African Journal of Agricultural Research

Volume 12 Number 27 6 July, 2017
ISSN 1991-637X

Academic Journals

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Vol. 12(27), pp. 2273-2282, 6 July, 2017
DOI: 10.5897/AJAR2017.12216
Article Number: 9EACF6465081
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Chlorophyll relative index for diagnosing nitrogen status in corn hybrids

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Received 10 February, 2017; Accepted 14 April, 2017

The aim of the current study was to determine the chlorophyll relative index (CRI) in different leaves and phenological stages of the crop in order to diagnose the status of nitrogen (N) in corn (*Zea mays* L.) hybrids as a function of N rates applied in bands correlating them with N content in the leaves and crop productivity. The field experiment consisted of two corn hybrids (P30R50 and AG8025) and 6 N rates applied in bands (0, 75, 150, 225, 300 and 375 kg N ha^{-1}), under a factorial 2 × 6 experimental design, arranged in a randomized block with 4 replications. The dose of 295 kg N ha^{-1} allowed estimating crop yields corresponding to 13.033 kg ha^{-1}. Hybrids and N rates influenced concomitantly CRI in several leaves and phenological stages. The chlorophyllometer is shown to be quite sensitive to nutritional status in corn hybrids as a response to N rates applied in bands since the early stages of the crop growing season for early diagnosis. At the end of the vegetative phase, as well as the reproductive phenological stage the chlorophyllometer performed well as an indicator of efficiency of nitrogen fertilizer application.

Key words: *Zea mays* L., chlorophyllometer, productivity, nitrogen fertilization.

INTRODUCTION

A high productive potential of the corn crop (*Zea mays* L.) is highly dependent on essential nutrients in the soil solution such as nitrogen (N), which is required by the plants in high quantities and provides a significant rise in crop yields. The amount of N absorbed taken up by the roots of the maize with the goal of reaching the maximum yield corresponds to 0.9% of the N present in dry phytomass of the sprouts (DPS) in compliance with Amado and Mielniczuk (2000), as well as to 1.17% of it according to Subedi and Ma (2005), showing therefore a high demand of the crop by such a macronutrient.

The complexity level and importance of N for the maize crop is consistent, mainly if we consider all the additional information needed to increase its efficiency. One of the alternatives to increase the efficiency of N is by synchronizing its application with the need of the plants. According to Rambo et al. (2007) and Holland and Schepers (2010), it is feasible to manage the amount of

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N to be applied in bands in compliance with the desired synchronism, enhancing its use efficiency by means of applications compatible to the needs of the plants. Such needs can be identified by the physiological parameter known as chlorophyll relative index (CRI) to be measured on the leaves of the crop by the chlorophyllometer throughout different phenological stages.

Measurements of chlorophyll rates (CR) on maize leaves, as well as other species, are relevant in studies carried out to examine the response of plants to nitrogen fertilization management when the aim is to increase crop photosynthetic efficiency (Amarante et al., 2010). In the same fashion, CRI correlates positively with chlorophyll rates and extractable N from the plants, as well as with crop yield. However, such a technique allows us to obtain reference values by means of a non-destructive procedure throughout different crop phenological stages. This will make possible the N monitoring and management whenever it is necessary in such a way as to obtain a positive correlation between the CRI and CR, regardless of the crop growing season and the maize hybrid in consideration.

The usage of indirect measurements aiming at estimating the CR by means of portable devices, which make use of optical principles and are based on the absorbance and/or reflectance of the light, turned out to be more straightforward and rapid, being able to be performed directly in the field without destroying the leaf tissues (Argenta et al., 2001b). CRI might also show a more practical applicability in agronomical trials owing to a high accuracy and low cost throughout its evaluation (Rambo et al., 2004).

Research manuscripts published by Argenta et al. (2002) and Hurtado et al. (2009) demonstrated a positive correlation between the CRI and maize yield, as well as between the N leaf level and productivity for diagnosing of the level of such element in the plants. Rambo et al. (2008) verified that the most precise characteristics for predicting the optimal doses of N to be applied to maize crops in bands are DPS and N accumulated in the plant, followed by the CRI in the leaf, which as a function of its practicability and ease of determination, shows a more pronounced potential for use. Therefore, in a sense, it is possible to increase the use efficiency of N in bands, either by means of a rise in yield or rationalization of nitrogen fertilization in bands, therefore decreasing the contamination of the soil and water, mainly due to nitrate lixiviation.

Owing to the climate and soil variability along with management practices existing in different production environments it is crucial to conduct regionalized research aiming at correlation studies between CRI readings and productive potential of maize hybrids, which will be expressed as a function of the climatic aptness for each region. Therefore, the aim of the current research paper was to determine the CRI and productive potential of maize hybrids in different leaves and throughout of different phenological stages with the purpose of diagnosing the nitrogen status in maize hybrids as a function of the amount of N applied to the plants in bands, correlating the content of N in the leaves with crop yield under field conditions in the State of Paraná, Brazil.

MATERIALS AND METHODS

The field experiment was conducted using a no tillage system at the region of Entre Rios, in the municipality of Guarapuava, PR, Brazil [latitude 25º32'S, longitude 51º28'W and altitude of 1,126 m] throughout the period between October 1st and March 20th of 2010. The climate of the site in the study according to the Köppen's classification is of the humid subtropical type without a dry season and with frequent severe frosts (Peel et al., 2007). Mean annual rainfall in the region is of 1.942 mm. Monthly mean air temperature is of 16.8°C with maximum and minimum values corresponding to 23.1 and 12.4^{o}C, respectively (Simepar, 2011).

Throughout of the crop growing season the overall amount of rainfall was above the 35 year historical average along with variations of 5.1 mm for the month of November and of 114.7 mm for the month of January. Crop rotations over the last year at the experimental area were for the winter/summer year season wheat/soybean (2006), oat/maize (2007), barley/soybean (2008), and in 2009 oat preceded maize crop. The type of soil prevailing in the experiment area is latossol with a depth of roughly 2 m, good physical conditions, and with a high potential for agricultural use. The chemical characteristics of the soil might be seen in the Table 1.

Treatments resulting from the combination of two simple hybrids classified as a precocious cycle for maize - Pioneer 30R50 and Agroceres 8025 (P30r50 and AG8025) - and 6 doses of N to be applied in cover (0, 75, 150, 225, 300 and 375 kg of N ha^{-1} in the form of urea), comprising a factorial design 2×6 designed in randomized blocks with four replications. Plots consisted of 8 lines 5 m long spaced 0.75 m occupying a total area of 30 m^{2}. Sowing was performed manually on October 1st of 2009 shortly after the incorporation of the 8-30-20 fertilizer formula plus 0.4% of Zn in order to reach a plant population of 69.722 plants ha^{-1}. Cultural practices were implemented according to its occurrence and recommendation for the crop in the field.

Nitrogen doses were applied manually in the total area in just one application on September 9th of 2009 with the plants at V_5 stage. Under the V_3 stage out of the two central lines 2 plants plot^{-1}were selected and identified for determination of CRI by means of the chlorophyllometer-chlorophy LOG.

Fifteen readings of CRI were made in compliance with the following sequence: 1st reading: Leaf 3 at stage V_3 (L_3V_3); 2nd reading: L_5V_5; 3rd reading: L_3V_5; 4th reading: L_5V_7; 5th reading: L_7V_7; 6th reading: L_7V_9; 7th reading: L_9V_9; 8th reading: L_5V_9; 9th reading: L_9 grain filling stage (L_9GFS); 10th reading: corn tassel emission L_{11}CTE; 11th reading: $L_{11}R_1$; 12th reading: $L_{13}R_2$; 13th reading: $L_{15}R_2$; 14th reading: $L_{13}R_3$; 5th reading: $L_{15}R_3$. For all analyzed leaves readings were taken in two different stages, except for the leaf 5, which were made in V_5, V_7 and V_9.

For each evaluation of CRI one single leaf plant^{-1} was used from four localized points in the central part of the leaf, between the edge and central nerve, obtaining the average from 8 readings per plot (2 plants). For the first assessment of CRI leaves were identified for the second reading, leaf 3 was the one taken into consideration for the identification of the other leaves.

Ten leaves per plot at the R_1 stage were used for the determination of leaf N content, always taking into account the below and opposite leaf oriented to the primary ear according to Malavolta et al. (1997). Leaves were stored in plastic bags, dried in

Table 1. Chemical characteristics of the soil in the experimental area.

Attributes	Unities	Depth (cm)		
		0-10	10-20	20-40
pH in $CaCl_2$		5.4	4.7	4.7
H + Al	$cmol_c\ dm^{-3}$	5.35	8.36	9.01
Al changeable	$cmol_c\ dm^{-3}$	0.0	0.3	0.4
Ca changeable	$cmol_c\ dm^{-3}$	6.9	4.0	3.1
Mg changeable	$cmol_c\ dm^{-3}$	2.4	1.5	1.3
K changeable	$cmol_c\ dm^{-3}$	0.57	0.38	0.25
P	$mg\ dm^{-3}$	22.9	6,0	2.4
C-organic	$g\ dm^{-3}$	32.0	21.0	19.0
CCC at pH 7,0	$cmol_c\ dm^{-3}$	15.22	14.24	13.66
CCC efetiva	$cmol_c\ dm^{-3}$	9.87	6.18	5.05
Sat. for bases (V)	%	64.8	41.3	34.0
Sat. for Al (m)	%	0.0	4.9	7.9
Sat. for Ca	%	45.3	28.1	22.7
Sat. for Mg	%	15.8	10.5	9.5
Sat. for K	%	3.7	2.7	1.8
Relation Ca/Mg		2.9	2.7	2.4
Relation Ca + Mg/K		16.3	14.5	17.6

H + Al: Buffer solution SMP; Al, Ca and Mg changeable: KCl 1 mol L^{-1}; P and K: Mehlich-1; C-organic: Walkley-Black. Source: Laboratory of Soil Fertility - State University of Ponta Grossa - UEPG.

a greenhouse with air circulation at 65°C up until the constant DPS was reached, then ground in a knife mill and analyzed by the semi-micro Kjeldhal method. Final productivity was assessed by means of the manual harvest of the ears from a useful area of 13.5 m^2, mechanical threshing, determination of DPS and extrapolation of the values for kg ha^{-1}, correcting it to a water content of 13%.

Experimental data obtained from each variable was subjected to analysis of variance through the SAS statistical program (SAS, 2008). Whenever the interaction between the hybrids and N doses was significant, and also when the effect of N doses was observed, a study of regression was carried out by means of illustrations provided by graphs made by an Excel program. The degree of correlation and agreement between the variables measured herein was expressed by the coefficient of the Pearson correlation.

RESULTS AND DISCUSSION

Interactions between the maize hybrids and doses of N to be applied in bands for the variable CRI occurred throughout the phenological stages: $L_{05}V_7$, $L_{07}V_7$, $L_{07}V_9$, $L_{09}V_9$, $L_{11}VT$, $L_{11}R_1$, $L_{13}R_2$, $L_{15}R_2$ e $L_{15}R_3$. Subedi and Ma (2005) obtained interactions only for the CRIs whenever N was applied in bands at different phenological stages of maize hybrids at V_8 four weeks after flowering.

A significant influence of the hybrids was observed over the $CRIL_{03}V_4$, $CRIL_{05}V_9$, $CRIL_{11}R_1$, $CRIL_{13}R_2$, $CRIL_{13}R_3$ and $CRIL15R_2$. However, this occurred with a higher frequency for the determinations performed throughout the reproductive phase. The only assessment of the CRI performed shortly before the application of N in bands was $CRIL_{03}V_4$. Nevertheless, the maize hybrid P30R50 showed a $CRIL_{03}V_4$ higher in 2.24 in relation to the AG8025 (Table 2). Average values obtained for the hybrids P30R50 and AG8025 was higher and similar to a CRI of 45.4% as cited by Argenta et al. (2003) at the stage V_3 to V_4, indicating that the content of N in the plant is quite adequate. The reading $CRIL_{03}V_4$ did not correlate with the N content in the leaf, as well as with yield (Table 3), corroborating with Argenta et al. (2002) and Hurtado et al. (2010).

The $CRIL_{03}V_5$ was remarkably affected by the doses of N applied in bands to the leaves, observing, therefore, an increasing linear effect (Figure 1a), occurring 4 days after its application (DAA). According to Godoy et al. (2007), a CRI of 46.6 between V_4 and V_5 triggered a more pronounced accumulation of DPS in maize. Once the mean value obtained for $CRIL_{03}V_5$ was of 46.87, such a value may be considered as perfect for the phenological stage in question.

The $CRIL_{03}V_5$ responded to the doses of N; however, during the assessment of the $CRIL_{05}V_5$ such a response did not show any statistical significance. This might be due to the fact that F_{05} is a very young phenological stage, suggesting then that the N added to the tissues will be utilized first to increase the number of cells of new leaves in conjunction with the cellular differentiation process. This final process will enhance the production of chloroplasts - the site of synthesis of chlorophyll - being confirmed during the evaluation of $CRIL_{05}V_7$ and $CRIL_{05}V_9$. Since the F_{03} was to be in a more advanced growth stage along with the fact that its final size was not

Table 2. Leaf chrophyll relative index at the leaf #3 at the phenological stage V_4 ($CRIL_{03}V_4$), $CRIL_{05}V_9$, $CRIL_{13}R_3$ and yield as a function of maize hybrid.

Hybrids	$CRIL_{03}V_4$	$CRIL_{05}V_9$	$CRIL_{13}R_3$	Yield (kg ha^{-1})
P30R50	48.07^a	63.56^a	64.58^b	10.929^b
AG8025	45.83^b	61.23^b	68.95^a	12.439^a

*Averages followed by the same letters do not differ among themselves by the F test at 5% confidence level.

Table 3. Correlation coefficients of the chlorophyll relative indices (CRI) at different leaves and phenological stages with leaf N content and yield.

Coefficients	Leaf N content (g kg^{-1})	Yield (kg ha^{-1})
$CRIL_{03}V_4$	0.24^{ns}	-0.10^{ns}
$CRIL_{03}V_5$	0.42**	0.36*
$CRIL_{05}V_5$	0.21^{ns}	-0.01^{ns}
$CRIL_{05}V_7$	0.40**	0.47**
$CRIL_{05}V_9$	0.72**	0.57**
$CRIL_{07}V_7$	0.39**	0.33*
$CRIL_{07}V_9$	0.58**	0.58**
$CRIL_{09}V_9$	0.41**	0.27^{ns}
$CRIL_{09}$GFS	0.73**	0.67**
$CRIL_{11}$CTE	0.63**	0.60**
$CRIL_{11}R_1$	0.67**	0.82**
$CRIL_{13}R_2$	0.65**	0.80**
$CRIL_{13}R_3$	0.69**	0.86**
$CRIL_{15}R_2$	0.71**	0.85**
$CRIL_{15}R_3$	0.71**	0.77**
Leaf N content (g kg^{-1})	-	0.69**

^{ns}, Not significant; *,** Significant at 0.05 and 0.01 probability level, respectively.

reached at all, its cells were at a more advanced differentiation stage, possessing more chloroplasts per cell and as a result of that having the synthesis of chlorophyll intensified by the supply of N. First of all N is used to assure cellular division plus cellular differentiation processes in the new leaves of the plants. Throughout the differentiation process cells get their perimeter enlarged, as well as the number organelles such as chloroplasts and photosynthesizing parenchyma. As a result of that, the content of chlorophyll rises concomitantly with cellular differentiation from tissue to organ. In this particular case, the organ in consideration is the leaf, being therefore incorporated into the membranes of the thylakoids as reaction centers called components of photo systems II and I (Taiz and Zeiger, 2013).

Mean values of $CRIL_{03}V_5$ (46.87) and $CRIL_{05}V_5$ (55.69) were to be very close to that one obtained by Hurtado et al. (2010) at V_4 to V_5 phenological stages, causing the maize crop to reach yields of 11.300 kg ha^{-1} found by such scientists, as well as by the current research (roughly corresponding to 11.684 kg ha^{-1}). However, either $CRIL_{05}V_5$ or mean yields were similar to the outcomes obtained by these authors. This might be explained by the fact that the amount of 32 kg ha^{-1} of N applied to the crop during the sowing date was sufficient to meet the initial physiological needs of the plants up to the stage of V_5, as well as owing to the fact that soil type in study possessed a content of organic matter capable of making available a higher amount of N to the soil solution. $CRIL_{03}V_5$ correlated positively with leaf N content and yield, showing coefficients equivalent to 0.42^{**} and 0.36^{*}, respectively, as opposed to the $CRIL_{05}V_5$ (Table 3). Similarly, Hurtado et al. (2010) observed a positive correlation between $CRIV_4$-V_5 with yield.

Nitrogen doses applied in bands resulted in a linear increase of $CRIL_{05}V_7$ (Figure 1b). Mean $CRIL_{05}V_7$ was of 63.7, regardless of the hybrid and N dose in consideration, revealing a superiority of 22.26% in relation to the CRI of 52.1 obtained by Argenta et al. (2003) for maize at V_6 to V_7, which corresponded to a rise of 6.7% in productivity. With a rise in availability of N an increment of $CRIL_{05}V_7$ occurred in conjunction with a rise in yield, a fact that might be firmed up by the positive

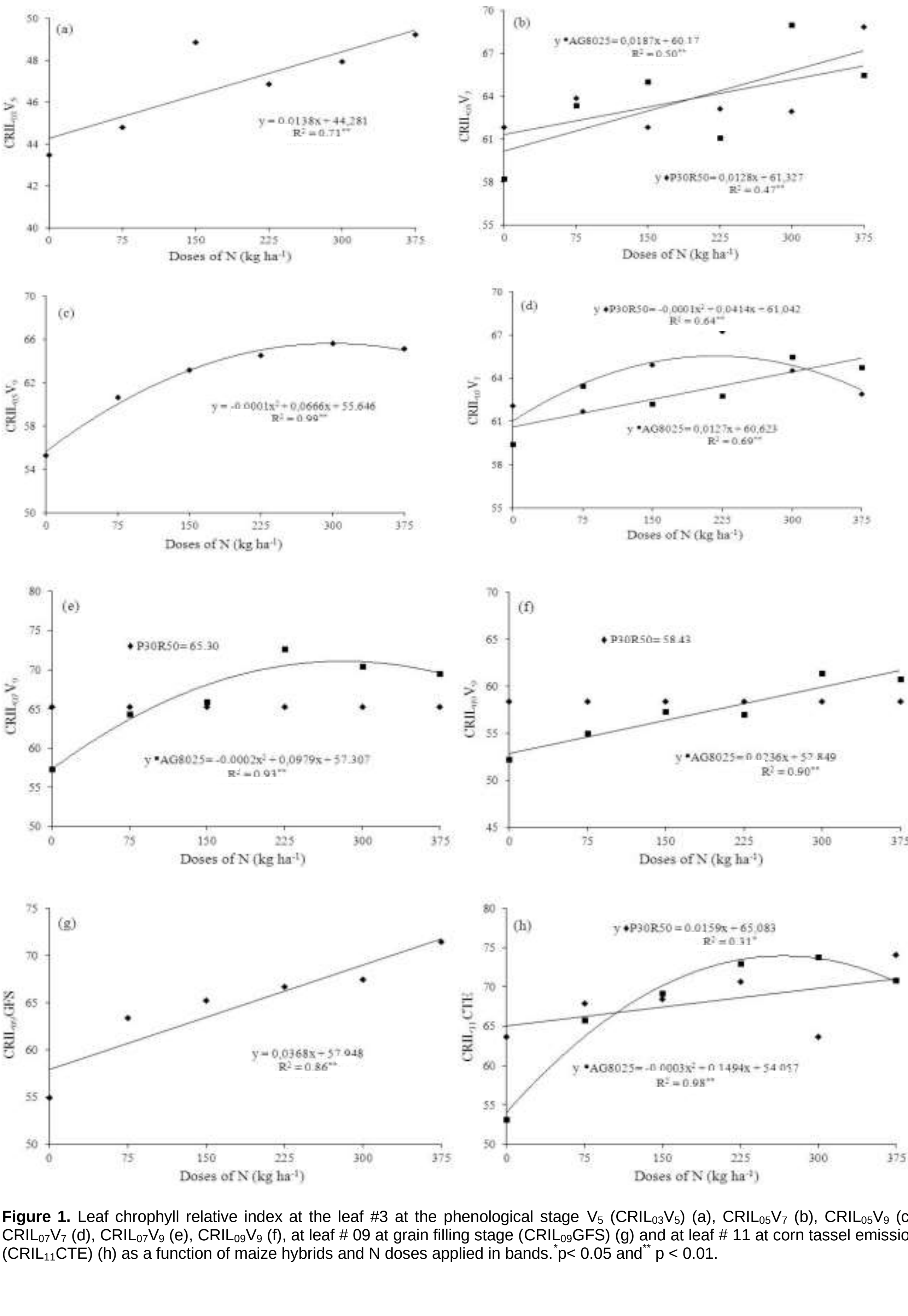

Figure 1. Leaf chrophyll relative index at the leaf #3 at the phenological stage V_5 ($CRIL_{03}V_5$) (a), $CRIL_{05}V_7$ (b), $CRIL_{05}V_9$ (c), $CRIL_{07}V_7$ (d), $CRIL_{07}V_9$ (e), $CRIL_{09}V_9$ (f), at leaf # 09 at grain filling stage ($CRIL_{09}GFS$) (g) and at leaf # 11 at corn tassel emission ($CRIL_{11}CTE$) (h) as a function of maize hybrids and N doses applied in bands. $^{*}p < 0.05$ and ** $p < 0.01$.

correlation established between $CRIL_{05}V_7$ readings and leaf N content, as well as productivity (Table 3).

The P30R50 hybrid showed a $CRIL_{05}V_9$ 3.8% higher in relation to the one yoked to the AG8025 hybrid, corresponding to a CRI difference of 2.33 (Table 2). Genetic variability between maize hybrids and discrepancies in chlorophyll synthesis was also observed by Sunderman et al. (1997) and Subedi and Ma (2005). In so far as N in bands increased up to an estimated dose of 325 kg of N ha^{-1} $CRIL_{05}V_9$ increased as well, resulting in a fraction of 66.73 of $CRIL_{05}V_9$ (Figure 1c). $CRIL_{05}V_9$ was associated to a higher coefficient of correlation concerning leaf N content (0.72^{**}) rather than with yield (0.57**) (Table 3).

Nitrogen doses unfolding for $CRIL_{07}V_7$ allowed to identify the differential responsiveness of hybrids to the N doses applied in bands, since the P30R50 hybrid responded well up to an estimated dose of 205 kg of N ha^{-1}, which would be corresponding to a CRI of 65.33, and the AG8025 hybrid responded linearly to the increasing N doses applied (Figure 1d). The estimated value for the hybrid P30R50 was superior to the mean value of 59.7, a fact that was evidenced also by Hurtado et al. (2010) between V_7 and V_8.The correlation of $CRIL_{07}V_7$ with leaf N content and yield was of 0.39^{**} and 0.33^{**}, respectively, being therefore quite below the one for $CRIL_{05}V_9$ (Table 3).

Faced with the unfolding of N doses applied in bands within maize hybrids for the variable $CRIL_{07}V_9$, P30R50 did not respond to the N doses, leading to a mean CRI of 65.3, whereas for the AG8025 hybrid there was a consistent rise in the $CRIL_{07}V_9$ up to an estimated dose of 245 kg of N ha^{-1} when CRI was expected to be of 69.29 (Figure 1e). The coefficient of correlation between $CRIL_{07}V_9$ and leaf N content and yield was the same (0.58^{**}) (Table 3).

The differentiated responsiveness of $CRIL_{07}$ for the hybrids at all N doses observed between V_7 and V_9stages might be ascribed to the fact that AG8025 hybrid was more productive than P30R50 (Table 2), once for such a genotype $CRIL_{07}$ between V_7 and V_9 was roughly the same. Such a similarity was established throughout the time and for the AG8025 hybrid an increment of 3.9 was achieved in such a fashion as to enhance the positive correlation between the CRI and leaf N content and yield at the stage V_9 in comparison to V_7 (Table 3). The difference between the CRI values estimated at F_{07} between the Stages V_7 and V_9 for the AG8025 hybrid demonstrated that there was an increase in the chlorophyll synthesis due to N incorporation into the tissues.

$CRIL_{09}V_9$ readings obtained for the P30R50 hybrid were not remarkably influenced by the N doses, showing an average of 58.43, whereas for the AG8025 hybrid $CRIL_{09}V_9$ expressed an increasing linear response to the N doses (Figure 1f). $CRIL_{09}V_9$ was strongly correlated to leaf N content in 0.41^{**} (Table 3). The lack of correlation between $CRIL_{09}V_9$ and yield might be attributed to the fact that CRI readings for the P03R50 hybrid were not influenced by the N doses, in spite of the linear responsiveness of the AG8025 hybrid. Nevertheless, low amplitude of $CRIL_{09}V_9$ among the maize hybrids was observed herein. Such a fluctuation translates that while for the P30R50 hybrid the mean value was of 58.43, the estimated $CRIL_{09}V_9$ in 61.7 by means of the fitted equation with a dose of 375 kg of N ha^{-1} would have been obtained for the AG8025 hybrid.

The mean value of $CRIL_{09}V_9$ was of 57.85 irrespective of the genotype and N dose to be applied, being higher in 9.15% to that one observed by Godoy et al. (2007) between V_8 to V_9, as well as lower in 8.03% of that obtained by Hurtado et al. (2010) between V_9 and V_{10}. The comparisons revealed that the indicator leaf of the phenological stage, no matter how young it is, usually does not respond to N applied in bands as to the CRI likely due to its utilization firstly for cellular division and differentiation. The $CRIL_{09}GFS$ regardless of the hybrid responded linearly to the increment of N applied in bands (Figure 1g). $CRIL_{09}GFS$ readings were positively correlated to both leaf N content and yield, showing coefficients of 0.73^{**} and 0.67^{**}, respectively (Table 3).

The AG8025 hybrid responded in an increasing manner up to the estimated dose of 250 kg of N ha^{-1} for the $CRIL_{11}CTE$, corresponding to a CRI of 72.66. The $CRIL_{11}CTE$ obtained for the P30R50 hybrid increased linearly with the increment of N applied in bands (Figure 1h). Since N takes part in the chlorophyll molecule with an overall of four atoms, it is well known that a rise in the requirements of N to the plants up to a certain point provides an increase in the tonality of the green color of the leaves, which will increase the chlorophyll content or CRI, as well as leaf N content and yield. Such a fact might be confirmed by means of the correlation analysis, once the coefficients between $CRIL_{11}CTE$ and leaf N content and yield were of 0.63^{**} and 0.60^{**}, respectively (Table 3).

Argenta et al. (2003) obtained a CRI of 58 in the leaves below and opposite to the stalks at the filling physiological stage of maize crop. Therefore, the mean $CRIL_{11}CTE$ of 67.84 was 16.96% higher. Chlorophyll synthesis is triggered by the light, being iron the co-factor of some enzymes, depending upon N, Mg and carbonic skeletons from the photosynthesis itself, in conjunction with the extrinsic factors (availability of N, Mg, Fe, water, solar radiation, photoperiod, management practices, etc.) and intrinsic factors (genetic variability). Water availability was an average of 8 mm day^{-1} throughout the month of December during the CTE stage. According to Matzenauer et al. (1998) such a water supply to the maize plants coincides with the crop needs and contributes to the plants in order to synthesize and maintain a high content of chlorophyll, expressed by the CRI.

The AG8025 hybrid is the one that would reach a

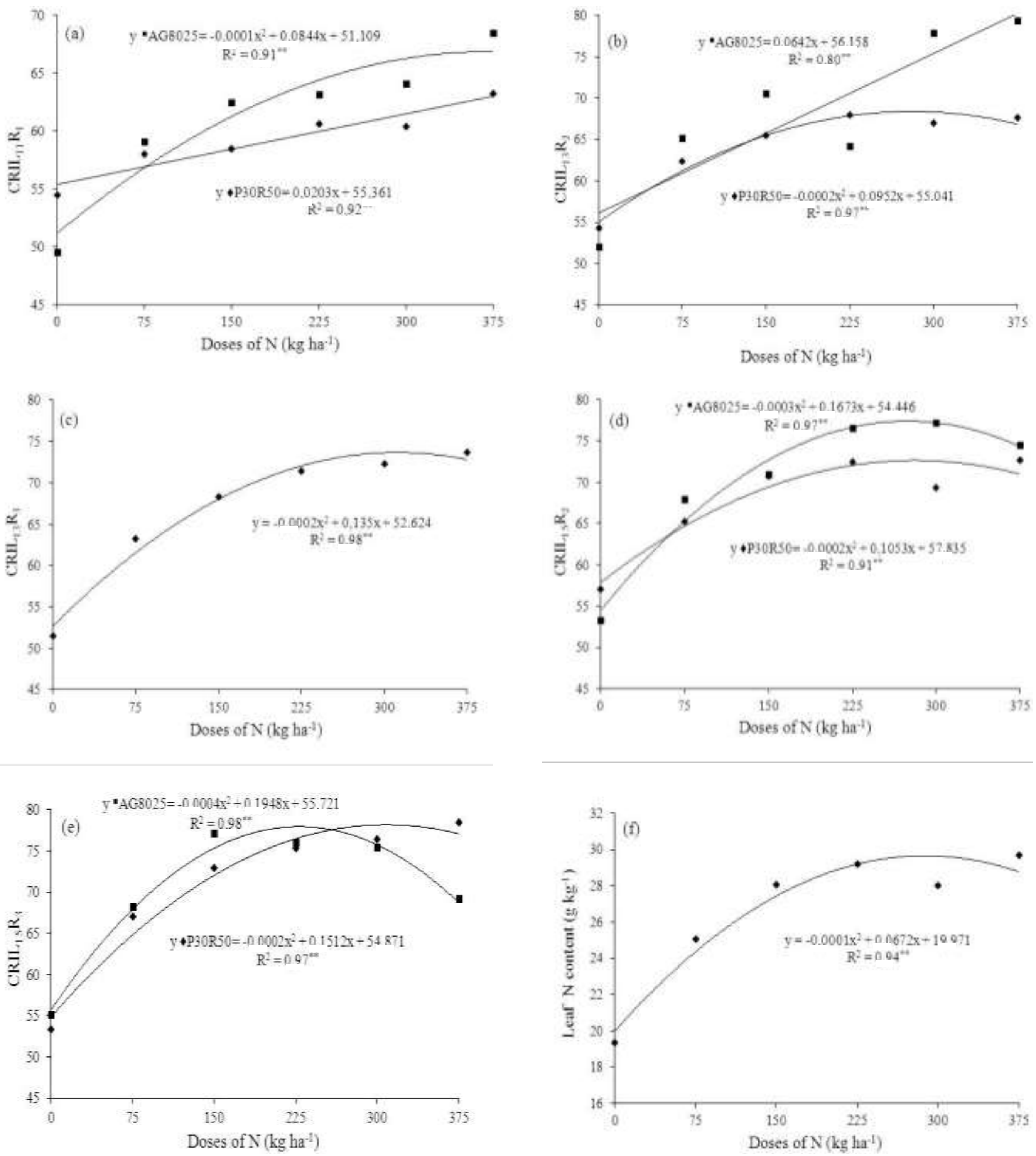

Figure 2. Leaf chlorophyll relative index at the leaf #11 at the phenological stage R_1 ($CRIL_{11}R_1$) (a), $CRIL_{13}R_2$ (b), $CRIL_{13}R_3$ (c), $CRIL_{15}R_2$ (d), $CRIL_{15}R_3$ (e) and leaf N content (g kg^{-1}) at reference leaf determined at phenological stage R_1 (f) as a function of maize hybrids and doses of N applied in bands. ** p< 0.01.

technical efficiency maximum dose (TEMD) in 375 kg of N ha^{-1} under an estimated $CRIL_{11}R_1$ of 68.70 (Figures 1h), and for the P30R50 hybrid there was an increment of the $CRIL_{11}R_1$ with N doses applied in bands (Figure 2a).

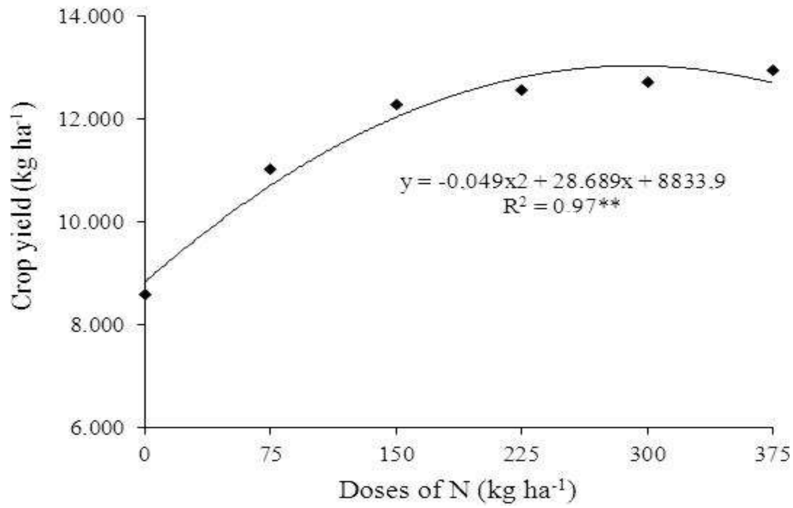

Figure 3. Maize crop yield (kg ha^{-1}) as a function of doses of N applied in bands. **p< 0.01.

The Pearson correlation coefficients between $CRIL_{11}R_1$ and leaf N content and yield were of 0.67^{**} and 0.82**, respectively (Table 3).

The mean value found for $CRIL_{11}R_1$ was of 60.13, an index higher than the mean CRI of 57.9 and lower than the maximum CRI of 68.9, as evidenced by Sunderman et al. (1997) for hybrids of maize at the same phenological stage under controlled water supply conditions. Such environmental conditions led to a mean yield of 14.455 kg ha^{-1}, showing a significant superiority to the maximum estimated yield observed in the current trial as 13.033 kg ha^{-1} (Figure 3).

For the variable $CRIL_{13}R_2$the unfolding of the interaction revealed a squared response for the P30R50 hybrid, having estimated a technical efficiency maximum dose in 235 kg of N ha^{-1} and a CRI in 66.37 (Figure 2b). The Pearson correlation coefficients between $CRIL_{13}R_2$ and leaf N content and yield were of 0.65^{**} and 0.80^{**}, respectively. The $CRIL_{13}R_3$ obtained for the AG8025 hybrid was higher in 4.37 for the P30R50 hybrid, corresponding to a value of 6.77% (Table 2). Regression analysis on the $CRIL_{13}R_3$ allowed to estimate the dose that would be quite conducive to the highest $CRIL_{13}R_3$ (75.4) as the one equivalent in 335 kg of N ha^{-1} (Figure 2c). In the current study, there was a positive correlation of the $CRIL_{13}R_3$ with the leaf N content given by a Pearson correlation coefficient of 0.69^{**}, along with a higher correlation coefficient of 0.86^{**} for yield. Hurtado et al. (2010) obtained in R_3 a CRI of 65.6, having been similar to the mean CRI of 66.76, as well as to the values obtained for each one of the hybrids in study (Table 2), but lower than the estimated value as a function of N doses (Figure 2c).

The highest $CRIL_{13}R_3$ obtained for the AG8025 hybrid evidences a more accentuate chlorophyll synthesis (Table 2). The fact that the plants show a higher content of chlorophyll, mainly of chlorophyll a, results in the highest photosynthetic rates. Consequently, yields will be the highest as shown in Table 3, for the AG8025 hybrid was the most productive. In order to reinforce such evidence in terms of physiological responsiveness, it may be observe in Table 3 that the highest Pearson correlation coefficient obtained for yield was just corresponding to the $CRIL_{13}R_3$, indicating that F_{13} is an important resource of photoassimilates for the grains.

Those doses estimated by means of equations that would be conducive to the maximum $CRIL_{15}R_2$ were of 260 and 275 of N ha^{-1} for the P30R50 and AG8025 hybrids, respectively, corresponding to a CRI of 71.69 and 77.77 (Figure 2d). The effect of N doses applied in bands for each hybrid on the $CRIL_{15}R_3$, shortly after the unfolding of the interaction, allowed to identify the doses of 375 and 245 kg of N ha^{-1} as being those that caused the P30R50 and AG8025 hybrids to present the highest values of $CRIL_{15}R_3$ (83.45 and 79.44, respectively) (Figure 2e).

CRI increased significantly throughout the maize crop growth season, corroborating the outcomes obtained by Argenta et al. (2010). Moreover, the results found in the current research, irrespective of the maize hybrid, leaf or phenological stage of the crop, showed higher CRI values than those obtained by Argenta et al. (2010), Godoy et al. (2007), Hurtado et al. (2009) and Hurtado et al. (2010). Fluctuations in the values of CRI reported by the literature might be ascribed to the equipment used, age of the leaf, position of the leaf, position reading on the leaf, phenological stage, cultural practices, and soil and climate conditions.

The mean leaf N content of 26.56 g kg^{-1} or 2.66% of the DPS determined in the reference leaf R_1 was found to be roughly above the adequate threshold (27.5 to 32.5 g kg^{-1}) in agreement with Malavolta et al. (1997). Ferreira et al. (2009) in a site under no-tillage system for 18 years observed for the AG9020, AG6018 and AG8021 hybrids mean leaf N contents of 23.17, 24.13 and 25.53 g kg^{-1}, respectively. Such indices were also above the range prescribed by Malavolta et al. (1997).

The leaf N content determined at R_1 was influenced by the N doses applied in bands (Figure 2f) without therefore expressing interactions with maize hybrids. Increments in N supplementation up to the estimated dose as TEMD was of 330 kg of N ha^{-1}, in which the reference leaf would reach a leaf N content of 31.26 g kg^{-1}. Hurtado et al. (2009) obtained a leaf N content of 30.25 g kg^{-1} by the time TEMD for yield was replaced with 242 kg of N ha^{-1}, corresponding therefore to a yield of 9.210 kg ha^{-1}. In the current work the estimated dose for maximum yields was of 295 kg of N ha^{-1}, culminating to a yield of 13.033 kg ha^{-1}. However, when such a dose was replaced with the one obtained by the fitted equation for the leaf N content (Figure 2f) in compliance with the proposition of Hurtado et al. (2009), the leaf N content to be obtained would be of 31.09 g kg^{-1}, reaching an index quite similar to that found by the aforementioned authors, but with a difference of 3.823 kg ha^{-1} in the grain yield.

By assessing four distinct field experiments, Argenta et al. (2010) obtained the following Pearson correlation coefficients between the readings of CRI performed in the reference leaf corresponding to the filling stage and maize yield: 0.69^{*}, 0.80^{*}, 0.87^{*} and 0.93^{*}. Table 3 reveals that readings of $CRIL_{11}CTE$, $CRIL_{11}R_1$, $CRIL_{13}R_2$ and $CRIL_{13}R_3$ were associated to Pearson correlation coefficients of 0.60^{**}, 0.82^{**}, 0.80^{**} and 0.86^{**}, respectively. The correlation coefficient of 0.69^{**} obtained between leaf N content and yield was lower than those coefficients reported by Argenta et al. (2002) in their four field trials (0.73^{*}, 0.76^{*}, 0.83^{*} and 0.91^{*}).

Argenta et al. (2010) obtained better correlations between CRI and leaf N content at more advanced phenological stages of the crop. Nevertheless, it can be noticed that such correlations get stronger with the physiological age of the leaves. This was observed during three evaluations at F_{05} for there was an increase of the correlation at the stage V_5 in relation to the V_7, as well from such stage to V_9 when the leaves 7, 9, 11 and 13 expressed the same behavior. This physiological responsiveness allows obtaining high correlations throughout the initial crop growth phases, such as vegetative phenological stage. Thus the observation on the age of the leaves for the assessment of CRI readings infers the efficiency of evaluation of N status in corn plants by means of CRI, regardless of the phenological stages related to vegetative and reproductive phases (Table 3).

The AG8025 hybrid productivity was higher than that of P30R50 in 1.510 kg ha^{-1}, corresponding to a difference of 13.8% (Table 2). Argenta et al. (2010) obtained a difference of 2.900 kg ha^{-1} between the P32R21 and Premium hybrids with final yields of 12.400 and 9.500 kg ha^{-1}, respectively. Discrepancies in productivity among maize hybrids were also found by Ferreira et al. (2009), whose outcomes report that hybrids such as AG9020 and AG8021 were more productive than AG6018.

The identification of the dose 295 kg of N ha^{-1} that would express maximum yields, equivalent to 13.033 kg ha^{-1} (Figure 3), corroborates the assumptions of Fontoura and Bayer (2009), since both scientists report that for yields above 12.000 kg ha^{-1} it is necessary an application within the range from 130 to 300 kg of N ha^{-1} in bands. The maximum yield estimated in 9.210 kg ha^{-1} obtained by Hurtado et al. (2009), which corresponded to 242 kg of N ha^{-1} in bands, resulted in a saving of 53 kg of N ha^{-1} causing therefore a reduction of 3.823 kg ha^{-1} in the productivity of grains. Different results reported by the literature might be attributed to differences in climate and soil conditions, management and cultural practices among studied sites in conjunction with genetic variability among hybrids. An inferior responsiveness was also found by Silva et al. (2005) with a TEMD estimated in 166 kg of N ha^{-1}, leading to a final yield of 6.709 kg ha^{-1}.

Over three years of evaluation, Holand and Schepers (2010) evidenced that the dose of 200 kg of N ha^{-1} brought about yields of 11.530, 12.110 and 13.660 kg of N ha^{-1} for maize crops. In the work published by Sangoi et al. (2009) the application of two doses of 100 kg of N ha^{-1} in bands at the phenological stages V_4 and V_{10} resulted in a yield of 12.634 kg of N ha^{-1} for the P30F53 simple hybrid. Such outcome was quite similar to that obtained for the AG8025 hybrid (Table 2) and for the maximum estimated yield with the dose of 295 kg of N ha^{-1} in bands (Figure 1) but with only one application at V_5. This appears to be an outstanding observation to surmise because P30F53 simple hybrid reveals an important participation in cultivated areas of the region of Campos Gerais of Paraná in order to assure the sustainability of agriculture in the southern regions of Brazil.

Conclusions

CRIs determined in the same leaf and at different phenological stages revealed that there is a rise in the values of such a physiological parameter, although the hybrids of maize did respond differently to the doses of N applied in bands. The correlation between CRI and leaf N content, as well as yield, increases with the age of the leaves, being useful to indicate the status of N in maize crop fields. A positive correlation between CRIs and leaf N content along with yield in different leaves and at different phenological stages made possible the identification of CRI for diagnosis of N status in maize hybrids with mean values ranging from 46.87 ($L_{03}V_5$) to 70.40 ($L_{15}R_3$). The necessity of N can be identified by means of CRI right on the initial stages of crop development (V_5, V_7 and V_9), causing its utilization to be rather feasible for precocious diagnosis of N status in maize crop. Moreover, when CRI determinations are performed at the end of vegetative stage, as well as throughout their productive phase it might indicate nitrogen fertilization efficiency in bands.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are very grateful to the State University of Ponta Grossa, Brazil, for the logistic support provided throughout the field trial. Special thanks are also devoted to the Conselho Nacional de Desenvolvimento Científico e Tecnológico, as well as to the Fundação Araucária for the concession of a productivity fellowship in research to the third and fourth authors of the current manuscript.

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academicJournals

Vol. 12(27), pp. 2283-2294, 6 July, 2017
DOI: 10.5897/AJAR2017.12375
Article Number: 29FBEC865084
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Growth patterns and Fulton's condition factor of the silver catfish *Chrysichthys nigrodigitatus* (Actinopterygii: Siluriformes: Claroteidae) from a sand-dragged man-made lake of Benin

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Received 12 April, 2017; Accepted 1 June, 2017

Notwithstanding the nutritional, economic and fisheries importance of *Chrysichthys nigrodigitatus*, and its relatively high abundance and propagation in Lake Ahozon (South Benin), nothing is known about the well-being of the species in this water body. Thus, this study investigated length and weight frequency distributions, length-weight relationships and condition factors of *C. nigrodigitatus*. A total of 1020 individuals of *C. nigrodigitatus* were sampled with seines, cast nets, gill nets and hooks during a 15 month period, from August 2014 to October 2015. Total length (TL) and body weight (W) frequency curves showed a unimodal distribution. The longest TL and highest W recorded were 324 mm and 456.1 g, respectively. In Lake Ahozon, *C. nigrodigitatus* population exhibited a significant ($P \leq 0.01$) isometric growth pattern, indicated by the power function ($W = 0.011TL^{2.99}$; r=0.96) and the log-transformed linear regression equation (LogW = 2.99 LogTL - 1.94; r=0.96). Growth models varied spatially, sexually, ontogenetically, and seasonally, while corresponding subpopulations showed allometric or isometric growth trends, with slopes *b* ranging between 2.47 and 3.21. The population showed conditions factors (K) ranging between 0.8 and 3.08 which significantly varied with habitats ($F_{1,1018}$ = 7.613, P = 0.006), seasons ($F_{2,1017}$ = 29, P = 0.0001) and life stage categories ($F_{2,1017}$ =5.583, P = 0.004). The active breeding that provided an important stock of offspring for recruitment, the isometric growth model and the relatively high condition factors indicated the perfect establishment of *C. nigrodigitatus* in the artificial lake of Ahozon. The sustainable exploitation of fisheries in this non-conventional medium-environment requires an integrated approach of lake management.

Key words: Claroteid, condition factor, establishment, fisheries, isometric growth, length-weight model, man-made lake.

INTRODUCTION

The silver catfish *Chrysichthys nigrodigitatus* (Lacepède, 1803) is widely distributed in the Western African inland waters, but also occurs to a lesser extent in Central, Northern and Southern Africa (Azeroual et al., 2010).

This catfish is a highly valuable resource for its numerous fisheries and commercial importance (Leveque, 1997; Andem et al., 2013), its intensive use in aquaculture (Kareem et al., 2015) and its nutritional role. In human health, *C. nigrodigitatus* provides highly nutritive proteins and nutrients, such as the omega-3 fatty acid, required by human body to maintain its cardiovascular health and to reduce tissue inflammation (Abowei and Ezekiel, 2013). Furthermore, *C. nigrodigitatus* is known for its ecological role in determining the dynamics and the structure of aquatic ecosystems (Abu and Agarin, 2016).

In the Benin freshwater and brackish water systems, *C. nigrodigitatus* is widely distributed (Van Thielen et al., 1987) and is among the most exploited fish species (Leveque et al., 1992; Laleye et al., 1995). Moreover, in Southern Benin, the species was introduced in a sand-dragged artificial lake of Ahozon where it constituted about 29.40% of the lake's fish biomass (Gbaguidi et al., 2016a, b). In Lake Ahozon, *C. nigrodigitatus* showed a sex-ratio of 1:2.1 and fecundities ranged between 350 and 26 040 eggs (Adité et al., 2017). In this special habitat, the species reproduced all seasons and exhibited a multiple spawner behavior, with sizes at first sexual maturity of 20 and 17 cm-TL for males and females, respectively. *C. nigrodigitatus* exhibited an opportunistic benthic food habit and fed mainly on aquatic insects, sand particles, detritus, seeds and algae (Lawal et al., 2010; Atobatele and Ugwumba, 2011; Gbaguidi et al., 2016). In the artificial lake of Ahozon, the species is sporadically exploited by a couple of migrant fishermen for domestic uses and sales.

Despite the nutritional, economic and fisheries importance of *C. nigrodigitatus*, and its relatively high abundance and propagation in Lake Ahozon, nothing is known about length-weight relationships and condition factors of the species in this water body. Length-weight models of fishes depict growth patterns that could be isometric when length and weight increase at identical growth rates, or rather allometric when these two morphometric traits increase at different growth rates (Adeyemi, 2010; Abowei, 2010b; Adeboyejo et al., 2016). Likewise, condition factor indicates the well-being and robustness of fishes and reflects interactions between biotic and abiotic parameters (Iyabo, 2015). Body condition of fishes is mainly affected by food availability (Ayoade and Ikulala, 2007), climate (Atobatele, 2013), gender, maturity stage, environment degradation and the overall ecological health of habitats (Bolarinwa, 2016). Indeed, domestic waste dumping, intrusion of agricultural pesticides and effluent discharges from various industries could alter water quality and modify the biological diversity with in particular, changes in the fish community structure and growth patterns. As reported by Seiyaboh et al. (2016), fish are able to bioaccumulate and biomagnify contaminants from aquatic ecosystems and both water quality alterations, and direct intrusions of contaminant in the body will negatively affect the growth and condition factors of fishes.

This investigation seeks to examine length-weight relationships and condition factors of *C. nigrodigitatus* to fill a gap in the current biological knowledge of the species, and to contribute to fisheries management and the valorization of numerous sand-dragged artificial lakes of Southern Benin.

MATERIALS AND METHODS

Study region

The study location is the artificial lake of Ahozon situated in Ouidah City in Southern Benin (Figure 1a). Lake Ahozon (06°22'52"N; 002°10'34"E) is a sand-dragged water body extending on about 0.165845 km^2 (Figure 1b) and lies between a freshwater body, Lagoon Toho-Todougba, and a brackish water, the Benin coastal lagoon. The study area showed a sub-equatorial climate with two wet seasons (April-July; mid-September-October) and two dry seasons (December-March; August-mid-September). Annual average rainfall reached 1310 mm (Akoegninou et al., 1993) and ambient temperatures varied from 25 and 33.6°C (ASCENA, 2003). Intense agriculture using pesticides dominates the study area.

Lake Ahozon is an unmanaged, neglected and abandoned water body that receives every wet season, an important volume of running waters (Adite et al., 2017). However, as reported by Gbaguidi et al. (2016), evaluation of water physicochemical parameters indicated that Lake Ahozon is of good quality and favorable for the development of aquatic organisms, including fish resource.

Dominant phytoplankton genera identified in Lake Ahozon were *Navicula, Peridinium, Scennedesmus, Pinnularia, Spirogyra, Cosmarium, Melosira, Synechocystis, Microcystis, Oscillatoria, Euglena, Phacus, Surirella* and *Lychmophora*, and zooplanktons were dominated by *Trichocerca, Keratella, Brachionus* and copepods. Benthic macro-invertebrates recorded were chironomid larvae and a Gasteropod mollusk, *Melanoïde tubercularis*. Aquatic vegetation was dominated by *Cyperus crassipes*, *Cyperus rotundus*, *Fuirena umbellata*, *Andropogon gayanus*, *Ludwigia perennis*, *Emilia praetermissa*, *Eleocharis complanata*, *Enydra fluctuans* and *Mariscus ligularis*. Six species, *Sarotherodon galilaeus*, *Oreochromis niloticus, Tilapia guineensis*, *Chrysichthys nigrodigitatus*, *Heterotis niloticus* and *Clarias gariepinus* (Gbaguidi et al., 2016a, b) composed the fish community of Lake Ahozon, and were sporadically exploited by a couple of migrant fishermen.

Sampling sites

In Lake Ahozon *C. nigrodigitatus* individuals were sampled on four sites including two in the "aquatic vegetation" habitat and two in the "open water" habitat (Figure 1b). The "aquatic vegetation" habitat, the edge of the lake, is shallow and characterized by a low water

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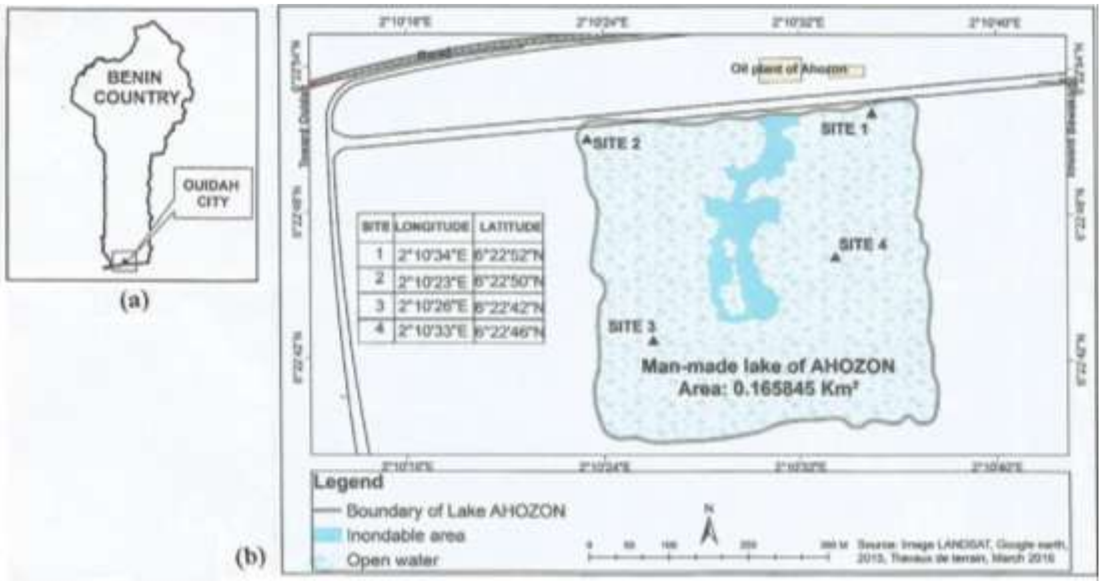

Figure 1. Map showing **(a)** Ouidah City (South-Benin) the study location and **(b)** Lake Ahozon along with the sampling sites.

velocity and relatively dense vegetation. The “open water” habitat is exempt of vegetation and exhibited a relatively high depth and high water velocity.

Fish samplings

Fish collections were done twice a month from August 2014 to October 2015. Samplings were performed both in the “aquatic vegetation” habitat and in the “open water” habitat using cast net (9.80 m-diameter, 4.90 m-height, 40 mm-mesh), seine (4.15 m-length × 1.77 m-width, 3 mm-mesh), hooks (90 m-length) and experimental gill nets (40 m × 1.05 m, 40 mm mesh). Seines were used at the lake’s edge whereas cast nets, gill nets and hooks were used in the “open water” according to Adite et al. method (2017). The two samplings per month and per habitat and the use of various fishing gears helped to get a relatively high sample size (1020 individuals) that reflects the population structure of *C. nigrodigitatus*.

Once collected, *C. nigrodigitatus* individuals were identified first *in situ* using references such as Needham and Needham (1962), Van Thielen et al. (1987) and Leveque et al. (1992). Individuals were then counted by habitat (open water, aquatic vegetation), gender (male, female), life stage (juvenile, sub adults, adults) and season (flood, dry, wet) to compute the relative abundance of each corresponding subpopulation. In addition, each of the 1020 individual was measured for its total length (TL) and standard length (SL) to the nearest 0.1 mm with a measuring board, and weighted to the nearest 0.1 g with an electronic scale. Fish samples were then preserved in 10% formalin and transported to the “Laboratoire d’Ecologie et de Management des Ecosystèmes Aquatiques (LEMEA)” and preserved in 90% ethanol.

Data analysis

Spatial, seasonal, sexual and ontogenetic biometric data including total length (TL), standard length (SL) and fish individual weight (W) were recorded in spreadsheets generated from SPSS computer software (Morgan et al., 2001). Range and average values of biometric data were also computed for *C. nigrodigitatus* population and for habitat, season, life stage, and gender subpopulations. The population size structure was examined by generating standard lengths (SL) frequency histograms of *C. nigrodigitatus*. Length-weight models were examined following Le Cren’s (1951) power function along with its logarithmic-transformed linear model:

$$W = aTL^{b} \quad \text{(Le Cren, 1951)} \qquad (1)$$

$$\text{LogW} = \text{Loga} + \text{b LogTL} \qquad (2)$$

where TL is the fish total length, W is the fish individual weight, a is the intercept, and b, the slope, is the allometry coefficient (Le Cren, 1951). One-way analysis of variance (ANOVA) was used to test significance of the regression. Fulton’s condition factors of *C. nigrodigitatus* population were computed following Tesch (1971) model:

$$K = \frac{W}{TL^3} \times 100 \qquad (3)$$

where K is the Fulton’s condition factor, W, the fish individual weight (g), TL the total length (cm), and $b = 3$ is the allometry coefficient. In addition, Fulton’s condition factors (K) and growth trends (length-weight relationships) were examined by habitat types (open water; aquatic vegetation), genders (male, female), life stage categories (juveniles, sub adults, adults) and seasons (flood, dry, wet).

RESULTS

Population structure

Abundance

In the sand-dragged man-made lake of Ahozon, the introduced silver catfish, *C. nigrodigitatus* exhibited

Table 1. Spatial, sexual, ontogenetic and temporal variations of the population structure of *Chrysischtys nigrodigitatus* from the artificial lake of Ahozon (South-Benin).

Subpopulations	Abundance (N)	Relative abundance (%)	SL mean (mm)	SL range (mm)	Weight mean (g)	Weight Range (g)
Habitat						
Aquatic vegetation	103	10.10	93.7	52-115	18.91	3.6-32.6
Open water	917	89.90	130	96-285	57.14	12.2-456.1
Sex*						
Male	322	31.57	134	52-285	60.79	3.6-456.1
Female	692	67.84	123	67-255	49.73	6.6-385.6
Life stage						
Juvenile	209	20.49	99.30	52-109	23.22	3.6-45.5
Sub-adult	608	59.61	122	110-135	40.6	22.5-99.9
Adult	203	19.9	169	136-285	122.18	12.2-456.1
Season						
Flood	236	23.14	125	70-190	44.76	9.7-171.4
Dry	170	16.67	135	67-285	85.76	6.6-456.1
Wet	614	60.19	125	52-262	47.57	3.6-344.2
Total	1020	100	126.5	52-285	53.22	3.6-456.1

*Genders were undetermined for small individuals (0.59% of the sample).

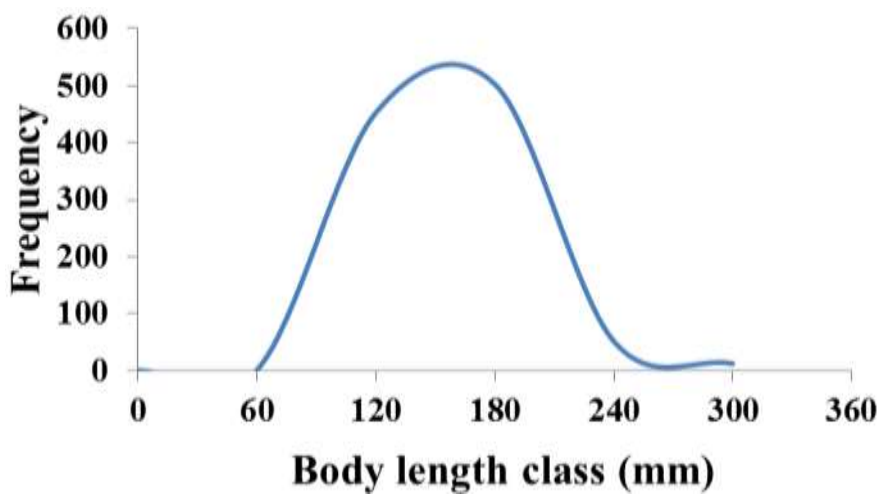

Figure 2. Length frequency distributions of *Chrysichthys nigrodigitatus* (N=1020) from the artificial lake of Ahozon (South-Benin).

significant ($P \leq 0.05$) spatial and seasonal variations of the species relative abundance with the "open water" habitat comprising alone 89.90% of the population and the "wet season" subpopulation making alone 60.19% (Table 1). Sexual variations of abundance were depicted with females dominating the population and numerically made up 67.84% of *C. nigrodigitatus* assemblages. Ontogenetically, aggregated juveniles (mean SL: 99 mm) and sub- adults (mean SL: 122 mm) made about 80.10% of the population.

Length and weight frequency distributions

Figures 2 and 3 show standard length (SL) and body weight (W) frequency distributions of *C. nigrodigitatus*

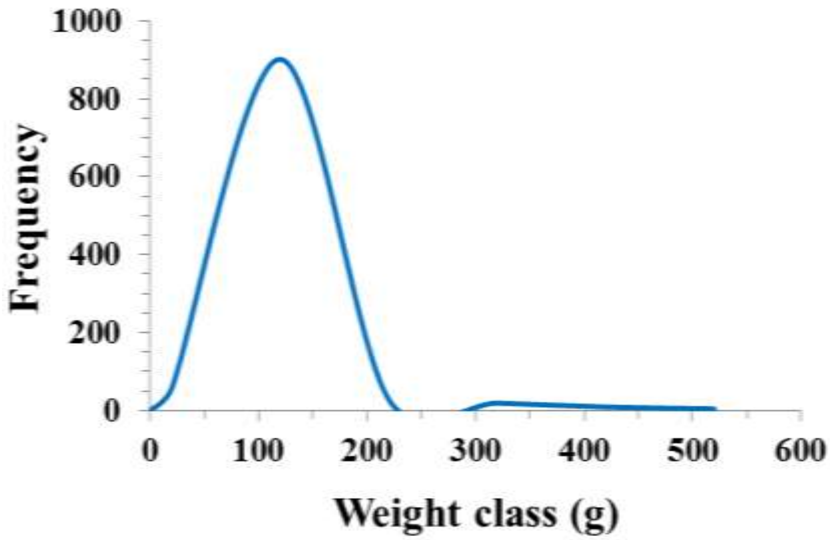

Figure 3. Weight frequency distributions of *Chrysichthys nigrodigitatus* (N=1020) from the artificial lake of Ahozon (South-Benin).

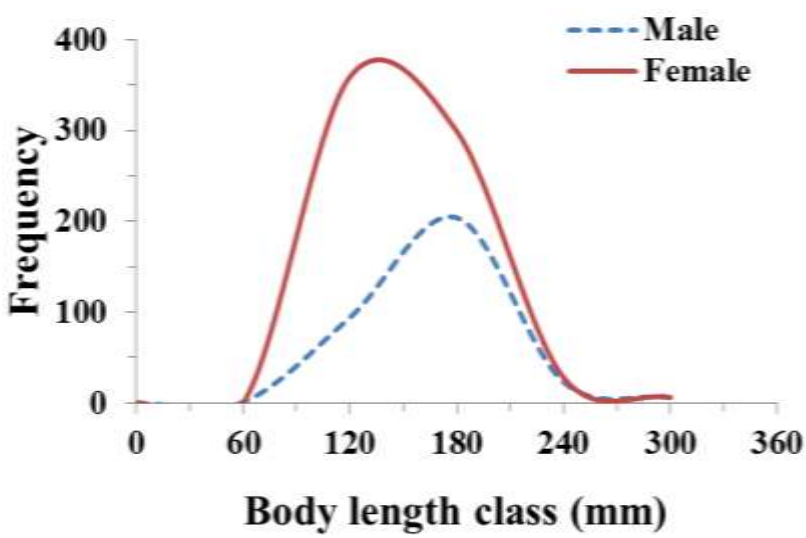

Figure 4. Length frequency distributions of *Chrysichthys nigrodigitatus* males (N=322) and females (N=692) from the artificial lake of Ahozon (South-Benin).

from Lake Ahozon. Overall, the population showed, SL varying between 52 mm (TL: 67 mm) and 285 mm (TL: 324 mm). The length-frequency curve established for the whole population exhibited a unimodal size distribution with the modal class (120 to 180 mm) showing the highest frequency occurrence of 503 individuals and class (0 to 60 mm) showing the lowest frequency occurrence of one individual (Figure 2).

Body weight ranged between 3.6 g (SL: 52 mm) and 456.1 g (SL: 246 mm) and like body length, the weight-frequency curve established for the whole population showed a unimodal distribution with the modal class (20 to 120 g) showing the highest frequency of 902 individuals and class (420 to 520 g) showing the lowest frequency of four individuals (Figure 3). Sexually, the same trends of unimodal distributions for length and weight frequency were recorded for both genders (Figures 4 and 5). Spatially, standard length (SL) frequency histograms showed unimodal size distributions for both “open water” habitat and “aquatic vegetation” habitat (Figures 6 and 7).

Length-weight relationships

Figures 8 and 9 show the length-weight models

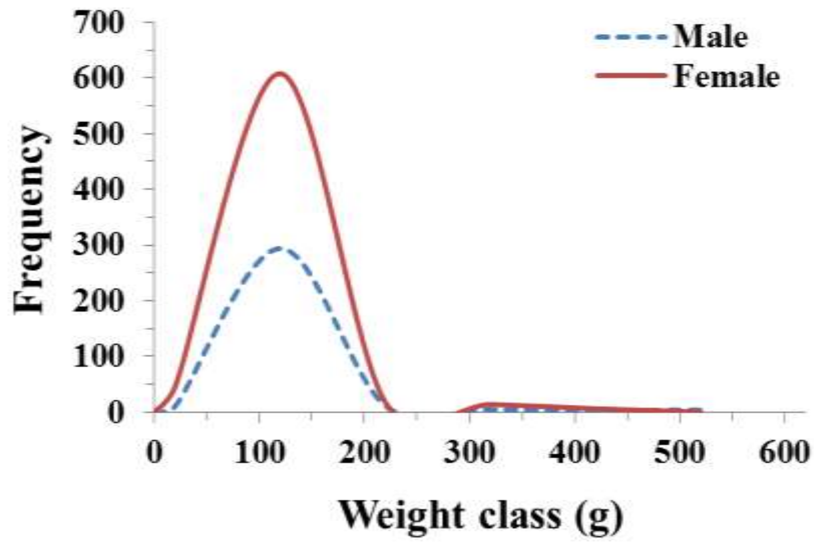

Figure 5. Weight frequency distributions of *Chrysichthys nigrodigitatus* males (N=322) and females (N=692) from the artificial lake of Ahozon (South-Benin).

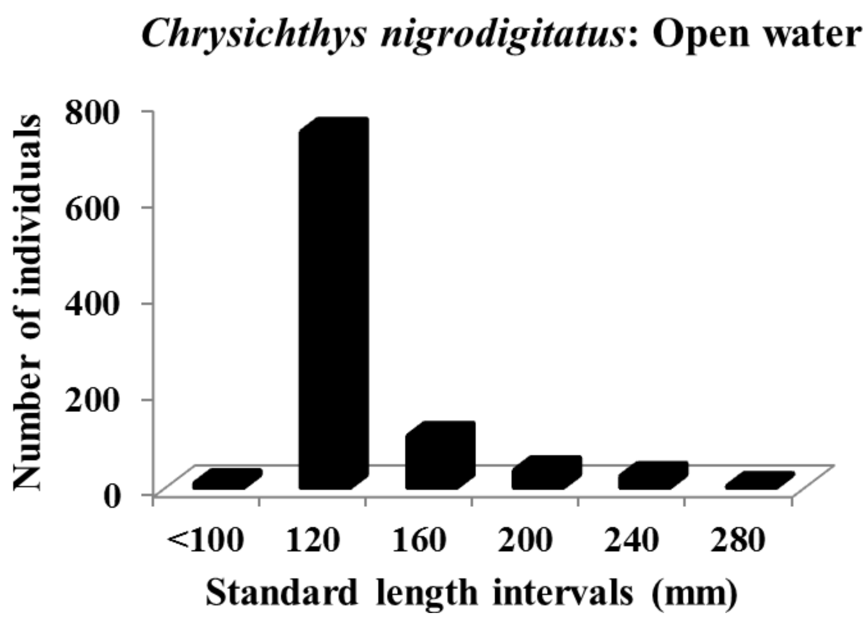

Figure 6. Size structure of *Chrysichthys nigrodigitatus* (N=917) from the "open water" habitat of the artificial lake of Ahozon (South-Benin).

established between *C. nigrodigitatus* total length (TL) and body weight (W). The resulting power function equation and the logarithm-transformed linear regression equation for the whole population were as follow:

$W = 0.011TL^{2.99}$ (Le Cren, 1951); (4)

Log W = 2.99 Log (TL) - 1.94 (5)

r = 0.96; N=1020

where W is the individual weight, TL is the total length, R is the correlation coefficient and N, the number of individuals.

In addition, the linear regression equations established for habitat type, gender, life category and season sub-populations gave positive slopes ranging between b=2.47 for the flood season sub-population and b=3.21 for the

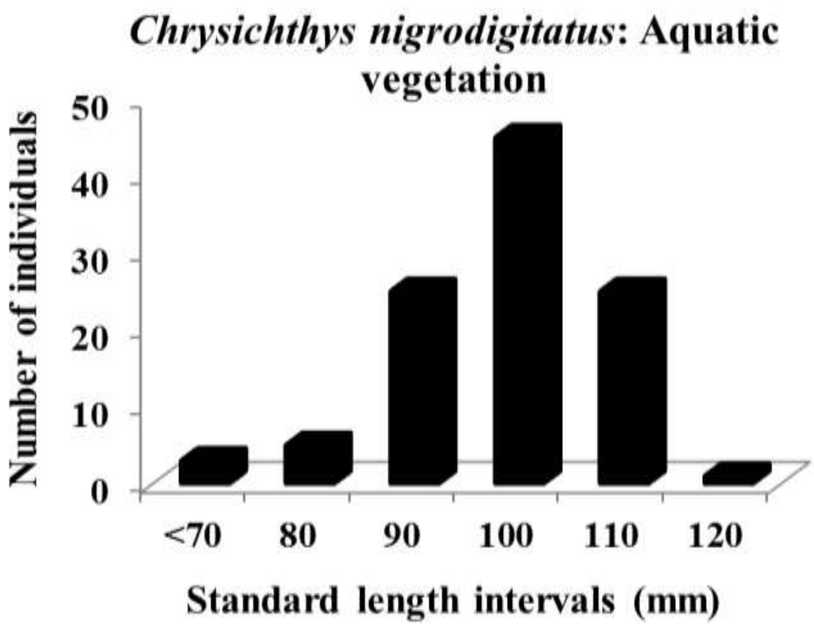

Figure 7. Size structure of *Chrysichthys nigrodigitatus* (N=103) from the "aquatic vegetation" habitat of the artificial lake of Ahozon (South-Benin).

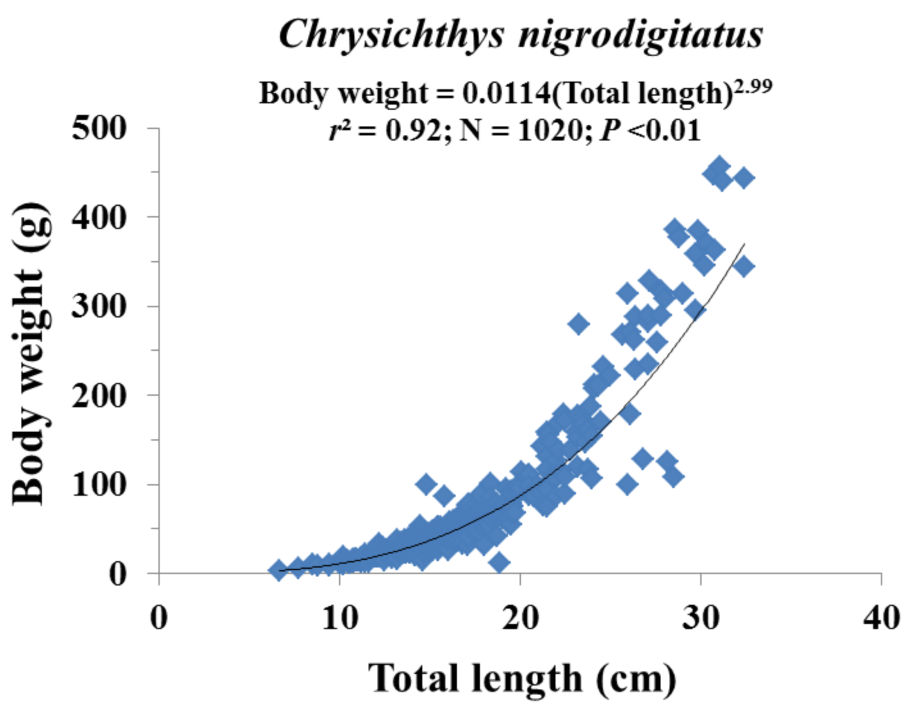

Figure 8. Power function of body weight (W) against total length (TL) of *Chrysischtys nigrodigitatus* from the artificial lake of Ahozon (South-Benin).

juvenile life stage sub-population. The associated correlation coefficients "*r*" were significant ($P \leq 0.05$) and varied between 0.77 and 0.99 (Table 2). The results consistently indicated that the sub-population of dry period showed higher slope b = 3.17 compared with flood and wet seasons. Ontogenetically, juvenile sub-population exhibited higher slope b = 3.21 compared to sub-adults and adults. Spatial variations of growth were

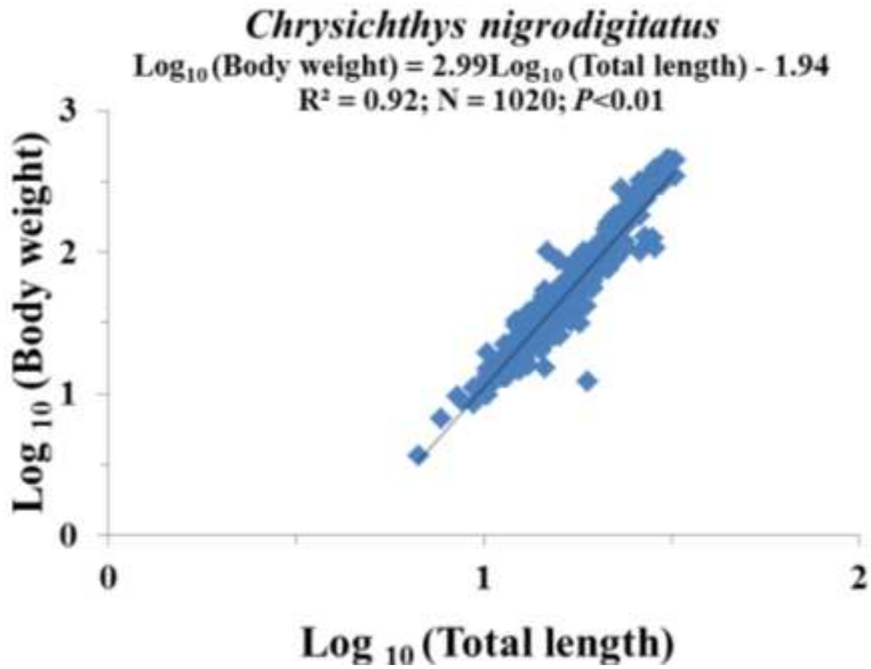

Figure 9. Linear relationship between body weight (W) and total length (TL) of *Chrysischtys nigrodigitatus* from the artificial lake of Ahozon (South-Benin).

Table 2. Spatial, sexual, ontogenetic and temporal variations of growth patterns of *Chrysischtys nigrodigitatus* from the artificial lake of Ahozon (South-Benin).

Subpopulations	Abundance (N)	Regression equation (LogW = Log a + bLogTL)	Correlation coefficient (*r*)	Growth pattern
Habitat				
Aquatic vegetation	103	LogW = -2.03 + 3.06LogTL	0.95	Isometry
Open water	917	LogW = -1.57 + 2.68LogTL	0.91	Allometry-
Sex*				
Male	322	LogW = -1.96 + 3.01LogTL	0.96	Isometry
Female	692	LogW = -1.93 + 2.97LogTL	0.95	Isometry
Life stage				
Juvenile	209	LogW = -1.93 + 3.21LogTL	0.93	Allometry+
Sub-adult	608	LogW = -1.56 + 2.92LogTL	0.89	Allometry-
Adult	203	LogW = -1.23 + 2.61LogTL	0.77	Allometry-
Season				
Flood	236	LogW = -1.32 + 2.47LogTL	0.85	Allometry-
Dry	170	LogW = -2.13 + 3.17LogTL	0.98	Allometry+
Wet	614	LogW = -1.84 + 2.89LogTL	0.95	Allometry-
Total	1020	LogW = -1.94 + 2.99LogTL	0.96	Isometry

*Genders were undetermined for small individuals (0.59% of the sample). Allometry-: Negative allometry; Allometry+: Positive allometry. P<0.01 for all regression slopes.

recorded with the “aquatic vegetation” sub-population showing higher slope $b = 3.06$, whereas sexually, male

Table 3. Spatial, sexual, ontogenetic and temporal variations of the condition factors (K) of *Chrysischtys nigrodigitatus* from the artificial lake of Ahozon (South-Benin).

Subpopulations	Abundance (N)	Mean condition factor (K)	Range (K)	±SD
Habitat				
Aquatic vegetation	103	1.16	0.75-1.81	0.17
Open water	917	1.11	0.18-3.08	0.006
Sex*				
Male	322	1.12	0.46-3.08	0.19
Female	692	1.10	0.18-1.64	0.18
Life stage				
Juvenile	209	1.15	0.54-3.08	0.26
Sub-adult	608	1.12	0.49-2.02	0.17
Adult	203	1.10	0.18-1.81	0.17
Season				
Flood	236	1.13	0.18-1.81	0.16
Dry	170	1.21	0.46-2.20	0.22
Wet	614	1.09	0.49-3.08	0.19
Total	1020	1.12	0.18-3.08	0.19

*Genders were undetermined for small individuals (0.59 % of the sample).

sub-population exhibited higher slope *b* = 3.005, corresponding to an isometric growth pattern.

Condition factors (K)

The body condition of *C. nigrodigitatus* from Lake Ahozon was evaluated through Fulton's condition factors (K) that were computed by season, life stage category, gender and habitat type. Overall, the population exhibited condition factors (K) ranging between 0.18 and 3.08 and averaging 1.12±0.19 (Table 3). Significant ($P\leq0.05$) variations of average K were also recorded seasonally, ontogenetically, sexually and spatially. Seasonally, the mean condition factor was higher during the dry period with K= 1.21±0.22 compared to those of wet and flood period. Ontogenetically, juvenile sub-population exhibited better condition (mean K= 1.15±0.26) compared to sub-adult and adult sub-populations, whereas sexually, male sub-population exhibited higher mean condition indices (1.12±0.19). Spatially, the "aquatic vegetation" sub-population showed higher condition factor (Mean K= 1.16 ±0.17), compared to the open water subpopulation.

DISCUSSION

Fisheries management of numerous abandoned sand-dragged artificial lakes distributed throughout Benin is badly needed to valorize fish resources (Laleye et al., 2004), to increase grass-root revenues (Gbaguidi et al., 2016), to lessen degradation pressures and to contribute to restore natural aquatic biota (Iyabo, 2015). Two years and half after its introduction in the sand-dragged man-made lake of Ahozon, *C. nigrodigtatus* efficiently exploited the existing food resources and spawned actively to become one of the prominent fishable species in this special water body (Adite et al., 2017). Management evaluation tools such relative abundance, length and weight frequency, length-weight relationships and condition factors globally indicated a perfect establishment of this catfish in Lake Ahozon.

Abundance, length/weight frequencies and growth patterns

In Lake Ahozon, *C. nigrodigitatus* made about 29.40% of the fish biomass and numerically accounted for 11.22% of the fish community (Gbaguidi et al., 2016). As further reported by Gbaguidi et al. (2016), the favorable physicochemical traits of Lake Ahozon which is within the tolerance ranges of *C. nigrodigitatus*, the high availability and utilization of food resources, the active breeding and the multiple spawner behavior accounted for the relatively high propagation of this claroteid in Lake Ahozon. In particular, the high abundance (80%) of aggregated juveniles and sub-adults indicated that *C. nigrodigitatus* provided an important stock of offspring for recruitment in this water body. These results are comparable to those recorded on natural coastal lakes and lagoons of Benin where *C. nigrodigitatus* biomass reached 26% of the fish productions (Direction des Pêches, 1996). These findings indicated that this claroteid is well-established in the man-made lake of Ahozon as it is observed in most aquatic natural biota of Benin. In contrast, Akpan (2013) reported for these species, a lower percentage biomass from Uta Ewa Creek of Niger Delta Region of Nigeria, probably because of overfishing and environmental degradation.

In Lake Ahozon, the maximum total length (TL = 324 mm) recorded, though relatively high, is lower than TL = 590 mm reported by Laleye et al. (1995) in Lake Nokoué in Benin and TL = 1090 mm reported by Andem et al. (2013) in the Itu Head Bridge, in Akwa Ibom State of Nigeria. Inversely, the maximum TL = 324 mm recorded in Lake Ahozon was higher than that reported by Iyabo (2015) in the Ebonyi River, South Eastern of Nigeria. According to King (1996), the maximum lengths attained by fishes depend on the species, habitat conditions and its variability, season and environmental stochasticity. For example, Lake Nokoué, the largest water body in Benin, exhibited a high productivity because of the seasonal flooding of Oueme and Sô rivers (both connected to Lake Nokoué) that discharge a huge quantity of nutrients in this coastal lake. Also, Lake Nokoué was invaded by “acadja” that fertilizes this water body and hence, increases its primary productivity. The “acadja” is a park of tree branches constructed in the shallow parts of the lake by local fishermen as a fishery method (Laleye et al., 1995). In contrast, Lake Ahozon is an isolated young man-made water body of modest productivity because exempt of local management tool such as “acadja” and lacking connection with other inland waters from which this lake could receive nutrients to boost its productivity and to improve the growth of fishes.

Regardless of habitat, season, gender and developmental stage, length-weight regression equations of *C. nigrodigitatus* population gave significant ($P \leq 0.05$) slopes b=2.99, indicating that, globally, this claroteid exhibited an isometric growth pattern (Table 2 and Figures 8 and 9). Consequently, length and weight increased approximately at identical growth rates and hence, during life cycles, the shape of the fish remained nearly constant. The high correlation coefficient (r=0.96) recorded indicated that there was a strong association between total length and body weight (Nwani, 2006). These results agree with those reported by Offem et al. (2008) and Bolarinwa (2015) that have reported an isometric growth trend for *C. nigrodigitatus* population in the forest-savana of the Cross River and in the Epe Lagoon of Nigeria, respectively. Inversely, several authors such as Adeboyejo et al. (2016), Iyabo (2015), and Abu and Agarin (2016) reported positive and negative allometric growths for *C. nigrodigitatus* in Badagry Creek of Lagos, in the Ebonyi River of South-East and in the Lower Reaches of the New Calabar River Niger Delta of Nigeria, respectively. Positive allometric growth, as noted by Tesch (1971) and Bagenal (1978), suggests that body weight increased faster than length whereas negative allometric growth implies that length increased faster than weight and hence, fish become more slender and less valuable. In this study, the isometric growth trends depicted in *C. nigrodigitatus* is the result of the favorable water quality of Lake Ahozon characterized by a moderate temperature (mean: 33.25±2.20°C), a high water transparency (mean: 36.63±11.18 cm), a high conductivity (mean: 240±118.40 µ/cm), an alkaline pH (mean: 7.51±0.78), a relatively high concentration of dissolved oxygen (mean: 5.43±2.52 mg/L) and a relatively high percent of saturation (mean: 82.69±41.52%) (Gbaguidi et al., 2016).

In Lake Ahozon, length-weight models varied with habitats (Open water, Aquatic vegetation), seasons (flood, dry, wet), genders (male, female) and life stage category (juvenile, sub adults, adults) with slopes varying between 2.47 and 3.21 (Table 2). Spatially, the aquatic vegetation subpopulation showed an isometric growth (b = 3.06) compared to the open water habitat that exhibited a negative allometric growth (b = 2.68) and where preys were probably less available and less concentrated (Gbaguidi et al., 2017). Though females are generally known to exhibited lower growth rates compared to male because females mobilized their metabolic energy to reproduce, in this study, males and females showed an isometric growth rate and exhibited nearly identical slopes b = 3.01 and b = 2.97, respectively. Probably, the relatively high and identical growth rates recorded in both genders were the results of the high availability of food resources along with their high exploitation and utilization by both genders. Ontogenetically, the sub-populations of the three life stage categories, juveniles, sub-adults and adults showed allometric growth patterns. Particularly, and in contrast with sub-adults and adults, juvenile sub-population exhibited a positive allometric growth trend with b=3.21, the highest slope recorded during this study. As reported by Gbaguidi et al. (2016), juveniles utilize their entire metabolic energy to grow, whereas adults are mostly involved in spawning and mobilized most of their energy for gonad maturation, egg laying, hatching, and parental care of larvae and hence, exhibited in this study, negative allometric growth with slope b<3, indicating that, as length increased the fish became thinner. Seasonally, dry period sub-population showed a positive allometric growth pattern with b=3.17, probably because of the water reduction that caused a high concentration of food resources more available for *C. nigrodigitatus*. In contrast, the wet and flood season sub-populations exhibited a negative allometric growth trend with slopes b <3. During these periods, food resources were probably more diluted, less concentrated and hence, less available for individuals.

Body condition

In this artificial medium-environment, *C. nigrodigitatus* showed relatively high condition factors (K) ranging between 0.8 and 3.08 and averaging 1.12±0.19. These findings agree with those reported by Abowei and Ezekiel (2013) in the Amassoma River flood plains of Nigeria. However, Iyabo (2015) reported lower condition factors ranging between 0.58 and 1.74 from the Ebonyi River in South Eastern Nigeria. In contrast, higher condition factors were reported by Bolarinwa (2016) in the Mahin lagoon of Nigeria and by Abu and Agarin (2016) in the Lower Reaches of the New Calabar River Niger Delta. The differential niche breadth, which could be relatively reduced in Lake Ahozon, a young water body, may have been the cause of these variations in the condition factors (K).

For example, in Lake Ahozon, the dominant species *Sarotherodon galilaeus* (Cichlidae) is a pelagic alguivorous specialist that showed a mean condition factor K= 2.26±0.84 (Gbaguidi et al., 2016) higher than that (1.12±0.19) recorded for *C. nigrodigitatus*, a benthic feeder that foraged mainly on aquatic insects, substrate particles and detritus. This gap in the condition factor between these two sympatric species could be attributed to the distinctive ecological niche that was probably more available to *S. galilaeus* than to *C. nigrodigitatus.* Indeed, except in the aquatic vegetation habitat where the bottom was mostly constituted of benthic preys, the bottom of the open water, the major habitat, was sandy with less benthic substrates because Lake Ahozon is a “young” water body isolated from rivers and floodplains from which it could receive nutrients to increase its primary production and to improve the body condition of *C. nigrodigitatus*, a benthic feeder.

The study also revealed spatial, ontogenetic and seasonal variations of body condition in the sand-dragged man-made lake of Ahozon (Table 3). Indeed, one-way ANOVA on K across habitats (open water, aquatic vegetation), seasons (flood, dry, wet), and life stages (juveniles, sub adults, adults) subpopulations showed significant (P<0.01) variations of condition indices. The computed F-values, along with degrees of freedom and P-values were $F_{1,1018}$ = 7.613, P = 0.006 for habitats, $F_{2,1017}$ = 29, P = 0.0001 for seasons, and $F_{2,1017}$ =5.583, P = 0.004 for life stages. Overall, condition factors were higher in the aquatic vegetation subpopulation where K averaged 1.16±0.17. As reported by Gbaguidi et al. (2017), foods are usually more available, more diversified and concentrated in aquatic vegetation habitats. Likewise, *C. nigrodigitatus* showed a higher well-being (mean K: 1.21±0.22) during the dry period when water volume was reduced and more concentrated in preys items. Ontogenetically, juvenile subpopulation exhibited a higher condition factors with K averaging 1.15±0.26, compared to sub-adults and adults showing lower values. Indeed, in general, juvenile mobilized its whole metabolic energy to grow whereas adults were mostly involved in spawning and consequently, mobilized their metabolic energy for reproduction and parental care (Murphy and Willis, 1996).

In contrast with habitats, seasons and life stages, one-way analysis of variance on K across males and females showed insignificant ($F_{1,1011}$ = 1.942, P = 0.164) sexual variation of condition factors, probably because preys were highly available to both genders that foraged *ad libitum* to compensate energy spent during spawning. Nevertheless, though insignificant, the well-being of male subpopulation tended to be higher than that of females.

Conclusion

The current study revealed that *C. nigrodigitatus* was well-conditioned and globally showed an isometric growth pattern, indicating that Lake Ahozon was suitable for the well-being and propagation of this valuable claroteid. The results showed spatial, seasonal and ontogenetic variations of growth factors. Length and weight frequency distribution evidenced the availability of fishable subpopulation composed of larger individuals along with an important stock of offspring for recruitment. Sustainable exploitation of Lake Ahozon fisheries requires an integrated management scheme including a periodic physico-chemical and biota monitoring of the water body.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

Logistic and financial assistance were provided by the “Laboratoire d’Ecologie et de Management des Ecosystèmes Aquatiques, Département de Zoologie, Faculté des Sciences et techniques, Université d’Abomey-Calavi”. We express our gratitude to Mr Doukpo Célestin, the owner of Lake Ahozon to allow us to conduct this investigation on the water body. We are also grateful to Mr Houessinon Geoffroy and Djihouessi Bernold for their assistance in fish sampling and laboratory works

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Vol. 12(27), pp. 2295-2309, 6 July, 2017
DOI: 10.5897/AJAR2017.12442
Article Number: 2EE2FF565086
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of temperature on sweet potato virus disease symptom expression

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Received 10 May, 2017; Accepted 6 June, 2017

The incidence and severity of sweetpotato virus disease (SPVD) was reported to be highly variable under different agroecological zones in Uganda, a situation that could be partly attributable to differences in temperature. This raised a need for understanding the effect of temperature on the biology of SPVD causative agents which ultimately influences disease development and symptom expression that undermines productivity among sweet potato cultivars. This study was carried out at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK). Initially clean sweet potato cultivars were inoculated with two viruses namely *Sweet potato chlorotic stunt virus* (SPCSV) and *Sweet potato feathery mottle virus* (SPFMV) that cause SPVD when co-infecting sweet potato and established at two temperature environments; field and glasshouse, followed by a weekly interval monitoring of the plants for symptom expression and growth response. Temperature differences significantly (p<0.001) influenced SPVD severity and the growth response of different sweet potato cultivars. Overall, the plants under field conditions where temperature was lower produced higher SPVD severity than under glasshouse where higher temperatures were recorded. SPVD severity for most of the cultivars was higher in the field than under glasshouse. Cultivar (cv.) Ejumula displayed the highest severity levels followed by cvs. Tanzania and Beauregard. Conversely, New Kawogo, Dimbuka and Naspot 1 showed none to mild severities particularly under the glasshouse conditions. Therefore temperature influenced the development of SPVD; low temperatures of 20 to 29°C produced more disease severities than high temperatures of 30 to 39°C. It is suggested that reasonably high temperatures under a controlled environment should be incorporated in any sweet potato seed production system for possible elimination of SPVD.

Key words: Temperature, disease symptom expression, viruses, *Ipomea batatas*, growth response.

INTRODUCTION

Sweet potato (*Ipomoea batatas*, family Convolvulaceae) is of great importance to Uganda and other African countries such as Rwanda and Burundi because of its various food and feed uses (Kpaka et al., 2013). The

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sweet potato (orange-fleshed varieties) also plays a key role in alleviating vitamins A, B and E deficiencies, which are rampant among children in Sub-Saharan Africa at more than 40 and 21.1% (127 million) of pre-school children and 5.6% (7.3 million) of pregnant women worldwide (HarvestPlus, 2012; Kpaka et al., 2013). According Kpaka et al. (2013), Uganda is the biggest producer of sweet potato in Africa but its low average yield of 4.58 t/ha (compared to experimental yield estimates of 25 t/ha) makes it impossible to satisfy the country's production demand. Major constraints such as pests and diseases explain the crop's current low yields (Kpaka et al., 2013; Sanginga and Mbabu, 2015). The most serious pest is the sweet potato weevil. The other pests include aphids (*Myzus persicae*), whiteflies (*Bemisia tabaci*), mites and caterpillars (Sanginga and Mbabu, 2015). The diseases which undermine the crop's productivity are mainly viral and some of these viruses are sweet potato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV); vectored by whiteflies and aphids, respectively (Wasswa, 2012). The two viruses synergize to produce sweet potato virus disease (SPVD) (Gibson et al., 2014). SPVD is the most important disease of the crop that threatens sweet potato in the tropics (Kpaka et al., 2013). It causes yield losses of up to 95%; there are no reports of immune cultivars (Adikini et al., 2016; Gibson et al., 2014; Sanginga and Mbabu, 2015). However, there exist good levels of SPVD resistance among the commonly grown sweet potato varieties in high infection areas (Kpaka et al., 2013). It is notable that some of the locally adapted cultivars like New Kawogo are more SPVD resistant than others (Gibson et al., 2014; Wasswa, 2012). Environmental conditions also seriously influence the productivity of sweet potato and these include temperature extremes (15 to 35°C for sweet potato growth), humidity and rainfall patterns and intensity, among other factors (Gibson et al., 2014; HarvestPlus, 2012). This study was focused on gaining an insight into the effect of temperature on the development of SPVD and growth of sweet potato.

Viruses that infect and replicate well in their hosts tend to decrease the survival of the hosts by affecting their growth and development (Gibson et al., 2014). In addition, the incidence and severity of pathogens is strongly influenced by the interaction of temperature, vectors, hosts, and pathogen genetics (Adikini et al., 2016; Mwanga et al., 2016). From one environment to another, climatic aspects namely rainfall patterns and intensity, relative humidity, wind speed and direction, and temperature tend to differ and fluctuate none equivocally (Mwanga et al., 2016; Sabaghnia et al., 2012).

Such changes influence the epidemiology of plant diseases and may also affect disease expression (Gibson et al., 2014; Wasswa, 2012). Previous studies reported that some areas such as central Uganda had higher SPVD incidences and severities than for instance the eastern region (Adikini et al., 2016). The two regions differ in average ambient temperature, among other climatic aspects; with the eastern being hotter than the central region. It has been suggested that high temperature favours recovery from SPVD (Adikini et al., 2016; Gibson et al., 2014). There are increasing reports on rise of SPVD outbreaks in traditionally disease free agro-ecologies in Uganda. The new epidemics could be associated with changes in climatic conditions especially temperature and humidity. The temperature range in which the sweet potato crop can grow is reportedly 15 to 35°C but most pathogens also thrive in the same temperature range (HarvestPlus, 2012; Seidl Johnson et al., 2014). An optimum temperature for the satisfactory sweet potato crop productivity and its recovery from the SPVD is not established (Gibson et al., 2014). There is also limited knowledge on the relationship between sweet potato growth response among cultivars and SPVD development at different temperatures. The main objective of this study was to generate knowledge about the effect of temperature on SPVD development. The specific objectives of this study were: (i) To determine the effect of temperature on the expression of sweet potato virus disease symptoms, and (ii) To characterize the growth and physiological response of sweet potato cultivars at different temperatures. Results from this study are expected to contribute towards improvement of sweet potato crop productivity through exploitation of high temperatures in controlled environments (such as the screen house) for SPVD management.

MATERIALS AND METHODS

Plant materials

Six sweet potato cultivars used in this study were collected from MUARIK and Namulonge (NaCRRI) research stations located in Wakiso district in Uganda. The cultivars selected for use in this study were based on their diverse attributes (Table 1). Different sweet potato cultivars were used in this study so as to ensure inclusion of genotypes of various attributes such as high yield, SPVD tolerance or resistance, nutritional value and farmer preference. Some varieties are more readily infected by SPVD than others even when exposed to similar amounts of inoculums. New Kawogo is reportedly more resistant to SPVD than other cultivars (Gasura and Mukasa, 2010; Mwanga *et al*., 2016). Beauregard is an orange – fleshed variety rich in beta carotene, a precursor for vitamin A; and is highly preferred in some countries like Australia (HarvestPlus, 2012). In the selection process, consideration was put to ensure the inclusion of orange and non-orange fleshed cultivars. These were New Kawogo, Dimuka, NASPOT 1, Beauregard, Ejumula and Tanzania. During the cultivar samples collection, only vines were obtained from the research stations since they are the locally common sweet potato propagation materials. Each of these cultivars were then delivered on the experimental site and established in an SPVD vector proof screen house.

Experimental design

Six sweet potato cultivars were collected for use in the field and

Table 1. List of attributes of sweet potato cultivars used in the study.

Cultivar	Origin of parent	Desirable/undesirable traits
New Kawogo	Uganda (landrace)	High dry matter content, white fleshed, resistant to SPVD, susceptible to *A. bataticola* blight
Dimbuka	Uganda (landrace)	High dry matter content, white flesh of storage roots, tolerant to SPVD
Ejumula	Uganda (landrace)	High dry matter content, orange flesh of storage roots, highly susceptible to SPVD
Tanzania	Uganda (landrace)	High dry matter, sweet taste, tolerant to SPVD
NASPOT 1	Uganda (bred clone)	High dry matter, orange flesh of storage roots, high root yield, *Alternalia bataticola* blight
Beauregard	CIP/Peru	Low dry matter content, orange flesh of storage roots, good root shape, susceptible to SPVD

Extracted and modified from Gasura and Mukasa (2010) and Mwanga et al. (2016).

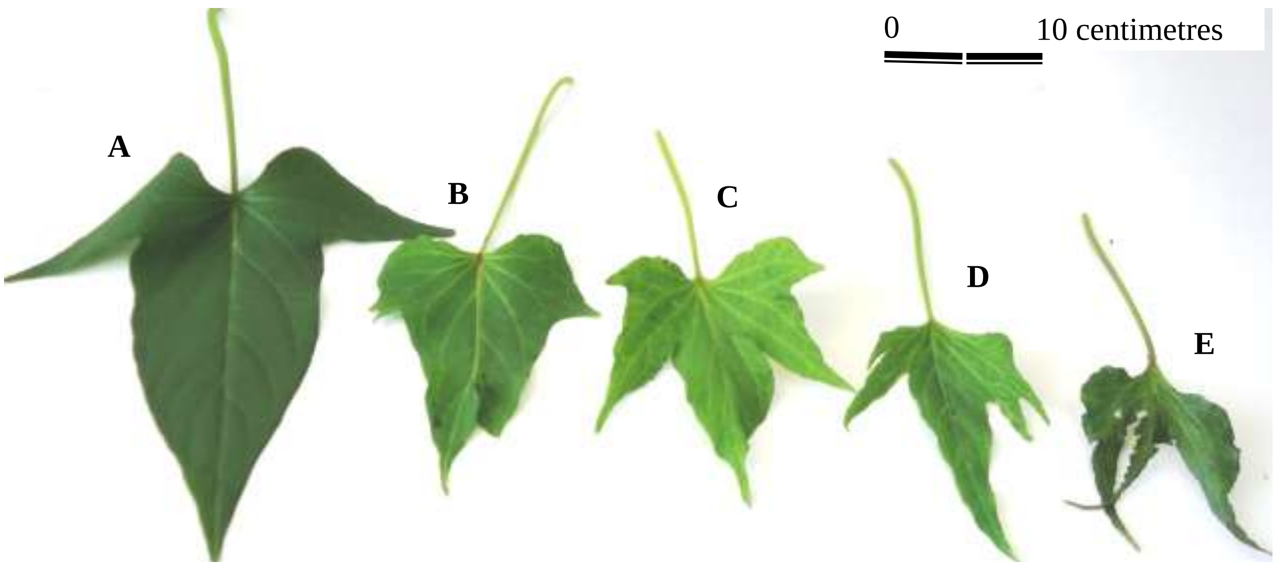

Figure 1. SPVD symptoms severity scoring at a scale of 1 to 5; where 1 (A) = no apparent symptoms, 2 (B) = <1/10 of the leaves/leaf surface has symptoms, 3 (C) = 1/10 to 3/10 of the leaves/leaf surface shows symptoms, 4 (D) = 3/10 to 1/2 of the leaves/leaf surface shows symptoms and 5 (E) = >1/2 of the leaves/leaf surface has symptoms.

glasshouse experiment at MUARIK. The samples were indexed for sweet potato viruses namely SPCSV and SPFMV using an indicator plant *Ipomoea setosa*. Vines in each cultivar found to be free from the said viruses were then multiplied under the vector proof screen house in order to raise a sufficient number of planting materials for the experiments. Five plants per cultivar for each temperature regime were graft inoculated with scions known for presence of SPCSV and SPFMV. Single isolates of SPCSV and SPFMV which were confirmed earlier using PCR technique by Wasswa et al. (2012) and kept in a vector-proof screen house at MUARIK were used from two respective plants of a common cultivar Ejumula. Before use in this study, indexing for verification was carried out by grafting on *I. setosa*. The plants were multiplied and bud grafting in which the scions were the infectious material was used. The inoculated plants were observed in two environments namely the field and an insect proof glasshouse at MUARIK. Plants which were inoculated were potted one week before planting of the controls. The controls were the non-inoculated plants planted on the day of inoculation. The plants were planted in pots of uniform size, with a soil volume of 3,234 cm^3. One plant was planted per pot. In the field, watering was done only when it had not rained for a period of four consecutive days to avoid desiccation. In the glasshouse, plants were watered regularly after every two days, in the morning hours to ensure that the soil remained saturated.

Data collection and analysis

Data collection on SPVD severity (on a most symptomatic leaf where applicable) and sweet potato plant growth response in the field and glasshouse was done at a 1 - week interval for 10 weeks, starting at 2 weeks after inoculation. The foliage of each plant was the observational unit. Disease expression was monitored based on visual virus symptoms. Disease incidence was recorded by counting the number of plants showing symptoms, and expressing it as a percentage. SPVD severity was recorded at a scale of 1 to 5 as modified from Gasura and Mukasa (2010), where: 1 = no apparent symptoms; 2 = $<{}^{1}/_{10}$ of the leaves/leaf surface has symptoms; 3 = ${}^{1}/_{10}$ to ${}^{3}/_{10}$ of the leaves/leaf surface shows symptoms; 4 = ${}^{3}/_{10}$ to ${}^{1}/_{2}$ of the leaves/leaf surface shows symptoms; and 5 = $>{}^{1}/_{2}$ of the leaves/leaf surface has symptoms (Figure 1). Field mercury thermometers were used to take record of daily temperature as it fluctuated both in the field and glasshouse, from which average weekly temperatures were computed for correlation with SPVD symptom expression. An analysis of variance (ANOVA) was carried out using GenStat 13th edition, at a 5% level

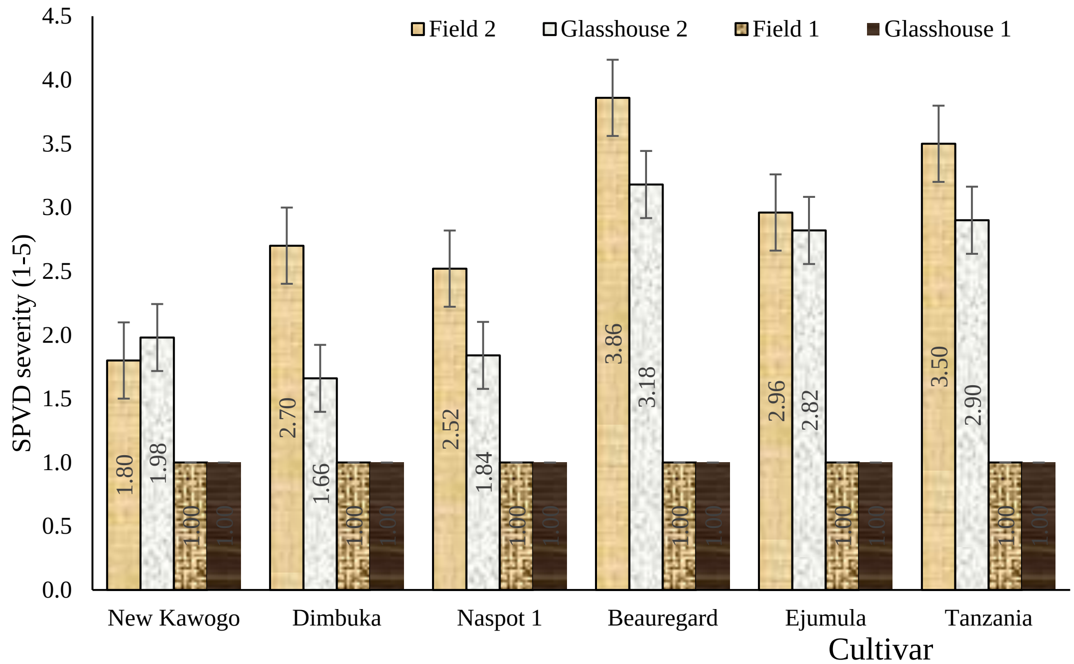

Figure 2. SPVD severities of different cultivars under the two environments of field and glasshouse averaged for the 10 weeks of the experiment.

of significance. Average temperature was recorded as the average of the maximum and minimum temperature of the day and at 8:00 am and 1:00 pm.

The data on growth parameters of length of internodes, stem length, number of auxiliary shoots, number of leaves and tuber yield was collected from the field and glasshouse. The length of internodes and stem length was recorded in centimetres (cm). Tuber yield was obtained at 14 weeks after planting by harvesting the plants in pots, washing the tubers off the soil and thereafter weighing. Yield data were recorded in grams per pot (g/pot), which was the same as grams per plant since each pot was planted with a single vine of uniform length, 10 cm. Means and probability values were generated by subjecting the internode length, stem length, number of auxiliary shoots, number of leaves and tuber yield data to ANOVA at 5% significance level. Field 1, Glasshouse 1, Field 2 and Glasshouse 2 were used to denote SPVD non-inoculated plants in field, SPVD non-inoculated plants in glasshouse, plants inoculated with SPVD viruses in field and plants inoculated with SPVD viruses in glasshouse, respectively the results section.

RESULTS

SPVD severity and temperature fluctuations

There was a significant difference ($p<0.001$) in SPVD symptom expression between the glasshouse and field environments. Less severity score averages were recorded in the glasshouse (2.397) than in the field (2.89). Overall, sweet potato cultivar Dimbuka in the glasshouse was the least severely affected whereas the highest SPVD scores were observed on Beauregard in the field. In the field, New Kawogo showed the lowest mean disease score (1.8) followed by Naspot 1, Dimbuka, Ejumula, Tanzania, and Beauregard with the highest disease core of 3.86 (Figure 2). In the glasshouse, Dimbuka displayed the lowest score (1.66) followed by Naspot 1, New Kawogo, Tanzania, Ejumula, and Beauregard with the highest score (3.18). Across the two environments, New Kawogo had the lowest SPVD score (1.89) followed by Dimbuka and Naspot 1 at score 2.18, Ejumula, Tanzania and Beauregard with the highest score (3.52).

As field temperature increased, glasshouse temperature increased. Similarly, as the field temperature decreased, the glasshouse temperature generally decreased in a corresponding manner. In the field, considering results during a period of three to ten weeks after planting, the lowest SPVD scores were obtained at different mean weekly temperatures. For instance New Kawogo showed its lowest disease score at 26.0°C (week 10); Dimbuka and Naspot 1 at 28.9°C (week 4), 25.4°C (week 7), and 25.4°C (week 8) though Naspot 1 showed a low score also at 21.3°C (week 5); Beauregard and Ejumula at 25.4°C (week 7); and Tanzania at 28.9°C (week 4). On a weekly interval, SPVD symptom scores generally varied from one cultivar to another as temperature also varied. For New Kawogo, it was observed that symptom development did not change systematically with plant age unlike the rest of the cultivars where severity scores generally increased with plant age, with Tanzania showing the clearest forward trend (Table 2).

SPVD symptoms on cv. Tanzania in the field increased

Table 2. Mean weekly severities of SPVD for different cultivars.

Cultivar	Weeks after planting										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	Mean
New Kawogo	1.0	1.0	1.9	2.1	2.5	2.5	2.6	2.1	1.7	1.5	1.89a
Dimbuka	1.0	2.0	2.2	2.5	2.3	2.5	2.3	2.2	2.4	2.4	2.18b
Naspot 1	1.2	1.9	1.9	2.0	2.1	2.7	2.5	2.2	2.5	2.8	2.18b
Ejumula	1.7	1.8	3.1	3.2	3.3	3.0	2.7	3.0	3.4	3.7	2.89c
Tanzania	1.3	1.4	2.5	3.2	3.3	3.5	3.7	4.2	4.2	4.7	3.20d
Beauregard	1.3	2.9	3.6	3.8	3.8	3.8	3.5	4.0	4.2	4.3	3.52e
Mean	1.25	1.83	2.53	2.80	2.88	3.00	2.88	2.95	3.07	3.23	2.643
s.e.d.	0.147	0.192	0.306	0.289	0.261	0.339	0.361	0.29	0.339	0.286	0.144
l.s.d (α = 5%)	0.296	0.385	0.614	0.580	0.526	0.682	0.725	0.580	0.682	0.575	0.283
CV %	26.3	23.4	27.0	23.1	20.3	25.3	28.0	21.9	24.7	19.8	38.5
F.pr.	<0.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Values followed by the same letter are not significantly different at 5% level of significance.

gradually from week 1 to week 10, irrespective of temperature fluctuations (Figure 3). A slightly similar trend was observed in the glasshouse but the rate of symptom development increased less steadily and to a lower maximum than in the field. In the glasshouse, the lowest SPVD scores after three weeks were obtained at 30.0°C (week 9), 30.0°C (week 9), 34.2°C (week 8), 34.2°C (week 7), 34.2°C (week 7) and 28.3°C (week 5) for New Kawogo, Dimbuka, Naspot 1, Beauregard, Ejumula and Tanzania respectively. The highest scores were observed at 28.3°C (week 5), 34.1°C (week 4), 34.0°C (week 4), 34.1°C (week 4), 28.3°C (week 5) and 33.3°C (week 10) for New Kawogo, Dimbuka, Naspot 1, Beauregard, Ejumula and Tanzania, respectively. SPVD scores for New Kawogo increased from week 1 to 5, remained constant up to week 7 and then it declined. A similar trend was observed for Dimbuka and Naspot 1. Generally, SPVD scores increased with plant age for Beauregard, Ejumula and Tanzania.

In the case of New Kawogo in the field, as plant age increased, SPVD severity increased less slightly from a score of 1 (no symptoms) at the first and second week to 2 ($<^1/_{10}$ of the leaves or leaf surface had symptoms), thereafter remained unchanged from week 3 up to week 6, beyond which the scores oscillated between 2.10 and 2.25, and declined to 1.5 at week 10. In the glasshouse, SPVD symptoms developed increasingly from the first week at score 1 to 3 at week 5, remained constant up to week 7 and then reduced decreasingly up to the 10th week (Figure 4).

Growth response

Length of internode per plant (cm)

There was a significant difference (p<0.001) in internode length between the two environments at a 5% significance level, with the highest mean for non-inoculated plants in glasshouse (3.23 cm) followed by inoculated plants in glasshouse, non-inoculated plants in field, and inoculated plants in field had the shortest internodes (1.91 cm) (Table 3). Significant still was the difference (p<0.001) in internode length among different cultivars. Generally, longer internodes were observed among non-inoculated plants than in presence of SPVD. Apart from New Kawogo cultivar whose plants had longest internodes in absence of SPVD in the field, the rest of the cultivars had highest internode lengths in absence of the disease in glasshouse. Plants with shortest internodes for New Kawogo were observed in presence of SPVD in glasshouse whereas for the remaining cultivars, shortest internodes are recorded from inoculated plants in the field. Across environments, Beauregard had the highest internode length (4.51 cm) followed by New Kawogo, Dimbuka, Naspot 1, Tanzania, and Ejumula with the shortest internodes (1.80 cm). Beauregard had the highest internode length across environments and SPVD treatments while Ejumula produced the shortest internodes in the field on SPVD inoculated plants.

On a weekly interval, internode lengths generally increased at a decreasing rate for all the cultivars. Cv. Beauregard, however, stood out with the longest internodes (Figure 5).The longest internodes were obtained in the glasshouse. Beauregard achieved the longest internodes among SPVD free plants in the glasshouse followed by SPVD inoculated plants in glasshouse, SPVD free plants in field and the shortest internodes were recorded from SPVD inoculated plants in the field (Figure 6).

Stem length per plant (cm)

The difference in stem length was significant (p<0.001)

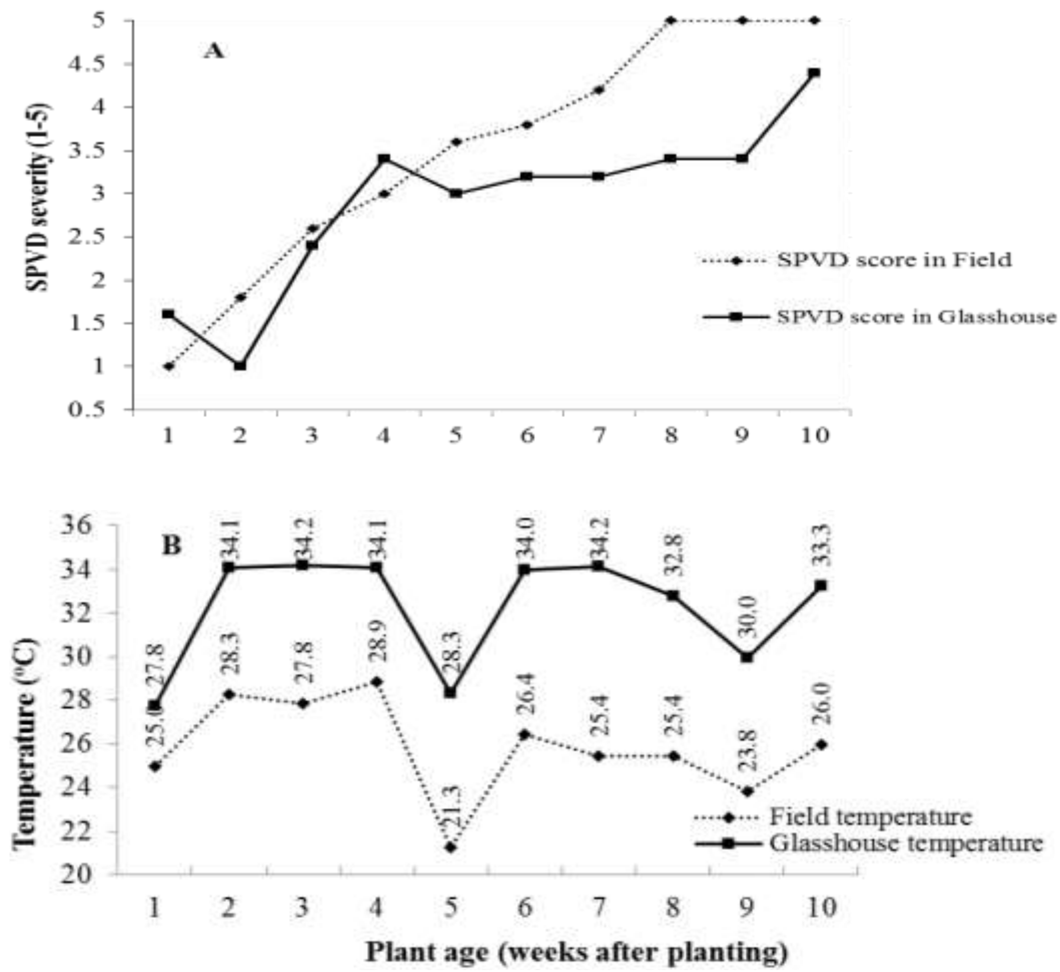

Figure 3. A comparative graph of changes in severity for cultivar Tanzania and weekly changes in temperature under the two environments. ***A*** = Changes in disease severity of cultivar Tanzania; ***B*** = Changes in temperature during the period of the experiment.

between the two environments, with a higher mean in the glasshouse on non-inoculated plants (55.1 cm) followed by glasshouse on SPVD inoculated plants, field on non-inoculated plants, and the shortest plants were obtained in the field in presence of SPVD (19.6 cm) (Table 4). The difference among cultivars was also significant (p<0.001). Similar to the trend of internode lengths, apart from New Kawogo which had its longest plants in field in absence of SPVD, the rest of cultivars registered their highest stem lengths in glasshouse in absence of the disease. Unlike in the trend of internode lengths, the shortest plants for all the six cultivars were observed in the field in presence of SPVD. Across environments, Beauregard had the tallest plants (83.2 cm) followed by New Kawogo, Tanzania, Dimbuka, Naspot 1, and Ejumula had the shortest plants (24.4 cm) (Figure 7). An in-depth description of stem length variation was based on Beauregard; and it further illustrated that the longest plants were observed in the glasshouse where temperatures were higher than in the field (Figure 8). SPVD free plants were longer than infected plants. Beauregard both inoculated and non-inoculated produced the longest plants in glasshouse while the shortest plants were observed in the field among SPVD inoculated plants, with the Ejumula being the shortest.

Overall, across environments and inoculation levels, stem length for all cultivars increased at constant rates throughout the time of the experiment. Cv. Beauregard had the highest rate of increase in stem length followed by New Kawogo. Tanzania, Naspot 1 and Dimbuka have similar curve trends. Cv. Ejumula's stem length was the shortest, having the lowest increase rate.

Number of shoots per plant

The highest shooting tendency was observed in the glasshouse on non-inoculated plants while the lowest number of shoots was recorded in the field on SPVD inoculated plants. The figure shows that Ejumula (both

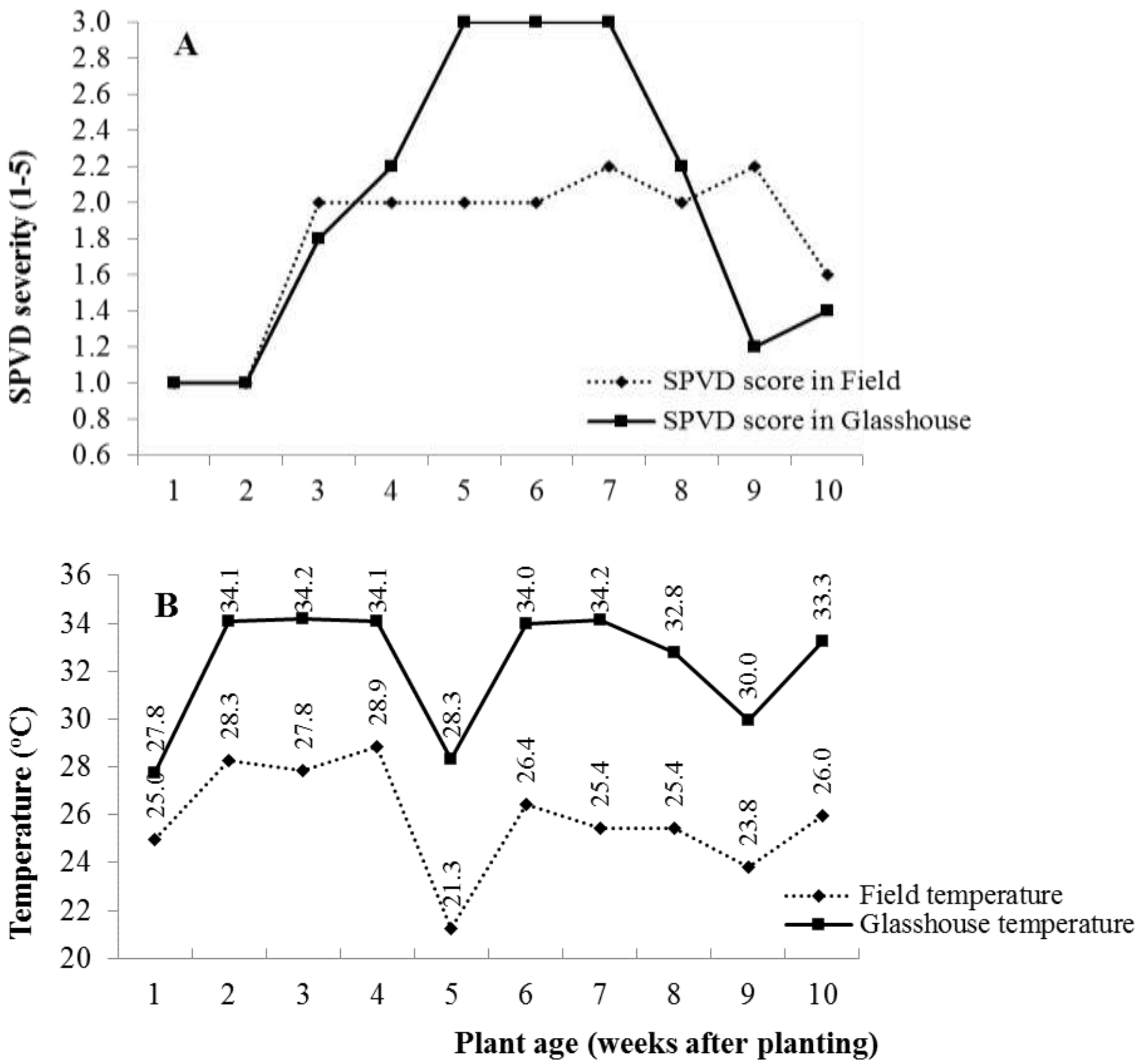

Figure 4. A comparative graph of changes in severity for cultivar New Kawogo over time.

Table 3. Mean internode length of sweet potato cultivars under the two environments.

Cultivars	Environment				Mean	s.e.d	l.s.d	c.v %	F.pr.
	Field 1	GH 1	Field 2	GH 2					
New Kawogo	2.54	2.46	2.43	2.08	2.38	0.145	0.286	31.0	0.019
Dimbuka	2.36	2.85	1.76	2.51	2.37	0.243	0.480	51.4	<0.001
Naspot 1	1.96	2.83	1.74	2.51	2.26	0.192	0.379	42.5	<0.001
Beauregard	3.91	5.75	2.96	5.44	4.51	0.493	0.973	54.6	<0.001
Ejumula	1.51	2.50	1.07	2.11	1.80	0.143	0.281	39.7	<0.001
Tanzania	1.75	2.97	1.52	2.64	2.22	0.168	0.331	37.8	<0.001
Mean	**2.34c**	**3.23a**	**1.91d**	**2.88b**	**2.59**	**0.107**	**0.209**	**50.4**	**<0.001**
s.e.d	**0.140**	**0.305**	**0.232**	**0.296**	**0.131**	**0.261**			
l.s.d.(α =5%)	**0.389**	**0.600**	**0.457**	**0.582**	**0.256**		**0.513**		
c.v %	**42.3**	**47.2**	**60.7**	**51.3**	**50.4**			**50.4**	
F.pr.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**				**<0.001**

Values followed by the same letter are not significantly different at 5% level of significance.

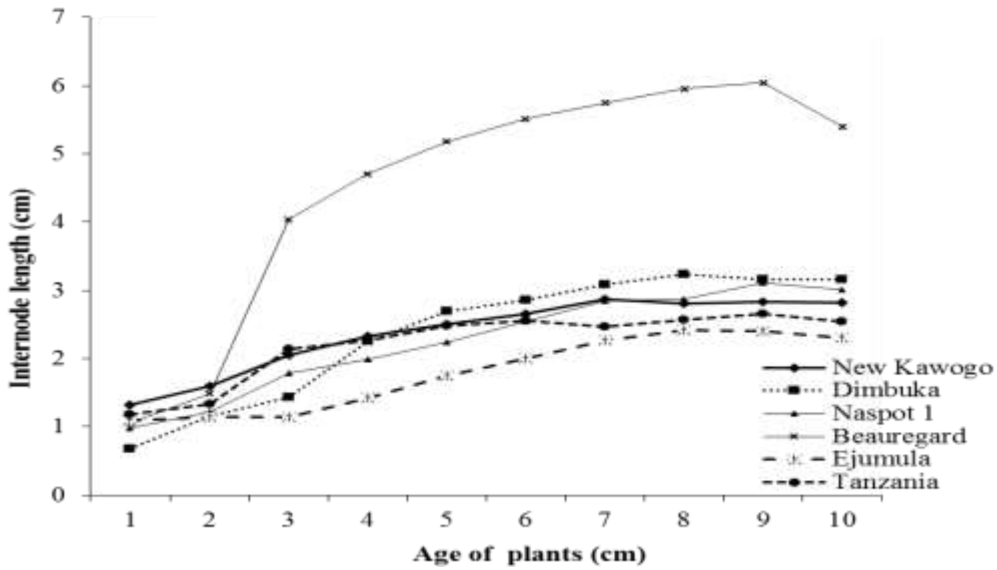

Figure 5. Variation of internode length among cultivars over time.

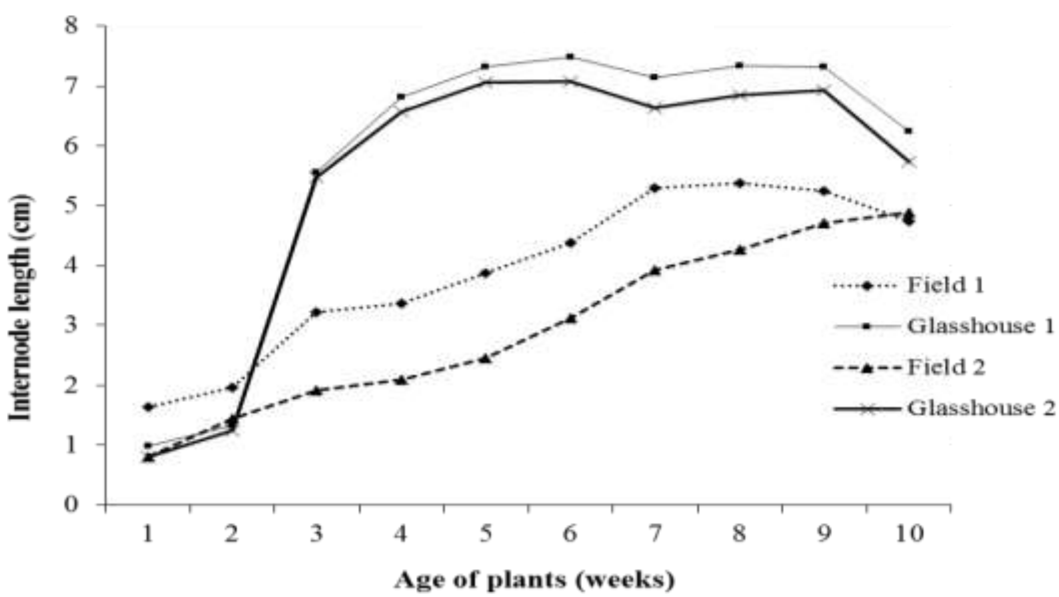

Figure 6. Variation of internode length of Beauregard plants under the two environments.

non- and inoculated in glasshouse) was leading followed by New Kawogo (both non- and inoculated in glasshouse) while Beauregard had the lowest number of shoots. The number of shoots differed significantly ($p<0.001$) between the two environments, with a higher mean number on non-inoculated plants in glasshouse (10.07) followed by inoculated plants in glasshouse, non-inoculated plants in field, and SPVD inoculated plants in the field had the lowest number of shoots (0.7) for non-inoculated plants (Table 5). Sweet potato cultivars also significantly differed ($p<0.001$) in the number of shoots produced. All the cultivars produced their highest numbers of shoots on non-inoculated plants grown in glasshouse while the least numbers of internodes were produced on SPVD inoculated plants in field. By the 10th week across environments and inoculation levels, Ejumula had the highest shooting tendency followed by New Kawogo, Dimbuka, Tanzania, Naspot 1 and Beauregard with the least number of shoots (Figure 9). Using the case of Ejumula, the highest shooting tendency was observed in the glasshouse in absence of SPVD followed by inoculated plants in the glasshouse, SPVD

Table 4. Mean stem lengths of sweet potato cultivars under the two environments.

Cultivars	Environment				Mean	s.e.d	l.s.d	c.v %	F.pr.
	Field 1	GH 1	Field 2	GH 2					
New Kawogo	48.2	48.1	30.4	45.1	42.9	5.209	10.27	62.2	0.004
Dimbuka	26.5	39.8	15.6	36.8	29.7	4.53	8.94	76.3	<0.001
Naspot 1	23.3	41.5	14.1	38.5	29.4	4.31	8.51	73.4	<0.001
Beauregard	59.7	120.0	36.7	116.5	83.2	15.63	30.82	93.9	<0.001
Ejumula	19.2	35.8	9.7	32.8	24.4	3.24	6.39	66.4	<0.001
Tanzania	24.6	45.2	11.2	42.3	30.8	3.97	7.83	64.4	<0.001
Mean	**33.6**	**55.1**	**19.6**	**52.0**	**40.1**	**3.06**	**6.01**	**93.5**	**<0.001**
s.e.d	**4.74**	**9.70**	**4.33**	**9.46**	**3.75**	**7.5**			
l.s.d.(α =5%)	**9.32**	**19.10**	**8.52**	**18.62**	**7.36**		**14.71**		
c.v %	**70.5**	**88.1**	**110.2**	**91.0**	**93.5**			**93.5**	
F.pr.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**				**<0.001**

Values followed by the same letter are not significantly different at 5% level of significance. Field 1 = SPVD non-inoculated plants in field; Glasshouse (GH) 1 = SPVD non-inoculated plants in glasshouse; Field 2 = plants inoculated with SPVD viruses and grown in field; and Glasshouse (GH) 2 = plants inoculated with SPVD viruses and grown in glasshouse.

Figure 7. Variation of stem length among cultivars over time.

non-inoculated plants in the field and plants and SPVD inoculated plants in the field had the lowest shooting tendency (Figure 10).

Number of leaves

There was a significant difference (p<0.001) in number of leaves between the two environments, with the highest mean leaf number in glasshouse on non-inoculated plants (21.82) followed by SPVD inoculated plants in glasshouse, non-inoculated plants in filed, and SPVD inoculated plants in the field produced the lowest number of leaves (10.37) (Table 6). The difference among cultivars was also significant (p<0.001). For all the six cultivars, their highest mean number of leaves were observed on non-inoculated plants grown in glasshouse while their lowest leaf numbers were recorded on SPVD inoculated plants grown in the field (Figure 11). Across environments, New Kawogo produced the highest mean number of leaves (21.45) followed by Beauregard, Ejumula, Tanzania, Naspot 1 and Dimbuka with the lowest number of leaves (14.30). The highest foliage number was observed on the SPVD non-inoculated New Kawogo in the glasshouse followed by SPVD inoculated New Kawogo in the glasshouse, and the lowest number

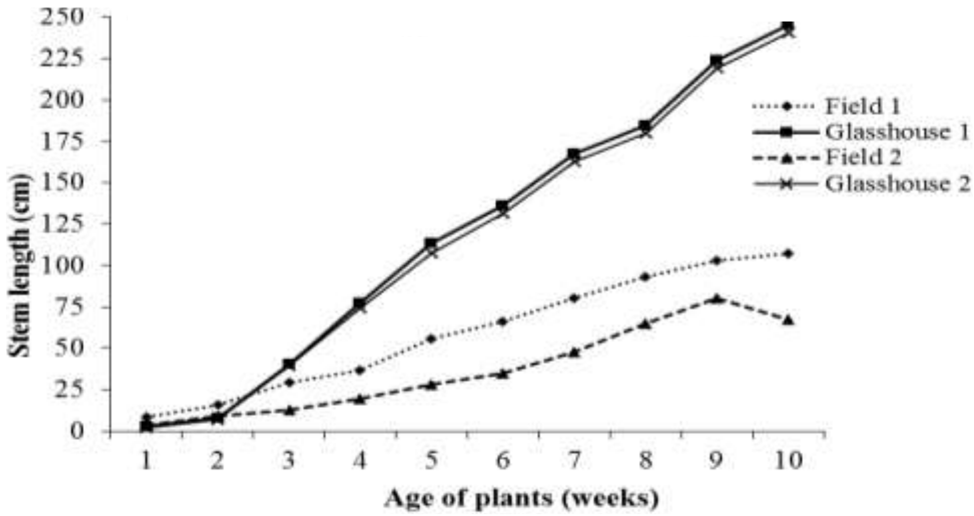

Figure 8. Variation of stem length of Beauregard plants under the two environments.

Table 5. Mean number of shoots of sweet potato cultivars under the two environments.

Cultivars	Environment				Mean	s.e.d	l.s.d	c.v %	F.pr.
	Field 1	GH 1	Field 2	GH 2					
New Kawogo	5.8	12.2	1.0	11.3	7.58	1.008	1.988	68.2	<0.001
Dimbuka	3.5	10.4	0.3	9.5	5.92	0.900	1.776	76.0	<0.001
Naspot 1	2.8	8.9	0.8	7.9	5.14	1.021	2.014	99.5	<0.001
Beauregard	1.6	5.9	1.4	4.9	3.48	1.730	3.412	56.6	<0.001
Ejumula	4.6	13.4	0.4	12.5	7.71	0.973	1.918	63.1	<0.001
Tanzania	7.9	9.6	0.2	8.8	6.63	0.781	1.539	58.9	<0.001
Mean	**4.37**	**10.07**	**0.70**	**9.15**	**6.07**	**0.37**	**0.727**	**74.7**	**<0.001**
s.e.d	**0.633**	**1.225**	**0.253**	**1.151**	**0.454**	**0.907**			
l.s.d.(α =5%)	**1.245**	**2.412**	**0.498**	**2.266**	**0.890**		**1.78**		
c.v %	**72.4**	**60.8**	**179.9**	**62.9**	**74.7**			**74.7**	
F.pr.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**				**<0.001**

Values followed by the same letter are not significantly different at 5% level of significance. Field 1 = SPVD non-inoculated plants in field; Glasshouse (GH) 1 = SPVD non-inoculated plants in glasshouse; Field 2 = plants inoculated with SPVD viruses and grown in field; and Glasshouse (GH) 2 = plants inoculated with SPVD viruses and grown in glasshouse.

on inoculated Ejumula in the field. By the 10th week, New Kawogo had the highest number of leaves followed by Beauregard, Ejumula, Tanzania, Naspot 1 and Dimbuka with the least leaf number (Figure 11). The highest foliage production was observed on SPVD free plants in glasshouse in which temperatures were higher than in the field; followed by diseased plants in the glasshouse, SPVD non-inoculated plants in field and the lowest foliage production was recorded on diseased plants in the field (Figure 12).

Yield per plant (g)

There was a significant difference in mean yield of different environments ($p<0.001$) and different sweet-potato cultivars ($p<0.001$) (Table 7). In the case of environments and SPVD inoculations, the non-inoculated plants in the field yielded the highest (236.2 g/plant) followed by inoculated plants in the field (182.9 g/plant), non-inoculated plants in glasshouse (38.5 g/plant) and inoculated plants in the glasshouse yielded the lowest (36.6 g/plant). Across environments, New Kawogo was the best yielder (199.3 g/plant) followed by Tanzania (171.0 g/plant), Beauregard, Naspot 1, Dimbuka, and Ejumula (47.8 g/plant).

For non-inoculated plants in the field, New Kawogo was the best yielder (398.2 g/plant) followed by Naspot 1 (332.0 g/plant), Tanzania (329.2 g/plant), Dimbuka (147.0 g/plant), Beauregard and Ejumula (104.9 g/plant). In the

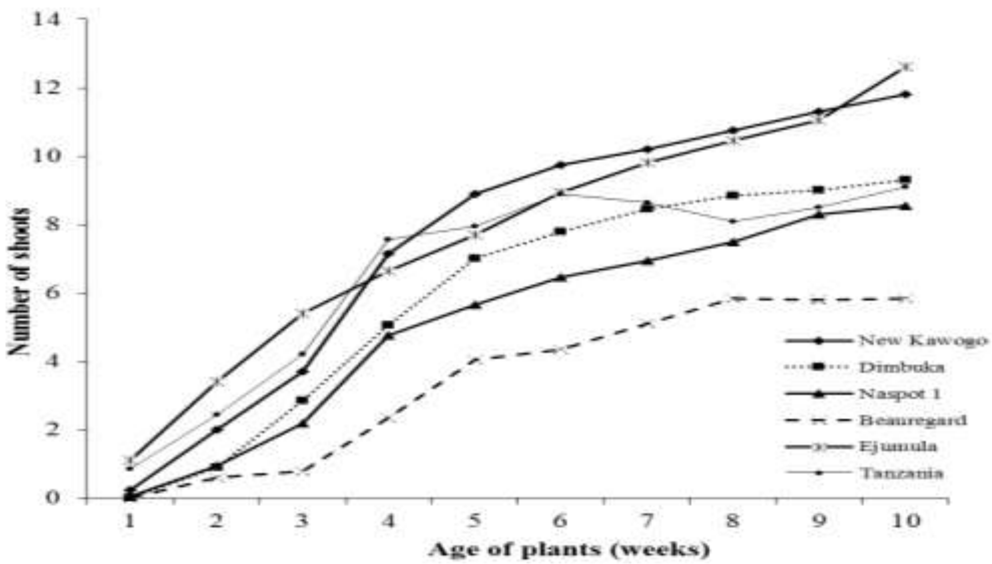

Figure 9. Variation of number of lateral shoots among cultivars over time.

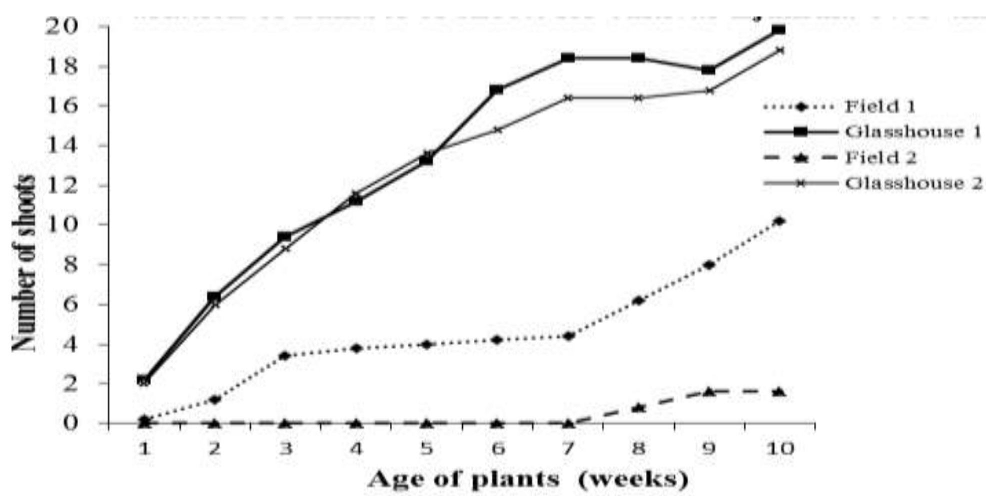

Figure 10. Variation of number of shoots of Ejumula plants under the two environments.

glasshouse in absence of the disease, Tanzania was the best yielder (93.2 g/plant) followed by Dimbuka (68.2 g/plant), Beauregard (44.0 g/plant), Naspot 1, Ejumula, and New Kawogo (3.5 g/plant). In the case of inoculated plants in field, New Kawogo performed better than the rest (394.7 g/plant), followed by Beauregard, Tanzania, Dimbuka, Ejumula, and Naspot 1 (26.7 g/plant). However, Beauregard was the best yielder (122.5 g/plant) followed by Tanzania (59.8 g/plant), Dimbuka (15.7 g/plant), Ejumula, Naspot 1, and New Kawogo (1.0 g/plant), among inoculated plants in the glasshouse.

Yield performance was best in the field. Non-inoculated New Kawogo performed the best in the field and this performance was not significantly affected by inoculation with SPVD. However, the same cultivar's performance was the worst in glasshouse. Naspot 1 was the second high yielder in the field in absence of SPVD, followed by Tanzania, Dimbuka, Beauregard and Ejumula. In the glasshouse in absence of the disease, Tanzania performed the best followed by Dimbuka, Beauregard, Naspot 1, Ejumula, and the poorest yielder was New Kawogo. For SPVD inoculated plants in the field, New Kawogo performed the highest followed by Beauregard, Tanzania, Dimbuka, Ejumula, and Naspot 1. For inoculated plants under glasshouse, Beauregard performed the best followed by Tanzania, Dimbuka, Ejumula, Naspot 1, and the poorest performer was New Kawogo.

Table 6. Mean number of leaves of sweet potato cultivars under the two environments.

Cultivars	Environment				Mean	s.e.d	l.s.d	c.v %	F.pr.
	Field 1	GH 1	Field 2	GH 2					
New Kawogo	21.92	25.66	14.37	23.86	21.45	2.157	4.254	51.5	<0.001
Dimbuka	14.50	18.06	8.36	16.30	14.30	1.490	2.938	52.1	<0.001
Naspot 1	14.62	20.10	8.22	18.20	15.28	1.730	3.412	56.6	<0.001
Beauregard	16.20	23.82	14.48	21.86	19.09	2.376	4.685	62.2	<0.001
Ejumula	14.88	22.56	8.18	20.60	16.55	1.642	3.238	49.6	<0.001
Tanzania	17.82	20.70	8.64	18.82	16.49	1.622	3.198	49.2	<0.001
Mean	**16.66**	**21.82**	**10.37**	**19.94**	**17.20**	**0.763**	**1.497**	**54.3**	**<0.001**
s.e.d	**1.574**	**2.272**	**1.347**	**2.124**	**0.934**	**1.869**			
l.s.d.(α =5%)	**3.098**	**4.471**	**2.651**	**4.181**	**1.833**		**3.666**		
c.v %	**47.2**	**52.1**	**64.9**	**53.3**	**54.3**			**54.3**	
F.pr.	**<0.001**	**0.014**	**<0.001**	**0.007**	**<0.001**				**0.429**

Values followed by the same letter are not significantly different at 5% level of significance. Field 1 = SPVD non-inoculated plants in field; Glasshouse (GH) 1 = SPVD non-inoculated plants in glasshouse; Field 2 = plants inoculated with SPVD viruses and grown in field; and Glasshouse (GH) 2 = plants inoculated with SPVD viruses and grown in glasshouse.

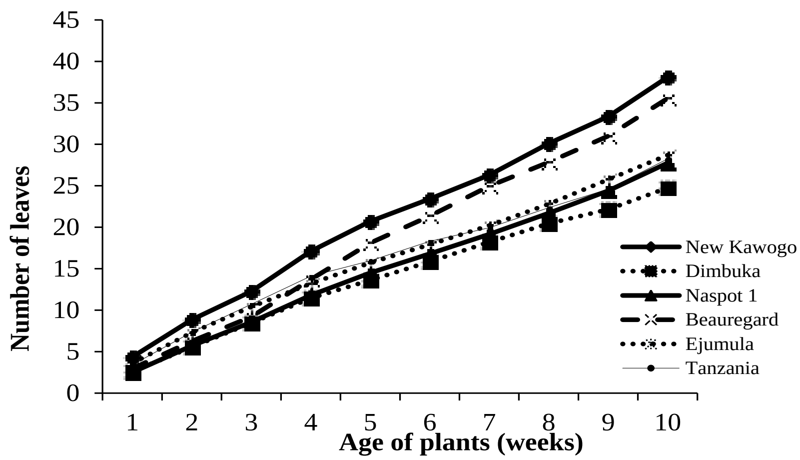

Figure 11. Variation of number of leaves among cultivars over time.

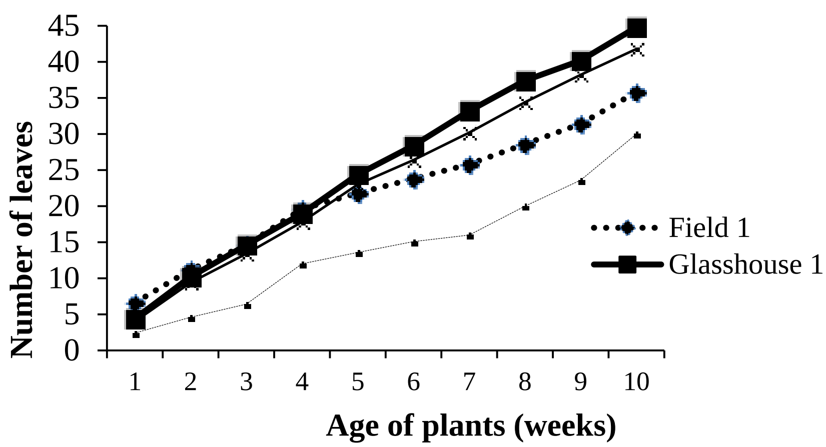

Figure 12. Variation of number of leaves of New Kawogo plants under the two environments.

Table 7. Mean yield per plant for different cultivars under the two environments.

Cultivars	Environment				Mean	s.e.d	l.s.d	c.v %	F.pr.
	Field 1	GH 1	Field 2	GH 2					
New Kawogo	398.22	3.47	394.71	0.97	199.34a	0.150	0.318	0.1	<0.001
Dimbuka	147.00	68.18	109.27	15.68	85.03e	0.464	0.984	0.9	<0.001
Naspot 1	332.03	13.67	26.74	5.46	94.47d	0.183	0.388	0.3	<0.001
Beauregard	105.81	44.04	298.54	122.45	142.71c	0.193	0.408	0.2	<0.001
Ejumula	104.89	8.38	62.49	15.30	47.77f	0.173	0.366	0.6	<0.001
Tanzania	329.21	38.48[c]	205.55	59.77	171.92b	0.280	0.593	0.3	<0.001
Mean	**236.19[a]**	**38.48[c]**	**182.88[b]**	**36.60[d]**	**123.54**	**0.108**	**0.214**	**0.3**	**<0.001**
s.e.d	**0.383**	**0.155**	**0.170**	**0.155**	**0.132**	**0.264**			
l.s.d.(α =5%)	**0.791**	**0.320**	**0.350**	**0.320**	**0.262**		**0.523**		
c.v %	**0.3**	**0.7**	**0.1**	**0.7**	**0.3**			**0.3**	
F.pr.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**				**<.001**

Values followed by the same letter are not significantly different at 5% level of significance. Field 1 = SPVD non-inoculated plants in field; Glasshouse (GH) 1 = SPVD non-inoculated plants in glasshouse; Field 2 = plants inoculated with SPVD viruses and grown in field; and Glasshouse (GH) 2 = plants inoculated with SPVD viruses and grown in glasshouse.

DISCUSSION

The lower severity of SPVD on inoculated plants in the glasshouse indicates that severity reduces at high temperature. Similarly, the higher severity of SPVD on inoculated plants in the field indicates that severity increases at low temperature. Therefore, SPVD severity changes with a change in temperature. It also suggests that recovery tendencies of sweet potato from SPVD are more likely at higher temperatures. It also signals the potential for inability of SPCSV and / or SPFMV to reproduce, and this can lead to their elimination (Gasura and Mukasa, 2010; Gibson et al., 2014). It is however, probable that these two viruses are influenced differently by temperature. If this supposition is true, a prevailing temperature regime would significantly influence the level of disease that is expressed. A study into the effect of temperature on the roles of the individual viruses of SPVD needs to be undertaken.

Different sweet potato cultivars were differently affected by SPVD at different temperatures. This implies that different cultivars exhibit differential responses to the disease as temperatures vary and this could suit them to different agroecologies. This is very likely because agroecological zones differ in a number of climatic conditions, temperature inclusive. A very low severity of SPVD in some cultivars like New Kawogo and very high disease scores for Ejumula, Tanzania and Beauregard at both environments of the field and glasshouse confirms the existence of SPVD resistant and susceptible varieties. This is particularly in agreement with Gasura and Mukasa (2010) who reported that cv. New Kawogo was resistant to SPVD. However, observations from this study indicate that resistance or tolerance potential of sweet potato to the disease is heightened at higher temperatures. The difference in internode length between plants in the field and glasshouse, suggests that temperature has an effect in the rate of cell multiplication and expansion in the internodes, with a higher temperature increasing the process. This is because the two environments experienced different temperature ranges. SPVD was also observed to play a role in length of the internodes in that presence of the disease could have limited the rate of cell growth around the internodes. Cultivar wise, some cultivars having manifested longer internodes in the field where temperatures were always lower than in the glasshouse suggests differential agro-ecological adaptations. This implies that cv. New Kawogo does well at lower temperature agro-ecologies than these rest of the cultivars included in this study. However, the effect of SPVD on internode lengths was very conspicuous in most cultivars, for instance under same conditions of the field, the SPVD inoculated plants of cv. Ejumula produced shorter internodes than the non-inoculated plants.

High temperatures of the glasshouse caused the plants to grow taller than those in the field where there were low temperatures. SPVD also negatively affected the stem lengths of plants. This emphasizes that the disease causes dwarfing symptoms. This trend is similar as in the case of internode lengths which implies that tall cultivars also had long internodes. An example of such cultivars is Beauregard which had the longest internodes and stems. The cv. Ejumula had the lowest internode and stem length both in glasshouse and in the field. This implies that this cultivar is very severely affected by SPVD. It can be argued that SPVD in combination with high temperature conditions cause increased shooting tendency. This assertion is based on the observations in glasshouse; most clearly with cv. Ejumula in which SPVD inoculated plants consistently produced more shoots than the non-inoculated ones. The high shooting tendency was also associated with dwarfing. For instance, whereas the longest plants were observed on cv. Beauregard, the highest number of shoots was recorded on cv. Ejumula.

High temperatures were observed to cause high vegetative growth at the expense of tuber yield. This is in agreement with the earlier observations by Wasswa (2010), Adikini et al. (2016) and Gibson et al. (2014) though most of these authors did not vary temperatures to wide ranges. Sweet potato infected with SPVD and grown under glasshouse conditions produce little or no tuber yield, and this observation concurs with that of Adikini et al. (2016). SPVD presence also influenced the number of leaves. Thus whereas the high temperatures of the glasshouse caused high vegetative growth, SPVD presence negatively affected the number of leaves per plant in all cultivars across environments. It is also evident that the vegetative growth response of different cultivars varies significantly at different environments. For the case of glasshouse conditions, cv. New Kawogo stood distinct from the rest of the cultivars in terms of very high leaf number however; this resulted into the lowest tuber yield. The cultivars having minimum leaf numbers produced a reasonable tuber yield under high temperatures of the glasshouse though in absence of SPVD. Such cultivars include Tanzania, Dimbuka and Beauregard. This suggests that different sweetpotato cultivars differently tolerate / resist temperature stresses. This tolerance or resistance has been based on tuber yielding ability because the East and Central African people grow this crop majorly for direct human food security (HarvestPlus, 2012). The development response of SPVD at temperatures higher than that in the glasshouse or lower than that in the field used in this study, is not yet well understood. This matter requires further investigation. A study on the effect of temperature on sweet potato virus load accumulation would help provide answers to this knowledge gap.

Conclusion

This study indicates that temperature influences the development of SPVD. Generally, as temperature increases SPVD development reduces, particularly with respect to symptom expression. Temperatures 20 to 29°C produce more disease severities than high temperatures of 30 to 39°C. Field conditions produce more disease severity than glasshouse conditions. SPVD also expresses differently among sweet potato cultivars at different temperatures. For instance, Ejumula is more severely affected by SPVD at high temperatures of the glasshouse than in the field whereas an opposite effect occurs with cv. Tanzania. New Kawogo followed by Dimbuka and Naspot 1 are more SPVD resistant, based on symptom expression, than the rest of the cultivars; Ejumula followed by Tanzania and Beauregard are the most SPVD susceptible according to this study. Beauregard, Tanzania and Dimbuka are more tolerant to the disease when it comes to tuber yield across temperature levels of the glasshouse and field than the rest of the cultivars. New Kawogo grows more vegetatively under high temperatures but with negligible or no tuber yield. High temperatures generally cause increased vegetative growth at the expense of tuber yield. Under high temperature and the generally uniform growth conditions of the glasshouse, there exist differences in cultivar growth responses. For instance, cv. Beauregard plants grow the tallest with very limited lateral shooting and relatively good tuber yield as compared to the rest of the cultivars. This suggests that some cultivars multiply better than other at high temperatures for delivery of vine cuttings to farmers. The development response of SPVD at a wider temperature range than that experienced during this experiment deserves further investigation. The results from this study suggest that reasonably high temperatures under a controlled environment should be incorporated in any sweet potato seed production system for possible elimination of SPVD. Further study into the effect of temperature on the SPVD is necessary.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The author acknowledges the Bio resource Innovations network for East Africa Development (BIO-INNOVATE) Project 2 on Potato Sweet potato and Cassava for funding this study, and the Makerere University Plant Tissue Culture Laboratory for the support.

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Vol. 12(27), pp. 2310-2318, 6 July, 2017
DOI: 10.5897/AJAR2017.12132
Article Number: 8ECB46565105
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Expediency of water and soil nutrients in irrigated and extreme drought conditions

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Received 9 January, 2017; Accepted 14 February, 2017

Climate change has become global issue for crops due to increase in temperature and less rainfall resulting in shortage of water and decrease in yield. The experiment consisted of five guar (*Cyamopsis tetragonoloba* L.) genotypes viz. S-5744, S-5824, S-5785, BR-90, BR-99 were cultivated under irrigated and drought stress (only soaking dose) conditions using Randomized Complete Block Design (RCBD) with 3 replications. The data for yield and its components were recorded at the time of maturity of guar genotypes. The two genotypes S-5744 and S-5824 showed better performance under both drought and irrigated conditions and gave maximum yield 900 and 1133.33 grams in drought and irrigated, respectively. All characters showed positive correlation with each other except number of cluster plant^{-1}. The soil analysis was done in order to check availability and utilization of nutrients in the presence of water (irrigated conditions) and water deficit conditions (drought). The above mentioned two genotypes were less affected by the water shortage compared to others and were comeback after rainfall in the months of July and August and have ability of better uptake of nutrients from the soil in drought and irrigated conditions. However, nutrient use was higher under irrigated conditions.

Key words: Utilization, nutrient uptake, soil and temperature, drought.

INTRODUCTION

On the global level, climate change has become an issue of severe and immediate concern having far-reaching effects not only on agricultural productivity but also on the demand for water and energy. During the past century, global temperatures have risen by nearly 1°C (due to burning of hydrocarbons and deforestation) and are expected to increase further by 1.4 to 5.8°C by the year 2100 (Naseer, 2013) .

In the past few years, the situation has worsened and signs of global warming are becoming evident. With

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climate change, in addition to higher average temperatures, there is an increased risk of rising sea levels, melting of glaciers, flooding and higher frequency of droughts. Consequently, crop yields are expected to decrease, affecting livelihoods and food production (Naseer, 2013). According to Jamshed Iqbal Cheema (Chairman: Pakistan Agricultural Scientists Association), per capita water availability at the time of independence was 5,600 m^3 against the current measure of 1,000 m^3 and the shortage is expected to rise to 31% of people's needs by 2025 (Naseer, 2013).

In this current situation of climate change and water shortage, guar crop plays an important role being highly drought resistant legume crop. Guar (*Cyamopsis tetragonoloba* L.) is a summer annual legume crop and commercially known as cluster bean (Kobeasy et al., 2011; Rao and Shahid, 2011). It is a highly drought tolerant and multi-used crop because it is used in gum industries, green manure, animal feed and fodder (Sharma and Gummagolmath, 2012). It is mainly cultivated in Pakistan and India as forage for cattle and vegetable for humans (Rao and Shahid, 2011) under arid/semiarid areas of the world preferring hot dry environment (Sharma and Gummagolmath, 2012; Sultan et al., 2012). Like other legume crops, guar is also an exceptional crop to enhance soil fertility as it can fix atmospheric nitrogen (Bewal et al., 2009; Sultan et al., 2012).

It has been observed that nitrogen fixation and nodulation are more sensitive to environmental stresses including water stress. Water stress has been reported to suppress O_2 flux in nodules or supply of photosynthates, consequently causing a decrease in nitrogen fixation (Silvente et al., 2012). Lotter et al. (2014) observed that net photosynthetic rate decreases under water limited conditions that can affect nutrient allocation and biomass in a legume *Aspalathus linearis*. Legume crops play an important role in the economy of arid and semiarid areas of the world as they are the major source of protein (Sohrawardy and Hossain, 2014). Legumes help in improving soil fertility because of their inherent capability to fix atmospheric nitrogen (Sohrawardy and Hossain, 2014).

Genetic diversity is one of the important factors to improve many crops including guar (Sultan et al., 2012). It was observed that little water requirements of guar help in showing more potential to salinity, consequently to obtained fast-growing high quality forage (Rao and Shahid, 2011). Stafford and McMichael (1991) reported that yield of guar plants was more affected than seeds/pod, seed weight and racemes/plant under water limited conditions.

Comeback of plants against stress depend upon the genetic makeup of cultivar, severity and period of the stress and vegetative and reproductive stage of plant (Khan et al., 2011; Razzaq et al., 2013). Due to stomatal closure, photosynthetic effectiveness of the majority of plants undergoes suppression under drought stress, which limits diffusion of CO_2 in leaf (Ali and Ashraf, 2011).

The objective of this study was to identify impact of extreme drought conditions and availability and utilization of nutrients by plants from drought soil, including its effects on yield and other characters such as Number of Cluster plant^{-1}, Number of Seeds pod^{-1} and Number of pods plant^{-1}. Soil and crop management practices to alleviate negative effects of drought and heat stresses are also discussed. Investigations involving determination and identification of most stress-tolerant plant genotypes are essential for understanding the complexity of the responses and for future plant breeding.

MATERIALS AND METHODS

The experiment was conducted at experimental field of Agricultural Research Station, Bahawalpur at latitude and longitude of 29.3957°N, 71.6833°E and altitude 461 m, respectively. The experiment consisted of two sets (drought and irrigated) with five genotypes including two check varieties for comparison. The experiment was sown in Randomized Complete Block Design (RCBD) with three replications keeping plot size of 2.7 x 7.2 m. In Drought set, only soaking dose was applied for proper germination followed by no irrigation during whole growing season while in irrigated set, 4 irrigations were applied at different stages of plant growth.

Eight soil samples were collected from experimented field i.e 4 samples before sowing and 4 samples after harvesting at 6 and 12 inches depth in order to check the utilization of nutrients by plants in irrigated and extreme drought soil conditions. The S1B3 and S2B3 are soil sample taken from drought field set before sowing and after harvesting of guar crop at 0 to 6 inches and 6 to 12 inches depth, respectively while S1B4 and S2B4 were soil sample taken from irrigated set at depth of 0 to 6 and 6 to 12 inches, respectively. The nutrients utilized by plants were calculated by subtracting the nutrients value obtain from soil analysis after harvesting to the nutrients value of soil analysis, before sowing of crop. Soil samples were got analyzed from Soil and Water Testing Laboratory, Bahawalpur in order to check the available utilized nutrients in soil before and after sowing of guar.

Procedure for soil nutrients analysis

Extraction of nitrogen (Nitrate NO_3^-) and organic matter

Turn on the balance, set a weigh boat on top, and zero the balance. Use a spatula to weigh out 10 g of soil (dried and sieved) and transfer to a labeled 100-mL beaker. Weigh out 0.1 g of calcium sulfate and transfer it to the beaker. Using a 25-mL graduated cylinder measure 20 mL of deionized water and transfer to the beaker. Repeat steps for each nitrogen soil sample. Thoroughly mix the contents of each beaker with a stir rod. Secure samples on a table-top shaker and shake for 1 min.

Extraction of phosphorus and potassium

Turn on the balance, set a weigh boat on the top, and zero the balance. Use a spatula to weigh out 2 g of soil (dried and sieved) and transfer into a labeled 100-mL beaker. Use a 25-mL graduated cylinder to measure 20 mL of Mehlich 2 soil extractant into the cylinder. Transfer to beaker. Repeat steps for each phosphorus and

Table 1. Soil analysis before sowing and after harvesting of guar crop.

S/No	Detail		Organic matter (%)			Nitrogen (%)			Available Phosphorus (ppm)			Available Potassium (ppm)		
	Acre No.	Depth (inches)	BS	AH	UB P	BS	AH	UB P	BS	AH	UB P	BS	AH	UB P
1	S1B3	0-6	0.98	0.31	0.67	0.51	0.16	0.34	7.1	7	0.1	169	125	44
2	S2B3	06-12	1.24	0.26	0.98	0.64	0.13	0.50	8.2	7.5	0.7	213	121	92
3	S3B4	0-6	1.29	0.46	0.83	0.67	0.23	0.43	7.3	6.3	1	249	80	169
4	S4B4	06-12	1.03	0.31	0.72	0.53	0.16	0.37	6.9	4.7	2.2	253	75	178

Note: BS (soil analysis before sowing of guar crop), AH (soil analysis after harvesting), UBP: [BS-AH](Nutrients utilized by plants).

potassium sample. Thoroughly mix the contents of each beaker with a stir rod. Secure samples on a table-top shaker table and shake for 5 min.

Nutrient extraction filtration - This step will be performed for all three analyses (nitrate, phosphate, and potassium)

Secure one end of the funnel hose onto a vacuum jet and the other to the side arm of the flask. Assemble the funnel by snapping together the cylinder and perforated top disk. Place the assembled funnel on the side-arm flask by inserting the rubber stopper on top of the flask, to secure the funnel and place 1 clean filter paper on top of the funnel. Turn on the vacuum jet.

Slowly pour soil extract solution into the funnel, allowing the extract to drain away from the soil into the bottom of the funnel flask. Pour filtered extract into a new, labeled 50-mL beaker. This filtrate will be analyzed as is. Remove funnel, discard filter paper, and rinse funnel and flask with deionized water. Use air jet to dry funnel and flask. Repeat steps for each soil sample.

The climatic data was also recorded from internet weather website of Bahawalpur location regarding maximum, minimum and average temperature, dew point, humidity and rainfall. One bag of DAP fertilizer was added to field before sowing of drought and irrigated sets. The data were recorded from five randomly selected plants of each genotype of the following traits i.e. Plant height (cm), number of clusters plant^{-1}, pod length (cm), number of seeds pod^{-1} and number of pods plant^{-1} from both irrigated and drought fields. Then, total and average five randomly selected plants from three replications were calculated. The average data of three replications was subjected to analysis of variance (ANOVA) and correlation among characters by statistical tool for agricultural research (STAR) version: 2.0.1 software.

RESULTS AND DISCUSSION

Nutrient uptake

The soil analysis was completed for drought and irrigated set before sowing and after harvesting of guar crop, which revealed that plants utilized available nutrients (Organic Matter, Nitrogen, Phosphorous and Potassium) present in the soil.

The organic matter utilized by plants was 0.67 and 0.98% in S1B3 and S2B3 while 0.83 and 0.72% in S1B4 and S2B4, respectively. The available soil Nitrogen, Phosphorous and Potassium were utilized by plants in drought set (0.34, 0.50%), (0.1 and 0.7 ppm) and (44, 92 ppm), at depths of 6 and 12 inches, respectively. S1B4 and S2B4 revealed that plants utilized maximum available of soil organic matter (0.83, 0.72%), Nitrogen (0.43, 0.37%), Phosphorous (1, 2.2 ppm), and potassium (169, 178 ppm) (Table.1). From these values it is clear that genotypes in irrigated conditions used more soil nutrients as compared to genotypes in drought conditions.

The maximum uptake of nutrients to plants is only possible with availability of water in soil and genotypes in drought set remained in stress during the May and June due to high temperature (41.52°C) and (41.83°C) and low precipitation (0.51 mm). Guar is known for its drought tolerance and grows without irrigation even in areas with as little as 250 mm of annual rainfall (Undersander et al., 1991). The plants in drought set were recovered in the months of July and August because of low temperature and rainfall (11.93 and 69.85 mm) as compared to May and June and uptake of the available nutrients in soil (Table 2). Two genotypes i.e. S-5744 and S-5824 performed well in both drought and irrigated conditions which gave maximum yield (1133.3 and 1300 g) as compared to other genotypes with yield 900 and 666.67 g.

Plant height (cm)

Genotypic Mean Square (GMS) for plant height (284.5507) and Genotypic F value showed significant results at 5% level of significance (24.50) in drought set while irrigated set also showed significant results for plant height with highest GMS (1930.6293) which showed significant results (1.74) in irrigated conditions, indicating the existence of variations among the

Table 2. Weather data of 7 months during guar crop season 2016.

Month	Temperature (°C)			Dew point (°C)			Humidity (%)			Precipitation (mm)
2016	High	Avg.	Low	High	Avg.	Low	High	Avg.	Low	Total
May	41.52	34.71	28.03	21	17.58	14.097	53.87	35.19	15.94	0.51
June	41.83	36.3	30.4	24.1	21.37	18.433	58.23	41.33	22.87	0.51
July	38.77	33.97	29.23	26.5	25.1	23.355	74.48	57.39	37.55	11.93
August	36.81	32.06	27.74	26.7	25	23.226	79.45	63.84	43.35	69.85
September	36.93	31.97	26.93	24.6	22.9	21.3	75.53	57.93	35.47	3.05
October	35.68	28	20.52	19.6	17.87	15.452	72.65	51.1	24.77	0

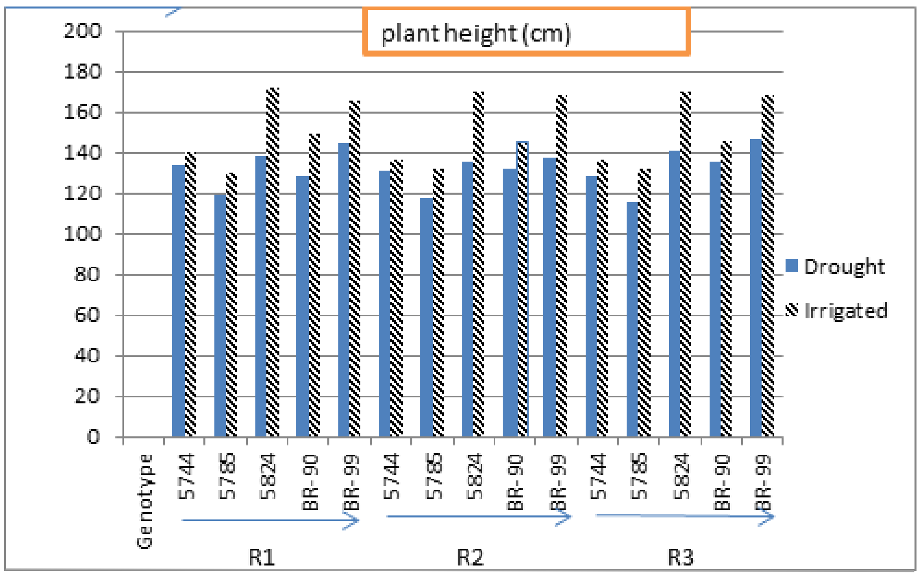

Figure 1. Comparison between drought and irrigated set for plant height (cm).

genotypes under study.

The plant height showed negative correlations with number of clusters plant^{-1} in drought (r^2=-0.23) and irrigated (r^2=-0.4385) conditions which means that as the Plant height of genotypes increases, the number of clusters plant^{-1} decreases while plant height showed positive correlation with other characters (Tables 5 and 6).

The two genotypes S-5824 and BR-99 were taller in both irrigated and drought conditions and performed well under both conditions, results are agreed with that of Ali et al. (2015), which stated that plant height of accessions BR99, BWP 5595, was less affected under water deficit conditions. On overall basis of all genotypes showed poor performance under drought conditions as compared to irrigated conditions (Figures 1 to 6).

Number of clusters plant^{-1}

The GMS for the number of clusters plant^{-1} was 29.9160 and Genotypic F value was 48.72 which showed highly significant results at 5% level of significance in drought set while in irrigated set, GMS (117.4893) and Genotypic F (2.58) showed highly significant results at 5% probability (Tables 3 and 4).

The Number of Clusters Plant^{-1} showed negative correlation with plant height (r^2=0.23), number of seeds pod^{-1} (r^2=0.5549), pod length (r^2=-0.5898) and number of seeds plant^{-1} (-0.3892) in drought set (Table 5). Under irrigated conditions number of cluster plant^{-1} had negative correlation with all characters except number of pods plant^{-1} (Table 6). The genotype BR-90 had maximum number of clusters plant^{-1} in drought set as compared to irrigated set while other four genotypes namely S-5744, S-5785, S-5824, BR-90 performed better in irrigated set and less number of clusters are formed in drought set.

Number of seeds pod^{-1}

The genotypes had highly significant differences with

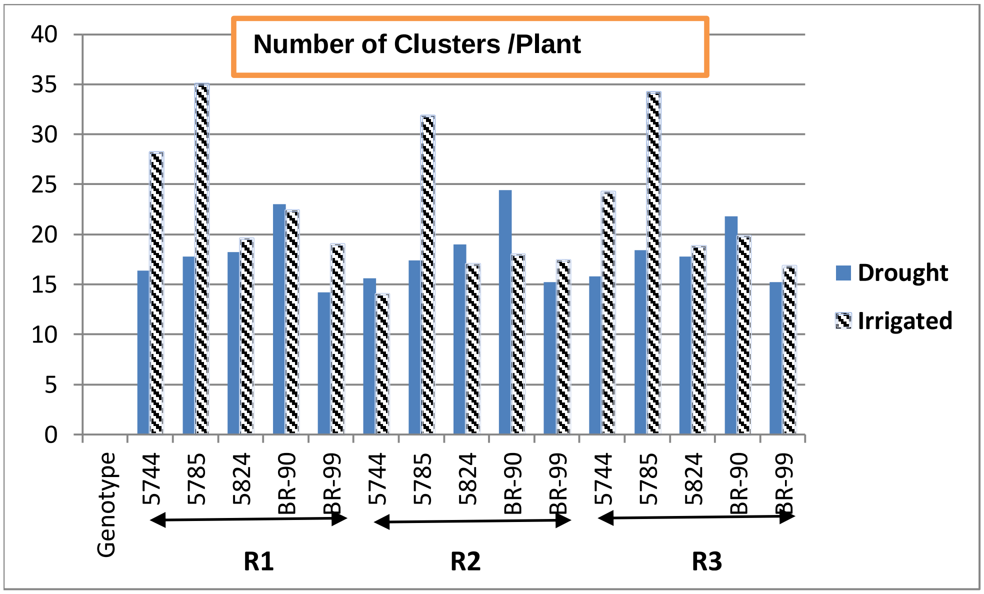

Figure 2. Comparison between drought and irrigated set for number of clusters plant^{-1}.

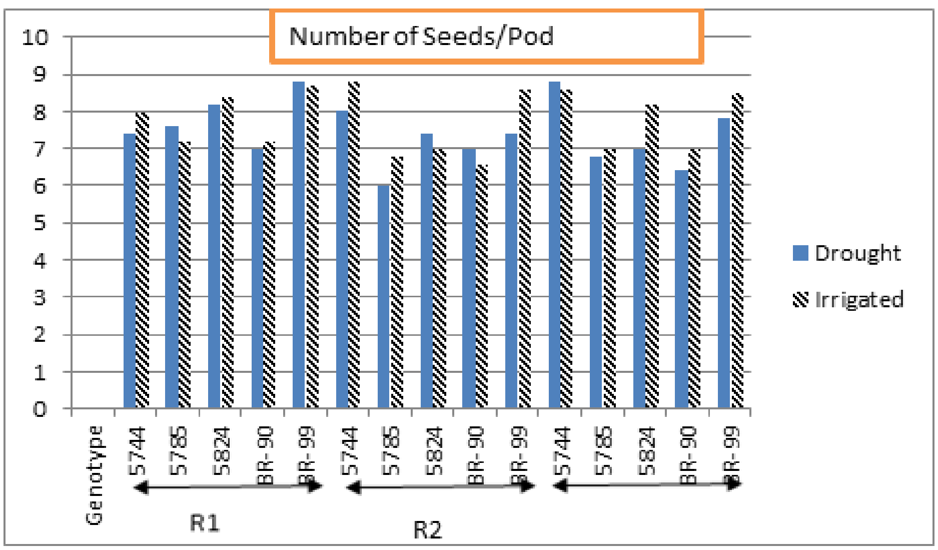

Figure 3. Comparison between drought and irrigated set for number of seeds pod^{-1}.

genotypic mean square value of (1.1507 and 873.5773) and Genotypic F value of 2.86 and 1.03 which is greater than probability value under both soil conditions, respectively (Tables 3 and 4).

The number of seeds pod^{-1} showed positive correlation with pod length (r^2= 0.9229) and number of pods per plant (r^2= 0.572) in drought set which means that an increase in pod length and number of pods plant^{-1} results in increase in the number of seeds pod^{-1} (Table 5), while irrigated set number of seeds pod^{-1} showed positive correlation with other two characters as shown in Table 6. The five genotypes performed well in the number of seeds pod^{-1} in three replications, BR99, S-5785, S-5824, BR-90 and S-5744 and showed maximum number of seeds pod^{-1} in drought condition at zero irrigation which only depend on rainfall and it means that, these genotypes will perform better in highly drought conditions at high temperature.

Pod length (cm)

The genotypes showed significant results for pod length and variations presents between the genotypes because

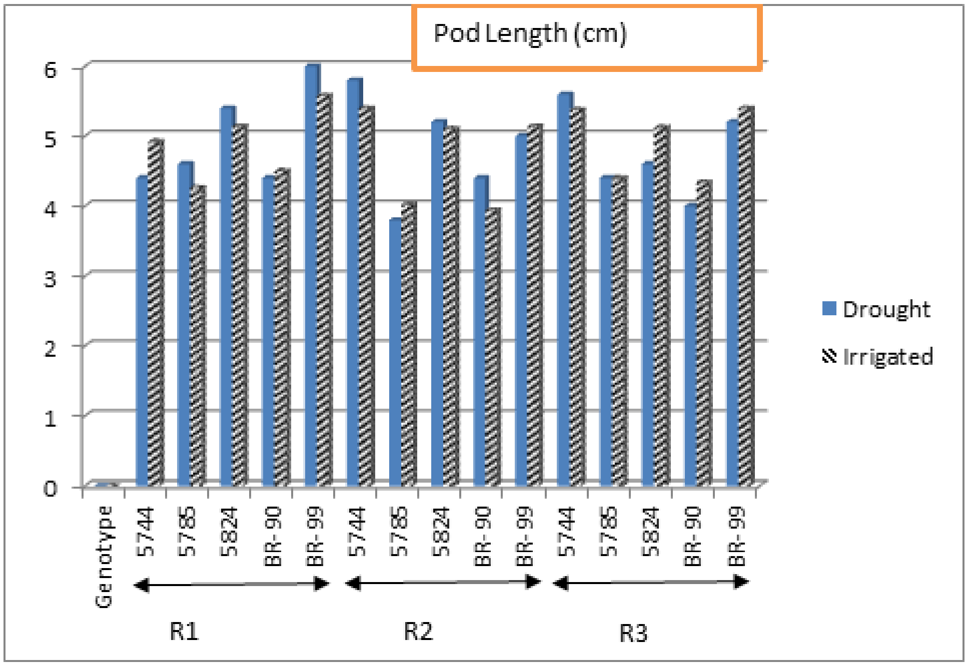

Figure 4. Comparison between drought and irrigated set for pod length (cm).

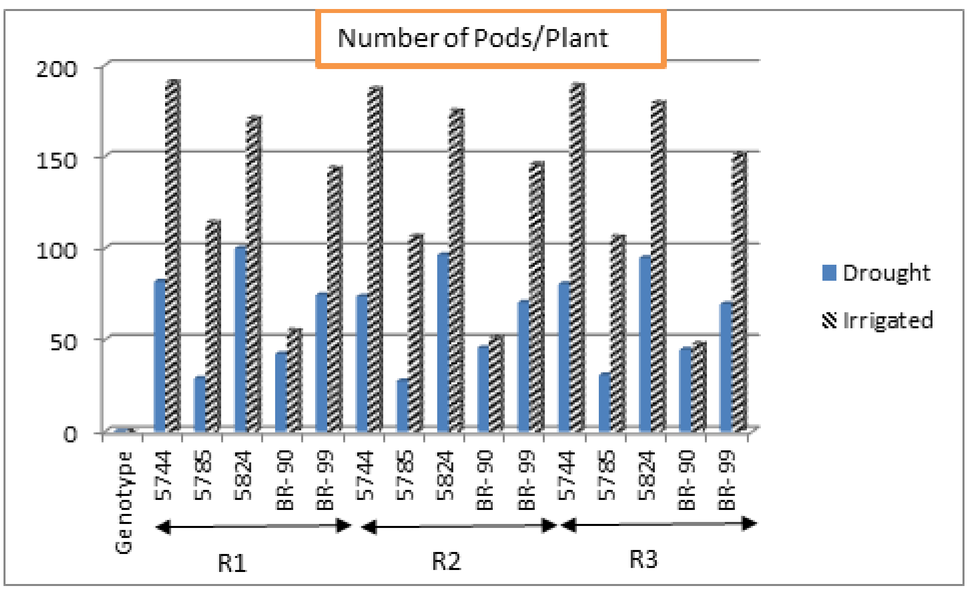

Figure 5. Comparison between drought and irrigated set for number of pods plant^{-1}.

of the significant Genotypic F value (3.00) obtained, which was greater than Probability F value in drought set (Table 3). Since the genotypes of irrigated set have highly significant results among the genotypes for pod length, variations were present in genotypes due to significant Genotypic F value 20.07 (Table 4). The positive correlation was found between pod length and number of pods plant^{-1} in drought and irrigated set (0.6331 and 0.7850) (Tables 5 and 6).

The comparison among the genotypes S-5785, S-5824, BR-99, S-5744, BR-90 performed better in drought conditions for pod length in three replications. These genotypes had the ability to perform well in drought conditions with no irrigation and depend only on rainfall.

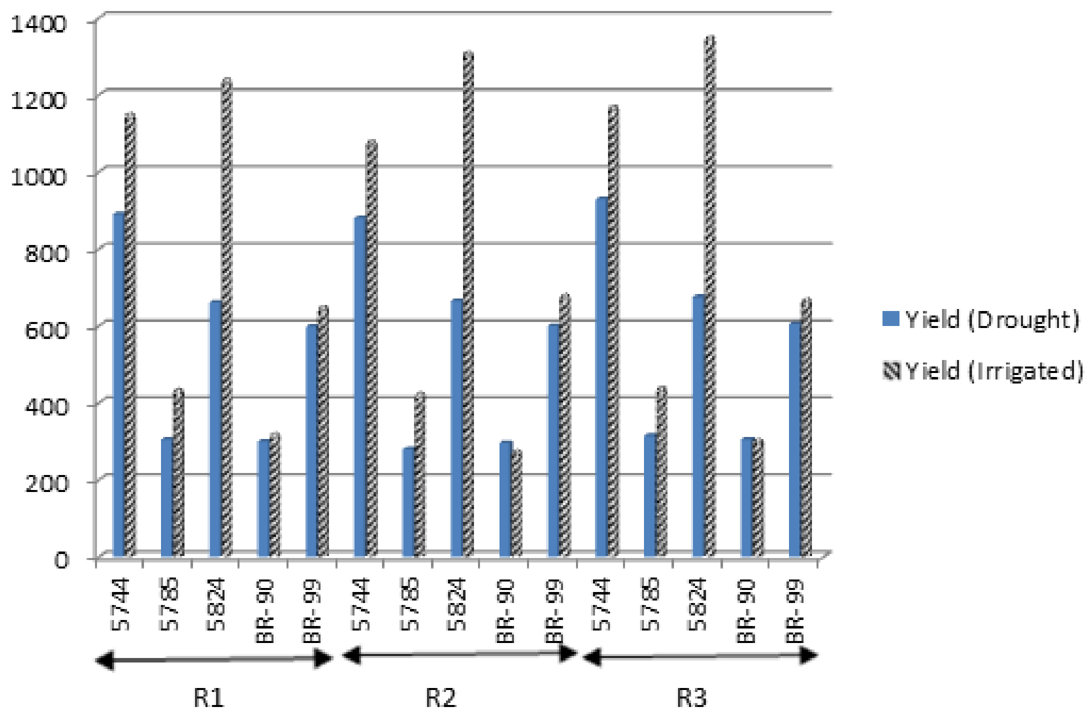

Figure 6. Comparison between drought and irrigated set for yield.

Table 3. ANOVA of five plant characters in drought set.

Trait	Replication mean square	Genotypic mean square	Error mean square	Replication F value	Genotypic F value	Probability value 5%
Plant height (cm)	10.3280	284.5507	11.6147	0.89	24.50**	0.4480 0.0002
No. of cluster plant^{-1}	0.3707	29.9160	0.6140	0.60	48.72**	0.5699 0.0000
No. of seeds pod^{-1}	0.5360	1.1507	0.4027	1.33	2.86**	0.3169 0.0964
Pod length (cm)	0.0507	0.9027	0.3007	0.17	3.00**	0.8478 0.0869
No. of pods plant^{-1}	10.0880	2233.8693	7.3313	1.38	304.70**	0.3065 0.0000
Yield (g/plot)	637.2667	197737.2667	121.0167	5.27	1633.97	0.034

Number of pods plant^{-1}

The genotypic mean square (2233.8693) and Genotypic F value (304.70) showed that, genotypes possessed highly significant and maximum variations which is a good sign for selection of better performing genotypes in drought set (Table 3). The genotypes in irrigated set also had significant results due to high value of Genotypic F (542.13), and showed more variations among the genotypes (Table 4).

Positive correlation was observed in number of Pods plant^{-1} with all other characters except the number of cluster plant^{-1} in drought conditions. Three genotypes had maximum number of pods plant^{-1} in irrigated conditions

Table 4. ANOVA of five plant characters in irrigated set.

Trait	Replication mean square	Genotypic mean square	Error mean square	Replication F value	Genotypic F value	Probability value
Plant height (cm)	803.8107	1930.6293	1106.7473	0.73	1.74**	0.5131 0.2331
No. of cluster plant^{-1}	8.8640	117.4893	45.5873	0.19	2.58**	0.8271 0.1185
No. of seeds pod^{-1}	842.2527	873.5773	847.9593	0.99	1.03**	0.4118 0.4478
Pod length (cm)	0.0593	0.9226	0.0460	1.29	20.07**	0.3269 0.0003
No. of pods plant^{-1}	4.1787	9251.6093	17.0653	0.24	542.13**	0.7885 0.0000
Yield (g/plot)	1581.6667	568333.3333	1142.0833	1.38	497.63	0.3045 0.0000

Table 5. Correlation between five plant characters in drought set.

Correlation	Plant height	No. of clusters plant^{-1}	No. seeds pod^{-1}	Pod length	No. of pods plant^{-1}
No. of clusters plant^{-1}	-0.2300	-	-	-	-
No. seeds pod^{-1}	0.4175	-0.5549	-	-	-
Pod length	0.4855	-0.5898	0.9229	-	-
No. of pods plant^{-1}	0.7079	-0.3892	0.5720	0.6331	-
Yield (g)	0.4310	-0.6252	0.6671	0.6731	0.8210

Table 6. Correlation between five plant characters in irrigated set.

Correlation	Plant height	No. of clusters plant^{-1}	No. seeds pod^{-1}	Pod length	No. of pods plant^{-1}
No. of clusters plant^{-1}	-0.4385	-	-	-	-
No. seeds pod^{-1}	0.2696	-0.7911	-	-	-
Pod length	0.4826	-0.2852	0.2289	-	-
No. of pods plant^{-1}	0.4213	0.0865	0.0894	0.7850	-
Yield	0.3665	0.1096	-0.0996	0.6944	0.9053

compared to drought conditions and the genotypes S-5744, S-5824 and BR-99 performed well in irrigated conditions which means that these genotypes are very sensitive in shortage of water and produce less number of pods plant^{-1}.

Yield (grams)

The Genotypic Mean Square (197737.2667) and Genotypic F value (1633.97) showed highly significant differences among the genotypes in drought set which illustrated the significant variation among genotypes (Table 3). The similar trend was observed for GMS (568333.3) and Genotypic F Value (497.63) in irrigated set (Table 4).

The comparison between drought and irrigated conditions for yield showed that two genotypes (S-5744 and S-5824) performed well in the drought and irrigated environment and gave maximum yield (900 and 666.67 g) in drought and (1133 and 1300 g) irrigated conditions, as compared to other genotypes (Tables 7 and 8). Yield showed positive correlation in drought conditions with all

Table 7. Five characters of genotypes in drought trial.

Characters	BR-99	BR-90	S-5744	S-5824	S-5785
Plant height (cm)	143.07	132.20	130.93	138.60	117.40
No. of clusters plant^{-1}	14.87	23.07	15.93	18.33	17.87
No. of seeds pod^{-1}	8.00	6.80	8.07	7.53	6.80
Pod length (cm)	5.40	4.27	5.27	5.07	4.27
No. of pods plant^{-1}	71.47	44.13	78.80	97.07	29.13
Yield (g/plot)	601	300	900	666.67	300

Table 8. Five characters of genotypes in irrigated trial.

Characters	BR-99	BR-90	S-5744	S-5824	S-5785
Plant height (cm)	168	147.30	137.30	172.10	129.50
No. of clusters plant^{-1}	17.73	21.13	25.80	19.73	33.67
No. of seeds pod^{-1}	8.60	6.90	8.50	8.20	7
Pod length (cm)	5.40	4.30	5.20	5.10	4.20
No. of pods plant^{-1}	146.9	51.33	189	175.1	109
Yield (g/plot)	666.67	300	1133.30	1300	433.33

characters except the number of clusters plant^{-1} which showed negative correlation while in irrigated conditions, yield possessed positive correlation except the number of seeds pod^{-1} which showed negative correlation (Tables 5 and 6).

Conclusion

From this experiment, it is concluded that two genotypes S-5744 and S-5824 performed well under drought and irrigated conditions and produced more number of seeds pod^{-1}**,** pods plant^{-1}, pod length and finally the grain yield, which means that these two genotypes were selected on the basis of graphical data and had better nutrients and water use efficiency under shortage of water and higher temperature/climatic conditions. These genotypes (S-5744 and S-5824) will be helpful for increase guar production in the country, if release for general cultivation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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INTRODUCTION

A significant reduction of economic growth occurred in many countries, as a result of the world crisis, which started in the United States in October of 2007. This happens, specially, in the European Union and in Japan, desencouraging international commerce and impacting in a generalized way the balance of payments and the economic growth of numerous countries, including Brazil in recent last years. The evolution of the Brazilian agricultural exports for the 2000 to 2014 period is presented in Table 1. As it can be observed, the total Brazilian exports started to change in value, significantly, from the year 2000, due to the effects of the new Brazilian commercial policy. The growth in Brazilian agricultural exports was notorious and exceeded the value of US$ 20 billion beginning a strong growth tendency. In 2005, when its annual exports was already up to US$ 32.21 billion, it went on into an expansion tendency upto the value of US$ 63.75 in 2010. In other words, a 98.92% increase in only five years. In the 2010 to 2014 period, the Brazilian agricultural exports presented a 29.62% increase.

Table 1. Brazilian agricultural exports, 2000 - 2015 period (in US$ billions).

Year	Value (US$)	Year	Value (US$)
2000	13.16	2008	58.36
2001	16.59	2009	54.83
2002	17.43	2010	63.75
2003	21.71	2011	81.80
2004	28.36	2012	83.41
2005	32.21	2013	84.18
2006	36.54	2014	82.63
2007	44.89	2015	-

Source: MDIC - SECEX (2015)

Among the major Brazilian agricultural exported products, in 2014, the soybeans complex with US$ 31.4 billion was the most important item with 13.95% of total exported value. Secondly, meat with a 7.74% participation followed by the sugar/ethanol complex with a 4.61% participation, as it can be seen in Table 2.

Table 2. Major Brazilian agricultural exported products, 2014 (in US$ millions).

Products	Value (US$)	Proportion (%)
Soybeans complex	31,403	13.95
Meat	17,429	7.74
Sugar/Ethanol complex	10,367	4.61
Forestry's products	9,951	4.42
Coffee	6,662	2.96
Tobacco and derivatives	2,502	1.11
Total	225,101	100.00

Source: MDIC - SECEX (2015)

In relation to the destination of Brazilian agricultural exports, China has been Brazil's major commercial partner with a 28.8% participation of the total exported value, followed by the United States with a 7.24%, Holland with 6.33% and Russia with 3.78% participation (Table 3).

Table 3. Major destinations of Brazilian agricultural exports, 2014.

Destination	Value (US$)	Proportion (%)
China	22,066,246.752	22.81
United States	6,999,951.209	7.24
Holland	6,128,300.884	6.33
Russia	3,652,839.330	3.78
Germany	3,475,948.021	3.59
Venezuela	3,045,359.172	3.15
Hong Kong	3,020,688.455	3.12
Total	96,747,880.752	100.00

Source: MDIC - SECEX (2015)

Therefore, the major objective of this paper was to analyze the effects of the exchange rate and the world income on the Brazilian agricultural exports for the 2000 to 2014 period. Additionally, its objective was also to verify if the shocks resulted from the exchange rate and world income represented significant oscillations in Brazilian agricultural exports along the study period.

In order to identify and to evaluate major variables that affect Brazilian exports, co-integration tests, as well the vector error correction model (VECM) were used for the short and long run, to verify how they react to changes in the long run equilibrium relations. To analyze how exchange rate and world income variations are transmitted to Brazilian agricultural exports along time a response- to impulse function was calculated, considering a twelve month period.

LITERATURE REVIEW

Major studies done in recent years, related to national and regional exports, in an aggregate manner or by products, giving emphasis to those that used vector auto regressive (VAR) and VECM models were reviewed as those used in this paper.

Castro and Cavalcanti (1998), using a VECM methodology, estimated total export and import equations for Brazil using annual value data (in US dollars) for the 1955 to 1995 period. The estimations were disaggregated by aggregated factor and category use. The authors included in the model, the following explained variables: real exchange rate, a proxy for the world income level, and an indicator for the domestic income level. The obtained parameters results were significant.

Brazilian agricultural products export supply functions were estimated by Barros et al. (2002). In their study, a theoretical model was developed to support the adjusted econometric models specifications for different products for which the export supply equation was derived from supply and domestic demand functions. The models were adjusted by the method of minimum ordinary least squares including the VECM in the case of co-integrated variables. The obtained elasticities presented coherent signs with the defined economic model. The exchange rate resulted to be of major importance for the Brazilian agricultural exports.

Alves and Bacchi (2004) estimated a Brazilian export sugar supply function using a vector auto regressive model with identification by the Bernanke process. The data used are for the October 1995 to the December 2001 period. The specified equation used to evaluate the impact variations on export conditions is fundamental for a theoretical model that assumes to have in part an exceeding domestic market. The integration and co-integration properties of the utilized series in the model were considered in the analysis. The results show that an increase in the export prices and exchange devaluations yield significant increase in Brazilian exports. On the other hand, an increase in domestic income and domestic prices has negative reflexes on the exported *quantum*. The more expressive effects of a same percent variation on the exports conditions of the sugar exported *quantum* happen in the case of the domestic income variable.

Resende and Godoy (2005) showed in their study that the developing economies and its investment rates are more sensitive to those type of fluxes. Therefore, their competitive gains and exports would be cycle functions of their international liquidity. It postulates that Brazilian exports are function of international liquidity cycles and of other variables traditionally contemplated in the Brazilian export equation. A Brazilian export equation specified in that way was estimated by the Engle-Granger (error correction mechanism) and Johnson methods. The results do not reject the hypothesis of the existence of a long run relationship between international liquidity and Brazilian exports.

Silva and Bacchi (2005) examined export equations for Brazilian gross sugar, with the objective to identify the export behavior determinants of this commodity. The authors used a VAR/VECM considering the integration and co-integration properties of the series analyzed in the study. The results clearly demonstrated that the Brazilian gross sugar exports depended on Russia's exchange rate, and on the domestic price. The last variable had no significant effect on the exported *quantum*.

The impact of the January 1999 change in the Brazilian exchange regime, and the following transformations experienced by the Brazilian agricultural sector were evaluated by Ferreira et al. (2006). Their study, specifically analyzed the Brazilian commercial balance in relation to the performance of the Gross National Product (GNP) or the agricultural product, world income and the real exchange rate for the 1980 to 2006 (up to the first trimester). The major conclusions were that the former exchange rate band policy, in the first five years of the Plano Real, had a negative influence in exports and favored imports. After the exchange liberation of 1999, even though the estimates were not statistically significant, the reverse indications of this scenario were positive.

Santos et al. (2010), using an Ordinary Minimum Square (OMS) model, estimated the export elasticity as a function of the world income, exchange rate, international and domestic prices. The results showed that in 1995 to 2009 period, Brazilian exports were significally influenced by the exchange rate and the world income.

Padrão et al. (2010) analyzed the determinants of the export coffee supply of the State of Minas Gerais for the July 1999 to December 2009 period. The econometric model used by the authors was the VECM, analyzing the impulse-response functions, as well as the variance decomposition of the prevision error. The results showed that quantity of exported coffee was affected, basically, by the international price and the real effective exchange rate.

Souza and Ferreira (2013) used a VECM and the Granger causality test in order to verify how the soybean international price and the exchange rate influence domestic price formation in the states of Mato Grosso, Paraná, Rio Grande do Sul and Goiás. The results demonstrated that variations in the international price of the soybean commodity and the exchange rate affected the soybean price in the four states and that the price of Mato Grosso did not suffer any influence from other states.

The study of Monte (2015), using a VAR/VECM methodology, had the objective of estimating the impact of the exchange rate and world income shocks on the exports of the state of Espírito Santo. The results indicated that the variables are co-integrated, while in the long run equation, the world income affected significantly the exports. The exchange rate presented a negative sign and was not significant. In the short run, the "export" variables revealed that short run disequilibrium is quickly corrected in relatively fast way, what does not happen to the "exchange rate" and "world income" variables. In the impulse-response functions, it was verified that a shock in the exchange rate had negative effects on the exports in almost all periods after the shock, as opposed to one would expect from economic theory, and the world income affects positively the exports.

METHODOLOGY

Proposed model

Several papers dedicated to the study of aggregate exports and trade products in an individual matter can be found in the literature of international trade. Among the studies focusing on export functions the most known are Morais and Barbosa (2006), Cavalcanti and Ribeiro (1998), Carvalho and De Negri (2000), Barros et al. (2002), Silva and Maia (2003), Alves and Bacchi (2004), Silva and Bacchi (2005), and Moraes and Barbosa (2006) are most known.

In this paper, part of the proposed model developed by Castro and Cavalcanti (1998) was used. Hence, the export equation can be expressed by:

$$x = e + y \qquad (1)$$

where x = exports real value; e = real exchange rate; y = total world imports, in real value, as a proxy for world income.

Positive shocks on the real exchange rate and on the world income are expected to yield positive impacts on Brazilian agricultural exports. Therefore, the econometric analysis used to evaluate the existence and the intensity effect among the Brazilian agricultural exports, world income and real exchange income, was done using a Vector Auto Regressive (VAR) model, which the main objective is to analyze variance decomposition and impulse response functions.

The VAR model can be expressed in the following way:

$$X_t = A_0 + A_1 X_{t-1} + ... + A_p X_{t-p} + B_0 Z_t + B_1 Z_{t-1} + ... + B_p Z_{t-r} + e_t \quad (2)$$

where $A_0 =$ is a $n \times 1$ vector of the intercepted terms; $A_1,.....,A_p$ = is a $n \times n$ matrix of coefficients that relate the lag values of the endogenous variables to the current values of those variables; $B_0,.....B_p$ = is a $n \times m$ matrix of coefficients that relate actual and lag values of exogenous variables to current values of endogenous variables; e_t = is a $n \times 1$ vector of error terms. Each one of the X and Z variables are explained by their lag variables.

In order to choose the best VAR model, the Schwartz Criteria (SC) and the Akaike Criteria (AIC) were used. This was done in view of the importance of the lag number determination to be included in the VAR model, since it takes in consideration the sum square of the residues, the number of observations and the parameter's estimators. Therefore, the smaller the values the better the estimated model. Hence, to test the stationary series, the Augmented Dickey – Fuller (ADF) (Dickey and Fuller, 1981) without structural breaking and the Zivot and Andrews with structural breaking (1992) were used in this paper to verify the integration order of the variables of interest, *that is,* since it was necessary to verify the existence or not of unitary roots in temporal series.

The next step was to test for the existence of co-integration among a set of economic variables using the Johansen and Juselius method (1990). This method is based on the following modified version of a VAR model:

$$\Delta y_t = \Gamma_1 \Delta y_{t-1} + ... + \Gamma_{p-1} \Delta y_{t-p+1} + \Pi y_{t-1} + \varphi d_t + \mu + \varepsilon_t \quad (3)$$

where $y_t =$ is a vector with k variables; $d_t =$ is a vector of dummy variables used to capture stationary variations; and $\varepsilon_t =$ is the random error.

If r is the rank of the matrix Π, then Π has r characteristic roots (eigenvalues) or auto values statistically different from zero. Three different situations may occur: (a) if r = k, then y_t is stationary; (b) if r = 0, then Δy_t is stationary; (c) if 0 < r < k, then the α and *β* matrices are such as $\Pi = \alpha\beta'$ and the βy_t vector is stationary. Where *α* represents the velocity of adjustment of the matrix parameters in the short run, while *β* is a co-integration matrix of coefficients in the long run.

The null hypothesis of the existence of a co-integrated vector is tested by using trace statistic (λ_{trace}) and the maximum auto value statistic ($\lambda_{\max}$). The trace test is given by:

$$\lambda_{trace} = -2\ln(Q) = -T\sum_{i=r+1}^{n}\ln(1-\lambda_i) \quad (4)$$

where Q = (maximum restricted likelihood function/maximum non restricted likelihood function).

The maximum auto value test is given by:

$$\lambda_{\max} = -T\ln(1-\lambda_{r+1}) \quad (5)$$

where λ_i are the estimated values of the characteristic roots obtained from the Π estimated matrix and T is the number of observations. If the calculated values of λ_{trace} and $\lambda_{\max}$ are greater than the critical values, then the null hypothesis of no co-integration is rejected.

The upto here described procedures were useful to determine the long run equilibrium relationship among variables. Hence, Engle and Granger (1987) demonstrated that even presenting a long run equilibrium relationship among no stationary variables (in level), it is possible to occur a disequilibrium in the short run, that is, the short run dynamic is influenced by the magnitude of the deviation in relation to the long run equilibrium. The mechanism that conducts the variables to equilibrium is known as the VECM, by which it is possible to determine the velocity that the disequilibrium in the short run are eliminated.

The data

The data utilized in the estimation of the model specified earlier were monthly series for the January 2000 to December 2014 period for the following variables:

(1) Total value of Brazilian agricultural exports (in millions of US$ - FOB), source: Sistema Alice Web do Ministério do Desenvolvimento, Indústria e Comércio Exterior MDIC;
(2) Real effective exchange rate, source: IPEADATA (2015); and
(3) World imports as proxy for world income, source: IPEADATA (2015), with the FMI as the original source.

The EVIEWS 8.0 was used to run the unitary root tests, co-integration and to estimate the VAR model.

Estimation of the Brazilian agricultural exports function

Given the objectives of this paper, the following Brazilian agricultural exports function was initially used:

$$Export_t = Const.Rmundial_t^{\alpha}.Cambio_t^{\beta}\varepsilon_t \qquad (6)$$

where *Export* is the monthly value of Brazilian agricultural exports, in US$; $Câmbio$ is the real exchange rate in R$-US$; $Rmundial$ is the total value of the world imports in US$ (proxy variable used). The α and β terms are the respective elasticity's, and ε_t is the random error or the stochastic disturbance.

The expected signs for the empirical tests are:

$$\frac{\partial Export}{\partial RMundial} > 0 \qquad \frac{\partial Export}{\partial Cambio} > 0$$

The world income and exchange variations are expected to affect positively the Brazilian agricultural exports.

In order to rationalize the estimation process a log-log model was used, since the extraction of variables logarithms with a stochastic component (ε_t) is necessary. Therefore, yielding the following function:

$$L\exp ort = Const + \alpha Lrmundial + \beta Lcambio + \varepsilon_i \qquad (7)$$

To capture the world income and the real exchange rate effects on the Brazilian agricultural exports, the analysis was done monthly for the 2000 to 2014 period, generating 174 observations.

EMPIRICAL RESULTS AND ANALYSIS

Unitary root tests

The first step in the time series analysis was to verify how did the present stochastic generator process behaved along time, that is, to identify if the utilized variables are or not stationary. First, a graphic analysis of the series with the objective to verify the existence of a structural break on each variable used in the model was done.

Observing the Lexport variable, it can be verified that its behavior in all interval values follows an increasing tendency with no structural break, presenting a stationary characteristic and with a deterministic tendency behavior (Figure 1).

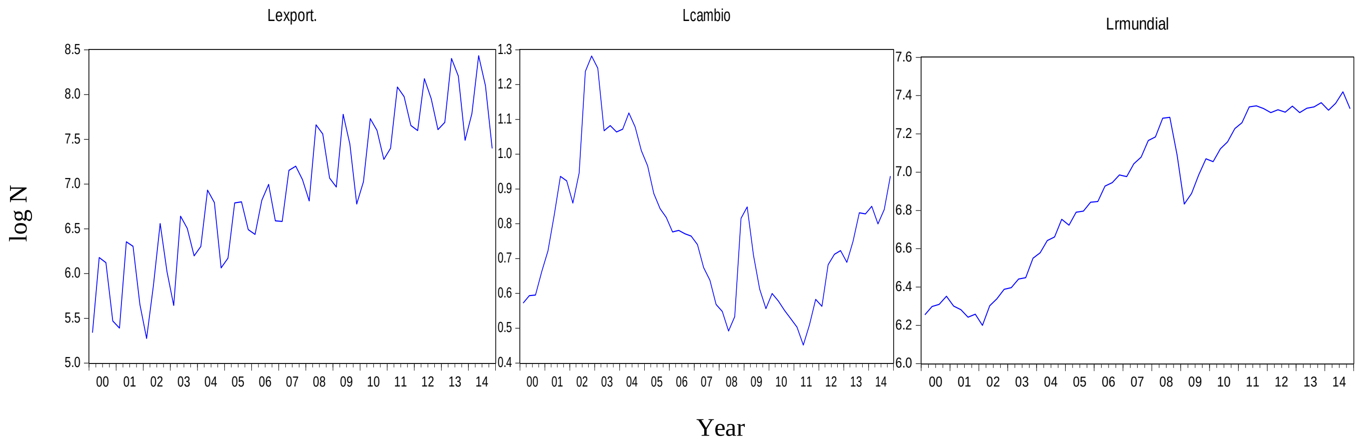

Figure 1. Graphic of Variables: Lexport, Lcambio and Lrmundial, January of 2000 to December of 2014 period.

For the real exchange rate variable (Lcambio), the presence of a structural break was detected, treated ahead when the unitary roots tests for the series were done. During the analysis of the world import variable (Lmundial), defined in the research as a proxy for world income, it can also be observed in Figure 1, that the variable presented a stationary deterministic tendency. However, with the presence of a level change outlier in the middle of the year of 2008, explained by the American crisis leading to an impact on international trade, resulting in a significant drop in world imports.

Hence, in order to begin the co-integration tests among the considered variables (Brazilian agricultural exports, world income, and real exchange rate) the stationary of the series was analyzed using the Augmented Dick-Fuller (ADF) test without structural break and the Zivot and Andrews test with structural break, with constant and tendency.

The results are presented in Tables 4 and 5 indicating that for the series in level, the unitary roots cannot be rejected at the 5% level of significance. Therefore, all series have unitary roots and are not stationary, that is, are not integrated in zero order I(0).

Table 4. Unitary root test (ADF).

Variable	Constant	Tendency	ADF Statistic	Critical value (5%)
Lexport	Yes	Yes	- 3.0802	- 3.4381
Lrmundial	Yes	Yes	- 2.4711	- 3.4380
Lcambio	Yes	Yes	- 2.1306	- 3.4360
DLexport[1]	Yes	Yes	- 4.8161	- 3.4381
Dlrmundial[2]	Yes	Yes	- 3.1839	- 3.1432[3]
Dlcambio[4]	Yes	Yes	- 7.6787	- 3.4363

Source: Based on the research results.

Table 5. Unitary root test with structural breaking: Zivot-Andrews.

Variable	Level (5%)	Critical (5%)	1^{st} Diference (5%)	Critical (5%)
Lexport	- 3.3465	- 5.08	- 5.4652	- 5.08
Lcambio	- 3.4580	- 5.08	- 7.4430	- 5.08
lrmundial	- 5.0550	- 5.08	- 5.1972	- 5.08

Source: Based on the research results.

[1] The letter D in the beginning of the variable refers to the first difference.
[2] The letter D in the beginning of the variable refers to the first difference.
[3] Significant at the level of 10%.
[4] The letter D in the beginning of the variable refers to the first difference.

The next step was to determine the lag number of the VAR model using the Akaike (AIC) and the Schwartz (SC) criteria. However, the Schwartz information criteria (SC) detected the smallest value for order two lag. The results are shown in Table 6.

Table 6. Lag number definition of the VAR model.

Lag	LogL	AIC	SC
0	-103.2602	1.2575	1.3131
1	501.3335	- 5.7909	- 5.5687
2	534.1781	- 6.0731	- 5.6842*
3	554.4133	- 6.2061	- 5.6505
4	566.2307	- 6.2394	- 5.5171
5	581.4068	- 6.3125*	- 5.4235

Source: Based on the research results.

Co-integration tests

To determine the number of co-integration vectors, trace tests indicated by λ_{trace} and eigenvalue by ($\lambda_{\max}$) were used, which results are presented in Tables 7 and 8.

Table 7. Determination of the number of co-integration vectors: trace test.

Null Hypothesis H_0	Alternative Hypothesis H_1	Test Statistic Λ_{trace}	Critical value 5%
R = 0	R = 1	49.6006	21.1316
R = 1	R = 2	7.2842	15.4947
R = 2	R = 3	1.2692	3.8415

Source: Based on the research results.

Table 8. Auto value maxim test.

Null Hypothesis H_0	Alternative Hypothesis H_1	Test Statistic (Λ_{tmax})	Critical value 5%
r = 0	R > 0	58.1540	29.7971
r ≤ 1	R > 1	8.5534	15.4947
r ≤ 2	R > 2	1.2692	3.8415

Source: Based on the research results.

Both tests suggest that the existence of a co-integration vector. The critical values at the 5% level of significance were adopted in realized tests (Tables 9 and 10; Figures 2 to 5).

Table 9. Long and short run coefficient estimates of the Vector Error Correction Model (VECM).

Variable	Short run coefficient (α)	Long run coefficient (β)
Lexport	- 0.3536	1.0000
Lrmundial	1.5042	1.8981
Lcambio	0.1684	0.2540

Author's estimation based on the research results.

Table 10. Variance decomposition of the exports prevision error.

Period	Lexport	Lcambio	Lrmundial
1	100.00	0.00	0.00
2	96.86	1.64	1.50
3	95.26	1.44	3.30
4	95.58	1.35	3.07
5	95.27	1.34	3.39
6	93.47	1.32	5.21
7	90.37	1.28	8.35
8	87.10	1.36	11.54
9	84.33	1.61	14.06
10	82.25	1.99	15.76

Source: Author's estimation based on the research results.

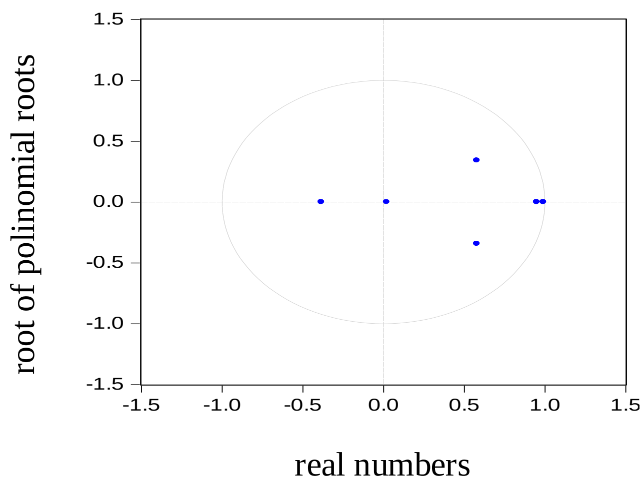

Figure 2. Inverse roots of the autoregressive characteristic polynomial

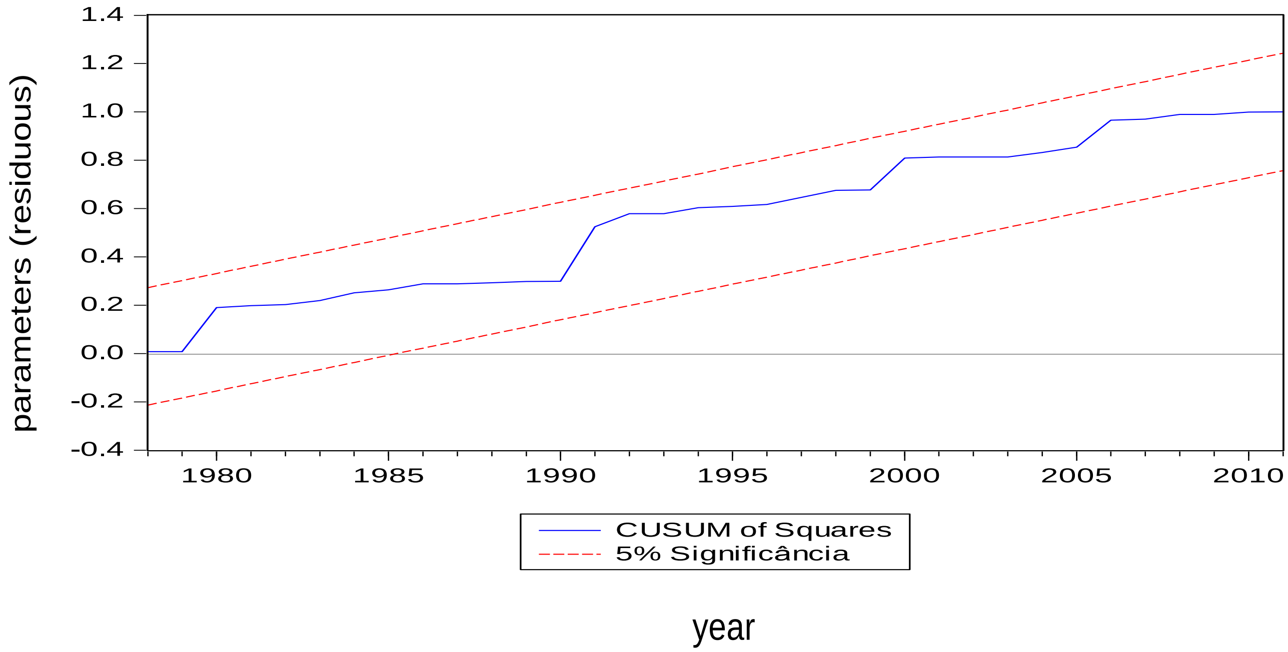

Figure 3. CUSUM test.

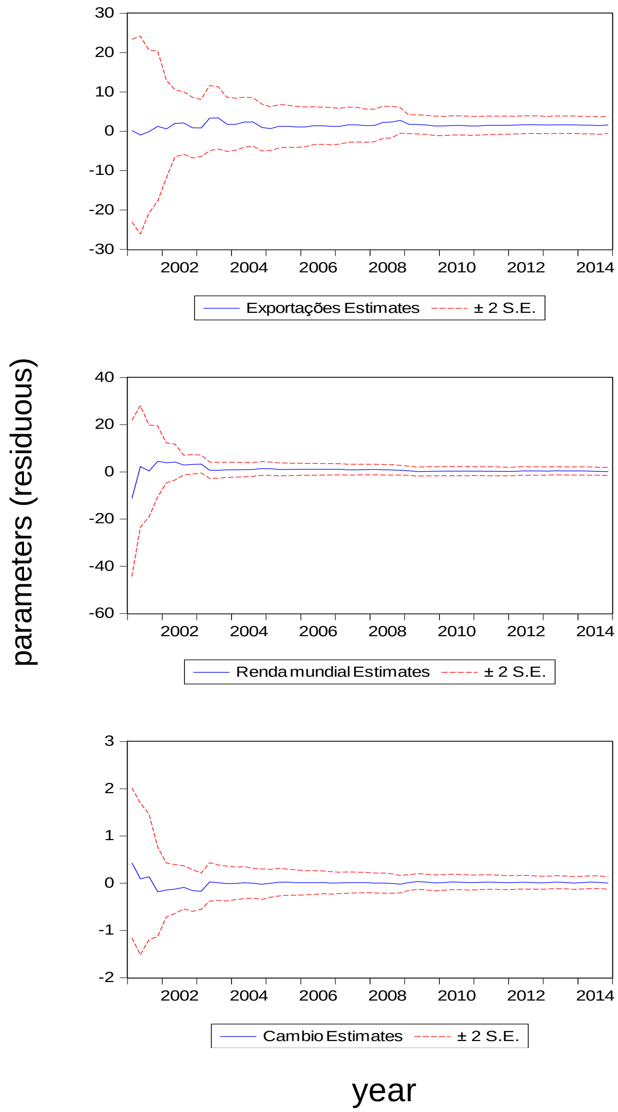

Figure 4. Parameters stability test: recursive residues

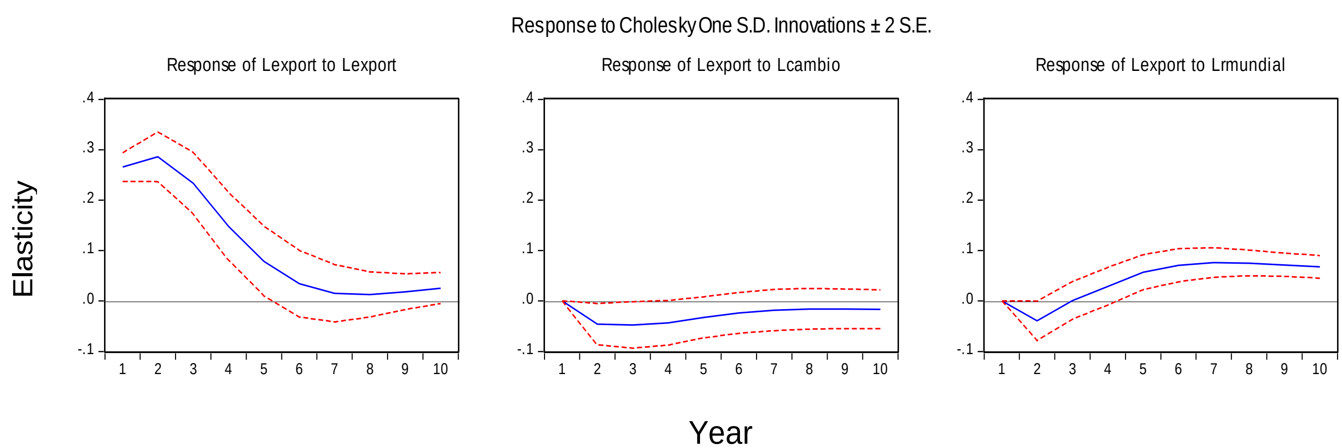

Figure 5. Impulse response functions and variance decomposition analysis.

DISCUSSION

The results showed that in the long run the coefficient value of Brazilian agricultural exports in relation to the exchange rate was inelastic (0.2450). However, the adjustment coefficient of the exported value in relation to the world income was 1.8981 (elastic). Therefore, if the real exchange rate variable is assumed constant, an increase of 10% in the world income in the long run should increase the exported value in about 20%.

In the short run, the adjust coefficients indicate a slow speed adjustment in the long run equilibrium direction for each variable. In other words, if disequilibrium occurs in any one of the model's variables, its correction towards the long run equilibrium will occur in a slow way.

Conclusions

The main objective of this paper was to analyze the short and long run effects of world income and effective real

exchange rate fluctuations on the value of Brazilian agricultural exports in the 2000-2014 periods (monthly).

The weightings set indicated a low speed of adjustment toward the long run equilibrium of each variable, that is, in the event of an imbalance in any one of the variables of the model, its own correction will slowly take place toward the long run equilibrium. In the variance decomposition analysis the results showed that the world income is more important than the exchange rate in the explanation of the variance error of the agricultural exports. It is interested to note that in the research done by Gonçalves Júnior (2005) the world income was the most important variable to explain the balance of payments outcome of the Brazilian agroindustrial complex with elasticity lightly higher than one.

The major conclusion of this paper is that both the exchange rate and the world income are relevant variables to explain the observed fluctuations values in the Brazilian agricultural exports during the analized period.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

African Journal of Agricultural Research