p≤0.05 by Tukey's HSD (honest significant difference) test.

Eigen values and percent variance of different yield attributing characters

Eigen values and corresponding proportions of percent variance extracted along with cumulative percentage of total variance studied is presented in Table 1. Data presented in Table 1 revealed that the first, second and third principal components explain about 68.18%, 13.00% and 12.42% of the total sample variance, respectively. The first three components containing the Eigen values greater than 1 have been retained for the study, so first three components explain the variance of the sample reasonably. Scree-plot test (Cattell, 1966) [18] which is based on the decreasing curve of eigen values, also gave a clear-cut visual aid for justification of retaining three components effectively (Fig. 3). The correlation of variables to the different principle components was presented in the form of the corresponding factor loadings after varimax rotation (Table 2). The PCA has extracted three factors based on the eigen value. The first factor consists of leaf nutrients i.e. N, P, K and B content. Second factor consists of leaf Zn content, fruit set, fruit drop and fruit retention percent. While third factor consists only leaf Cu content. The factors like leaf N, P, K and B content of guava showed highest loadings in PC 1. It was evident that the first factor was closely associated with traits having significantly higher correlation with guava fruit yield. The first factor extracted in this PCA accounts for the maximum amount of total variance in the observed variables (68.18%). However, the second factor accounted for a maximum amount of variables that was not accounted for by the first and third component. Thus B concentration in guava leaves recorded the largest squared cosine value (0.952) as because it loaded heavily on the first factor. Thus it can be assumed that B has most significant role in regulating fruit yield. It may be due to the beneficial effect of boron in fruit set, pollen grain germination and pollen tube development (Ganie *et al.*, 2013; Wojcik *et al.*, 2008) [19-20] and also in promotion of several metabolic processes such as carbohydrate transport (Marschner, 2012; Mengel *et al.*, 2001) [21-22] etc. which ultimately increased fruit set and retention in guava.

Table 1: Total variance explained for each component based on different yield attributing characters in rainy season guava (Based on pooled data of 2013-14 and 2014-15).

	Factors						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Eigenvalue	6.14	1.17	1.12	0.34	0.16	0.05	0.03
Variability (%)	68.18	13.00	12.42	3.73	1.75	0.56	0.36
Cumulative %	68.18	81.18	93.60	97.33	99.08	99.64	100.00

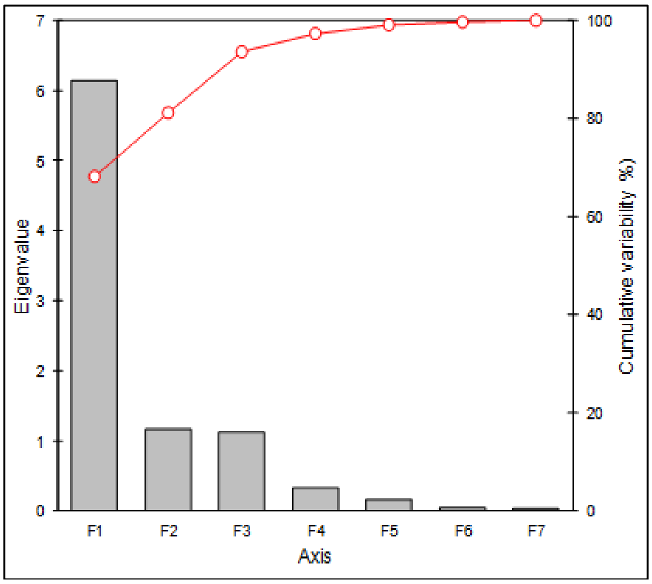


Fig 3: Scree-plot for different yield attributing characters influencing rainy season guava (Based on pooled data of 2013-14 and 2014-15).

Table 2: Principal factor matrix after Varimax rotation (Kaiser Normalization) for different yield attributing characters of rainy season guava (Based on the pooled data of 2013-14 and 2014-15)

Parameters	**Unit**	**Factors**		
		F1	**F2**	**F3**
N	%	0.461	0.398	0.075
P	%	0.404	0.093	0.328
K	%	0.693	0.256	0.000
B	ppm	0.952	0.000	0.002
Zn	ppm	0.002	0.909	0.004
Cu	ppm	0.001	0.006	0.971
Fruit set	%	0.237	0.717	0.013
Fruit drop	%	0.409	0.432	0.086
Fruit retention	%	0.344	0.594	0.035

N = Total Nitrogen (%) concentration of leaf; P = Total Phosphorus (%) concentration of leaf; K = Total Potassium (%) concentration of leaf; B = Total Boron (ppm) concentration of leaf; Zn = Total Zinc (ppm) concentration of leaf, Cu = Total copper (ppm) concentration of leaf.
Values in bold correspond for each observation to the factor for which the squared cosine is the largest

Diversity Analysis

Biplot analysis was used to identify the mostly correlated set of traits associated with the individual observation (treatment) (Banerjee *et al.*, 2017) [23]. The scattered plot matrix score clustered the different yield attributing traits into groups showing superiority with a set of associated micronutrient treatments (Fig. 4). Among the micronutrient treatments B_1Zn_1 was the most effective in regulating most of the yield attributing traits of guava. From this biplot, it was clear that, this two treatment (B_1Zn_1) was closely associated with this yield attributing traits (leaf nutrient content, fruit set % and fruit retention %) while sole application of copper (Cu_1) and control ($B_0Zn_0Cu_0$) positively associated with fruit drop % and total phosphorus content (%) of guava leaf. This may be due to synergistic relationship between zinc and boron (Shukla, 1983) [24] in guava. This classification is not in exact, but in agreement with the cluster analysis generated employing single linkage using the same data (Biniam *et al.*, 2015) [25].

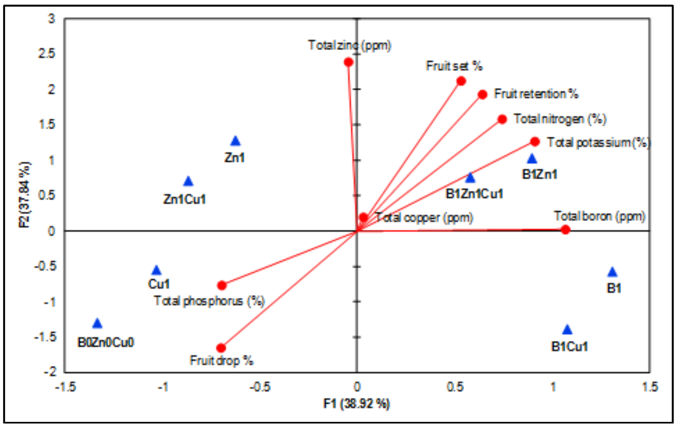


Fig 4: Biplot for different yield attributing traits of rainy season guava as influenced by varied micronutrient fertilization (Based on pooled data of 2013-14 and 2014-15).

Conclusion

Considerable variations were observed in yield attributing traits and leaf nutrient status of guava plant upon foliar fertilization of B, Zn and Cu. From PCA it can be assumed that leaf B status has most significant role in regulating fruit yield followed by leaf Zn concentration. The highest fruit yield was obtained with combined foliar application of B_1Zn_1. Results of Biplot-analysis also revealed that combined application of B_1Zn_1 has the most effective in improving yield attributing traits of guava. On the basis of study, it can be concluded that application of micronutrient fertilizers (0.2% H_3BO_3 + 0.5% $ZnSO_4$) along with RDF of NPK can be adopted to maximize the yield of rainy season guava in West Bengal.

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