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African Journal of Plant Science

Full Length Research Paper

Multivariate analysis of sugar yield contributing traits in Sugarcane (*Saccharum officinarum* .L), in Ethiopia

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Knowledge on performance of genotypes and interrelationships among traits is very important for sugarcane breeding programmes. The objectives of this study were to assess the phenotypic relationship among 49 sugarcane genotypes and the inter-relationships among traits considered. The cluster analysis demonstrated that the 49 sugarcane genotypes studied were clustered into nine groups and were highly different for Pol in juice, cane yield (tons $ha^{-1}m^{-1}$), number of tillers (ha^{-1}), purity% and milleable stalk population (ha^{-1}). The relationship among sugarcane genotypes was not dependent on geographic origin, suggesting that a high proportion of total genetic variation was retained within the groups of origin and active genetic ex-change was found between different origins. The principal component analysis indicated that cane yield, milleable stalk height and milleable stalk diameter were highly correlated with sugar yield while the correlation of quality traits with sugar yield was weak. In contrary, path and multiple regression analysis revealed that cane yield, recoverable sucrose percentage (%) and Pol contribute more to the variability of sugar yield; these are very important traits for high sugar yield that should be considered in sugarcane breeding programmes. Moreover, milleable stalk height and milleable stalk population via cane yield and Brix, Pol, purity and number of internodes via recoverable sucrose percentage had high indirect effects on sugar yield suggesting these traits should also be given consideration during selection for high sugar yield. Generally, similar and adequate information was generated following the use of cluster, principal component, linear discriminant, path coefficient and multiple regression analyses indicating the use of multivariate analyses was successful and results of the study were more substantial to give concrete recommendation.

Key words: Clusters, genotypes, multiple regression, path coefficient, phenotypic correlation.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important industrial crop and global major source of energy and is a major crop in most parts of tropical and subtropical regions (Khan et al., 2013). The increasing multiple use

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of sugarcane necessitates a strong breeding program that generates gene pools that enables identification of sugarcane genotypes with multiple uses. Hence, to have successful genetic improvement of sugarcane genotypes for multiple purposes, efficient and diversified selection procedures have to be followed. Selection will be efficient if the procedures consider many traits simultaneously during evaluation of sugarcane genotypes. As the appropriate methods that provide accurate evaluations and estimation of genetic diversity depend on genetic variation, sampling methods, the magnitude of data sets, and the statistical tools applied in the data analysis (Mohammadi and Prasanna, 2003), multivariate statistical analysis techniques like principal component analysis (PCA) and cluster analysis techniques are very important to study genetic diversity of sugarcane.

However, prior to starting selection, the genetic diversity among the genotypes need to be assessed using morphological and agronomic traits as the genetic diversity assessment is a basic tool to determine whether there could be enough genetic pool that enables generating desirable genes and genotypes. The information about the status of the genetic pool of a crop (introduction, existing commercial varieties and germplasm) using cluster and other analyses, determines the success of selection efficiency of breeding sugarcane (Malik et al., 2010). The quantified gene pool could be used for future breeding purposes such as combination and introgression of genes (Mohammadi and Prasama, 2003).

After the magnitude and pattern of existing genetic base of the crop determined for traits of interest, the most important thing most sugarcane breeding programs deserves to do is to follow efficient selection procedures that utilize both direct and indirect methods to improve quantitative traits. The method of path coefficients was first used for yield component analysis by Dewey and Lu (1959), and subsequently has become a common method to examine breeding strategy in the 'whole variety' context. As yield is affected by numerous components and is a complex resultant character, the internal adjustments between components causes' increment in one component and causes decrement in the other, causing no change in resultant yield (Wen and Zhu, 2005) and causal pathways existed when independent variables are co-related (Kozak et al., 2007).

Ong'ala et al. (2016) recommended PCA and linear discriminant analysis to identify representative traits for phenotypic characterization of sugarcane, and thereby to select superior clones in the breeding process. During phenotypic evaluation of sugarcane clone, many traits are simultaneously evaluated, which are often genetically linked. It is costly to evaluate all the traits which probably may be interrelated and does not ensure optimal selection gains. Path coefficient analysis is one of the most important tool that enable breeders to handle both selection methods simultaneously (Sidwell et al., 1976). Moreover, it enables to have an insight in to the correlation of these effects with the actions of additive and non-additive genes that govern the traits of interest.

High sugar yield are obtained from cane yield and sucrose content (Terzi et al., 2009) and therefore cane yield and sucrose content and their interaction are important parameters for developing superior genotypes (Zhu et al., 2000; Chohan et al., 2007). Several reports clarify about the relationship of cane yield components with cane yield and cane quality traits. For example, Ahmed et al. (2010) reported positive correlation between cane yield and its components (number of milleable stalks/m^2, milleable stalk height internodes/stalk and single weight) but negative association with milleable stalk diameter, Pol in juice and purity. Similarly, Tyagi and Lai (2007) reported that the weight of milleable stalks contributed high direct effect on cane yield followed by milleable stalk, height, number and thickness. Ei-Shafi and Ismail (2006) reported to use multiple regressions model and reported that the main contributors for sugar yield were cane yield, sugar recovery percentage and milleable stalk diameter. Generally, the results of different studies showed discrepancies to the level of sugar yield and cane yield. This phenomenon necessitates successive studies to be conducted to determine the relationship and association of the traits to increase the efficiency of selection.

One of the major sugarcane production constraints in Ethiopia is the lack of high yielding and stable sugarcane varieties across sugar estates. Based on these problems, the Ethiopian Sugarcane Research Sector is introducing, collecting and recycling sugarcane materials to increase the genetic base and efficient use of gene pool of the crop. However, under Ethiopian sugarcane research conditions, the existing diversity among most of the materials is not assessed and selection strategies that help to increase the selection efficiencies of traits have not been well developed. Moreover, multivariate analysis that helps to develop efficient selection strategies have not been efficiently exploited. Therefore, the objectives of this study were to assess the magnitude of genetic divergence among sugarcane genotypes and to study the interrelationships among traits using multivariate techniques.

MATERIALS AND METHODS

Description of experimental materials

Forty-three sugarcane genotypes along with six commercial varieties were grown across Ethiopian Sugar Estates (Wonji, Metahara and Finchaa) and Projects (Tendaho and Belles) over two successive plant cane and first ratoon crops in 2013 to 2016 production years (Table 1). Of which, 21, 3, 5, 7 and 7 genotypes were introduced from France, Philippines, Barbados, USA and Cuba, respectively. The rest 6 varieties were from commercial varieties which had been introduced into Ethiopia from India, South Africa and Barbados before 50 years and were included in this study for comparison purposes. Out of the introduced materials from France, those whose name starts with PG, are clones that

Table 1. Description of 49 sugar cane genotypes analyzed for sugar yield contributing traits.

Code	Genotypes	Origin	Code	Genotypes	Origin
1	PSR97 092	PHILSURIN (Philippines)	26	VMC95 212	USA
2	DB70047	WICSCBS (Barbados)	27	NCO-334	South Africa
3	DB66 113	WICSCBS (Barbados)	28	FG03 418	Cirad (France)
4	FG06 700	Cirad (France)	29	CO449	India
5	FG06 729	Cirad (France)	30	FG03 204	Cirad (France)
6	PSR97 087	Cuba	31	FG02 553	Cirad (France)
7	PSR97 051	PHILSURIN (Philippines)	32	FG03 103	Cirad (France)
8	HO95 988	USDA (Louisiana)	33	FG03 318	Cirad (France)
9	Cp99 1534	USDA (USA)	34	FG04 708	Cirad (France)
10	FG04 829	Cirad (France)	35	FG04 705	Cirad (France)
11	DB71 060	Cirad (France)	36	FG02 551	Cirad (France)
12	TCP93 4245	USDA (Texas/Canal Point)	37	FG03 173	Cirad (France)
13	CP001 252	USDA (USA)	38	FG04 187	Cirad (France)
14	VMC95 173	USA	39	FG03 372	Cirad (France)
15	FG03 447	Cirad (France)	40	FG03 214	Cirad (France)
16	CO 740	India	41	C86-56	Cuba
17	CP99 1894	USDA (USA)	42	SP70-1284	Cuba
18	FG03 425	Cirad (France)	43	C86-165	PHILSURIN
19	FG05 408	Cirad (France)	44	B78-505	Barbados
20	FG03 520	Cirad (France)	45	C132-81	Cuba
21	FGo4 754	Cirad (France)	46	C86-12	Cuba
22	FG04 466	WICSCBS (Barbados)	47	C90-501	Cuba
23	FG03 526	Cirad (France)	48	B52-298	Barbados
24	Mex54/245	Mexico	49	CO- 678	India
25	FG03 396	Cirad (France)	-	-	-

have been screened half way at the sugarcane breeding scheme in Cirad (France) and those varieties whose name starts with other than PG are advanced breeding clones at the final testing stage before a possible commercial release.

Experimental design and layout

The experiment was implemented with partially balanced square lattice design and was repeated (replicated) three times. Plot size for a genotype per replication was 8.7 × 6 m (52.5 m^2) with four test rows and two guard rows. Moreover, the design contains 7 blocks per replication and each block had an area of 8.7 m (width) × 48 m (length) = 417.6 m^2 and the experimental area per location was 0.78 hectares. Each replication was defined as replication nested in each location because the replications were unique for location and each block was nested within both replications and location. At planting, 18 two budded sets were laid on a furrow with 5 m length and cane was harvested at 17 and 13 months cane age for plant cane and ratoon crops, respectively. All recommended agronomic and cultural practices were uniform to raise the crop across all the sugar estates.

Morphological and agronomic characteristics

Cane and cane yield components

Sprout percentage: The percentage of setts which sprout 45 days after planting was calculated as the numbers of setts sprouted divided by the numbers of setts planted per plot and multiplied by 100, while the number of tillers the (ha^{-1}) per plot (from the central test rows) was counted 4 months after planting and was converted on hectare basis. For average numbers of internodes per stalk, milleable stalk diameter and stalk height (cm), 20 randomly selected milleable stalks per plot were considered and only the average values were reported. For estimation of cane yield (tons $ha^{-1}m^{-1}$), all milleable stalks from the central four rows per plot were hand trashed to remove the leaves and hand topped at the natural breakpoint of sugarcane stalk. The milleable stalks were then weighted using digital scale balance to the weights per plot and was extrapolated to tons ha^{-1} m^{-1}

Sugar yield and yield quality traits

Recoverable sucrose percentage refers to the total recoverable sugar percent in the cane and was calculated as recoverable sucrose percentage = [Pol% - (Brix - Pol%) 0.61] 0.75) as described by Berg (1972), where 0.61 = non-sugar factor, representing the amount of sucrose lost in final process and 0.75 = cane factor, representing the correlation factor between theoretical yields of molasses mixed juice and primary juice for the same genotype and the same cut of cane determined by milling test. Pol and Brix in cane refers to Pol and Brix percentage in cane and were determined as Pol in juice × (100-(fiber%+5))/100 and Brix in juice × (100-(fiber%+3))/100, respectively. Moreover, sugar yield (ton/ha) was estimated as the product of cane yield per hectare and average estimated recoverable sucrose percentage, and was computed as sugar yield = [Cane Yield (t/ha) × Recoverable

Table 2. Combined analysis of variances for 49 sugar cane genotypes (G) evaluated across 12 test environments (Location × Crop Years).

Parameter	Sources of variation						CV	Mean
	Environment	Genotype	GxE	Rep (Env)	Block (Rep*Env)	IBE		
DF	**11**	**48**	**528**	**24**	**144**	**432**		
Traits								
Sprout%	51676.93**	1117.7**	799.67**	1090.17**	108.1ns	97.25	15.13	65.17
TN	1695948.57**	1.04E+^{12}*	7954.96**	5159.09**	1.9x10^{7}*	8.9x10^{7}	14.37	207900
DM	8.18**	0.59**	0.17**	0.62**	0.05**	0.14	7.18	2.67
MSH	94.11**	1.29**	0.39**	0.61*	0.09ns	0.08	11.63	2.39
MSP	76279.85**	2985.5**	1206.57**	605.99ns	442.3**	219.4	14.79	100150
NIPS	2167**	71.45**	22.44 ns	59.46*	17.08ns	20.41	17.03	26
CYLD	1646.65**	40.95**	16.36**	13.58**	4.27ns	3.82	19.83	9.85
Pol%	141.65**	12.74**	9.98**	7.79ns	3.34**	2.38	8.59	18.16
Brix%	242.36**	9.54**	5.08*	2.81ns	1.44**	3.45	5.67	20
Purity%	1068.96**	22.64**	14.43**	10.72ns	4.99**	3.8	2.21	90.02
RS%	70.74*	6.33**	3.56**	2.64ns	1.22**	0.68	6.62	12.47
SYLD	22.06**	0.60*	0.28**	0.18*	0.08ns	0.065	20.87	1.22

**Highly significant at p<0.01; *Significant at P<0.05; ^{ns}Non-significant; rep: Replications; IBE: intra block error; DF: degree of freedom; DM: Diameter; NT: number of tillers (ha^{-1}); Pol: Pol in juice (%); RS%: recoverable sucrose percentage (%); Brix: Brix in juice (%); MSH: milleable stalk height (m); DM: milleable stalk diameter (mm); MSP: milleable stalk population (ha^{-1}); NIPS: numbers of internodes per stalk; SYLD: sugar yield (t ha^{-1}m^{-1}); CYLD: cane yield (t ha^{-1}m^{-1}).

sucrose percentage] / 100. As the plant cane crops and ratoon crop were harvested at 17 and 14 months age of cane, respectively, data for cane and sugar yield were converted to t ha^{-1}m^{-1} (tons per hectares per month) to bring the crops types to the same productivity unit.

Statistical analysis

The data collected for each trait were subjected to combined analysis of variances (ANOVA) and using SAS program and data quality was checked to meet the assumptions of ANOVA and block effects were using SAS software package, 2009. Genotypic means were adjusted for the lack of orthogonality (intra or inter block) in the data depending on the relative magnitude of the block variance relative to the residual error as suggested by Federer et al. (2001) and the adjusted means were used for multivariate analyses. For cluster analysis, average linkage was obtained by specifying METHOD=AVERAG as adopted by Sokal and Michener (1958), while Euclidean distance and linear discrimination analysis were computed using Minitab v. 17. Moreover, multiple regressions were analyzed using GENSTAT (Edition 13th), while the path coefficient analysis was done using the SAS software package (SAS, 2009) and SAS program of PROC MATRIX and PROCIML as suggested by Kang (1994).

RESULTS AND DISCUSSION

The pooled analysis of variance (Table 2) showed that the variances for genotypes were highly significant (P<0.01) for all traits, suggesting there was an ample genetic variability among the genotypes. As the variability among genotypes was highly significant for all traits studied, conducting of multivariate analyses using these traits will be relevant to generate further analysis. Hence, means for sprout percentage (%), number of tillers (ha^{-1}), milleable stalk height (m), milleable stalk diameter (cm), milleable stalk population (ha^{-1}), numbers of internodes per stalk, cane yield (tons ha^{-1} m^{-1}), Brix in juice, Pol in juice, purity, recoverable sucrose percentage and sugar yield (t ha^{-1} m^{-1}) were adjusted. The adjusted means of the traits studied were subjected to cluster, linear discriminant, principal component, path coefficient and multiple regression analyses (Table 3).

Cluster analysis

Based on the adjusted means of 12 sugarcane traits presented in Table 3, results of the cluster analysis indicated that the 49 sugarcane genotypes formed 10 distinct groups or clusters where 3 of the groups contained a single genotype (Table 4). Starting from left to right of the Dendrogram (Figure 1), clusters number II comprised much of the genotypes studied (17 genotypes) followed by cluster V (8) and I (7). Cluster 1 consisted of 7 genotypes that have different origins but introduced from France. Cluster II consists of 17 genotypes (9, 36, 14, 29, 40, 13, 37, 23, 31, 42, 41, 44, 32, 13, 30, 43, 18, 45 and 48) which were a mixture of commercial varieties and introduced genotypes. It was also observed that most of the commercial varieties were grouped in one cluster except genotypes 24 and 49 form another separate group. Genotypes 7, 19 and 34 were ungrouped, suggesting these genotypes were outliers for lower or higher mean values of the traits studied.

Based on the grouping, the relationships observed

Table 3. Adjusted means for 12 traits in 49 sugarcane genotypes used for multivariate analyses.

Genotypes		Traits											
Cd	**Name**	**Sprout%**	**NT**	**Pol %**	**Purity**	**RS%**	**Brix%**	**Cyld**	**HT**	**DM**	**MSTP**	**NIPS**	**SYLD**
1	PSR97 092	61.6	184034.4	17.69	89.46	12.08	19.63	12.7	2.643	3.03	87813.33	14.9	1.521
2	DB70047	64.5	159785.84	18.92	90.52	13.26	21.06	9.38	2.639	2.79	86312.57	16	1.226
3	DB66 113	80.5	242950.29	17.25	89.66	11.91	19.13	11.9	2.436	2.66	118483.1	12.89	1.409
4	FG06 700	58.6	241346.91	18.5	90.08	12.52	20.1	8.35	2.483	2.22	105329.5	17.52	1.023
5	FG06 729	62.7	183176.18	19.67	91.44	13.46	21.05	9.4	2.703	2.67	86440.39	17.65	1.262
6	PSR97 087	71.6	172225.55	18.75	89.45	12.65	20.48	9.81	2.305	2.97	73518.3	17.26	1.229
7	PSR97 051	86.6	251177.41	19.02	89.65	13.05	21.04	10.6	2.38	2.78	91003.73	15.19	1.37
8	HO95 988	70.2	216704	18.65	91.56	12.98	20.29	8.94	2.242	2.26	137152.6	16.21	1.154
9	Cp99 1534	55.3	185746.53	17.71	90.12	12.36	19.87	8.75	2.107	2.63	95732.89	14.42	1.078
10	FG04 829	58.1	196837.72	19.3	90.11	13.26	21.27	9.31	2.203	2.49	116522.9	16.91	1.232
11	DB71 060	56.5	181090.09	18.63	90.18	12.8	20.52	10.8	2.235	3.12	75663.11	15.03	1.377
12	TCP93 4245	71.7	202129.37	19.82	91.84	13.65	21.19	9.42	2.625	2.47	104872.6	18.98	1.291
13	CP001 252	61.3	200516.75	17.93	91.2	12.54	19.67	9.37	2.451	2.59	100506.4	13.87	1.171
14	VMC95 173	63.7	207430.05	17.41	90.42	12.03	19.12	9.27	2.546	2.62	87714.31	14.36	1.128
15	FG03 447	49.7	233412.46	18.47	91.2	12.94	20.46	9.79	2.614	2.54	105984.5	15.4	1.245
16	CO 740	55.5	200983.62	17.47	89.28	11.78	19.07	11.2	2.257	2.8	107522.9	12.25	1.31
17	CP99 1894	75.3	245477.14	18.56	88.87	12.69	20.78	7.17	2.131	2.39	108191.2	16.71	0.91
18	FG03 425	59.1	205614.96	17.41	87.82	11.73	19.68	9.13	2.441	2.71	94010.43	15.28	1.064
19	FG05 408	55	236234.46	18.72	93.04	13.23	20.03	8.33	2.01	2.66	97390.73	15.37	1.121
20	FG03 520	78.5	229228.81	18.04	88.84	12.41	20.15	12	2.954	2.77	96965.76	15.14	1.49
21	FGo4 754	67.2	214073.9	17.78	88.75	12.28	20.16	11.5	2.322	2.94	95710.55	13.18	1.399
22	FG04 466	53.3	172227.5	18.38	90.15	12.66	20.25	11.8	2.284	2.84	101854.3	12.68	1.475
23	FG03 526	65.4	203918.56	18.55	90.8	12.82	20.33	9.47	2.434	2.7	100986.7	13.77	1.21
24	Mex54/245	54.4	172808.67	17.07	88.48	11.63	19.16	10	2.832	2.58	94102	13.36	1.145
25	FG03 396	74.7	194664.81	19.19	90.93	13.31	21	9.82	2.448	2.63	99619.4	15.21	1.309
26	VMC95 212	77.3	257855.02	17.85	90.03	12.21	19.57	10.9	2.354	2.58	110613.5	14.24	1.32
27	NCO-334	65.6	237816.99	16.96	88.34	11.51	19	11.1	2.548	2.44	136396.9	13.76	1.271
28	FG03 418	69.8	184731.11	19.06	91.23	13.24	20.79	11.7	2.527	2.64	108611.4	16.52	1.54
29	CO449	63.4	181656.15	17.83	90.31	12.27	19.59	8.88	2.477	2.62	100092.2	13.29	1.08
30	FG03 204	54.9	191504.07	18.21	91.06	12.62	19.9	10.2	2.63	2.69	98034.82	15.83	1.274
31	FG02 553	67	202959.95	17.96	90.29	12.34	19.77	9.67	2.124	2.65	102663.2	14.43	1.17
32	FG03 103	68.1	225035.79	17.71	90.35	12.31	19.57	10.7	2.643	2.65	90884.98	14.41	1.314
33	FG03 318	56.2	163633.75	17.92	89.55	12.29	19.9	10.2	2.725	2.78	82001.93	14.27	1.268
34	FG04 708	77.8	301267.25	18.61	89.41	12.73	20.69	6.45	1.676	2.53	113309.4	14.48	0.826
35	FG04 705	75.7	263476.38	19.18	89.91	13.23	21.23	8.85	2.199	2.49	103371.2	17.48	1.156

Table 3. Contd.

36	FG02 551	55.3	160240.66	17.46	90.2	12.21	19.55	9.42	2.281	2.71	97298.79	14.72	1.147
37	FG03 173	62.3	217494.25	18.21	90.65	12.59	20.01	9.25	2.277	2.56	99489.64	13.13	1.164
38	FG04 187	84.1	232984.5	17.14	88.46	11.62	19.2	13.1	2.669	2.58	113411.6	13.04	1.506
39	FG03 372	65.4	169686.89	18.53	90.05	12.75	20.45	11.3	2.844	2.66	97016.87	14.9	1.436
40	FG03 214	64.2	189855.88	17.64	89.49	12.12	19.56	9.03	2.445	2.52	99992.32	13.44	1.091
41	C86-56	71.5	241200.44	17.33	90.41	12.13	19.46	9.92	2.227	2.72	103595.6	12.77	1.199
42	SP70-1284	67.1	240728.83	17.91	90.17	12.3	19.74	9.32	2.143	2.75	109318.3	14.9	1.135
43	C86-165	50.7	221037.66	17.49	91.33	12.17	19.26	10.3	2.278	2.6	102467.5	14.98	1.25
44	B78-505	60.2	224108.15	17.93	90.23	12.39	19.74	9.82	2.376	2.9	93891.2	14.47	1.195
45	C132-81	71.4	198182.45	17.45	88.71	11.94	19.49	9.76	2.132	2.94	86807.38	13.65	1.152
46	C86-12	65	164182.53	18.76	90.34	12.75	20.36	9.74	2.23	2.77	97970.94	18.4	1.244
47	C90-501	68.2	167499.59	20.55	90.36	12.74	20.32	9.81	2.135	2.76	94214.57	16.49	1.256
48	B52-298	73	252441.62	17.18	88.46	11.67	19.28	9.04	2.148	2.62	100737	13.22	1.044
49	CO- 678	48.2	168277.54	16.36	87.9	11	18.46	10.3	2.688	2.69	95908.11	12.62	1.123

*NT: Number of tillers (ha^{-1}); Pol: Pol in juice (%); RS: recoverable sucrose percentage (%); Brix: Brix in juice (%); MSH: milleable stalk height (m); DM: Milleable stalk diameter (mm); MSTP: milleable stalk population (ha^{-1}); NIPS: numbers of internodes per stalk; SYLD: sugar yield (t $ha^{-1}m^{-1}$); CYLD: cane yield (to $ha^{-1}m^{-1}$); CD: code.

Table 4. Clusters of 49 sugar cane genotypes based on traits contributing to sugar yield.

Clusters	Genotypes	Freq.	%
I	1, 21, 22, 16, 33, 39, 20	7	14.28
II	9, 36,14, 29, 40, 13, 37, 23, 31, 42, 41, 44, 32, 13, 30, 43, 18, 45, 48	17	34.69
III	24, 49	2	4.08
IV	3, 26 38, 27	4	8.16
V	2, 5, 25, 28, 12, 10, 46, 47	8	16.32
VI	7	1	2.04
VII	6, 11	2	4.08
VIII	19	1	2.04
IX	4, 17, 35, 8	4	8.16
X	34	1	2.04

*Freq: Frequency; 1-49=codes of the genotypes and are given in Tables 1 and 3.

among these genotypes had no any correspondence with the geographic origin (from where they were introduced). This suggested that the genotypes of different geographic origin had genetic similarity and genotypes of the same geographic origin had different genetic background,

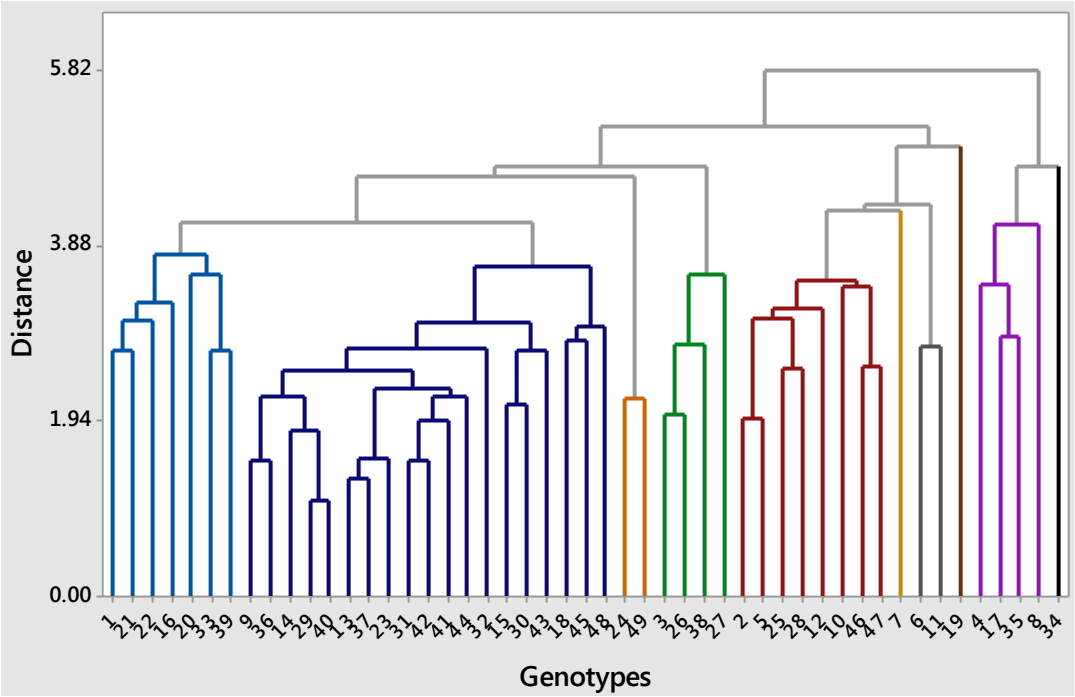

Figure 1. Dendrogram of 49 sugar cane genotypes based on Euclidean distance.

Table 5. Euclidean distances between cluster groups.

CL	I	II	III	IV	V	VI	VII	VIII	IX	X
I	0	4.1265	3.7805	5.7723	3.4236	4.3103	2.8287	5.8858	4.285	8.158
II	4.12653	0	6.0381	3.7624	3.6033	3.7174	3.8664	4.21976	7.0447	7.0159
III	3.78046	6.0381	0	5.5644	6.4863	5.193	3.8829	6.99782	4.9947	7.4587
IV	5.77226	3.7624	5.5644	0	6.0394	4.7534	3.909	4.33017	7.0749	4.3024
V	3.42356	3.6033	6.4863	6.0394	0	4.1943	3.964	5.32772	6.3177	7.8924
VI	4.31028	3.7174	5.193	4.7534	4.1943	0	4.5438	5.78581	7.6557	7.6557
VII	2.82874	3.8664	3.8829	3.909	3.964	4.5438	0	4.04076	4.0382	5.989
VIII	5.8858	4.2198	6.9978	4.3302	5.3277	5.7858	4.0408	0	7.685	5.8337
IX	4.28496	7.0447	4.9947	7.0749	6.3177	7.6557	4.0382	7.68504	0	8.9809
X	8.15798	7.0159	7.4587	4.3024	7.8924	7.6557	5.989	5.83368	8.9809	0

*Cl: Clusters.

suggesting that a high proportion of total genetic variation was retained within the groups of origin and active genetic ex-change was found between different origins. This relationship suggests the introduction strategy was successful in terms of improving the base of the crop in Ethiopia and increases the chances of selection efficiency during parental selection in the future using the traits that contributed more to the existed phenotypic diversity. Similar results were also observed by Ram and Hemaprabha (1998) and Tahir et al. (2013) in which they found the progenies of a cross clustered independently of their parents. Hence, our introduction strategy was appropriate in terms of broadening the narrow genetic pool of sugarcane in Ethiopia.

Euclidean distances between clusters groups and contributions of variable (Traits) to diversity

Distances between clusters groups based on the Euclidean Distances statistic (Table 5) revealed that groups I, II, III, V, VI and IX had the highest distances to group X with a single genotype (34) suggesting the genotype was an outlier. Moreover, cluster group IX was

Table 6. Step wise order inclusion of variables in the discriminant analysis.

Step number	Multivariate statistics					Statistic	
	Trait	Trait entered	R-Square	Partial R-Square	Trait removed	Wilks' Lambda	Pillai's Trace
1	Pol	Pol	0.7790**	-	No	0.0022**	0.7798**
2	Pol	Pol	0.7790**	0.7676**	No	0.0615**	1.4974**
	Cyld	Cyld	0.7352**	0.7215**			
3	TN	TN	0.6629**	0.6438**	No	0.0219**	2.0788**
	Pol	Pol	0.7790**	0.7610**			
	Cyld	Cyld	0.6426**	0.7083**			
4	TN	TN	0.6529**	0.6066**	No	0.0107**	2.5482**
	Pol	Pol	0.7790**	0.7619**			
	Cyld	Cyld	0.7352**	0.6602**			
	MSP	MSP	0.6424**	0.5086**			
5	TN	TN	0.6629**	0.6190**	No	0.0055**	2.9653**
	Pol	Pol	0.7790**	0.7545**			
	Purity	Purity	0.4923**	0.4830*			
	Cyld	Cyld	0.7352**	0.6862**			
	MSP	MSP	0.6424**	0.5218*			
6	Sprout	No		0.2130ns	No further steps possible		
	RS			0.1711ns			
	Brix			0.1918ns			
	Ht			0.1623ns			
	DM			0.1926ns			
	NIPS			0.2843ns			
	SYLD			0.2530ns			

**Highly significant at P<0.01; *Significant at P<0.05; ^{ns}Non-significant; DM: Diameter; NT: number of tillers (ha^{-1}); Pol: Pol in juice (%); RS%: recoverable sucrose percentage (%); Brix: Brix in juice (%); MSH: milleable stalk height (m); DM: milleable stalk diameter (mm); MSP: milleable stalk population (ha^{-1}); NIPS: numbers of internodes per stalk; SYLD: sugar yield (t ha^{-1}m^{-1}); CYLD: cane yield (t ha^{-1}m^{-1}).

more distanced from cluster groups II (7.016), III (7.461), IV (7.070), VI (7.651) and VIII (7.680); genotypes of each group had ample diversity and can be crossed with genotypes in groups IX. On the contrary, the smallest distance was observed between cluster groups I and VII, the diversity between the groups was narrow. Generally, the smallest and larger distances among cluster groups suggest high probability of getting divergent genotypes that are useful for crossing purposes.

A step wise discriminant analysis by minimizing the Wilk's criteria (Table 6) resulted in significant F-values for Pol%, cane yield, number of tillers; Purity% and milleable stalk population, suggesting that these traits contributed more to the discrimination among the groups.

Results of different studies demonstrate that the linear discrimination function is a usefully tool for screening and evaluating variation among sugarcane genotypes studied. Moreover, the step wise discrimination procedure provided in Table 6 indicated that Pol in juice, cane yield (t ha^{-1}m^{-1}), number of tillers (ha^{-1}), purity% and milleable stalk population (ha^{-1}) significantly explained total variability (R^2) of 77.90, 73.52, 66.29, 64.24 and 49.23%, respectively; revealing these traits contribute more to the diversity which existed among the 49 sugarcane genotypes. This result was inconsistent with findings reported by Kang et al. (2013) in which Brix and juice contents contributed more to divergence among genotypes. It can be concluded that the 49 sugarcane genotypes were diversified for Pol in juice; cane yield (t ha^{-1}m^{-1}), number of tillers (ha^{-1}), purity% and milleable stalk population (ha^{-1}).

Principal component analysis

Biplot of principal component analysis based on

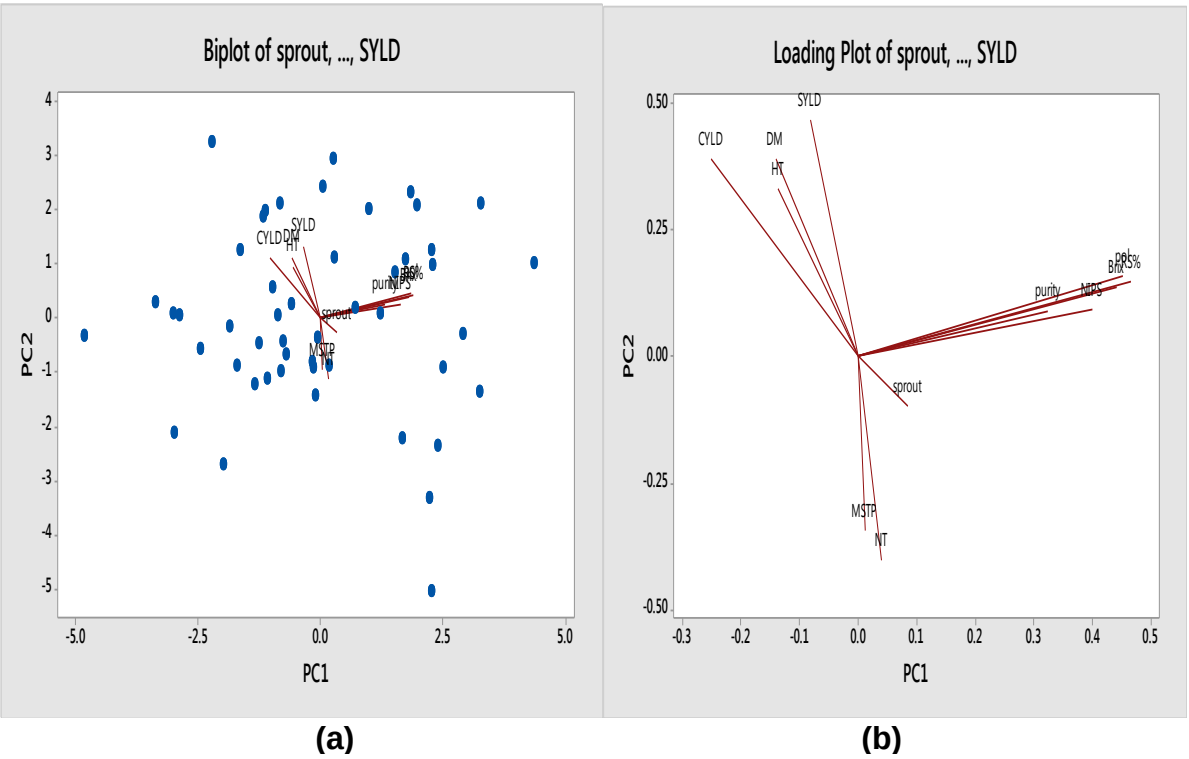

Figure 2. (a) Biplot distribution of 49 sugar cane genotypes displaying 12 traits and (b) loading plot distribution of 12 traits. DM: Diameter; Sprout NT: number of tillers (ha^{-1}); Pol: Pol in juice (%); RS: recoverable sucrose%; Brix: Brix in juice (%); HT: milleable stalk height (m); DM: milleable stalk diameter (mm); MSTP: milleable stalk population (ha^{-1}); NIPS: numbers of internodes per stalk; SYLD: sugar yield (t $ha^{-1}m^{-1}$); CYLD: cane yield (t $ha^{-1}m^{-1}$).

correlation matrix is depicted in Figure 2. It was sufficient enough to show correlation among variables considered. 58, 96 and 100% of the variation existed among the genotypes was explained by the first two, eight and ten principal components, respectively (Figure 2a). The Biplot and loading plot (Figure 2b) were able to separate cane yield and its components (large PC2 score) from the quality traits (large PC1) highlighting characterization of genotypes in terms of traits would be possible using principal component analysis.

As far as the relationships (correlation) existed among the traits is concerned, cane yield, milleable stalk height, diameter and sugar yield were characterized with large PCA2 score and were more correlated (angles among the specified traits were acute) which agreed with reports of Rewati and Joshi (2005) in which milleable stalk height and diameter were positively correlated with cane yield. Selection of sugarcane genotypes based on cane yield, milleable stalk height and diameter increased sugar yield which is consistent with the results reported by Khan et al. (2013) and Masri et al. (2015). On the contrary, quality parameters such as purity%, pol in juice, Brix in juice and recoverable sucrose percentage were characterized with large PCA 1 and had small angles among themselves; indicating strong and positive correlation. Moreover, milleable stalk population and tiller number were characterized with small PCA score and were negatively correlated with cane yield and sugar yield which disagreed with the results of Punia et al. (1983). Sprout percentage was positively correlated with milleable stalk population which was consistent with reports of Sahu et al. (2008) in which germination% showed a positive and significant correlation with number of millable canes.

Weak and positively correlation was observed between sugar yield Pol%, Purity%, Brix and numbers of internodes per single stalk. Furthermore, milleable stalk diameter made obtuse angle with numbers of internodes per stalk implying negative correlation disagreed with report of Kumar and Kumar (2014). The inconsistencies of the results might be attributed to the nature of quantitative traits which are more affected by environment and sampling error. Generally, weak correlation existed between sugar yields and recoverable sucrose percentage which is not expected as recoverable sucrose percentage is the main component of sugar yield. Thus, additional analyses such as path coefficient analysis, which enable to compute indirect effects of secondary traits on dependent trait, should be used.

Results obtained from PCA analysis were supported by linear discriminant analysis in that Pol% and cane yield which contributed more to the total variability in the linear discriminant analysis had long vectors in the Biplot

Table 7. Phenotypic path coefficients showing direct (diagonal) and indirect effects (off diagonal) of 11 sugarcane traits on sugar yield.

Traits	Sprout%	Tiller	Pol	Purity	RS%	Brix	CYLD	Height	MSP	DM	NIPS
sprout	**0.01358**	-0.00722	0.003191	-0.0001	0.058078	-0.01348	0.08917	-0.000157	0.000323	-0.00373	-0.00048
Tiller	0.006932	**-0.01414**	-0.00165	-0.00016	-0.01341	0.000183	-0.23835	-0.000519	0.001292	-0.00822	0.000398
Pol	0.002567	0.001379	**0.016882**	2.71E-05	0.378162	-0.04761	-0.26173	-0.000217	0.000231	0.001669	-0.00373
Purity	-0.00247	0.001003	0.00939	0**.000933**	0.300446	-0.02188	-0.2226 -	-0.000156	0.000549	-0.0005	-0.002
RS%	0.001891	0.000455	0.015309	0.000375	**0.41702**	-0.04938	-0.29027	-0.000172	0.000372	0.001119	-0.0035
Brix	0.003425	4.85E-05	0.015042	5.85E-05	0.385327	**-0.05344**	-0.2809	-0.000174	0.000223	0.00166	-0.00355
CYLD	0.001178	0.003278	-0.0043	-0.0002	-0.11771	0.014596	**1.028372**	0.0007413	-0.00146	0.000587	0.001556
Height	-0.00152	0.005261	-0.00262	-0.0001	-0.05149	0.006641	0.545963	**0.0013964**	-3.3E-05	0.003156	3.85E-05
MSP	-0.00134	0.005588	-0.00119	-0.00016	-0.04739	0.003649	0.45961	0.0000142	**-0.00327**	0.011761	0.001077
DM	0.002948	-0.00677	-0.00164	2.71E-05	-0.02719	0.005165	-0.03515	-0.000257	0.002239	**-0.01717**	0.000358
NIPS	0.001305	0.001134	0.012663	0.000375	0.293461	-0.03824	-0.32222	-0.000011	0.000709	0.001238	**-0.00497**

*Coefficient of determination=0.99 and residual=0.06; NIPS: number of internodes per stalk; CYLD: cane yield; DM: milleable stalk diameter; TN: numbers of tillers; SYLD: sugar yield; MSP: milleable stalk population; HT: milleable stalk height.

(Figure 2a) and loading plot (Figure 2b), indicating these traits contributed more to the total variation explained by the first two dimensions. This suggests that the PCA and linear discriminate analysis were similar in identifying the traits which were dominant in explaining the existing variability among the genotypes. Moreover, these traits can be further used to discriminate cluster groups and are helpful for parent selection in sugarcane breeding programs as the variation existed among the genotypes was highly significant for these traits (Table 2).

Path coefficient analysis

The gap in principal component or correlation analysis with respect to causal relationships among traits necessitates path coefficient analysis to be used in selection to utilize both direct and indirect relationships among traits (Kang, 2015). Thus, the use of path coefficient analysis will be mandatory to increase the efficiency of our selection. The path coefficient analysis presented (Table 7) indicated that the highest positive direct effect on sugar yield was exerted by cane yield (1.028) followed by recoverable sucrose percentage (0.417) and Pol (0.016) which is consistent with report of Khan et al. (2013). In the contrary, small negative direct effects on sugar yield were exerted by number of tillers, Brix, stalk diameter, milleable stalk population and numbers of internodes per stalk. Furthermore, milleable stalk height and milleable stalk population exerted indirect effect of 0.546 and 0.459, respectively via cane yield, while Brix% in juice, Pol% in juice, Purity% and number of internodes had indirect effects of 0.385, 0.378, 0.300 and 0.293, respectively via recoverable sucrose percentage. Hence, these traits should be given due consideration during selection for high sugar yield. This result was similar with reports of Khan et al. (2012) in which higher number of tillers, good weight, endowed with better available sugar in the cane (Pol%), commercial cane sugar (CCS)% and purity% were the important characters which should be considered in selection of higher sugar yield in sugarcane genotypes.

Selection of sugarcane genotypes on the basis of cane yield and recoverable sucrose percentage (%) would be beneficial for increasing sugar yield in sugarcane. Our result was in agreement with report of Hussein et al. (2012) except for the effect of number of milleable stalks to sugar yield in our study was negative and negligible. Moreover, the coefficient of determination and the residual effect in this study were 0.990 and 0.061, respectively suggesting that most of the variability in sugar yield was best explained by the traits studied (causal factors) and the error was negligible and thus, no additional traits is necessary to be included in selection. Generally, the path coefficient analysis, in the present study was sufficient enough to increase the efficiency of selection. For example, the weak correlation between sugar yield and recoverable sucrose%,

Table 8. Multiple linear regression model to explain sugar yield variation using its related characters.

Variable name	Estimate	Standard error
Sprout (%)	0.000244	0.000255
Numbers of tillers (ha^{-1})	-6.96E-08	7.26E-08
Pol%	0.00271	0.00540
Purity%	0.00092	0.00957
Recoverable sucrose%%	0.1080*	0.0421
Brix%	-0.0099	0.0258
Cane yield	0.12199**	0.00256
Milleable stalk height (m)	0.0008	0.0118
Milleable stalk populations(ha^{-1})	-0.000000212	0.000000262
Milleable stalk diameter (cm)	-0.0026	0.0218
Number of internodes per stalk (ha^{-1})	-0.00039	0.00157
Intercept	-1.232	0.883
Model significance	<001	-
R^2	0.995	-
R^2 of eliminated traits	0.00413	-

**Highly significant at p<0.01;*Significant at p<0.05.

in the principal component analysis, was ruled out by path coefficient analysis in such a way that it was able to compute the direct effect of recoverable sucrose percentage (0.417) and its highest indirect effects via Brix% in juice (0.385), Pol% in juice (0.378) and Purity% (0.300).

Multiple linear regressions analysis

Although path coefficient analysis provides a picture of the pattern of association, it cannot construct a prediction equation for dependent variable using its components (El-Shafi and Ismail, 2006). For this reason, multiple regressions were used to develop the regression model. SY (Sugar yield) =-1.232+0.000244 (sprout %) +-6.96E-08 (Tiller Numbers) +0.00271 (Pol %) +0.00092 (Purity%) +0.1080 (Yield %) +-0.0099 (Brix %) +0.12199 (cane yield) + 0.0008 (stalk height) + -0.000000212 (stalk population) + -0.0026 (stalk diameter)-0.00039 (number of internodes). The result presented in Table 8 demonstrated that 99.5% of the variability is explained as R^2 and the rest 0.41% is attributed to unknown variation. Furthermore, the multiple linear regressions indicated that recoverable sucrose percentage and cane yield significantly contributed to sugar yield which is similar to the results reported by Hussein et al. (2012) in which recoverable sucrose percentage and stalk weight contributed more to sugar yield.

Conclusion

The multivariate analysis generates relevant information about the performance of the genotypes, relationship among genotypes and interrelationships among traits which is very important for sugarcane breeding programmes. The cluster analysis demonstrated that the 49 sugarcane genotypes studied were clustered into ten groups and were highly significantly different for Pol in juice, cane yield (t $ha^{-1}m^{-1}$), number of tillers (ha^{-1}), purity% and milleable stalk population (ha^{-1}). The relationship existed among sugarcane genotypes studied was not related to their geographic origin, suggesting that a high proportion of total genetic variation was retained within the groups of origin and active genetic ex-change was found between different origins indicating that the introduction strategy was successful.

Generally, regarding with the interrelationships among the traits, more information was generated following the use of principal component, linear discriminant, path coefficient and multiple regression analyses indicating the use of multivariate analyses was successful. Path coefficient was unique in generating information about the indirect effects of traits on sugar yield which was very important to provide substantial information about indirect effects of traits that are very relevant to increase selection efficiency in sugarcane plant breeding programs.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Determination of planting spacing for improved yield and yield components of Dekoko (*Pisum sativum* var. *abyssinicum)* at Raya Valley, Northern Ethiopia

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Dekoko is a cool-season food legume cultivated in Tigray, Northern Ethiopia. It is highly appreciated by the local people for its taste and high market value. Yields of Dekoko, however, are limited by improper planting spacing. Thus, an experiment was conducted in 2013 and 2014 cropping seasons to determine the appropriate planting spacing of Dekoko that maximizes its productivity under rain fed conditions. Treatments comprised combinations of three plant spacing (10, 15 and 20 cm) and three levels of row spacing (40, 50 and 60 cm) and broad casting were done in a randomized complete block design with three replications. Plant spacing influenced plant height, grain yield and biomass yield. The greatest plant height (50.63 cm) was obtained at a spacing of 60x20 cm while the maximum mean grain (544.58 kg ha^{-1}) and biomass yields (1562.65 kg ha^{-1}) were obtained at spacing of 40x15 cm in both cropping seasons. A planting spacing of 40 x 15 cm is recommended for the growers in the study area.

Key words: Dekoko, plant spacing, row spacing, yield, yield components.

INTRODUCTION

Ethiopia is the largest producer of cool-season food legumes (CSFLs) in Africa. The CSFLs are largely produced by subsistence farmers and serve as supplementary protein sources and soil fertility restorers. Among the CSFLs, a pea variety locally called Dekoko (*Pisum sativum* var. *abyssinicum*) is a unique crop developed and cultivated in Ethiopia (Haddis et al., 2015). It is restricted to highland regions of Ethiopia (South Tigray and North Wollo) (Yemane and Skjelvag, 2002).

Dekoko is well appreciated for its taste and obtains a premium price in local markets as compared to field pea or 'Ater' (*Pisum sativum var. sativum*) (Yemane and Skjelvag, 2002). Farmers and consumers call it the "Dero-Wot of the poor". This may be due to its good taste and high nutritional value. Most often, the dry seeds of

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Dekoko are decorticated and split ('split peas') before boiling. According to Sentayehu (2009), in Ethiopia, the annual consumption per person of field pea including Dekoko seeds is estimated at 6 -7 kg. Both field pea and Dekoko are considered as protein supplement in the cereal based diets of Ethiopia. The work of Yemane and Skjelvag (2002) showed that due to its favorable amino acid profile, it can be a suitable complementary protein source for a cereal based diet. Moreover, its early maturation can make it an important crop in areas where the growing season is too short for other CSFLs and yield losses caused by terminal droughts are common. The CSFLs are soil fertility restorers for subsistence farmers in Ethiopia (Yemane and Skjelvag, 2002).

Optimum plant population has a promising impact in improving the productivity of legumes. According to Pawar et al. (2007), dry weight of green bean was increased with increased row spacing (30 cm) as compared to narrow row spacing (22.5 cm). Wider row spacing (60 and 45 cm) gave significantly higher number of pods plant^{-1} as compared to 30 cm row spacing (Mohammed et al., 1984). This is supported by Kakiuchi and Kobata (2004) who concluded that lower plant density increased the pod number plant^{-1} and the higher plant density, decreased the pod number plant^{-1}. Samih (2008) reported that high yield was observed in the case of high plant populations (20x30 cm) over that of low plant population (60x30 cm) of bush beans. Similarly, Gan et al. (2007) have shown increase of grain yield at higher plant density in chickpea. The use of high plant density usually increases seed yield of chickpea in areas with a short growing season (Gan et al., 2003), but the magnitude of the yield increase depends on environmental conditions. However, Parihar (1996) indicated that row spacing had no significant effect on seed yield. Other studies by Nawaz et al. (1995) and Felton et al. (1996) concluded that dry matter production and plant height of chick pea were higher in higher plant populations (60 plants m^{-2}), but a population of 40 plants m^{-2} gave the maximum grain yield.

Dekoko is the most neglected pulse crop in the Tigray Region. Research has not yet been done on improved management practices for yield improvement of Dekoko. Productivity is low because of lack of improved varieties, low soil fertility, little or no application of fertilizers, insect pests and lack of improved agronomic practices including seeding rate and row spacing.

An experiment was conducted on planting spacing of Dekoko in Raya Valley, Northern Ethiopia. The objective of this study was to identify the optimal planting spacing of Dekoko under rain fed conditions.

MATERIALS AND METHODS

Description of the experimental area

The experiment was carried out under rain fed conditions in 2013 and 2014 cropping seasons at Mehoni Agricultural Research Center testing site (12°41'50" N and longitudes of 39°42'08" E). It is 678 km north of Addis Ababa. The area is situated at an altitude of 1578 m above sea level (m.a.s.l) with mean annual rainfall of 750 mm and minimum and maximum annual temperature is 18 and 25°C, respectively. The textural class of the soil was clay loam with a pH value of 7.9 at a soil depth of 0-30 cm (Haileslassie et al., 2015).

Treatments and experimental procedures

The experiment consists of combinations of three intra row spacing (10, 15 and 20 cm) and three inter row spacing (40, 50 and 60 cm), and a broad casting planting pattern. The treatments were arranged in a randomized completed block design (RCBD) with three replications having a plot size of 6 x 5 m. The spacing between blocks and plots was 1.5 and 0.5 m, respectively. Urea and triple super phosphate (TSP) were used as source of N and P, respectively. 20 kg P ha^{-1} in the form of P_2O_5 was applied at planting as band for row planting and broad casted application for broad casting method of planting. Similarly, 23 kg ha^{-1} of N was applied as a starter at planting. Local variety of Dekoko was used as a test crop. The other crop management practices like weeding (first weeding was done three weeks after planting and second weeding was six weeks after planting), thinning and chemical spraying were applied uniformly to all plots as per recommendations in field pea.

Data collection and statistical analysis

Data on days to 90% maturity, plant height (cm), pod number plant^{-1}, seed number pod^{-1}, grain yield (kg ha^{-1}), biomass yield (kg ha^{-1}) were collected and analyzed. The data were collected from middle rows of a net plot area where the two outer most rows of each treatment were left as border effects. In addition, 0.10, 0.15 and 0.20 m length in both ends for 10, 15 and 20 cm intra row spacing, respectively, of each harvestable row were also left as border effects. Moreover, the net harvestable area for broad casting method of planting was 5.5 by 4.5 m. Five plants from the net plot area were pre tagged to collect data of plant height, pod number plant^{-1} and three pods per each of these plants with a total of fifteen pods were considered to determine seed number pod^{-1}. Dry matter was measured using an electronic balance after the net plot area plants had been harvested and oven dried at 70°C until constant dry weight was attained. Similarly, shelled seed yield was weighed using electronic sensitive balance from the harvested plants of net plot area.

The collected agronomic data were subjected to the analysis of variance (ANOVA) using the SAS software computer package version 9.1 (SAS Institute, 2004) and significance difference among the treatment means was computed with least significant difference (LSD) at 5% probability level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Days to 90% physiological maturity

Days to 90% physiological maturity did not differ due to planting spacing (Table 1). This lack of significance difference could be most probably due to less competitive effect of the associated Dekoko plants for limited growth resources until physiological maturity. Generally, it matured at a range of 79.67 – 81.33 days starting from its planting time (Table 1).

Table 1. Effect of planting spacing on mean values of days to 90% maturity and plant height of Dekoko.

Treatments	Days to 90% maturity			Plant height (cm)		
	2013	2014	Mean	2013	2014	Mean
Broad casting	80.67	80.00	80.33	40.33	44.33e	42.33d
40 X 10 cm	80.33	80.00	80.17	41.00	47.73de	44.37cd
50 X 10 cm	80.33	79.67	80.00	41.00	49.33cde	45.17bcd
60X10 cm	80.33	79.67	80.00	42.00	50.67cd	46.33bcd
40X15 cm	80.33	80.00	80.17	41.67	54.67ac	48.17abc
50X15 cm	81.33	80.33	80.83	42.00	53.67acd	47.83abc
60X15 cm	82.00	80.33	81.17	42.67	56.00a	49.33ab
40X20 cm	80.33	80.00	80.17	43.67	54.67ac	49.17ab
50X20 cm	81.33	80.67	81.00	41.67	55.40ac	48.53abc
60x20 cm	81.00	80.33	80.67	42.00	59.27a	50.63a
CV (%)	1.59	0.76	1.00	8.58	6.91	5.17
LSD (0.05)	NS	NS	NS	NS	6.23	4.18

Means with the same letter (s) in the same column are not significantly different at P<0.05; NS= non-significant; LSD= least significant difference; CV= coefficient of variance.

Plant height

Plant height was not significantly affected by planting spacing in 2013 cropping season, but highly significantly varied (P<0.01) in 2014. In 2014 cropping season, the highest plant height (59.27 cm) was obtained from 60 x 20 cm, while the lowest (44.33 cm) was from broad casting method of planting. Similarly, the pooled mean result indicated that the greatest (50.63 cm) and least (42.33 cm) plant height was recorded from 60 x 20 cm and broad casting methods, respectively. The greatest plant might be most probably due to availability of free access of environmental resources (water, nutrients and light) for the plants in the wider spacing. In line with this result, Shirtliffe and Johnston (2002) reported that plant height of different cultivars of field pea was significantly affected by row spacings. Similarly, Yayeh et al. (2014) showed that highest plant height for Sefinesh field pea variety was obtained under higher inters and intra row spacing. However, contrasting findings were achieved by Derya (2013) who indicated that denser plant population of pea increased plant height due to competition among plants.

Number of pods plant^{-1}

Concerning pods plant^{-1}, it was not significantly influenced due planting spacing in both cropping seasons. All the treatments were significantly at par. The pooled mean result, though non-significant, showed that slightly high number of pods (21.83) was obtained at spacing of 40 x 15 cm while the lowest result (18.83) was gained from broad casting method of planting (Table 2). Number of pods plant^{-1}, an important primary yield component, ranged from 17.00 to 20.33 during 2013 and 19.00 to 23.67 during 2014 with maximum average pods plant^{-1} of 21.33. Yayeh et al. (2014) found that number of pods plant^{-1} of field pea was not significantly affected by intra and inter row spacing, which was concurrent to the current finding. Similarly, in an experiment on peas, Biabani (2008) found that the effect of density on the pods plant^{-1} was not significant, while Biabani (2010) and Khandan et al. (2010) reported that influence of the density on the number of pods plant^{-1} was significant on chickpea

Number of seeds pod^{-1}

According to Table 2, spacing did not significantly affect the number of seeds pod^{-1} of Dekoko in both seasons. The number of seeds pod^{-1} of Dekoko ranged from 3.33 to 4.33. In agreement with this finding, Yayeh et al. (2014) reported that seeds pod^{-1} of field pea was not significantly influenced by planting spacing, and number of seeds pod^{-1} ranged from 4.4 to 4.9. Moreover, Ali et al. (2012) reported that, influence of plant density on number of grains pod^{-1} of peas was non-significant, the grains pod^{-1}ranged from 6.3 to 7.4. This result also confirmed the findings of Derya (2013).

Grain yield

With respect to grain yield of Dekoko, it was highly significantly (P<0.01) affected by planting spacing in both seasons. Accordingly, in 2013 cropping season, the highest grain yield (549.90 kg ha^{-1}) was obtained at a spacing of 40 x 15 cm, whereas the lowest numerical

Table 2. Effect of planting spacing on mean values of pod number plant^{-1} and seed number pod^{-1} of Dekoko.

Treatments	Pod number plant^{-1}			Seed number pod^{-}1		
	2013	2014	Mean	2013	2014	Mean
Broad casting	18.67	19.00	18.83	4.00	3.33	3.67
40 X 10 cm	20.33	21.33	20.83	4.33	4.00	4.17
50 X 10 cm	17.33	21.67	19.50	3.67	4.00	3.67
60X10 cm	18.67	21.33	20.00	4.33	3.67	3.67
40X15 cm	20.00	23.67	21.83	3.67	4.33	4.33
50X15 cm	17.00	22.00	19.50	3.33	3.33	3.50
60X15 cm	20.00	20.33	20.17	3.67	4.33	3.83
40X20 cm	18.00	21.67	19.83	3.67	3.67	3.67
50X20 cm	18.67	21.33	20.00	3.67	4.00	3.83
60x20 cm	19.00	23.67	21.33	3.67	4.00	3.83
CV (%)	11.93	8.86	7.22	12.72	15.34	10.30
LSD (0.05)	NS	NS	NS	NS	NS	NS

NS= Non-significant; LSD= least significant difference; CV= coefficient of variance.

Table 3. Mean values of grain and biomass yields of Dekoko as influenced by planting spacing.

Treatments	Grain yield (kg ha^{-1})			Biomass yield (kg ha^{-1})		
	2013	2014	Mean	2013	2014	Mean
Broad casting	463.22bcd	458.58bcd	460.90bcd	1420.97acd	1405.04bc	1413.00bcd
40 X 10 cm	493.22abc	514.48ab	503.85ab	1510.02a	1474.17ab	1492.10ab
50 X 10 cm	502.18ab	486.06abc	494.12bc	1445.65ac	1437.12ab	1441.39bc
60X10 cm	456.22bcd	447.98cd	452.10cd	1429.56ac	1386.56bc	1408.06bcd
40X15 cm	549.90a	539.25a	544.58a	1576.11a	1549.18a	1562.65a
50X15 cm	457.33bcd	448.61cd	452.97cd	1384.21cde	1372.70bc	1378.46cde
60X15 cm	434.94d	419.08d	427.01d	1339.23cde	1335.19c	1337.21cde
40X20 cm	482.11bcd	434.41cd	458.26cd	1366.29cde	1324.39c	1345.34cde
50X20 cm	442.00cd	416.11d	429.06d	1263.41de	1349.29bc	1306.35de
60x20 cm	426.23d	409.38d	417.81d	1243.85e	1307.30c	1275.57e
CV (%)	7.03	7.72	5.51	6.77	5.60	4.70
LSD (0.05)	56.77	60.55	43.88	162.33	133.87	112.66

Means with the same letter(s) in the same column are not significantly different at P<0.05; LSD= least significant difference; CV= coefficient of variance.

result (426.23 kg ha^{-1}) was recorded from a spacing of 60 x 20 cm (Table 3). A similar result was also obtained in 2014. With reference to Table 3, the highest grain yield was produced at a spacing of 40 x 15 cm where it gave 31.72% yield advantage over 60 x 20 cm spacing. Moreover, the pooled mean result indicated that planting spacing of 40 x 15 cm produced the highest yield of Dekoko. The lowest grain yield production could be due to extreme wider spacing at which the required population density ha^{-1} could not be accommodated and this in turn results in production of low grain yield because of minimum population density. This confirmed the previous findings of Yayeh et al. (2014) who showed that further increase in intra and inter row spacing together for small seeded field pea cultivars (Megeri) result in yield penalty. Likewise, Ali et al. (2012) and Derya (2013) found that seed yield of field pea was significantly affected due population density.

Biomass yield

Like grain yield, biomass yield was significantly influenced (P<0.05) by planting spacing in both seasons (Table 3). In 2013, the highest biomass yield (1576.11 kg ha^{-1}) was produced at a spacing of 40 x 15 cm which gave 26.73% more biomass yield than 60 x 20 cm at which the lowest result was obtained. Correspondingly, in 2014, 40 x 15

cm, which gave the highest biomass yield, was statistically at par with 40 x 10 and 50 x 10 cm. Nevertheless, the lowest value (1307.30 kg ha^{-1}) was obtained from 60 x 20 cm which was 15.61% lower than the biomass yield of 40 x 15 cm. In addition, the pooled mean result revealed that the maximum yield was observed at spacing of 40 x 15 cm, while the minimum was from 60 x 20 cm. This could be attributed to sparse density of plants in wider spacing to have appropriate density and which resulted in low biomass yield. This result is consistent with the work of Ali et al. (2012) who reported that biological yield of peas was significantly affected by plant density where the highest density was obtained from 70 plants m^2.

Conclusions

Optimum planting spacing has a promising impact in improving the productivity of Dekoko. According to the results of this experiment, plant height, grain yield and biomass yield were significantly affected by planting spacing of Dekoko. The greatest plant height was obtained at a spacing of 60 x 20 cm. Moreover, the maximum grain and biomass yields were obtained at spacing of 40 x 15 cm in both cropping seasons as compared to the other treatments. It is, therefore, concluded that planting spacing of 40 x 15 cm can be recommended for the growers in the study area to improve Dekoko productivity. Moreover, it can be recommended from the findings that further investigation on different varieties together with different fertilizer levels, soil types, utilization and quality aspects, can be a step forward to identify best technology on the growth and yield improvements of Dekoko.

Conflict of interest

The authors have not declared any conflict of interest

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Full Length Research Paper

Diazotrophic bacteria inoculation associates with acids and nitrogen in corn

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A successful application of humic acids and diazotrophic bacteria in corn represents a potential that collaborates to break the current energetic consumption paradigm, which is based on unsustainable fossil sources. Thus, this study aimed to quantify the contribution of diazotrophic bacteria in association with humic acids and nitrogen (N) in corn, in an experiment conducted under controlled conditions in a greenhouse. The experiment was carried out at the Federal Institute of Rondônia, Campus of Colorado do Oeste-RO, Brazil. The experimental design was completely randomized with four replicates and the treatments consisted of: control; inoculation of *Azospirillum brasilense*; 80 kg ha^{-1} of N; inoculation of *A. brasilense* + humic acid; inoculation of *A. brasilense* + 80 kg ha^{-1} of N; and inoculation of *A. brasilense* + 80 kg ha^{-1} of N + humic acid. At 40 days after emergence, plants were collected, divided into shoots and roots, and the variables were analyzed. According to the results, the joint use of plant growth-promoting bacteria and humic acids increased in plant height, stem diameter and root length and volume. Inoculation of *A. brasilense* combined with 80 kg ha^{-1} of N and humic acid increased N use efficiency in corn plants by 60%, while inoculation of *A. brasilense* combined with 80 kg ha^{-1} of N increased shoot N contents in corn plants.

Key words: *Zea mays* L., *Azospirillum brasilense,* humic substances, biological nitrogen fixation (BNF).

INTRODUCTION

Brazil occupies the third position in the ranking of corn grain production, after UAE and China. Corn planted area in the 2014/2015 season is estimated at 15,769 million hectares with production of 78,554 million tons of corn (Conab, 2015). Despite its high photosynthetic rate, corn is influenced by problems of environmental stress, such as those related to low fertility of soils, which mostly have nitrogen (N) deficiency (Araujo et al., 2014).

Identifying, selecting and using corn genotypes more tolerant to N deficiency and more efficient to absorb this nutrient constitute an important strategy (Reis Junior et al., 2008). Thus, the search for genotypes that form more-efficient associations with diazotrophic bacteria must be considered. Besides the capacity of biological N

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fixation, diazotrophic bacteria associated with grasses are known to act directly on the production of phytohormones (Radwan et al., 2004; Creus et al., 2004); solubilization of phosphates (Rodriguez et al., 2004); increase in nitrate reductase activity, when occur endophytically (Cassan et al., 2008); and indirectly on the biological control of pathogens and synthesis of siderophores (Correa et al., 2008; Vessey, 2003).

Currently, endophytic diazotrophic bacteria, from the most different genera and species, have been reported in association with a large number of grasses, from both tropical and temperate climates (Reis Júnior et al., 2008). In addition, the possibility of occurrence of significant increases in yield and N availability through biological nitrogen fixation (BNF) in corn has been described by many authors. Among the diazotrophic microorganisms found in association with cereals and grasses, the species of *Azospirillum* constitute, currently, one of the most studied groups. Although the number of researches involving endophytic bacteria has increased in the last years in Brazil, little is known about the effects of using endophytic diazotrophic bacteria combined with humic substances. Humic substances, the main component of soil organic matter, can promote increase in the population of endophytic diazotrophic bacteria, acting as a physical-chemical conditioner, as well as stimulating the increase in the establishment of the bacterial inoculum inside the plant. This can be hypothetically explained as part of the effects of humic substances to increase in the number of lateral roots, which constitute the major site of infection of the host plant by endophytic bacteria (Marques Júnior et al., 2008).

However, a successful application of humic acids and diazotrophic bacteria in corn represents a potential that collaborates to break the current energetic consumption paradigm, which is based on unsustainable fossil sources. The use and the knowledge on the potentialities of these bacteria, which supply N through biological fixation and increase fertilizer use efficiency, as an alternative for N nutrition, as well as the application of humic substances, represent an economically viable, ecologically sustainable strategy. As given earlier, this study aimed to quantify the contribution of diazotrophic bacteria in association with humic acids and N in corn, in an experiment conducted under controlled conditions in a greenhouse.

MATERIALS AND METHODS

The experiment was carried out under controlled conditions, in a greenhouse, from February 2015 to March 2015, at the Plant Production Sector of the Federal Institute of Education, Science and Technology of Rondônia, Campus of Colorado do Oeste-RO, Brazil (13° 06' S; 60° 29' W; 407 m). According to Köppen's classification, the climate in the region is Awa, hot and humid tropical, with two well-defined seasons. The soil used in the study was classified as Red Yellow Argisol of very clayey texture (Embrapa, 2013) and collected in the layer of 0 to 20 cm. The soil chemical analysis before the experiment showed the following results: O.M., 10.00 g dm^{-3}; pH ($CaCl_2$), 5.30; P, 1.10 mg dm^{-3}; K, 0.14 cmolc dm^{-3}; Ca, 5.56 cmolc dm^{-3}; Mg, 1.15 cmolc dm^{-3}; Al, 0.0 cmmolc dm^{-3}; H+Al, 2.25 cmolc dm^{-3}; SB, 6.90 cmolc dm^{-3}; CEC, 9.10 cmolc dm^{-3}; and base saturation, 75.30%. Granulometric analysis showed 199 g kg^{-1} of sand, 166 g kg^{-1} of silt and 635 g kg^{-1} of clay.

The experiment was set in a completely randomized design, with four replicates, and the treatments were: 1) control; 2) inoculation of *Azospirillum brasilense*; 3) 80 kg ha^{-1} of N; 4) inoculation of *A. brasilense* + humic acid; 5) inoculation of *A. brasilense* + 80 kg ha^{-1} of N; and 6) inoculation of *A. brasilense* + 80 kg ha^{-1} of N + humic acid, totaling 24 experimental units.

Based on the results of soil chemical analysis, basal fertilization was performed in order to guarantee the establishment of the crop, by mixing the soil with 110 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O, as single superphosphate and potassium chloride, respectively. Micronutrients were applied based on crop requirements, in the form of a solution, using deionized water and salts (A.R.), according to Epstein and Bloom (2006). N fertilization was performed with the dose 80 kg ha^{-1} of N, as urea (45%), by applying 40 kg ha^{-1} of N at sowing and 40 kg ha^{-1} of N as top-dressing, 15 days after plant emergence (DAE).

The experimental units consisted of plastic pots with capacity for 6 dm^{-3}, filled with air-dried soil, sieved through a 4-mm mesh. The moisture in the pots was daily controlled through weighing, in order to maintain the soil at 60% of field capacity. Irrigation was performed using distilled water.

The experiment used seeds of 'BRS Caatingueiro' corn, previously inoculated with a product containing a combination of two strains of *A. brasilense* (Ab-V5 and Ab-V6), in inoculant with peat formulation, produced by the Total Biotecnologia company. The dose used was 100 g of the peat inoculant and 50 ml of the sugar solution (10% of sugar concentration) for 50 g of seeds, mixed with the seeds, in order to cover them completely. After that, seeding was performed. Seeds germinated directly in the pots and at 8 DAE, thinning was performed, leaving only one plant in each experimental unit.

Humic acids were extracted and provided by the Biotechnology Laboratory of the Norte Fluminense State University (UENF), established in the Campus of Goytacazes-RJ, Brazil. Humic acids were isolated from vermicompost, according to Canellas et al. (2010). The material was previously dissolved in water, in the proportion of 13.5 mg L^{-1}. Then, plants were sprayed using 20 ml per pot, at the beginning of the stem elongation stage, at 15 DAE.

At 40 DAE, plant height and stem diameter were determined. Plant height was obtained through the measurement from the basis to the apical meristem of the plants, using a ruler. Stem diameter was determined using a digital caliper, at height of 2 cm from the soil surface. Then, plants were collected and divided into roots and shoots. All the collected plant material was washed in running water, HCl solution at 0.1 mol L^{-1} and deionized water, respectively.

Root length was determined using a ruler and root volume through the graduated cylinder method, in which roots are submerged in a graduated cylinder containing a known volume of water and root volume is determined by the difference between the initial and final volumes in the cylinder. After that, samples were placed in paper bags and dried in a forced-air oven at temperature of 65°C for 72 h. After drying the plant material, its dry matter was weighed and ground in a Wiley-type mill and the samples were subjected to sulfuric acid digestion, for the determination of N contents in the different plant parts (roots and shoots), according to the methodology described in Embrapa (2009).

N absorption efficiency, ratio between total N content in the plant and root dry matter, was calculated according to Swiader et al. (1994). N transport efficiency, ratio between shoot N content and total N content in the plant, and N use efficiency, ratio between the total dry matter production and total N accumulation in the plant, were calculated according to Siddiqi and Glass (1981).

Table 1. Plant height (PH), stem diameter (SD), root length (RL), root volume (RV), shoot dry matter (SDM), root dry matter (RDM) and dry mass of corn plants in response to the inoculation of *Azospirillum brasilense* in association with humic substances and nitrogen (Colorado do Oeste-RO, Brazil, 2015).

Treatment	PH (cm)	SD (mm)	RL (cm)	RV (cm^3/planta)	SDM (g)	RDM (g)	TDM (g)
Control	40.50^{b}	6.19^{b}	44.62^{b}	8.50^{b}	0.20	5.12	5.32
Inoculation	52.25^{ab}	6.62^{b}	48.75^{ab}	12.00^{ab}	0.60	5.29	5.90
80 kg ha^{-1} N	46.75^{ab}	6.03^{b}	57.00^{ab}	10.50^{ab}	0.41	5.20	5.61
Inoculation + Humic acids	57.00^{a}	7.22^{a}	66.25^{a}	16.00^{a}	1.10	5.59	6.69
Inoculation + 80 kg ha^{-1} N	55.62^{ab}	6.59^{b}	62.75^{ab}	11.25^{ab}	0.68	5.28	5.97
Inoculation + 80 kg ha^{-1} N + Humic acids	51.75^{ab}	6.94^{b}	55.12^{ab}	9.75^{ab}	0.71	5.29	6.01
Medium	50.64	6.60	55,75	11.33	0.62	5.29	5.91
Test F	0.03*	0.04*	0,01*	0.05*	0.15^{NS}	0.11^{NS}	0.07^{NS}
CV (%)	13.43	15.73	14,24	26.88	71.30	4.04	9.57

* and ^{NS}Significant 5% probability and not significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between themselves by Tukey test, the 5% probability. CV: Coefficient of variation.

The results were subjected to analysis of variance and the means were compared by Tukey test at 0.05 probability level, using the statistical program Sisvar (Ferreira, 2000).

RESULTS AND DISCUSSION

There was significant difference (p≤0.05) for plant height, stem diameter and root length and volume in response to *A. brasilense* inoculation associated with humic substances and N (Table 1).

Plant height and stem diameter of corn showed the highest values in the treatment with *A. brasilense* inoculation associated with humic acids, statistically differing from the control (without inoculation and without N) (Table 1). There were increases of 40.74 and 16.63% in plant height and stem diameter, respectively, in relation to the control. It should be pointed out that higher stem diameter is directly related to the increase in production, since it acts in the storage of soluble solids that will be used later for grain formation (Fancelli and Dourado Neto, 2008).

The inoculation of *A. brasilense* in association with humic acids influenced the root length and volume in corn plants (Table 1). Plants inoculated and under the application of humic acids showed increases of 48.47% in root length and 88.23% in root volume, compared with the control (not inoculated), but did not differ from the other treatments. This effect of increase in root length and volume is due to the production of auxins by the bacteria, which stimulates the growth of secondary roots, thus increasing the specific area of absorption of water and nutrients by plants (Radwan et al., 2004). Similar results were reported by Canellas et al. (2013), who observed increase in root area of corn plants when inoculated with *Herbaspirillum seropedicae* combined with humic substances.

The positive responses of the association of Ab-V5 and Ab-V6 + humic acid may have been due to what was found by Marques Júnior et al. (2008), under controlled conditions. These authors observed that inoculation of bacteria from the genus *Herbaspirillum* + humic substances in heat-treated seed pieces of sugarcane (variety 'RB72454') showed effects of inoculation, combined or not with humic substances, on the increase in the population of the inoculated bacteria, as well as on the increment in root growth, induced by both, inoculation of the selected bacteria and humic acids, which suggests new models of utilization of diazotrophic bacteria in plants. However, based on the results, it can be inferred that inoculation of *A. brasilense* associated with humic acids is capable of providing the N necessary for corn growth and development, allowing a reduction in the use of synthetic N fertilizers and consequently, a reduction in production costs.

N contents in the shoots, roots and in the plant were higher in the treatment with inoculation + 80 kg ha^{-1} of N, not differing statistically from the control and from those treatments with inoculation and inoculation + humic acids (Table 2). It should be pointed out that, commonly, in grasses, there is greater contribution of inoculation associated with N fertilization. According to Baldani et al. (1996), the inoculation of diazotrophic bacteria in the presence of small N doses proves to be more efficient for the plant-bacteria system, in comparison to the isolated use of bacteria. This is due to the fact that the amount of organic compounds excreted, deposited and/or exuded in the rhizosphere by the plant in the presence of small N doses produces intense microbial activity and interactions, which allow these bacteria to colonize, that is, it allows the emission of signals to the microorganisms.

As observed in the present study, Dobbelaere et al. (2002) reported that the effect of inoculation of *A. brasilense,* strain Sp 245, and *Azospirillum irakense*, strain KBC1, was higher when associated with N doses. Dalla Santa et al. (2004), in tests with corn, using the *Azospirillum* species strains RAM-7 and RAM-5, observed that the use of these strains was able to reduce

Table 2. Shoot nitrogen content (SNC), root nitrogen content (RNC), total nitrogen content (TNC), shoot nitrogen accumulation (SNA), root nitrogen accumulation (RNA) and total nitrogen accumulation (TNA) in corn in response to the inoculation of *Azospirillum brasilense* associated with humic substances and nitrogen. Colorado do Oeste-RO, Brazil (2015).

Treatment	SNC (g kg^{-1})	RNC (g kg^{-1})	TNC (g kg^{-1})	SNA (mg)	RNA	TNA
Control	39.40^{ab}	14.80	53.97^{ab}	16.52	77.89^{b}	298.94
Inoculation	37.35^{ab}	15.40	53.77^{ab}	24.81	78.35^{b}	319.90
80 kg ha^{-1} N	30.65^{abc}	19.22	48.85^{ab}	20.69	101.45^{a}	319.42
Inoculation + Humic acids	29.30^{ab}	17.42	47.42^{ab}	32.29	97.37^{ab}	318.66
Inoculation + 80 kg ha^{-1} N	39.65^{a}	20.27	59.92^{a}	18.16	105.19^{a}	291.59
Inoculation + 80 kg ha^{-1} N + Humic acids	24.67^{c}	18.95	39.00^{b}	14.74	99.96^{ab}	234.33
Medium	33.48	17.67	50.35	21.20	93.37^{ab}	297.14
Test F	0.09*	0.12	0.01*	0.23	0.04*	0.19
CV (%)	13.33	17.18	13.83	69.15	14.95	17.05

* and ^{NS}Significant 5% probability and not significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between themselves by Tukey test, the 5% probability. CV: Coefficient of variation.

Table 3. Nitrogen absorption efficiency (NAE), nitrogen transport efficiency (NTE) and nitrogen use efficiency (NUE) by corn plants in response to the inoculation of *Azospirillum brasilense* associated with humic substances and nitrogen (Colorado do Oeste-RO Brazil, (2015).

Treatment	NAE (mg g^{-1})	NTE (%)	NUE (mg g^{-1})
Control	58.39	5.49	0.10^{b}
Inoculation	60.40	7.09	0.11^{ab}
80 kg ha^{-1} N	61.39	8.42	0.09^{b}
Inoculation + Humic acids	56.82	9.60	0.14^{ab}
Inoculation + 80 kg ha^{-1} N	55.11	6.96	0.12^{ab}
Inoculation + 80 kg ha^{-1} N + Humic acids	44.26	6.00	0.16^{a}
Medium	56.06	7.26	0.12
Test F	0.17	0.12	0.00*
CV (%)	16.51	52.16	17.57

* and ^{NS}Significant 5% probability and not significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between themselves by Tukey test, the 5% probability. CV: Coefficient of variation.

by 40% the amount of N fertilization recommended. Araújo et al. (2014) observed that the inoculation of the strain Z-94 of *H. seropedicae* combined with 80 kg ha^{-1} of N promoted an increase of about 25.74% in shoot N contents of corn plants, in comparison to the control, fertilized with 80 kg ha^{-1}. These authors reported that the higher N content in inoculated plants is the result of both BNF and the mechanisms of root growth promotion, which can increase the capacity of plants to absorb this nutrient.

N use efficiency increased by approximately 60% with the inoculation of *A. brasilense* associated with 80 kg ha^{-1} of N and humic acids, in relation to the control (Table 3). This shows the beneficial effects of bacteria on N assimilation by corn plants when associated with humic substances and small N doses, since the dose recommended for corn under field conditions and clayey soils is 120 kg ha^{-1} of N to supply its requirement. Araujo et al. (2015) observed that plants fertilized with 30 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae* showed the highest percentage of N use efficiency. On average, in these treatments, nitrogen use efficiency was equal to 84.65%, against 64.63% in treatments fertilized with 120 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae.* Studies on N use efficiency in production systems are essential, because as the applied amount exceeds the capacity of plants to absorb the nutrient for production, N can be leached or accumulated in the tissues, reducing its use efficiency (Araujo et al., 2015).

However, despite not causing increments in dry matter production, the combined use of plant growth-promoting bacteria and stabilized organic matter (humic acid) should be more studied because, based on the other analyzed variables, it was promising for the corn crop.

Conclusions

The joint use of plant growth-promoting bacteria and

humic acids promoted increase in plant height, stem diameter and root length and volume.

Inoculation of *A. brasilense* combined with 80 kg ha^{-1} of N and humic acids increased N use efficiency of corn plants by 60%, while the inoculation of *A. brasilense* combined with 80 kg ha^{-1} of N increased N content in the shoots of corn plants.

Conflict of Interests

The authors have not declared any conflict of interests.

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