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Effect of plant spacing on growth, yield and fruit quality of some introduced banana (*Musa* AAA) clones

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ABSTRACT

A field experiment was conducted at the Experimental Farm of the National Institute for the Promotion of Horticultural Exports, University of Gezira, Sudan, during 2009/10 and 2010/11. The objective of the study was to determine the effects of plant spacing on the vegetative growth, crop earliness, yield components, total yield and fruit quality of five introduced banana clones under Gezira conditions, Sudan. Treatments consisted of three banana clones introduced from Austria, namely, Williams hybrid 172 (WH 172), Williams hybrid 1366 (WH 1366) and Grand Nain 1824 (GN 1824) and two clones introduced from South Africa, namely, Zelig and Bio. These five introduced clones were compared with the local clone Dwarf Cavendish (DC). Plant spacing was 2x2, 2x3 and 3x3 m. Treatments were arranged in a split plot design with three replicates. Plant spacing was assigned to the main plots and clones to the subplots. Results showed that vegetative growth parameters, yield and yield components were significantly affected by banana clones and plant spacing. Generally, the introduced banana clones had more vigorous vegetative growth than the local clone DC. The most vigorous vegetative growth was obtained by WH 172 followed by WH 1366 and GN 1824. Plant spacing of 2x3 m and 3x3 m resulted in significantly higher growth parameters values than 2x2 m. The earliest clone was GN 1824 and the latest were Bio and DC. Bio clone took the longest time from shooting to harvesting and both WH clones and Zelig took the shortest. Plant spacing of 3x3 m resulted in significantly the shortest time from shooting to harvesting compared to the others. The highest yield and yield components were obtained by WH 172, WH 1366 and GN 1824 and the lowest were obtained by the local clone DC. Plant spacing of 3x3 m resulted in the highest yield components but the lowest total yield.

However, the close spacing of 2x2 m produced the lowest yield components but the highest total yield due to the large number of bunches per unit area. Total soluble solids were comparable in all clones, however, GN 1824 and WH 1366 had a better taste than the other clones. Plant spacing of 2x3 m and 3x3 m resulted in significantly higher TSS and taste values than 2x2 m. It is recommended to grow the banana introduced clones WH 172, WH 1366 and GN 1824 at a spacing of 2X3 m for the highest yield and best fruit quality.

INTRODUCTION

Banana is one of the most popular fruits in Sudan. It is highly nutritious, cheap and available all year round. Although the Sudan has a great potential to be one of the leading banana producers in the world, yet, banana production can hardly satisfy the demand of the local markets. In order to establish a successful export banana industry in Sudan, the choice of the right cultivars and the improvement of cultural practices are indispensable prerequisites.

The predominant banana clone grown in Sudan is the Dwarf Cavendish (DC), which is a low yielder, has small-sized fingers and does not meet international market standards. Therefore, some of the most internationally popular clones have been introduced by the National Institute for the Promotion of Horticultural Exports and evaluated compared to the local clone DC (Mahmoud and Elkashif, 2003; Elkashif and Mahmoud, 2005; Elkashif *et al.*, 2005; 2010; Elsiddig *et al.*, 2009)

Elkashif and Mahmoud (2005) and Mahmoud *et al.* (2010; 2011) evaluated some introduced irradiated clones of Grandnain (GN) and Williams Hybrid (WH) and found that they had significantly higher bunch weight, total and exportable yields and longer fruit green life, but slightly lower total soluble solids (TSS) content than the local clone DC. Similarly, Elsiddig *et al.* (2009) and Ahmed *et al.* (2012) reported that introduced clones of GN and WH had the best vegetative growth and the highest exportable and total yields. However, the local clone DC had the least vegetative growth and yield components but recorded the highest TSS content.

Banana spacing is one of the most important cultural practices because it determines plant population, number of bunches per unit area and, hence, total yield. Therefore, the most appropriate plant spacing should be chosen, depending on cultivar, soil type and management of the plantation. Generally, wide plant spacing results in vigorous vegetative growth, large-sized bunches and fingers, high exportable yield but low total yield. Close spacing, on the other hand, results in taller and slender plants, low exportable yield due to small-sized fingers and high total yield due to the large number of bunches per unit area (Elsiddig *et al.*, 2009; Mahmoud *et al.*, 2010;) Also, wide plant spacing results in an early crop maturity and *vice versa* (Ahmed *et al.*, 2012)

Mahmoud *et al.* (2010) studied the effects of plant spacing and number of suckers on growth and yield of bananas. They found that plant spacing of 3x3 m (1111 plants/ha) with two suckers resulted in the best vegetative growth, the largest fingers and the highest exportable yield. However, it produced the lowest total yield. The close spacing of 2x2 m (2500 plants/ha) produced the highest total yield, but the smallest fingers and the lowest exportable yield. This data indicates that there is a need to find out the optimum plant spacing that would give high total yield of high quality large fingers suitable for export. Although the previous study has determined the optimum plant spacing, yet, there are newly introduced banana clones that may be affected by plant spacing. Therefore, the objective of this study was to investigate the effects of plant spacing on growth, yield components, total yield and quality of selected introduced banana clones.

MATERIALS AND METHODS

A field experiment was conducted at the Experimental Farm of the National Institute for the Promotion of Horticultural Exports, University of Gezira ,Sudan, at Hantoub area along the eastern bank of the Blue Nile, lat. 14 5'N and long. 33 4'E, during 2009 to 2011. The objective of the study was to determine the effects of plant spacing on the vegetative growth, crop earliness, yield components, total yield and fruit quality of five introduced banana clones under Gezira conditions, Sudan. Treatments consisted of three banana clones introduced from Austria, namely, Williams hybrid

172 (WH 172), Williams hybrid 1366 (WH 1366) and Grand Nain 1824 (GN 1824) and two clones introduced from South Africa, namely, Zelig and Bio.

These five introduced clones were compared with the local clone Dwarf Cavendish (DC). Plant spacing treatments were $2x^2$, $2x^3$ and $3x^3$ m. Treatments were arranged in a split plot design with three replicates. Plant spacing was assigned to the main plots and clones were to the sub-plots.

Growth Measurements

Growth measurements were taken at shooting from two randomly selected plants from each plot. Growth measurements consisted of pseudostem height (cm), pseudostem girth (cm), number of leaves and leaf area (m^2). Leaf area was calculated as the product of leaf length and width multiplied by a factor of '0.8' (Murray, 1960). Number of days from planting to shooting and from shooting to harvesting were recorded.

Determination of yield components

Banana bunches were harvested at the mature green, "full three quarters" stage, weighed and total yield (kg/ha) was determined. The bunches were then dehanded and number of hands per bunch, number of fingers per hand and per bunch were counted. Finger length (cm) was determined for the upper, middle and lower parts of bunches.

Determination of fruit quality

Banana fruits were dipped in Ethrel solution at a concentration of 2ml/l for two minutes and then ripened at 20° C. Fruit colour, taste and total soluble solids (TSS) were determined at the ripe stage. Colour change was rated on a scale of 1 to 7 according to Chiquita Company colour chart, as follows: 1, dark green; 2, light green; 3, pronounced yellow shading; 4, predominantly yellow; 5, yellow with green tips; 6, full yellow and 7, yellow with brown flecks.

Total soluble solids were measured according to Wills (1981). Thirty grams of pulp were blended with 90ml water for two minutes. Total soluble solids of the juice were measured using Kruss hand refractometer (Model HRN-32).

Taste of banana fruits was evaluated by a taste panel. Ten panelists were asked to evaluate the taste of the fruits from the different treatments according to a scale of 1 to 5 as follows: 1, unacceptable; 2, slightly acceptable; 3, acceptable; 4, sweet and 5, very sweet.

Statistical analysis

Data were statistically analysed using the standard analysis of variance procedure. Mean separation was done according to Duncan's Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Vegetative growth

The height of the pseudostem of banana plants is an important characteristic which affects many management practices. Tall banana plants are usually vulnerable to wind damage and most often they fall down due to their weak pseudostems, unless they are protected with wind breaks. When fruit bearing banana plants fall down, immature bunches are lost and mature ones are subjected to pronounced physical injury and bruising which render them unmarketable. Hence, thick pseudostems are important in supporting banana plants against strong winds and preventing them from tumbling down.

The main effects of banana clones on vegetative growth parameters at shooting were significant (Table 1). Generally, the vegetative growth of the introduced clones was more vigorous than that of the local clone DC. However, within the introduced clones, WH 172 had the tallest and thickest

pseudostems and the largest leaf area compared to the others. These results were consistent with those reported by Mahmoud and Elkashif (2003) and Elkashif *et al.* (2005; 2010).

The main effects of plant spacing on vegetative growth parameters were significant (Table 2). The spacing of 2x3m and 3x3m resulted in the most vigorous vegetative growth manifested in pseudostem diameter, number of leaves and leaf area as compared to 2x2m. However, it resulted in the shortest pseudostems. The spacing of 2x2m, on the other hand, resulted in the tallest and thinnest pseudostems, which was probably due to the high competition between plants for sunlight at the closer spacing. Since there was no significant difference in vegetative growth between 2x3m and 3x3m, this suggests that plant spacing can be reduced and hence, plant population can be increased, without any adverse effects on vegetative growth. These results were in agreement with the findings of Elsiddig *et al.* (2009) and Mahmoud *et al.* (2010; 2011) who reported that banana plants

grown at a close spacing were taller and slender than those grown at a wider spacing. Also, similar results were obtained by Mohamed (2012) who reported that wide plant spacing resulted in short banana plants with thick pseudostems.

DC152.3 f52.1 cd13.0GN 1824184.5 c53.2 c14.72WH 172197.4 a62.7 a15.22WH 1366193.1 b55.2 b15.14Zelig180.2 d47.0 e15.02	eaf area	of	stem]	Pseudo	Pseudostem	
DC152.3 f52.1 cd13.0GN 1824184.5 c53.2 c14.72WH 172197.4 a62.7 a15.22WH 1366193.1 b55.2 b15.14Zelig180.2 d47.0 e15.02	(cm^2)	ves	cm)	girth	height (cm)	Clones
GN 1824184.5 c53.2 c14.72WH 172197.4 a62.7 a15.22WH 1366193.1 b55.2 b15.12Zelig180.2 d47.0 e15.02	3123.2 d	.2	cd	52.3	168.5 e	Bio
WH 172197.4 a62.7 a15.252WH 1366193.1 b55.2 b15.142Zelig180.2 d47.0 e15.042	851.3 f	.0	cd	52.1	152.3 f	DC
WH 1366193.1 b55.2 b15.1Zelig180.2 d47.0 e15.0	8947.2 c	.7	c	53.2	184.5 c	GN 1824
Zelig 180.2 d 47.0 e 15.0	5118.6 a	.2	a	62.7	197.4 a	WH 172
6	535.9 b	.1	b	55.2	193.1 b	WH 1366
Sig. level ** ** NS	2918.1 e	.0	e	47.(180.2 d	Zelig
	***	S		**	**	Sig. level
CV (%) 15.7 13.4 13.9	16.5	.9	1	13.	15.7	CV (%)

Table 1. Effects of banana clones on growth parameters.

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

,* and NS indicate significance at P=1%, 0.1% and not significant, respectively.

Spacing (m)	Pseudostem	Pseudostem	No. of	Leaf area (cm ²)			
	height (cm)	girth (cm)	leaves				
2x2	111.9 b	48.1 b	13.5 b	6476.2 c			
2x3	152.1 a	54.9 a	15.6 a	8805.5 b			
3x3	155.5 a	56.2 a	15.8 a	8898.7 a			
Sig. level	***	**	***	***			
CV%	17.4	12.5	13.1	18.4			

Table 2. Effects of plant spacing on growth parameters.

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

,* and NS Significant at P=1% and not significant, respectively.

Crop earliness

Table 3 shows the main effects of banana clones on number of days from planting to shooting and from shooting to harvesting. Results showed highly significant differences between banana clones on number of days from planting to shooting and from shooting to harvesting.

The number of days from planting to shooting ranged from 276 days to 301 days and the number of days from shooting to harvesting ranged from 94 days to 107 days in all tested clones. The clones Zelig and GN1824 took the shortest duration of time from planting to shooting and Bio, DC and WH 1366 took the longest. The clones GN 1824, WH 172 and Zelig took the shortest duration of time from shooting to harvesting, whereas Bio took the longest. This data indicate that the earliest clones were GN 1824 and Zelig and the latest were Bio, DC and WH 1366. Mahmoud and Elkashif (2003) evaluated different introduced banana clones and reported that the latest were irradiated clones of the cultivars Williams Hybrid and Grandnain.

The effects of plant spacing on number of days from planting to shooting and from shooting to harvesting were significant (Table 4). The closer plant spacing of 2x2 m resulted in the longest duration of time from planting to shooting and from shooting to harvesting, whereas the wider spacing of 2x3m and 3x3m resulted in the shortest. This was most probably due to the competition between plants at the closer spacing for water, nutrients and sunlight. There were no significant differences in crop earliness between 2x3m and 3x3m plant spacing, which suggests that the traditional spacing of 3x3m can be reduced to 2x3m without having any deleterious effects on crop earliness.

Clones	No. of days from	No. of days from
	Planting to shooting	shooting to harvesting
Bio	301.0 a	107.8 a
DC	300.3 a	104.1 b
GN 1824	276.0 c	96.5 c
WH 172	298.2 b	99.3 c
WH 1366	301.0 a	103.4 b
Zelig	279.7 с	94.4 c
Sig. level	**	**
CV (%)	8.7	9.5

Table 3. Effects of banana clones on number of days from planting to shooting and from shooting to harvesting.

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

** Significant at P=1%.

Table 4. Effects of plant spacing on number of day	s from planting to shooting and from shooting to
harvesting.	

Plant spacing (m)	No. of days from	No. of days From
	planting to shooting	shooting to harvesting
2x2	315.6 a	104.6 a
2x3	290.6 b	98.7 b
3x3	288.9 b	95.5 b
Sig. level	*	*
CV%	8.3	6.7

Means in columns followed by the same letter(s) are not significantly different

at P≤0.05 according to Duncan's Multiple Range Test.

* Significant at P=5%.

The interaction effects of banana clones and plant spacing on number of days from planting to shooting and from shooting to harvesting were significant (Table 5). The earliest clones were GN1824 and Zelig planted at a spacing of 3x3 m, whereass the latest clone was DC grown at 2x2 m spacing. Gezira j. of agric. sci. 13 (2) (2015)

Regardless of banana clones, the wider spacing of 3x3 m always resulted in an early crop and the closer one in a late crop. This was most probably due to the fact that the wider spacing resulted in vigorous vegetative growth with high photosynthetic efficiency which hastened flowering and shortened the required time for bunch maturation.

	Spacing (m)	No. of days from	No. of days from
Clones		planting to shooting	shooting to harvesting
Bio	2x2	304.7 bc	100.7 de
	2x3	301.3 bc	96.0 fg
	3x3	297.0 fg	86.3 i
DC	2x2	311.7 a	119.0 a
	2x3	296.0 gh	101.3 d
	3x3	293.3 h	97.7 de
GN 1824	2x2	296.0 gh	105.0 c
	2x3	266.0 j	101.0 d
	3x3	262.7 ј	92.0 h
WH 172	2x2	302.7 bc	98.7 de
	2x3	297.7 de	93.0 h
	3x3	292.4 h	86.7 i
WH 1366	2x2	305.0 bc	108.3 b
	2x3	300.3 de	101.7 d
	3x3	297.7 fg	100.3 d
Zelig	2x2	298.5 ef	102.7 c
2x3		284.0 g	97.3 ef
	3x3	272.3 ј	94.0 gh
Sig.		***	***
CV%		12.3	16.7

Table 5. Interaction effects of banana clones and plant spacing on number of days from planting to shooting and from shooting to harvesting.

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

*** Significant at P = 0.1%.

Yield and yield components

The main effects of clones on yield components and total yield were highly significant (Table 6). Clones WH 172, WH 1366 and GN 1824 produced the highest bunch weight, number of fingers per hand, number of fingers per bunch, number of hands per bunch and total yield. However, the local clone DC resulted in the lowest yield parameters. These yield results are consistent with the vegetative growth data (Table 1) which showed that the clones WH 172 and GN 1824 had the most vigorous vegetative growth as compared to DC. These results were in agreement with those reported by Gubbuk *et al.* (2004) who reported that WH and GN clones were superior to Dwarf Cavendish in all vegetative growth and yield parameters. Also, similar results were obtained by Mahmoud and Elkashif (2003), Elkashif *et al.* (2005; 2010), Elsiddig *et al.* and Mahmoud *et al.* (2010; 2011) who reported that irradiated clones of GN and WH cultivars resulted in the best vegetative growth and the highest yield components and total yield compared to the local clone DC.

Plant spacing had significant effects on yield components and total yield (Table 7). Plant spacing of 3x3 m resulted in the highest yield components but the lowest total yield due to the low plant population per unit area. However, the spacing of 2x2 m produced the lowest yield parameters but the highest total yield due to the large plant population. The number of plants at the spacing of 2x2 m was 2500 plants/ha compared to 1666 and 1111 plants/ha at the spacing of 2x3 m and 3x3 m, respectively. The high yield obtained at the close spacing of 2x2 m was mostly of small-sized fingers (Table 7) which do not meet the standards of international markets. On the other hand, the relatively low yield obtained at the wider spacing of 3x3 m was mostly of large-sized fingers which are suitable for export. Yield components obtained at the spacing of 2x3 m were comparable to those obtained at 3x3 m. However, there was no significant difference in total yield between 2x2 m and 2x3 m , which indicates that plant population can be increased to 1666 plants/ha and, hence, total yield, without having any adverse effects on fruit size. Therefore, in order to produce high yields of good quality banana fruit suitable for export, the spacing of 2x3 m should be used.

	No. of	No. of	No. of	B wt	H wt	Yield
Clones	H/B	F/B	F/H	(kg)	(kg)	(t/ha)
Bio	7.3 c	106.7 e	13.8 c	15.8 b	1.8 bc	29.6 c
DC	7.2 c	97.4 f	13.4 c	11.7 d	1.6 c	21.3 e
GN1824	9.0 a	133.1 b	15.0 b	17.0 a	2.2 ab	31.3 b
WH172	8.7 a	135.0 a	15.4 ab	17.5 a	1.9 ab	32.8 a
WH1366	8.1 b	126.1 c	15.8 a	17.3 a	2.2 a	32.6 a
Zelig	8.1 b	110.7 d	13.8 c	13.3 c	1.7 c	25.0 d
Sig.	***	***	**	***	***	***
CV (%)	11.2	9.7	9.3	8.2	11.6	10.4

Table 6. Effects of banana clones on yield components and total yield.

Means in columns followed by the same letter(s) are not significantly different at P \leq 0.05 according to Duncan's Multiple Range Test. B=bunch, F=finger and H=hand.

** and *** Significant at P=1% and 0.1%, respectively.

1 au	able 7. Effects of plant spacing on yield components and total yield.						
	Plant	No of	No of	No of	Bwt	Hwt	Total yield
	Spacing	H/B	F/B	F/H	(kg)	(kg)	(t/ha)
	(m)						
	2x2	7.9 b	106.3 c	13.5 c	11.3 c	1.5 c	28.3 a
	2x3	7.9 b	121.2 b	14.9 b	16.5 b	2.0 b	27.5 a
	3x3	8.4 a	124.6 a	15.2	18.2 a	2.3 a	20.2 c
				а			
	Sig.	*	***	**	***	**	***
	CV%	10.9	9.2	8.7	10.0	12.9	10.9

Table 7. Effects of plant spacing on yield components and total yield.

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test. B=bunch, F=finger and H=hand.

*,** and *** Significant at P=5%, 1% and 0.1%, respectively.

Fruit quality attributes Finger length

The main effects of banana clones on finger length of the upper, middle and lower parts of the bunch were significant (Table 8). Generally, the longest banana fingers are produced in the upper part of the bunch, followed by the middle and shortest fingers are produced in the lower part of the bunch. Banana fingers of 20.3cm or more in length are considered exportable (Kesavan *et al.*, 2001). The introduced clones GN1824, WH 172 and WH 1366 produced the longest fingers whereas DC produced the shortest. These results are in agreement with those reported by Mahmoud and Elkashif (2003) who showed that introduced banana clones were

superior in finger length than the local clone DC. The length of banana fingers is an important characteristic which is considered as a yardstick for suitability for export. This data indicates that the previously mentioned clones are good candidates for the start of a competitive banana industry in Sudan.

Plant spacing had highly significant effects on finger length (Table 9). Plant spacing of 3x3 m and 2x3 m resulted in the longest fingers in all parts of the bunch compared to 2x2 m. This was because this plant spacing resulted in the most vigorous vegetative growth (Table 2) and the highest yield parameters (Table 8) which was reflected in long fingers. Since there was no significant difference in finger length between 3x3 m and 2x3 m plant spacing, this indicates that plant spacing can be reduced to 2x3 m and plant population and total yield can be increased without any significant reduction in finger size.

	0	0	,
Clones	Fin	ger length (cm)	
	Upper	Middle	Lower
Bio	19.6 bc	17.5 d	16.0 de
DC	18.7 d	17.0 e	16.6 bc
GN1824	20.9 a	19.1 a	17.2 a
WH172	20.7 a	18.7 ab	16.9 ab
WH1366	20.3 ab	18.6 ab	16.7 ab
Zelig	18.9 cd	17.8 cd	15.7 e
CV (%)	8.6	7.3	7.8
Sig.	*	*	*

Table 8. Effects of banana clones on finger length of the upper, middle and lower parts of the bunch .

Means in column followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

* Significant at P=5%.

	Finger length (cm)				
Plant spacing (m)	Upper	Middle	Lower		
2x2	18.5 b	17.8 b	16.0 b		
2x3	20.8 a	19.1 a	17.2 a		
3x3	21.4 a	19.6 a	17.8 a		
Sig.	***	***	**		
CV%	10.1	5.9	6.0		

Table 9. Effects of plant spacing on finger length of the upper, middle and lower parts of the bunch .

Means in column followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

and * Significant at P=1% and 0.1%, respectively.

Fruit colour, TSS and taste

Banana clones varied significantly in their fruit colour and taste (Table 10). The clones Bio and Zelig recorded the best colour, whereas GN 1824, WH 172 and WH 1366 recorded the best taste. Banana clones were not significantly different in their total soluble solids (TSS) content, however, GN 1824, WH 172 and WH 1366 had higher values compared to the other clones. These results are in agreement with those reported by Elkashif and Mahmoud (2005) and Elkashif *et al.* (2005) who found that introduced banana clones had better sensory quality attributes than the local clone DC.

Plant spacing had significant effects on TSS and taste (Table 11). The spacing of 2x3 m and 3x3 m resulted in fruits of higher TSS values and better taste than 2x2 m. This was because the wider spacing produced well-developed, fully mature fruits which ripened normally to give excellent aesthetic characteristics.

In conclusion, it is recommended to grow the introduced banana clones GN 1824, WH 172 and WH 1366 at a spacing of 2x3 m for the production of high yield of good quality fruit.

		,	
Clones	Colour	TSS	Taste
Bio	4.3	19.5	2.6 c
DC	3.8	19.9	1.5 d
GN1824	3.9	21.6	3.6 a
WH172	3.9	20.4	2.6 c
WH1366	3.9	21.4	3.4 ab
Zelig	4.1	20.9	3.1 b
Sig.	*	NS	**
CV (%)	11.7	9.4	8.5

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

*,** and NS Significant at P=5%, 1% and not significant, respectively.

Table 11. Effects of	plant spacing o	on colour, TSS and taste of fruits.	

Spacing(m)	Colour	TSS	Taste
2x2	3.8	19.6 c	2.9 b
2x3	3.9	20.5 b	3.3 a
3x3	4.0	21.8 a	3.7 a
Sig.	NS	*	*
CV%	10.4	8.2	9.4

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$ according to Duncan's Multiple Range Test.

* and NS Significant at P= 5% and not significant, respectively.

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